Trans. 45
(In full)
By:
I. Antik

451

Lepeshinskaia, O. B.

[Unsound criticism of T. D. Lysenko's work on species [formation] by N. V. Turbin and N. D. Ivanov.]

Bot. Zhur. (38):386-388
May/June 1953. 451 R923

(In Russian)

Nedobrokachestvennaia kritika N. V. Turbina i N. D. Ivanova raboty T. D. Lysenko o vide

A scientific, substantiated criticism is a factor which encourages progress in science. But a criticism with a tendency and a goal of discrediting, rightly or wrongly, a scientist, one who deservedly became a leading scientist, spiteful criticism based at best on misunderstanding of the teaching which is being criticised and at worst - on inventing things which do not exist in the teaching being criticised, on distortion of facts, on the aim to justify false, harmful teachings, advocated by the critics - such criticism is not scientific, it is detrimental. There should not be a place for such criticism in our country of socialism, in a country where progressive science has the support of our party and always used to have the personal support of comrade Stalin.

Reading the criticism of N.V. Turbin and N.D. Ivanov of T.D. Lysenko's article "New in the Science of Biological Species", I remembered, because of its similarity, the criticism by the 13 Leningradians in the "Meditsinskii rabotnik" /medical worker/ of my book "Origin of cells from a living substance and the role of the living substance in the organism". What a striking similarity, what a likeness between Turbin's and Ivanov's criticism and that of the 13 Leningrad morphologists! But one should be just; these

13 critics honestly realized their mistake and admitted it publicly and in the press. We hope that the criticism of Lysenko's critics will help them to realize their mistakes as it was in the case of the 13 Leningradians.

And what are their mistakes?

Are Academician Lysenko and the partisans of the new teaching on species really right when they maintain, that Darwin's Theory of Evolution is basically metaphysical, denying qualitative changes in the development of the living Nature? Are they really right, when they maintain that only the new teaching of species gives the sole correct, dialectically - materialistic solution of the problem of formation of species?

Lysenko approached the problem of formation of species as a materialist-dialectician and in complete accord with I.V. Stalin, who writes: "... Darwinism rejects not only Cuvier's cataclysms, but also the dialectically understood development which includes the revolution, while from the point of view of the dialectical method, evolution and revolution, quantitative and qualitative changes - are the two necessary forms of the same movement."[1)]

The critics are doubtful: is Darwinism really negating qualitative changes in the development of the living Nature? And they themselves answer this question with the words: "Yes, it is quite impossible to agree with T.D. Lysenko's statements that Ch. Darwin's theory of evolution is basically metaphysical."

But Darwin's theory of evolution recognizes only quantitative accumulation without qualitative changes. This is a sign of metaphysics.

---

1) I.V. Stalin, Complete Works, vol. 1, p. 309.

Where is, then, the real difference between Darwin's theory of evolution and T.D. Lysenko's new outlook on the species?

Darwin tells in details that the appearance of new species takes place by way of gradual and consecutive quantitative increments and thus it results that there are no borderlines between species, while T.D. Lysenko maintains that the formation of new features under the influence of changes in the surroundings, of a new species substance from which successively a new species originates - has a leaping character.

T.D. Lysenko says on the matter: "Species are links of the living Nature, they are stages of a qualitative distinctiveness, steps in the gradual historical development of the organic world"; and further: "The primary cause of the appearance of some species from others, as well as the cause of the appearance of intra-species variety of forms, is the change in conditions of life of plants and animals, changes of the metabolism type. Origin and development of new species is connected with such changes of metabolism type in the process of development of organisms, which concern their species specification." 1)

Not at all considering I.V. Stalin's opinion on Darwinism the critics maintain, that Darwinism with its theory of evolution of formation of species, with the theory of natural selection, is not in contradiction with dialectic materialism.

According to the opinion of the critics "the problem of deciding which

---

1) T.D. Lysenko, New in the science of the biological species. In the collection "Philosophycal problems of contemporary biology", 1951, p. 10.

of the two theories on formation of species - Darwinism or the new outlook on species - reflects more adequately the process of historical development of organic forms, should be solved only by way of comparing one and the other theory with known facts characteristic for the given process." But the facts show, that Lysenko, who stands on correct methological positions, and develops Michurin's teaching in practice, demonstrated the formation of new species under the influence of changes in the surroundings. In his work is demonstrated the appearance of new species from an old one (rye from wheat) and the role of the living substance in this problem.

Turbin asks the question: "How, if not by the effect of selection, can be explained the development of complex forms of adjustment in the behavior of animals, of their instincts?" and he answers: "Darwin's theory of evolution, theory of natural selection gives a more than satisfactory answer to this question, and the "New teaching on species" leaves it without any explanation" (p. 307). Let us note that Lysenko has no work "New teaching on species". He develops Michurin's biological teaching. That is why Lysenko says that the appearance of new species is the result of influence of the surroundings and not a conscious adjustment, i.e. he reveals the process of appearance of new species - a problem which was not touched by Darwin. Lysenko demonstrates these concrete conditions in changes of species and explains how the surroundings influence the development stage, as well as the development of species, and explains why particular conditions of the surroundings are needed for each stage of development.

Turbin doubts whether Lysenko's point of view reflects Michurin's outlook on formation of species and quotes Michurin's well known excerpt:

"man's interference makes it possible to force each form of animal or plant to change more rapidly and in the direction desirable for man."

But then it is Lysenko and no one else, who develops a new outlook on the process of formation of species and reveals concrete forms in changes of species in the direction desirable for man (his theory of phases, vernalisation, winter-resistance and his last works), i.e. he develops Michurin's ideas of these problems.

Turbin writes that "Academician T.D. Lysenko himself comparatively recently defended and developed Darwinism, the point of view of Darwin and Timiryasev. And then, not only did he not see any contradiction with Michurin's teaching, but on the contrary, admitted that the latter is a development of the basic materialistic nucleus of Darwin's teaching." Lysenko actually accepted and accepts now, in accordance with pronouncements of the classics of Marxism-Leninism, the basic nucleus of Darwin's teaching, his theory of development, but never agreed to all the theses of his teaching. And it is seen from the pronouncements of the critics, that they take Darwin entirely, without pointing out the erroneous statements of his teaching.

N.V. Turbin expresses doubts about T.D. Lysenko's basic experiments on appearance of new species under the influence of changes in the surroundings, proclaiming that the manifestation of these new species is a result of "pereopylenie" repollination [out-pollination?], i.e. he doubts the basic methods of Lysenko's work. But Lysenko, who anticipated the possibility of such arguments, warned that inter-species hybrids possess intermediate features and are fruitless, and that specimens of one species appearing in the offspring

of another species, have no intermediate features and are completely productive.

Turbin's statement that Darwinism satisfies all the requirements of the dialectical - materialistic theory of development, that characteristic for it is not only a gradual development but leaps as well - such a statement is not correct and is in contradiction with comrade I.V. Stalin's opinion on this problem.

All the other statements on the treatment of the theory of formation of species which Turbin brings up in the summary, are incompetent, which was told in the text of the article. As to Turbin's statement that "there are no bases for replacing Darwin's theory of evolution by the theory of natural selection, new theory of formation of species introduced by T.D. Lysenko," - against this statement speak all the results obtained by adapting of T.D. Lysenko's theory in the practice of agriculture, when, by changing the surroundings, the basic features of the organism were changed (theory of development in stages, theory of formation of species, breeding of winter-resistant plant varieties).

I consider it my duty to give an advice to critics: to approach the evaluation of each eaching from the point of view of practice and theory of the dialectical materialism, which was not done by Turbin. It is necessary to know well the teachings of Marx, Engels, Lenin, Stalin. In criticising and rejecting what is not suitable, it is necessary to distinguish and use the positive, which the critics did not do in relation to Darwin's theory.

In the 36th year of the Soviet government, it is time to renounce the

defense of any metaphysical ideas and the attempt to justify under the pretense of criticism, one's own mistaken, pseudo-scientific tendencies.

As to Ivanov's criticism it is so without foundation, either methodologically or scientifically, and it is so discrediting for itself, that there is no need to argue against it.

Trans. 452
(In full)
By:
R. Adelman

Hassebrauk, K.

Untersuchungen über die Einwirkung von Sulfonamiden und Sulfonen auf Getreideroste

[Studies on the effect of sulfonamides and sulphones on cereal rust. I: Influence on capacity for fructification].
Phytopathologische Zeitschrift 17(2):384-400
April 1951 464.8 P562

(In German)

I. Introduction

Gassner and Hassebrauk (3), in 1936, reported on attempts to increase rust resistance by spraying various organic substances over the soil of nursery containers [flats]. Of the compounds discovered which rendered the plants more or less immune without causing any externally visible injury, picric acid was the most noteworthy. Sempio (12) reported the same year partly successful experiments to increase rust resistance of beans and wheat grown in nutritive solutions by means of supplemental [feeding] of metallic salts or alkaloids. Continuing our earlier experiments, I later on was able to identify further rust decreasing nitro-groups or sulphur containing organic substances of which the o- and p-toluolsulphonamide proved to be especially effective (5), even though only in greenhouse experiments. These results fundamentally coincide with the older findings of the IG-Farbenindustrie-A.G., which indicate that amides of aromatic

sulphonic acids or of their substitutes and derivatives can be used successfully in the control of rust diseases of cultivated plants (DRP 617 899 of 1931). Hart and Allison (4) as well as Straib (13) were able to confirm the rust inhibiting effect of picric acids and toluolsulphonamide by repeating the tests. Lings' (8) observation that an attack of Procystis occulta on rye can be reduced with picric acid also deserves to be mentioned here. It is true, however, that the effective dosages used in his experiments proved to be very damaging to the test plants. In the years that followed, a few more experiments became known which aimed at increasing fungi resistance in higher plants by application of chemicals. As far as inorganic salts were used in these cases, a discussion of them is being omitted. However, in view of our own tests, special notice should be accorded a report submitted by Polyakew (9)[17] on the increase of rust resistance in wheat achieved by means of different chemicals.[1] According to Polyakew, the most effective substances were the ones containing thio- or amino-groups, as well as cyan derivatives. The results of Polyakew's [experiments] are in accord with our own observations inasmuch as we, too, were able to prove that of all the substances tested, the toluol sulphonic amides containing a NH_2SO_2-group produced by far the strongest rust reducing effect. Hence, in continuing

---

[17] [See also Poliakov]

[1] Regardless of all efforts, I have been unable thus far to obtain [Polyakov's] work in the original. Nor can it be ascertained whether the available provisional report has been followed up with a more detailed one in the meantime.

the tests, it appeared advisable to test primarily the adequacy of substances of a similar constitution even if the toluol sulphonic amides had, for unknown reasons, failed in the field (6,7), and even though they exhibit the disadvantage of a relatively unfavorable chemotherapeutic index.

In considerations of this sort it was important to pay attention, principally, to substances known simply as sulphonic amides, which in the last two decades had attained a revolutionizing importance as chemotherapeutants in human medicine, after Domagk (1) succeeded, for the first time in 1932, in exerting a chemotherapeutic influence upon experimental streptococcus infection in mice and rabbits with azo-compounds containing sulphonic amides. Besides the real sulphonic amides, a few more sulpho-compounds were included in the tests.

The substances were, obligingly, made available to me largely by the different firms of the pharmaceutical industry, which is here once more gratefully acknowledged. I further am especially indebted to Professor Dr. Awe, Director of the Institute for Applied Pharmacy of the Technical University, as well as to Docent Dr. Bersch and Pharmacist Karbe, whose ready assistance and advice I have enjoyed at all times.

II. Method of the Principal Experiments

For tests, in general, the same method was used which had justified itself already in our earlier experiments. To begin with, all experiments

with cereal shoots were conducted in a greenhouse under normal temperatures, chiefly during the summer months of 1950. Test plants were grown in garden soil in flower pots (height: 7 cm, top diameter: 7.5 cm.) and about six days after sprouting shoots, they were inoculated with fresh uredospores in the usual manner. Having been covered with glass globes for 48 hours, they were allowed to stand free in damp peat bog in the greenhouse.

The substances to be tested were (fed (given to the test plants, at first, a few days before the inoculation, and later on the very day of the inoculation. Since sulphonamides are preponderantly little water-soluble, they were mixed with quartz sand in order to ensure an even distribution of the very small amounts available over the ground. Soluble substances were applied in water solutions. In several, especially emphasized cases, solutions in $NaHCO_3$ were applied. For reasons (5) explained above, the dosages furnished always had to cover a surface of 100 sq. cm. Appropriate control measures were instituted to enable detection of a possible effect of gaseous separations.

Most of the experiments were conducted with Puccinia triticina on Michigan Amber. For reasons of comparison with a few concentrations, P. simplex was tested for barley of Fong Tien, P. coronata for Flaeming's Golden oats /Gold hafer/ and P. dispersa for Petkus winter rye. The experimental varieties displayed high susceptibility (type IV) to the rust-biotypes utilized. Inasmuch as other varieties were being used, this is particularly emphasized.

In compiling the table of the experimental results, I am using the same symbols as those used in the earlier publications. Here they are explained once more briefly:

0000 = rust completely suppressed

0000 = rust almost completely suppressed, some traces of a pustule

000+ = rust strongly suppressed, isolated pustules

000+ = clearly visible reduction of rust, isolated pustules to slight infection

00++ = still a visible reduction of rust, slight infection

00++ = moderate reduction of rust, slight to irregular infection

0+++ = insignificant reduction of rust, scattered infection

0+++ = very slight reduction of rust, almost total infection

++++ = no effect on rust, strong uniform infection the same as on control plants

III. Experimental Results

A. Results of spraying substances over soil of experimental vessels

The results obtained with different sulphonamides and sulphones in the experimental series in which the soil surface of the cultivation vessels was sprayed with the preparations according to the method just described, are compiled in table 1. All experiments with the highly susceptible varieties mentioned above were repeated several times. A few of the results not listed in the table are analyzed in the text.

Concerning the results, primarily the secondary effect of the preparations upon the growth and other aspects of appearance of the host-plant, the following must be stated in detail:

1. Chemodyn, Prontalbin (= p-Aminobenzolsulphonamide). The rust inhibiting effect is most noteworthy. A ratio of 25 mg. of substance per 100 sq. cm. of surface is enough to cause considerable loosening /reduction/ of brown rust infection in wheat. However, the phytotoxic secondary effects are likewise relatively strong. From about 50 mg. on, there can be observed in the growth-zone of the subsequent leaves a more or less pronounced paling of the leaf green, increasing growth inhibition, and necrosis at the tips of the leaves. During the first stages of development, shortly after sprouting shoots, the plants are very sensitive. Of the cereal varieties, oats suffers the most. 250 mg. produced in oat shoots chlorotic and necrotic damages to such an extent that the experiment could no longer be evaluated. Rye, on the contrary, proved to be very resistant, and a quantity of 250 mg. brought about only a secondary effect in the form of growth inhibitions. As compared to toluolsulphonamides, the rust inhibiting effect of chemodyn is slight, since the first have, in part, suppressed rust attacks almost completely with as little as 5 mg. It is true that disproportionately stronger concentrations of chemodyn or prontalbin can be endured before substantial damages become apparent.

Table 1. Testing of Sulphonamides and Sulphones As to Their Rust Inhibiting Effect on Cereal Shoots After Spraying over the Soil of the Experimental Vessels. For experimental details, See Text.

No.	Preparation	Influencing the Infection Caused By												
		Puccinia triticina							P. simplex		P. coronata		P. dispersa	
		Ratio of Doses (mg/100 sq. cm.)												
		50	100	125	150	250	1250	2500	125	250	125	250	125	250
1	(Chemodyn / Prontalbin)	0000	0000	0000	0000	0000			0000	0000	0000	--	0000	0000
2	Albucid... (Acetamide)	0000	0000		0000					0000	0000	--	0000	0000
3	Prontosil....					++++		++++	++++	++++				
4	Marfanil .... (p-Toluenesulphonamide)	++++		++++		++++		++++	++++	00++			++++	
5	(Sulfapyridine / Eubasin and / Eubasin-Na)	++++	++++		++++	++++				00++	++++	++++		
6	Uliron..... (Sulfanilanilide)	++++		++++		++++	++++			++++				
7	Uliron C (Sulfanilanilide)	++++		++++		++++	++++			++++				
8	Neo-Uliron (Sulfanilanilide)	++++		++++		++++	++++			++++				
9	Sadignal (Urea)	++++	++++		++++				++++	++++	++++	++++		
10	Marbadal:...	++++	++++	++++	++++	000+	0000		++++	00++				
11	Suprenal....	++++	++++		0+++				++++	++++	++++	++++		

(Continued on 6b.)

Table 1. Testing of Sulphonamides and Sulphones As to Their Rust Inhibiting Effect on Cereal Shoots (Contd.) After Spraying Over the Soil of the Experimental Vessels. For experimental details, See Text.

No.	Preparation	Influencing the Infection Caused By												
		Puccinia triticina							P. simplex		P. coronata		P. dispersa	
		Ratio of Doses (mg/100 sq. cm.)												
		50	100	125	150	250	1250	2500	125	250	125	250	125	250
12	Eleudron..... /Sulphathiazole/	++++	++++		++++				++++	++++	++++	++++		
13	Debenal /Sulphadiazine/	0000		0000		0000	0000	0000	0000	0000	++++	++++	++++	++++
14	(Methylpyrimal) (Methyldebenal) (Na-Methyldehenal)	0000		0000		0000			00++	0000	++++	++++	++++	++++
15	Aristamid.....	++++	0+++	000+		0000	0000	0000		0000	0000	0000		++++
16	Protocid.....			++++		++++	++++			++++	++++	++++		
17	(Sulfaguanidine) (Resulfon) /Sulphaguanidine/	++++	++++		++++					++++	++++	++++		
18	Globucid..... /Sulphanilamide/	0+++	00++		000+					++++	++++	++++		
19	Ladogal.....	++++	0+++		00++	000+	0000			000+	++++	0000	++++	++++
20	Baludon.....	0+++		00++		00++	0000		++++	++++	++++	++++		0+++
21	Tibatin /"N,N'-digalactoside" under Aniline/			++++		++++	++++	++++			++++	++++		
22	Saccharin.....	000+		000+		0000		0000	++++				++++	

2. Albucid (= Acetylsulphanilamide). The rust inhibiting effect is about equal to that of p-Aminobenzolsulphonamide. The damaging secondary effects are generally slight; they appear chiefly in the form of increasing chlorosis, primarily in the zone of growth. Barley, following an application of 250 mg., displays infection of the 0 type with chlorosis in the zone of infection, while growth remains entirely normal. Rye is again relatively robust; oats, on the contrary, is very susceptible and 125 mg. suffice to inhibit its growth, while the damage caused by 250 mg. is irreparable. - Solutions of albucid in $NaHCO_3$ are almost neutral. Their rust diminishing effect is increased. In wheat, 50 mg. cause infection of the 0 type with very weak chlorosis in the zone of infection. In point of growth, the plants are furthered as compared to control plants. As much as 125 mg. is required to cause a slight growth inhibition.

3. Prontosil (= 4-Sulphonamide-2,4-diaminoazobenzol), in tested concentrations, evinces no effect whatever on susceptibility to rust, unless it be that, now and then, it appears to have increased. Neither were there established any other secondary effects.

4. Marfanil (= hydrochloric acid salts of 4-Aminomethylbenzolsulphonamide). Despite repeated tests, it was not possible to clarify the effect of marfanil satisfactorily. Twice the plants were completely immunized with dosages of 125 mg., yet after

application of stronger dosages type IV was again observed. In a series of repetitions [Wiederholungsreihen] this result, however, could not be confirmed. Slight chloroses and necroses of leaf tips were not surprising in view of the acidity of the substance. After dissolving marfanil in Na_2CO_3, up to 125 mg. no immunizing effect was noted.

5. Eubasin, Sulphapyridine (= a-p-Aminobenzolsulphonamido-pyridine) proved to be generally ineffective in every respect. The same is true of sodium salt of Eubasin. In a series of tests with barley, "sulphapyridine" produced a slight rust inhibiting effect; "Eubasin," however, did not. Oats reacts to eubasin with a slight inhibition of growth and chlorosis, but there is not the slightest change in its degree of rust.

6. Uliron (= 4- [4' Aminobenzolsulphonamido]-benzolsulphondimethylamide). No effect whatever.

7. Uliron C (= 4- [4' Aminobenzolsulphonamido] - benzolsulphonamide). No effect whatever, which is surprising in view of the good effect produced by p-aminobenzolsulphonamide.

8. Neo-Uliron (= 4 - [4' Aminobenzolsulphonamido]- benzolsulphonmonomethylamide). Without any effect.

9. Badional (= 4-Aminobenzolsulphothiocarbamide). From 100 mg. or more, the plants suffer from increasing growth inhibition, again oats in particular. If subjected to prolonged action, the susceptibility to rust seems to be rather increasing.

10. Marbadal (= Marfanil salt of badional), without a doubt, has an immunizing effect on wheat if applied in larger doses. Growth inhibition is relatively strong. Plants which have received 500 mg. are half the height of control plants at the time experiments are terminated. A striking [phenomenon] is the frequently recurring very dark-green coloring of the original leaves, probably to be attributed to the components of carbamide.

11. Supronal (= Combination of marbadal and debenal M [see No. 14]). An influence on rust infection cannot be established with certainty - a surprising circumstance in view of the unmistakable effect of marbadal and the strong immunizing effect of methyldebenal (No. 14). There were likewise no secondary effects to be noted. Only oats show once more increasingly an inhibition of growth.

12. Eleudron (= p-Aminobenzolsulphonamidothiazol). Effect on rust is not observed. Only stronger doses cause a slight growth inhibition - in the case of oats from 250 mg. on.

13. Debenal (= 2-p-Aminobenzolsulphonamido-pyrimidine). Debenal belongs to those sulphonamides which embody the combination of a strong immunizing effect and harmlessness. Wheat and barley, up to the largest dosages of 2500 and 1250, respectively, exhibited the i-O type of infection during vigorous growth. The growth zone of subsequent leaves is slightly chlorotic. Oats again exhibit a more or less strong chlorosis of all leaves and inhibition of growth.

14. Methylpyrimal, methyldebenal and sodium-methyldebenal. The data concerning debenal applies essentially also to the methylated form of debenal and its sodium salt. Oat damages are even stronger. The negative results cited here concerning rust suppression do not indicate quite accurately the actual observations insofar as in one experiment total rust suppression was established once, after application of 250 mg., accompanied, to be sure, by a very strong inhibition of growth and rather strong chlorosis.

15. Aristamide (= 6-Sulphanilamido-2,4-dimethylpyrimidine). As regards an effect on the susceptibility to rust as well as on the habits of experimental plants, there is no essential difference from debenal. Solutions in $NaHCO_3$ do not differ from the original substance where effect is concerned.

16. Protocid (= Methylpyrimal + p-aminobenzolsulphonamidoa-ethylthiodiazol). This combination of the highly effective methyl-pyrimal with ethylester of the ineffective eleudron produces no effect whatever on the state of rust or on the appearance of experimental plants. Not even oats is influenced.

17. Sulphuguanidine, Resulphon (= p-Aminobenzolsulphonyl-guanidine). Without any effect.

18. Globucid (= Sulpha-ethyl-thiodiazol). The moderately immunizing effect observed in wheat is accompanied by increasing chlorosis in the growth zone and by increasing growth inhibition.

In barley and oats only the accompanying damages are noted. Dosages of 125 mg. dissolved in $NaHCO_3$ do not alter the state of rust, but do promote growth.

19. Ladogal (= Aminobenzolsulphonoxymethylamide-N^4-d-glucoside sulfonic acid sodium). Ladogal has a very positive immunising effect and it safeguards treated plants against attacks without causing real chlorosis in the infection zone (type i-0 [1]). The required effective amounts are, to be sure, relatively high; total suppression of rust can be expected only from approximately 500 mg. In rye total immunity was achieved only with dosages of 1250 mg., this concentration, however, caused no noticeable damages of any kind. This perfect innocuousness [compatibility] makes up for the disadvantage of the relatively large amounts required. Wheat and barley show the first weak symptoms of retarded growth after the use of 1250 mg., but the crops are still strong and healthily green. Oats are more susceptible here, too, and react to 250 mg. with slight [leaf] tip necrosis and minor growth inhibition.

20. Baludon (= 4,4-Diaminodiphenylsulphon-diacetaldehydbisulphite-Sodium) had a good rust preventive effect on P. triticina if stronger dosages were used, but its tested dosages were ineffective on other types of rust. Thus, it required 1000 mg. or more to achieve a very slight reduction in the fructification of dwarf rust. Innocuousness is relatively good. In most cases it takes 1000 mg. to cause slight growth inhibitions and chlorosis of the

subsequent leaves. A small portion of rust reduction brought about with baludon may possibly be attributed to the effect of isolated gaseous matter..

21. Tibatin (= Digalactoside of 4,4-diaminodiphenylsulphon), in the concentrations applied, influenced neither rust nor the appearance of the experimental plants.

22. Saccharin. It was to be expected that saccharin, as a higher degree of oxydation of o-Toluolsulphamide, would produce a rust reducing effect. The table shows that rust suppression is indeed quite strong, at least as far as P. triticina is concerned. However, a dosage of but 50 mg. of saccharin causes simultaneously tip chlorosis and growth inhibition; damages resulting from 125 mg. are very severe and become irreparable if dosages are further increased. Infection by P. simplex was not reduced, despite the damage caused to the host-plant.

Results of a series of experiments with several other sulphones are not entered on the table. They either did not affect rust, or the rust reducing effect was accompanied by extremely severe damages, e.g., in the case of the Fewa washing powder. It seemed in part, e.g., with sulphonal and methylsulphonal, that gaseous isolations exerted a decisive influence on the rust reducing effect as well as on the secondary toxic phenomena, depending on the degree of temperature.

If we examine the results, it must be emphasized once more that the findings discussed apply only to the experimental types cited. It will be proved later on that in working with other types, we must always be prepared for more or less deviating results. With this reservation, it can be established that, besides a large number of quite ineffective substances, there are a few which exhibit a strong rust reducing or rust inhibiting effect. They are primarily chemodyn and prontalbin, albucid, marbadal, debenal and its derivatives, ladogal as well as baludon and saccharin. While chemodyn and prontalbin, albucid, marbadal and saccharin, especially when used in large amounts, cause increasing damages to the experimental plants, debenal, methylpyrimal, aristamid, ladogal and baludon are entirely harmless.

If we exclude oats which proved just as susceptible as in earlier experiments, the phytotoxic secondary effects produced by sulphonamides and baludon are generally slight, as compared with damages caused earlier by other organic substances. Tip necrosis, e.g., which appears readily following an application of toluolsulphonamide, is observed seldom and mostly to a much lesser degree. Growth inhibitions, too, occur normally within the limits of endurance, though it is true that experience as to what the situation might be under prolonged experiments is still

lacking. The frequently observed pale coloring of the growth zone in secondary leaves is striking.

The observation that the influence of effective sulphonamides does not result merely in a minor or major decrease of the spread of pustules, but that often, if not always and not very markedly, there is to be noted a chlorotic pale coloring in the infection zone, deviates somewhat from our earlier findings. Thus, the infection type 0 is frequently found on treated leaves.

Rye inoculated with P. dispersa exhibited in several series of experiments, under the influence of certain concentrations of sodium-methyldebenal and aristomide, which did not suppress rust, on more or less extended areas in the infection zone of isolated leaves, very small, scattered, pale golden-yellow uredorsori, in addition to the normal dark brown pustules, which looked very much like P. glumarum. These uredospori had lost all their aggressiveness and have thus far refrained from further reproduction. Whether here a change is taking place in the race of rusts induced, probably, by sulphonamides, is subject to further tests.

In view of the rust inhibiting effect of sulphonamides with respect to the different types of rust, there evidently exists a certain specificity, as indicated on table 1. We had observed such a diverse effect already in our earlier tests and we then formed the opinion that these differences are conditioned by the various reactions of the rust varieties, and not by those of the

hosts. Such conclusion cannot be drawn from the tests finished thus far, because they were not conducted with different types of rusts on the same variety of host. Since, in addition, the host varieties used, especially oats, exhibited quite different degrees of susceptibility to sulphonamides, the question in the given case must be left open.

To gain the first insight as to whether differences in the effect of sulphonamides or sulphones are due to the biotypes of rusts used up to now, or to the use of a host variety of the same type, corresponding investigations were conducted, which, however, require further study.

Testing of effective concentrations of preparations No. 1, 2, 13, 14, 15 and 19 for Michigan Amber /wheat variety/ inoculated with another strain of P. triticina, which also causes infection of type IV in control plants, led to a total suppression of fructification in all cases concerned, which was in accord with observations made during the main experiments. There were, however, deviations following the application of baludon. For the existing degree of rust, 250 mg. proved totally ineffective. Therefore, the question of the effect of approved substances on different biotypes of rusts requires further study.

The results of a varietal experiment with P. triticina are compiled on table 2. A different system was used for the compilation of these results, since the varieties tested included not

only those of high susceptibility, but also varieties with resistant types of infection. This time, besides the type of infection and symbols for chlorosis, necrosis and degree of fructification, the table shows also the length of secondary leaves as an important external symptom of the effect of sulphonamides or sulphones. Sulphonamides utilized were ladogal (625 mg/100 sq. cm.) and sodium-pyrimal (125 mg./100 sq. cm.) dissolved in water, and albucid (50 mg./100 sq. cm.) dissolved in $NaHCO_3$. The water soluble baludon was selected from sulphones at the rate of 250 mg./100 sq. cm. Of each experimental variety at least 20 plants were tested in each series.

The results of these varietal experiments are noteworthy in many respects. If we consider, first of all, the effect of the preparations on rust infection on the first five varieties which exhibit high susceptibility to the biotype used, then, on the whole, the experiences gained earlier with Michigan Amber as the main experimental variety are confirmed. Usually a total suppression of the spread of pustules is brought about. Occasionally chlorosis occurs in the infection zone (type 0 /2/) to a limited extent. The difference between a very scattered pustule spread or development of isolated pustules (type IV- and IV =) and complete immunity (i or 0), observed after the application of 250 mg., are not significant, but in view of the consistent results, they seem to be (inevitable certain for all experimental plants. According to this,

the extent of rust suppression in these susceptible varieties ought to depend, to a limited extent, on the host variety used at the time.

In the last three varieties listed on the table, we have fairly strong resistance on untreated control plants. Here, the tested substances produced a surprisingly diverse effect. While ladogal here, too, suppresses any formation of pustules, sodium-pyrimal is in one case, Peines Défiance, ineffective, and albucid unequivocally increases infection in Triticum polonicum. An increase in the susceptibility of this variety caused by baludon is only a likelihood. Fructification is reduced, but, due to the absence of necrotic and stronger chlorotic changes, the type of infection must be designated as III. In view of the connection which we presumed to exist between these symptoms and the occurrence of resistance, the changes in chlorosis and necrosis in the three resistant varieties are of particular interest. In general they are more or less reduced or they disappear. Only ladogal effects in Tr. polonicum solely the prevention of fructification, without influencing the chlorosis and, especially, the necrosis in this variety.

As regards the capacity of endurance in relation to tested preparations, there no doubt exist important varietal differences. Tr. compactum var. icterinum proved to be especially susceptible.

Table 8.

Effect of several sulphonamides and one sulphone on Puccinia triticina in different wheat varieties (For experimental details, See Text)

	Variety	Series	Infection Type and Growth Length [Laengenwachstum] (Control = 1) Following Application of									
			--		Ladogal 625 mg		Na-Debenal 125 mg		Albucid 50 mg		Saludon 250 mg	
			Type	Growth	Type	Growth	Type	Growth	Type	Growth	Type	Growth
WW	Michigan Amber	Tr. aestivum	IV ±	1	i	1	i	0,8	i	0,8	IV -	1
WW	Gray Emmer	Tr. dicoccum var. atratum	IV ±	1	i-0(;)	0,8	i-0(;)	0,5	i-0(;)	0,6	IV =	1
WW	Hohenheim white Kolbendinkel	Tr. spelta var. album	IV ±	1	i-0(;)	0,9	i-0(;)	0,7	0(;)	0,7	IV -	i
WW	--	Tr. compact. var. icterinum	IV ±	1	i-0(;)	0,5	IV =	0,5	i-0(;)	0,5	0 (;)	0,7
WW	Rye wheat	Tr. vulgare milturum	III-IV±	1	i	0,9	i	0,9	0(;)	0,8	i	0,9
SW	Barley wheat, loose	Tr. durum hordeiforme	II;-	1	0(;)	1	i	0,7	0(;)	0,7	i-0(;)	1
SW	--	Tr. polonic. var. laevissimum	I-II.;-	1	0.;	0,7	i-0(;)	0,7	III;∓	0,5	III =	1
WW	Peines Défiance	Tr. turgidum var. dinurum	II;-	1	0(;)	0,7	II;-	0,7	0(;)	0,6	i	1

Symbols: ; = Chlorosis, . = Necrosis
± = Normal Postule spread, = = Isolated pustules, ∓ and - corresponding intermediate stages

In isolated variations, the preparations best endured in the required effective dosages are ladogal and baludon, which coincides with the experiences gained earlier with Michigan Amber.

B. Results Obtained Following Suspension of Inoculated Plants in Solutions of Substances to be Tested.

Every experiment striving to find a plausible explanation for the rust reducing effect of several sulphonamides must be initiated, first of all, on the precondition that no essential change takes place when the causal agent enters the zone of infection. There is nothing to be expected, if we furnish the plants organic substances via the soil in which they probably are subject to diverse uncontrolable decomposition [Abbau] or selection processes. I, therefore, in testing several water soluble sulphonamides and baludon as to their rust reducing effect, am using the process which we have already used successfully when we had to prove that mineral salts and other elements were absorbed via the leaf surface (2; 3). In accordance with the method described elsewhere, the inoculated cereals, 24 hours after the sprouting of sporangia, were suspended for five consecutive nights with the surface parts in solutions of the substances to be tested. Henceforth they stood unprotected in damp peat in the greenhouse without further treatment. Michigan Amber, serving as an experimental variety, was inoculated with a biotype of P. triticina which under normal conditions causes the highest degree (type IV) of infection. The results of this experiment are compiled on table 3.

Table 3

Influencing P. triticina infection by suspending inoculated leaves in aqueous solutions of some sulphonamides and sulphones. Date of inoculation: August 9, 1950. Suspended: August 10-14, 1950. Termination of experiment: August 19, 1950. Concentration of experimental solutions: 5%o. (For further experimental details, See Text.)

Suspended in	Rust Infection
Water	++++
Ladogal	0000
Na-Methyldebenal	0000
Prontalbin	0000
Baludon	++++

Thus, the tested sulphonamides, having been fed through the leaf surface, also effected total suppression of the spread of pustules. We, therefore, may assume that they are absorbed unchanged also by the roots via the soil and are thus fed to the leaves. It is noteworthy that they are still capable of strong rust inhibition even if they are applied some 30 hours following the inoculation. Baludon failed in tests using this method. This may be so for various reasons. At one time, it is possible that the aforementioned partially present effect of gaseous isolations

is preponderant, in which case it no longer is capable of exerting an influence after the inoculation has been accomplished. At another time, it has to be considered whether the application of baludon via the soil does not cause a splitting off of its effective components, which does not occur in aqueous solutions.

Ladogal produced no secondary effects in this experiment: the experimental plants exhibited a rather better development than the control ones. The suspension in sodium-methyldebenal produced an even chlorotic pale /green/ color, suspension in prontalbin--a 1 cm. large necrosis on leaf tips. Baludon, similar to ladogal, rather furthered the development slightly.

C. Research Concerning the Importance of Seed Treatment With Sulphonamides and Sulphones in Rust Control

Traub (14), following the treatment of the seed of Phaseolus vulgaris with sulphonamide solutions, observed in the plants resulting from them strong morphological changes which are attributed to polyploidy. Even though, as it will have to be debated later on, the rust inhibiting effect of sulphonamides could, primarily, hardly be affected by the dividing anomalies of the cytoblast, the ascertainment of such deep-set deviations demands that the question be examined as to whether presoaking of the seed in sulphonamide solutions could not possibly effect a change in the rust resistance of the shoots.

For the clarification of this question, Michigan Amber wheat was soaked in solutions of ladogal, sodium-methyldebenal, prontalbin and baludon from 9 o'clock until 18 o'clock in room temperature, and were immediately after placed for germination in Petri dishes on filter paper moistened with tap water. The concentrations of the solutions consisted of: 5‰, 2.5‰, 1.25‰ and 0.6‰.

In 48 hours all grain had germinated 100%. Development of roots and shoots was somewhat inhibited by the stronger ladogal concentration, but not by the weaker ones. Baludon produced altogether a little severer inhibition of development. Presoaking in sodium-methyldebenal and prontalbin deterred shoots and roots in the first stages of development. The greatest damage was incurred by the roots which eventually died off when their base changed to a glassy brown, caused by rather weak concentrations.

At the expiration of another 48 hours, all plants treated with ladogal, and plants pretreated with the two weakest concentrations of baludon were stuck [pikiert] in the ground and, after the expiration of another 48 hours, they were inoculated for P. triticina. The infection in each case was similar to that found in control plants presoaked in water only. Thus, preliminary treatment of seed with a sulphonamide or sulphon solution exerts no influence whatever on the degree of rust in the subsequent shoots. However,

the development of the plants from the experimental series with 1.25% and 0.6% of ladegal exhibited a definite increase as compared with control plants.

The shoots from other experimental series could not be stuck in the ground because, as stated above, their development ceased completely. Only the plants pretreated with 2.5% baludon had, after 14 days, developed in isolated areas brownish green primary leaves, a few centimeters long but very thick, while growth of the roots was greatly inhibited. The appearance, especially that of the roots, did not reveal polyploidy such as Traub had observed in his beans.

IV. Examination of the Results

In the given investigations, in part, a fairly strong rust inhibiting effect of several sulphonamides was unequivocally established under the selected experimental conditions. But a whole series of questions could not yet be clarified or not clarified exhaustively. In my earlier experiments, e.g., the type of soil in which the experimental plants were planted was of considerable importance for the degree of rust inhibition. It is entirely possible that this factor also exerts a decisive influence on the processes of the substances concerned. This assumption is justified by the result of a fairly extensive experimental series

available at the time the manuscript was being finished; in these experiments the plants were erroneously planted in a grass-plot permeated with humus components instead of in the garden soil used heretofore. Plants treated with aristamid and ladogal in dosages designated by experience as optimally effective, exhibited a rust reduction so insignificant or uneven that the experiment, which was to serve as proof of substances with an antisulphonamide effect, could not be evaluated. Other open questions are the importance of temperature, of the age of experimental plants, and of the influence of natural conditions of environment. The inadequately clarified dependence of rust inhibition on the biotypes and host varieties used at the time must also be tested extensively. And finally, it is most important to investigate how long and to what degree the effect of sulphonamides on rust and on plant development lasts.

The references made to these limitations do not change the important finding that a number of sulphonamides render cereal shoots more or less immune to rust. Premature optimism, misled by the great therapeutic successes achieved with sulphonamides in human medicine, might expect from these results future gains for the practice of agriculture. We had, however, pointed out emphatically in our earlier research that from observations such as these, gained from shoots under greenhouse conditions, no

conclusions whatever may be drawn as to a possible practical utilization of the immunizing substances. Just how justified my reticent attitude was, was proved by the field experiments conducted by me at the time, which ended in a complete failure of the toluol-sulphonamides tested at the greenhouse (6, 7).

Yet, from another point of view, the results of the experiments are of increased interest. In our first investigations (Gassner and Hassebrauk 3) we were at a disadvantage, because we could test organic substances as to their rust inhibiting effect only purely empirically. The isolated successes which we had to record eluded any causative consideration.[2] In the investigations (5) which I conducted later on, it at least was possible, on the basis of available experience, to select the substances to be tested. But even from the effective substances found here, nothing could be said concerning the mechanism of the rust inhibiting effect. Only one conclusion is valid for all the earlier as well as the present investigations, to wit, that the prevention of the fructification cannot be explained primitively by the damage caused to the host plant. There is no relation between the extent of the damages and the suppression of rust.

---

[2] Straib (13) tries to explain the rust inhibiting effect of picric acid simply by its acid nature. Such an explanation is so obviously misleading that there is no use in taking a more closely defined position in regard to it.

If we, now again, were to bring up the question of how the described rust suppression, achieved by means of a number of sulphonamides, is to be explained, we now, in contrast to the unsatisfactory situation in our earlier research, could lean on a wealth of material containing important knowledge as to the far-reaching consistent interference of sulphonamides in the substance utilization [stoffhaushalt] of organisms. In connection with the downright revolutionary role which sulphonamides performed in human medicine, the chemism of their bacteriostatic effect has been investigated by numerous authors. The regularities revealed were surprising and they have had a fruitful effect in the sphere of growth substance research. Equipped with the knowledge or experience gained heretofore in sulphonamide research, it is not only possible to study more closely the question pertaining to the mechanism of the rust inhibiting effect of several sulphonamides observed by us, but, beyond this, there is hope that perhaps by this means some insight will be gained into the process of metabolism in obligate parasites which heretofore has been largely hidden from us.

Today, there can no longer be any doubt that, at least to a considerable extent, the inhibiting effect which sulphonamides have on many microorganisms is due to the displacement of the structurally similar, vital p-aminobensoic acids from which are derived other important complex [compounds], among them the

important folic acid [Folsäure] (see Rudolph 10). According to this, there exists a pronounced antagonism between sulphonamides and p-aminobenzoic acid which was proved in numerous investigations to be prevalent also in higher objects. Since the fundamental features of vitamin and antivitamin study exhibit an extended consistency with respect to plant organisms (see Schopfer and Anker 11), the hypothesis has to be considered as to whether or not the rust inhibiting action of several sulphonamides manifests itself through its obstruction of the p-aminobenzoic acid, in which case it would have to be supposed that p-aminobenzoic acid is a growth substance essential to the development of uredinia [Uredineen]. The first results of the experimental series initiated for the clarification of this question, at least, do not seem to contradict this assumption. Following the termination of the investigations, they will be discussed in connection with an exhaustive evaluation of the whole intricate question in a future report. The question arising from considerations of this sort, as to whether p-aminobenzoic acid or its derivatives of a higher complex could, possibly, be responsible for the obligate parasitism of the uredinia which, probably, are unqualified for their synthesis, was also being debated.

The objection raised to a general evaluation of the sulphonamides, which we tested in connection with the rust problem, on

the grounds that by no means all of them had produced rust inhibition, although their chemotherapeutic capacity in human medicine had, likewise, excelled more or less, is not valid, because, as regards its (approval (admissibility, it proceeds from dissimilar preconditions. Differences in permeability, dissolving ratio, root isolations, absorption processes in the soil as well as in chalcedony, and may other physical as well as chemical unknown factors may here perform a role that, at first, is entirely invisible.

V. Summarizing the Results

1. A number of sulphonamides and sulphones used in human or veterinary medicine were tested as to their rust inhibiting effect on cereal shoots under greenhouse conditions. While some preparations had no influence over the fructification of rusts, several others reduced rust to a greater or lesser extent.

2. In a highly susceptible wheat variety P. triticina infection was totally suppressed by the following substances absorbed through the roots: (1) p-aminobenzolsulphonamide; (2) acetylsulphanilamide; (3) marbadal; (4) saccharin;(5) 2-p-aminobensolsulphonamido-pyrimidine and its methylized derivatives; (6) p-aminobenzolsulphonoxymethylamide-N^4-d-glucoside sulphonic acid sodium, as well as (7) 4,4-diaminodiphenylsulphone-di-acetaldehydbisulphite-sodium. The phytotoxic secondary effects produced by fairly large dosages of the first three

sulphonamides are relatively big, those produced by saccharin are considerable. The ones produced by the last three substances are slight. While the first six preparations unequivocally exert their influence following their absorption through the roots, in the case of preparation No. 7 a secondary effect of gaseous isolations is not excluded.

3. Total rust suppression was achieved also in experiments with P. triticina, if the leaves were suspended for five nights following the inoculation in 0,5‰ solutions of p-aminobenzolsulphonamide, 6-sulphanilamido-2,4-dimethylpyrimidine and p-aminobenzolsulphonoxymethylamid-N^{4}-d-glucoside sulphonic acid sodium.

4. The tested substances exhibited preponderantly the same rust inhibiting effect against another biotype of P. triticina. As regards effectiveness against other types of rust on other host varieties (P. simplex, P. coronata, P. dispersa), it is for the most part basically consistent, yet occasionally deviates more or less.

5. It was proved in a varietal experiment with P. triticina that the extent of the rust reducing effect of several substances can be modified for some varieties.

6. Presoaking of seed in solutions of {approved / tested} sulphonamides did not produce any change in the amount of rust on shoots as long as no unbearable injury was caused.

7. Under the influence of single or double methylated 2-p-aminobenzolsulphonamido-pyrimidine, on the leaves of rye infected with P. dispersa, there appeared repeatedly non-infectious bright yellow uredosporangia alongside of the normal dark-brown pustules.

8. The results obtained do not yet permit to draw conclusions as to the feasibility of practical utilization of sulphonamides. It is being indicated what importance they might assume in a more detailed study of the rust problem.

VI. References

1. Domagk, G., Chemotherapy of bacterial infections. Angew. Chemie [applied chemistry] 48, 657, 1935.

2. Gassner, G. and Hassebrauk, K., Effect on susceptibility to rust produced by suspension of inoculated leaves in solutions of mineral salts and other substances. Phytopath. Ztschr. [periodical], 5, 323, 1933.

3. Gassner, G., and Hassebrauk, K., Investigations of the problem of cereal rust control with chemicals. Phytopath. Ztschr. 7, 427, 1936.

4. Hart, H., and Allison, J. L., Toluene compounds to control plant disease. Phytopath. 29, 978, 1939.

5. Hassebrauk, K., Further investigations of cereal rust control with chemicals. Phytopath. Ztschr. 11, 14, 1938.

6. Hassebrauk, K., Contributions to chemical control of rust on cultivated plants. Angew. Botan. 20, 366, 1938.

7. Hassebrauk, K., Final investigations of feasible field utilization of p-toluolsulphonamide as effective internal therapy in cereal rust control. Phytopath. Ztschr. 12, 509, 1940.

8. Ling, L., Factors affecting infection in rye smut and subsequent development of the fungus in the host. Phytopath. 31, 617, 1941.

9. Polyakow, I. M., A new method of controlling agricultural crop diseases. (Vorl. Mttlg. [preliminary report]). Bull. Pl. Prot. (USSR) 1, 92, 1941. - Ref.: Chem. Abstr. 36, 2073, 1942.

10. Rudolph, W., Growth substances and antigrowth substances, Bern, 1948.

11. Schopfer, W. H. and Anker, W., Effect of sulphonamides and antisulphonamides on the growth of Pisum roots in sterilized organic culture. Experientia (Basel) 5, 117, 1949.

12. Scurpio, C., Influence of various substances on parasitic diseases: rust on kidney-beans, rust and white disease in wheat. Rev. Path. Veg. 26, 201, 1936.

13. Straib, W., Effect of organic compound sprays in the control of rust fungi infection. Ztbl. Bakt. [Journal of Bacteriology], II, 103, 73, 1941.

14. Traub, H. P., Effect of sulphonamide and other sulfa compounds on nuclear condition in plants. Journ. Hered. 32, 157, 1941.

Trans. 300
(In full)
By:
R. Adelman

Sysoev, A. T.

O vozmozhnosti sochetaniia biologicheskogo i khimicheskogo metodov v bor'be s vrediteliami sel'skokhoziaistvennykh kul'tur.

[Feasibility of combining the biological and chemical methods in controlling pests of agricultural crops]

Vsesoiuzn. Akad. Sel'skokhoz. Nauk im. V. I. Lenina. Dok. 18(7):26-31, 1953. 20 Akl

(In Russian)

(Submitted by the Section of Plant Protection of the All-Union Academy of Agricultural Sciences, Order of Lenin, for whom it is named)

Mealybugs - grape (Pseudococcus citri Risso), citrus (Pseudococcus gahani Grech.) and seaside (Pseudococcus maritimus Ehr.) - are the most dangerous pests of citrus and many other subtropical fruit, technical and ornamental crops. Cryptolaemus (Cryptolaemus montrauzieri Muls.) is used extensively for the biological control of these pests. This beetle destroys 90-95% of mealy bugs anywhere on the surface of the infested plants and during all phases of their development. Cryptolaemus, however, is frequently destroyed by various poisons used in the control of pests and plant diseases such as mites, scale insects, Oidium, etc. The chemical treatment of plants with sulfur, ISO (?), Bordeaux mixture, anabasine sulfate, DDT, etc., coincides with the use of Cryptolaemus against mealybugs. Hence the instances of its death by sulfur and DDT dust.

For the purpose of studying the feasibility of combining the use of Cryptolaemus with chemical treatment of plants, the Sochi base of the All-Union Scientific Research Institute of Plant Protection (VIZR) conducted during 1950-1952 field experiments at the village of Lazarevsk, Region of the City of Sochi, Krasnodar Territory, and laboratory tests at the insectary of VIZR in the village of Lazarevsk.

For laboratory tests watermelons and squash heavily infested with the grape mealybugs were employed. The method of reproducing mealybugs on squash plants was developed by the Sochi Base of VIZR. All experiments were repeated twice. Twenty beetles and twenty second stage [vtorogo vozrasta] larvae were used for each experiment. Cryptolaemus was released on the plants in gauze breeders [sadki] at intervals of 1, 5, 15 and 30 days following their treatment with chemicals. Records were kept by tabulating the living and dead individuals in each breeder at intervals of 5, 10, 20, and 30 days. Control breeders were situated at a distance from the experimental ones treated with chemicals. While the work was being conducted the temperature in the insectary fluctuated between 23 and 29° C, and outdoors - between 22 and 35° C. The relative humidity varied between 60 and 75%.

As a result of dusting the plants with sulfur used in mite control, under laboratory conditions considerable mortality of beetles, released in the breeder one day following the treatment of the plants, was observed as early as on the 6th solar day (table 1). On the larvae the effect of sulfur became evident somewhat later.

Table 1.

Survival of Cryptolaemus on plants dusted with sulfur

(Under laboratory conditions, in percentages)

Number of solar days between dusting and release	Number of solar days following release of							
	5		10		20		30	
	larvae	beetles	larvae	beetles	larvae	beetles	larvae	beetles
1	80	45	45	20	5	0	0	0
Control	100	100	100	100	100	80	100	60
5	95	85	50	55	No record kept			
Control	100	100	100	100	--	--	--	--
15	60	70	40	50	0	0	0	0
Control	90	100	90	80	80	70	80	70
20	75	75	60	45	5	0	0	0
Control	100	100	100	100	100	100	100	100

During the entire life span of Cryptolaemus on the dusted plants, its beetles and larvae scarcely ate any of the mealybugs. Cryptolaemus females laid no eggs. The high rate of mortality of the predator was observed already after 10 days. On the 21st solar day, Cryptolaemus was totally destroyed without having produced any progeny. Only in the experiment conducted 37 days after dusting was there noted an insignificant number of emerged larvae. Sexual reproduction of Cryptolaemus did not exceed an average of 1.4 individuals per female. Besides, the young were inactive and underdeveloped. In the control, however, a mass hatching of progeny began 20 days after Cryptolaemus was released. Sulfur produced no effect on the mealybugs.

It was established that in a closed room, dusting with sulfur has a destructive effect on Cryptolaemus for an extended period. Thus, it is expedient to release Cryptolaemus on plants dusted with sulfur in hothouses, greenhouses, etc., only after the sulfur is completely decomposed and removed from the plants and the soil by means of watering, soil aeration, etc.

On open plantings [pantatsiiakh], sulfur affects Cryptolaemus considerably less. This has been confirmed by special experiments.

In the summer of 1950 and 1951, Cryptolaemus was released on citrus plantings infested with the seaside mealybugs and likewise on grapes infested with the grape mealybug, at intervals of 15 and 30 days following the sulfur dusting of the plants. On citrus plants, 25 beetles were released per tree, on grapes - 1 beetle and 2 larvae per shrub. Observations disclosed that beetles released on lemon trees 15 days following the dusting, leave these plants at once regardless of their high rate of infestation with mealybugs.

If, however, Cryptolaemus were released on the same lemon trees 30 days after their dusting with sulfur (June 29), the beetles remained on the trees and began to lay eggs; larvae developed in large numbers. When a count was taken on August 1, the lemon trees had been freed of mealybugs. On the farm, "Malyi Akhun" (Sochi zone), larvae and beetles of Cryptolaemus were released on July 16, 1951, onto the grape varieties Katalog, Chaus, and Shaslia dusted with sulfur one month prior to that date. By September 1st, the grapes were freed of the infestation by mealybugs on the areas where

Cryptolaemus had been released. Consequently, Cryptolaemus must not be released on plants dusted with sulfur before the expiration of 30 days following the treatment.

Influence of ISO spray (0.5°B) upon Cryptolaemus. Laboratory experiments, attempting to clarify how Cryptolaemus is influenced by ISO spray used in mite control, disclosed that in releasing the predator onto plants one solar day following their treatment with this preparation, the beetle mortality hardly exceeds that on control plants (table 2). Only a short delay (of 10 days) is observed in the appearance of the predator's larvae, as compared with that on control /plants/. Similarly, the survival of mealybugs is not influenced by ISO. The sexual reproduction of Cryptolaemus released at different times onto treated plants shows hardly any noticeable deviation from that on control /plants/ (table 3).

T a b l e 2.

Survival of Cryptolaemus released onto plants 24 hours following their spraying with ISO (0.5°B)

Solar days following release of Cryptolaemus	Survival of Cryptolaemus (in %)			
	Experiment		Control	
	larva	beetles	larva	beetles
5	95	95	100	100
10	95	95	100	100
20	80	95	90	100
30	80	85	90	90

Table 3.

Effect of ISO spray (0.5°B) upon the reproduction of Cryptolaemus

Solar days between spraying and release of Cryptolaemus	Average number of larvae from 10 beetles		
	I Repetition	II Repetition	Control
1	495	510	569
5	415	470	491
15	529	506	554
30	392	483	462

Analogous results were obtained under natural conditions in July of 1951 at the small village of Matsesta.

The experiments conducted show that during the summer Cryptolaemus may be released onto plants sprayed with ISO (0.5°B) as early as 15 days after the spraying. In sheltered soil (greenhouses, hothouses, etc.) dusting sulfur must of necessity be replaced with ISO spraying.

The use of Cryptolaemus has shown that freeing a medium-size citrus tree and a full-grown cultivated grape shrub of mealybugs, by releasing beetles at the ratio of 25 per tree and 3 per grape shrub, requires a period of 30-45 days. During the space of time indicated the plants must not be dusted with sulfur or sprayed with ISO.

If the release of Cryptolaemus onto citrus were initiated during the normal period, i. e., during the period of the first generation of the seaside and citrus mealybugs (at end of May - beginning of June), when control measures against the silvery mite are not yet being applied, then

Cryptolaemus is able to free the trees of mealybugs by the beginning of July. To safeguard the crop against mite injuries, it is necessary, at this time, to spray ISO or to dust with sulfur. When Cryptolaemus is released against the II generation of the same mealybugs (at end of July), it is recommended that ISO be sprayed during the first ten days of July and that it be repeated at the beginning of September. On sectors of grapes infested with mealybugs, sulfur dusting is permissible until mid-June, in case Cryptolaemus is being applied. If necessary, the dusting may be repeated at the end of August.

Influence of 1% Bordeaux mixture spray upon Cryptolaemus. Under laboratory conditions, in releasing Cryptolaemus 24 hours after spraying with Bordeaux mixture, a slightly negative effect appeared on the fifth day (table 4). At the end of the experiment, the difference in the mortality rate between the experiment and control did not exceed 10-15%.

T a b l e 4.

Survival of Cryptolaemus released on plants 24 hours following the spraying with 1% Bordeaux mixture

Solar days following release of Cryptolaemus	Survival of Cryptolaemus (in %)			
	Experiment		Control	
	Larvae	Beetles	Larvae	Beetles
5	70	85	100	100
10	65	85	70	100
20	60	65	70	80
30	60	45	70	60

The larvae and beetles of Cryptolaemus did not abandon the areas sprayed with Bordeaux mixture, and they ate the mealybugs. Reproduction and development of the beetles ran their normal course. Development of the mealybugs also proceeded unchanged, since Bordeaux mixture does not stay on the wax-like surface of their bodies.

The data on table 5 show that the offspring of Cryptolaemus released the first day following the spraying with Bordeaux mixture was not quite so numerous as that in control. The later the beetles were released following the spraying, the more offspring did they yield. The same results were obtained under field conditions. Thus, for example, we, on August 16, 1951, in the City of Sochi, sprayed with Bordeaux mixture 6 mature Lenkoran Acacia trees which housed a large amount of Cryptolaemus larvae and beetles released earlier for the control of mealybugs. These trees were kept under observation until September 26, 1951. During this period no dead Cryptolaemus larvae or beetles were found either on the trees or on the ground beneath the Acacias. The beetles developed normally and by mid-September they cleared the Acacias of mealybugs. An analogous picture was observed on the other trees subjected to Bordeaux mixture spray.

Thus, Bordeaux mixture spray has neither a fatal effect on Cryptolaemus, nor does it repel them. On the strength of this, it is permissible to release Cryptolaemus onto plants sprayed with 1% Bordeaux mixture beginning with the second day after the spraying.

Table 5.

Influence of 1% Bordeaux mixture spray upon the reproduction of Cryptolaemus

Solar days elapsed between sprinkling and release of Cryptolaemus	Average number of larvae from 10 beetles		
	I Repetition	II Repetition	Control
1	498	411	584
5	467	498	593
15	512	471	549
30	582	570	604

Influence of anabasine-sulfate spray upon Cryptolaemus. In spraying plants with an anabasine-sulfate solution (40 g. of 40% anabasine-sulfate and 80 g. of soap to 10 l. of water) under laboratory conditions, there is observed a slight (not over 10%) Cryptolaemus larvae and beetle mortality (table 6).

Table 6.

Survival of Cryptolaemus released onto plants one solar day following their spraying with anabasine-sulfate (40 g. of anabasine-sulfate and 80 g. of soap to 10 l of water)

Solar days following Cryptolaemus release	Survival of Cryptolaemus (in %)			
	Experiment		Control	
	Larvae	Beetles	Larvae	Beetles
5	95	90	100	100
10	85	85	100	100
20	80	75	90	90
30	80	65	90	70

The development of Cryptolaemus continued normally. If the release of Cryptolaemus was begun on the fifth day following the spraying, their reproduction was not affected by anabasine-sulfate (table 7). Anabasine-sulfate spraying was not reflected in the vigor of the mealybugs.

The same results were obtained in field experiments. Thus, on July 26, 1951, in the City of Sochi, 3 mature Lenkoran Acacia trees infested with seaside mealybugs were sprayed with anabasine-sulfate. At the moment the trees were being sprayed there was on them a large amount of Cryptolaemus larvae. On July 14, 1951, twenty-five Cryptolaemus beetles had been released on each of these trees. In July and August 1951, Cryptolaemus was developing normally on all Acacias. No dead Cryptolaemus larvae were found either on the trees or under them. At the end of August the trees were cleared of mealybugs. It was found simultaneously that spraying plants with anabasine-sulfate not only did not kill Cryptolaemus, but it did not even repel them.

The data obtained show that Cryptolaemus may be released onto plants sprayed with the usual dosages of anabasine-sulfate, beginning with the fifth day after the spraying.

Influence of "2% butyrous emulsion on copper vitriol /sulphate/" upon Cryptolaemus. Through observations of the after-effect produced on Cryptolaemus by industrial treatment of plants with 2% butyrous emulsion, it was established that the latter has no negative effect upon it.

After-effect of plant fumigation with hydrogen cyanide upon the survival of Cryptolaemus. For the clarification of this question, in September 11, 1951, squash, with a III and IV degree infestation of grape

mealybugs, were fumigated with hydrogen cyanide. The fumigant, an ordinary sodium cyanide, was used at the rate of 15 g. per 1 cu. m. The fumigation took place under a thick canvas, at a temperature of 22° C, with a 45 minute exposure. After the fumigation the mealybugs were still alive. The next day the fumigated squashes were placed in gauze breeders and left under natural conditions. Cryptolaemus beetles were released into the breeders. In 8 days larvae appeared and in a month - young beetles. The Cryptolaemus larvae in the breeders containing fumigated squashes developed in as large numbers as those in control and within the same space of time. In the first replicate, 412 Cryptolaemus larvae were obtained from 10 beetles, in the second - 386, and in control - 401.

T a b l e 7.

Influence of anabasine-sulfate spray upon the reproduction of Cryptolaemus

Solar days between spraying and release	Average number of larvae from 10 beetles		
	I Repetition	II Repetition	Control
1	461	427	520
5	481	465	537
15	506	511	541
30	538	560	544

If beetles were released onto plants in the open, on the second day following the fumigation with hydrogen cyanide, no deaths were observed among them.

If the fumigation is conducted indoors (greenhouses and hothouses), it is permissible to release Cryptolaemus after the hydrogen cyanide, absorbed by the indoor surface of the room and the plants, is completely eliminated. This requires spraying abundantly, with water, the walls, ceiling, floor and the plants subjected to gas treatment, and a subsequent airing of the rooms for 5-6 hours. The following day Cryptolaemus may be released in these rooms.

Influence of DDT and hexachlorcyclohexane /GKHTSG - Geksakhlortsiklogeksan/ dust upon Cryptolaemus. For the experiment were used squashes infested with grape mealybugs dusted with DDT and GKHTSG. In gauze breeders in the insectary, if released on the second day following the dusting with these chemicals /dustami/, the Cryptolaemus beetles died within 5 hours and the larvae within 12. Having been released 5, 15, and 30 days after the dusting, Cryptolaemus was destroyed just as quickly as it was after sulfur dusting (within 20 days). Cryptolaemus produced no progeny. In the laboratory, during two months of observations, Cryptolaemus beetles remained at a distance from squashes dusted with 5% GKHTSG. Dusting with GKHTSG destroys 20% of the grape mealybugs mainly in the 1st and 2nd stages of larvae, during the first 10 days following the dusting. Thus, of the 500 individuals recorded, 191 grape mealybugs, including 168 larvae in 1st and 2nd stages, were destroyed. We lacked the opportunity to establish in the open the after-effect of DDT and GKHTSG dusts upon Cryptolaemus. We suppose that the after-effect of these dusts in the open will be less severe than in a laboratory.

Influence of thiophos spray (0.1% concentration) upon Cryptolaemus.

Thiophos produces good results in the control of mealybugs. Cryptolaemus, in turn, destroys mealybugs in areas inaccessible to chemical treatment (in fissures and under the bark) and also on fruit-bearing crops. Therefore, it is expedient to apply, concomitantly, thiophos, Cryptolaemus and agrotechnical measures which will ensure a total elimination of the breeding places of mealybugs.

Our observations conducted in March of 1952 revealed that Cryptolaemus may be released on plants 20 days following the spraying of these plants with thiophos (0.1% solution). In our experiment the grape mealybugs developed well on squashes if they were inoculated 15 days after the spraying. Cryptolaemus beetles released on these squashes 5 days after the inoculation (March 16, 1952) produced as much progeny as those on control [plants], and in the same space of time. On the average, by April 1, 1952, there were 319 thriving Cryptolaemus larvae obtained from 10 beetles in the first replication, in the second - 280, in control - 296.

Everything outlined above justifies the assertion that, in mealybug control, the use of Cryptolaemus can be combined with the basic measures applied for the protection of subtropical crops against pests and diseases: spraying with ISO (0.5°B), 2% butyrous emulsion, 1% Bordeaux mixture, ordinary solution of anabasine-sulfate, and normal fumigation with cyanogas. By observing the safe interval in agrotechnics of subtropical crops, Cryptolaemus may be applied even after the use of sulfur spray.

All-Union Scientific Research Institute of Plant Protection

Received at Editorial Office April 14, 1953

Trans. 454
By:
A. Antik

Novotel'nova, N. S.

Vliianie temperatury i vlazhnosti na prorastanie Konidii Phytophthora infestans etc.

[Influence of Temperature and Humidity on the Germination of the Conidia of Phytophthora infestans].

Zashchita Rastenii 12: pp. 79-86, 1937
421 P 942

(In Russian)

454

INFLUENCE OF TEMPERATURE AND HUMIDITY ON GERMINATION OF PHYTOPHTHORA INFESTANS CONIDIA

Since the time of the initial study of Phytophthora infestans De Bary (1861), the influence of temperature and air humidity on germination of conidia was recorded (Jones, Giddings and Lutman, 1912; Melhus, 1915; Murphy, 1922 and others).

There are few data in regard to the influence of air humidity; they are based mostly on observations. Experimental data on the influence of temperature are known more or less in general.[1]

In order to develop the prognosis indicators of development of Phytophthora infestans, the purpose of this work was to study the peculiarities of spore germination and to establish cardinal points of temperature and humidity.

Methods. We kept the culture of Phytophthora infestans, isolated from potato leaves, for a year on fresh potato slices. All the experiments were conducted with a 7-day culture. Conidia from the potato slices were transferred with a platinum needle to a hanging drop in a moist chamber (depression slide). Water from the tap was taken, but it was heated up to required temperature before the experiment.

[1] After the completion of this work, a voluminous work by Crossier (1934) appeared in the press, in which, with sufficient fullness, is presented the problem of the influence of meteorological factors on germination of spores Phytophthora infestans.

Experiments on spore germination under different temperatures were conducted in Kiuster's multi-incubators; for continuous observations Leitz's apparatus "Nuttle" proved extremely convenient, it is an electric water-bath incubator with a glass front wall.[2] Inside the "Nuttle" is placed a microscope, the upper part of its tube emerging. Such an apparatus enables continuous observation of the spores under a microscope at any set temperature. In experiments on the influence of air humidity the spores were kept in dessicators with various concentrations of sulfuric acid. Calculations were made according to the Regnault[3] scale. The following variants of relative air humidity were tested: 100, 83, 62, 48, 34 and 21%.

Similar to the procedure during the experiments on the influence of temperature, conidia were transferred to cover glasses, not in a water drop but on dry surface. Then these cover glasses were placed, on special supports inside the dessicators, drops of water were deposited on the conidia and the glasses which were examined under the microscope were placed on depression slides, after which the moist chambers were placed in the multi-incubator at an 18° temperature.

All the tests continued for 48 hours, because preliminary experiments showed that if, during 48 hours, the spores did not germinate, further changes do not take place; besides, bacteria develop later on which upset observations. The germination percentage was calculated on the basis of a microscopic examination of not less than a hundred spores, but usually their number was considerably higher. Characteristic stages were drawn with the help of Leitz's drawing ocular [camera Lucida?].

1. Influence of temperature on the manner of germination of conidia

We studied, in our experiments, temperatures from 0 to 35° C. in 1-2° intervals. Drawings of peculiarities in spore germination observed under conditions of various temperatures are given in fig. 1.

---

[2]We want to use this opportunity to express our thanks to the director of the National roentgenological-radiological and cancer institute, Prof. Nemenov, for the permission to use this apparatus.

[3]Landolf-Bornstein, Physikalisch-chemische Tabellen.

Fig. 1. Influence of temperature on manner of germination of spores P. infestans.

The following types of germination of conidia were observed: (1) forming of germ tubes (so-called direct germination); (2) originating of zoo-spores (the so-called indirect germination); (3) the so-called combined germination which was observed at higher temperatures. The first type of germination was observed within the 4-30° C. range. The second, within the 6-21° C. range. Crossier indicates a lower temperature minimum -3°.

The formation of zoo-spores took place as follows: at first the contents of conidia is divided mostly in 6 parts which after awhile emerge, moving with the help of flagella; then they stop, become round, grow in size and germinate. At low temperatures, such as 6-8°, the zoo-spores, as a rule, germinated inside conidia and then their germ tubes emerged from the top of the conidia as well as from any point of its wall. In one case, when at a low temperature, conidia germinated to form zoo-spores, the following phenomenon was observed: on zoo-spores were formed short germ tubes with a swelling at the end similar to secondary zoo-spores. Being transferred to more favorable temperature, the "secondary zoo-spores" germinated and the germ tube reached normal size.

The third manner of germination is the so-called combined germination. The germ tube which is formed at the beginning does not develop later on, it produces a thickening which changes then into a secondary conidium; the latter can germinate like an ordinary conidium, producing a shoot or zoo-spores.

The secondary conidia usually differ morphologically from the normal ones by larger size and a more distinctly papilla; but generally speaking their form is highly variable. As a rule they are formed - and that in a large quantity - at temperatures of 24° to 30° C., but single instances were sometimes observed at lower temperatures as well. The higher the temperature, the longer the time of their originating, and at low temperatures this period decreases. Thus at 30° C. secondary conidia are disclosed after 72 hours, and sometimes even after 96 hours. At 26° and 24° they appear after 48 and sometimes even 24 hours.

There are hypotheses that secondary conidia represent a resting stage, which appears under the influence of unfavorable conditions. Blackwell and Waterhouse observed that during early development

stages the secondary conidia can produce a germ tube and zoo-spores, but in dried-out media with inadequate nutrient substances, they exist for weeks and months forming chains of conidia which gradually diminish in size. In the old artificial culture on potato agar (20 days old and older) we also used to find secondary conidia but, according to drawings which we have, they have little similarity to the secondary conidia obtained in water.

Murphy observed secondary conidia only in water when oxygen was gradually decreasing, and he calls them hydro-conidia. De Bary (1863) indicates that secondary conidia are formed only on the surface of a water drop, but if they are put into water, a normal production of zoo-spores begins.

Szymanek (1927) refers the formation of secondary conidia to anomalies of direct germination. The author finds that secondary conidia discovered, after 4 months, during the study in a drop of glucose in Van-Tigem cell, were absolutely identical with bodies which he found in infected tissues of potatoes and that they are the resting stage of the fungus.

At a 26-28° temperature, the secondary conidia germinate in 48 hours and produce tertiary conidia, which in turn germinate into germ tubes at the end of which thickenings may be formed and they are then transformed into conidia. Due to the fact that the secondary conidia being formed have walls no thicker than those of normal conidia, that no formation of additional nutrient substances is observed in them and, finally, due to just as rapid a formation in them of zoo-spores as in normal conidia--we have no foundation to refer the secondary conidia to the resting stage of the fungus. In order to solve this problem definitely, it is necessary to study the viability of secondary and tertiary conidia during a longer period of time.

2. Critical points of temperature in spore germination

The type of germination into germ tubes has a small range of temperatures, with the lower point at 4°C. and a germination percentage at 9.2.

(p. 82) Fig. 2 /Caption/ Influence of temperature on germination percentage of spores of P. infestans.

The higher the temperature, the higher the germination percentage, which reaches its maximum at a 10-15° temperature (100%). This is an optimum for formation of zoo-spores. Still higher temperatures begin to depress formation of zoo-spores and the germination percentage decreases.

The low germination percentage at 19-20° can be explained by the fact that the formation of zoo-spores is already depressed and the germination into tubes only begins to prevail. But at 21° the germination percentage begins to increase again due to formation of germ tubes until it reaches the second optimum at 25° (93%). This temperature is optimal for formation of germ tubes. Between 25-30° a new decrease in germination percentage takes place and at 32° there is no germination.

The percentage of zoo-spore germination was not recorded because it is difficult to distinguish in the mass the non-germinated zoo-spores; usually none of them germinate. 21° is a maximum temperature for formation but not germination of zoo-spores, because their germination takes place at 30° C. Crossier (1934) mentions a slightly lower temperature at which germination was still noticed--28°. It is necessary to emphasize that a 100% germination takes place at temperatures optimal for formation of zoo-spores (10-15°), while the formation of germ tubes never reaches 100%.

The results which we obtained do not coincide with data by Jones, Giddings and Lutman, who indicate 10-20° as an optimal temperature for formation of zoo-spores and do coincide with the optimum which they established for germ tubes (about 25°). Crossier also sets the optimum at 24°. Agreement is noted with data by Melhus and Murphy whose optimum temperature for formation of zoo-spores is 10-13° and 10-15°, according to Crossier--13°. Melhus considers the optimal temperature for formation of germ tubes to be 22-23°, i. e., lower than that obtained in our tests. The minimum temperature for zoo-spores is indicated as 5-6°, for germ tubes--above 6°; we observed an opposite phenomenon.

Germination of conidia into zoo-spores took place in a much shorter time period than germination into germ tubes; thus only one hour is needed for formation of zoo-spores at an optimum 15° temperature, while 5 hours are needed for formation of a germ tube at an optimal temperature. This is what Melhus writes about it: "The time necessary for germination varies in relation to temperature, namely because to produce a germ tube more energy is needed than to produce zoo-spores." In the experiments of the author, at a 12-13° temperature, the observed germination time was 1-8 hours. The originating of zoo-spores is connected with the maturity of conidia. In our tests zoo-spores were formed only in young cultures, not older than 7 days. Melhus maintains, also, that young spores produce zoo-spores more frequently and germinate into germ tubes less frequently.

Caption of fig. 3. Dependence of the length of the germ tube of P. infestans on temperature. (p. 83)

The length of the germ tube also depends on temperature. They are longest at 22° C. (813 microns) which coincides with the highest termination percentage as indicated above at corresponding temperatures. The connection between the germination percentage and the length of the germ tube can be established in older (9-days old) cultures. In these cases the germination percentage and the greatest length of the tubes are approximately at the same temperature (23°). And in cases where there is germination into zoo-spores, the highest percentage of germination cannot possibly coincide with the greatest length of the tube. The obtained data are confirmed by Crossier: rapid lengthening of the germ tubes is observed at 21-24° and the highest germination percentage--at 21-26°.

In the light of the obtained results we come to the conclusion that in the methods of recording germination of conidia Phytophthora infestans, the germination should be judged according to the germination percentage and not the speed of elongation of the germ tube. The criterion of germination percentage is conditioned by biological peculiarities of this fungus.

The results of the experiment in regard to the manner of spore germination and to cardinal points of temperature are given in table 1.

Table 1 (p. 83)

Manners of germination	Temperatures - Celsius			
	Limits	Optimum	Maximum	Minimum
Formation of zoo-spores	6-21	10-15	20	6
Formation of shoots [7]	4-30	25	30	4
Formation of secondary conidia	24-30	24-28	30	24

3. Change of temperatures.

The following test was conducted in order to clarify the effect of temperatures--which are beyond the critical ones--on spores; whether they destroy the latter's viability or only inhibit the process of their germination. For this purpose the spores were kept a certain time at high temperatures and then were transferred to optimal temperatures. Below is given a table with results of the change of temperature from 35 to 18° (table 2).

Table 2 (p. 84)

Effect of super-maximum temperatures on conidia.

Temperature-Centigrade	Time of exposure	% of germination of conidia	Length of germ tubes in microns
35	24	0	0
35	4	93	99,8
35	1	100	168,0
35	30	77,7	197,1

It was brought out that a 24-hour exposure to a 35° temperature caused destruction of conidia, while a 4-hour exposure to the same temperature was even stimulating for germination in the sense of increase of germination percentage. Blackwell and Waterhouse (1931) state a hypothesis that heating accelerates the maturing of conidia. One hour of heating strongly stimulates germination, while 30 minutes are insufficient for stimulation. Effect of high temperature of short duration is often not lethal, while a longer exposure at considerably lower temperature destroys conidia.

When transferred, after 48 hours, from 32° to 13°, a formation of secondary conidia (after 48 hours) was observed. At high temperatures, in the majority of cases a side germination of conidia took place. Secondary conidia obtained at 26° formed zoo-spores after a transfer to 15°, similar to the normal conidia. When the temperature was alternating from 20 to 30°, secondary conidia were also obtained. Zoo-spores obtained at 9°, when transferred to 35° (24 hours), stopped moving and did not germinate. Neither did the zoo-spores germinate after the return transfer to a 9° temperature. At a low temperature of 1-2° during 48 hours, germination is lacking, but with a change of temperature to 10°, conidia germinate in a direct and indirect manner. Thus it appears that sub-minimum temperature is more readily endured by conidia than the supra-maximum temperature.

4. Influence of air humidity.

The spores of Peronospora are particularly sensitive to air humidity. Doran's experiments with Peronospora viticola, Ravaz and Verge's (1912)—with Plasmopora viticola and Arens's with the same organism, confirm the high sensitivity of these organisms to air humidity. According to McAlpine (1910), drying out of conidia, even for a very short period, completely destroys their power to germinate.

Melhus does not indicate the amount of air humidity but he says that conidia did not germinate after a 6-hour stay in the laboratory.

By:
A. Antik

Lohnis [7] points out that conidia do not survive when the drying out process is fast.

In our experiments conidia do not germinate without water even at a 100% air humidity. The next problem was that of the effect produced by various percentages of air humidity on conidia which are in a dry state. We tested their viability, or its loss, by placing conidia in water after a certain exposure to various air humidities. At the beginning of the experiment a 24-hour exposure was chosen. But since the first tests already showed that at this procedure the spores lost completely their viability, it was necessary to change to shorter exposures of 6, 4 and 2 hours. Later on it became clear that even these exposure periods are destructive to conidia. Only tests with a half-hour exposure at a 100% humidity gave positive results, i. e., subsequent addition of water resulted in a small percent of germination of conidia. Therefore conidia of Phytophthora infestans are extremely non-resistant and lose very rapidly their viability when they fall from the conidia-bearers. These results coincide with results obtained by many researchers who observed the influence of dry air on spores of perenosporaceae. Discrepancy is found with experiments by Crossier. The author indicates that at 20-40% air humidity the conidia lost their viability after 1-3 hours and in humid air (50-80%)--after 5-15 hours.

5. Age of culture.

Numerous studies of spore germination among Perenosporaceae indicate that the younger the conidia the greater their power of germination. Thus Rosenbaum (1917) observed the formation of zoo-spores of Phytophthora cactorum only in very young cultures. Uppal (1926) obtained zoo-spores of Phytophthora colocasiae in cultures not older than 7 days. According to Ashby (1920) in Phytophthora palmivora the percentage of zoo-spore formation decreases with the increase of age of culture. Zattler (1931) writes on Pseudoperenospora humuli that the higher the age of spores the more they deviate from the optimum in their germination. Blackwell and Waterhouse (1931), on the basis of their observations of Phytophthora colocasiae and Phytophthora cactorum, when zoo-spores were formed in eight- and ten-day culture (and not in twelve-day ones), attribute the cause of such a phenomenon to deficiency in oxygen and accumulation of products of metabolism as factors accompanying greater age.

Doran (1922) also explains the inhibition in the germination process by oxygen deficiency. However, there is more than one opinion on the influence of oxygen on germination. Murphy (1922) and Wals (1868) maintain that oxygen stimulates formation of zoo-spores and according to Uppal oxygen is necessary to direct germination and not

By:
A. Antik

for formation of zoo-spores. According to Uppal's conclusions, the process of zoo-spores formation is only a reconstruction of protoplasm, while the formation of the germ tube requires energy and represents growth.

In observing spore germination we frequently came across the phenomenon that under similar conditions conidia germinated either in larger numbers or singly and the difference concerned the length of the shoots [?] as well. We contributed such variations to difference in the degree of maturity of spores. For this purpose experiments were carried out on germination of spores of 7- and 9-day cultures. The germination percentage in a 9-day culture lagged considerably behind the germination of conidia of a 7-day culture and the germ tubes were considerably shorter. It is interesting to note, that in a 9-day culture there was usually no formation of zoo-spores. In this regard there is a complete analogy with the results obtained by Rosenbaum with Phytophthora cactorum, by Uppal with Phytophthora colocasiae and Blackwell and Waterhouse with the same fungi. The more dubious seems the fact that DeBary obtained zoo-spores from conidia Phytophthora infestans, which persisted on slowly drying out leaves of the plant-host during three weeks after maturing. It is more probable that on these leaves continued formation of new conidia which produced zoo-spores.

SUMMARY [published in English on p. 667

. . . . . .

In conclusion I consider it my duty to express my appreciation to N. A. Naumova for constant guidance and advice during the work process and to Prof. N. A. Naumova for valuable direction and criticism.

Gorlenko, M. V.

Bolezni Rastenii i Vneshniaia
Sreda

[Plant Diseases and Environment].

Moskov. Obshch. Ispyt. Prirody,
Moscow, 1950. 120 p. 464 G87

(In Russian)

COURSE OF CHANGES IN SPECIES MAKE-UP OF CAUSAL AGENTS OF DISEASES OF CULTIVATED PLANTS

Considerable change took place during the last 50 years in the make-up and development of diseases of cultivated plants grown in the USSR. These changes took place partly due to appearance of new diseases and, on the other hand, due to a complete or partial disappearance of some widespread diseases and, finally, due to increased development of diseases little known before.

For example, ergot on rye lost, during recent years, its significance as a disease very harmful for crops of this plant. It happened because a systematic cleaning of the grain began to be adopted, adequate crop rotations were introduced and weed control in the fields was carried out in the agricultural production of the USSR. But at the same time ergot became a most important disease of forage grasses. It occurred due to specific properties of these plant crops, as well as the difficulties in cleaning the grass seeds from sclerotia of ergot. The difficulty in cleaning consists of the fact that the size and weight of grass seeds and of sclerotia are very similar.

During recent years in various parts of the USSR was noted a very serious damage by ergot of the following cultivated grasses in production fields: timothy, miscellaneous orchard grass, red fescue grass, meadow fescue grass, awnless brome grass, "bekmaniia" [?], Kentucky bluegrass, rye grass, white bent grass, rootless couch grass.

Some grasses were so seriously affected that 20-70 ergot spurs were formed in one spike.

Trans. 455
(In part)
pp: 21-33
By: A. Antik

There are different ways in which new diseases of cultivated plants can originate. The following are the basic ones:

1) importation of diseases from other countries or other areas of the same country;

2) transfer of parasites from some plant species to others (widening of the plant-host circle);

3) change of saprophytes to a parasitic mode of life.

It could probably be added that many diseases are frequently referred to as new because the species make-up of diseases of one or another crop are not yet known. Probably the following diseases could be mentioned as once incorrectly referred to the group of new diseases:

1. Bacterial canker of tomatoes (Aplanobacter michiganese Sm.), discovered in 1936 in the Crimea and later found in many other places; at the present time it is one of the most harmful tomato diseases.

2. Black rot of carrots (Alternaria radicina M. D. et E.) The disease was discovered in 1933 (T. L. Dobrozrakova, 1935) in the Leningrad area. At the present time it is one of the causes of mass destruction of carrot seeds in many areas of the USSR.

3. Brown spottiness and "streak" of tomatoes were once considered as new diseases. Now they are very widespread.

4. Mosaic of winter wheat is considered to be a very widespread disease. However it was not described until 1936 (V. K. Zashurilo and M. V. Gorlenko, 1937).

5. Typhulosis of wheat (Typhula Itoana Imai) was first found by L. M. Kozhevnikova (1947) in the Penza oblast. In reality this disease was more widespread.

All the enumerated diseases were known for a long time, but they were attributed to wrong causes. Thus it was considered that the reason for the dwarfing of wheat lies in the freezing of the tillering node. In reality this disease is caused by a specific virus, Triticum virus 8 Zaghurilo et Sitnicova.

No doubt that Typhula of wheats was earlier confused with the destruction of winter crops by Sclerotinia, which has similar symptoms.

(In part)
pp: 21-33
By: A. Antik

In 1942, in the Arkhangel'sk oblast', N. A. Naumov (1948) described a new disease of rye caused by the fungus Sclerospora secalina N. N. The symptoms of the disease are very similar to other causal agents of spottinesses of rye. Undoubtedly the disease was overlooked up to now. It was not found in any other places and the narrow specificity of the agent does not make it possible to consider its crossing to rye from other plants. Apparently this disease is of local importance and perhaps its area of natural distribution is limited.

Due to insufficient study of diseases of coriander and anise, N. A. Riakhovskii once (1935) referred to the cercosporosis (Cercospora coriandri N. Rjach.) and rust (Puccinia pimpinella Mart.) as new diseases of these crops.

Quite frequently it is not possible to judge the origin of some disease or other from published data. It happens because some authors limit themselves to statements of the existence of a disease, not going any deeper into the analysis of conditions accompanying its appearance. For example, A. M. Eremeeva (1938) describes in an interesting note a new disease of cotton caused by the fungus Robillardia gossypii Erem. According to the author it is a species new to science. However it is not clear how it became established on cotton. It could be supposed that we are dealing with the adjustment of a fungus to a new host. Apparently, however, the fungus develops on weakened and even dying plants. In any case, A. M. Eremeeva found it among plants perishing from Rhizoctonia bataticola (Taub.) Butl.

Similarly, N. A. Cheremisinov (1939), describing new species of fungi which cause spottiness of leaves of tau saghyz, did not even compare them with fungi of related wild-growing plants. At the same time species of the same genera parasitize wild species of Scorzonera (Pyllosticta scorzonera Pas. and other species).

In this regard, of great value are the works of K. E. Murashkinskii (1935, 1947), in which he analyzes the origin and also follows up the fate of all the newly appearing plant diseases in Siberia. Similarly valuable are the works of A. A. Babaian (1949) on new plant diseases in Armenia.

(In part)
pp: 21-33
By: A. Antik

Importation of diseases from other countries or from some areas of the USSR into others.

We can point out the following diseases which, among others, were imported at different times into our country: American mildew of gooseberries (1900), mildew of spindle trees (1900), mildew of oak (1910), rust of sunflowers (1866-1869), infectious drying out of lemons (1936), Phytophthora of potatoes.

All these diseases entering new areas became very widely spread.

From other areas of our country were imported anthracnose of the gourd family into the Omsk and Moscow oblasts, common smut of corn (Omsk oblast'), smut diseases of brome grass and American couch grass, twisting of cotton leaves (Armenia, A. A. Babaian, 1949), spottiness of pea leaves (Septoria pisi Westd.), apple scab (Omsk oblast', 1939-1940) and many others. P. N. Davydov points out the importation to the Altai of the fungus from the family of Phallaceae (Clathrus cancellatus Tournef.), probably with fragments of soil which clung to the planting material received by the Altai fruit and berry station[1]. Without continuing this list it is seen clearly that cases of transfer of diseases from some areas into others is a frequent occurrence. Therefore serious attention should be paid in transporting seed and planting material to its phytopathological examination, taking measures for disinfection of seeds or liquidation of diseased seedlings[2].

Transfer of parasites from some species of plants to others.

The establishing of facts of transfer of parasites from some species of plants to others is important not only from point of view of explanation of appearance of new diseases, but also for the study of the problems connected with the evolution of parasitism. Unfortunately there are not enough experimental data. Nevertheless it is frequently possible to prove the fact of crossing over by analyzing the conditions of disease appearance and the course of diseases of closely related plants.

---

1) Davydov, P. N.--Foreign guests. Priroda, 6, 1950. The fungus was found in hothouses.

2) I. V. Michurin also pointed out the danger of importing diseases and pests. He wrote in one of his works: "We take a risk of transferring into our gardens the contamination by many pests, as it took place with the mildew Sphaerotheca mors-uvae, which affected all the varieties of gooseberries in our gardens." (Selected writings, 1948, p. 395).

(In part)
pp: 21-33
By: A. Antik

Experimentally proven is the transfer of the rust fungus (Puccinia verruca) from the wild Centaurea Scabiosa to safflower. Numerous are the cases when rust fungi crossed from wild-growing grasses to cultivated ones. K. E. Murashkinskii (1935) describes a case of transfer of Uromyces astragali to Sophora japonica imported to the Omsk oblast'.

A. A. Babaian (1949) describes several cases of transfer of mildew fungi from one species of plants to others. Thus according to his opinion mildew fungi crossed from other plant species to potatoes, peanuts, flax.

A. S. Bondartsev (1945) observed damage to potatoes by "openki" [a brown edible mushroom] Agaricus malleus in sections previously occupied by timber. K. E. Murashkinskii (1935-1947) describes migration of Ascochyta pinodes from peas to alfalfa, Daldinia concentrica - from timber to apple trees, mildew - to strawberry from some other plants.

The crossing from some species of plants to others is conditioned by particularly favorable conditions which act depressingly on the host but not on the parasite. We described earlier (1946) that wheat varieties resistant to mildew become highly susceptible to it when there is a deficiency of soil moisture. These conditions cannot be always reproduced in an experiment (perhaps it is not even always possible to determine them exactly). Therefore, the experimental checking frequently does not confirm the fact of migration. It seems to us that serious outbreaks of brown rust of wheat in the Zapoliar'ie [?] described by L. S. Gutner and M. K. Khokhriakov (1940) are due to such a crossing of the fungus from wild-growing grasses. It is hard to assume the migration of uredospores of this fungus into Zapoliar'ie from areas farther south.

This explains also the appearance of mildew on cotton in Middle Asia in 1929 and in 1946-1947. In 1929 the first case of crossing of fungus to cotton was observed. However, it [fungus] did not establish itself on the new host. Then a second independent transfer took place, not connected with the first one, and it led to an outbreak of the disease in 1946.

Mildew of potato belongs to the same disease group. The fact of its appearance on potatoes was registered by S. Iu. Shembel' as early as 1915 and 1925 in the Astrakhan' oblast'. The disease was not developed there any further. Such a long interval between two cases of appearance of the fungus is due each time to new crossing over of the fungus from one host to another.

In 1944 mildew on potatoes appeared in Armenia. It developed there only in the conidial stage. A. A. Babaian assumes that it crossed to potatoes from other plants.

Recently E. A. Osnitskaia (1950) described a new (for the Moscow oblast') cabbage disease - "yellows" which, according to her opinion, is caused by the fungus Fusarium conglutinans Wr. Pointing out that cabbage yellows appeared in hot-houses, where rubbish from the city dumps was used for fertilizer, E. A. Osnitskaia assumes that ". . . it is just rubbish which presents a source of infection."

However, Fusarium conglutinans on cabbage is not registered anywhere in the USSR. If that is the case, how did it get into the rubbish? It should be pointed out that the same species (but a different form) affects asters in the Moscow oblast'. It is possible that the fungus crossed to cabbage from asters or other plants. It can be also supposed that Fusarium conglutinans occurred before on cabbage but was overlooked. This is indicated in the work by N. N. Vladimirskaia (1940) who studied fusariosis on cabbage seedlings. We note that the fungus which she isolated affected a series of other plants besides cabbage.[1]

A widening of the host cycle of broom-rape takes place all the time. These plants began lately to parasitize tau- and kok-saghys (I. G. Beilin, 1947), anisette [anise ?] (K. I. Osipov, 1936).

We think that the transfer from certain species of plants to others takes place quite extensively, even among narrowly-specialized parasites. This crossing is conditioned by exterior factors which bring the plants into a state of susceptibility and deprive them of their natural protective properties.

Change of saprophytes to a parasitic mode of life

In nature a process takes place all the time of adjustment of saprophytic organisms to feeding on living plants. It takes place among bacteria as well as among fungi. The following cases of plants parasitized by saprophytic bacteria are known:

---

1) Not excluded is also the possibility that in the given case we are dealing with a saprophytic form of Fusarium which adjusted itself to parasitism.

A nearly parasitic species of Melanospora betae Panas was disclosed in hollows of sugar beets together with Fusarium (V. T. Panasenko, 1938). But generally speaking the representatives of this genus belong to saprophytes which develop in the soil or on rotting remains of plants[1]. Probably in this case we have the first stage of adjustment of the fungus to parasitizing living plants[2].

Thus the process of adjustment of saprophytes to parasitic mode of life is rather widely spread. This transition is furthered, first of all, by surrounding factors.

* * *

The fate of newly appearing plant diseases can vary. It depends on outside factors, favorable or unfavorable, for the development of the disease, on man's activity, on resistance of the plant-host. Following cases are possible:

1. Newly appeared diseases can disappear again. This can occur because man liquidated the nucleus of this disease, or because the parasite did not find conditions favorable for its development.

In 1930 O. B. Natal'ina (1931) discovered a very dangerous flax disease - "pasmo" /Septoria linicola (Speg) Garis/. The nucleus of this disease was liquidated and it was not discovered anywhere else in the USSR.

In 1937 we found in the Voronezh oblast' damage to wheat by the fungus Dilophospora alopecuri Fr. All the diseased spikes were destroyed. During the following years (1938-1941) the disease was not found anymore.

In 1930 R. Dzhelalov (1935) discovered wet smut of barley in Azerbaidzhan (Tilletia paniciі Bub. et Ranojevic). After that no one found this fungus either there or in other places.

---

[1] One of species of this genus is parasitizing fungi (M. parasiticus) and the M. intera causes rotting of roots and stem centers of tomatoes. S. F. Morochkovskii (1948, p. 45, 188) thinks that M. betae parasitizes not the beet, but a fungus of the genus Fusarium.

[2] Apparently it is not possible to draw a sharp line between parasites and saprophytes. Almost every saprophytic species, under certain conditions, can go over to feeding on living tissues, i.e., become a parasite. Destruction of young growths of wheat, corn and cotton from fungi of the genus Penicillium - obvious saprophytes - has been known.

(In part)
pps 21-33
Bys A. Antik

In 1946, also there, a focus of tuberculosis disease of olives (Bact. Savastanoi Sm. et Tows.) was destroyed[1].

To such once-occuring diseases could be referred also the brown bacteriosis of wheat (Bact. nigrofaciens Chod.) described by N. A. Khodakovskii in the Volga region and not found anywhere else. It is not quite clear what explains the lack of information on spreading of this disease in other places. It is possibly connected with localization of the parasite in only this area or it can be explained by negligence of the explorers. This disease is noticeable only on seeds after the threshing.

It is also known that a change of varietal composition of one or another crop can lead to disappearance of diseases spread among them. Very interesting data pertaining to this problem are given by A. I. Solov'ieva (1949). In 1948 A. A. Babaian and D. N. Teterevnikova-Babaian discovered in Middle Asia a disease of the Asiatic cotton (Gossypium hirsutum [according to Callaham]) caused by the fungus Fusarium bucharicum Jacz. When the "guza" - Asiatic cotton - was not cultivated any more, this disease disappeared.

Several years ago in Tadshikistan and Uzbekistan appeared a new disease of the Egyptian cotton - the Fusarium wilt. After replacing nonresistant varieties by varieties resistant to this disease - the latter has almost disappeared.

2. Newly discovered diseases can take a mass character by becoming widely spread diseases of one or another crop. To such can be referred the Macrosporium lallemantiae Chochr., which was described in 1934 by M. K. Khokhriakov and which at the present time became an agent of an epidemic disease of "lallemantia".

Probably in the thirties the infectious drying out of lemons (Deuterophoma tracheifilla) was imported into our country. By the middle of the forties the disease became one of the most harmful ones for this crop (L. A. Konchaveli and K. G. Gikashvili, 1947).

To the same group could be referred also the American mildew of gooseberry, imported here in 1900, which very rapidly almost entirely eliminated the cultivation of this plant. It should be pointed out that during recent years an adjustment of gooseberries to the fungus took place and in connection with it its damage by mildew decreased. The adjustment process seems to have taken place in the following manner: at the time when the fungus came to this country it found

---

[1] Illustrated manual on pests and diseases of external quarantine. 1948, p. 218.

gooseberry varieties very susceptible to it. This led to a most severe flare-up of the disease and a mass destruction of nonresistant plants. But during the process of aggressive attack of the fungus on plants, a natural selection of more resistant varieties took place, aided by man who destroyed the most seriously affected bushes. All the viable plants were preserved for 50 years and since at that time the viability was determined by the resistance to mildew, - they were also the more resistant ones. At the same time all the susceptible plants were perishing. It led finally to a less serious affection of gooseberries at the present time. In addition to all that, reliable control measures were developed (works by A. S. Bondartsev, I. E. Barbarin, S. L. Strelin, M. V. Gorlenko) which are very effective at the present time because they are applied to more resistant varieties. It is known that every measure is more effective the more the variety being protected is resistant.

Mass development of newly imported parasites is altogether a quite general occurrence and is connected with the fact that the parasite comes in contact with plants not adjusted to it.

In introducing new plants or varieties for cultivation a mass development can take place of diseases which up to then were not known or not widely spread. For example, rust of safflower (Puccinia verruca Thum.), which crossed to this crop from the pink cornflower, became an epidemic disease of this plant. We were convinced of it when we examined the safflower fields in Kazakhstan.

Greatly developed on branched wheat is the fungus Septoria nodorum Berk., which only slightly affects other wheat species.

3. Newly appeared diseases do not develop extensively, but do not disappear either. They go over to the group of rarely found diseases of one or another crop. Sometimes more or less considerable flare-ups of these diseases can take place. There are many diseases belonging to this group, for example, Phoma solanicola Pr. et Del. on potatoes develops only during certain years. 1944 was such a year. In other years it could be found only by carrying out special searches. Here belong probably also perforated spottiness of beets, caused by bacteria, mildew of flax, scab of cherries, spottiness of apple leaves caused by the fungus Hendersonia mali Thum., pseudo-mildew of beets.

* * *

Of tremendous interest is the problem of study of make-up of diseases of plants being introduced for cultivation in one or another area. Such kind of material opens to a certain degree the curtain over the process of formation of pathogenic flora of one or another cultivated plant.

(In part)
pp: 21-33
By: A. Antik

When a plant moves into new areas or wild-growing plants are introduced to cultivation, the process of formation of pathogenic flora can proceed by the following ways:

1. Part of the diseases is imported with the seeds or planting material from areas of old cultivation or from places of natural growth. The development degree of the imported diseases in new areas depends on external factors. They might either disappear or, to the contrary, become more harmful than in old areas.

2. If in the new cultivation areas closely related plants are grown or are in a wild-growing state, then the crossing of part or all the disease agents from these plants to the newly introduced ones is possible.

3. If the plants find conditions unfavorable for them in the new cultivation areas, then it is possible that a series of saprophytic species will make an adjustment to parasitize them.

The more unfavorable the conditions are, the more the plant will suffer from saprophytes and parasites from other plants which will be adjusting themselves to the new plant.

This can be illustrated by a series of examples.

During the thirties, root rubber-yielding plants tau-saghyz and kok-saghyz, which up to then were known only in a wild state, were introduced to cultivation.

Almost 20 years of studying the diseases of these plants make it possible to establish the origin of each one of them and therefore to visualize the general picture of formation of the pathogenic flora of these plants.

Diseases of tau-saghyz, spread at the present time, are of following origin:

1. Diseases imported with seeds or by some other way from natural growing places:

a) Black necrosis (Bact. necrosis Kalin.) spread widely with seeds wherever tau-saghyz settled. The disease is found under natural growth conditions and damages the crop greatly.

b) Mildew (Leveillula taurica F. tau-saghysi N. Cherem.) is so far imported only to fields located in areas adjoining the natural

growths and is widely spread in the latter as well. The damage in some sections is very great (N. A. Cheremisinov, 1939).

2. Diseases transferred from other plants.

a) Rhizoctoniosis (Rhizoctonia violacea Tul). The fungus crossed to tau-saghyz from other crops, possibly from the sugar beet. A. M. Shvorneva (1940) found on tau-saghyz a perfect stage of this fungus - Helicobasidium purpureum (Tul.) Pat. Serious damage by Rhizoctonia took place in a number of plantations in Kazakhstan.

b) Sclerotinia (Sclerotinia Libertiana Fuckl.). The fungus causes root rot. It crossed to tau-saghyz from the sunflower where it is one of the most important parasites. That the transfer is really from this crop is indicated by the circumstance that the distribution areas of the sclerotinia root rot of tau-saghyz coincide with areas of sunflower cultivation (N. A. Cheremisinov, 1939).

c) Broom rape (Orobanche cumana Wallr. and O. ramosa L.) crossed to tau-saghyz - the first species from sunflowers, the second from hemp. It is potential as a mass parasite of tau-saghyz (T. G. Beilin, 1940).

d) Septoriosis (Septoria tau-saghysi N. Cherem.) and phyllostictosis (Phyllosticta tau-saghysi N. Cherem.). It is possible that they crossed over from wild species of Scorzonera. At least S. F. Rostovtsev (1908) points out Phyllosticta Scorzonerae Pass. on wild plants.

3. Diseases originating due to unfavorable conditions of cultivation. Here belong root rot or maceration of root. Cause of this disease - inadequate aeration of soil and light freezing of the root neck [7] (see the details of this disease on p. 112).

Formation of disease make-up of kok-saghyz proceeded in the following manner:

1. Diseases imported from natural growths:

a) Black necrosis (Bact. necrosis Kalin). Spreads everywhere with kok-saghyz cultivation (M. V. Gorlenko, 1947);

b) Rust (Puccinia taraxaci f. kok-saghysi Zaiceva). A. A. Zaitseva (1948) considers rust on kok-saghyz to be a microorganism specie for this plant which came to the plantation from areas of natural growth where the disease is spread. However, a mass distribution of the disease in a short time in new places speaks rather of a local character of the infection. Yu. Smarods (1943) refers it to the species Puccinia taraxaci Plowr., not separating it as an individual variant. The problem is subject to checking.

Trans. 455
(In part)
By: A. Antik
pp: 21-33

2. Diseases transferred from other plants:

a) Ragwort (Senecio jacoboea, acc. to Callaham) [?"zheltukha" ?] of kok-saghyz crossed to this plant from dandelion on which it is very widespread. Its significance is very great;

b) Spottiness of leaves - ramulariosis (Ramularia taraxaci) is of the same origin as the preceding species. Its significance is slight;

c) Mildew (Sphaerotheca fuliginea f. taraxaci) is widely spread on the common and other dandelions from which it crossed to kok-saghyz;

d) Gray rot of heads - result of enlarging of the host range of the multi-toxic fungus Botrytis cinerea Pers.;

e) "Zarazikha" [broom rape ?] (Orobanche cumana, O. ramosa) came to kok-saghyz from sunflowers or hemp. So far the parasite has no mass development on kok-saghyz (I. G. Beilin, 1947).

3. Diseases connected with unfavorable conditions for plant development.

Infertility of heads with the ensuing population of the dead baskets with various fungi, chiefly Botrytis cinerea Pers., Alternaria sp. Since kok-saghyz is pollinated by insects, in their absence the seeds do not germinate and the heads die off. When there are large plantations of kok-saghyz and the number [of flights ?] of insect is inadequate, for examole, during rainy weather, then there are not enough insect-pollinations, part of the heads not pollinated, dies off and is populated by fungi. The fewer pollinators there are the more infertile baskets.

* * *

During recent years, in the non-black-earth zone of the USSR, the cultivation of Cucurbitae - watermelons and melons began. Up to that time, in the same areas, only pumpkins and cucumbers of the gourd family were cultivated. It was natural to expect a transfer of a number of diseases from these crops to melons and watermelons; and it took place. In 1940 V. F. Izrail'son carried out at our suggestion an investigation of diseases of Cucurbitae crops in the Moscow oblast'. As a result of this work the following diseases of melons and cucumbers were exposed: (p.29)

(In part)
pp: 21-33
By: A. Antik

Name of disease	Name of causal agent	Origin of disease
Anthracnose	Colletotrichum oligochaetum	Imported with seeds
Root rot	Fusarium oxysporum	Transferred from other plants
Fusariosis of fruits	Fusarium solani	Transferred from other plants
Brown spottiness of fruits	Scolecotrichum melophtorum Prill. et Del.	Transferred from cucumbers
White rot of fruits	Scl. rotinia Libertiana Linck.	Transferred from other plants
Mildew (only on melons)	Erysiphe cichoracearum Sphaerotheca fuliginea	Transferred from cucumbers and pumpkins
Mosaic	-	Transferred from cucumbers and pumpkins

Almost all the melon and watermelon diseases in the Moscow oblast' are of local origin.

The analysis of melon and watermelon seeds arriving in the Moscow oblast' from different spots in the USSR and carried out by V. F. Izrail'son in the spring of 1949 and 1950, showed that there were no pathogenic organisms (in the catches which were analyzed). With the only exception of anthracnose. A. S. Pimenova (1949) assumes correctly ". . . that anthracnose of melons and cucumbers penetrates into areas farther north simultaneously with these crops.

The imported character of anthracnose is indicated by the fact that it is found only in two (of the investigated) points which are close to each other. On the gourd family spread in the Moscow oblast' (cucumbers, pumpkin, squash) anthracnosis is not found (A. S. Pimenova, 1949; B. A. Gerasimov and E. A. Osnitskaia, 1948). K. E. Murashkinskii (1947) describes cases of importation of anthracnose with seeds into the Omsk oblast'. Later on the disease disappeared there.

The following facts prove the local origin of almost all the diseases of melons and cucumbers which are spread in the Moscow oblast'.

1. Fusarium solani u. Sclerotinia Libertiana are multitoxic micro-organisms which develop in various substrata. There is no basis to assume that specific forms of these fungi will develop on fruits of melons.

(In part)
pp. 21-33
By A. Antik

2. The possibility of infection of various fruits of the gourd family with the fungus Scolecotrichum melophtorum is proved by experiments of Yu. I. Schneider (1949).

3. Cross inoculation with mildew of cucumbers, melons, pumpkins and squash was carried out in our laboratory by E. S. Bazanova. According to her data only watermelon was not infected by mildew taken from cucumbers. The disease was not found on them in nature either.

4. Mosaic of the gourd family readily infected all the representatives of these families (A. M. Vovk, 1942).

It is not clear from which plants Fusarium, which causes root rot, crossed to melons. There is no doubt that the disease is of local origin. It is indicated by its general spreading and mass affection of plants the very first years after sowing of melons and watermelons, as well as by the lack of this fungus on seeds which arrive in the Moscow oblast' from other areas. However, on cucumbers and pumpkins in the Moscow oblast' the Fusarium root rot was not widely spread. Besides that, the fungus species which V. F. Izrail'son isolated and which causes root rot (Fus. oxysporum), is different from species which affect similar plants in other places (S. A. Avakian, 1949; Bodigin, 1934).

Fusarium oxysporum affects potatoes and is found on these plants in the Moscow oblast'. But generally speaking it is a multi-toxic species, its forms - discovered on melons and watermelons - are parasites of seedlings of pine, tomato and many other plants. It is possible that the fungus crossed over from the melon to potatoes.

V. F. Izrail'son's experiments showed that Fus. oxysporum can live in the soil like a saprophyte. That probably accounts for this assumption: while in the soil the fungus found a particularly susceptible host and caused its mass disease.

Usually the root rot and wilt of the gourd family is caused by Fusarium bulbigenum from which the species which affects the gourd family in the Moscow oblast' differs in less abundant spores.

There are no widely spread representatives of the gourd family in the wild-growing flora of the Moscow oblast'. Therefore, the formation of the composition of diseases of melons and watermelons was due basically to their crossing from related cultivated plants.

* * *

When the sugar beet moved to the eastern areas of the USSR, the make-up of diseases of this crop in the new areas was different from that in the basic areas of its cultivation. Mildew took the first

place among diseases of grown plants. In the European USSR this disease develops sporadically (V. P. Murav'iev, 1938). Therefore, it is hardly possible to suppose an imported character of this disease. It could rather be considered that the disease passed over to sugar beets from some local cultivated or wild-growing plants. Similar is the origin of this disease in Turkmenia (A. A. Bogoiavlenskii, 1944).

The principal cause of disease of sugar beet sprouts is the fungus Rhizoctonia Adercholdii widespread in the soils of Middle Asia and which causes root rot of cotton sprouts and of some weeds. Even the make-up of organisms causing rotting of sugar beets in the "Kagat" /mounds for storing tubers/ in Middle Asia is different from that in the old areas of beet-sowing. First of all the main agent of "Kagat" rot is missing - the fungus Botrytis cinerea. The climatic conditions turned out to be unfavorable for its development. To replace it came species of the genus Fusarium (probably local forms) [1] and Rhizopus nigricans. The rot caused by the latter fungus is widely spread in Uzbekestan, probably because this parasite likes heat very much.

Apparently the make-up of diseases of sugar beet in Middle Asia is also of local origin. Its difference from the make-up of diseases of the same plant in other areas of our country is determined by specific climatic conditions which do not allow the development of many widely spread diseases of sugar beets (for example cercosporiosis, caused by the fungus Cercospora beticola).

Local parasites will frequently present a much greater threat to newly introduced crops in one or another area, than the ones they brought with them. The former can cause right away a mass affection on a large territory. This makes the control of them rather difficult from the very beginning. On the contrary, the imported diseases form at first small loci and it is quite possible to liquidate them. Extreme vigilance and attention have to be exercised in these cases as well, particularly during the first stages of introduction of a crop. Of great importance is a thorough examination of the sowing and planting material which makes it possible to liquidate the disease before it strikes the field.

A. T. Solov'ieva (1949) is quite right when she writes: "Renouncing old notions - to study only the harmful diseases - the phytopathologists have to carry out most profound research on new diseases and have to pay particular attention to factors which accompany the reproducing parasites. This allows to determine the danger potentialities of the new disease and to liquidate or suppress it at the very beginning of its appearance". [2]

---

1) Panfilova, T. S. - "Kagat" rot of sugar beet in Uzbekestan, Dokl. AN UzSSR. 5, 1948

2) XIXth plenum of the plant protection section at the VASKhNIL. Theses of reports, III, Stalinabad, 1949, p. 31.

BIBLIOGRAPHY

Avakian, S. A. On a new disease of apricots. Dokl. AN Arm. SSR, III, 1945, p. 113-116.

Avakian, S. A. Root rot of gourd crops in the Armenian SSR. Mikrobiolog. sbornik, 3, 1949, p. 123-139.

Babaian, A. A. Mildew on potatoes. Arm. n.-issl. inst. tekhn. kul'tur. sbornik trudov po zashchite rastenii [collect. of writings on plant protection], 2, 1949, p. 93-101.

Babaian, A. A. New data on fungal diseases of cultivated plants in the Arm. SSR. Sbornik trudov po zashch. rast., 2, 1949, p. 102-111.

Babaian, A. A. Discoveries of the virus rolling [twisting] of cotton leaves and of the bacterial canker of tobacco ["stolbur"] in the Arm. SSR. Sborn. trudov pozashchite rastenii, 2, 1949, p. 112-118.

Babaian, A. A. and Teterevnikova-Babaian, A. A. On disease of Asiatic cotton caused by the fungus Fusarium buharicum Jacz. Materialy po mikol. i fitopatol, VIII, no. 1, 1929, p. 205-218.

Beilin, I. G. On control of broom rape on sunflowers. Vestn. zashchity rastenii, 3, 1940, p. 94-104.

Beilin, I. G. Broom rape and their control. 1947, p. 1-76.

Beilin, I. G. Evolution of parasitism among flower plants. Biull. Mosk. obshch. ispyt. prirody, otd. biol. LIII, 3, 1948, p. 55-65.

Bogoiavlenskii, A. A. From investigation material of agricultural plants in Turkmenia in 1943, "Sov. botanika", 3, 1944, p. 46-47.

Bondartsev, A. S. On affection of potatoes by "openki" [brown edible mushrooms] "Sov. botanika", 5, 1945, p. 28

Vladimirskaia, N. N. Fusariosis of cabbage seedlings and use of condensate in control of it. Vestn. zashch. rastenii, 5, 1940.

Vovk, A. M. Mosaic disease of cucumbers. Mikrobiologiia, XI, no. 4, 1942, p. 169-177.

Gerasimov, B. A. and Osnitskaia, E. A. Pests and diseases of vegetable crops. 1948.

Gorlenko, M. V. Influence of exterior factors on the development of mildew of wheat. Dokl. VASKhNIL, 9-10, 1946, p. 13-15.

Gorlenko, M. V. Essay on geographical distribution of bacterial plant diseases in the USSR. Biull. Mosk. obshch. ispyt. prirody, otd. biol. LII, 2, 1947, p. 61-70.

Gorlenko, M. V. Principal plant diseases spread in the central zone of the USSR. Kalendar' prirody SSSR, II, 1949, p. 57-80.

Gutner, L. S. and Khokhriakov, M. K. Material on diseases of cultivated plants of the Kol'skii peninsula. "Vestn. zashch. rastenii", 1940.

Dzhalalov, R. New species of smut on barley. "Na zashchitu urozhaia", 3, 1935, p. 25.

Dobrozdrakova, T. L. Black rot of carrot seeds. "Na zashchitu urozhaia", 3, 1935, p. 26-27.

Eremeiev, A. M. A new species from genus Robillardia on cotton. Materialy otd. spor. rast. Bot. inst. AN SSSR, IV, no. 10-12, 1938, p. 34-35.

Zazhurilo, V. K. and Gorlenko, M. V. Mosaic of wheats in the Voronezh oblast'. Itogi rabot VIZR za 1936, part I, 1937, p. 129-131.
Zaitseva, A. I. On biology of the rust agent of kok-saghyz. Dokl. Mosk. s.-kh. akad. im. Timiriazeva, no. 7, 1948.
Kalashnikov, K. Ia. Trichothecium roseum Link. on cucumber plants in a protected ground. "Zashchita rastenii", 7, 1935, p. 136-138.
Konchaveli, L. A. and Gikashvili, K. G. Malsecco--disease of lemons, in the Georgian SSR. XVIth plenum of the plant protection section VASKhNIL. Tezisy dokladov, 1, 1947, p. 34-37.
Kozhevnikova, L. M. On the problem of destruction of winter crops in some areas. Dokl. AN SSSR, LV, 1, 1947.
Morochkovskii, S. F. Fungal flora of the "kagat" [?] rot. 1948.
Murav'iev, V. P. Diseases of sugar beets and control measures against them. Sveklovodstvo. III, 1938, p. 218.
Murashkinskii, K. E. New diseases of cultivated plants of Western Siberia. Trudy Omsk. SKhI, I (XIV), no. 6, 1935, p. 1-28.
Murashkinskii, K. E. New "pirenomitset" [? pyrinomycet ?] on millet. Trudy Omsk. SKhI, III (XVIII), 1938, p. 103-104.
Murashkinskii, K. E. From summaries of studies of phytopathogenic microflora in the Omsk oblast', in 1946. Thesis and autoreport. Dokl. nauchu. Konfer. Omsk. SKhI, 1947, p. 1-3.
Natal'ina, O. B. Preliminary report on flax diseases caused by fungus Phlictiena linicola. "Zashchita rastenii", 8, 2, 1931.
Natal'ina, O. B. New on diseases of our gardens. "Na zashchitu urozhaia", 7, 1935.
Naumov, N. A. On new plant diseases caused by new or little known fungi. "Sov. botanika", 3, 1939, p. 75-84.
Naumov, N. A. Disease of winter rye caused by a new species of the fungus Perenospora secalina N. N. Sborn. trudov Vsesoiuz. inst. zashch. rast., no. 1, 1948, p. 51-52.
Osipov, K. I. Broom rape of anisette [?]. "Sov. botanika", 1, 1936, p. 130.
Osnitskaia, E. A. Yellowness of cabbage. "Sad i ogorod", 2, 1950, p. 59.
Panasenko, V. T. On a new species of Melanospora. Bot. mater. Otd. spor. rast. Bot. inst. AN SSSR, IV, no. 10-12, 1938, p. 31-33.
Pimenova, A. S. Melon and watermelon diseases in northern areas. "Sad i ogorod", 6, 1949, p. 72-75.
Riakhovskii, N. A. New species for Voronezh oblast' of parasitic fungi. "Bot. zhurn. SSSR", XX, 5, 1935, p. 473-474.
Smarods, Iu. Fungi latvici exsiccati. Fasc. XXII, 1056, Riga, 1943, p. 399.
Solov'ieva, A. I. Virus and other diseases of cotton. XIX plenum sektsii zashch. rastenii VASKhNIL. Tezisy dokladov, III, 1949, p. 29-31.
Khodakovskii, N. A. Report on a new bacteriosis of wheat. Rezul'taty otch. issl. Nem. STAZRA za 1928-1930. Pokrovsk na Volge, 1931.
Khokhriakov, M. K. New species of fungal parasites on new olive plants. Collect. Diseases and pests of olive crops. Trudy VNIMNK, no. 1, 1934.
Cheremisinov, N. A. Mycoses of tau-saghyz. Trudy Voronezh. gos. univers. XI Bot. otd. no. 1, 1939, p. 7-29.

(In part)
pp: 21-33

Shembel', S. Iu. Survey of diseases of cultivated plants in the Astrakhan' gub. (former Astrakhan. stentsiia zashch. rastenii, 1927).

Shisholova, N. A. The role of proto-pectinase in the infection process of plants. Mikrobiologiia, XVII, 3, 1948, p. 252-262.

Shneider, Iu. I. Etiology of brown (green) spottiness of cucumbers. Mikrobiologiia, XVIII, 1949, p. 62-66.

(In part)
pp: 109-111
By:
A. Antik

Gorlenko, M. V.

Bolezni rastenii i Vneshniaia Sreda.

[Plant Diseases and Environment]

Moskov. Obshch. Ispyt. Prirody,
Moscow, 1950. 120 p.
464 G67

(In Russian)

Notes on biology of rust fungi on cereals.

A very great deal has been written about rust fung. on grain cereals and it would seem difficult to establish anything new concerning their biology. However, biological peculiarities of an organism are not something permanent, set once and for always. They will change depending on changes in surrounding conditions. And in talking about parasitic organisms--there always takes place an adjusting of the fungus to biological peculiarities of the plant-host. That it is really so, we showed with sufficient detail in relation to the causal agent of mildew of wheat (see the preceding section).

In the present section we wanted to show the adjustment properties of fungi which cause brown rust of wheat and stem rust of rye.

* *
*

In 1926, L. F. Rusakov established for the first time the fact of over-wintering of brown rust in winter crop plantings. Subsequant works carried out by a number of researchers demonstrated that such over-wintering process of the mentioned fungus is almost unique. The intermediate host of this rust - "vasilistnik" (*) -, discovered in 1923 by A. M. Eremeeva, plays an insignificant role in the development of this rust. Biological analysis of "editsii" developing on "vasilistnik", which we carried out in the Voronesh oblast and V. A. Bryzgalova in East Siberia, showed that in the majority of cases they belong to species not affecting wheat.

(*) Thalictrum sp.

(**) misprint in original, should be "aecidia"

(In part)
pp: 109-111
By:
A. Antik

As to hibernation in winter crop plantings--the highest development of brown rust takes place in areas of greatest concentration of winter wheat.

Thus in areas where there are winter and spring wheats, the development of this fungus is quite satisfactorily explained without the presence of the intermediate host. However, we still have areas where there were almost no winter wheats. Nevertheless a development of brown rust took place there. How then does the annual regeneration of the fungus occur there?

V. A. Bryzgalova's works (1937) indicate, that there is in East Siberia a specific intermediate host of brown rust - Isopyrum fumarioides. In the cycle of its development the fungus passes necessarily through the formation of the aecidial stage on the mentioned plant. Such difference in the biology of the same species depends on various peculiarities in the development of the plant-host.

In areas of winter wheat cultivation the most viable forms are those which develop only on the main host. "Vasilistnik" is found rarely in the fields and therefore could not be the cause of mass rust development.

In areas of only spring wheat cultivation there were no possibilities for a mass development of the fungus in uredo-stage only. There forms are more viable which form abundantly teleuto-stage and develop with transmissal through an intermediate host. Even more--the place of mass formation of "editsii" /aecidia/ proved to be not the "vasilistnik", but a weed "leshchitsa" (Isopyrum fumarioides). Apparently the adjustment properties of the fungus became sufficiently clearly outlined.

* *
*

Among the many forms of stem rust of cereals, that which affects rye is most widely spread. And the development of the stem rust of rye is not connected with the presence of the intermediate host-barberry - in the same degree as it is in the fungus which affects wheat. We expressed a hypothesis on over-wintering of this species on rye and on weeds which are affected by the same form as rye.

In 1949-50 the student L. N. Borisova was engaged in studies of this problem in our laboratory. Her work demonstrated that

Trans. 455
(In part)
pp: 109-111
By:
A. Antik

the uredo-spores of stem rust of rye retain viability during the entire winter period of hibernating stems of rye and couch grass. These data appear in the figure on p. 111.

Thus the uredo-spores of the rye form, adjusted themselves to preservation of viability at low temperatures. Therefore this form occurs so widely. But that is not all. Study of specialisation of various forms of this same species showed that rye can be affected by all the forms of stem rust which are spread in the Moscow oblast. (table 1).

Table 1 (p. 110)

Specialization of various forms of stem rust in the Moscow oblast (data by L. N. Borisova, 1949).

Forms of rust	Affected cereals									
	Secale cereale	Triticum vulgare	Avena sativa	Agropyrum repens	Dactilis glomerata	Phleum pratense	Poa pratensis	Bromus inermis
f. secalis from rye	+	-	-	+	-	-	-	-
f. secalis from couch grass	+	-	-	+	-	-	-	-
f. avenae from oats	+	-	+	-	+	-	-	-
f. avenae from misc. orchard grass and weak	+en!	-	+	-	+	-	-	-
f. phlei pratensis	+	-	-	-	-	+	-	-
f. tritici	+	+	-	-	-	-	-	-

A number of plants mentioned in the table are perennials, and hibernation of rust on them is also possible.

Rye can be affected by any form of the rust fungus which hibernated to some degree on the above-mentioned cereals. Of greatest danger in this respect are forms which develop on couch grass, miscellaneous orchard grass, timothy.

Trans. 456
(In part)
pp: 109-111
By:
A. Antik

The development of stem rust on rye proceeds probably as follows: the fungus appears first on wild grasses and then crosses over to rye. Therefore various forms of the same species develop differently on various plants. In this respect these data coincide with those which we obtained on mildew.

Fig. (p.111) October, November, December, January, February, March, April

Number of germinated spores of Puccinia graminis (in %):
1 - from rye; 2 - from couch grass (1 mm = 1%)

Biological peculiarities of one parasite in various localities makes it imperative to approach in a different manner the organization of control measures. Therefore the necessity of biological study of parasites under concrete conditions of distribution is confirmed once more.

Bibliography

Bryzgalova, V. A. On a new intermediate host of brown rust. Trudy po zashchite rastenii vsst. Sibiri, 5, 1937

Gorlenko, M. V. Rust of cereals and control measures. Sel'khozgiz, 1948

Eremeeva, A. M. On aecidial stage of Puccinia triticina Eriks, "Bolezni rastenii", 3-4, 1923

Rusakov, L. F. On the problem of hibernation of cereal rust. Materialy po mikologii i fitopatologii, no. 1, 1926

Trans. 456
(In full)
By:
A. Antik

Nilova, V. P., Svoiskaia, V. D., and
Ikonnikova, A. M.

Tirozin, aktivnost' tirozinazy i immunitet pshenitsy k buroi rzhavchine (Puccinia triticina Erikss)

[Tyrosine, activity of tyrosinase and wheat immunity to Puccinia triticina].

Vsesoiuzn. Akad. Sel'skokhoz. Nauk im. V. I. Lenina. Dok. 15(1):38-42. 1948. Ref. 20 Akl

(In Russian)

There are many works dedicated to study of the bio-chemical essence of plant immunity. The authors connect the varying degree of plants' resistance to diseases either with the varying activity of enzymes or with varying content of some substances or others (1,2,3).

It has been established that susceptible wheat varieties are characterized by a greater activity of catalase and peroxidase (2). Besides that, a number of authors showed that resistance of plants correlates relatively well with their contents of phenols (4,5,6). Fedotova (7), who applied the serological method, demonstrated that plant immunity is connected with various properties of albumen existing already in the seed.

All these disconnected facts do not explain the causes of plant resistance. They only indicate, that varieties varying in degree of resistance, vary in the serological properties of albumen, quantitative contents of phenols, activity of oxidizing--regenerating ensymes and, in particular, of enzymes which oxidize phenols.

The present work is an attempt to establish connection between facts mentioned in literature.

Tyrosinase--an enzyme occurring extensively in the plant world--belongs to the group of phenolases.

We conducted studies of activity of this enzyme in wheat varieties of varying resistance to leaf rust (table 1).

Table 1 (p. 39)

Resistant	Resistant depending on age	Susceptible
	Trit. Vulgare Spring Wheat	
Klein 31 (Argentina)		Novinka Lintestsens 062
	Winter Wheat	
Democrat (U.S.A.) Mediterranean	Lesostepka 074	Ukrainka Kooperatorka Gussap
	Trit. Durum Spring Wheat	
Gordeiforme 010		

Taken for analysis were seeds from yields of 1937, 1938, and 1944 which were preserved uhtil 1945 and seeds of the 1945 yield. Besides that the activity of these enzymes was examined on 5-6 day old seedlings obtained from the same seeds.

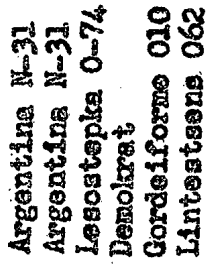


Fig. 1. Activity of Tirosinase of wheat varieties (% of tirosin oxidized by tyrosinase from 1 g. of absolutely dry seed substance). p-40

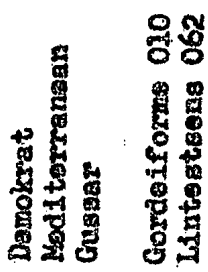


Fig. 2. Activity of tirosinase of wheat varieties.
a. % of tirosin oxidized by tirosinase from absolutely dry substance of seedlings
b. amount of tyrosin (in G.)oxidized by tyrosinase from 1 g. of absolutely dry substance of seedlings. p-40

It is seen from figures 1 and 2, that the tyrosinase activity in susceptible varieties is considerably higher than in the resistant ones,

in seedlings as well as in seeds. Thus tyrosinase behaves analoguous to peroxidase and catalase. It should be pointed out, that this difference in activity of enzymes in resistant and susceptible varieties is manifest considerably sharper in both phenolases than in catalase.

As mentioned above, a number of authors point out that varieties different in their resistance are different in the quantitative contents of phenols. Accordingly, there are many attempts of qualitative identification of phenols in resistant and susceptible varieties. Resistance is linked with the presence of combinations of the protocate acid type, flavones etc. However, the establishing of strict quantitative correlations was not achieved (4,5,6). It is necessary to note, that methods of quantitative determination of phenols, which are developed for chemical preparations, are of little use in the work with vegetative plant objects.

It is known that the substrate of tyrosinase is tyrosin--amino acid which is oxidized under the influence of this enzyme until /it becomes/ melanines. This amino acid is interesting because the phenol group is part of it and at the same time it is an obligatory component of most of the vegetative albumen.

In connection with the above mentioned facts a question arose, whether or not tyrosin might be one of phenols, which condition plant resistance, and the relatively strong activity of the enzyme, which oxidizes this phenol in susceptible varieties--one of the causes of non-resistance?

We were determining the content of free tyrosine in seeds and seedlings of wheat varieties of varying susceptibility.

Tyrosine was determined by method of bromination at cold temperatures. Presence of tyrosine in a plant substrate was confirmed by a positive reaction with a "mialonovyi" /?/ reagent. The obtained results are presented in figures 3 and 4.

It is seen from the obtained data, that the contents of free tyrosine in seedlings and seeds of resistant varieties is higher than that of susceptible ones.

Besides tyrosine which is present in a free state, some amount of it can be liberated by breaking down of albumen, because tyrosine is a component of almost all the albumens. Therefore we found it necessary to carry out comparative determinations of tyrosine in the

flour of varieties different in their resistance, before and after auto-lysis. In fig. 5 are shown amounts of tyrosine liberated by auto-lysis.

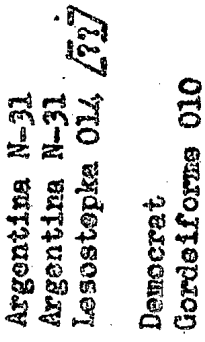


Fig.3 Content of free tyrosine in wheat varieties (in % per 1 g. of absolutely dry seed substance).

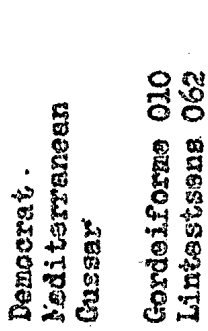
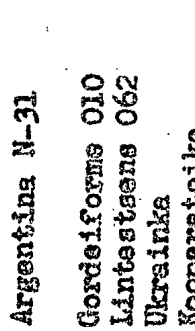


Fig.4 Content of free tyrosine in wheat varieties (in % per 1 g. of absolutely dry substance of seedlings).



(p.41)

Fig.5 Difference in content of free tyrosine before and after auto-lysis (in % per 1 g. of absolutely dry seed substance).

It is seen from obtained data that after auto-lysis of flour more tyrosine is discovered in resistant varieties than in susceptible ones. It might be caused either by a comparatively greater liberation of tyrosine in hydro-lysis of albumen of resistant varieties, or be a result of a more energetic oxidation of tirosin in auto-lysate [7] of susceptible varieties, which, as we indicated, poses a more active tyrosinase. The established facts--higher content in resistant varieties of free tyrosine, of tyrosine found after auto-lysis and lesser activity of tyrosinase--make an assumption possible, that in the process of life activity under similar conditions, the concentration of substances with phenol groups is higher in resistant varieties than in susceptible ones.

Invasion of parasite into plant tissues is accompanied by a breakdown of albumen and other processes which take place during auto-lysis. It can therefore be expected that with the invasion of a parasite more phenols will be found in resistant varieties than in susceptible ones.

A number of authors point out that phenols, which in small quantities are harmless for a parasite, are very toxic at a certain concentration. It is possible that in resistant varieties the concentration of phenols reaches the limits at which they suppress the development of the parasite.

Trans. 456
(In full)
By:
A. Antik

Conclusions

1. Wheat varieties susceptible to fugal diseases (to leaf rust) differ from resistant varieties by higher activity to tyrasinase in seeds as well as in shoots.

2. The content of free tyrasine in seeds and shoots of resistant varieties is considerably higher than that of susceptible varieties.

3. As a result of auto-lysis of flour, greater increase in tyrosine content is discovered in resistant varieties than in the susceptible ones.

4. The intensiveness of usual life processes in various varieties is different. It represent a result of a greater or lesser activity of enzymes, in particular, of the oxidizing-regenerating ones.

5. Varying intensiveness of proteo-lysis and oxidation of products of albumen break-down of the tyrosine type, condition in varieties the varying amounts of substances which at a certain concentration are toxic for parasites.

From the above results the unequal resistance of wheat varieties to the brown rust of leaves.

Bibliography

2. Kudriavtseva, M. A. - Vestnik zashchity rastenii. M.-L. No. 3, 1940
3. Sukhorukov, K. T. & Ovcharov, K. E. - Doklady AN SSSR, V XIV No. 6 392, 1937

6. Kargopolova, N. P. - Trudy po prikladnoi botanike, genetike, selektsii Seriia II No. 11, 1937

7. Fedotova, T. I. - Vestnik zashchity rastenii, No. 4, 1940

Vses. N. - i. inst. zashchity rastenii Received 21/IV 1946

Trans. 457
(In full)
By:
A. Antik

Kleshnin, A.

Znachenie razlichnykh oblastei spektra fiziologicheskoi radiatsii dlia rosta i razvitiia rastenii

[Importance of various spectral sections of physiological radiation for the growth and development of plants]

Akad. Nauk SSSR. Dok 70:891-894.
Ref. Feb. 11, 1950. 511 P444A
(In Russian)

Comparative study of plants grown under conditions of relative monochromatic light was undertaken repeatedly (1,2). However the methods of these studies were far from being satisfactory: the intensity of radiation was expressed either in units of exposure or was not uniform. The obstacle to carrying out of more thorough studies was in the lack of monochromatic sources of radiation which provide intensity sufficient for a normal plant development. With the appearance of luminescent lamps it will be possible to fill this gap. In the present report are presented the results of a study in this direction conducted in 1948-49.

The plants -- Moscow lettuce, strawberry "Komsomolka," "patissony" (?), cucumbers, cauliflower, cabbage no. 1, soya 2173, Amaranthus retroflexus etc. -- were grown in a dark room in special chambers entirely under artificial light obtained with the help of luminescent lamps of red (CdB_2O_5), green ($ZnSiO_3$) and blue ($MgWO_4$) light, manufactured to special orders by the Moscow electric bulb (?)/ lamp (?) plant. The spectral characteristics of these lamps are given in the book by A. P. Ivanov(3). The lamps 15 volt strong and representing glass pipes 45 cm. long with a 2.5 cm. diameter, were placed like a horizontal lattice (15 lamps per 1 m. or 450 volt (m^2) above the plants, 5-10 or 20-25 cm. high. The intensiveness of the physiological radiation (4) was equal in all variants and fluctuated depending on the position of the plants and time period of lamp burning within the limits of 25000-40000 "erg"(?) [energy(?)]/cm^2 sec. The temperature was also uniform, about 25°.

The plants were grown in clay pots containing about 750g. of soil with peat added. The results of the experiments are given in tables 1, 2, 2 and fig. 1, 2, 3.

457

It is seen from data in table 1 and fig. 1 and 2, that in plants which are in a vegetative condition or depend little upon the spectral composition of radiation during the transition to blossoming (cabbage, turnip, kohlrabi, cucumbers etc.), the accumulation of dry mass, the growing of leaves, stems and roots proceeds most intensively under orange-red, and least intensively under green rays. Under blue-violet rays these processes take place either with the same speed as under green rays or slightly more intensively, but considerably less so than under red rays. In other words we meet here a typical photo-synthetic spectral curve. A considerably more complex picture is observed in plants, the transition to blossoming of which is determined by the spectral composition of radiation, such as lettuce (fig. 3, table 1). At first, while the plant is in a vegetative state, the accumulation of dry mass takes place here as in the first group of plants. However with the progress in development processes, which proceed particularly intensively under red rays, the plants in the latter case begin gradually to lag behind in accumulation of dry mass and then stop this process entirely, while the plants in the blue light continue the vegetative growth and form a luxurious leaf rosette (fig. 3). As a result considerably stronger plants are obtained in the blue light than in the red or green.

Table 1 (p. 892)

Dry weight of plants grown with the help of luminescent lamps with various spectral content (composition?) of radiation (in g. per one plant)*

Plants	Age in days	Leaves			Stems			Roots			Total Weight		
		K	3	C	K	3	C	K	3	C	K	3	C
Cabbage, white-head, no. 1	60	5,88	2,39	2,30	2,30	1,00	0.70	3,32	1,02	0,90	11,50	4,41	13,9
Moscow lettuce	20	0,066	0,03	0,03	0,02	0,01	0,01	-	-	-	0,08	0,04	0,04
	34	0,27	0,09	0,25	0,15	0,06	0,04	-	-	-	0,42	0,15	0,29
	64	0,72	0,34	3,36	1,45	0,28	1,22	0,45	0,08	1,90	2,62	0,69	6,48

* K — red light, 3 — green light, C — blue light.

Fig. 1. Head cabbage No. 1. 1 — in red, 2 — in green, 3 — in blue light. (p.892)

Fig. 2. Cucumbers. 1 — in blue light (intensiveness of radiation twice as high as in other variants), 2 — in blue, 3 — in green, 4 — in red light.

(p.393)

Fig. 3. Moscow lettuce. 1 — in red light (blossoming), 2 — in green light (the plant has no rosette form), 3 — in blue light (rosette, beginning of arrow-forming(?))

At the basis of this phenomenon is, doubtless: 1) the process of photosynthesis — the only source of organic substance and the basic supplier of all kinds of substances which determine growth, development and formation of plants, 2) the development process which finally leads to aging of plants, and 3) the process of leaf formation (table 2, fig. 1 and 2), which determines the working area of the photosynthetic apparatus. All the processes mentioned take place most intensively under blue-violet rays reaching a minimum of about 500 mm(4). The general appearance of the plant and the accumulation of the dry substance are determined, in rough approximation, by the relationship between the speeds of these processes.

Experiments with cabbage, radishes, lettuce and other plants demonstrate that the elongation of the stem takes place mainly under the influence of green rays. Under these rays many rosette forms (lettuce, cabbage, radishes) do not at all form a rosette, acquiring right away the appearance of a stem plant (fig. 3). The above is in conformity with data of literature, according to which the most active, in the sense of preventing elongation of stem, are the orange-red rays with a maximum about 623 mm. (5)

In table 3 and fig. 3 are given the data on the process of transition of plants from a vegetative state to a reproductive development among long-day plants (lettuce, strawberry, cauliflower etc.). The transition to blossoming takes place most intensively under orange-red and least intensively under blue-violet and green rays. Among short-day plants (Amaranthus retroflexus etc.) the picture is slightly more complicated: under conditions favorable for passing through the light stage (short day), they also begin to blossom earliest in the red, then blue and, finally, green light. However in a long day the short-day plants start blossoming first in the blue and then in the red light. The latter, besides Amaranthus, is established also for soya, cucumbers and other short-day plants. This at first somewhat unexpected result becomes understandable, taking into consideration that in the photoperiod process of the long-day as well as short-day plants the most active are the orange-red rays (6-8) and therefore, under long-day conditions, they should inhibit more intensely the transition to blossoming in short-day plants than the blue-violet rays.

It is interesting that vegetative reproduction of plants apparently is also more intensive under red, than blue or green rays.

In experiments with strawberries, tendrils were formed better in red light and less well under lamps producing blue or green light.

Table 2 (p.894)

Leaf surface of plants grown with the help of luminescent lamps with varying spectral composition of radiation.

Plants	Age in days	Mean sizes of leaf in cm^2			Total leaf surface in cm^2			Number of leaves on plant		
		red	green	blue	red	green	blue	red	green	blue
Radishes 230 pink-red	17	75,0	29,6	41,4	448,2	148,1	248,1	—	—	—
Cucumbers	in 4-leaf stage	104,0	37,9	94,7	622,4	227,6	568,6	—	—	—
Moscow lettuce	64	24,7	12,7	66,5	370,0	190,0	298,9	15	15	45

Development of plants grown with the help of luminescent lamps with varying spectral composition of radiation.

Table 3 (p.894)

Plants	Number of days between germination and budding		
	red	green	blue
Moscow lettuce	33	51	45
Strawberry Komsomolka	38®	59®	47®
Cauliflower	115	∞	150
Amaranthus retroflexus			
in a short day (8 hours)	47	69	51
in a long day (24 hours)	89	-	72

®Number of days from beginning of exposure of young plants transplanted as cuttings from flower beds.

Thus orange-red rays should be recognized as the most active among physiological radiation. Under their influence the development processes of short-day as well as long-day plants take place particularly intensely under conditions corresponding to/with favorable day length. Orange-red rays are a factor which inhibits the development of short-day plants under conditions of unfavorable for them long days. The red light appears as a factor which causes formation of organs of vegetative reproduction in strawberry and is the most favorable for growing of plants if under its influence the latter do not develop and become old too rapidly — to the detriment of the yield. In this case takes place the best formation of leaves and other organs and a particularly intensive accumulation of dry substance.

For plants which under orange-red rays pass extrerely fast to the reproducing development (lettuce) the blue light is the most favorable.

Inst. of plant physiology
imeni K. A. Timiriazev, A.N. USSR

Received 10.XII 1949

Quoted literature

3) Ivanov, A. P. Electrical sources of light, 1948
4) Kleshnin, A. F. Izv, A. N. SSSR, ser. fizich, 13, no. 2, 294, 1949
6) Katunskii, V. P. DAN, 15, 501, 1937
7) Kleshnin, A. F. DAN, 40, 237, 1943; 52, 819, 1946

Trans. 457
(In full)
By:
A. Antik

Kleshnin, A.

Znachehie razlichnykh oblastei spektra fiziologicheskoi radiatsii dlia rosta i razvitiia rastenii

[Importance of various spectral sections of physiological radiation for the growth and development of plants]

Akad. Nauk SSSR. Dok 70:891-894.
Ref. Feb. 11, 1950. 511 P444A
(In Russian)

Comparative study of plants grown under conditions of relative monochromatic light was undertaken repeatedly (1,2). However the methods of these studies were far from being satisfactory: the intensity of radiation was expressed either in units of exposure or was not uniform. The obstacle to carrying out of more thorough studies was in the lack of monochromatic sources of radiation which provide intensity sufficient for a normal plant development. With the appearance of luminescent lamps it will be possible to fill this gap. In the present report are presented the results of a study in this direction conducted in 1948-49.

The plants -- Moscow lettuce, strawberry "Komsomolka," "patissony" (?), cucumbers, cauliflower, cabbage no. 1, soya 2173, Amaranthus retroflexus etc. -- were grown in a dark room in special chambers entirely under artificial light obtained with the help of luminescent lamps of red (CdB_2O_5), green ($ZnSiO_3$) and blue ($MgWO_4$) light, manufactured to special orders by the Moscow electric bulb (?)/ lamp (?) plant. The spectral characteristics of these lamps are given in the book by A. P. Ivanov(3). The lamps 15 volt strong and representing glass pipes 45 cm. long with a 2.5 cm. diameter, were placed like a horizontal lattice (15 lamps per 1 m. or 450 volt (m^2) above the plants, 5-10 or 20-25 cm. high. The intensiveness of the physiological radiation (4) was equal in all variants and fluctuated depending on the position of the plants and time period of lamp burning within the limits of 25000-40000 "erg"(?) [energy(?)]//cm^2 sec. The temperature was also uniform, about 25°.

The plants were grown in clay pots containing about 750g. of soil with peat added. The results of the experiments are given in tables 1, 2, 2 and fig. 1, 2, 3.

It is seen from data in table 1 and fig. 1 and 2, that in plants which are in a vegetative condition or depend little upon the spectral composition of radiation during the transition to blossoming (cabbage, turnip, kohlrabi, cucumbers etc.), the accumulation of dry mass, the growing of leaves, stems and roots proceeds most intensively under orange-red, and least intensively under green rays. Under blue-violet rays these processes take place either with the same speed as under green rays or slightly more intensively, but considerably less so than under red rays. In other words we meet here a typical photo-synthetic spectral curve. A considerably more complex picture is observed in plants, the transition to blossoming of which is determined by the spectral composition of radiation, such as lettuce (fig. 3, table 1). At first, while the plant is in a vegetative state, the accumulation of dry mass takes place here as in the first group of plants. However with the progress in development processes, which proceed particularly intensively under red rays, the plants in the latter case begin gradually to lag behind in accumulation of dry mass and then stop this process entirely, while the plants in the blue light continue the vegetative growth and form a luxurious leaf rosette (fig. 3). As a result considerably stronger plants are obtained in the blue light than in the red or green.

Table 1 (p. 892)

Dry weight of plants grown with the help of luminescent lamps with various spectral content (composition?) of radiation (in g. per one plant)*

Plants	Age in days	Leaves			Stems			Roots			Total Weight		
		K	3	C	K	3	C	K	3	C	K	3	C
Cabbage, white-head, no. 1	60	5,88	2,39	2,30	2,30	1,00	0.70	3,32	1,02	0,90	11,50	4,41	13,9
Moscow lettuce	20	0,066	0,03	0,03	0,02	0,01	0,01	-	-	-	0,08	0,04	0,04
	34	0,27	0,09	0,25	0,15	0,06	0,04	-	-	-	0,42	0,15	0,29
	64	0,72	0,34	3,36	1,45	0,28	1,22	0,45	0,08	1,90	2,62	0,69	6,48

* K — red light, 3 — green light, C — blue light.

Fig. 1. Head cabbage No. 1. 1 — in red, 2 — in green, 3 — in blue light. (p.892)

Fig. 2. Cucumbers. 1 — in blue light (intensiveness of radiation twice as high as in other variants), 2 — in blue, 3 — in green, 4 — in red light.

(p.393)

Fig. 3. Moscow lettuce. 1 — in red light (blossoming), 2 — in green light (the plant has no rosette form), 3 — in blue light (rosette, beginning of arrow-forming(?))

At the basis of this phenomenon is, doubtless: 1) the process of photosynthesis — the only source of organic substance and the basic supplier of all kinds of substances which determine growth, development and formation of plants, 2) the development process which finally leads to aging of plants, and 3) the process of leaf formation (table 2, fig. 1 and 2), which determines the working area of the photosynthetic apparatus. All the processes mentioned take place most intensively under blue-violet rays reaching a minimum of about 500 mm(4). The general appearance of the plant and the accumulation of the dry substance are determined, in rough approximation, by the relationship between the speeds of these processes.

Experiments with cabbage, radishes, lettuce and other plants demonstrate that the elongation of the stem takes place mainly under the influence of green rays. Under these rays many rosette forms (lettuce, cabbage, radishes) do not at all form a rosette, acquiring right away the appearance of a stem plant (fig. 3). The above is in conformity with data of literature, according to which the most active, in the sense of preventing elongation of stem, are the orange-red rays with a maximum about 623 mm. (5)

In table 3 and fig. 3 are given the data on the process of transition of plants from a vegetative state to a reproductive development among long-day plants (lettuce, strawberry, cauliflower etc.). The transition to blossoming takes place most intensively under orange-red and least intensively under blue-violet and green rays. Among short-day plants (Amaranthus retroflexus etc.) the picture is slightly more complicated: under conditions favorable for passing through the light stage (short day), they also begin to blossom earliest in the red, then blue and, finally, green light. However in a long day the short-day plants start blossoming first in the blue and then in the red light. The latter, besides Amaranthus, is established also for soya, cucumbers and other short-day plants. This at first somewhat unexpected result becomes understandable, taking into consideration that in the photoperiod process of the long-day as well as short-day plants the most active are the orange-red rays (6-8) and therefore, under long-day conditions, they should inhibit more intensely the transition to blossoming in short-day plants than the blue-violet rays.

It is interesting that vegetative reproduction of plants apparently is also more intensive under red, than blue or green rays.

In experiments with strawberries, tendrils were formed better in red light and less well under lamps producing blue or green light.

Table 2 (p.394)

Leaf surface of plants grown with the help of luminescent lamps with varying spectral composition of radiation.

Plants	Age in days	Mean sizes of leaf in cm^2			Total leaf surface in cm^2			Number of leaves on plant		
		red	green	blue	red	green	blue	red	green	blue
Radishes 230 pink-red	17 in 4-leaf stage	75,0	29,6	41,4	448,2	148,1	248,1	—	—	—
Cucumbers		104,0	37,9	94,7	622,4	227,6	568,6	—	—	—
Moscow lettuce	64	24,7	12,7	66,5	370,0	190,0	298,9	15	15	45

Development of plants grown with the help of luminescent lamps with varying spectral composition of radiation.

Table 3 (p.394)

Plants	Number of days between germination and budding		
	red	green	blue
Moscow lettuce	33	51	45
Strawberry Komsomolka	38@	59@	47@
Cauliflower	115	∞	150
Amaranthus retroflexus			
in a short day (8 hours)	47	69	51
in a long day (24 hours)	89	-	72

@Number of days from beginning of exposure of young plants transplanted as cuttings from flower beds.

Thus orange-red rays should be recognized as the most active among physiological radiation. Under their influence the development processes of short-day as well as long-day plants take place particularly intensely under conditions corresponding to/with favorable day length. Orange-red rays are a factor which inhibits the development of short-day plants under conditions of unfavorable for them long days. The red light appears as a factor which causes formation of organs of vegetative reproduction in strawberry and is the most favorable for growing of plants if under its influence the latter do not develop and become old too rapidly — to the detriment of the yield. In this case takes place the best formation of leaves and other organs and a particularly intensive accumulation of dry substance.

Trans. 457
(In full)
By:
A. Antik

For plants which under orenge-red rays rass extrerely fast to the reproducing develonment (lettuce) the blue light is the rost favorable.

Inst. of plant physiology
imeni K. A. Tirirlazev, A.N. USSR

Received 10.XII 1949

Quoted Literature

3) Ivanov, A. P. Electrical sources of light, 1948
4) Kleshnin, A. F. Izv, A. N. SSSR, ser. fizich, 13, no. 2, 294, 1949
6) Katunskii, V. P. DAN, 15, 501, 1937
7) Kleshnin, A. F. DAN, 40, 237, 1943; 52, 819, 1946

Nilova, V. P., and Egorova, G. N.

Aktivnost' katalazy i peroksidazy i
immunitet pshenitsy k buroi rzhavchine
(Puccinia triticina Erikss)

[Activity of catalase and peroxidase
and wheat immunity to Puccinia triticina].

Vsesoiuzn. Akad. Sel'skokhoz. Nauk im.
V. I. Lenina. Dok 13(1):33-38. 1948.
Ref. 20 Akl

(In Russian)

The development of the diagnostic method of plant resistance is unthinkable without the knowledge of the essence of the nature of plant immunity.

Taking into consideration the enormous variety of parasites and hosts, it is hard to assume that the processes of their interrelations would have a uniform character. However our opinion is that there is a possibility to disclose some properties characteristic for plants resistant to individual disease groups.

At the present time most researchers are inclined to think that plant immunity is the extreme degree of resistance.

From the bio-chemical point of view it means the following. The processes of conversion of substances of resistant as well as of susceptible wheat varieties proceed in one direction, but their intensity varies greatly in individual varieties. Therefore the parasite getting at approximately the same dates on all the varieties, finds external bio-chemical media varying in their degree of favorableness for its development. Besides, in the process of interaction of parasite and host some shift or other can originate in the process of conversion of substances. These shifts are similar in character for all the varieties but they differ in degree.

As a result, particularly unfavorable conditions for the development of the parasite are created in some varieties making them immune.

Trans. 458
(In full)
By:
A. Antik

It is known that conversions of substances in plants are conditioned and are directed by the work of certain enzyme groups. A specific role is played by the oxidizing-regenerating enzymes, which are connected with the principal physiological processes in the plant - photo-synthesis and respiration. The connection between the activity of these enzymes and the degree of susceptibility of the plant to one or another disease was noted by many authors (1, 2, 3). Catalase belongs to the number of enzymes which continue and sustain oxidizing-regenerating processes.

It is known that the action of catalase consists in splitting of peroxides into water and molecular oxygen. It is considered that the biological significance of catalase consists in making harmless the surplus amount of peroxides. The oxygen liberated at that time is used up as an acceptor in oxidizing-regenerating processes. Therefore it is possible that the saturation of tissues with oxygen depends on the activity of catalase. Rust is a strictly obligate and extremely oxygen-requiring parasite. Many authors attempted to establish a connection between the activity of catalase and the susceptibility of wheat to brown rust.

Strictest correlations were obtained by Kudriavtseva (4) who demonstrated that in leaves of wheat susceptible to Pue. triticina the activity of catalase is considerably higher than in leaves of resistant varieties.

We studied the activity of catalase in varieties contrasting in their susceptibility, in seeds and in 5-6 day old shoots. For characteristics of the studied varieties in regard to resistance were used the data of the laboratory for immunity at the All-Union Institute for Plant Protection (table). Table (p.35)

Resistant	Resistant depending on age	Susceptible
	Trit. Vulgare	
	Spring Wheats	
Klein 31 (Argentina)	Pionerka	Liutestsens 062
		Diamant
		Novinka
		Tulun 3A/32
		Milturum 321
	Winter Wheats	
Mediterranian	- - - -	Ukrainka
Voroshilovka	- - - -	Kooperatorka
Democrat (U.S.A.)	- - - -	Gussar (U.S.A.)
		Malakhov (U.S.A.)
	Trit. durum	
Gordeiforme Olo		

Trans. 458
(In full)
By:
A. Antik

In seedlings we studied separately the shoots, roots and endosperms.

It was disclosed that a correlative connection can be established only between the activity of catalase in resting seeds and shoots, that is, in the parts of seedlings from which the green mass of plants develops. It was not possible to establish strict correlations according to activity of catalase in rootlets and germinated endosperm.

The results are given in diagrams (fig. 1, 2).

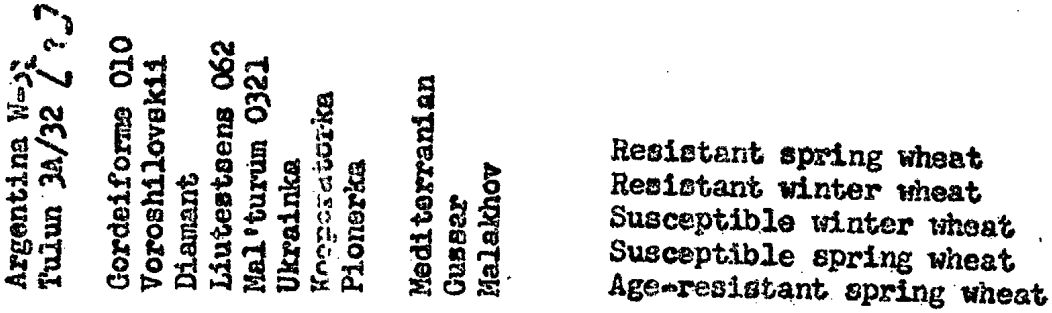


Fig. 1. Activity of catalase of seeds (in ml. O_2 per 1g. of absolutely dry substance during 20 min.)

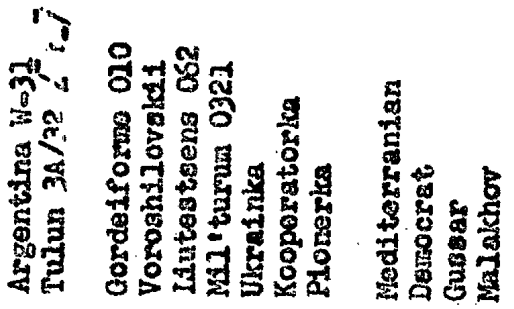


Fig. 2. Activity of catalase of shoots (in ml. O_2 per 1g. of absolutely dry substance during 20 min.)

It is seen from the diagrams that absolute values which denote the catalase activity of seeds from different years harvests, which were preserved for various time periods - vary. Thus the catalase activity of seeds from the 1918 yield, which were preserved for 7 years, is considerably lower than the catalase activity of seeds

from the 1945 crop. But in comparing the catalase activity of resistant and susceptible varieties of the same age, similar regularity is observed - susceptible varieties possess more active catalase. Similar results are obtained in seedlings grown from these seeds.

It is necessary to point out the behavior of this enzyme in the group of the so-called age-resistant varieties. To this group belong such varieties as Pionerka, Lesostepka, etc. According to data of the laboratory for immunity at the VIZR, these varieties are affected during early development stages, but in aging they acquire properties of resistance. According to our data, during the stage of seedlings and even of resting seeds, this group of wheat varieties behaves in the same manner as the group of varieties susceptible during all the development stages. Apparently the change in the chemism, in the direction of resistance, takes place in them during later development stages or is conditioned by other causes.

It can be concluded on the basis of the obtained data, that the high catalase activity of susceptible varieties is potentially already in the wheat seed itself.

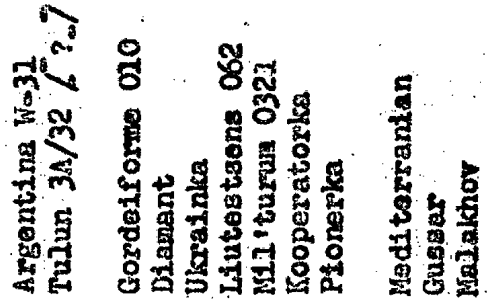


Fig. 3. Activity of peroxidase of seeds in the count per 1g. of absolutely dry substance.

We consider it necessary to call attention to the Voroshilovskii variety. It was bred and released for reproduction as a variety resistant to Pue. triticina. But according to data of the laboratory for immunity at the VIZR, its physiological herterogenaity reached 30% by 1937. In 1941, in some areas it was affected by rust similarly to susceptible varieties. It is necessary to point out, that according to our data, it manifests itself as a variety closer to the group of susceptible ones. Apparently the physiological

heterogeneity in the sample at our disposition was so considerable, that it casts doubt on the correctness of referring this variety to the group of resistant ones.

The importance of the functional significance of peroxidase for a living plant organism served as a basis for including this enzyme in the sphere of our studies. It is known that peroxidase is a catalyzer of one of reactions in the respiratory process. Therefore it is incorporated with other oxidases in the group of respiratory enzymes.

Pointed out, in the literature, is the complete parallelism between the activity of peroxidase and the intensity of respiration (5). Noted also is the fact that parallelism is disturbed in assimilating tissues.

Peroxidase oxidizes the diphenols by way of shifting hydrogen to peroxides. The diphenols are converted at that time into quinones, which are, according to Palladin, respiratory chromogens (6).

It was mentioned before that phenols are the substrate for peroxidase. There are indications of the existence of a connection between a high content of these combinations and plant resistance to fungal diseases (7, 8). Since the presence of phenols in plants has to be connected with the activity of the phenol, it can be assumed, that the activity of these enzymes has to correlate with plant resistance. The activity of peroxidase in relation to plant resistance was studied by many authors, but they did not succeed to discover definite laws (4, 9).

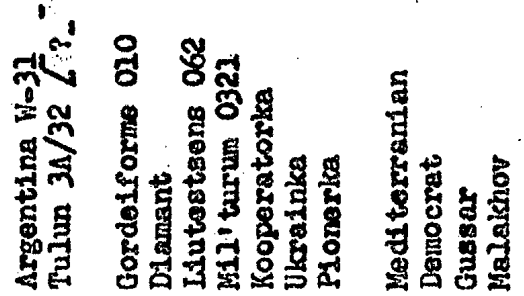


Fig. 4. Peroxidase activity of rootlets of young seedlings in the count per 1g. of absolutely dry substance.

The reason is probably in the fact that the green mass of the plant was examined - the orgeus which are directly affected by rust.

As was mentioned before, the parallelism between the activity of peroxidase and the intensity of respiration in assimilating tissues, is disturbed. Therefore the result of peroxidase activity in these tissues is obscured by some other processes.

Trying to find diagnostic symptoms in the earliest stages of plant development, we subjected to examination the seeds which are in the state of rest and young 5-6 day old seedlings, separating them according to organs, as indicated above.

It thus appeared that no regularity was discovered in the peroxidase activity of green shoots of 5-6 day old seedlings.

A different picture was obtained when rootlets and seeds in the state of rest were analysed (fig. 3 and 4). It became clear that the peroxidase activity in rootlets of susceptible varieties is always higher than that of resistant varieties of similar age.

Here, as in catalase, the peroxidase activity in seeds, which were preserved during considerable time periods, changes in absolute value, but the indicated relation remains.

The analogy should be mentioned in the behavior of catalase and peroxidase in varieties resistant depending on age - during the state of seeds and seedlings they behave similar to particularly susceptible varieties.

Conclusions

1. The activity of catalase in shoots, young seedlings and seeds in a state of rest is higher in susceptible varieties than in shoots and seeds of resistant varieties.

2. The activity of peroxidase in rootlets of young seedlings and in seeds in a state of rest is considerably higher in susceptible varieties than in rootlets and seeds of resistant varieties.

3. The high activity of oxidising-regenerating enzymes inherent to wheat varieties susceptible to brown rust exist potentially in the seed itself.

4. High activity of catalase in tissues of wheat varieties

Trans. 458
(In full)
By:
A. Antik

susceptible to brown rust creates apparently conditions favorable for its (rust) development.

5. The high activity of peroxidase in tissues of wheat varieties susceptible to leaf rust, creates apparently an environment with lower concentration of substances harmful for development of rust.

Bibliography

1. Stroganov, B. - Doklady AN SSR, v, XV, no. 9, 1937.
3. Sukhorukov, K. T. - Zhurn. agr. Tu. V. VIII, 11, 1930.
4. Kudriavtseva, M. A. - Vestn. Zashch, rastenii, M.-L. no. 3, p. 105-115, 1940.
5. Diachkov - Biokhimiia, v. 6, no. 4-5, 1941.
6. Palladin, V. T. - The role of enzymes in living and destroyed plants, 1913.
9. Kargopolova, N. N. - Trudy po prikladnoi cotanike, genetike i selektsii, seziia II, no. 11, 1937.

(In full)
By:
A. Antik

Ivanova, G. F.

Vliianie ammiachnogo i nitratnogo pitaniia na obmen veshchestv i rost rastenii

[Effect of ammoniacal and nitrate (nitrogen) nutrition on metabolism and growth of plants].

Moskov. Ordena Lenina Sel'skokhoz Akad. im. K. A. Timiriazeva. Dok. 11:180-187. 1949. 20 M857

(In Russian)

Important in assimilation by plants of ammonium and nitrate nitrogen is the amount of calcium and potassium in the nutrient solution, as well as the ratio between calcium and potassium.

It is known that the role of the calcium and potassium ratio in assimilating nitrogen depends in a high degree on physiological peculiarities of the plant, the form of nitrogen and the pH of the nutrient media.

We are meeting constantly with various combinations of these factors in the practice of applying fertilizers. Thus, there are many soil varieties some of which are acid-poor in calcium and potassium, others are neutral or weakly alkaline-rich in potassium and especially in calcium.

Besides that, in adding lime to acid soils we enrich them with calcium and in this case the introduction of potassium fertilizers will play an important role not only in raising the level of potassium nutrition but also in reaching a favorable ratio between these elements.

Our studies were conducted on tobacco, "makhorka" [low grade of tobacco] and corn in water cultures at pH 6.5. As a nutrient solution in tests with tobacco and "makhorka" served the somewhat changed Gel'rigel's compound in which as a nitrogen source was used, on one hand, sulfate of ammonium, on the other hand--calcium or sodium salpeter [nitrate].

In experiments with tobacco and "makhorka" the effect was studied of does of ammonium and nitrate nitrogen on metabolism and growth of plants. The doses of nitrogen in the tobacco test-0.5, 1 and 1.5 of the Gel'rigel norm and in the "makhorka" test-1 and 4 Gel'rigel norms.

459

The amount of calcium was equal in both nitrogen sources. "Makhorka" and tobacco did not react uniformly on the change in the content of ammonium nitrogen in the nutrient solution (tables 1 and 2).

It is seen from table 1 that there is no sharp difference in the yield of the "ammonium" and "nitrate" plants after a 0.5 dose of nitrogen.

With the increase of nitrogen the tobacco yield from the nitrate source of nitrogen increases and from the ammonium source, on the contrary-decreases sharply.

Table 1 (p.181)

Tobacco yield (Trapezond 93) g/1 plant

Scheme of the test			NO_3	NH_4
			Fresh weight	Fresh weight
1.	Gel'rigel's compound	0.5N	46.8	44.0
2.	" "	1 "	70.1	34.4
3.	" "	1.5"	76.6	27.0

Table 2 (p.181)

"Makhorka" yield (Vysokoroslaia Zelenaia) g/1 plant

Scheme of test			Fresh weight
1.	Gel'rigel's compound	1N/$Ca(NO_3)_2$/	53.2
2.	" "	4N "	69.5
3.	" "	1N-$(NH_4)_2SO_4$	38.9
4.	" "	4N "	33.3

From table 2 it is also seen that with the increase of the nitrate nitrogen dose the "makhorka" yield increases and with the increase of the ammonium nitrogen dose, on the contrary-decreases. But it must be pointed out that even after a quadruple dose of ammonium nitrogen there is no such sharp decline in "makhorka" yield as after a one and a half dose in the tobacco test.

The chemical analysis of tobacco leaves (as well as "makhorka" leaves) and determination of activity of oxidizing enzymes, (catalase and peroxidase showed (table 3) that under the conditions of ammonium nutrition, the direction of biochemical processes and metabolism in plants are slightly different than under conditions of nitrate nutrition.

Thus the activity of oxidizing enzymes (catalase and peroxidase) in "ammonium" plants is, as a rule, higher than in the "nitrate" plants. Increase in activity of the enzymes, when ammonium nutrition is applied, is accompanied by lesser accumulation of organic acids and simultaneously by greater accumulation of soluble carbohydrates and various forms of nitrogenous combinations. And decline in activity of oxidizing enzymes, when nitrate nutrition is applied, is accompanied by a greater accumulation of organic acids and lesser accumulation of carbohydrates and nitrogenous combinations.

Thus it is possible that with ammonium nutrition the plants rebuild their enzyme apparatus in order to make up for the deficiency in active oxygen of nitrates. In order to confirm the regularities in the metabolism which were disclosed in grown plants and to disclose the most favorable ratios between calcium and potassium in the nutrient media, we conducted a series of experiments with corn seedlings (12-day old plants). In these experiments we followed the line of simplification of the nutrient media. As a substrate in the tests served a mixture of distilled and tap water to which ammonium or nitrate source of nitrogen was added and a varying amount of calcium and potassium. The total sum of cations of calcium and potassium was equal to 9M. ekw/l.

In each vessel (2.5l. volume) 70 plants were grown. The results of the experiments indicate that with the increase of the potassium fraction in the sum of cations or when in equal ratio, better conditions are created for the use of the ammonium as well as nitrate nitrogen. The increased activity of the synthetic processes in Plants, judged according to the accumulation of albumen nitrogen, takes place at the ratio of Ca:K=4.5:4.5. The maximum accumulation of albumen nitrogen is accompanied by high activity of catalase and low-of peroxidase, by a relatively large accumulation of organic acids, ash elements and by lesser accumulation of carbohydrates,- with an ammonium as well as a nitrate source of nitrogen (tables 4 and 5).

Regularities in biochemical processes observed in mature plants (tobacco, "makhorka") are confirmed also in data of experiments with seedlings. Thus the activity of oxidizing enzymes in plants, expecially the catalases, is higher with ammonium than with nitrate nutrition. With nitrate nutrition, larger amounts of organic acids, ash elements are contained in corn seedlings as compared with ammonium nutrition. However the content of carbohydrates in "ammonium" plants is not always higher in comparison with the "nitrate" ones, but only when they grow in media rich in potassium.

In media poor in potassium the "nitrate" plants appear to be richer in carbohydrates.

From data in tables 4 and 5 it follows also that the content of carbohydrates in plants with either source of nitrogen is in inverse dependence upon the content of organic acids (fig.1) and the content of organic acids-in inverse dependence upon enzyme activity of peroxidase (fig.2).

Such dependence was observed in all the experiments which we conducted. it is possible, that when there is a more active peroxidase, the oxidation of organic substances in plants does not stop at the stage of organic acids and goes deeper (to CO_2).

Thus, by way of a correct combining of ammonium and nitrate nitrogen with such elements of ash nutrition as calcium and potassium, we can change the course of biochemical processes, direct metabolism in plants with the purpose of increasing the yield and changing its quality in direction needed for us.

(p.186)

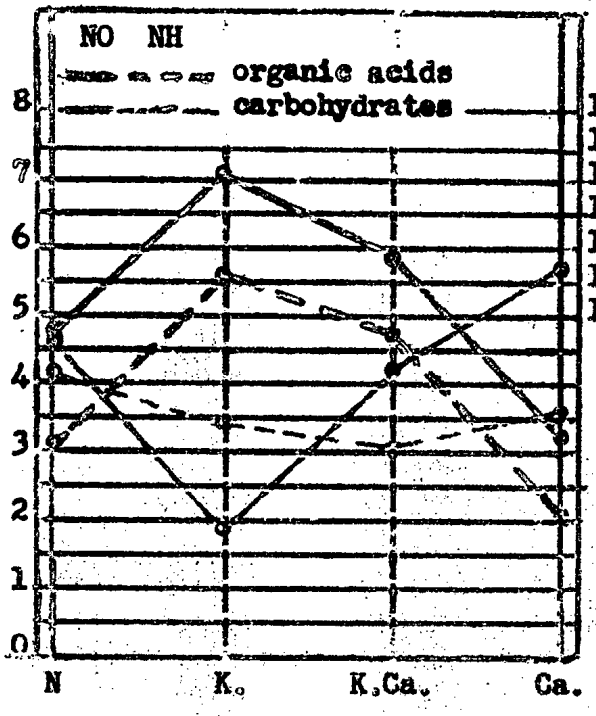


Fig.1. Effect of Ca and K on the content of soluble carbohydrates and organic acids. (% per air dry substance).

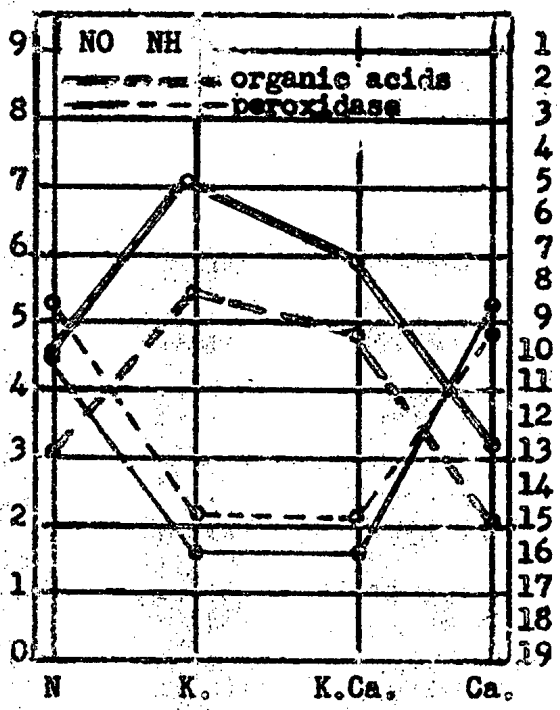


Fig.2. Effect of Ca and K on the content of organic acids and the activity of peroxidase in corn.

(p.183) Table 3

Chemical composition of tobacco leaves and activity of oxidizing enzymes in them

Variants	Activity of enzymes		Chemical composition of tobacco leaves-% (per air dry substance)							
			Carbohydrates			Nitrogen				
	Catalase ml. 0.1n $KMnO_4$	Peroxidase sec.*	Reducing	Total	Sum of organic acids	Ammonium	Asparegina	Amino-acids	Albumen	Total
Gel'rigel's combination 0.5 NO_3	24.0	15.0	4.16	7.74	3.9	0.11	0.28	0.16	2.04	2.59
" " 1. "	34.0	14.2	4.12	7.0	7.9	0.11	0.30	0.24	2.48	3.13
" " 1.5 "	39.0	14.0	3.25	6.0	9.4	0.12	0.28	0.25	2.52	3.17
" " 0.5 NH_4	40.5	7.5	9.15	11.9	3.3	0.12	0.28	0.19	2.12	2.71
" " 1 "	48.9	4.0	10.8	13.2	3.5	0.15	0.42	0.36	2.52	3.45
" " 1.5"	52.5	2.0	14.77	15.5	2.6	0.28	0.72	0.36	2.63	4.02

*The more seconds the less active is peroxidase

Trans. 469
By
A. Antik

Table 4 (p. 184)

Scheme [outline 2] of the experiment	Weight of air dry mass of corn	Activity of enzymes		Chemical composition of the above-ground part of corn (mg/vessel)						
		Catalase ml. O. ln. KMnO4	Peroxidase esc.	Carbohydrates: Reducing	Carbohydrates: Sum	Organic acids	Albumen nitrogen	Total nitrogen	K_2O	SaO / CaO
1. N-6 M.-EKV. $NaNO_3$	6.7	68	10.0	564.1	629.4	306.0	194.3	245.5	65.0	16.7
2. N-6 M.-EKV. $NaNO_3$ + K_9 M.-E	8.0	123.5	15.7	292.4	296.8	564.8	208.0	252.8	440	12.8
3. N-6 M.-EKV. $NaNO_3$ + K_8 M.-E Ca_1 M.-E	8.4	122.0	11.6	224.2	285.5	---	218.4	261.2	445.2	10.0
4. N-6 M.-EKV. $NaNO_3$ + K_6 M.-E Ca_3 M.-E	7.3	120.5	11.2	324.8	339.4	---	204.4	247.4	387.0	11.9
5. N-6 M.-EKV $NaNO_3$ + $K_{4,5}$ M.-E $Ca_{4,5}$ M.-E	7.9	128.0	15.7	515.1	665.9	456.6	221.2	272.5	441.0	11.4
6. N-6 M.-EKV $NaNO_3$ + K_3 M.-E Ca_6 M.-E	7.5	116.0	12.6	637.5	828.0	---	195.0	255.0	285.0	18.8
7. N-6 M.-EKV. $NaNO_3$ + K_1 M.-E Ca_8 M.-E	6.4	112.5	9.9	670.1	826.2	---	185.6	250.0	166.4	21.7
8. N-6 M.-EKV $NaNO_3$ + Ca_9 M.-E	4.6	102.0	8.4	417.2	517.4	144.9	136.0	204.2	39.1	27.6

Trans. 459
Eys
A. Antik

Table 9 (p. 169)

Scheme [outline ?] of the experiment		Weight of air dry mass of corn	Activity of enzymes		Chemical composition of the above-ground part of corn (mg/vessel)						
			Catalase ml. 0.1n KMnO4	Peroxidase sec.	Carbohydrates: Reducing	Carbohydrates: Sum	Sum of organic acids	Albumen nitrogen	Total nitrogen	K_2O	CaO [?] CaC
1.	N-6 M.-EKV. $(NH_4)_2SO_4$	6.8	150.5	8.4	365.1	556.8	213.5	190.4	252.9	45.6	6.8
2.	N-6 M.-EKV. $(NH_4)_2SO_4$ / K_9 M.-E.	8.1	175.0	14.6	364.5	548.3	442.3	211.6	278.6	428.5	7.3
3.	N-6 M.-EKV. $(NH_4)_2SO_4$ / K_6 M.-E. Ca_1 M.-E.	8.6	164.5	13.1	428.2	677.6	-----	213.3	278.6	455.8	8.8
4.	N-6 M.-EKV. $(NH_4)_2SO_4$ / K_6 M.-E. Ca_3 M.-E.	7.3	163.5	12.5	341.6	505.1	-----	186.9	259.9	391.8	8.0
5.	N-6 M.-EKV. $(NH_4)_2SO_4$ / $K_{4.5}$ M.-E. $Ca_{4.5}$ M.-E.	7.7	179.5	14.7	271.4	460.0	364.2	223.9	301.8	382.6	9.2
6.	N-6 M.-EKV. $(NH_4)_2SO_4$ / K_3 M.-E. Ca_6 M.-E.	7.5	160.0	10.2	472.9	699.7	-----	198.0	277.5	204.7	13.9
7.	N-6 M.-EKV. $(NH_4)_2SO_4$ / K_1 M.-E. Ca_8 M.-E.	7.0	160.0	10.4	374.5	549.4	-----	199.5	281.4	96.6	14.0
-.	N-6 M.-EKV. $(NH_4)_2SO_4$ / Ca_9 M.-E.	6.5	150.5	9.3	297.1	438.2	126.5	201.5	289.2	32.5	26.0

Trans. 460
(In full)
By:
A. Antik

Fedorinchik, N. S.

Virulentnost' i kul'tury parazita vzhavchiny Darluca filum (Biv.) cast.

[Virulence and effectiveness of the Culture of a parasite detrimental to the rust fungus].

Mikrobiologiia 21(6):711-717. Nov./Dec. 1952.
448.3 M582

(In Russian)

According to the literature the fungus Darluca filum sometimes appears as a serious biotic factor which limits the development of rust fungi of many species, among them rust of grain crops.

There is indication in the literature that it was possible to obtain a pure culture of D. filum. However neither methods for its accomplishment nor the nutrient media for cultivation of the parasite are given.

We tried to select the nutrient media empirically. We obtained spore formation of D. filum on potato agar (with a content of 20% potato, 3% agar, 0.01% citric acid) and on pea agar (7% pea and 3% agar).

Isolation of the fungus in pure culture consisted in the following: the newly-formed and not yet matured (which had no time yet to become dark) py nidia were sowed in one of the mentioned nutrient media in Petri dishes or test tubes directly from the natural substrate. The latter were uredo-spores of brown rust on wheat leaves in which pycnidia of D. filum were contained. Py nidia formed in artificial nutrient media were no different in exterior characteristics from those which are usually observed in a natural substrate.

Then other nutrient media were also tested: corn, soya, wheat, carrot, beet, rice and other agar media as well as slices of carrot, potato, turnip, table and beet sugar, grains of corn, wheat, oats, pea, rice, barley, etc.

Repeated sowings demonstrated that in various nutrient media, under similar conditions, the fruiting of D. filum appeared with unequal intensity. We conducted a corresponding experiment with 23 nutrient media. This experiment established that py nidia are formed most abundantly on slices of carrot, grains of oats and

corn as well as in corn, potato, carrot, and wheat agar.

In nature in a natural substrate within the 12° - 28° temperature range, the period of D. filum development is 5 - 7 days. In order to find out the time needed for the development of the parasite in artificial nutrient media, we tested 10 of the most suitable for pycnidia-formation nutrient media within the 8° - 24° temperature range.

The media were prepared partly in chemical test tubes, partly in small flasks, 150 ml. in volume. The parasite culture was taken from slices of carrot. The sowing was done with aqueous suspension of spores.

The experiment was repeated three times for each media and assigned temperature - in four parallel test tubes and in four flasks. The results are given in table 1.

Table 1 (p. 712)

Speed in formation of pycnidia Darluca filum, in days in various nutrient media, depending on temperature.

Nutrient Media	Temperature								
	8°	11°	13°	15°	17°	18°	21°	22°	24°
Grains of Soya	12	—	8	—	7	—	—	—	—
" " Corn	12	—	10	—	6	—	—	6	—
Slices of Potato	12	—	—	6	—	—	6	5	6
Grains of Wheat	12	—	6	—	—	—	—	—	—
Slices of Carrot	—	10	9	—	—	—	7	—	6
Grains of Oats	—	—	9	—	7	—	6	6	—
Corn Agar	—	—	12	11	—	9	—	—	6
Wheat "	—	—	19	18	—	13	—	9	—
Potato "	—	—	—	—	—	8	—	7	—
Agar - oats bran	—	—	—	—	—	8	7	—	—
Natural substrate	13	—	4	5	5	—	—	6	—

The data given in table 1 indicate that no sharp difference is observed in speed in fruiting in the nutrient media which we selected as compared with the natural substrate. A certain inhibition in formation of pycnidia is noted in the wheat and corn agar. All the other tested media produced almost no deviations.

The selecting of nutrient media suitable for mass accumulation of parasite could not yet be considered a basis for judgment on the virulence of the fungus culture obtained in one or another nutrient media. Accordingly it was necessary to evaluate the media from the point of view of virulence of the fungus culture bred in them. We carried out such evaluation in regard to 9 nutrient media in the following procedure. Seeds of the Ukrainka wheat were sowed into containers. During the stage of the fourth leaf a suspension of uredospores of brown rust mixed with a suspension of D. filum spores was placed on plants. Tests were conducted separately in each of the mentioned media. For testing each of the nutrient media 10 containers and an additional control container for each were used; the control containers were inoculated only with suspension of rust spores.

Before the introduction of suspension of spores of both fungi, as well as after, the plants remained in the green-house under conditions which approximate natural condition during July.

A month after the introduction of suspension on the plants, records were taken of the development of rust and its parasite. The rust was recorded according to the changed scale No. 2. D. filum was recorded by visual estimation of the percent of pustules affected by the parasite. The results are given in table 2.

The most virulent was the culture taken from carrot slices. High virulence was disclosed in cultures bred on oats and oats-agar. Fungus cultures from the rest of the media showed lesser virulence.

Table 2 (p. 712)

Virulence of Darluca filum culture obtained from various nutrient media

Nutrient media in which the culture was grown	Development of Puccinia triticina in %	% of pustules affected by D. filum	True percent of rust development
Carrot	16	78	3,5
Control	50	0	50,0
Oats	18	65	6,3
Control	40	0	40,0
Corn	18	53	8,4
Control	25	0	25,0

Table 2 (p. 712) (con.)

Virulence of Darluca filum culture obtained from various nutrient media.

Nutrient media in which the culture was grown	Development of Puccinia triticina in %	% of pustules affected by D. filum	True percent of rust development
Corn Agar	24	49,6	12,1
Control	40	0	40,0
Oats Agar	10	42	5,8
Control	25	0	25,0
Wheat Agar	15	36	9,6
Control	32	0	32,0
Soya Agar	13	15	11,0
Control	23	0	23,0
Potato Agar	22	19	17,8
Control	40	0	40,0

Effectiveness of Darluca filum culture

In the above described experiment the suspension of spores of rust and of its parasite was introduced simultaneously on the plants. Under natural conditions the intermittent impingement of plant by spores of two fungi is more frequent. In some cases the spores of the parasite fall on the plant at the moment when the rust has already appeared, in others - a few days before the rust spores fall on the plant. In order to clarify at what dates of striking the plant the spores of the parasite are most effective on rust, we conducted an appropriate experiment in two series.

It was hoped to clarify, by the first series of the experiment, the effectiveness of the culture of the parasite when its spores are introduced into the plants within 1-10 days after the inoculation with rust.

For that purpose seeds of the Ukrainka Wheat were sowed in 50 containers. During the fourth leaf stage all the plants were simultaneously inoculated with uredospores of Puccinia triticina. Immediately after introducing the suspension of rust spores onto the plants, all the containers were distributed in 10 sub-groups, 5 containers in each: 4 containers of each sub-group were later on inoculated with the suspension of the D. filum spores and one remained for control. Onto plants of the first sub-group the suspension of the D. filum spores was introduced the same day and into plants of the remaining sub-groups similar suspension was introduced at one day intervals between each next sub-groups. Thus into plants of the last sub-group the suspension was introduced on the tenth day after inoculation of the first sub-group.

The second series of tests differed from the first only by having a reverse order: first the spores of the D. filum were introduced into the plants and then, as in the first series, the suspension of the P. triticina spores was introduced in one-day intervals.

Suspension of D. filum spores consisted of a mixture taken from various nutrient media (carrot, oats, corn, potato).

The experiment was conducted in a green-house where the temperature regime was kept within the optimum range for both fungi.

The results of the test of the first series are given in table 3 and of the second - in table 4.

Table 3 (p. 714)

Results of introduction into plants of Darluca filum spores after former were inoculated with Puccinia triticina.

Number of sub-group and control	Date of inoculation of plants with P. triticina	Date of introduction of D. filum on plant leaves	Rust development in %	% of pustules affected by D. filum	True percent of rust development
1st Sub-group	8.V	8V			
2nd Sub-group		9V	10	74	2,6
Control		—	65	0	65,0
3rd Sub-group		10V	10	98	0,2
Control			65	0	65,0

By:
A. Antik

Table 3 (p. 714)(con.)

Number of sub-group and control	Date of inoculation of plants with P. triticina	Date of introduction of D. filum on plant leaves	Rust development in %	% of pustules affected by D. filum	True percent of rust development
4th Sub-group		11V	25	97	1,0
Control			40	0	40,0
5th Sub-group		12V	23,7	94	1,4
Control			65	0	65,0
6th Sub-group		13V	26,7	95,7	1,1
Control			65	0	65,0
7th Sub-group		14V	32,5	70,5	9,6
Control			85	0	85,0
8th Sub-group		15V	15	98,0	0,3
Control			65	0	65,0
9th Sub-group		16V	20	86,5	2,9
Control			65	0	65,0
10th Sub-group		17V	56	91,7	5,0
Control			65	0	65,0

Table 4 (p. 714)

Results of introduction into plants of Darluca filum spores after /sic-before/ the former were inoculated with Puccinia triticina.

Number of sub-group and control	Date of introduction on plants of D. filum culture	Date of inoculation of plants with P. triticina	Rust development in %	% of pustules affected by D. filum	True percent of rust development
1st Sub-group	9V	9V	22,5	94	1,4
2nd Sub-group		10V	25	95	1,2
3rd Sub-group		11V	22,5	90	2,2
4th Sub-group		12V	22,5	80	4,5
5th Sub-group		13V	38,7	70	11,7
6th Sub-group		14V	26,2	63,7	12,4
7th Sub-group		15V	8,7	45	4,9

Table 4 (p. 714)(con)

Number of sub-group and control	Date of introduction on plants of D. filum culture	Date of inoculation of plants with P. triticina	Rust development in %	% of pustules affected by D. filum	True percent of rust development
8th Sub-group		16V	18,7	56,2	8,4
9th Sub-group		17V	10	60	4,0
10th Sub-group		18V	13,7	93,5	1,1

It can be seen from table 3 that, in the above-described experiment, cultures of D. filum which were introduced into wheat on the first as well as the tenth day after its inoculation with rust appeared to be highly virulent. Differences depending on the dates of introduction of spore suspensions were insignificant.

The data of the second series of the test (table 4) are similar to those of the first series.

Examining the results obtained only from the point of view of the degree of effect of the parasite on rust, without concern about the plant condition, the following conclusion can be made: independent of whether the spores of the parasite strike the plant within 10 days before or after their inoculation with rust, the effectiveness of the D. filum culture remains sufficiently high. One is readily convinced of it by examining the column "true percent of rust development" (tables 3 and 4). Thus, for example, in the 3rd sub-group (table 3), the degree of rust development in the case of inoculation with D. filum reached only 10% while in the control - 65%. The difference of 55% is an indicator that the parasite depressed the mycelium of the rust already inside the leaf tissue and as a result pustules were not formed. Besides that, of the portion of pustules (10%) which succeeded in forming, 98% were filled with the parasite. Thus there were only 0.2% of unharmed pustules which are capable of spreading the rust infection.

Even if the 10th sub-group (table 3) be taken, where the degree of development of rust in the test reaches 56% and the difference with the control amounts to only 10%, even in this case 91.7% of the pustules are affected by the parasite and 5% remained free from it.

Trans. [illegible]
(In full)
By:
A. Antik

Therefore the parasite can appear as a powerful biotic factor which is not only able to lower the rust development to an economically unnoticeable volume, but can, besides, bring to a minimum the accumulation of rust infection.

The mentioned indicators of the effectiveness of the D. filum are the result of tests carried out only in green-houses where the basic factors of the media could be relatively easily regulated. Therefore, we look at the described experiments only as a presumptive solution, of the probelm of applying the culture of the parasite obtained in artificial nutrient media. Carrying over these tests under field condition will require a series of additional studies mainly in connection with conditions of existence and development of the parasite in the nature.

Reaction of the Wheat plant

In the preceding section we examined the effectiveness of the action of the D. filum on rust without concern about the condition of the plant itself. At the same time it is probably not immaterial to the plant whether only one fungus-parasite inhabits it or whether another fungus is added, though the latter does not develop directly at its expense.

Observations which we carried out revealed the following. In cases when the culture of the parasite is introduced into the plants before the appearance of rust, i.e. during the period of its incubation, the manifestation of D. filum can be of three types.

First type: the leaves of the plant are of a normal green color and the sporulation of rust on them is completely lacking. Fruiting of the parasite appears as individual pycnidia scattered on the surface of the leaf blade, sometimes they are entirely lacking. Such a manifestation of D. filum is observed more frequently when its culture is introduced in plants one-two days after their inoculation with rust.

Second type: on the plant leaves appear yellow spots. Fruiting of rust takes place in way of single pustules. Fruiting of the parasite - in way of individual pycnidia and in yellowed sections- pycnidia in dense groups. This type of manifestation is observed more frequently when the culture of the parasite is introduced into plants one-two days before the appearance of rust.

Third type: the plant leaves lose the normal color, they have the appearance of a complete necrosis and become yellow. Pustules of rust are single and in groups. Pycnidia of the parasite cover densely either the entire surface of the leaf blade or its greater part. This type of

manifestation is observed usually when the culture of the parasite is introduced into the plant after the appearance of rust pustules.

In cases when the culture of the parasite is introduced into the plants one-two and more days before their inoculation with rust, the manifestation of the parasite corresponds to the first type.

And in cases when the culture of the parasite is introduced into plants after the rust appears in various degrees and sporulates, we have two additional types of parasite manifestation. First type: the plant leaves lose the normal coloring and have the appearance of a solid necrosis and single yellowed sections dry out. Single pustules free of the parasite are found. The basic mass of pustules is overcrowded with pycnidia of the parasite. Second type: the plant leaves become yellow and dry out. The leaf blade is covered solidly with rust pustules which in turn are filled almost entirely with pycnidia of the parasite. Pustules free from the parasite are found either singly or in small groups.

The state of plants at all the described manifestations of both fungi was compared with the state of control plants which were inoculated only with rust. As a result of such a comparison it became clear that the yellowing and drying out of the leaf blade of the plants on which both fungi, develop, as a rule, takes place earlier than on control plants (in all the described types, except the first). In other words, the fight between two fungi, which takes place in the plant itself, has a more serious negative effect on the latter in regard to the speed in drying out of the leaf blade than when it takes place in the presence of the basic parasite - rust. It is true that such occurrence is observed only in cases when the parasite of the rust, due to various causes, was not able to suppress it in the early stage of development, i.e. at the beginning of the incubation period.

More serious suppression of the plant in the process of the fight on it of two fungi than in the presence of only one basic parasite - the rust, can be explained as follows. It is known that the rust as well as its parasite D. filum form a mycelium inside the tissue of the plant leaf. Fruiting of one as well as the other fungus takes place on the surface of the leaf blade. It is quite understandable that if the rust succeeds in forming a rigorous mycelium and even fruiting, then by this same fact the parasite is provided with unlimited nutrition and therefore with the possibility of forming, in its turn, a vigorous mycelium and abundant sporulation. In this case the destruction of the plant's assimilating apparatus will take a more intensive course and will lead to a more rapid drying out of leaf blade.

We observe an entirely different phenomenon in cases when the parasite succeeds in destroying the rust mycelium at the beginning of its development. Here the fight of the two fungi is on a very limited scale.

By:
A. Antik

By suppressing the rust mycelium the parasite thus deprives itself of nutrient media and accordingly it succeeds in forming only an unsignificant amount of pycnidia singly scattered on the surface of the leaf blade. In this case the leaf does not experience a noticeable depression and develops quite normally.

From all this we must make the following conclusion. Use of parasite for control of rust can be rectified under the condition of the latter's suppression at the beginning of its development, which coincides with the first stages of plants' growing. Inhibition of rust during this period of a less (relatively more rapid, than in the case of affection by rust alone) of first appearing young leaves and thus preservation of healthy leaves of subsequent formations in subsequent stages of plant growing - more than compensate for the negative effect of D. filum on the state of the plant.

Methods of mass accumulation of fungus culture of Darluca filum in artificial nutrient media.

Since our fungus is an "obligate" parasite, it is understandable that in artificial nutrient media it adjusts itself considerably less than any other accidental saprophyte fungi or bacteria. Therefore penetration of foreign organisms even in the slightest amounts rapidly gets the upper hand.

A specifically conducted test on methods of resowing of the D.filum culture made it possible to establish that the best initial media for the subsequent resowing are the grains of oats: For transferring them from a test-tube to a test-tube or flask or other container it is most convenient to use lightly springy forceps.

We carried out the sowing mainly into matt-flasks ("Ru flasks). They are portable (in the 1000 ml. volume) and were very convenient for mass accumulation of fungus culture. The following wing nutrient media appeared, as a result of tests, to be the most suitable for mass accumulation of fungus culture: slices of carrot and potato, grains of oats and corn.

Cleaned and washed carrot and potato were cut into $1cm^3$ cubes. These cubes are once more thoroughly washed in plain water and placed in a flask to about a 1/3 of its volume. Then the flasks are corked and they undergo sterilization in an autoclave for 40 min. under a 1.5 atmospheric pressure. Grains of oats and corn occupy about 1/4 of the flask volume and to it about two volumes of water are added. The sterilization is carried out in an autoclave for 15-20 min. with flowing steam and then one hour under a 1.5 atmospheric pressure.

It is better to carry out sowing into freshly prepared media cooled to room temperature. After sowing it is necessary to shake the flask well in order to have fruit-bearing started simultaneously in the entire nutrient media. After the sowing, depending on how soon abundant fruiting has to be obtained, the flasks are kept at a corresponding temperature. At a 18-24° temperature about 1½ - 2 months are required for a mass formation of fruit-bearing. In flasks the fungus can develop successfully without losing its virulence during 8 - 10 months. This makes it possible to start preparing the culture ahead of time.

In a case when it is necessary to transport the culture somewhere, it can be done in the following manner: the culture is extracted from the flasks together with the nutrient media and dried to an air-dry condition at a normal room temperature or at a 24-26° (but not higher) temperature /C. ?/, 3-4 days are needed for it if the culture is spread in a thin layer. After such drying the culture, can be easily transported in a non-breakable container.

Conclusions.

1. A number of inexpensive nutrient media are selected (slices of carrot and potato, grains of oats and corn etc.) in which the rust parasite Darluca filum develops successfully.

2. Simple and widely accessible methods of mass accumulation of the culture of the D. filum fungus in nutrient media are developed.

3. Under conditions of a green-house experiment it has been demonstrated, that the D. filum fungus grown in nutrient media retains its virulence and is capable of harming up to 98% of rust pustules (regardless of the development degree) at a belated introduction on a plant and suppresses the rust almost completely during the mycelium stage, when the culture of the rust parasite is introduced in time on plants inoculated with rust.

All-Union scientific-research
institute for plant protection
Leningrad

Received 3, VIII 1951

(S) 46' Trans. 6
(In full)
By:
A. Antik

Sidorov, K.

Nochnoe opylivanie
Khlopchatnika

[Night Dusting of Cotton].

Kolkhoz. Proizvodstvo 10(6):45. June 1950
281.8 K83

(In Russian)

Many pests of agricultural plants reproduce very rapidly and produce several generations during the summer. As a rule their control has to be carried out in a short time in all the area affected. And at the same time it should be considered that the effectiveness of treatment of plants with chemical preparations depends on the hours of the day at which it takes place. At noon, when the air humidity is lower and the wind stronger, the effect of poison dusts is considerably weaker than in the morning. Therefore, it is usually recommended that dusting of plants be conducted only in the morning and the evening. In such a limited time the kolkhozes were not able to treat the fields or had to dust through the entire day.

In order to use more fully the chemical preparations and the equipment of the kolkhozes and to complete the treatment of the plantings in shortest time, the station of plant protection of the All-Union scientific-research institute of cotton industry, carried out experiments last year, with dusting cotton at night. These experiments were very successful. At night the plant leaves are covered with dew, the air humidity increases, the wind usually dies down. All this creates the best conditions for a chemical treatment of plantings.

Especially large concentrations of cotton were treated at night in the kolkhozes "Kadyrlik" and "Azamat", Ordzhonikidze rayon, Uzbek SSR, where there were many cobweb mites in the cotton fields.

It was noted, beforehand, where and in what amounts to apply the poisons, how to place the people [workers?], stations were organized for repair of equipment. Serious attention was paid to the control of work quality. On moonlit nights there was no need for additional light. "Bat" lamps were used only when there was need in regulating or repairing of equipment.

461

Trans. 461
(In full).
By:
A. Antik

The results of night dusting were good. The entire contaminated area of cotton was cleared from pests. The time needed for treatment of plantings was reduced in half. Observations demonstrated that night dusting equals in value that of the morning, is 20-22% higher than dusting during the day and 8-17% higher than that of the evening.

K. Sidorov
Scientific Worker
All-Union scientific - research
institute of cotton industry.

Trans. 402
(In full)
By:
A. Antik

Paikin, D. M.

Rezul'tat ispytaniia tiofosa v bor'be s vrednoi cherepashkoi

[Results of testing thiophos in the control of Eurygaster integriceps].

Vsesoiuzn. Akad. Sel'skokhoz. Nauk im V. I. Lenina. Dok. 15(7):33-35. 1950
20 Akl (In Russian)

As a result of testing of numerous organic compounds, the exceptionally high contact effect of some of the phosporous-organic combinations was established and, in particular, of the preparation "thia-phos" which we obtained from the Scientific institute of fertilizers and insecto-fungicides imeni Ya. V. Samoilov. This preparation represents a one-per cent talc dust of diethyl-p-paranitrophenyl-thiophosphate.

In laboratory tests new generation insects, Eurygaster integriceps (harmful Eurygaster), known as the most resistant to poisons, among them DDT-were dusted with thiophos at the rate of 1.5 and 3g. per $1m^2$, after which they were transferred to breeding places and kept there under temperature and humidity conditions optimal for the development of insects. During 24 hours a complete destruction of Eurygasters treated with the preparation took place (it was repeated three times with 100 insects each time).

Laboratory-field tests were carried out with winter wheat before harvesting and in stubble fields in the kolkhoz imeni I. V. Michurin, Krasnoar-Meiskii raion, krasnodarskii krai.

Plots 30 x 50 m^2 in size, with great density of bugs were dusted in the rate of 15 and 30 kilogram per hectare. Part of the bugs were selected for breeding for subsequent observation under laboratory conditions. Besides that records of living and dead bugs were made in the treated plots. Together with the one-percent dust, diluted dusts were also tested in laboratory-field tests.

The results are presented in the table.

It follows from the data given, that with a consumption of the active element in the preparation, from 120 grams per hectare and up,

---

1. The work was carried out in 1949 by the brigade of the All-Union institute of plant protection, under the leadership of I. M. Poliakov.

Trans. 46
(In full)
By:
A. Antik

destruction takes place rapidly. But if they stay even for a long time in a soil treated with thiophos-there is no notable destruction percentage.

It is interesting to note the sharp decline in toxicity of thiophos when it is applied in form of dust suspension in water while it is constantly being stirred. When sprinkled with the suspension, at the rate of 30 kg. of dust and 500 liters of water per hectare, the death rate of Eurygaster declined almost twice in comparison with the death rate reached by dusting with the same amount of dust without water. Whether the loss of toxicity is the result of hydrolysis of the active element or the result of some other cause-can not be decided without special studies.

Very important is the problem of duration of thiophos's action. It has been established recently, that such synthetic preparations as DDT and GKhTsG (hexachlorocyclohexane) lose toxicity under field conditions due to the effect of light, in particular of the ultra-violet part of the spectrum-and of temperature. Popov (NIUIF) points out the faculty of thiophos to lose toxicity. In studying the effects of light and heat on break down of thiophos, we established that the decisive factor is the temperature.

We assume that loss of toxicity by thiophos is the result of evaporation of the active element; the higher the temperature-the faster this process.

Not only thiophos but other phosphorous-organic combinations as well have a future in the control of agricultural pests. It was established in our laboratory tests for determination of toxicity of some of the phosphorous-organic preparations obtained from the UIUIF, that the 5-percent dust of dimethyl-paranitro-phenyl-thiophosphate does not differ from thiophos in its effect on Eurygasters. Other phosphorous-organic combinations (dipropyl, paranitro-phenyl-thiophosphate, diiso-propyl-paranitro-phenyl-thiophosphate, ethyl-paranitro-phenyl-thiophosphate), though not as effective as thiophos are nevertheless of considerable toxicity and could be used in the control of pests of agricultural crops.

Received 19 IV 1950

All-Union scientific-research
institute for plant protection

Trans. 462
(In full)
By:
A. Antik

the complete destruction of Eurygaster takes place on the second day after dusting. At a 60-75 gram per hectar consumption the bugs are destroyed completely during the following 3-4 days.

There was no known preparation with such a small consumption of the active element and such a high effectiveness and it seems to us that its importance can be hardly overestimated. The use of thiophos promises exceptionally high practical results for the control of the harmful Eurygaster, especially by airplane method, with the help of which small doses of dust can be applied up to 5 kg. or less, which contain 75-100 g. of active element-diethylparanitro-phenyl-thiophosphate.

Tests showed that the thiophos dust caused no harm to wheat, millet, corn, pumpkin, kidney beans and young shoots of apple-trees. Non-resistant to thiophos were cucumbers, the larves of which were injured.

Table (p.34)

Effectiveness of thophos in dusting the harmful Eurygaster.

concentration of active element in the dust, %	Doses of dust on conversion to one hectar in kg.	consumption of active element in g. per hectar	death rate of harmful Eurygasters, %			
			after 24hrs.	after 48hrs.	after 72hrs.	after 96hrs.
1	15	150	74,0	100,0	—	—
1	30	300	100,0	—	—	—
0,5	15	75	48,5	97,0	100,0	—
0,5	30	150	95,5	100,0	—	—
0,4	15	60	30,0	91,8	95,0	100,0
0,4	30	120	81,2	100,0	—	—
0,3	15	45	13,3	88,3	98,0	100,0
0,3	30	90	49,3	98,5	100,0	—
control	-	-	2,9	4,0	5,7	11,4

Crude-laboratory experiments showed also high toxicity of thiophos against aphids, grain mites, granary curculionidae, caterpillars, lesser apple worm and beetles of the beet curculionidae. With expenditure of 1.5 and 3 g. of dust per square meter of area, the complete destruction of pests took place in 24-48 hours.

In regard to the beet curculionidae one peculiarity was discovered which is of essential importance in using thiophos on old "sveklianitse" (beet plants). In dusting curculionidae the

Trans. 40
(In full)
By:
A. Antik

Sveshnikova, N. M.

Opyt primeneniia preparatov ditio-
Karbaminovoi kisloty dlia bor'by
s nematodami-parazitami rastenii

/ Attempt of Using Dithiocarbamic
Acid Derivatives in Fighting Nematodes
which function as Plant Parasites /.

Trudy Zoologicheskogo instituta
Akademii Nauk SSSR, IX, no. 2, pp 462-475,
1951. 410.9 L543 (In Russian)

EXPERIMENTAL APPLICATION OF DITHIOCARBAMIC ACID DERIVATIVES FOR CONTROL OF PLANT PARASITIC NEMATODES

Among the nematodes causing economic losses to national economy of the USSR particularly damaging are: the root-knot, potato and wheat nematodes.

Root-knot nematode--Heterodera marioni (Cornu), which parasitizes roots of over 1,500 species of most diversified plants--vegetables, technical, medicinal and ornamental plants and some fruit trees and palms--depresses them, deforms the roots, inhibits the development and decreases the yield. Root-knot nematode is disclosed on plants by the presence of swellings on roots--galls, which vary in size from a pin head to a walnut, depending on the host plant, degree of soil contamination with root-knot nematode and other conditions.

The females of the root-knot nematode, which are found in galls, lay hundreds of eggs into the external surroundings. In the spring the larvae which developed in the eggs emerge into the soil, find young plants and penetrate their roots for further development until the sexual maturity stage of male and female. The root-knot nematode is distributed predominantly in the South of the USSR where in a number of localities it makes cultivation of vegetables and other susceptible plants quite impossible. Besides that, the root-knot nematode, as it was found by the author (1949), can inhabit the open ground and be harmful for non-rotated cultivation of vegetable plants in the central belt of the USSR. The root-knot nematode is very harmful also in farms with covered ground / greenhouses /.

Potato nematode--Heterodera rostochiensis Wr., discovered in some points of the Baltic coast of the USSR (Sveshnikova, 1948), causes great losses to potatoes decreasing the yield in seriously affected sections up to 14-27 g. / grams? / per plant. Potato nematode parasitizes the roots of potato plants into which it penetrates in the spring during the larval

By: A. Antik

stage after hatching from eggs which hibernated in the soil in the dead female-cyst. The larvae make a mass attack on young potato rootlets; pentrating into them they feed on plant sap; undergo a number of "linek" [molts] after which they reach the stage of sex maturity of female and male. The harmfulness of the potato nematode is manifest in the destruction of the potato rootlets: around the penetrated larvae a necrosis of root cells takes place. Inasmuch as the number of larvae in the root frequently exceeds 10 per lcm., a dying of roots takes place. Many larvae are destroyed together with roots, but the attack by larvae is repeated several times and the plant becomes exhausted by wasting material for regeneration of roots, the leaves develop weakly, they dry out soon and therefore the tubers formed are small or they are not formed at all.

Wheat nematod —Anguina tritici (Steinbuch) is a serious parasite of cereals; mainly of wheat. Larvae of the wheat nematode affect the plant at the beginning of its germination: the plants are dwarfed in size, the stems are thickened and have crimped leaves (fig. 8). With the formation of the spike, the larvae infect the ovary and reach there their sex maturity stage. After fertilization the females lay masses of eggs (up to 15 thousand) from which larvae hatch and undergo molts. From the ovary in affected spikes there are formed, instead of grain, galls with thick walls which protect the larvae within the galls. Galls contaminate the seed grain and enter the soil where the larvae crawl out and attack young plants. The harmfulness of the wheat nematode is manifest in the fact that part of the affected plants are destroyed while young; in mature plants the infected spiklets do not blossom, instead grain-galls are formed in them, which are filled with larvae, and in the grain gathered from a greatly contaminated field the amount of galls might reach up to 1,000 and more per 1kg., i. e. up to 3% of the net grain weight. On certain farms the loss caused by the wheat nematode is quite considerable. The wheat nematode is distributed in Central Asia and in a number of southern localities of the USSR, as well as in Belorussia; it was recorded also in Siberia (Kirianova, 1941). The author carried out experimental inoculation and obtained formation of galls near Moscow.

In search for measures for decontamination of the soil of the mentioned three species of nematodes, the researchers had in mind affecting the larvae of the second stage which migrate in the soil looking for a plant-host.

It was suggested, as control measures against the gall nematode, to steam the soil up to a 60- 65° C. temperature, at a 20-25cm depth, as well as to apply a series of agro-technical measures as, for example, replowing in fields where the soils dry out, flooding of soil for long periods, crop rotation with plants resistant to root-knot nematodes (Filipyev, 1934). However, in many cases it is not possible to carry

out these measures and in some parts of the Azerbaidzhan, Crimea and Black Sea Coast of the Caucasus, the root-knot nematode has reproduced intensely. According to Selivonchik's data (1838) [1938], the vegetable yield on the Apsheron peninsula is partly destroyed by root-knot nematode. Radical measures are required for their complete extermination or, at any rate for a decrease in contamination to a degree where growing of vegetable - Cucurbitae and other crops, susceptible to nematode infection, should be possible. The opinion existing in the literature, that root-knot nematode does no harm under conditions of temperate climate, in particular in the central belt of the USSR - is not correct. In 1944, the author observed in the Moscow oblast' a complete destruction of carrot yield in a focus of root-knot nematode in a garden near a farm house, during the third year of cultivation of garden plants: with potatoes in 1942 and 1943, and with carrots in 1944. The carrots were affected so severely by the root-knot nematode that the yield was not suitable for use (Sveshinikova, 1949).

Among chemical control measures tested in the USSR by A. A. Ustinov (1934) effective were: carbon bisulfide at a dose of 500g. per $1m^2$ and chleropierin at a dose of 100-150g. per $1m^2$. The carbon bisulfide in the indicated dose resulted, under conditions of Sukhumi, in 100% destruction of the root-knot nematodes in plots: however, this preparation is poisonous, explosive and therefore not convenient for extensive use. Chloropicrin in indicated doses is effective, however, a 100% destruction of root-knot nematode, under conditions of severe soil contamination in Sukhumi, was not obtained, therefore, its dose per $1m^2$ has to be increased. Besides that, application of highly poisonous fluid chemicals requires use of injecters, work with them is dangerous and has to be conducted by specifically trained people in gas maska and protective suits and due to all that the process becomes too cumbersome and expensive.

Therefore, the author conducted in 1945-1949 a test for the control of root-knot, wheat and potato nematodes with powdered preparations suggested by the Scientific Institute for Fertilizers and Insectofungicides imeni Ia. V. Samoilov. Tested were the following derivatives of dimethyl-dithiocarbamic acid in dust form and manufactured on the basis of: methyl ester of dimethyl - dithiocarbamic acid (10% dust, patented preparation "cystogon", similar dust of Soviet manufacture with another filler (of "cystogon" type), 20% dust on the basis of the same toxic element ("forbiat" [?], 20% dusts of ethyl (no. 23), butyl (no. 35), isoamyl (no. 34) and propyl (no. 33) of dimethyl - dithiocarbamic acid: esters of diethyl - dithiocarbamic acid: ethyl (no. 31), butyl (no. 30) and propyl (no. 32) also manufactured as 20% dusts. The rest of the preparations which did not show a sufficiently nematodocidal action are not mentioned here since they are indicated in the collated table 4.

(Inf[illegible])
By:
A. Antik

Methods of application were as follows: in the spring the preparations were scattered by hand in a 1:3 ratio mixture with dry sand or road dust, into adequately friable soil which was replowed or redug and after that the preparation was thoroughly plowed under at a 20-25cm depth. Part of the preparations, due to their small amounts, were tested in flower pots. In the latter case the chemicals were introduced into the soil before filling the containers, without adding sand and they were mixed evenly with the entire volume of soil. During the work in the open air gas masks were not used. Rubber gloves or canvas mittems were used in mixing the preparations with sand and in scattering them. No other special safety measures are required. Contact of the preparation with mucous membrane of eyes, mouth and nose should be avoided. Therefore, smoking and eating during the work was prohibited. After the work, face, hands and feet (if work was done barefooted) were washed with water.

In 1946, the author conducted tests with "cystogon" against root-knot nematode under conditions of natural foci of the parasite in open ground in the Moscow oblast' in an individually owned garden (Sveshnikova, 1949). Well prepared sandy soil was dug up with a shovel to a 25cm depth and then broken up with a rake. The introduction of the preparation took place on May 8, at a 14° C. air temperature and a 8° C. soil temperature at a 20cm depth. The soil humidity constituted 17.22% when recalculated in absolutely dry soil. "Cystogon" was taken at a ratio of 185g. per $1m^2$. It was introduced in a mixture with dry sand for a more uniform distribution in the soil, it was scattered by hand through a soil sieve. After the introduction of the preparation the soil was again dug with a shovel and stirred with a rake. The soil in the control area was cultivated in the same manner. In large areas it is recommended to introduce the preparation into the soil with a drill for fertilizers and to finish up with a horse-drawn rake. Seven days later, i.e. on May 15, carrots of the Danvers variety, with a few white carrots added were sown in the experimental beds. On May 30, normal carrot shoots (line) were observed in the treated as well as in the control area which did not differ in density or size. Further growing was normal. Caretaking - stirring up of the ground and weeding - was done by the owner of the garden. On September 9, the experiment was terminated. Examination of 124 carrot roots from the treated plot disclosed on the thin roots of 26 of them single galls discernable only with binocular glasses /magnifying glass/ (21.4%). The remaining roots were absolutely free from infection (fig.11). The roots free from infection as well as the affected ones from the treated plot were of quite normal size and shape and suitable for food, while in 1945, the owner of the garden discarded the yield of this bed because the disease incidence was too great. From the control bed were taken 180 roots. All (100%) of the plants were entirely deformed by root-knot nematodes as was the case in the preceding year (fig. 1)

Trans. 463
(In full)
By:
A. Antik

(P. 465)
Fig. 1 - Caption. Left - carrot from the plot treated with "Cystogon" /?/ in the dose of 185g, per $1m^2$: right - carrot from the control plot affected with gall nematode.

The second part of the experiment was conducted in heavy clay soil in another area of concentration of root-knot nematedes in the Moscow oblast'. The soil was not "mature" i.e. it did not crumble, but was compacted (humidity 19.65%) and it was not possible to break it up finely. "Cystogon" was introduced by similar methods in a dose of 200g. per $1m^2$, with consideration of the stickiness of the soil. The Danvers variety carrots were sowndirectly after soil treatment in order to find out about the effect of the poison on sprouting. Germination was normal - on the 15th day young growth of uniform density was observed in the treated as well as the control area. Here the results were less successful: of the 94 plants taken from the treated plot, 29, i.e. 30.7%, were affected. The degree of infection was from 1-5 gal 1-5 galls per plant. From the control area 60 plants were taken, of which 42, i.e. 70%, were affected. Degree of infection - from 1 to 100 galls per plant. The results of the test were undoubtedly influenced by the soil structure.

On the basis of the preliminary experiment with the "Cystogon" against root-knot nematode carried out in 1946 (Sveshnikova, 1949), the author decided to try out in 1948 another preparation - "forbiat" /?/- for decontamination of soil from the root-knot nematode under conditions of intensive contamination which exists on the Apsheron peninsula. Prior to the author's tests, "forbiat" was applied as an insecticide, but no one tried it as a nematocide. The experiment was conducted in Baku with the cooperation of the director of the Azerbaidzhan Quarantine Laboratory, S. L. Popov and the entomelegist L. I. Shapieva. Soil treatment was carried out on two farms with different soils: in the village Shuveliany in sandy soil and in the Armenikendskii nursery of the Baku-soviet - in clayey loam soil. Methods of poison introduction were similar to those for "Cystogon": digging as deep as possible, loosening of the ground and thorough mixing of the chemical with the soil. The "forbiat" was tested in doses of 70, 85, 100 and 110 g. per $1m^2$. The experiment was conducted during the second decade of May in three plots $5m^2$ in size. A week after the treatment cucumber seeds of the Chinese variety were introduced.

During the growing period the control plots showed very great sparsity: in treated plots there were almost no bare spots, the plots were covered with strong vines and produced a yield shown in table 1. In the second part of August, the plants were dug out and records were taken of infection of their roots with root-knot nematode. Almost all

Trans. 463
(In full)
By:
A. Antik

the plants were affected with it, but to various degrees (fig. 2). While in control plants the roots were absolutely deformed as a result of parasitizing by the root-knot nematode, in plants from treated plots there were considerable less galls and the higher the dose of poison, the fewer galls (fig. 2). When the dosage was 100 and 110g. per $1m^2$, the galls appeared singly and were most frequently distributed at a depth of less than 20cm. Numerical data of recorded state of roots are presented in table 1.

Table 1 (p. 466)

Effect of soil treatment with "forbiat" against gall nematode.

Dose of "forbiat" per $1m^2$ (in g.)	Shuveliany		Armenikend	
	Average % of root infection	yield (in kg.)	average % of root infection	yield (in kg.)
Control (without treatment)	100 (At the end of the experiment all the plants were destroyed)	0	100	11.2
70.	70.5	5	84.4	13.3
85.	50.1	5	88.1(1)	23.1
100.	38.5	8.7	67.1	29.8
110.	36.1	9.1	54	30.3

The tested doses of "forbiat" did not entirely destroy the root-knot nematodes in the soil, but they gave the plants the possibility of growing normally.

In order to develop a 100% effectiveness of "forbiat" an experiment was conducted in flower pots with a clayey loam soil artifically inoculated to a high degree with root-knot nematodes. The soil was thoroughly mixed with "forbiat" in the ratio of 150, 250 and 350g. per $1m^2$ for each three pots.

1) High percentage of infection is caused by defects in the agrotechnique

Radishes were taken as test plants. Two weeks after the planting, depression of plants in control (untreated) pots became noticeable and toward the middle of the second month of growing, a picture of better plant condition in treated pots as compared with the control ones was apparent.

The result of examination of plant roots are in table 2.

Table 2(p. 467)

Results of soil treatment with "forbiat" against gall nematode (flower pots)

Dose per $1M^2$ (in g.)	Total number of plants	Number of affected plants	% of infection
Control (without treatment)	25	25	100
150.	25	2(1)	8
250.	25	1(1)	4
350.	25	0	0

Thus it was established that the effectiveness of the preparation depends on the degree of soil contamination and on the dose, and, mainly, on the thoroughness in mixing the preparation with the soil.

(P. 467)

Fig. 2 - Caption. Roots of cucumbers from plots treated with "forbiat". From left to right: control, "forbiat" in doses: 70, 85, 100 and 110g. per $1m^2$.

On the basis of our works which demonstrated high nematocidal properties of preparations of dithiocarbamic acid, in 1949, a test was conducted with a wider assortment against potato nematode which possesses a higher resistance against unfavorable conditions of the envirement. According to data in the literature, its cysts can remain viable in the soil up to 10 years. Up to recent years the potato nematode was not known in the USSR, therefore, prior to our research, - none was conducted. The experiments were conducted on the Base of the Lithuanian Quarantine Inspection with the cooperation of the agronomist E. S. Mikhnova.

1) Intensiveness of invasion - 1 gall per entire plant.

By:
A. Antik

The following preparations were tested under field conditions: 10% dust of the "Cystegon" type (methyl ester) in doses of 200 and 300g. per $1m^2$, 20% dust of the "forbiat" type in doses of 168g. per $1m^2$ and ethyl ester (preparations no. 23) in doses of 100 and 185g. per $1m^2$. Soil contamination reached up to 2500 cysts of nematodes per kg. of soil. Preparation of the "Cystogon" type was tested in plots of $100m^2$, preparation no. 23 and "forbiat" - in $15m^2$ plots, depending on the amount of chemicals. It was possible to establish during the process of potate development that the plants' germination did not suffer from the effects of the preparations being tested, with the exception of a 300g. dose per $1m^2$ of preparation of the "Cystogon" type which somewhat inhibited germination, though within the potate norm. The effect of decontamination of soil with preparations was clearly expressed in the exterior aspect of the leaves, which looked quite normal in treated plots (fig. 3, 4, 5 and 6). The yield of plants in treated plots was 4-10 times heavier than in the control plots. It should be pointed out that these data pertain to the 15th of August, when the yield did not yet correspond to the norm and besides that we intentionally omitted manuring, in order to demonstrate more clearly the influence of plant decontamination from potato nematodes. The results of the treatment are shown in table 3.

[redacted] naturally contaminated with [illegible]e background - soil treated with preparation no. 23 in the dosage of 100g. per $1m^2$.

Table 3 (P. 469)

Results of soil treatment with esters of dithiocarbamic acid for control of potato nematodes.

Name of preparation	Dose per $1m^2$ (in g.)	Average height of bushes (in cm.)	Average weight of tubers per bush (in g.)	Average % of large tubers	% of affected roots	Number of cysts on a bush
Ethyl ester (no. 23) 20% dust.	100	26	79	57	52	Single
Same. . . .	185	32	108	59	36	"
Methyl ester 20% dust - "forbiat". .	168	51	141	68	4	"
Methyl ester, 10% dust. .	200	27	110	70	40	"
Same. . . .	300	39	113	60	36	"
Control . .	without treat-ment	14	11.5	18	100	Up to 15 per 1cm. of root

Trans. 463
(In full)
By:
A. Antik

(P. 469)
Fig. 4 - Caption. Plots treated with "Cystogon" in dosage of 200-300g. per $1m^2$.

Damage to plants following treatment with "forbiat" decreased 96%, following treatment with preparation no. 23 in a 100g. dose - 46% and no. 23 in a 185g. dose - 64%, as compared with the control, where 100% of potato bushes were affected to a high degree. The exterior aspect of the plants and the yield are presented in fig. 6.

The remaining 12 esters of dithiocarbamic acid (see table 4) were obtained in amounts not exceeding 100g., therefore tests of them were carried out in flower pots (three times) and the natural contamination of soil was 1200-1700 cysts per 1 kg. The soil was treated with dust in the ratio of 150g. per $1m^2$. Two days after treatment potato tubers of the "Vel'tman" variety, about 100g. in weight which were taken from a farm free from potato nematodes were planted in all the containers and were placed on racks in a green-house with a roof of wire screen, i.e. under climatic conditions of open ground in Vilnus, and they were held there until the moment of record-taking of the experiment. Potato shoots were noticed on the 14-24th day, i.e. within the norm for this crop, though in the control containers shoots were observed on the 11-13th day. Further on a certain difference in development was observed; in pots with soil treated with chemicals, the growth of bushes was more powerful and on July 18, budding started: in the control containers the plants were weaker, shorter, they did not blossom and towards the middle of July began wilting and dropping flower leaves. It is seen from table 4, that preparations no. 25-29 had no decontaminating effect: there were cysts of potato nematode - 1 - 10 per 1cm. of root, i.e. up to a degree of affection observed in the control, in the soil there were, besides the brown, also white cysts of the 1949 generation.

(P.470)
Fig. 5 - Caption. Plot treated with "forbiat" in dosage of 160g. per $1m^2$.

Esters of carbamic acid no. 30, 32, 34, 35 and 23 have an undoubtedly nematodocidal effect: they freed the roots completely from cysts. Therefore, the potato Heterodera did not develop in these containers during the growing period. Absent from the soil also were light-colored cysts of the 1949 generation. At the same time it should be pointed out, that the soil in these five containers retained a strong odor of the preparations until the very moment of record-taking of the experiment.

Trans. 483
(In full)
By:
A. Antik

Table 4 (P. 471)

Results of treatment of soil inoculated with potato nematode, with preparations of dithiecarbamic acid (in flower pots).

No. of test	No. of Preparation	Name of preparation	Degree of infection with cysts per 1 cm of root	Number of cysts in the soil in 50g. White	Brown	Total	in 1kg. Average before treatment	Average after treatment	Remarks
1	25	Carbisoamyldimethyl of dithiocarbamate . . .	1-3	32	38	70	1300	1400	
2	26	Carbethoxydimethyl of dithiocarbamate . . .	1-4	42	23	65	1120	1300	
3	27	Carbpropyloxidemethyl of dithiocarbamate . . .	1-3	16	33	49	1466	980	
4	28	Carbisobutoxydimethyl of dithiocarbamate . . .	1-10	3	23	26	1686	520	
5	29	Carbisobutyloxidimethl of dithiocarbamate . . .	1-2	13	46	59	1293	1180	
6	30	Butyl ester of diethyl-dithiocarbamic acid .	0	0	46	46	1640	920	Soil with strong odor of preparation
7	31	Ethyl ester of diethyl-dithio carbamic acid .	Single (1)	2	31	33	1406	660	
8	32	Propyl ester of diethyl-dithiocarbamic acid . .	0	0	55	55	1413	100	Same as no. 6
9	33	Propyl ester of dimethyl dithiocarbamic acid . .	Single (1)	0	35	35	1270	700	" "
10	34	Lsoamyl ester of dimethyl-dithiocarbamic acid . .	0	0	26	26	1686	520	" "
11	35	Butyl ester of dimethyl-dithiocarbamic acid . .	0	0	50	50	1740	1000	" "
12	23	Ethyl ester of dimethyl-dithiocarbamic acid . .	0	0	27	27	1706	540	
13		Methyl ester of dimethyl-dithio-carbamic acid . . ("Cystogon" type). . . .	0	0	18	18	1560	360	
		Same prepared with sand	(1)	3	15	18	1480	420	
		Control (without treatment)	10	32	53	85	1556	1700	

(1) Single cysts per entire bush.

Table 5 (474)

Results of tests of "forbiat" and "Cyatogen" for destruction of wheat nematode in the soil

No. of Plots	Poison introduced (in g.)	GROWN SPIKES Total	Among them: Healthy Number	Healthy %	With Galls Number	With Galls %	Average Infection	Intensiveness of infection
				FORBIAT				
7	control	101	90	89.1	11	10.9	15.4	1.6
9	control	100	80	80	20	20	-	1-18
13	75	208	201	96.1	7	3.9	1.9	1-3
16	75	240	240	100	0	-	-	-
10	100	182	180	98.9	2	1.1	0.5	1
11	100	177	177	100	0	-	-	-
14	125	277	276	99.6	1	0.4	0.2	3
15	125	339	339	100	0	-	-	-
				CYSTOGON				
17	control	59	57	96.6	2	3.6	-	1-2
20	150	198	195	98.4	3	1.6	-	1
19	175	398	398	100	0	0	-	-
18	200	310	310	100	0	0	-	-

Trans. 463
(In full)
Bys
A. Antik

As to the remaining esters (no. 31,33) and the preparation of the "Cystogon" type prepared with sand, their nematodocidal action appeared to be weaker because there were single cysts of the 1949 generation on roots and in the soil. The results of the experiment are given in fig. 7 and table 4.

Fig. 6 - Caption (p. 472) Potato bushes grown in plots treated with various preparations: A - "forbiat"; B and V - preparation no. 23; G and D - "Cystogon"; E-control.

Fig. 7 - Caption (p. 472) Potato bushes grown in soil treated with esters of dithiocarbamic acid (preparations no. 33, 34, 35, and 23). The extreme right is the control.

Fig. 8 - Caption (p. 473) Wheat plants: left - normal, center - infected with wheat nematode, right - grown in soil decontaminated with "forbiat".

As to the preparation of the "Cystogon" type, when prepared with kaolin, it was quite effective, the roots and soil were free of cysts of the 1949 generation, but prepared with sand it was less effective since infection of roots and soil with cysts of the 1949 generation was observed. In the control containers the roots were seriously infected by cysts - up to 10 cysts per 1 cm. of root and there was a large amount of cysts, light-colored and brown, which contained live larvae of nematode.

Control of wheat nematodes is carried out by cleaning the grain of galls by machines; by the wet method for elimination of galls which rise to the surface of fluids with a heavy specific gravity, or simply water; liveration of soil from larvae, which remained in the soil when the grain shattered, especially from the lower spikes which are not picked by the harvesting machines, is achieved by crop rotations when wheat is not returned to the contaminated field before 2 - 3 years have passed. But it is not always possible to carry out this measure and menas for decontamination of soil have not yet been tested by anyone. Therefore the author tested the action of "forbiat" and Cystogon" in decontaminating soil from larvae of wheat nematode. The soil was treated with "Cystogon" in doses of 150, 175, and 200 g. per $1m^2$ and with "forbiat" in doses of 75, 100, and 125 g. per $1m^2$. Viable larvae of wheat nematodes, inclosed within galls with a slightly cracked wall, previously moistened were simultaneously introduced into the soil. Seven days after the treatment the plots were sown with spring wheat of the Surkhak variety. It is mentioned in the literature (Goffart, 1941) that "Cystogon" has an effect on germination of cereals. According to our observations it appeared that the germination in treated plots is indeed retarded by 1 - 2 days as compared with the control. Subsequent growing demonstrated that the condition of wheat plants in treated plots is considerably better than in the control plots. This was due to the decrease damage to plant by wheat nematodes, as well

Trans. 463
(In full)
By:
A. Antik

as to the lesser effect of the insect-fauna which was chased away from the plots by the odor of "forbiat" and "Cystogon". The condition of plants in the control plots was noticeably worse. During threshing the number of infected spikelets was counted. Table 55 gives an idea of the effect of the preparations "forbiat" and "Cystogon" on the degree of damage to plants by wheat nematodes. It is obvious from the data that both preparations decrease the percentage damage of plants by wheat nematodes: "forbiat" - to 0.2% with a 100-125 g. per $1m^2$ dose (from 15% in the control), "Cystogon" - to zero with a 150-175 g. per $1m^2$ dose (from 3.6% in the control). The experiment was conducted on a small scale in one-meter plots.

As a result of works carried out on testing powdered chemical preparations for control of root-knot potato and wheat nematodes, the following was established:

1. Methyl ester of dimethyl-dithiocarbamic acid manufactured as a 10% dust with kaolin ("Cystogon" type) and a 20% dust ("forbiat" type), when introduced into the soil, has undoubted nematocidal properties against gall, potato, and wheat nematodes and particularly good results are obtained from the 20% dust.

2. I

2. Though as a result of soil treatment with the preparations mentioned in doses of 175-200-300 g. per $1m^2$ (10% dust) and 110-168 g. per $1m^2$ (20% dust), under field conditions, no 100% of destruction of root-knot and potato nematodes in the soil was obtained with a single introduction, nevertheless the development of the growing mass of crops (cucumbers, potatoes) and the yield capacity increased sharply and the infection of plants by nematodes (cucumber, carrots, potatoes) decreased drastically.

3. High nematocidal properties were manifest by the ethyl, butyl, and propyl esters of the diethyl-dithiocarbamic acid in 20% dusts applied at a 150 g. per $1m^2$ dose (under flower pot conditions).

Trans. 463
(In full)
By:
A. Antik

4. Taking into consideration the simplicity and convenience in the use of the tested effective preparations as compared with liquid fumigants, the dusts manufactured on the basis of esters of dithiocarbamic acid should be considered as having a future in agriculture for control of the potato and root-knot nematode.

BIBLIOGRAPHY

Kirianova, E. S. 1935. Nematode Diseases of Potatoes. Zasheh rast., 6, p. 86-98.

Kirianova, E. S. 1941. Nematode of Grain Crops. Ils. Vysshikh Kursov Prikladnoi Zoologii i Fitopatologii. Vol. XII, NKL SSSR. L.

Sveshnikova, N. M. 1948. Potato Nematode. Illustr. sprav. posredit. i bol. S.-Kh. Rastenii Vneshn, Karantina. Ild. Minist. Selhsk. Khoz. SSSR, M.

Sveshnikova, N. M. 1949. On Use of the "Cystogon" Preparation for Control of Gall Nematode. Dokl. Akad. S.-Kh. Nauk, im. Timiriazeva. M. VIII.

Sveshnikova, N. M. 1949. On Study of Basic "Heteroderosis" (?) of Agricultural Plants in the USSR. Auto lecture of the dissertation. Ild. Tsentr. laboratorii po karantinu rastenii i Akal. S.-Kh. Nauk im. Timiriazeva, M.

Selivonchik, E. V. 1938. Results and Measures Against Root-knot Nematode in Azerbaidzhan, Baku.

Ustinov, A. A. 1934. Root (gall) Nematode, Sukhumi, Abgiz.

Filipev, I. N. 1934. Nematodes Harmful and Useful in Agriculture, Selkhozgiz, M.

Goffart, H. 1941. A New Measure for Control of Nematodes, Mitteil, Biolog. Reichsanstalt Land und Forstwirtshaft. H. 64.

Trans. 463
(In full)
By:
A. Antik

Sveshnikova, N. M.

Opyt primeneniia preparatov ditio-Karbaminovoi kisloty dlia-bor'by s nematodami-parazitami rastenii

[Attempt of Using Dithiocarbamic Acid Derivatives in Fighting Nematodes which function as Plant Parasites].

Trudy Zoologicheskogo instituta Akademii Nauk SSSR, IX, no. 2, pp 462-475, 1951. 410.9 L543 (In Russian)

EXPERIMENTAL APPLICATION OF DITHIOCARBAMIC ACID DERIVATIVES FOR CONTROL OF PLANT PARASITIC NEMATODES

Among the nematodes causing economic losses to national economy of the USSR particularly damaging are: the root-knot, potato and wheat nematodes.

Root-knot nematoda-Heterodera marioni (Cornu) which parasitizes roots of over 1,500 species of most diversified plants--vegetables, technical, medicinal and ornamental plants and some fruit trees and palms--depresses them, deforms the roots, inhibits the development and decreases the yield. Root-knot nematode is disclosed on plants by the presence of swellings on roots--galls, which vary in size from a pin head to a walnut, depending on the host plant, degree of soil contamination with root-knot nematode and other conditions.

The females of the root-knot nematode, which are found in galls, lay hundreds of eggs into the external surroundings. In the spring the larvae which developed in the eggs emerge into the soil, find young plants and penetrate their roots for further development until the sexual maturity stage of male and female. The root-knot nematode is distributed predominantly in the South of the USSR where in a number of localities it makes cultivation of vegetables and other susceptible plants quite impossible. Besides that, the root-knot nematode, as it was found by the author (1949), can inhabit the open ground and be harmful for non-rotated cultivation of vegetable plants in the central belt of the USSR. The root-knot nematode is very harmful also in farms with covered ground [greenhouses].

Potato nematode--Heterodera rostochiensis Woll., discovered in some points of the Baltic coast of the USSR (Sveshnikova, 1948) causes great losses to potatoes decreasing the yield in seriously affected sections up to 14-27 g. [grams?] per plant. Potato nematode parasitizes the roots of potato plants into which it penetrates in the spring during the larval

(In full)
By:
A. Antik

stage after hatching from eggs which hibernated in the soil in the dead female-cyst. The larvae make a mass attack on young potato rootlets, pentrating into them they feed on plant sap, undergo a number of "linek" [molts] after which they reach the stage of sex maturity of female and male. The harmfulness of the potato nematode is manifest in the destruction of the potato rootlets: around the penetrated larvae a necrosis of root cells takes place. Inasmuch as the number of larvae in the root frequently exceeds 10 per 1cm., a dying of roots takes place. Many larvae are destroyed together with roots, but the attack by larvae is repeated several times and the plant becomes exhausted by wasting material for regeneration of roots, the leaves develop weakly, they dry out soon and therefore the tubers formed are small or they are not formed at all.

Wheat nematode--Anguina tritici (Steinbuch) is a serious parasite of cereals, mainly of wh[illegible] Larvae of the wheat nematode affect the plant at the beginning o[illegible]s germination: the plants are dwarfed in size, the stems are thic[illegible]d and have crimped leaves (fig. 8). With the formation of the spike, the larvae infect the ovary and reach there their sex maturity stage. After fertilization the females lay masses of eggs (up to 15 thousand) from which larvae hatch and undergo molts. From the ovary in affected spikes there are formed, instead of grain, galls with thick walls which protect the larvae within the galls. Galls contaminate the seed grain and enter the soil where the larvae crawl out and attack young plants. The harmfulness of the wheat nematode is manifest in the fact that part of the affected plants are destroyed while young; in mature plants the infected spiklets do not blossom, instead grain-galls are formed in them, which are filled with larvae, and in the grain gathered from a greatly contaminated field the amount of galls might reach up to 1,000 and more per 1kg., i. e. up to 3% of the net grain weight. On certain farms the loss caused by the wheat nematode is quite considerable. The wheat nematode is distributed in Central Asia and in a number of southern localities of the USSR, as well as in Belorussia; it was recorded also in Siberia (Kirianova, 1941). The author carried out experimental inoculation and obtained formation of galls near Moscow.

In search for measures for decontamination of the soil of the mentioned three species of nematodes, the researchers had in mind affecting the larvae of the second stage which migrate in the soil looking for a plant-host.

It was suggested, as control measures against the gall nematode, to steam the soil up to a 60- 65° C. temperature, at a 20-25cm depth, as well as to apply a series of agro-technical measures as, for example, replowing in fields where the soils dry out, flooding of soil for long periods, crop rotation with plants resistant to root-knot nematodes (Filipev , 1934). However, in many cases it is not possible to carry

Trans. 463
(In full)
By:
A. Antik

out these measures and in some parts of the Azerbaidshan, Crimea and Black Sea Coast of the Caucasus, the root-knot nematode has reproduced intensely. According to Selivonchik's data (1938) [1938], the vegetable yield on the Apsheron peninsula is partly destroyed by root-knot nematode. Radical measures are required for their complete extermination or, at any rate for a decrease in contamination to a degree where growing of vegetable - Cucurbitae and other crops, susceptible to nematode infection, should be possible. The opinion existing in the literature, that root-knot nematode does no harm under conditions of temperate climate, in particular in the central belt of the USSR - is not correct. In 1944, the author observed in the Moscow oblast' a complete destruction of carrot yield in a focus of root-knot nematode in a garden near a farm house, during the third year of cultivation of garden plants: with potatoes in 1942 and 1943, and with carrots in 1944. The carrots were affected so severely by the root-knot nematode that the yield was not suitable for use (Sveshnikova, 1949).

Among chemical control measures tested in the USSR by A. A. Ustinov (1934) effective were: carbon bisulfide at a dose of 500g. per 1m2 and chloropicrin at a dose of 100-150g. per 1m2. The carbon bisulfide in the indicated dose resulted, under conditions of Sukhumi, in 100% destruction of the root-knot nematodes in plots: however, this preparation is poisonous, explosive and therefore not convenient for extensive use. Chloropicrin in indicated doses is effective, however, a 100% destruction of root-knot nematode, under conditions of severe soil contamination in Sukhumi, was not obtained, therefore, its dose per 1m2 has to be increased. Besides that, application of highly poisonous fluid chemicals requires use of injecters, work with them is dangerous and has to be conducted by specifically trained people in gas masks and protective suits and due to all that the process becomes too cumbersome and expensive.

Therefore, the author conducted in 1945-1949 a test for the control of root-knot, wheat and potato nematodes with powdered preparations suggested by the Scientific Institute for Fertilizers and Insectofungicides imeni la. V. Samoilov. Tested were the following derivatives of dimethyl-dithiocarbamic acid in dust form and manufactured on the basis of: methyl ester of dimethyl - dithiocarbamic acid (10% dust, patented preparation "cystogon", similar dust of Soviet manufacture with another filler (of "cystogon" type), 20% dust on the basis of the same toxic element ("forbiat" [7], 20% dusts of ethyl (no. 23), butyl (no. 35), isoamyl (no. 34) and propyl (no. 33) of dimethyl - dithiocarbamic acid: esters of diethyl - dithiocarbamic acid: ethyl (no. 31), butyl (no. 30) and propyl (no. 32) also manufactured as 20% dusts. The rest of the preparations which did not show a sufficiently nematocidal action are not mentioned here since they are indicated in the collated table 4.

(On [illegible])
By:
A. Antik

Methods of application were as follows: in the spring the preparations were scattered by hand in a 1:3 ratio mixture with dry sand or road dust, into adequately friable soil which was replowed or redug and after that the preparation was thoroughly plowed under at a 20-25cm depth. Part of the preparations, due to their small amounts, were tested in flower pots. In the latter case the chemicals were introduced into the soil before filling the containers, without adding sand and they were mixed evenly with the entire volume of soil. During the work in the open air gas masks were not used. Rubber gloves or canvas mittens were used in mixing the preparations with sand and in scattering them. No other special safety measures are required. Contact of the preparation with mucous membrane of eyes, mouth and nose should be avoided. Therefore, smoking and eating during the work was prohibited. After the work, face, hands and feet (if work was done barefooted) were washed with water.

In 1946, the author conducted tests with "cystogon" against root-knot nematode under conditions of natural foci of the parasite in open ground in the Moscow oblast' in an individually-owned garden (Sveshnikova, 1949). Well prepared sandy soil was dug up with a shovel to a 25cm depth and then broken up with a rake. The introduction of the preparation took place on May 8, at a 14° C. air temperature and a 8° C. soil temperature at a 20cm depth. The soil humidity constituted 17.22% when recalculated in absolutely dry soil. "Cystogen" was taken at a ratio of 185g. per $1m^2$. It was introduced in a mixture with dry sand for a more uniform distribution in the soil, it was scattered by hand through a soil sieve. After the introduction of the preparation the soil was again dug with a shovel and stirred with a rake. The soil in the control area was cultivated in the same manner. In large areas it is recommended to introduce the preparation into the soil with a drill for fertilizers and to finish up with a horse-drawn rake. Seven days later, i.e. on May 15, carrots of the Danvers variety, with a few white carrots added were sown in the experimental beds. On May 30, normal carrot shoots (line) were observed in the treated as well as in the control area which did not differ in density or size. Further growing was normal. Caretaking - stirring up of the ground and weeding - was done by the owner of the garden. On September 9, the experiment was terminated. Examination of 124 carrot roots from the treated plot disclosed on the thin roots of 26 of them single galls discernable only with binocular glasses /magnifying glass/ (21.4%). The remaining roots were absolutely free from infection (fig. 1). The roots free from infection as well as the affected ones from the treated plot were of quite normal size and shape and suitable for food, while in 1945, the owner of the garden discarded the yield of this bed because the disease incidence was too great. From the control bed were taken 160 roots. All (100%) of the plants were entirely deformed by root-knot nematodes as was the case in the preceding year (fig. 1)

Trans. 463
(In full)
By:
A. Antik

(P. 465)
Fig. 1 - Caption. Left - carrot from the plot treated with "Cystogon" [?] in the dose of 185g. per $1m^2$: right - carrot from the control plot affected with gall nematode.

The second part of the experiment was conducted in heavy clay soil in another area of concentration of root-knot nematodes in the Moscow oblast'. The soil was not "mature" i.e. it did not crumble, but was compacted (humidity 19.65%) and it was not possible to break it up finely. "Cystogon" was introduced by similar methods in a dose of 200g. per $1m^2$, with consideration of the stickiness of the soil. The Danvers variety carrots were sowndirectly after soil treatment in order to find out about the effect of the poison on sprouting. Germination was normal - on the 15th day young growth of uniform density was observed in the treated as well as the control area. Here the results were less successful: of the 94 plants taken from the treated plot, 29, i.e. 30.7%, were affected. The degree of infection was from 1-5 galls per plant. From the control area 60 plants were taken, of which 42, i.e. 70%, were affected. Degree of infection - from 1 to 100 galls per plant. The results of the test were undoubtedly influenced by the soil structure.

On the basis of the preliminary experiment with the "Cystogon" against root-knot nematode carried out in 1946 (Sveshnikova, 1949), the author decided to try out in 1948 another preparation - "forbiat" [?] - for decontamination of soil from the root-knot nematode under conditions of intensive contamination which exists on the Apsheron peninsula. Prior to the author's tests, "forbiat" was applied as an insecticide, but no one tried it as a nematocide. The experiment was conducted in Baku with the cooperation of the director of the Azerbaidzhan Quarantine Laboratory, S. L. Popov and the entomologist L. I. Shapieva. Soil treatment was carried out on two farms with different soils: in the village Shuveliany in sandy soil and in the Armenikendskii nursery of the Baku-soviet - in clayey loam soil. Methods of poison introduction were similar to those for "Cystogon": digging as deep as possible, loosening of the ground and thorough mixing of the chemical with the soil. The "forbiat" was tested in doses of 70, 85, 100 and 110 g. per $1m^2$. The experimsnt was conducted during the second decade of May in three plots $5m^2$ in size. A week after the treatment cucumber seeds of the Chinese variety were introduced.

During the growing period the control plots showed very great sparsity: in treated plots there were almost no bare spots, the plots were covered with strong vines and produced a yield shown in table 1. In the second part of August, the plants were dug out and records were taken of infection of their roots with root-knot nematode. Almost all

Trans. 463
(In full)
By:
A. Antik

the plants were affected with it, but to various degrees (fig. 2). While in control plants the roots were absolutely deformed as a result of parasitizing by the root-knot nematode, in plants from treated plots there were considerable less galls and the higher the dose of poison, the fewer galls (fig. 2). When the dosage was 100 and 110g. per $1m^2$, the galls appeared singly and were most frequently distributed at a depth of less than 20cm. Numerical data of recorded state of roots are presented in table 1.

Table 1 (p. 466)

Effect of soil treatment with "forbiat" against gall nematode.

Dose of "forbiat" per $1m^2$ (in g.)	Shuveliany		Armenikend	
	average % of root infection	yield (in kg.)	average % of root infection	yield (in kg.)
Control (without treatment)	100 (At the end of the experiment all the plants were destroyed)	0	100	11.2
70.	70.5	5	84.4	13.3
85.	50.1	5	88.1(1)	23.1
100.	38.5	8.7	67.1	29.8
110.	36.1	9.1	54	30.3

The tested doses of "forbiat" did not entirely destroy the root-knot nematodes in the soil, but they gave the plants the possibility of growing normally.

In order to develop a 100% effectiveness of "forbiat" an experiment was conducted in flower pots with a clayey loam soil artificially inoculated to a high degree with root-knot nematodes. The soil was thoroughly mixed with "forbiat" in the ratio of 150, 250 and 350g. per $1m^2$ for each three pots.

1) Higher percentage of infection is caused by defects in the agrotechnique

Trans. 463
(In full)
By:
A. Antik

Radishes were taken as test plants. Two weeks after the planting, depression of plants in control (untreated) pots became noticeable and toward the middle of the second month of growing, a picture of better plant condition in treated pots as compared with the control ones was apparent.

The result of examination of plant roots are in table 2.

Table 2(p. 462)

Results of soil treatment with "forbiat" against gall nematode (flower pots)

Dose per 1M² (in g.)	Total number of plants	Number of affected plants	% of infection
Control (without treatment)	25	25	100
150.	25	2(1)	8
250.	25	1(1)	4
350.	25	0	0

Thus it was established that the effectiveness of the preparation depends on the degree of soil contamination and on the dose, and, mainly, on the thoroughness in mixing the preparation with the soil.

(P. 467)
Fig. 2 - Caption. Roots of cucumbers from plots treated with "forbiat". From left to right: control, "forbiat" in doses: 70, 85, 100 and 110g. per 1m².

On the basis of our works which demonstrated high nematocidal properties of preparations of dithiocarbamic acid, in 1949, a test was conducted with a wider assortment against potato nematode which possesses a higher resistance against unfavorable conditions of the envirement. According to data in the literature, its cysts can remain viable in the soil up to 10 years. Up to recent years the potato nematode was not known in the USSR, therefore, prior to our research, - none was conducted. The experiments were conducted on the Base of the Lithuanian Quarantine Inspection with the cooperation of the agronomist E. S. Mikhnova.

1) Intensiveness of invasion - 1 gall per entire plant.

By:
A. Antik

The following preparations were tested under field conditions: 10% dust of the "Cystegon" type (methyl ester) in doses of 200 and 300g. per $1m^2$, 20% dust of the "forbiat" type in doses of 168g. per $1m^2$ and ethyl ester (preparations no. 23) in doses of 100 and 185g. per $1m^2$. Soil contamination reached up to 2500 cysts of nematodes per kg. of soil. Preparation of the "Cystogon" type was tested in plots of $100m^2$, preparation no. 23 and "forbiat" - in $15m^2$ plots, depending on the amount of chemicals. It was possible to establish during the process of potato development that the plants' germination did not suffer from the effects of the preparations being tested, with the exception of a 300g. dose per $1m^2$ of preparation of the "Cystogon" type which somewhat inhibited germination, though within the potato norm. The effect of decontamination of soil with preparations was clearly expressed in the exterior aspect of the leaves, which looked quite normal in treated plots (fig. 3, 4, 5 and 6). The yield of plants in treated plots was 4-10 times heavier than in the control plots. It should be pointed out that these data pertain to the 15th of August, when the yield did not yet correspond to the norm and besides that we intentionally omitted manuring, in order to demonstrate more clearly the influence of plant decontamination from potato nematodes. The results of the treatment are shown in table 3.

(P. 468)

Fig. 3 - Caption. Plot naturally contaminated with potato nematodes; in the background - soil treated with preparation no. 23 in the dosage of 100g. per $1m^2$.

Table 3 (P. 469)

Results of soil treatment with esters of dithiocarbamic acid for control of potato nematodes.

Name of preparation	Dose per $1m^2$ (in g.)	Average height of bushes (in cm.)	Average weight of tubers per bush (in g.)	Average % of large tubers	% of affected roots	Number of cysts on a bush
Ethyl ester (no. 23) 20% dust.	100	26	79	57	52	Single
Same. . . .	185	32	108	59	36	"
Methyl ester 20% dust - "forbiat". .	168	51	141	68	4	"
Methyl ester, 10% dust. .	200	27	110	70	40	"
Same. . . .	300	39	113	60	36	"
Control . .	without treatment	14	11.5	18	100	Up to 15 per 1sm. of root

Trans. 463
(In full)
By:
A. Antik

(P. 469)
Fig. 4 - Caption. Plots treated with "Cystogen" in dosage of 200-300g. per $1m^2$.

Damage to plants following treatment with "forbiat" decreased 96%, following treatment with preparation no. 23 in a 100g. dose - 46% and no. 23 in a 185g. dose - 64%, as compared with the control, where 100% of potato bushes were affected to a high degree. The exterior aspect of the plants and the yield are presented in fig. 6.

The remaining 12 esters of dithiocarbamic acid (see table 4) were obtained in amounts not exceeding 100g., therefore tests of them were carried out in flower pots (three times) and the natural contamination of soil was 1200-1700 cysts per 1 kg. The soil was treated with dust in the ratio of 150g. per $1m^2$. Two days after treatment potato tubers of the "Vol'tman" variety, about 100g. in weight which were taken from a farm free from potato nematodes were planted in all the containers and were placed on racks in a green-house with a roof of wire screen, i.e. under climatic conditions of open ground in Vilnus, and they were held there until the moment of record-taking of the experiment. Potato shoots were noticed on the 14-24th day, i.e. within the norm for this crop, though in the control containers shoots were observed on the 11-13th day. Further on a certain difference in development was observed; in pots with soil treated with chemicals, the growth of bushes was more powerful and on July 13, budding started; in the control containers the plants were weaker, shorter, they did not blossom and towards the middle of July began wilting and dropping of lower leaves. It is seen from table 4, that preparations no. 25-29 had no decontaminating effect: there were cysts of potato nematode - 1 - 10 per 1cm. of root, i.e. up to a degree of affection observed in the control, in the soil there were, besides the brown, also white cysts of the 1949 generation.

(P.470)
Fig. 5 - Caption. Plot treated with "forbiat" in dosage of 160g. per $1m^2$.

Esters of carbamic acid no. 30, 32, 34, 35 and 23 have an undoubtedly nematocidal effect: they freed the roots completely from cysts. Therefore, the potato Heterodera did not develop in these containers during the growing period. Absent from the soil also were light-colored cysts of the 1949 generation. At the same time it should be pointed out, that the soil in these five containers retained a strong oder of the preparations until the very moment of record-taking of the experiment.

Trans. 483
(In full)
By:
A. Antik

Table 4 (P. 471)

Results of treatment of soil inoculated with potato nematode, with preparations of dithiocarbamic acid (in flower pots).

No. of test	No. of Preparation	Name of preparation	Degree of infestation with cysts per 1 cm of root	Number of cysts in the soil					Remarks
				in 50g.			in 1kg.		
				White	Brown	Total	Average before treatment	Average after treatment	
1	25	Carbisoamyldimethyl of dithiocarbamate . . .	1-3	32	38	70	1300	1400	
2	26	Carbethoxydimethyl of dithiocarbamate . . .	1-4	42	23	65	1120	1300	
3	27	Carbpropylamidomethyl of dithiocarbamate . . .	1-3	16	33	49	1466	980	
4	28	Carbisobutoxydimethyl of dithiocarbamate . . .	1-10	3	23	26	1686	520	
5	29	Carbisobutylamidimethl of dithiocarbamate . . .	1-2	13	46	59	1293	1180	
6	30	Butyl ester of diethyl-dithiocarbamic acid .	0	0	46	46	1640	920	Soil with strong odor of preparation
7	31	Ethyl ester of diethyl-dithio carbamic acid .	Single (1)	2	31	33	1406	660	
8	32	Propyl ester of diethyl-dithiocarbamic acid . .	0	0	55	55	1413	100	Same as no. 6
9	33	Propyl ester of dimethyl dithiocarbamic acid . .	Single (1)	0	35	35	1270	700	" "
10	34	Isoamyl ester of dimethyl-dithiocarbamic acid . .	0	0	26	26	1686	520	" "
11	35	Butyl ester of dimethyl-dithiocarbamic acid . .	0	0	50	50	1740	1000	" "
12	23	Ethyl ester of dimethyl-dithiocarbamic acid . .	0	0	27	27	1706	540	
13		Methyl ester of dimethyl-dithio-carbamic acid . ("Cystogen" type). . .	0	0	18	18	1560	360	
		Sand prepared with sand	(1)	3	15	18	1480	420	
		Control (without treatment)	10	32	53	85	1556	1700	

(1) Single cysts per entire bush.

Table 5 (474)

Results of tests of "forbiat" and "Cystogen" for destruction of wheat nematode in the soil

No. of Plots	Poison introduced (in g.)	GROWN SPIKES					Average Infection	Intensiveness of infection
		Total	Among them					
			Healthy		With Galls			
			Number	%	Number	%		
			FORBIAT					
7	control	101	90	89.1	11	10.9	15.4	1.6
9	control	100	80	80	20	20	-	1-18
13	75	208	201	96.3	7	3.9	1.9	1-3
16	75	240	240	100	0	-	-	-
10	100	182	180	98.9	2	1.1	0.5	1
11	100	177	177	100	0	-	-	-
14	125	277	276	99.6	1	0.4	0.2	3
15	125	339	339	100	0	-	-	-
			CYSTOGON					
17	control	59	57	96.6	2	3.6	-	1-2
20	150	198	195	98.4	3	1.6	-	1
19	175	398	398	100	0	0	-	-
18	200	310	310	100	0	0	-	-

As to the remaining esters (no. 31,33) and the preparation of the "Cystogon" type prepared with sand, their nematodocidal action appeared to be weaker because there were single cysts of the 1949 generation on roots and in the soil. The results of the experiment are given in fig. 7 and table 4.

Fig. 6 - Caption (p. 472) Potato bushes grown in plots treated with various preparations: A - "forbiat"; B and V - preparation no. 23; G and D - "Cystogon"; E-control.

Fig. 7 - Caption (p. 472) Potato bushes grown in soil treated with esters of dithiocarbamic acid (preparations no. 30, 32, 34, 35, and 23). The extreme right is the control.

Fig. 8 - Caption (p. 473) Wheat plants: left - normal, center - infected with wheat nematode, right - grown in soil decontaminated with "forbiat".

As to the preparation of the "Cystogon" type, when prepared with kaolin, it was quite effective, the roots and soil were free of cysts of the 1949 generation, but prepared with sand it was less effective since infection of roots and soil with cysts of the 1949 generation was observed. In the control containers the roots were seriously infected by cysts - up to 10 cysts per 1 cm. of root and there was a large amount of cysts, light-colored and brown, which contained live larvae of nematode.

Control of wheat nematodes is carried out by cleaning the grain of galls by machines; by the wet method for elimination of galls which rise to the surface of fluids with a heavy specific gravity, or simply water; liberation of soil from larvae, which remained in the soil when the grain shattered, especially from the lower spikes which are not picked by the harvesting machines, is achieved by crop rotations when wheat is not returned to the contaminated field before 2 - 3 years have passed. But it is not always possible to carry out this measure and means for decontamination of soil have not yet been tested by anyone. Therefore the author tested the action of "forbiat" and Cystogon" in decontaminating soil from larvae of wheat nematode. The soil was treated with "Cystogon" in doses of 150, 175, and 200 g. per $1m^2$ and with "forbiat" in doses of 75, 100, and 125 g. per $1m^2$. Viable larvae of wheat nematodes, inclosed within galls with a slightly cracked wall, previously moistened were simultaneously introduced into the soil. Seven days after the treatment the plots were sown with spring wheat of the Surkhak variety. It is mentioned in the literature (Goffart, 1941) that "Cystogon" has an effect on germination of cereals. According to our observations it appeared that the germination in treated plots is indeed retarded by 1 - 2 days as compared with the control. Subsequent growing demonstrated that the condition of wheat plants in treated plots is considerably better than in the control plots. This was due to the decrease damage to plant by wheat nematodes, as well

Trans. 463
(In full)
By:
A. Antik

as to the lesser effect of the insect-fauna which was chased away from the plots by the odor of "forbiat" and "Cystogon". The condition of plants in the control plots was noticeably worse. During threshing the number of infected spikelets was counted. Table 5 gives an idea of the effect of the preparations "forbiat" and "Cystogon" on the degree of damage to plants by wheat nematodes. It is obvious from the data that both preparations decrease the percentage damage of plants by wheat nematodes: "forbiat" - to 0.2% with a 100-125 g. per $1m^2$ dose (from 15% in the control), "Cystogon" - to zero with a 150-175 g. per $1m^2$ dose (from 3.6% in the control). The experiment was conducted on a small scale in one-meter plots.

As a result of works carried out on testing powdered chemical preparations for control of root-knot potato and wheat nematodes, the following was established:

1. Methyl ester of dimethyl-dithiocarbamic acid manufactured as a 10% dust with kaolin ("Cystogon" type) and a 20% dust ("forbiat" type), when introduced into the soil, has undoubted nematocidal properties against gall, potato, and wheat nematodes and particularly good results are obtained from the 20% dust.

2. Though as a result of soil treatment with the preparations mentioned in doses of 175-200-300 g. per $1m^2$ (10% dust) and 110-168 g. per $1m^2$ (20% dust), under field conditions, no 100% of destruction of root-knot and potato nematodes in the soil was obtained with a single introduction, nevertheless the development of the growing mass of crops (cucumbers, potatoes) and the yield capacity increased sharply and the infection of plants by nematodes (cucumber, carrots, potatoes) decreased drastically.

3. High nematocidal properties were manifest by the ethyl, butyl, and propyl esters of the diethyl-dithiocarbamic acid in 20% dusts applied at a 150 g. per $1m^2$ dose (under flower pot conditions).

Trans. 463
(In full)
By:
A. Antik

4. Taking into consideration the simplicity and convenience in the use of the tested effective preparations as compared with liquid fumigants, the dusts manufactured on the basis of esters of dithiocarbamic acid should be considered as having a future in agriculture for control of the potato and root knot nematode.

BIBLIOGRAPHY

Kirianova, E. S. 1935. Nematode Diseases of Potatoes. Zasheh rast., 6, p. 86-98.

Kirianova, E. S. 1941. Nematode of Grain Crops. Ils. Vysshikh Kursov Prikladnoi Acologii i Fitopatologii. Vol. XII, NKL SSSR. L.

Sveshnikova, N. M. 1948. Potato Nematode. Illustr. sprav. posredit. i bol. S.-Kh. Rastenii Vnesha, Karantina. Ild. Minist. Selhsk. Khoz. SSSR, M.

Sveshnikova, N. M. 1949. On Use of the "Cystogon" Preparation for Control of Gall Nematode. Dokl. Akad. S.-Kh. Nauk, im. Timiriazeva. M. VIII.

Sveshnikova, N. M. 1949. On Study of Basic "Heteroderosis" (?) of Agricultural Plants in the USSR. Auto lecture of the dissertation. Ild. Tsentr. laboratorii po karantinu rastenii i Akal. S.-Kh. Nauk im. Timiriazeva, M.

Selivonchik, E. V. 1938. Results and Measures Against Root-knot Nematode in Azerbaidzhan, Baku.

Ustinov, A. A. 1934. Root (gall) Nematode, Sukhumi, Abgis.

Filipev, I. N. 1934. Nematodes Harmful and Useful in Agriculture, Selkhozgiz, M.

Goffart, H. 1941. A New Measure for Control of Nematodes, Mitteil, Biolog. Reichsanstalt Land und Forstwirtshaft. H. 64.

Trans. 463
(In full)
By:
A. Antik

4. Taking into consideration the simplicity and convenience in the use of the tested effective preparations as compared with liquid fumigants, the dusts manufactured on the basis of esters of dithiocarbamic acid should be considered as having a future in agriculture for control of the potato and root knot nematode.

BIBLIOGRAPHY

Kirianova, E. S. 1935. Nematode Diseases of Potatoes. Zashch rast., 6, p. 86-98.

Kirianova, E. S. 1941. Nematode of Grain Crops. Ils. Vyshikh Kursov Prikladnoi Aoologii i Fitopatologii. Vol. XII, NKL SSSR. L.

Sveshnikova, N. M. 1948. Potato Nematode. Illustr. sprav. posredit. i bol. S.-Kh. Rastenii Karantina. Ild. Minist. Selhsk. Khoz. SSSR, M.

Sveshnikova, N. M. 1949. On Use of the "Cystogon" Preparation for Control of Gall Nematode. Dokl. Akad. S.-Kh. Nauk, im. Timiriazeva, M. VIII.

Sveshnikova, N. M. 1949. On Study of Basic "Heteroderosis" (?) of Agricultural Plants in the USSR. Auto lecture of the dissertation. Ild. Tsentr. laboratorii po karantinu rastenii i Akal. S.-Kh. Nauk im. Timiriazeva, M.

[redacted]livonchik, E. V. 193[redacted] Results and Measures Against Root [redacted] matode in Azerbaidzhan, Baku.

Ustinov, A. A. 1934. Root (gall) Nematode, Sukhumi, [redacted]

Filipev, I. N. 1934. Nematodes [redacted]ful and Useful in [redacted] ture, Selkhozgiz, M.

Goffart, H. 1941. A New Measur[redacted] Control of Nematodes. Mitteil. Biolog. Reichsanstalt Land und [redacted]wirtshaft. H. 64.

464

Trans. 464
(In full)
By:
A. Antik

Nichiporovich, A. A.

"Fotosintez rastenii"

Priroda 41(4):37-46 April 1952
410 P933

(In Russian)

PHOTOSYNTHESIS OF PLANTS

In the known lecture "On Physiological Significance of Chlorophyll," K. A. Timiriazev wrote:

"There is hardly any process on the surface of the earth which deserves the same degree of general attention as the not yet elucidated process which takes place in a green leaf when sunlight falls on it. Examined from a chemical point of view it is the process in which the inorganic substances, carbonic acid and water, are converted into an organic substance.

Examined from the physical, dynamic point of view, it is the process in which the energy of sunlight is converted into chemical energy, into stored energy. Examined from both points of view it is a process on which, in final analysis, depend all the manifestations of life on our planet, and therefore-the welfare of all humanity." [1]

K. A. Timiriazev's brilliant research in this field opened wide perspectives for further study of light nutrition of plants--photosynthesis--on a truly scientific materialistic basis.

But the flaming tribune of science for the people--K. A. Timiriazev--not only conducted experimental work, he also popularized with unexcelled mastery, the knowledge of photosynthesis. Many Russian scientists who followed the road indicated by K. A. Timiriazev, made a rich contribution to the study of this most important problem of the present-day natural science.

It is enough to remember the words of such great Russian scientists

1. K. A. Timiriazev, On Physiological Significance of Chlorophyll, Selected writings, Vol. I, Sel'khozgiz, 1948, P. 257.

as A. N. Bakh, M. S. Tsvet, K. O. Purievich, V. V. Lapozhnikov, F. . Krasheninnikov, A. A. Rikhter, V. N. Liubimenko, S. P. Kostychev, in order to have an idea of the merits of Russian science in this field.

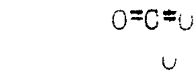
However, the significance of this problem is such that even at the present time, it is necessary to continue the work started by K. A. Timiriazev, to expand it and to popularize widely the knowledge of photosynthesis. In these works must be united the efforts of "representatives of most diversified fields of natural science"[1] - biologists, physicists, chemists, physico-chemists.

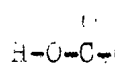
It is the more necessary inasmuch as, since the time of works by K. A. Timiriazev and his direct disciples, many notions on photosynthesis have changed radically.

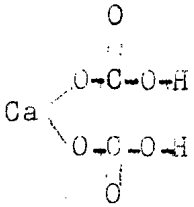
Photosynthesis is a process of carbon nutrition of green plants, which is realized with participation of light. Carbon is part of organic substances; its content in the mass of the dry substance of bodies of living organisms (of plants as well as of animals) reaches 40-45%.

Carbon is present on the Earth in two groups of combinations. The first group is the inorganic substances containing carbon. The basic combination of this group is carbon dioxide (CO_2) which is present in a gaseous state in the air and in a dissolved state in the water of rivers, seas, oceans. The derivatives of carbon dioxide are carbonic acid (H_2CO_3) and salts of the latter: carbonates and bicarbonates.

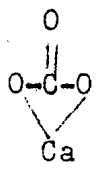
Carbon, which is part of these combinations, is completely oxydized, it is combined only with atoms of oxygen, for example:


Carbon dioxide (CO_2)


carbonic acid (H_2CO_3)


Calcium bicarbonate $Ca(HCO_3)_2$

1. In the same book /preceding foot-note/, p. 276.



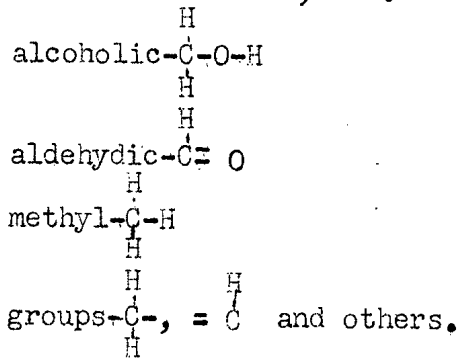
Calcium carbonate ($CaCO_3$)

The major part of carbon on the Earth is contained in carbon dioxide and in carbonates.

The second part of substances containing carbon are organic combinations. In a vast majority of cases organic combinations represent complex molecules consisting of chains or rings with two, three and more (attaining up to hundreds) interconnected carbon atoms. Besides that, the latter annex atoms of hydrogen, oxygen, sometines-of nitrogen and sulfur.

Combination of carbon with hydrogen is the most characteristic peculiarity of organic compounds and indicates that the carbon of organic compounds, unlike the oxydized carbon of non-organic substances, is restored.

Characteristic groupings of carbon, which is part of substances most important in the world, are:



It is true that in organic combinations can also be found groupings with connections C-C, C-O or C-O-H, in which connections of carbon with hydrogen are absent. Among those the most important ones are the carboxylic-C-O-H, characteristic for organic acids and the ketonic -C-, characteristic for ketones. However, these groups are contained in the chains with the restored carbon and thus, on the whold, one or another organic combination is a restored combination containing restored carbon.

Thus, the basic and cardinal distinction between inorganic and organic combinations of carbon (not speaking of coal, graphite, diamond which consist of pure carbon) consists in the fact that in the former the carbon is completely oxydized and in the latter it is reduced to some degree or other (i.e., at least partly liberated from connections with oxygen or combined with hydrogen).

Because of that inorganic compounds are poor in chemical energy, are inert and stable in a chemical sense. On the contrary, the organic combinations are rich in accumulations of potential chemical energy, are capable of acting as reagents, are unstable in a chemical sense and, in particular, are readily oxydized and can burn when from their molecules are removed hydrogen atoms and oxygen is added.

As a result of a complete oxydization of organic combinations, inorganic substances are formed: water-as a product of oxydization of hydrogen and carbon dioxide-as a product of oxydization of carbon. When organic substances are oxydized, the accumulations of potential chemical energy are liberated and are usually expressed as heat energy.

Thus organic substances containing carbon and hydrogen are readily broken down, oxydized with formation of inorganic combinations of carbon and hydrogen (CO_2 and H_2O). On the contrary, the inorganic combinations (carbon dioxide and water) can be converted into organic only with expenditure of energy from outside which partly has to be connected and stored as a potential chemical energy of organic combination.

Organic substances serve as a basic material in the organization, in the support of life, in the metabolism of living organisms. Just because of that carbon nutrition and metabolism in living organism are the foundation of their life.

However, various organisms accomplish carbon nutrition in a different manner and in a different manner they create the organic substances which are part of the make-up of their bodies. The fundamental theoretical difference consists in the fact that some organisms can feed on inorganic carbon-containing combinations and form from them organic substances necessary for life. And others cannot form organic substances from inorganic ones and are bound to feed on existing organic substances.

The first group of organisms is called <u>autotrophic</u>, the second-<u>heterotrophic</u>.

Trans. 464
(In full)
By:
A. Antik

Autotrophic organisms must possess the faculty of using some sources of hydrogen and to direct it to reduction of carbon dioxyde. Besides that they must possess a specific apparatus for utilizing energy from outside sources in order to convert it with the help of CO_2 and store it as energy of chemical bonds in organic substances being newly formed.

Several types of bacteria which bring about chemosynthesis and photo-reduction possess such peculiarities.

As sources of hydrogen for reduction of carbon dioxide they utilize ammonia ($N H_3$), hydrogen sulfide (H_2S), molecular hydrogen (H_2), some organic combinations; as sources of energy for the synthesis of organic substances-either the energy of oxydization of a number of inorganic (ammonia, nitrate, ferrous iron, hydrogen sulfide, sulfur), or the energy of oxydization of organic substances, or even the light energy (bacteria which contain bacteriochlorophyll).

However, in the general process of the carbon cycle and of new formation of organic substances, these organisms play a very insignificant role: of too limited a character are also the sources of hydrogen and of energy which they utilize for reduction of CO_2 and for formation of organic substances.

And the basic group of autotrophic plants which primarily synthesize organic substances in enormous quantities, are green plants capable of bringing about photosynthesis.

The source of carbon for them is carbon dioxide gas which is in the air (for land plants) or dissolved in water (for water plants). As a source of hydrogen for reduction of carbon dioxide serves water and as a source of energy-sunlight absorbed by chlorophyll and used for carrying out of chemical work.

Carbon dioxide, and water, and sunlight are distributed everywhere. Due to that, green plants are the predominant group of living organisms on the Earth.

The process of photosynthesis is remarkable in many respects: as a result of this process the green plants create on Earth enormous accumulations of organic substance and chemical energy, necessary for their own life as well as for the life of all the other organisms; just as a result of a photosynthesis crops of agricultural plants are created. Thus the system of agriculture is essentially a system directed to the best use of the photosynthetic faculty of green plants, for the increase of its productivity.

Trans. 464
(In full)
By:
A. Antik

During millions of years, in the process of photosynthesis the green plants enriched the atmosphere of our planet with oxygen and they sustain its contents in the air on a level which makes the existence of living organisms possible.

The most remarkable aspect of photosynthesis consists in its physical and chemical foundations: in the process of photosynthesis under ordinary conditions of surroundings the plants carry out such complex reaction, which so far it is not possible to achieve artificially, even by subjecting CO_2 and H_2O to strongest and most complex stimuli.

* * *

Accepting that the basic product of photosynthesis of plants can be sugar, glucose, we can express the summary results of photosynthesis by the following equation:

$$6CO_2 + 6H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} C_6H_{12}O_6 + 6O_2 - 674 \text{ kkal. } [\text{kilo-calories}]$$

This equation speaks about 6 molecules CO_2 and H_2O which in the process of photosynthesis under the influence of light absorbed by chlorophyll enter reactions which lead to formation of one molecule of sugar, to liberation of six molecules of free oxygen and as a result of this in the gram-molecule of sugar are stored 674 kilo-calories of energy, due to conversion of sunlight into energy of chemical connections.

However, giving a quite general notion of initial products and final results of photosynthesis, this equation does not reveal many important aspects of the chemistry and mechanism of this process.

Thus on the basis of this equation it is not possible to draw a conclusion on the form of participation of chlorophyll in the photosynthesis and on the nature of photochemical reactions of photosynthesis. It does not say either what the nature and the subsequent stages are of interrelation between carbon dioxide and water, it does not reveal the formation processes of final products-carbons and oxygen. Neither does this equation explain the nature of conversion of light energy into chemical energy.

So far in the majority of cases it is not possible to give definitive answers to all these questions though intensive research is conducted in this field. But many important particulars of the process

of photosynthesis are now adequately disclosed. We shall try to expose some of them.

One of the most important discoveries for the understanding of the "mechanism" of photosynthesis was made by Russian scientists A. P. Vinogradov and R. V. Teis.

In studying the isotope content of oxygen which is liberated in the process of photosynthesis, A. P. Vinogradov established that it corresponds with isotope content of oxygen of water and not of carbon dioxide. Thus it can be assumed that during photosynthesis, the oxygen is liberated not from carbonic acid but from water. These works are of great significance because they indicate that one of the first steps of the photosynthesis is decomposition of water which leads to liberation of oxygen and to the use of ~~oxygen~~ H for reduction of CO_2.

These conclusions were confirmed by other no less important experiments.

Chlorophyll is concentrated in cells of photosynthesizing plant organs in specific "grains"--chloroplasts. If leaves of green plants are exposed to light in the presence of carbon dioxide, then in chloroplasts starch is formed--one of the basic products of photosynthesis. Chloroplasts are those intra-cellular formations in which the basic reactions of the process of photosynthesis take place.

From leaves of some plants the chloroplasts are isolated quite readily and they can be obtained as an aqueous suspension. It is quite natural that a question arose whether it is possible to force the chloroplasts in such suspensions to reduce in the light the carbon dioxide and to synthesize organic substances. However, experiments demonstrated that under these conditions the carbon dioxide is not assimilated. But if some oxidizers more active than CO_2 (for example ferric oxide, quinones, etc.) are added to the suspension then in this case the chloroplasts, as the photosynthesizing leaf, liberate in the light free oxygen. Tests with adding water containing heavy isotope of oxygen (O^{18}) into the suspension, showed that in this case oxygen liberated in the light is of aqueous origin. In the same experiments another important detail was established: together with liberation of oxygen in suspensions of chloroplasts in the light, there takes place also a restoration of active oxydizers added to them (for example, ferric oxide into ferrous iron, quinone into hydroquinone, etc.).

Trans. 464
(In full)
By:
A. Antik

This research is extremely important because it demonstrates that the action of light and the photochemical reaction of photosynthesis are connected with decomposition of water while the oxygen is liberated in a free state and the hydrogen is used for restoration of some oxydizers.

In the given case it can be said that the water itself is oxydized and it reduces with hydrogen other substances which in the end transmit the hydrogen also to reduction of carbon dioxide.

Approximately the schematic meaning of these conclusions can be expressed as follows:

$2H_2O$
light and chlorophyll

4(H) .... O_2

Transmission of hydrogen to restoration of carbon oxide or other oxydizers.

Liberation of oxygen in a free state.

As to reduction of carbon dioxide--there is a series of data indicating that transmission of hydrogen to it is carried out without participation of light as a result of reactions which take place with the participation of specific regulators of biochemical processes in living organisms--enzymes.

This is proved, in particular, by E. A. Boichenko's experiments. In isolating, with special precautions, chloroplasts from plant leaves she extablished that in an atmosphere of hydrogen they are capable to reduce even in darkness the carbon dioxide with formation of organic substances. But this takes place only when chloroplasts are in an atmosphere of free hydrogen. In the given case this hydrogen takes place of hydrogen which in the usual photosynthesis is mobilized from water with the help of light.

Let us remember also, that many chemosynthesizing organisms (for example, nitrifying bacteria, iron bacteria) reduce carbon dioxide at the expense of energy of oxidation, also without participation of light. Therefore, we can consider it trustworthy that in the same manner, i. e., with the help of enzymes and without participation of light, the transmission of hydrogen to carbon oxide is accomplished in the process of photosynthesis as well. A. A. Krasnovskii's as well as E. A. Boichenko's experiments provide a foundation for considering that in the transmission of hydrogen to reduction of carbon dioxide participate enzymes--transmitters of hydrogen--"dehydrase".

Important work for the understanding of the nature of photosynthesis was carried out during recent years by A. A. Krasnovskii. He established that chlorophyll oxidizes in the light the ascorbic acid taking hydrogen away from it, at the same time the chlorophyll is being restored. Then in darkness the chlorophyll can again be oxidized transmitting the hydrogen either back to the oxidized form of ascorbic acid or to other oxidizers.

All this permits the assumption that photosynthesis represents a process which consists of a series of successive oxidation-reduction reactions. Some substances are reduced by taking upon them hydrogen and oxidizing others and then they transmit hydrogen to other oxidizers restoring them and becoming oxidized themselves until some transmitter transmits the hydrogen to CO_2.

At a certain stage chlorophyll serves as such an acceptor of hydrogen and it carries out the reaction of oxidization--reduction with the participation of sun energy, connects and transmits it together with the transmittable hydrogen for reduction of CO_2.

In order to have a more complete notion of the general character of the process of photosynthesis, it is necessary to point out the following circumstances as well. It has been established by studies with the radio-active isotone of carbon C^{14}, that carbon dioxide enters the cycle of photosynthetic conversions not in a free state but joining organic substances which are already in the cells and forming carboxylic groups characteristic for organic acids. Schematically this reaction can be expressed as follows: [1]. $RH - CO_2 \rightarrow RCOOH$. Thus in the process of photosynthesis not directly carbon dioxide is reduced but the carboxalic group.

Probably the same can be said also about water: in the reaction of photosynthesis it enters not in a free form but as a complex combination. Liberation of oxygen during photosynthesis takes place probably through stages of formation of some peroxide (as it was supposed already by A. N. Bakh).

To sum up, we can present photosynthesis by a scheme (see p. 43) in which this process is expressed as a process of restoration of carbon dioxide with hydrogen of water with a transmission of hydrogen from water to CO_2 through a series of transmitters which participate in reversible oxidizing--reduction reactions, among them as one of the links takes place the reversible oxidation-reduction of chlorophyll with the help of light. In the given scheme indicated in parentheses are complex combination, with symbol Chl-oxidized and symbol HChl-reduced form of chlorophyll, with

1. By symbol RH is presented the organic substance containing hydrogen.

symbol Z-intermediate transmitter of hydrogen of which there might be several.

Each pair of adjoining arched arrows indicates the direction of the oxidizing-reduction reaction in which one component is being oxidized transmitting the hydrogen to another component, and the other one, in turn, receives the hydrogen for itself and becomes reduced.

Many details of the process exposed in this scheme are not yet clarified. However, it presents correctly the nature of photosynthesis emphasizing that it includes the following links.

1. Process of photo-oxidation of water with liberation of oxygen and primary mobilization fo hydrogen, probably in chlorophyll.

2. Transmission of hydrogen from water to CO_2 in a series of oxydizing-reduction reactions.

3. Primary fixation and reduction of CO_2 with formation of intermediate and final products of photosynthesis.

* * *

It is accepted in the given scheme, that for reduction of one molecule of carbon dioxide, four molecules of water are needed. This circumstance is not accidental and its meaning consists in the following:

As a result of photosynthesis, organic substances are formed with a level of reduction of carbons, i. e., such that for each atom of hydrogen there are on the average two atoms of hydrogen and one atom of oxygen. At the same time the reaction between carbon dioxide and the water has to take place according to the equation: [1]

$$CO_2 + 2H_2O^* \rightarrow (CH_2O) + O_2^* + H_2O$$

In other words, for reduction of one molecule CO_2, at least two molecules of water have to undergo photooxidation and four atoms of hydrogen have to be mobilized, i.e., four H-O bonds to be broken.

From the number of the indicated four atoms of hydrogen, two have to be used for taking away the molecule CO_2 of the oxygen atom with a secondary formation of water, and two--for a direct reduction of carbon or carboxilic group by way of addition to carbon. It is more likely

1. Symbol O^* represents oxygen which is or was in water.

that the four atoms of hydrogen necessary for it, are mobilized from four molecules of water, and the four remaining after it--radicals (O-H) form peroxide (conditionally indicated by H_2O_2) from which is already liberated oxygen with a partial regeneration of water:

$$2(H_2O_2) \rightarrow 2H_2O + O_2$$

Thus the most likely equation of photosynthesis can be expressed as:

$$CO_2 + 4H_2O^* \rightarrow (CH_2O) + H_2O + 2H_2O^* + O_2^*$$

This found a reflection in the given scheme of photosynthesis. From it we see also the complexity of the restoration process of carbon dioxide and the carboxylic group: for that each molecule has to be supplied at once or one after another with at least four atoms of hydrogen. It follows from here that the reduction process of CO_2 to the level of carbon reduction has to be complex and many-staged. And it has also to be remembered, that besides the CO_2 reduction itself, in the process of photosynthesis the CO_2 molecules have to be added to each other in succession, in order to create a carbon chain of organic substance.

* * *

Having analyzed these problems we approach the evaluation of the most important, most complex and, unfortunately, least studied problem of the process of photosynthesis, we approach its principal "secret".

We have in mind the problem of the nature of photochemical reaction of photosynthesis, of the nature of assimilation, combination or as K. A. Timiriazev used to say, "storing for the future" of sunlight energy by plants.

According to present-day notions the radiation of light is carried out in certain particles--quanta. The energy value of light quanta depends on the length of the wave: it is inversely proportional to the length of the wave (λ)[1] Thus the energy of a quantum of red light (λ = 660mμ) is one and a half times less than the energy of a quantum of blue light (λ = 440mμ).

In simple systems the photochemical reactions are carried out in such a manner that each reacting molecule absorbs one quantum of energy.

1. The length of the light wave is expressed in millimicrons (mμ). 1 mμ = 1/1,000,000 of a millimeter.

At the same time a gram-molecule of the reacting substance consisting of 6×10^{23} molecules, absorbs 42 kilo-calories of energy if the process takes place under the action of red rays and time and a half more, i. e., 63 kilo-calories, if the process is under the action of blue rays.

Subsequent absorption of two or several quanta of light with summarizing of their action in simple systems (ordinary solutions, suspensions) is hardly probable.

However, the study of energy characteristics of photosynthesis indicates that in the given case we are dealing with a more complex process.

As we saw, in order to reduce one molecule CO_2 it is necessary to liberate from the water four atoms of hydrogen. For this purpose four O-H bonds have to be broken.

The O-H bonds between hydrogen and oxygen in water molecules are very strong (110 kilo-calories) and for the rupture of one O-H bond should be spent the energy of at least three quanta of red light which is absorbed best by chlorophyll and is the most active for photosynthesis.

However, a more detailed examination of the problem indicates that not a simple but a very complex photochemical process takes place during photosynthesis. Many works on determination of the so-called quantum yield or quantum consumption of photosynthesis give us the right to conclude that under the most favorable conditions in the process of photosynthesis for the break of one O-H connection are spent not three quanta as would follow from the above mentioned, but only two or even one quantum.

Apparently the water in the living leaf which enters the cycle of photosynthetic conversions, undergoes already prior to that some influences as a result of which the strength of O-H connections is greatly weakened and in the end they can be broken with application of energy of only one or two quanta.

Such weakening of O-H bonds can be reached by various ways: at the expense of entering of H_2O molecules into some complex combinations, at the expense of concentration of such complex combinations in places with very high oxidizing potentials, at the expense of a temporary effect on this connection of the energy which is already inside the system.

In reality probably the one and the other conditions are taking place. And in the end the plants readily accomplish this reaction which it is not yet possible to accomplish in simple systems in non-living media. The study of conditions providing the possibility of a photochemical decomposition of water, is one of the most important tasks in the works on clarification of the nature of the process of photosynthesis. When this problem is solved we shall probably understand the mechanism of such photochemical processes where in one reaction, in the breaking of one chemical connection is summed up the action of several quanta of energy and where this energy is not lost in the reverse course of reaction, but is preserved in the system.

Having studied the photochemical stage of photosynthesis we shall also understand the mechanism of such preparatory reactions as a result of which strong connections of chemically stable substances become weaker and can break under the action of a considerably smaller amount of energy entering from outside than under usual conditions.

All this opens up enormous practical possibilities for us.

* * *

No less important results can be obtained in connection with the study of peculiarities of transmission of hydrogen for reduction of CO_2 and of chemism of the formation process of products of photosynthesis.

We already spoke about the fact that water is a relatively inactive reducer. Therefore, the oxidation of water with removal of hydrogen can be realized only in the presence of very active oxidizers and with the action of additional energy.

At the same time carbon dioxide and the carboxylic group are very inert oxidizers. Their reduction can be realized only in the presence of very active reducers and also with the action of additional energy.

Thus assuming that the transition of hydrogen from water to carbon dioxide consists of several stages, we have to consider that hydrogen passes gradually from a zone with a high oxidizing potential and a low energy level to zones of constantly higher reduction potential and high energy level.

Such tendency of many-staged process presents one of the most surprising aspects of photosynthesis. It is interesting because it differs drastically from the usual tendency in the course of chemical processes: at a simultaneous formation in the process of photosynthesis of active

oxidizers (up to free oxygen) and active reducers (up to transmitters of hydrogen which are able to restore such an inert oxidizer as carbon dioxide or the carboxylic group), they are protected against a reaction among them and against losses of the energy which accumulates in reduced products.

The process of transmission of hydrogen and energy to carbon dioxide is completed by the fact, that in the final products of photosynthesis are connected and accumulated about 112 kilo-calories of energy per each gram-atom of carbon or per one gram-molecule of reduced carbon dioxide. This energy equals that of three quanta of red light.

For realization of such a complex process very specific conditions are needed. Among them of great importance is the presence of non-homogeneous structure of the photosynthetic apparatus of plants with a simultaneous existence within the microscopic space of the chloroplasts of zones with drastically different energy conditions and oxidizing-reduction potentials.

An important condition is the presence of a strict specificity of the course of transmission of hydrogen from water to CO_2 and of a protection system of hydrogen being converted by oxidization with any oxidizers more active than carbon dioxide or the carboxylic group.

In connection with this it is interesting to point out the results of experiments conducted at the Institute of Plant Physiology imeni K. A. Timiriazev, Academy of Sciences USSR, with the participation of the author of the present article. It was known that exposure to light of plant leaves increases considerably the reduction of nitrates contained in them, i.e., of nitrites which serve as a basic source of nitrogenous plant nutrition and in which nitrogen is fully oxidized being connected only with oxygen (for example, in potassium nitrate--KNO_3). It would be assumed that for the reduction of nitrogen of nitrates such hydrogen is partly directed which is mobilized as a result of photo-oxidation of water and which is used in its major part for reduction of CO_2. At the same time it was considered probable that the removal of carbon dioxide from the air surrounding the leaf, can increase the reduction of nitrates: They would not have a competitor in obtaining of hydrogen from intermediate transmitters.

However, despite this assumption it appeared that reduction of nitrates in leaves proceeds most intensely when light and carbon dioxide are present.

It is clear from the above that hydrogen transmittable /being transmitted/ from water to CO_2 is well protected from being intercepted on the way by other oxidizers even more active than carbon dioxide, and only having reduced CO_2 and having become part of the content of the organic substance, can this hydrogen play an active role in the subsequent restoration of other oxidizers.

But if no CO_2, basic acceptor of hydrogen, enters the leaf, then the process of photo-decomposition of water also stops and stops the process of primary mobilization of hydrogen.

Thus the process of photosynthesis in the living leaf consists of strictly conjugated reactions. If but a single link falls out of the chain of these reactions, the entire process as a whole stops.

This peculiarity of photosynthesis from the biological point of view is quite understandable: the process of photosynthesis exists for assimilation by green plants of carbon in CO_2, by way of its reduction. If the hydrogen, which is mobilized for it, could be intercepted by other oxidizers many of which are more active than CO_2 and are present in large amounts in the plants, then it would be extremely difficult to perform the basic functions and tasks of photosynthesis.

Thus the specificity and strictly outlined course of the ways of hydrogen transmission just for reduction of CO_2 and not of other restorers, are one more remarkable characteristic of photosynthesis.

Many details of basic reactions of photosynthesis, details of structure and organization of the photosynthetic plant apparatus, are not yet clear and known to us. But nevertheless we now already have a notion of the basic principles of realization of this process. Already we can see how important and essential are the regularities which are at the basis of this process. Already now we can imagine how limitlessly will be enriched our possibilities in carrying out industrial syntheses of various organic substances and in utilization for industrial purposes of sunlight energy, when all the details of the process of photosynthesis will be completely known.

The importance of study of this field will be even more understandable if we shall take into consideration the following circumstance.

Up to the present time there still exists a widely spread opinion that carbons are the only direct product of plant photosynthesis. And it

is also considered that the distinction of plants according to metabolism and to inherited properties is conditioned by distinctions in secondary conversions of basic products of photosynthesis--carbons.

However, already at the end of the last century, a pupil of K. A. Timiriazev, Professor V. V. Sapozhnikov conducted experiments which led him to believe, that in the process of plant photosynthesis, albumens are formed besides carbons.

This point of view was not widely accepted for a long time. Only in recent times and, in particular, in the photosynthesis laboratory of the Institute for Plant Physiology at the Academy of Sciences, USSR, were quite convincing data obtained which confirmed V. V. Sapozhnikov's point of view. It became clear in these works that the quantitative relations between the albumens and carbons being formed change greatly depending on the age of plants and leaves, on nutritional conditions, on intensity and quality of light, on types and species of plants.

All this proves, that the process of photosynthesis is not a purely physico-chemical process. It is first of all a physiological process, a process the realization of which itself and the work tendency of which are closely connected with the specification of organization of plants as living organisms with their life functions and physiological state.

On the other hand, the process of photosynthesis itself, which changes quantitatively and qualitatively, determine decisively the physiological state, direction and the results of life processes of plants.

Thus, for example, in the light of various quality and intensiveness, the plants develop with various speed, form in a different manner various organs (bulbs, tubers, heads /of cabbage, etc/, fruit-bearing organs etc.) and acquire various morphological peculiarities.

Apparently it is conditioned to a considerable degree by the fact that under various conditions the photosynthetic plant apparatus does not work uniformly, not only quantitatively, but qualitatively as well, thus creating a varying tendency in metabolism of plants.

And metabolism is the foundation of life processes, and if its tendency varies, then the direction of the basic life processes varies as well.

All this opens new possibilities for work on organization of crops, their quality and inherited peculiarities of plants.

Trans. 464
(In full)
By:
A. Antik

Works carried out at the Institute of Biochemistry at the Academy of Sciences, USSR, by N. M. Sisakian, demonstrated, that chloroplasts of green plants are the concentration place of numerous and most important enzymes.

This serves as an additional and indisputable proof of a great biochemical activity of chloroplasts and of their faculty to carry out a complex and manifold work in the process of photosynthesis.

* * *

The characteristics of the process of photosynthesis which distinguish it in many ways from other processes, known to us, make the interest quite understandable which is shown toward it by specialists of various branches. This interest is understandable because the knowledge of the nature of the photosynthetic process promises new and enormous possibilities in most diversified fields of our activity: in the field of rational direction of photosynthesis in plants and, therefore, direction of their yields and improvement of their quality; and in the field of development of new principles and methods of industrial utilization of sun radiation--this inexhaustible source of energy; and in the field of manufacturing of various artificial syntheses of valuable organic substances with the use of carbon dioxide or carbonates (chalk, limestones) as a raw material and of sunlight as a source of energy.

Anon.

Trans. 465
(In full)
By:
A. Antik

Novyi vklad v sovetskuiu biologicheskuiu nauku.

/A new contribution to Soviet biological science/.

Biokhimiia v. 15, no. 4, pp. 297-298, July-August 1950. 385 B523

(In Russian)

A NEW CONTRIBUTION TO SOVIET BIOLOGICAL SCIENCE

It is inherent to the science of the Soviet country to fight against all the varieties of idealism and metaphysics, to review courageously the dogmas when they become outlived, when from a support of science they become of its further development.

One of such dogmas in biological sciences, one which dominated almost a hundred years in the field of science of the cell and tissues of organism, was the metaphysical and idealistic "Virkhovianskaia" /?, by Virkhov?/ dogma of Virkhov, which introduced as an incontestable immovable statement--that of: "each cell from a cell." This theory that a cell can be formed only from a similar cell as a result of the latter's division, is the basis of the entire system of opinions which in pathology found its expression in the famous "Virkhovskaia" /?/ /by Virkhov?/ conception of the "cellular pathology"; on the other hand, notions of continuous cellular chains interlocked most directly with the Veismann-Morgan notions of the continuity of the germ plasma.

It is quite obvious that the notion of the living world, which follows from these opinions, as of a system of uninterrupted cell chains, is in an unreconcilable contradiction with the advanced progressive dialectical-materialistic understanding of regularities in the development of the living world which is represented by Michurin's biology.

Virkhov's /?/ postulate on the origin of cell necessarily from other similar cells by way of division, limits and constricts the possibilities and conditions of changeability of cell types, makes the correct understanding of development processes in the living nature more difficult. Uncritically accepted by wide circles of biologists and medical men, Virkhov's /?/theory inhibited the development of a series of most important problems which are in a direct relation to the study of the essence of life.

In particular in the field of biochemistry, the idealistic tendencies of the /Virkhov's theory?/ created a breach between the study of processes of metabolism which characterize a living substance and morphology which rejects the possibility of presence of life outside the cells.

New perspectives in the field of effective development of a series of biological problems of radical importance are open by results of many years of research by O.B. Lepeshinskaia and her co-workers, in connection with problems of non-cellular forms of life and of cell origin from a living substance. The conference summoned in May by the Division of biological sciences at the Academy of Sciences USSR with participation of representatives of the Academy of Medical Sciences USSR and a number of other scientific organizations, was dedicated to listening and discussing of these works.

It follows from O.B. Lepeshinskaia's research that a new formation of cells is possible not /? only ?/ by division of the previously existing cells but from a living substance which has no cellular structure and, as a result of a development of this substance which takes place in a certain manner. Origination of cells from a structureless living substance was demonstrated on chicken egg yolks (or embryos) on hydra tissues, fish eggs which were pulverized until they lost completely the cellular structure; finally, the structure formation of the cellular type was discovered in the structureless albumen of the chicken egg.

These works lead out of the state of stagnation and open wide possibilities for further development in a series of fields of biological sciences. Though the significance of new notions is particularly apparent and direct for cytology, histology, embriology and other morphological disciplines, not less important new lines of research are being opened also for biochemistry; there, very distinctly arise problems for a profound study of metabolism in non-cellular forms of existence of a living substance and as further goals--problems for study of processes of the synthesis of a living substance.

Engels gave a classical determination of life as a form of existence of albumen bodies"--an essential moment of which is a constant metabolic exchange with the surrounding exterior nature and with the cessation of this metabolic exchange life also stops" *--this definition emphasizes forcefully the significance of metabolic exchange as a paramount obligatory attribute of living substance. In the light of this statement, becomes clear the significance which will be acquired by the study of metabolism processes taking place not only in cellular and tissue formations which served formerly as objects of biochemical studies, but in non-cellular forms of a living substance as well. Forward moves the problem of foremost importance--of criteria of the "living

* F. Engels, Dialectics of Nature, Gospolitizdat, 1948, p.246

substance" themselves, and here no doubt the decisive word will belong to results of biochemical studies. What processes are at the basis of existence of a structureless living substance, what are the changes in the character of metabolism processes which have to take place during the transition from a structureless state to a cellular organization--all these are problems which, beside the direct biochemical significance, are linked most closely with fundamental problems of morphological order as well, touching directly upon the entire field of inter-relations among chemism and the fine structure of living formations.

The Soviet biochemistry is already in possession of a series of achievements which acquire a special significance in the light of the outlining new problems of research. The dependence of the tendency of enzyme processes on the participation of factors of a structural order, [2] the already realized reconstruction from products of deep segmentation [?] of albumen of initial albumen molecules which possess antigenic and catalytic (fermentative) properties characteristic for biological formations, [3] disclosure of deep conversions of amino acids and albumens in the structureless blood plasma,--all this acquires a new importance as supporting points in the study of non-cellular life forms.

The resolution accepted by the conference speaks of necessity of a universal spreading of research work in the study field of development of cells and non-cellular forms of life and it suggests to biologists of various special fields to engage directly into development of this progressive field of the science of life and at the same time to carry on an irreconcilable fight against all the antiquated notions of Virkhov the "Virkhovianstvo" and other idealistic currents in biology.

It is quite obvious that in realization of these indications an essential role is to be played by our biochemical science. Any success on newly traced roads will be important not only for solving of fundamental problems of theoretical order, but will also arm our practice with new means for the control of diseases, for the fight for reorganization of the living Nature, on the basis of Michurin's principles for the benefit of our great country and of humanity.

Trans. 466
(In full)
By:
A. Antik

466

Marland, A. G.

Zakonomernosti vasvitiia koronchatoi rzhavchiny ovsa v zavisimosti ot meteorologicheskikh faktorov

[The effect of meteorological conditions on the development of crown rust of oats].

Itogi N-I Rabot Vses. Inst. Zashchity Rastenii. Part 1, 168-170, 1937
423.92 L541 1936 pt. 1

[In Russian]

The effect of meteorological conditions upon the development of crown rust of oats Puccinia coronifera Kleb.

The present work is a continuation of research lf 1935. Following problems were the subject of the 1936 study:

1. Peculiarities in rust development on buckthorn.

2. Peculiarities in rust development on oats: a) Speed of infection of oats with aecidia-and uredo-spores; b) effect of temperature and air humidity on the duration of the incubation period when oats are infected (inoculated) with aecidia-and uredo-spores during the germination and the flowering stages.

Peculiarities in rust development on buckthorn

In spite of numberous attempts to speed up the germination of teleuto-spores which hibernated in the plot, by treating them with a 1% solution of citric acid, their germination took place only on 5-7V (May 5-7), which coincided with their germination in Nature.

In the green house 20 experiments were conducted on artificial inoculation of buckthorn leaves with basidio-spores. Positive results were obtained only in four tests; they are presented in table I.

Table I (p.168)

Artificial inoculation of buckthorn with basidio-spores (P. coronifera Kleb.)

Date of inoculation	Appearance of spermogonia	Duration of the incubation period in days	Appearance of aecidia-spores	Total duration of the incubation period in days	Temperature during the incubation period (inoculation appearance of aecidia)		
					Minimum	Mean	Maximum
9/V	20/V	11	3/VI	25	7,8	12,6	17,8
11/V	20/V	9	3/VI	23	8,4	12,6	17,8
14/V	24/V	10	7/VI	24	8,3	13,4	18,8
14/V	24/V	10	9/VI	26	8,7	13,8	19.1

It is seen from table I, that the appearance of the aecidia-spores was extremely slowed down. It can be apparently explained by insufficiently favorable conditions for the development of the buckthorn, which was cultivated in Vagner containers in the greenhouse.

Peculiarities in rust development on oats.

The dynamics in the development of crown rust of oats depend basically on meteorological factors. It is important for a prognosis, to establish temperatures which are critical for infection of oats, as well as time needed for spore germination and infection of plants in relation to temperatures. It was established in 1935 that uredo-spores of P. coronifera germinate within a wide temperature range from 3 to 35° (1) and the aecidio-spores - within the 5-31° range (V.D. Kuprianova's data). Temperatures close to the critical ones inhibit the growth of shoots as well as decrease the percentage of spore germination.

A. Speed of infection of oats with aecidia-and uredo-spores. Our experiments for establishing the speed of infection of oats were conducted five times on sprouts of the "Zolotoi dozhd" ("Golden rain") oats. Aecidia- and uredo-spores (of 4-5 day age) were introduced in a drop of river water into sprouts which were in the second leaf stage. The border line of the infection drop which was introduced was marked with India ink. Then the plant was placed in a suitable chambers of a pölyincubator and held there for various time periods: 2, 3, 4, 6, 8, 10 and 24 hours. At the end of the mentioned time intervals, the respective plant groups were taken out, the infected parts of the leaves were cut off and placed into Carnoy's fixing solution.

(1) "Ttogi nauchno-issled rabot Vses.Inst.Zashchity Rastenii 2a 1935g." p.65.

Microscopic examinations of the cuts of the infected leaves gave following results presented in table 2. There were no differences in this respect between the aecidia-and uredo-spores, therefore, the results of tests with both types of spores are compiled together.

Table 2 (p.169)

Speed of penetration of shoots into the leaf tissue at various temperatures (aecidia-and uredo-spores) (1)

Duration of observation	Temperature 11°(2)	12°	17,5--18°	20--22,8°	28--30°	33°
2 Hours	—	—	—	—	—	—
3 "	—	—	—	≠	—	—
4 "	—	—	—	≠	≠	—
5 "	≠	≠	≠	≠	≠	—
6 "	≠	≠	≠	≠	≠	—
24 "	≠	≠	≠	≠	≠	—

It is seen from table 2, that the inoculation of oats sprouts, i.e. penetration of the shoot into the leaf tissue takes place within the II-30° temperature range after 5 hours. At the optimum temperature (20-22.8°) the penetration takes place even after 3 hours.

Table 3 (p.169)

Speed of infection of oats sprouts at various temperatures (aecidia-and uredo-spores)

Duration of observation	Temperature 4,5-5,5°	6°	7-12°	14-16°	17-27°	30-31,4°	33°
3 Hours	—	—	—	—	—	—	—
4 "	—	—	—	—	—	—	—
5 "	—	—	—	—	≠	—	—
6 "	—	—	—	≠	≠	—	—
8 "	—	—	—	≠	≠	—	—
10 "	—	—	≠	≠	≠	—	—
12 "	—	—	≠	≠	≠	≠	—
18 "	—	—	≠	≠	≠	≠	—
20 "	—	≠	≠	≠	≠	≠	—
24 "	≠	≠	≠	≠	≠	≠	—

(1) Symbol ≠ indicates the presence of shoots penetrated into tissues, symbol — indicates their absence.

(2) Due to technical causes temperature below 11° was not tested.

In 1936 the methods of this experiment were changed. At the end of the indicated time intervals the plants were removed from chambers of the poly-incubator, the water drops with the spores were dried out with the help of filter paper and then the plants were transferred into t hot-house where they were held until the appearance of pustules.

The results of the experiments are compiled in table 3.

It is seen from table 3 that the infection of oats occurs within the 14-27° temperature range after 5-6 hours. At temperatures below 14 and above 27° the infection takes place after a longer time interval (10-12 and more hours).

Table 4. (p.170)

Duration of the incubation period at natural fluctuations of temperature (during the sprouting stage)

Duration of the incubation period	Temperature during the incubation period		
	Minimum	Mean	Maximum
7 days	14,6	19,3	25,1
8 "	13,5	18,7	25,4
9 "	11,5	15,7	21,8
10 "	9,4	13,0	18,0
11 "	8,0	10,7	18,9
12 "	7,5	10,2	18,4
13 "	7,7	17,7	14,7
14 "	7,2	10,7	14,3

The reported data indicate that completion of infection of oats with aecidia-and uredo-spores (i.e. such which results in appearance of rust) requires longer time than it is necessary for only penetration of shoots i into the tissue. Thus penetration of shoots is possible already after three hours and a complete infection at similar temperatures, one which produces later on pustules, is possible only after 5-6 hours. There are even more differences between temperature limits within the range of which spore germination and complete infection are possible. Thus germination of uredo-spores at an optimum temperature reaches noticeable intensiveness already after 1½ hours and a completion of infection is possible at the same temperature only after 5-6 hours. In order to make a prognosis it is, of course, necessary to be guided only by the speed of infection and to consider as acceptable only the second of the applied methods.

B. Effect of temperature and air humidity on the duration of the incubation period where oats areinoculated with aecidia-and uredo-spores during the germination and the flowering stages. The 1936 experiments were conducted on a wider scale as compared with the 1935 experiments and they confirmed the relation, which we disclosed earlier, between the duration of the incubation period and temperature; this is presented in table 4 which is an abstract of the known data.

More extensive material which we obtained is being statistically organized.

When plants were inoculated with aecidia-and uredo-spores at various development stages (sprouting, flowering), in some cases a slight difference in duration of the incubation period was observed. Thus in the case of inoculation with aecidia-spores during the sprouting stage, the incubation period was 6 days, and during the flowering stage 6-9 days. With the 7 day incubation period in sprouts corresponded a 6-8 day period during flower stage. Similar picture was observed with uredo-spore inoculation. With aecidia- and uredo-spore inoculation during the samp plant development stage, there was no difference in duration of incubation period in relation to the type of spores.

Trans. 467
(In full)
By:
A. Antik

Boichenko, E. A.

Aktivirovanie molekuliarnogo vodoroda gidrogenazoi khloroplastov

(Activation of molecular hydrogen by the hydrogenase of chloroplasts).

Biokhimiia, vol. 13, no. 3, pp: 219-224
May/June 1948 385 B523

(In Russian)

ACTIVATION OF MOLECULAR HYDROGEN BY THE HYDROGENASE OF CHLOROPLASTS

by

E. A. Boichenko

Soon after Bakh, Palladin and other authors discovered oxidation-reduction (reducing) in plant cells, a search began for a connection between their action and the most powerful oxidation-reduction (reducing) process of the bio-sphere--photosynthesis. One of the first works in this direction were Liubimchenko's experiments with anti-oxidase in which, even before Vil'shtetter introduced the conception of "assimilating enzyme", photosynthesis was considered as being based "on a series of subsequent oxidations and reductions of a definite enzyme system."(1) Further works on differentiation of light and dark reactions of the photosynthesis, on bacterial photosynthesis, on photoreduction in green plants and, finally, on dark reduction (in darkness?) of carbonic acid -- confirmed Bakh and Liubimchenko's ideas on photosynthesis as an oxidation-reduction (reduction) process. But even though the participation of enzymes in reactions of photosynthesis does not arouse at the present time any doubt and is accepted in all the schemes of the assumed course of this process, nevertheless concrete data about what actual enzymes are connected with the reduction of carbonic acid by green plants, were almost absent until recent time. Gaffron discovered reduction in darkness of carbonic acid in green algae and showed that it can take place by an oxidation of the molecular hydrogen as well as by oxidation of other (hydrogen) "donators" of the glucose type. On the basis of these experiments Gaffron assumed the participation of the enzyme of hydrogenase which activates molecular hydrogen (2.3) in the assimilation of carbonic acid by green plants. A problem arose to isolate from the cell a corresponding enzyme system in order to recreate outside the organism a reaction which is at the basis of all the organic substances.

For this purpose the author of this article used preparations of chloroplasts from leaves of various green plants. The most convenient object appeared to be white clover; basically the subsequent work was conducted with it.

Preparations of chloroplasts were obtained by method described in the preceding work (4). Especially thorough was the washing off of sugar traces (until samples gave negative test with a-napthol); because if carbohydrates were present in the preparation, an absorption of hydrogen could take place instead of its secretion. In each test 25 mg. of chloroplasts were taken. Measurements were carried out in differential manometers of Barkroff. They were altered in such a manner as to make it convenient to let through a flow of gas or to introduce additional gas during the test. For that purpose an additional stopcock was fixed on the side to the small vessels. Through the manometer was passed a flow of hydrogen which passed first through permanganate and alkaline pyrogallol. From time to time the containers were tested with alkaline pyrogallol for absence of oxygen. When all the air was let out of the containers, the films with chloroplasts were lowered into the solution and measurements took place of gas-exchange followed by calculations of the coefficients Q (number of u l /microliters/ of gas absorbed or secreted, at a ratio of 1 mg. of dry weight per one hour). In tests with reduction of methylene blue and oxygen, there was alkali in the central small container for elimination of carbonic acid; and in tests with carbonic acid its determination was carried out at the end of the experiment by connecting the small container with alkali to the side stopcock (2). The tests were conducted at a 25° /centigrade/ temperature.

Tests for hydrogenase in one or another organism or preparation are conducted most frequently on reduction of methylene blue in atmosphere of hydrogen. While the control with boiled enzyme does not discolor the dye, with hydrogenase the usual transition time to leuco-color is 5-15 minutes. A flow of hydrogen was let through the containers of the manometer. The time for discoloring of the methylene blue (1:50 000) with hydrogen was determined for 25 mg. of the preparation in 1 ml. of phosphate buffer with pH = 7, because previous experiments indicated that this pH is the best for reduction.

The hydrogenase appeared to be widely spread among green plants. It could be disclosed with more or less work in all the species which we examined with more or less work, depending on degree of preservation of chloroplasts being isolated. Plants, the chloroplasts of which can stand well the isolation, like white cloter, Chenopodium, can serve as a good material for obtaining of hydrogenase. On the contrary, easy breaking up into "grans" as in an aster, decreases the activity of the preparation.

Trans. 467
(In Full)
By:
A. Antik

Table 1(P. 220)

Discovery of hydrogenase in plants of various families

Species	Family	Time for restoration of methylene Blue, in min.
Aspidistra elatior	Liliaceae.....	26
Trifolium repens	Leguminosae.....	15
Chenopodium album	Chenopodiaceae.....	16
Callistephus chinensis	Compositae.....	43

Participation of hydrogenase in reduction of oxygen by molecular hydrogen was observed by many authors in various objects.

And in almost all the cases the high percent of oxygen in combination with hydrogen led to a rapid inactivation of the enzyme, but for individual hydrogenases this percent was very different. Thus one absorbed well the hydrogen at 10% of oxygen, others - at 5%, in Azotobacter at 2.5 - 0.5% while the hydrogenase of Clostridium could work only with complete lack of oxygen. According to Gaffrou's data, the work of hydrogenase of green alga was suppressed by 2% of oxygen in the media.

The containers of the manometers were filled with hydrogen as in the preceding experiment. After that a certain percent of oxygen was introduced by adding drops of hydrogen peroxide into the solution of permanganate and sulfuric acid. Readings of the manometer were taken before and after the introduction of oxygen. Then every 5 minutes gas consumption was recorded. When it was finished the remaining amount of oxygen was determined by absorption with alkaline pyrogallol. From the total amount of absorbed gas, oxygen was subtracted and the ratio of absorbed H_2O_2 was determined. It was close to one unit in all the tests, being usually slightly higher.

Table 2 (P. 220)

Restoration of oxygen by molecular hydrogen 25 mg chloroplasts; 25°

% O_2	Absorbed O_2 in µl	Absorbed H_2 in µl	Ratio H_2/O_2
0,6	75	88	1,17
0,6	75	88	1,17
1,3	163	175	1,06
1,4	175	200	1,14
2,0	250	0	--
2,3	288	138	0,48

Restoration of oxygen proceeds completely only if its content in combination with hydrogen is about 0.5 - 1.5%. With a higher content of O_2 an inactivation of enzyme takes place - first the absorption of hydrogen decreases which slows down the absorption of oxygen and, finally, when the oxygen is about 10%, the preparations hardly absorb any gas at all. After such keeping under abnormal conditions, the films, if used in a new test, might at first absorb more or less of hydrogen than corresponds with the H_2/O_2 =1 ratio. Therefore only after the balance was established at the beginning of the experiment and there was no absorption of hydrogen, is oxygen introduced into the media. Under such conditions the ratio of H_2/O_2 almost never deviated from 1.

It is seen from the above exposition that though it is possible to obtain, if desired, proportions of H_2/O_2 higher and lower than 1, the normal restoration of oxygen by hydrogen proceeds at the ratio of $H_2:O_2$= 1. This coincides with Gaffron's data for living plants.

The fermentative reduction of carbonic acid by molecular hydrogen was disclosed in living cells of various bacteria. And depending on the organism the reduction proceeded either up to formic acid or to methane. In a case of green plants Gaffron giving the ratio H_2/CO_2= 2, does not indicate the direct product of reduction. Nevertheless, on the basis of all the known data, already beforehand it was difficult to expect a formation of methane by hydrogenase of chlorplasts (5). One could rather expect the formation of a carboxylized combination of the type of formic acid.

The filling of containers with hydrogen was conducted as in experiments with reduction of oxygen. Instead of introducing oxygen from hydrogen peroxide and permanganate, carbonic acid was obtained by adding drops of sulfuric acid into solution of bicarbonate. The manometer readings were also recorded before its introduction and after. At the end of the experiment the remaining carbonic acid was determined with absorption by alkali.

Trans. 467
(In Full)
By:
A. Antik

Table 3 (P. 221)

Reduction of carbonic acid by molecular hydrogen.
25 mg of chloroplasts; 25°.

% CO_2	Absorbed in μL (microliters)			Ratio $H_2/O_2 + CO_2$
	O_2	CO_2	H_2	
2,0	без O	250	238	0,94
2,7		338	413	1,15
3,0		375	388	1,03
0,6	25	75	100	1,00
1,6	13	200	200	0,94
3,0	0	375	263	0,70

Absorption of carbonic acid and hydrogen took place also when oxygen was absent. It contradicts Gaffron's data for living plants for which the presence of oxygen was necessary. Oxidation of hydrogen with oxygen presented a peculiar respiration of cells under conditions of his experiment and had inevitably an effect on assimilative processes. But when hydrogenases was isolated outside the organism, such connection was not preserved. It should be remembered that reduction of carbonic acid by hydrogen, by bacteria cells with formation of methane as well as formic acid, proceeded with complete absence of oxygen (6,7). Nevertheless the introduction of oxygen into manometric containers noticeably speeds up the reaction. This speeding up tells mainly on the absorption of carbonic acid and is little reflected on its subsequent hydrogenation. The oxygen speeds up the reduction of carbonic acid but is itself almost not absorbed. In case of low percentages of carbonic acid it is being slightly reduced, but with larger amounts of carbonic acid being absorbed it appears not to have room on the surface of the enzyme.

For comparison of the speed in absorption of gases at various concentrations of carbonic acid, in table 4 are given data for the first 5 minutes. It is seen from the figures of the table, that the increase of concentration of carbonic acide up to 6% speeds up the process. With its higher percent in the media, though some absorption of carbonic acid does take place, there is, however, no reduction of it by hydrogen. Final ratio of absorbed hydrogen and carbonic acid in all cases of reduction was close to unity. Generally speaking, when oxygen is absent this ratio is characterized by great consistency.

Trans. 467
(In Full)
By:
A. Antik

Table 4 (P. 222)

Speed in reduction of carbonic acid depending on its concentration.

% CO_2	Absorbed during 5 min. of $H_2 + CO_2$	Final Ratio H_2/CO_2
0,6	12,5	1,16
1,6	50	1,00
3,0	175	1,10
4,9	325	1,12
6,0	375	1,02
10,0	75	Only CO_2

The speed of reduction depends also on temperature. With a 10° increase of temperature within the range of 20 to 35°, the reduction is twice as fast. Above 35° begins injury of hydrogenase and reduction soon stops. It is interesting to point out that reduction of methylene blue proceeds under similar temperatures almost with the same speed. Such difference in the course of reductions of individual acceptors of hydrogen was observed for other hydrogenases as well.

Effect of temperature on speed of reduction of hydrogen and methylene blue. CO_2 3%

Table 5 (P. 222)

Temperature in °C.	Absorbed during 5 min. of $H_2 + CO_2$	Time for reduction of methylene blue in min.
20	125	15
25	175	16
30	250	16
35	362,5	17

In reducing carbonic acid as when reducing oxygen, the absorbed hydrogen after a certain time begins to be liberated. At 35°, after a rapidly passing reduction, begins an even more rapid and complete liberation of both gases. At about 30°C the conversion does not proceed anymore to the end. At 25° only hydrogen is liberated back; at lower temperatures under

conditions of hydrogen atmosphere there is no conversion at all. Therefore, for the study of hydrogen liberation by hydrogenase it is necessary to work at a temperature above 30° and it is more convenient to study the oxidation at below 30° temperature when the conversion takes place more slowly. To prevent completely the conversion in order to study the reduction products the experiment should be conducted at 20°.

We determined the character of the formed product in two ways. Since the absorption of gases proceeded in all the tests in the $H_2:CO_2 = 1:1$ ratio, and this proportion remained at a complete conversion of reduction, it was to be assumed that formic acid would be formed: $H_2 + CO_2 = HCOOH$. Confirmation of it is in the reduction of nitrate silver, corrosive sublimate and other substances. However, in testing the solution, no reduction products were found in it. It appears that these products are preserved on the films themselves. Thus after reduction of carbonic acid the film was rapidly removed from the manometer, washed with a buffer solution and it appeared capable to reduce a neutral solution of nitrate silver and also, when boiled with sodium sulfite, to discolor methylene blue in 2 minutes. The control films, which did not conduct reduction of carbonic acid, were deprived of such reducing properties. Tests on formation of calomel from corrosive sublimate were carried out by heating in a boiling aqueous bath during $1\frac{1}{2}$ hours with a 5% solution of corrosive sublimate and hard sodium acetate in an amount about 30 times higher than the expected amount of formic acid. The residue of calomel was washed in a 5% solution of hydrochloric acid, dried up to a constant weight at 90° and then weighed.

Table 6 (P. 223)

Products of reduction of carbonic acid

№ of experiment	Absorbed $H_2 + CO_2$ in μl	Formed HgCl in mg
1	425	5,5
2	662,5	8,0
3	762,5	9,4
4	950	12,0
5	1125,0	13,8

The data of reduction of corrosive sublimate corresponds quite closely with the manometric reading. Nevertheless, the obtained product is not a free formic acid but is connected with a preparation. This connection

remains when washing with buffer solution takes place. The reduction itself of corrosive sublimate procedds slowly. Besides that and particularly important under conditions of experiment, hydrogenase can not decompose the formates because formation of hydrogen from them is suppressed by hydrogen atmosphere. And the product of reduction of carbonic acid by hydrogenase, as mentioned before, can at a certain temperature serve again as a donator of hydrogen. The reducing carboxylizing which takes place here on the surface of the preparation makes reversible liberation and absorption of gas possible also in a hydrogen atmosphere. However, a more complete reduction than that corresponding to $H_2/CO_2 = 1$, by hydrogenase under conditions of our experiments was not accomplished. This reaction is only a beginning of conversions of carbonic acid on the way to carbohydrates or other products. It reminds of reducing carboxilizing in Escherichia coli which also proceeds with the $H_2/CO_2 = 1.07 - 1.13$ ratio. It is interesting that Lebedev, who was the first to discover the anaerobic reduction of carbonic acid in bacteria by molecular hydrogen, found that ratio $H_2CO_2 = 0.91 - 1.30$ (8).

For further reduction of carbonic acid it is necessary, in addition to hydrogenase, to have participation of other enzymes. On the basis of Gaffron's data on utilization of glucose instead of molecular hydrogen, as a donator in reducing CO_2 (2), and of data from the previous work of the author on the connection of the work of hydrogenase with glucosodehydrase in decomposing glucose to carbon dioxide and hydrogen (4), - it is possible to suppose that in its synthesis participate similar enzymes. Though the glucosodehydrase of green plants decreases the activity when there is an atmospheric content of oxygen, nevertheless this enzyme stands well a larger amount of oxygen than hydrogenase (from 5 to 10%). The work of glucosodehydrase could serve as a connecting link between the first anaerobic stage of reduction and the formation of final products. Thus in regard to individual oxidation-reduction reactions of assimiliation of carbon a succession is outlined which reminds of individual links in the breathing process. Common to both processes is the dependence of participation of some enzyme systems or other on the oxidation-reduction potential of the media.

The author considers it his pleasant duty to express his appreciation to the member-correspondent of the Academy of Sciences USSR - A. P. Vinogradov, in whose laboratory the present work was conducted.

CONCLUSIONS

1. The hydrogenase of chloroplasts is liberated from plants of various families; it reduces with molecular hydrogen methylene blue, oxygen and carbonic acid.

2. The ratio of the absorbed gases during these reductions was $H_2:O_2$ = 1:1 and $H_2:CO_2$ = 1:1.

3. Reduction of carbonic acid is possible also in absence of oxygen. The product which is thus obtained remains related to the preparation and possesses the properties HCOOH.

4. All the reductions of hydrogenase are reversible.

Received: 30. VIII, 1947

Institute of geochemistry and
analytical chemistry im. V. T.
Vernadskii, AN SSSR, Moscow.

Trans. 467
(In full)
By:
A. Antik

Boichenko, E. A.

Aktivirovanie molekuliarnogo vodoroda gidrogenazoi khloroplastov

(Activation of molecular hydrogen by the hydrogenase of chloroplasts).

Biokhimiia, vol. 13, no. 3, pp: 219-224
May/June 1948 385 B523

(In Russian)

ACTIVATION OF MOLECULAR HYDROGEN BY THE HYDROGENASE OF CHLOROPLASTS

by

E. A. Boichenko

Soon after Bakh, Palladin and other authors discovered oxidation-reduction (reducing) in plant cells, a search began for a connection between their action and the most powerful oxidation-reduction (reducing) process of the bio-sphere, photosynthesis. One of the first works in this direction were Liubimchenko's experiments with anti-oxidase in which, even before Vil'shtetter introduced the conception of "assimilating enzyme", photosynthesis was considered as being based "on a series of subsequent oxidations and reductions of a definite enzyme system."(1) Further works on differentiation of light and dark reactions of the photosynthesis, on bacterial photosynthesis, on photoreduction in green plants and, finally, on dark reduction (in darkness?) of carbonic acid -- confirmed Bakh and Liubimchenko's ideas on photosynthesis as an oxidation-reduction (reduction) process. But even though the participation of enzymes in reactions of photosynthesis does not arouse at the present time any doubt and is accepted in all the schemes of the assumed course of this process, nevertheless concrete data about what actual enzymes are connected with the reduction of carbonic acid by green plants, were almost absent until recent time. Gaffron discovered reduction in darkness of carbonic acid in green algae and showed that it can take place by an oxidation of the molecular hydrogen as well as by oxidation of other (hydrogen) "donators" of the glucose type. On the basis of these experiments Gaffron assumed the participation of the enzyme of hydrogenase which activates molecular hydrogen (2.3) in the assimilation of carbonic acid by green plants. A problem arose to isolate from the cell a corresponding enzyme system in order to recreate outside the organism a reaction which is at the basis of all the organic substances.

For this purpose the author of this article used preparations of chloroplasts from leaves of various green plants. The most convenient object appeared to be white clover; basically the subsequent work was conducted with it.

Preparations of chloroplasts were obtained by method described in the preceding work (4). Especially thorough was the washing off of sugar traces (until samples gave negative test with a-napthol); because if carbohydrates were present in the preparation, an absorption of hydrogen could take place instead of its secretion. In each test 25 mg. of chloroplasts were taken. Measurements were carried out in differential manometers of Barkroff. They were altered in such a manner as to make it convenient to let through a flow of gas or to introduce additional gas during the test. For that purpose an additional stopcock was fixed on the side to the small vessels. Through the manometer was passed a flow of hydrogen which passed first through permanganate and alkaline pyrogallol. From time to time the containers were tested with alkaline pyrogallol for absence of oxygen. When all the air was let out of the containers, the films with chloroplasts were lowered into the solution and measurements took place of gas-exchange followed by calculations of the coefficients Q (number of u l /microliters/ of gas absorbed or secreted, at a ratio of 1 mg. of dry weight per one hour). In tests with reduction of methylene blue and oxygen, there was alkali in the central small container for elimination of carbonic acid; and in tests with carbonic acid its determination was carried out at the end of the experiment by connecting the small container with alkali to the side stopcock (2). The tests were conducted at a 25° /centigrade/ temperature.

Tests for hydrogenase in one or another organism or preparation are conducted most frequently on reduction of methylene blue in atmosphere of hydrogen. While the control with boiled enzyme does not discolor the dye, with hydrogenase the usual transition time to leuco-color is 5-15 minutes. A flow of hydrogen was let through the containers of the manometer. The time for discoloring of the methylene blue (1:50 000) with hydrogen was determined for 25 mg. of the preparation in 1 ml. of phosphate buffer with pH = 7, because previous experiments indicated that this pH is the best for reduction.

The hydrogenase appeared to be widely spread among green plants. It could be disclosed with more or less work in all the species which we examined with more or less work, depending on degree of preservation of chloroplasts being isolated. Plants, the chloroplasts of which can stand well the isolation, like white cloter, Chenopodium, can serve as a good material for obtaining of hydrogenase. On the contrary, easy breaking up into "grans" as in an aster, decreases the activity of the preparation.

Table 1(P. 220)

Discovery of hydrogenase in plants of various families

Species	Family	Time for restoration of methylene Blue, in min.
Aspidistra elatior	Liliaceae.....	26
Trifolium repens	Leguminosae.....	15
Chenopodium album	Chenopodiaceae.....	16
Callistephus chinensis	Compositae.....	43

Participation of hydrogenase in reduction of oxygen by molecular hydrogen was observed by many authors in various objects.

And in almost all the cases the high percent of oxygen in combination with hydrogen led to a rapid inactivation of the enzyme, but for individual hydrogenases this percent was very different. Thus one absorbed well the hydrogen at 10% of oxygen, others - at 5%, in Azotobacter at 2.5 - 0.5% while the hydrogenase of Clostridium could work only with complete lack of oxygen. According to Gaffrou's data, the work of hydrogenase of green alga was suppressed by 2% of oxygen in the media.

The containers of the manometers were filled with hydrogen as in the preceding experiment. After that a certain percent of oxygen was introduced by adding drops of hydrogen peroxide into the solution of permanganate and sulfuric acid. Readings of the manometer were taken before and after the introduction of oxygen. Then every 5 minutes gas consumption was recorded. When it was finished the remaining amount of oxygen was determined by absorption with alkaline pyrogallol. From the total amount of absorbed gas, oxygen was subtracted and the ratio of absorbed H_2O_2 was determined. It was close to one unit in all the tests, being usually slightly higher.

Table 2 (P. 220)

Restoration of oxygen by molecular hydrogen 25 mg chloroplasts; 25°

% O_2	Absorbed O_2 in μl	Absorbed H_2 in μ	Ratio H_2/O_2
0,6	75	88	1,17
0,6	75	88	1,17
1,3	163	175	1,06
1,4	175	200	1,14
2,0	250	0	--
2,3	288	138	0,48

Restoration of oxygen proceeds completely only if its content in combination with hydrogen is about 0.5 - 1.5%. With a higher content of O_2 an inactivation of enzyme takes place - first the absorption of hydrogen decreases which slows down the absorption of oxygen and, finally, when the oxygen is about 10%, the preparations hardly absorb any gas at all. After such keeping under abnormal conditions, the films, if used in a new test, might at first absorb more or less of hydrogen than corresponds with the H_2/O_2 =1 ratio. Therefore only after the balance was established at the beginning of the experiment and there was no absorption of hydrogen, is oxygen introduced into the media. Under such conditions the ratio of H_2/O_2 almost never deviated from 1.

It is seen from the above exposition that though it is possible to obtain, if desired, proportions of H_2/O_2 higher and lower than 1, the normal restoration of oxygen by hydrogen proceeds at the ratio of $H_2:O_2$= 1. This coincides with Gaffron's data for living plants.

The fermentative reduction of carbonic acid by molecular hydrogen was disclosed in living cells of various bacteria. And depending on the organism the reduction proceeded either up to formic acid or to methane. In a case of green plants Gaffron giving the ratio H_2/CO_2= 2, does not indicate the direct product of reduction. Nevertheless, on the basis of all the known data, already beforehand it was difficult to expect a formation of methane by hydrogenase of chlorplasts (5). One could rather expect the formation of a carboxylized combination of the type of formic acid.

The filling of containers with hydrogen was conducted as in experiments with reduction of oxygen. Instead of introducing oxygen from hydrogen peroxide and permanganate, carbonic acid was obtained by adding drops of sulfuric acid into solution of bicarbonate. The manometer readings were also recorded before its introduction and after. At the end of the experiment the remaining carbonic acid was determined with absorption by alkali.

Trans. 467
(In Full)
By:
A. Antik

Table 3 (P. 221)

Reduction of carbonic acid by molecular hydrogen.
25 mg of chloroplasts; 25°.

% CO_2	Absorbed in μl (microliters)			Ratio $H_2/O_2 + CO_2$
	O_2	CO_2	H_2	
2,0	без O	250	238	0,94
2,7		338	413	1,15
3,0		375	388	1,03
0,6	25	75	100	1,00
1,6	13	200	200	0,94
3,0	0	375	263	0,70

Absorption of carbonic acid and hydrogen took place also when oxygen was absent. It contradicts Gaffron's data for living plants for which the presence of oxygen was necessary. Oxidation of hydrogen with oxygen presented a peculiar respiration of cells under conditions of his experiment and had inevitably an effect on assimilative processes. But when hydrogenases was isolated outside the organism, such connection was not preserved. It should be remembered that reduction of carbonic acid by hydrogen, by bacteria cells with formation of methane as well as formic acid, proceeded with complete absence of oxygen (6,7). Nevertheless the introduction of oxygen into manometric containers noticeably speeds up the reaction. This speeding up tells mainly on the absorption of carbonic acid and is little reflected on its subsequent hydrogenation. The oxygen speeds up the reduction of carbonic acid but is itself almost not absorbed. In case of low percentages of carbonic acid it is being slightly reduced, but with larger amounts of carbonic acid being absorbed it appears not to have room on the surface of the enzyme.

For comparison of the speed in absorption of gases at various concentrations of carbonic acid, in table 4 are given data for the first 5 minutes. It is seen from the figures of the table, that the increase of concentration of carbonic acide up to 6% speeds up the process. With its higher percent in the media, though some absorption of carbonic acid does take place, there is, however, no reduction of it by hydrogen. Final ratio of absorbed hydrogen and carbonic acid in all cases of reduction was close to unity. Generally speaking, when oxygen is absent this ratio is characterized by great consistency.

Table 4 (P. 222)

Speed in reduction of carbonic acid depending on its concentration.

% CO_2	Absorbed during 5 min. of H_2 + CO_2	Final Ratio H_2/CO_2
0,6	12,5	1,16
1,6	50	1,00
3,0	175	1,10
4,9	325	1,12
6,0	375	1,02
10,0	75	Only CO_2

The speed of reduction depends also on temperature. With a 10° increase of temperature within the range of 20 to 35°, the reduction is twice as fast. Above 35° begins injury of hydrogenase and reduction soon stops. It is interesting to point out that reduction of methylene blue proceeds under similar temperatures almost with the same speed. Such difference in the course of reductions of individual acceptors of hydrogen was observed for other hydrogenases as well.

Effect of temperature on speed of reduction of hydrogen and methylene blue. CO_2 3%

Table 5 (P. 222)

Temperature in °C.	Absorbed during 5 min. of H_2 + CO_2	Time for reduction of methylene blue in min.
20	125	15
25	175	16
30	250	16
35	362,5	17

In reducing carbonic acid as when reducing oxygen, the absorbed hydrogen after a certain time begins to be liberated. At 35°, after a rapidly passing reduction, begins an even more rapid and complete liberation of both gases. At about 30°C the conversion does not proceed anymore to the end. At 25° only hydrogen is liberated back; at lower temperatures under

conditions of hydrogen atmosphere there is no conversion at all. Therefore, for the study of hydrogen liberation by hydrogenase it is necessary to work at a temperature above 30° and it is more convenient to study the oxidation at below 30° temperature when the conversion takes place more slowly. To prevent completely the conversion in order to study the reduction products the experiment should be conducted at 20°.

We determined the character of the formed product in two ways. Since the absorption of gases proceeded in all the tests in the $H_2:CO_2 = 1:1$ ratio, and this proportion remained at a complete conversion of reduction, it was to be assumed that formic acid would be formed: $H_2 + CO_2 = HCOOH$. Confirmation of it is in the reduction of nitrate silver, corrosive sublimate and other substances. However, in testing the solution, no reduction products were found in it. It appears that these products are preserved on the films themselves. Thus after reduction of carbonic acid the film was rapidly removed from the manometer, washed with a buffer solution and it appeared capable to reduce a neutral solution of nitrate silver and also, when boiled with sodium sulfite, to discolor methylene blue in 2 minutes. The control films, which did not conduct reduction of carbonic acid, were deprived of such reducing properties. Tests on formation of calomel from corrosive sublimate were carried out by heating in a boiling aqueous bath during $1\frac{1}{2}$ hours with a 5% solution of corrosive sublimate and hard sodium acetate in an amount about 30 times higher than the expected amount of formic acid. The residue of calomel was washed in a 5% solution of hydrochloric acid, dried up to a constant weight at 90° and then weighed.

Table 6 (P. 223)

Products of reduction of carbonic acid

№ of experiment	Absorbed $H_2 + CO_2$ in μl	Formed HgCl in mg
1	425	5,5
2	662,5	8,0
3	762,5	9,4
4	950	12,0
5	1125,0	13,8

The data of reduction of corrosive sublimate corresponds quite closely with the manometric reading. Nevertheless, the obtained product is not a free formic acid but is connected with a preparation. This connection

remains when washing with buffer solution takes place. The reduction itself of corrosive sublimate procedds slowly. Besides that and particularly important under conditions of experiment, hydrogenase can not decompose the formates because formation of hydrogen from them is suppressed by hydrogen atmosphere. And the product of reduction of carbonic acid by hydrogenase, as mentioned before, can at a certain temperature serve again as a donator of hydrogen. The reducing carboxylizing which takes place here on the surface of the preparation makes reversible liberation and absorption of gas possible also in a hydrogen atmosphere. However, a more complete reduction than that corresponding to $H_2/CO_2 = 1$, by hydrogenase under conditions of our experiments was not accomplished. This reaction is only a beginning of conversions of carbonic acid on the way to carbohydrates or other products. It reminds of reducing carboxilizing in Escherichia coli which also proceeds with the $H_2/CO_2 = 1.07 - 1.13$ ratio. It is interesting that Lebedev, who was the first to discover the anaerobic reduction of carbonic acid in bacteria by molecular hydrogen, found that ratio $H_2CO_2 = 0.91 - 1.30$ (8).

For further reduction of carbonic acid it is necessary, in addition to hydrogenase, to have participation of other enzymes. On the basis of Gaffron's data on utilization of glucose instead of molecular hydrogen, as a donator in reducing CO_2 (2), and of data from the previous work of the author on the connection of the work of hydrogenase with glucosodehydrase in decomposing glucose to carbon dioxide and hydrogen (4), - it is possible to suppose that in its synthesis participate similar enzymes. Though the glucosodehydrase of green plants decreases the activity when there is an atmospheric content of oxygen, nevertheless this enzyme stands well a larger amount of oxygen than hydrogenase (from 5 to 10%). The work of glucosodehydrase could serve as a connecting link between the first anaerobic stage of reduction and the formation of final products. Thus in regard to individual oxidation-reduction reactions of assimiliation of carbon a succession is outlined which reminds of individual links in the breathing process. Common to both processes is the dependence of participation of some enzyme systems or other on the oxidation-reduction potential of the media.

The author considers it his pleasant duty to express his appreciation to the member-correspondent of the Academy of Sciences USSR - A. P. Vinogradov, in whose laboratory the present work was conducted.

Trans. 467
(In Full)
By:
A. Antik

CONCLUSIONS

1. The hydrogenase of chloroplasts is liberated from plants of various families; it reduces with molecular hydrogen methylene blue, oxygen and carbonic acid.

2. The ratio of the absorbed gases during these reductions was $H_2:O_2 = 1:1$ and $H_2:CO_2 = 1:1$.

3. Reduction of carbonic acid is possible also in absence of oxygen. The product which is thus obtained remains related to the preparation and possesses the properties HCOOH.

4. All the reductions of hydrogenase are reversible.

Received: 30. VIII, 1947

Institute of geochemistry and
analytical chemistry im. V. T.
Vernadskii, AN SSSR, Moscow.

Trans. 468
(In full)
By:
A. Antik

Meisel', M. N. and Pomoshchnikova, N. A.

Prostoi mikrobiologicheskii metod opredeleniia piridoksina (vitamina B_6)

[Simple microbiological method of determining Pyridoxine (Vitamin B_6)]

Biokhimiia vol. 17, no. 5, pp. 593-597, 1952, 385 B523

[In Russian]

468

Simple microbiological method of determining pyridoxine (vitamin B_6)

Most precisely, simply and rapidly pyridoxine (vitamin B_6) can be disclosed and quantitatively determined by the microbiological methods. Suggested chemical methods are reliable only when pure solutions of this vitamin are being studied. Biological methods using animals are very lengthy and cumbersome. This apparently explains the circumstance that the micro-organisms which need to obtain from outside the pyridoxine or its derivatives (pyridoxal and pyridoxamine) were widely tested as indicators of this vitamin.

For this purpose, at different times, bacteria such as Lactobacillus casei and Streptococcus faecali's (Snell, 1950) were suggested. Even though it is possible with the help of bacteria to decompose pyridoxine, pyridoxal and pyridoxamine, the artificial media for cultivation of these indicator organisms is so complex and requires such a large selection of pure amino acids, purine and pyrimidine bases and vitamins, that these methods could not become widely spread (see Trufanov, 1948; Terusalimskii, 1949; Mardashev, 1951). For determination of pyridoxene variant Neurospora sitophila (Tatum etc.) which needs this vitamin, was suggested. But this organism, as well, appeared to be not quite convenient for work and besides that, the method produces results after a relatively prolonged time interval. The most suitable indication organism for determination of pyridoxene (as well as pyridoxamene and pyridoxal) was yeast which belongs to various species of the genus Saccharomyces. Suggested were yeast organisms: Sacch. cerevisiae (Williams and others, 1941. Sacch. carlsbergensis (Atkins and others, 1943). Sacch. oviformis (Burkholder, 1943). Yeast methods of vitamin determination excell considerably in simplicity and accessability. The bacterial methods, however, as they stand developed now - they have essential shortcomings. To those belong first of all the insufficient capacity of yeast to react by increased growth and

reproduction not only to some vitamins or others, but also (when vitamins are in the media) to some amino acids, purine and pyrimidine bases. Therefore the artificial sugar-mineral media suggested as a basic media for yeast methods of vitamin determination is very frequently physiologically not of full-value. Further, the suggested yeast indicator organisms need, for their normal development and life activity, to obtain from outside not only the vitamin which interests us - pyridoxine, but also other vitamins (especially biotin and pantothenic acid), which therefore have to be added to the basic sugar-mineral media. Thus the requirements in nutrient substances in yeast appeared to be not simple at all; therefore the yeast methods of vitamin determination cannot be either considered as easily realisable.

We decided to revise the yeast methods of vitamin determination in order to eliminate the above mentioned shortcomings. First of all it was necessary to look over the indicator forms and to select from them the most reliable ones. Then a physiologically full-value nutrient media was to be combined from the simplest natural substrata and, finally, the vitamin which had to be determined with the help of the indicator culture, had to be removed from this nutrient media. The most complicated task is just the removal from the nutrient media of the sought for vitamin without essential changes in the contents of other necessary vitamins and amino acids. Such removal can be accomplished by a selective destruction of the given vitamin with physical or chemical reactions, its sedimentation or absorption, or, finally, its inactivation with an antagonistically acting factor. In regard to pyridoxin we applied exposure to ultra-violet light, which, as it is known, leads to a rapid destruction of this vitamin.

As an indicator yeast organism we used the culture of Saccharomycodes ludwigii KM, which we applied earlier for a quantitative determination of pantothenic acid (Meisel', Pomoshchnikova and Trofimova, 1949). As we found out, this organism develops well in Rider's sugar-mineral media.

Contents of Rider's media in % (p.594)
Saccharose . . . . 2 etc.

The media is prepared on water from the faucet with added biotin, pantothenic and nicotinic acids and pyridoxine. Other vitamins and amino acids are not able to replace these four vitamins. Adding of hydrolysate of casein on this substrate has no supplementary effect; this presents a proof that the basic amino acids Saccharomycodes ludwigii can synthesize independantly from the selection of vitamins necessary for this organism, only pyridoxin is sensitive to light, the others are light resistant. It was therefore necessary to try to isolate this vitamin more or less selectively with the help of light from natural media. We succeeded to accomplish it as follows. We took a 10% yeast autolysate in which, as it is known, are contained all the necessary vitamins, amino acids and other biologically active substances, we neutralized it with a NaOH solution up to pH7, poured it in a thin layer in a Petri

dish (10 ml of autolysate in a dish 10 cm in diameter) and exposed it in an open dish to a quarts ultra-violet lamp (PRK-2) (PK-2).

The distance from the burner (U V filament) to the dish was 25 cm. Exposure lasted 4 hours. This time was sufficient to destroy the pyridoxin which is present in yeast autolysate. During the process of exposure to light takes place a considerable evaporation of water from the autolysate; distilled water should be added from time to time into the evaporating autolysate in order to restore the total volume of fluid.

It was, of course, necessary to find out, whether or not, during exposure to light , the other vitamins and biologically active substances besides pyridoxin which are needed for the indicator organism Sacch. ludwigii - are being destroyed. It was also to be established, what amounts of autolysate which have been exposed to light, can produce, in the presence of pyridoxin, a full-value development of indicator culture. For clarification of these problems the following experiments were conducted. To Rider's sugar-mineral media which was poured into test tubes were added 4 vitamins (biotin, pantothenic and nicotinic acids and thiamin) necessary for Sacch. ludwigii, or instead of them - and in some tests together with the vitamins-yeast autolysate which was or was not exposed to light, in various combinations with pyridoxin. Equal inoculation of indicator culture took place in the test tubes. After 48 hours the growth of the culture was recorded and its intensiveness marked with crosses (see table).

It follows from the data in the table, first - that in the autolysate having been exposed to light, of the substances necessary for the indicator organism only pyridoxin appeared destroyed. Second - that in 0.1 - 0.2 ml. of autolysate a sufficient amount of biotin, thiamin, pantothenic and nicotinic acids is contained and preserved to provide a normal growth of culture in 5 ml. of media. Third - that when autolysate is exposed to light, no formation takes place of any kind of toxic substances depressing the development of the culture. All this confirms the possibility and expediency of using yeast autolysate which was exposed to light as a source of vitamins and other biolcgically active substances in combining a nutrient media for Sacch. ludwigii for determination of pyridoxin.

Table I (p. 595)

Development of Sacch. ludwigii culture depending on the presence of vitamins and yeast autlysate.

Contents of media	Growth of Culture without pyridoxin	Growth of Culture with added pyridoxin
Basic media* + 4 vitamins ** + 0.1 ml. autolysate having been exposed to light.	±	+++
Same, without vitamins + 0.1 ml. autolysate having been exposed to light.	+	+++
Same, + 4 vitamins + 0.1 ml. autolysate not having been exposed to light	+++	+++
Same, without vitamins + 0.1 ml. autolysate not having been exposed to light	+++	+++
Same, + 4 vitamins + 0.2 ml. autolysate having been exposed to light	±	+++
Same, without vitamins + 0.2 ml. autolysate having been exposed to light.	+	+++
Same, + 4 vitamins + 0.2 ml. autolysate not having been exposed to light.	+++	+++
Same, without vitamins + 0.2 ml. autolysate not having been exposed to light.	+++	+++
Same, + 4 vitamins, without autolysate	—	+++
Same, without vitamins, without autolysate	—	—

*Basic media-Rider's sugar-mineral media
** Vitamins Biotin, thiamin, panthothenic and nicotinic acids.

Further we had to establish the correlative character between the quantitative pyridoxin content in the media and the accumulation of the mass culture "yield" of the indicator organism. (p.595)

For that purpose Rider's sugar-mineral media with a 2% yeast autolysate which has been exposed to light, was poured into small conie retorts (100 ml volume), 10 ml in each, and increasing amounts of pyridoxin were being added. The retorts were inoculated with very small amounts of indicator culture (Sacch. ludwigii and kept in an incubator at 38° during 40 hours. Then the content of the retorts was filtered through previously weighed

Standard curve of weight increase of culture (in mg of dry substance) in 10 ml of media during 40 hours, depending on content of pyridoxin in media (in mu g/ml)

membrane filters no. 3. The latter were dried up to a stable weight and weighed.

The experiments demonstrated that there is a complete and distinct dependence between the increase of culture and the content of pyridoxin in the media. On the basis of the obtained numerical data (mean of four tests) we built a standard vurve which can serve for a quantitative determination of pyridoxin according to the dry weight of Sacch. ludwigii (fig.). Analysis of the standard curve shows, that most reliably the concentrations of pyridoxin can be determined in the interval between 0.0001 to 0.01 u g/ml, i.e., that the suggested method is sufficiently sensitive in a rather wide range of vitamin concentrations. Some increase in the Sacch. ludwigii culture in the control without pyridoxin is apparently to be explained by the presence in the media or introduction into it with the inoculation of negligeable amounts of pyridoxin. However, this circumstance does not hinder the accuracy of the determination .

On the basis of the obtained results we can suggest the following scheme for pyridoxin (vitamin B_6) determination.

1. As an indicator organism is used the yeast organism (Saccharomycodes ludwigii KM. This organism is sustained in wort-agar. From a 2-3 day culture a highly deluted suspension is prepared with sterile water from the faucet (hardly noticeable cloud)for the inoculating of the indicator media. 1-2 drops of such suspension are introduced into a retort with indicator media.

2. The indicator media is Rider's (see above) media to which 1-2% of yeast autolysate is added which was previously exposed during 4-6 hours to light from a mercury-quartz lamp. In such an autolysate the pyridoxin is practically inactivated while the other vitamins and biologically active substances necessary for indicator culture, are preserved.

3. The indicator media is poured into 100-ml conic retorts, 10 ml in each, or into 250 ml retorts - 25 ml in each. To the retorts are added solutions being analyzed for pyridoxin content (biological fluids, autolysates, hydrolysates) in various dilutions, with the consideration that the concentration of pyridoxin being determined should be finally between 0.0001 and 0.01 ug/ml. The retorts are inoculated with an equal amount of indicator culture (1-2 drops of highly diluted suspension) and kept at 28° during 40 hours.

4. The content of the retorts is filtered through membrane filters (previously weighed) arranged in Neitts' apparatus; the filters are dried and weighed.

5. According to the dry weight of the culture - using the standard curve- the pyridoxin content in a milliliter of indicator media (of sample being tested) is determined. Knowing the degree of dilution, the content of pyridoxin in the test sample is calculated.

6. In determining pyridoxin in a new not yet studied substrate, it is expedient to subject part of the test sample to exposure to the light of a mercury-quartz lamp and to establish whether this substrate contains, after the destruction of pyridoxin, any substances activating or depressing the development of the indicator culture. In case such are disclosed - the fact has to be taken into consideration for final calculations.

CONCLUSIONS

A new simple microbiological method is developed for quantitative determination of pyridoxin (vitamin B_6). As an indicator organism the yeast organism Saccharomycodes ludwigii is suggested. The principle of the new method consists in adding of yeast autolysate in which pyridoxin is selectively inactivated with the help of exposure to ultra-violet light,-to a simple sugar-mineral nutrient media. Such nutrient media is quite sufficient for an accurate quantitative determination of pyridoxin and does not require an additional introduction of any kind of vitamins or amino acids.

The suggested method is strictly specific and provides no possibility to determine pyridoxin in concentrations of 0.00001 to 0.01 ug/ml.

Received: 7.VI.1952

Institute of Microbiology
Academy of Sciences USSR, Moscow

BIBLIOGRAPHY

1. Terusalimskii, N.D. Nitrogen and Vitamin Nutrition of Microbes. Publ. by AN SSSR, Moscow, 1949.
2. Mardashev, S.R. Microbiological and enzymatic methods of quantitative determination of amino acids. Uspekhi biolog.Khimii (year book) 1,281,1950
3. Meisel, M.N., Pomoshchnikovo, N.A. and Trofimova, N.P. Biokhimiia, 14,361,1949
4. Trufanov, A.V., Usp. sovi.biolog. 25,19,1948.

Trans. 468
(In full)
By:
A. Antik

Meisel', M. N. and Pomoshchnikova, N. A.

Prostoi mikrobiologicheskii metod opredeleniia piridoksina (vitamina B_6)

[Simple microbiological method of determining Pyridoxine (Vitamin B_6)]

Biokhimiia vol. 17, no. 5, pp. 593-597, 1952, 385 B523

[In Russian]

Simple microbiological method of determining pyridoxine (vitamin B_6)

Most precisely, simply and rapidly pyridoxine (vitamin B_6) can be disclosed and quantitatively determined by the microbiological methods. Suggested chemical methods are reliable only when pure solutions of this vitamin are being studied. Biological methods using animals are very lengthy and cumbersome. This apparently explains the circumstance that the micro-organisms which need to obtain from outside the pyridoxine or its derivatives (pyridoxal and pyridoxamine) were widely tested as indicators of this vitamin.

For this purpose, at different times, bacteria such as Lactobacillus casei and Streptococcus faecalis (Snell, 1950) were suggested. Even though it is possible with the help of bacteria to decompose pyridoxine, pyridoxal and pyridoxamine, the artificial media for cultivation of these indicator organisms is so complex and requires such a large selection of pure amino acids, purine and pyrimidine bases and vitamins, that these methods could not become widely spread (see Trufanov, 1948; Terusalimskii, 1949; Mardashev, 1951). For determination of pyridoxene variant Neurospora sitophila (Tatum etc.) which needs this vitamin, was suggested. But this organism, as well, appeared to be not quite convenient for work and besides that, the method produces results after a relatively prolonged time interval. The most suitable indication organism for determination of pyridoxene (as well as pyridoxamene and pyridoxal) was yeast which belongs to various species of the genus Saccharomyces. Suggested were yeast organisms: Sacch. cerevisiae (Williams and others, 1941. Sacch. carlsbergensis (Atkins and others, 1943). Sacch. oviformis (Burkholder, 1943). Yeast methods of vitamin determination excell considerably in simplicity and accessability. The bacterial methods, however, as they stand developed now - they have essential shortcomings. To those belong first of all the insufficient capacity of yeast to react by increased growth and

reproduction not only to some vitamins or others, but also (when vitamins are in the media) to some amino acids, purine and pyrimidine bases. Therefore the artificial sugar-mineral media suggested as a basic media for yeast methods of vitamin determination is very frequently physiologically not of full-value. Further, the suggested yeast indicator organisms need, for their normal development and life activity, to obtain from outside not only the vitamin which interests us - pyridoxine, but also other vitamins (especially biotin and pantothenic acid), which therefore have to be added to the basic sugar-mineral media. Thus the requirements in nutrient substances in yeast appeared to be not simple at all; therefore the yeast methods of vitamin determination cannot be either considered as easily realisable.

We decided to revise the yeast methods of vitamin determination in order to eliminate the above mentioned shortcomings. First of all it was necessary to look over the indicator forms and to select from them the most reliable ones. Then a physiologically full-value nutrient media was to be combined from the simplest natural substrata and, finally, the vitamin which had to be determined with the help of the indicator culture, had to be removed from this nutrient media. The most complicated task is just the removal from the nutrient media of the sought for vitamin without essential changes in the contents of other necessary vitamins and amino acids. Such removal can be accomplished by a selective destruction of the given vitamin with physical or chemical reactions, its sedimentation or absorption, or, finally, its inactivation with an antagonistically acting factor. In regard to pyridoxin we applied exposure to ultra-violet light, which, as it is known, leads to a rapid destruction of this vitamin.

As an indicator yeast organism we used the culture of Saccharomycodes ludwigii KM, which we applied earlier for a quantitative determination of pantothenic acid (Meisel', Pomoshchnikova and Trofimova, 1949). As we found out, this organism develops well in Rider's sugar-mineral media.

Contents of Rider's media in % (p.594)
Saccharose . . . . 2 etc.

The media is prepared on water from the faucet with added biotin, pantothenic and nicotinic acids and pyridoxine. Other vitamins and amino acids are not able to replace these four vitamins. Adding of hydrolysate of casein on this substrate has no supplementary effect; this presents a proof that the basic amino acids Saccharomycodes ludwigii can synthesize independantly from the selection of vitamins necessary for this organism, only pyridoxin is sensitive to light, the others are light resistant. It was therefore necessary to try to isolate this vitamin more or less selectively with the help of light from natural media. We succeeded to accomplish it as follows. We took a 10% yeast autolysate in which, as it is known, are contained all the necessary vitamins, amino acids and other biologically active substances, we neutralized it with a NaOH solution up to pH7, poured it in a thin layer in a Petri

dish (10 ml of autolysate in a dish 10 cm in diameter) and exposed it in an open dish to a quarts ultra-violet lamp (PRK-2) (PK-2).

The distance from the burner (U V filament) to the dish was 25 cm. Exposure lasted 4 hours. This time was sufficient to destroy the pyridoxin which is present in yeast autolysate. During the process of exposure to light takes place a considerable evaporation of water from the autolysate; distilled water should be added from time to time into the evaporating autolysate in order to restore the total volume of fluid.

It was, of course, necessary to find out, whether or not, during exposure to light , the other vitamins and biologically active substances besides pyridoxin which are needed for the indicator organism Sacch. ludwigii - are being destroyed. It was also to be established, what amounts of autolysate which have been exposed to light, can produce, in the presence of pyridoxin, a full-value development of indicator culture. For clarification of these problems the following experiments were conducted. To Rider's sugar-mineral media which was poured into test tubes were added 4 vitamins (biotin, pantothenic and nicotinic acids and thiamin) necessary for Sacch. ludwigii, or instead of them - and in some tests together with the vitamins-yeast autolysate which was or was not exposed to light, in various combinations with pyridoxin. Equal inoculation of indicator culture took place in the test tubes. After 48 hours the growth of the culture was recorded and its intensiveness marked with crosses (see table).

It follows from the data in the table, first - that in the autolysate having been exposed to light, of the substances necessary for the indicator organism only pyridoxin appeared destroyed. Second - that in 0.1 - 0.2 ml. of autolysate a sufficient amount of biotin, thiamin, pantothenic and nicotinic acids is contained and preserved to provide a normal growth of culture in 5 ml. of media. Third - that when autolysate is exposed to light, no formation takes place of any kind of toxic substances depressing the development of the culture. All this confirms the possibility and expediency of using yeast autolysate which was exposed to light as a source of vitamins and other biolcgically active substances in combining a nutrient media for Sacch. ludwigii for determination of pyridoxin.

Table I (p. 595)

Development of Sacch. ludwigii culture depending on the presence of vitamins and yeast autlysate.

Contents of media	Growth of Culture without pyridoxin	Growth of Culture with added pyridoxin
Basic media* + 4 vitamins ** + 0.1 ml. autolysate having been exposed to light.	+	+ + +
Same, without vitamins + 0.1 ml. autolysate having been exposed to light.	+	+ + +
Same, + 4 vitamins + 0.1 ml. autolysate not having been exposed to light	+ + +	+ + +
Same, without vitamins + 0.1 ml. autolysate not having been exposed to light	+ + +	+ + +
Same, + 4 vitamins + 0.2 ml. autolysate having been exposed to light	+	+ + +
Same, without vitamins + 0.2 ml. autolysate having been exposed to light.	+	+ + +
Same, + 4 vitamins + 0.2 ml. autolysate not having been exposed to light.	+ + +	+ + +
Same, without vitamins + 0.2 ml. autolysate not having been exposed to light.	+ + +	+ + +
Same, + 4 vitamins, without autolysate	—	+ + +
Same, without vitamins, without autolysate	—	—

*Basic media-Rider's sugar-mineral media
** Vitamins Biotin, thiamin, panthothenic and nicotinic acids.

Further we had to establish the correlative character between the quantitative pyridoxin content in the media and the accumulation of the mass culture "yield" of the indicator organism. (p.595)

For that purpose Rider's sugar-mineral media with a 2% yeast autolysate which has been exposed to light, was poured into small conie retorts (100 ml volume), 10 ml in each, and increasing amounts of pyridoxin were being added. The retorts were inoculated with very small amounts of indicator culture (Sacch. ludwigii and kept in an incubator at 38° during 40 hours. Then the content of the retorts was filtered through previously weighed

Standard curve of weight increase of culture (in mg of dry substance) in 10 ml of media during 40 hours, depending on content of pyridoxin in media (in mu g/ml)

membrane filters no. 3. The latter were dried up to a stable weight and weighed.

The experiments demonstrated that there is a complete and distinct dependence between the increase of culture and the content of pyridoxin in the media. On the basis of the obtained numerical data (mean of four tests) we built a standard vurve which can serve for a quantitative determination of pyridoxin according to the dry weight of Sacch. ludwigii (fig.). Analysis of the standard curve shows, that most reliably the concentrations of pyridoxin can be determined in the interval between 0.0001 to 0.01 u g/ml, i.e., that the suggested method is sufficiently sensitive in a rather wide range of vitamin concentrations. Some increase in the Sacch. ludwigii culture in the control without pyridoxin is apparently to be explained by the presence in the media or introduction into it with the inoculation of negligeable amounts of pyridoxin. However, this circumstance does not hinder the accuracy of the determination .

On the basis of the obtained results we can suggest the following scheme for pyridoxin (vitamin B_6) determination.

1. As an indicator organism is used the yeast organism (Saccharomycodes ludwigii KM. This organism is sustained in wort-agar. From a 2-3 day culture a highly deluted suspension is prepared with sterile water from the faucet (hardly noticeable cloud)for the inoculating of the indicator media. 1-2 drops of such suspension are introduced into a retort with indicator media.

2. The indicator media is Rider's (see above) media to which 1-2% of yeast autolysate is added which was previously exposed during 4-6 hours to light from a mercury-quartz lamp. In such an autolysate the pyridoxin is practically inactivated while the other vitamins and biologically active substances necessary for indicator culture, are preserved.

3. The indicator media is poured into 100-ml conic retorts, 10 ml in each, or into 250 ml retorts - 25 ml in each. To the retorts are added solutions being analyzed for pyridoxin content (biological fluids, autolysates, hydrolysates) in various dilutions, with the consideration that the concentration of pyridoxin being determined should be finally between 0.0001 and 0.01 ug/ml. The retorts are inoculated with an equal amount of indicator culture (1-2 drops of highly diluted suspension) and kept at 28° during 40 hours.

4. The content of the retorts is filtered through membrane filters (previously weighed) arranged in Neitts' apparatus; the filters are dried and weighed.

5. According to the dry weight of the culture - using the standard curve - the pyridoxin content in a milliliter of indicator media (of sample being tested) is determined. Knowing the degree of dilution, the content of pyridoxin in the test sample is calculated.

6. In determining pyridoxin in a new not yet studied substrate, it is expedient to subject part of the test sample to exposure to the light of a mercury-quartz lamp and to establish whether this substrate contains, after the destruction of pyridoxin, any substances activating or depressing the development of the indicator culture. In case such are disclosed - the fact has to be taken into consideration for final calculations.

CONCLUSIONS

A new simple microbiological method is developed for quantitative determination of pyridoxin (vitamin B_6). As an indicator organism the yeast organism Saccharomycodes ludwigii is suggested. The principle of the new method consists in adding of yeast autolysate, in which pyridoxin is selectively inactivated with the help of exposure to ultra-violet light,-to a simple sugar-mineral nutrient media. Such nutrient media is quite sufficient for an accurate quantitative determination of pyridoxin and does not require an additional introduction of any kind of vitamins or amino acids.

The suggested method is strictly specific and provides no possibility to determine pyridoxin in concentrations of 0.00001 to 0.01 ug/ml.

Received: 7.VI.1952

Institute of Microbiology
Academy of Sciences USSR, Moscow

BIBLIOGRAPHY

1. Terusalimskii, N.D. Nitrogen and Vitamin Nutrition of Microbes. Publ. by AN SSSR, Moscow, 1949.
2. Mardashev, S.R. Microbiological and enzymatic methods of quantitative determination of amino acids. Uspekhi biolog.Khimii (year book) 1,281,1950
3. Meisel, M.N., Pomoshchnikovo, N.A. and Trofimova, N.P. Biokhimiia, 14,361,1949
4. Trufanov, A.V., Usp. sovi.biolog. 25,19,1948.

Trans. 469
(In Part)
By:
A. Antik

Naumov, N. A.

Rzhavchina khlebnytkh zlakov v SSSR.

[The rusts of cereals in the USSR]
(A monographical study)

Moskva, Sel'khozgiz, 1939. 401 p.
p. 343-398. 464.02 N223R
(Assisted by E. E. Geschele and A. A. Shitikova-Roussakova)

(In part - pp. 63-92; pp. 225-231)

[In Russian]

469

The Rust of Cereals in the USSR.

Chapter VI, pp. 63 - 87

Relation of rust development to exterior conditions.

Circumstances preceding and accompanying the development of the aecidial stage. -- Puccinia graminis Pers. -- Over-wintering of teleuto-spores and their /secondary/ maturing. -- Effect of temperature and of other factors. -- Latest dates of teleuto-spore germination. -- Period when teleuto-spores retain their viability. -- Germination of teleuto-spores and formation of basidio-spores. -- Effects of temperature and humidity. -- Speed of germination. -- Germination of basidio-spores and infection of the intermediate plant. -- Influence of exterior conditions. -- Formation of aecidia, formation of aecidio-spores and distribution of the latter. -- Viability of aecidio-spores. -- Optimum conditions for germination of aecidio-spores. -- P. triticina, P. glumarum, P. coronifera, P. dispersa, P. anomala. -- Circumstances accompanying the development and germination of uredo-spores (and development of teleuto-spores). -- General deliberations. -- Puccinia graminis Pers. -- Morphology of germination. -- Periods of preservation of viability by uredo-spores. -- Destruction of uredo-spores during the winter and necessary conditions for their successful over-wintering. -- Effect of light. -- Influence of other exterior conditions. -- P. triticina, P. glumarum, P. coronifera, P. dispersa, P. anomala. -- Summarizing table of termperature norms for various species of rust fungi.

There is no doubt that appearance and development of rust on cultivated cereals depend to a considerable degree on exterior conditions: the character of its manifestation (the discussion here is about varieties which possess some degree of susceptibility) is determined first of all by the influence of weather conditions and of all the factors characterizing the weather as: temperature, humidity, light, not only at the moment

of the visible appearance of rust on cereals, but also long before that, during the entire cycle of parasite development, which, as it is seen in Chapter II, extends over a considerable period.

Therefore for a correct idea of the process of rust manifestation, and even more -- for possession of the needed material for a prognosis of further developments, it is absolutely necessary to know how to evaluate the role and influence of each of the elements in the surroundings of the plant, in the form in which they are manifest during the development of the parasite itself. An analogous problem of relation between rust appearance and exterior conditions in the field, is discussed in the next chapter.

I. Circumstances preceding and accompanying the development of the aecidial stage.

Study of the development cycle of the rust fungus indicates that the formation of the spring (aecidial) stage of its development is a consequence of formation and further germination of a basidio-spore and infection of the intermediate host. Thus the first (according to time of formation) is the stage of basidio-spores (therefore the designation of aecidia as "first" or "spring" stage is not justified. If in regard to a primary infection of cereals there can be doubts in each particular case about the origin of the infection -- from aecidio-spores or uredo-spores (and then, whether from local origin or imported ones), then about the infection of the intermediate plant there cannot be such doubts: its source is necessarily in the basidio-spores of local origin, which are formed shortly before (minutes, hours, less frequently -- tens of hours), during the germination of teleuto-spores. Due to all that the success of infection of each intermediate plant depends on factors which reflect on germination of teleuto-spores and basidio- spores of the given rust fungus. In adding to this that the teleuto-spores, during their germination, can be extremely capricious (this term became attributed to them since Erickson's time) and that the ability to form basidio-spores depends on condition of their maturing, which continues from the fall to the spring of the following year, -- it will be evident that weather and other conditions which took place long before the infection of the given plant species itself, reflect considerably on the infection character of the intermediate host.

Here is again confirmed the correctness of the statement about the mutual biological importance of the infection for the cereal and the intermediate host: serious infection of one has to entail, theoretically speaking, a serious infection of the other, just as, in the reverse direction, infection of the intermediate plant conditions infection of the cereal, but an infection of the intermediate plant is impossible without a preceding infection of the cereal. How true is it all under concrete conditions of each given area? The answer can be only that the regulators of the infection process are local conditions, among them the weather factors.

One can hear from many sources that high incidence of infection of the aecidial host does not necessarily entail a severe infection of the cereal; here should be noted the up-to-date material which characterizes the influence of metereological and other conditions on the following moments in the life of the fungus:

1. Over-wintering of teleuto-spores and their /secondary/ maturing.
2. Germination of teleuto-spores and formation of basidio-spores.
3. Germination of basidio-spores and infection of the intermediate plant.
4. Initiation of aecidia, formation of aecidio-spores and distribution of the latter.

It is necessary to clarify these circumstances separately for each of the rust species, taking into consideration that they can differ from each other in their ecological characteristics. At the same time it should be pointed out that the information is more extensive on Puccinia graminis species which has been more thoroughly studied.

1. Puccinia graminis Pers.

Over-wintering of teleuto-spores and their /secondary/ maturing.

It is a firmly established fact that a successful over-wintering of teleuto-spores of this species, (but then of the majority of others as well) is possible only under natural conditions, i.e. in a case when during the entire winter period (or a considerable part) they are exposed to the effects of the combined weather conditions: humidity, low temperature etc. Teleuto-spores which over-wintered even "in the backyard", but under a roof cover do not acquire an ability for further germination. In regard to the given species one can speak of its exceptional demands to conditions of over-wintering, because according to statements by Melkhes /Melhus?/, Diurrel' and Kerbi /Kirby?/ (1920), incapable of further germination also the teleuto-spores which, though over-wintered in the field -- were on spread straw, while a maximum germination is observed only in teleuto-spores which hibernated on standing cereal stems.

Apparently for USSR conditions this latter observation has no completely categorical significance.

Conditions necessary for /secondary/ maturing of teleuto-spores of P. graminis and partly of other species, can be formulated on the basis of L. F. Rusakov's data as follows.

In areas where frosts are uninterrupted for a number of months or where the snow cover remains uninterruptedly all through the winter (and these conditions are characteristic for the greater part of the USSR), the /secondary/ maturing of teleuto-spores takes place only in the spring, but in the south-west corner of the European USSR it can frequently take

place in winter as well. The most important factor furthering this is the high precipitation at higher temperatures. Spring maturing of teleuto-spores is not connected either with the date of disappearing of the snow cover or with reoccurrence of days with mean temperature of 8° for the early-spring period. Of great importance for the germination of teleuto-spores are fogs, spring rains and abundant dew. After a severe winter with an uninterrupted snow cover, 6 - 8 warm rains are necessary, not less than 1 mm each. If the spring is cold and without a rapid and continuous thawing of snow, more than 10 such rains are needed in order to bring the number of germinated teleuto-spores up to 60%. If the spring starts out intensively, and continues on its way with precipitation and later-on, dry and hot days begin, then the teleuto-spores succeed in germinating in a mass prior to the opening of the foliage of the intermediate host. Micro-ecological factors, as distribution of sori of teleuto-spores on the stubble, affect greatly the speed of teleuto-spore germination: those located in the upper part can be 10 days late in their germination as compared with those which are below.

Even though the total of the above mentioned weather factors was not analyzed in detail, according to present day opinion, shared by V. A. Transhel', as a decisive factor should be recognized not the drop in temperature but the humidity and at that -- the intermittent character of humidification. It seems that among all the researchers Lambert alone (1929) thinks differently. In any case, the maturing of teleuto-spores is a long process and it is not possible to speed it up by any means: not by the action of chemical stimulants, not by the effects of some temperatures or humidity; a frequent change of drying out and humidification only raises the degree of their germination and soaking in a weak solution of citric acid -- 1% - 15 min.-- acts similarly, while a great number of tested reagents did not lead to any positive results (data by Lambert, 1929). The latter author supposes also, that the temperature is not the principal factor which determines the character of maturing of teleuto-spores. In any case, in regard to the average climatic conditions of the European USSR, as well as of Western Europe and of many states of the U.S.A., it can be maintained that in Nature the moment of final maturing of the teleuto-spores coincides in time with the moment when, due to weather conditions, their germination is possible, i.e. approximately at the beginning of May, not any earlier. All attempts to cause their premature germination by creating, artificially, absolutely favorable conditions (in the laboratory), usually lead to negative results or bring about an insignificant germination percent, which is smaller the earlier the corresponding experiment is conducted. The earliest date in Western Europe is considered to be March (Ditel', 1911-1921), in Sweden -- April-May (Erikson), in Australia -- September (beginning of the spring) (MacAlpine, 1906), in the U.S.A. -- April (Mel'khes [Melhus?] and co-workers, 1920). The other conditions, as light (Lambert, 1929) have no importance in the process of [secondary] maturing of teleuto-spores. There is no doubt that this process touches the deepest fundamentals of teleuto-spores and Prais [Price?] (1914) is quite right when he says that the essence of changes which they suffer at that time, has as its basis the protoplasm in which molecular changes take place with the introduction of new properties in its colloid structure.

In connection with all the above mentioned it is necessary to make clear how all these processes of maturing of teleuto-spores proceed under climatic conditions when there are no winters and on the other hand -- where the winters are severe and there are no thaws, when the snow cover is negligible.

In regard to the first question, answers should be expected from authors who observed the behavior of rust in tropical countries (Gassner, Meta and MacAlpine).

In regard to the second question -- not much is known. According to Bryzgalova's (1935) observations, teleuto-spores of this species under conditions of East Siberia are almost never formed, but about how the germination of those formed and preserved until spring -- there are no data in her work.

Not without interest are A. A. Iachevskii's observations showing that teleuto-spores of this species which were gathered in the fall and kept under conditions of laboratory storage until February, then removed for 1½ months in Nature-- nevertheless germinated successfully.

As a parallel to all this, it is interesting to point out that rust species on many other plants, for example P. helianthi, can be readily preserved during the entire winter in the laboratory without any damage to them. Uord /Ward?/ (1889) indicates even (which is quite rightly doubted by A. A. Iachevskii) that after 3 years of storing teleuto-spores of P. graminis in the laboratory, it was possible to observe their germination.

Together with the question asked above about the earliest dates of teleuto-spore germination, it would be useful to find out what the latest dates of their germination are during the summer following their formation, as well as what the longest periods for their preservation of viability are.

First of all should be pointed out, that the processes of maturing and germination of teleuto-spores of P. graminis, as of all the rust species on cereals (and probably also of the majority of Uredinales in general), have to be considered mass processes in the true sense of the word, which spread at once over all the specimens at hand; thus the maturing of all of them proceeds simultaneously (within the given "statsiia" /station?/) and just as simultaneously takes place for them the germination process. Certain deviations towards speeding up as well as towards slowing down can be observed due to differences of a micro-climatic character even within the borders of the same station. Thus as soon as there are favorable conditions for germination (humidity) they germinate immediately and, as a rule, there is no remaining percentage of ungerminated teleuto-spores (an essential characteristic of biology of such parasites as Plasmodiophora, Spongospora and Orobanche). But the pointed out differences in micro-climate can occur to be so essential (for example, different levels on which the teleuto-sori are located on a very long and tall straw, as it almost always happens on Agropyrum, then -- differences

in micro-relief etc.), that even for one station, with all the mass character of the germination process, delays can be observed (not due to slowed down germination, which is not found in rust) and part of teleuto-spores not infrequently germinate later than the general germination date for the given station as a whole. Even though there are no direct observations of delayed germination of teleuto-spores of this species, there is indirect information about it; thus our personal observations of rust development in Nature led us to the necessity of recognizing existence of a late germination of teleuto-spores of linear rust; in 1913 we observed development of aecidia on barberry in Experimentalfaltet (Sweden) on July 9, and in 1933 -- on Elagin island (Leningrad) in the beginning of September; apparently they took place as a result of a recent teleuto-spore germination (keeping in mind that the basidio-spores are short-lived).

At the same time it is absolutely clear (and it has been proved by Kotter's direct tests, 1932) that when conditions necessary for germination are lacking(humidity and temperature), the teleuto-spores of this species retain their viability very long -- during one year (and even 1½ yrs.) after maturity, or 1½ - 2 years -- counting from the moment of their formation; and conditions which hinder a timely germination of teleuto-spores and which provide them with a long viability period are low temperature and low humidity.

This property of teleuto-spores is used adequately in research work for preservation of stores of viable teleuto-spores during the entire summer and the following winter.

As to information on over-wintering of teleuto-spores of the species being studied, in various areas of the USSR -- there is very little of it.

Thus for the entire Trans-Volga it is considered that the problem of the initial source of regeneration of linear /stem/ rust is unsolved (Sakharov, report 1935). In the Far-East krai, according to Rusakov's data (1935), as well as in East Siberia, according to Bryzgalova's data (1935), the severe winters without thaws hinder the /secondary/ maturing of teleuto-spores, and according to statements by the latter author, they, as a rule, are not even formed and here the rust over-winters in a different way, apparently with participation of uredo-spores (the material here concerns P. graminis f. secalis on rye and on Agropyrum). As a result the usually observed delay in formation of aecidia takes place.

According to Rusakov's data (1926), no uredo-spore over-wintering is observed in the entire European USSR while teleuto-spores over-winter adequately and mature fully.

According to Iachevskii's data, in high-mountain areas the germination of teleuto-spores and formation of aecidia takes place always late -- in July - August; the same probably occurs in the high elevated spots of the Caucasus.

Germination of teleuto-spores and formation of basidio-spores.

The morphology of this process is known for so long and has been described so thoroughly in text-books and other quite accessible publications, that there is no need of describing here in detail the emerging of the germ tube (basidium) and its further destiny. We shall note only that as a result of recent study by Kredzhi /Craigie/ (1927 - 1931) it can be stated that of the four basidio-spores which are formed here, the two upper ones are of one sex, the two lower ones-- of the other.

And the basidio-spores are formed on pointed, short sterigmata and at the moment of achieved complete maturity they are actively thrown off. 15 seconds prior to that, at the basis of the basidio-spore begins to gather (apparently by oozing) a small droplet smaller than the basidio-spore itself. Distance of the throw has the limit of about 1 mm.(Mel'khes /Melhus/, Diurrel' /Durrell/, Kerbi /Kirby/ 1920). All this takes place very rapidly, the basidio-spores are thrown off one after the other.

The factors which are important at that time are, first of all, the humidity of air (about 100%) and partly of the substrate, which has to be moistened prior to that with drop-liquid water or has to absorb humidity from the air; second -- temperature (see below). Light, on the contrary, is devoid of any importance, just as its action does not reflect on the process of /secondary/ maturing of teleuto-spores and on their retention of viability.

Specifying the data given here we notice, that the optimum temperature for teleuto-spore germination is considered to be 18° ($12 - 18^{\circ}$), according to Lambert (1929); $15 - 20^{\circ}$ ($5 - 25^{\circ}$), according to Mel'khes /Melhus/ and others (1920); 22° ($9.5 - 23^{\circ}$), according to Ditel' (1912); and the optimum air humidity is 95.6 - 100% (Kotter, 1932).

For teleuto-spores of P. graminis f. avenae, according to Novotel'-nova (1935), under our conditions the temperature optimum for germination is at 22°, minimum -- at $9 - 12^{\circ}$, maximum -- at 29°.

Quite correct will be the general statement that all the exterior factors, with the exception of those just mentioned -- temperature and humidity, are of no importance at the moment of /secondary/ maturing of teleuto-spores or of their germination; neither light nor chemical stimulants, or any other tested causal agents can speed up the germination process. This means that in Nature their germination depends only on humidity (rain or fog) and temperature.

Time needed for teleuto-spore germination is very short and most frequently such germination can be disclosed already 3 - 4 hours after placing the material in a moist chamber. Some authors make these figures more precise: according to Ditel', in May 2 h. 45 m. are needed for it, in June -- 4 h. 30 m., while the not completely mature teleuto-spores require for the start of germination 30 hours (apparently this number comprises time for completion of the maturing process) as it occurs in regard to teleuto-spores transferred in March to the laboratory.

If in the process of germination (mass) during 2 days the teleuto-spores will be caught in a 1 - 10 day period of dryness, then, when favorable conditions are restored, they continue to germinate further and the

practical degree of infection of barberry, under these conditions, does not decrease in the experiments, as it was established for P. graminis f. tritici and f. secalis (Kotter, 1932); unfortunately, the mechanism of this phenomenon remained undisclosed to the end, because it is not clear whether the infection continues as result of earlier-formed basidio-spores or of such formed anew, or (which is less probable according to the meaning of the text) whether on barberry the infection which took hold /of it?/ before the drying continues to develop.

The process itself of formation of a basidio-spore at the end of sterigmata takes 20 minutes (Buller, 1924). But for its complete maturing here are required additional 30 - 60 min.; only after that and at optimum conditions can it be thrown off.

Germination of basidio-spores and infection of the intermediate plant.

The ejected basidio-spores can cause at once an infection of barberry. They withstand readily migration by air, particularly in humid air. Their maximum life span is several tens of hours, but with increase of temperature, decrease in humidity etc. -- it is still shorter.

In germinating they form a shoot which is able to penetrate independently through the cuticle dissolving it at any point (distinction from aecidio-spores and uredo-spores).

Same conditions play a role during their germination as during teleuto-spore germination, i.e. temperature and humidity, the other factors of the exterior surroundings are almost devoid of importance. In any case it is important to point out that the germination process is not dependent on the properties of the barberry foliage: basidio-spores can germinate on any substrate and sometimes they start also germinating directly on the basidium (if there is an excess of humidity -- Lambert). Of essential importance is the indication by the same researcher, that basidio-spores can germinate also in solid substrate, independent of the presence here of drop-liquid water. In Nature they usually germinate fully, without any remaining.

Optimum temperature for basidio-spore germination is 15 - 20° with the optimum between 18 - 20° (Lambert).

According to Novotelova (1935), the optimum germination temperature for P. graminis f. avenae is 17°, minimum -- 6 - 9°, maximum 27 - 30°.

As to the infection of barberry, this process represents a continuation of the germination process with the formation of a germ tube. Penetration of the latter, as indicated above, does not depend on the presence here of water in free form.

Optimum temperature for this is considered to be 17 - 18°(Lambert), 12 - 14° and 19 - 21° (Kotter, 1932); the latter author assumes on the basis of his experiments that infection is possible within a wide range of temperatures: from 8 - 10° and up to 24 - 35°. Brief reductions in air humidity (after barberry stayed in a humid atmosphere for two days) do not affect in any way substantially the results of inoculation.

Here as well the light is not only of no direct but even indirect importance, because the process of inoculation and incubations proceeds just as successfully in darkness (which seems to be slightly unexpected for rust as an obligate parasite). Anyway, the "spermogonia" /pycnidia/ are initiated and are formed normally. According to Lambert's data it seems that quantitatively the infection process is more intensive in darkness, while qualitatively (judging by the intensity of damage) it proceeds more actively in the light (on leaves as well as on petioles) which is undoubtedly in direct relation to the plants requirements of fresh supplies of assimilates.

Time necessary for completion of the infection process is excessive as compared with the rapidity of basidio-spore germination, it requires 50-70 hours, while according to Kotter's data, 17-42 hours are not sufficient. This is confirmed by his experiments with barberry plants exposed to falling basidio-spores (method of "little net") for 1½, 2, 3, 8, 24 hours and longer.

The susceptibility degree of barberry leaves depends on many circumstances and, first of all, on plant characteristics themselves; thus there can be discerned susceptible and rust resistant species and forms of barberry. Dealing here so far only with the former, the importance of their age should be pointed out first, since generally speaking the young leaves are the most susceptible ones; but then no great difference is observed in their relation to infection during the first 16 days of their life. Older leaves (16 - 20 days old) become quite rapidly non-susceptible.

The duration of the incubation period here is 9 days (prior to formation of aecidia), at optimum condition. Among the latter should be mentioned the 20 - 30° temperature (though development of the fungus at this stage is possible even at 10° and lower). The relative humidity of the surrounding air, character and amount of air are of no significance and even transferring of plants into darkness does not increase the time needed for aecidia formation. Cooling of barberry down to 0° is of no particular importance: rust development renews with added speed as soon as the temperature comes back to normal; and, with all that, freezing of the plant with freezing of leaves stops the development of the parasite. Moistening of barberry leaves or removing of the wax film from them does not speed up the infection course.

As to species of barberry (and of other plants) which can be affected by P. graminis during the aecidia stage, information on that is in chapter X.

Initiation of aecidia, formation of aecidio-spores and distribution of the latter.

The incubation period of basidio-spores on barberry, as indicated above, is on the average 50-70 hours, the pycnidial spot becomes noticeable in 4-6 days, aecidia are formed on the 9-10th day, maturing of chains of aecidio-spores requires a few additional days. Growing of chains with a gradual liberation of maturing and falling off aecidia-spores, continues for 36-40 days (Kotter) and the regulating factor here is humidity; a high humidity degree is necessary and when it is lacking, during dry periods,

the growth of chains can be completely interrupted, renewing later at the beginning of humid weather. Each cell at the basis of an aecidium which liberates aecidia-spores, can give a start to a very large number of them, up to 100. Humidity is important also during the formation of peridium - in humid air the peridium is always longer, it acquires the shape of a narrow long pipe.

The nuclear processes which take place during the initiation of an aecidium and the formation of aecidio-spores, are not described here in detail.[1] Information on a maximum number of aecidio-spores originating in one aecidium is given in chapter III. It has been taken into consideration that in one aecidium two biological rust forms can be presented simultaneously; 11 such cases are mentioned by Kotter.

Aecidio-spores are thrown off just as actively, the distance in calm weather is 4 - 5 mm. They are liberated more frequently in groups than singly, and then only when air humidity is bordering on complete saturation.

Distribution of the aecidia-spores is subject to the general regularity established by K. M. Stepanov (1935); their number per unit of air volume or the number which is deposited in a certain area, decreases rapidly with the increase of distance from the infection source, according to the 1935 formula: $Y = C + \frac{a}{S.X}$

where: a - is the constant, which depends on the properties of the fungus spores and conditions of observation; C - is the same; Y - is the distance between the distribution point and the point of falling; X - is the unknown number of spores; S - is the area.

Therefore the number of spores which are observed 10m from the shrub, will be hundreds of times smaller than that 3 - 5 m away, and at a 100 m distance thousandth and ten thousandth fractions will be encountered (at each given moment) of the amount which is liberated at this moment. According to data by Melhus, Durrell and Kirby (1920), presence of barberry at a 200 feet (about 60 m.) distance, does not influence further the cereal's (Agropyrum) infection capacity; as a distance beyond which there is no danger for the state of cereals, the laws of various countries give numbers of the order of 160 - 250 m.

With all that it should not be overlooked that distribution of aecidio-spores is a process which takes place in time and therefore its results are additive (are capable to be summed up), due to which small amounts of spores which per unit of area in some distance or other can be summed up causing a quite definite biological effect.

---

1. See Kursanov, Mikologiia /Micology/, 1933, p. 56, 380.

Viability of aecidio-spores which determines the distance of effective distribution, is of about 1½ months (46 days, according to Kotter, 1932), and that under green-house conditions; under natural conditions, which are often characterized by a lower degree of humidity, it has to be even more prolonged (at least in the maximum). Besides cases of direct loss of germination, others can be observed, when their viability is not harmed, but when nevertheless the infection by them of their host plant is prevented (example - forcing of aecidia-spores to the earth by a pouring rain).

The aecidio-spores complete their development normally by germinating on the cereal stem (less frequently on the leaf) by way of penetration of the shoot through the stoma.

Those are the optimum conditions for germination of aecidio-spores: temperature 5 - 18° (-22 - 24°), presence of drop-liquid water (or 100% air humidity). Beginning of germination is noticeable already after 1 h. 30 m. - 2 hours after their transfer to such conditions. Nevertheless cases of hampered germination are frequently observed; then, according to opinions of Erikson and Iachevskii, it is necessary to cool them during 2 - 3 hours down to 3 - 0°, after which the germination increases at once up to 100%.

As a conclusion of this section should be noted, that none of the development stages of linear [?] rust connected with formation of aecidia, takes place at a temperature above 22 - 23°, this is important as a powerful limiting factor.

2. Puccinia triticina Erikss

Over-wintering of teleuto-spores

Earlier indications in regard to over-wintering of teleuto-spores of this species (A. A. Iachevskii, 1909) lead to the same general conclusions as for the P. graminis, namely, to establishing of obligatory over-wintering of teleuto-spores with their germination in the following spring. The same follows from direct words by Erikson dedicated to this species on wheat (at that time P. dispersa and P. triticina were still united under the name of P. dispersa) (Erikson, 1896).

There is no doubt that the over-wintering period for teleuto-spores of this species under conditions of our climate is as obligatory as for P. graminis.

As to the more detailed information on the border-line temperatures which they can stand, on the significance of alternating periods of thaws and frosts etc., we can find some of it in Rusakov's works. As a critical moment in the entire development cycle of brown rust this author considers the period of the end of snow thawing, when, due to unfavorable conditions (development of semi-parasites etc.) a destruction of over-wintering

infection can take place(apparently meaning basically the uredo-stage).

In passing it is necessary to point out that the presence of affected leaves during the winter directly on the surface of the soil does not at all hinder a normal germination of teleuto-spores of P. triticina in contrast to what is known for linear rust.

Germination of teleuto-spores and formation of basidia-spores

Erikson (1896) remarks on a characteristic morphological peculiarity of basidia of this species, which makes it possible to distinguish without fail the teleuto-spores from those of the P. glumarum: in yellow rust they are filled with a yellow content, while in the given species they are absolutely colorless.

As to the optimum conditions in regard to temperatures, --there are no data on that.

Neither is there information on optimum conditions of humidity, but there is no doubt whatever, that we may refer fully to this species as to the subsequent ones, all the data which were obtained for P. graminis, namely-- that their germination is possible either at 95.6 - 100% air humidity or when the teleuto-spore layer is covered with a thin water film. It seems that in regard to this, the teleuto-spores of absolutely all the species of rust have a similar behavior.

As to concrete indications on behavior of teleuto-spores of this species under natural conditions in various areas of the USSR, the data of this kind are also very limited. It is known that under the conditions of East Siberian areas, which are characterized by unsignificant amounts of fall precipitation and by usual aridity of spring months -- April, May and June --, by severe winters without thaws, -- the /secondary/ maturing of teleuto-spores of P. triticina takes place not every year and here this rust species can be preserved differently -- as uredo-spores (see chapter XI).

Germination of basidia-spores and infection of intermediate hosts.

The basic information on this problem was obtained by Dzhekson /Jackson/ and Mains (1921) when they were working on the problem of existence of the aecidia stage in this rust species.

When V. A. Bryzgalova (1933 - 1935) determined a new aecidial host of this rust species (Isopyrum (Leptopyrum) fumarioides), inoculations of this plant with basidio-spores of wheat were not carried out,[1] therefore data on germination of teleuto-spores, formation of basidia-spores

1. Only in 1936 was such an experiment conducted by V. A. Bryzgaleva and with a positive success.

and their subsequent behavior are lacking as are the data on the role of exterior conditions in this process.

Initiation of aecidia, formation of aecidio-spores, distribution of the latter.

In regard to this species, the problem of dynamics of aecidial infection is not sufficiently developed.

3. Puccinia glumarum Erikss. et Henn.

The behavior of this rust species differs considerably from that of other species which parasitize cereals in the sense that the aecidial host has not been disclosed yet, therefore it is too early to speak of the relation between the development of the aecidial stage and the exterior conditions. But since the existence of the aecidial stage is not entirely out of question, some attention should be paid to circumstances which can be considered as preceding the moment of aecidia formation, especially since getting acquainted with them will make the search for the aecidial stage itself easier as well.

Over-wintering of teleuto-spores and their /secondary/ maturing.

Another characteristic peculiarity of yellow rust is that its teleuto-spores do not need an obligatory rest period since they possess the capacity of germinating already in the fall, soon after their formation; on the other hand, over-wintering under the snow, under usual conditions of teleuto-spore hibernation, does not deprive them of this capacity; thus the plasticity of this organism, from point of view of conditions for preservation of teleuto-spores, is incomparably wider. Finally, a very peculiar circumstance which distinguishes this rust species from others is the fact that the teleuto-spore germination does not depend on conditions of preservation in winter and thus they appear to be capable of germinating next year even in a material which was not preserved in the open, but in a laboratory. Already Erikson (1896) and Iachevskii (1909) had this information. The latter author confirms these data with his personal experiments and observations carried out in our country (seemingly in the former Smolensk district).

Nevertheless the influence of /exterior/ surroundings cannot be considered here as being entirely out of question, and the basic regularities derived from observations of other rust species -- such as the importance of excess humidity -- remains in force here as well. Germinated teleuto-pustules can be observed in the field already after a few rainy days. More accurate information on border-line and optimum temperatures and on degrees of humidity are lacking.

Germination of teleuto-spores and formation of basidia-spores.

The typical peculiarity of basidia of P. glumarum which makes it possible to distinguish teleuto-spores of this species from those of P. triticina, is their yellow coloring which is due to the presence in its contents of numerous drops of oil. Such are also the basidio-spores themselves.

The conditions of teleuto-spore germination are mentioned above; more detailed data on this problem are lacking.

Basidio-spores germinate immediately after they originate, having apparently few demands for conditions of the /exterior/ surroundings, which however is also not sufficiently investigated. The germ tube produced at that time is also characterized by a yellow content.

Inasmuch as accidia of this species are not known, there is nothing to report here on other problems.

4. Puccinia coronifera Kleb.

Over-wintering of teleuto-spores and their /secondary/maturing.

This species in its behavior within a given development stage reminds one tremendously of the linear /stem/ rust by its requirement for an obligatory winter rest period during which the maturing of the teleuto-spores takes place (Kherner, 1921). At the same time the effect of the usual metereological factors inherent to the fall, winter and spring, are an obligatory condition. According to Kherner (1921) the teleuto-spores of this species placed for hibernation on May 2 (is it in the northern hemisphere?) cannot germinate either. In regard to the earliest germination dates, there are reports by Iachevskii who observed the germination of adequately overwintered teleuto-spores not earlier than in March. Maximum germination in Nature is observed (applicable to the greater part of territory of Europe) in May; it stops naturally due to germination of all the teleuto-spores during earlier periods -- toward the beginning of July.

Therefore the retention of their viability longer than for one year is impossible as it is for teleuto-spores of other rust species.

Germination of teleuto-spores and formation of basidia-spores.

Conditions of their germination have been studied by V. D. Kupriianova, who established that the needed air humidity is 100%, and the minimum, optimum and maximum temperatures are respectively -- 4; 17 - 21 and 31.5°; adequately overwintered teleuto-spores form their basidio-spores already after 3 hours. Here the basidia as well as the basidio-spores have an orange colored contents.

Discharge of mature basidio-spores is observed mostly at temperatures optimal for germination, at which time the highest ejection reaches 950 m.

Germination of basidio-spores and infection of the intermediate plant.

According to Kupriianova, the cardinal temperatures here are as follows: 3,5; 12 - 22; 30.0°. At an optimum temperature they germinate already after a half hour. The penetration of the germ tube into the leaf tissue of buckthorn takes place independently, i.e. due to "drilling" through, by way of dissolving the cuticle. Following this takes place the transfusion of the basidio-spore content into the part of the shoot which already penetrated the plant tissue. Erikson points out that an analogous picture can be observed also when basidio-spores are placed on a barberry leaf, but he did not have a chance to bring the started observations to an end. Iachevskii who repeated his experiments obtained negative results.

When possibility of infection is lacking the basidia-spores of this species as well produce a secondary basidia-spore.

Initiation of aecidia, formation of aecidia-spores and distribution of the latter.

The incubation period (prior to formation of the spermogonial spot) is given as 6 days by Erikson, 9 - 11 days by Marland (1936). Aecidia are formed 7 days or more after that (according to Marland -- up to 14 - 16 days). The time for discovering the latter in Nature is the same as for aecidia of P. graminis, i.e. approximately during the last ten days in May. It is interesting to note that the same date is true for the tropics: in India, according to Barclay's observations (1887, 1889) the aecidia on Rh. dahurica achieve complete maturity towards the second part of May — to the middle of July.

Nothing is known on details of initiation of aecidia on buckthorn and, in particular, on the significance of regulating factors (temperature, humidity). It can be assumed that the basic relationships established for P. graminis retain their significance here as well.

According to Frezer /Frazer?/ and Ledingam's /Ledingham?/ data (1933), the aecidio-spores of P. coronifera (= P. coronata f. avenae) retain their germinating capacity for 180 days, if kept in a refrigerator.

5. Puccinia dispersa Erikss. et Henn.

Over-wintering of telento-spores and their /secondary/ maturing.

Here even more than for the yellow rust (P. glumarum) is true the statement that the hibernation period is not obligatory and that the

/secondary/ maturing of teleuto-spores takes place already while the plant is growing during the last summer months. There is also another indication by Erikson (1896), that the teleuto-spores which are kept in a protected place (in a shed, in a room) preserve perfectly their germinating capacity until the fall of the next year (but we did not find in Erikson's works the statement ascribed to him by Iachevskii, that teleuto-spores which over-wintered in the open appear to be absolutely incapable of germinating.) There is no doubt that the teleuto-spores which, due to insufficient humidity, did not germinate in the fall (unlikely as it is) will be still capable of germinating in the following year. Such a statement can be found in Meins and Dzhekson /Jackson/ (1924) as well.

Optimum temperature and humidity conditions for maturing as well as germination have not been established experimentally.

Germination of teleuto-spores and formation of basidio-spores.

The morphology of germination does not present anything particular in comparison with the preceding species not counting the fact that here the basidium is colorless in contrast to what is known about the crown, yellow and, partly, — linear /stem/ rust. This process has not been studied in detail.

Germination of basidio-spores and infection of the intermediate plant.

There are no direct data based on an experiment on this problem, as well as in regard to the following section : "Initiation of aecidia, formation of aecidia-spores and distribution of the latter".

II. Circumstances accompanying the development and germination of uredo-spores (and development of teleuto-spores).

Knowledge of all the details in the development of a parasite is absolutely necessary for a correct notion of its behavior in Nature, under field conditions where individual components of these conditions can govern the subsequent development — which determines the results of inoculation — one way or another, favorable or not favorable to the development of the parasite. Thus as a basis for the prognosis of rust development should be taken, undoubtedly, the data which are characteristic for the behavior of the fungus at the beginning of the relationship with the host plant, i.e. just at the beginning of spore germination of the parasite. Once the infection occurs — its consequences will be anyway manifest sooner or later, the question is only one of time.

The evaluation of exterior conditions, which accompany germination, has to take into consideration temperature, humidity, light, as factors which are capable of influencing most strongly the development of the

fungus. And then not only conditions which result in high germination of spores should be considered as favorable, but also those which further their maximum germination during a minimum time period (Shtreide /Strode?/, 1933).

In order to secure an absolutely precise evaluation of the role of the external factors, it is also necessary to pay adequate attention to individual properties of the spores being tested, as for example to their age; this is of great importance since the study of spore behavior near the time limits of their life span, can lead to wrong notions and the time limits of life span themselves can be very different for similar spore forms of various fungi. In cases when spore germination takes place on a plant leaf, the germination process can be influenced to some degree, besides the above-mentioned factors, also by a stimulating effect of the living substrate itself. It is very difficult to tell on what such capacity depends (if it is at all there) -- on the acidity (pH) degree of the cell sap, on properties of nitrogen combinations (proteins, amino-acids etc.), or on the presence of toxins (manifestation of a negative chemo-tropism in case of resistant plants); in any case it is certain that even if such an influence of the living leaf is observed, it must be conditioned by soluble substances capable of diffusing.

Anyway the difference in the degree and speed of uredo-spore germination of the majority of rust species on susceptible and, correspondingly, resistant varieties, is extremely slight. It is more noticeably manifest when the germination takes place in the juice squeezed from the plant; in this case the difference is so obvious that it can be used for diagnostic purposes when it is desired to determine the degree of resistance of the cereal without having recourse to artificial inoculation. Similar experiments (Ezekiel, 1930) proved this way that it was possible to catch the interesting, for us, differences in the susceptibility of plant-hosts and thus they substantiated the possibility of using this property of the squeezed juice and of the spores as a laboratory method of diagnostics.

As a result of a detailed study of the problem, mentioned here, of biology and ecology of spore germination of rust fungi, the entire needed material should be obtained according to the complete scheme of approximately such content (and that separately for each of the six rust species being studied).

Germination characteristics of each of the spore species (basidio-spores, aecidio-spores, uredo-spores, teleuto-spores) must be studied with consideration of the effects of temperature, humidity, light, age of spores, their numbers and characteristics of the environments.

Parallel to this, it is necessary also to touch upon the related problem of the capacity of spores to preserve their viability during lengthy time intervals. Finally, for uredo- and teleuto-spores all these data have to be accompanied by a study of over-wintering— whether it is possible for these spores, or obligatory, and what are the characteristics of the over-wintered spores.

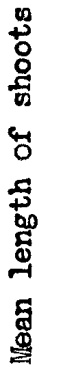
Fig. 20. Various germination types of uredo-spores of rust fungi. (According to Ezekiel.) 1-- Puccinia graminis f. tritici, bio-type 19, 2-- same, bio-type 18, 3-- same, bio-type 18 /?/, 4-- same, biotype 14, 5-- same, bio-type 1, 6-- same, bio-type 23.

Unfortunately at the present time a lack of information is evident on many of the mentioned problems in relation to many of the rust fungus species, therefore there might occur considerable gaps in the selection of material based on facts which appears below.

1. Puccinia graminis Pers.

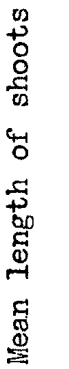
Basic information on germination of uredo-spores of this species can be found in studies by Tiulan /Tulasne/ (1854), De Bari /Bary/ (1865), Erikson (1896), Sapien-Truffi (1896-1897), Plourait /Plowright/ (1898), Ezekiel (1930), Stok (1931) and others.

Beginning of germination is frequently observed 2 - 3 hours after sowing /inoculation of plates/ (but sometimes, as Erikson indicates, only after 3 - 4 days). Usually the maximum growth of germ tubes is observed already after 24 hours; after that, even if they become longer-- it is very negligible. If their germination is observed in a liquid medium (in particular-- in a hanging drop), then germ tubes of various length and various branching character are formed and in this can be seen the manifestation of characteristics of various bio-types (Ezekiel's work, 1930, on bio-types 18 and 19 of f. tritici). Under these conditions the germination goes through 2 (or 3) stages, but one of the germ tubes formed rapidly overtakes the other. The more spores there are in the drop (to a certain limit), the longer are the germ tubes. Thus/the sowing of bio-type 1, when there were 15 spores per drop, the length of the tubes was determined as 45 m, when there were 2100 spores it was 597 m. Such is the state of affairs with bio-types 14, 18 and 19 (same author).



Number of spores in the drop.

Fig. 21 /p. 77/. Correlation between the number of sowed uredo-spores of Puccinia graminis f. tritici and the length of their germ tubes. (According to Ezekiel.)



Number of spores in the drop.

Fig. 22 /p. 77/. Correlation between the number of sowed uredo-spores of Puccinia graminis f. tritici and the speed of their germination. (According to Ezekiel).

The protoplasm in its densest part migrates gradually towards the end of the shoot, where frequently a widening of its diameter takes place and even a formation of end swellings which resemble appressoria.

In nutrient agar the spores germinate as well (if not better), resulting in good germination and formation of long shoots, -- not infrequently almost up to 1000 m.

In extracts from wheat leaves the relation between the parasite and the plant is very obvious in the length of germ tubes, as has been proven by Ezekiel for Kanred (susceptible to bio-type 18 and resistant to 19) and Mindum (susceptible to bio-type 19 and resistant to 18).

The effect of temperature on the germination process is very obvious, disclosing a minimum at 2°, optimum at 20° and maximum at 31°. In all these cases the evaluation of germination takes place not only quantitatively (as percentage of germinated spores), but also with consideration of germination speed (achieving of a certain percent during a certain time period), as well as the length of germ tubes obtained.

As to the light it has a clearly noticeable inhibiting influence on germination not only altering the direction of the originating hyphae away from the source of light (negative photo-tropism), but also inhibiting the germination process (Stok, 1931). In this respect the given species behaves differently than P. dispersa, P. coronifera and P. triticina.

In cases when the spores are not in liquid media but in humid air, the degrees of its humidity, /even/ including 99% humidity, are not sufficient, because the spores germinate exclusively at 100 % relative humidity.

The acidity of the media, as for all the other rust species which parasitize cereals, has its pH optimum ≅ 6.

The spores of the given species germinate quite well at pH ≅ 3.1 (unlike P. triticina) and even at pH ≅ 9.0, but with low acidity — less well than all the other rust species (Stok, 1931).

As to the length of time during which the uredo-spores of linear /stem/ rust retain their viability -- it is well known that under adequate storing conditions (low temperature, sufficient dryness) they can remain viable for a long time, and under humid conditions, as usually takes place under natural conditions, they perish quite rapidly, in any case having a shorter period of viability preservation than the shortest winter period in any point of the USSR.

As an illustration can serve Pel't'e's /Pelletier?/ observations of uredo-spores of P graminis f. tritici bio-type 21, which were stored at various temperatures (5; 10; 15; 20°) and humidities (29.5 and 70.4%). And at the same time it was disclosed as in many experiments of the same

kind, that regardless of the humidity degree at which the spores are stored, they lose their germination capacity faster, the higher the temperature is. Longest periods of successful storing are observed at medium humidity degrees of the surrounding air, at a 49% optimum. A more rapid drop in germination capacity is observed at high temperatures than at low ones. During a one year storing of uredo-spores of this species at 5° and 49% of humidity, their germination capacity was preserved up to 30% (Pel't'e, 1925 /Pelletier?/).

Destruction of uredo-spores of rust fungi during the winter is not always caused by the effect of low temperature as has been proved by Melander's (1935) experiments. The fact is that when the uredo-spores are exposed directly to the effect of low temperatures (below 0°), their destruction takes place in the majority of cases within 24 hours. However if the same uredo-spores underwent the effect of "hardening" (exposure at 0° for 10 days and longer), then their frost resistance increases considerably and such forms as P. graminis f. tritici and f. phlei-pratensis appeared to be capable of enduring without harm being exposed to −29° up to −40° during 45 days and P. graminis f. avenae -- 40 days.

Apparently the uredo-spores which originated in the fall at a low temperature are themselves more frost resistant than the summer uredo-spores.

Fluctuating temperatures within the above mentioned limits below 0° are not more damaging for uredo-spores (which did not undergo hardening) than the steadily supported temperatures, but on the other hand the uredo-spores which underwent hardening perished under these conditions more rapidly than the unhardened ones (P. graminis f. tritici).

The incubation period of linear /stem/ rust at low temperatures is extremely long. Thus already at 10° its duration is 7 days longer than at 20° (for formation of uredo-pustules) and for graminis f. tritici bio-type 35 the formation of uredo-pustules took 70 days at a 0° to +1° temperature, but the bio-type 15 did not produce any spore-bearing even after 80 days, in spite of the fact that the plants were exposed to near-zero temperatures only after 48 hours.

The mycelium of at least some of the linear /stem/ rust forms can stand temperatures as low as those which the corresponding host plants can stand. Thus P. graminis f. tritici, bio-type 15, which was sown on the entire selection of differential /?/ plants and was maintained 2 days at 20° with a subsequent retention for 80 days at 0° and was then placed in a green-house at 20° -- produced (it is true, only in one case) uredo-pustules on the 8th day. Maintaining of similar inoculated plants for 90 days at 0 - 1° caused complete destruction of mycelium in their tissues. Analogous great resistance showed the mycelium of P. graminis f. avenae, bio-type 2, P. graminis f. tritici bio-type 35 and P. graminis f. secalis bio-type 7.

Temperature conditions observed during rust development influence little or almost not at all the type of affection of the corresponding cereal (not counting deviations exceptional in degree). Thus at 10° the pustules develop in exactly the same manner as at 20°, but for the temperature of 0 - 1° the difference was very considerable because P. graminis f. tritici bio-type 15 and bio-type 35 produced here on corresponding varieties type 1, while at 20° normally develops type 3 or 4. Transferring of plants into a green house with more suitable temperature made the manifestation of type 3 possible at once.

P. graminis f. tritici bio-type 36 and P. graminis f. secalis bio-type 7 are able to form normally uredo-spore-bearing at 0 - 1°; bio-types 15 and 35 of the first species form under these conditions few small uredo-pustules which suggest infection type 1.

Low temperatures from 0 to 1° stimulate formation of teleuto-pustules in P. graminis f. secalis bio-type 7 and P. graminis f. tritici bio-types 15 and 35 did not form them there but instead rapidly started forming teleuto-spores after being transferred to a warm green house where usually teleuto-spores are not initiated in abundance.

Light is of no essential significance in rust development and only decreased intensity of light can inhibit, though very slightly, the formation of uredo-pustules, thus for P. graminis f. tritici bio-type 15 and P. graminis f. avenae bio-type 1 - 2 days more were needed for it when the light intensity was 162 - 301 light/feet, as compared with 294 light/feet.

Intensity of light and quality of the radiated light can reflect on the size and shape of uredo-spores; thus it is seen from Melander's (1935) work, that P. graminis f. tritici bio-type 15 forms longer and narrower uredo-spores at light intensiveness of 301 light/feet at 20° than those formed under other light conditions.

Uredo-spores can stand not only the effect of low temperatures but also their fluctuations, particularly while in a dry state. Therefore the supposition is quite probable that the destruction of uredo-spores which takes place in winter in countries of temperate climate is not conditioned either by the effect of low temperatures as such, or by fluctuations in their low or even medium levels, until the uredo-spores themselves are in a dry state, but perish most likely during the change in temperature conditions at the time of thaws (Melander, 1935).

Light affects the rust in a very definite manner during the process of plant infection as Khart [Hart] and Forbes (1935) demonstrated on the example of P. graminis f. tritici, contrary to what is known about all the other rust species on cereals. Thus with inoculation in light the number of successful infections for the mentioned species fluctuated within the 70.0 to 90.1% limits (and for the resistant variety Khop when inoculated with bio-type 49 -- it was 54.8%); for all the other species

under similar conditions (by light) it varied from 89.8 to 100.0%. In darkness the number of successful infections went down for P. graminis f. tritici to 53.1 - 9.8%. The decrease in infection of the resistant variety Markillo /Marquillo/ was 11.3% for bio-type 21 and 9.8% for bio-type 49.

According to Ueston's /Weston/ (1931, 1932) research, different parts of the sun spectrum have a different effect upon the spores during their storing and germination as well as on the young mycelium according to the same author. In any case, the germination of uredo-spores of P. graminis f. tritici in sun light is inhibited, which should not be considered as due to the effect of ultra-violet rays, as was assumed earlier, but due to the least refractive part of the visible spectrum. Thus spores germinated well in his tests under green and light blue Wratten's light filters, but did not germinate hardly at all under red, orange and yellow light filters. Similar results were obtained in another series of tests -- with breaking up of sun light with a prism.

And in regard to ultra-violet sun light this /?/ author remarks that the dark-colored spores having a wall which incloses protective pigments,-- possess a greater resistance to ultra-violet rays. Thus uredo-spores of white strains of P. graminis f. tritici are much more susceptible to this kind of radiation than the normal red and even gray spores.

2. Puccinia triticina Erikss.

In order to illustrate the relation of uredo-spores of P. triticina to temperature at the moment of germination, it is expedient to show Stok's data (1931).

/p. 80/

	Temperature in °C							
	2.5	5.0	10.0	15.0	25.0	27.5	30.0	32.5
Number of germinated uredo-spores in % . . .	1.1	30.7	89.2	98.2	90.4	80.2	30.0	0.0

Contrary to what is known about the stem rust on cereals during the uredo-spore stage, the other three species (P. triticina, P. glumarum, P. dispersa) are quite indifferent to light effect during spore germination. But in regard to air humidity they all behave absolutely identically, not germinating even at high degrees of relative air humidity (including up to 99%) and being able to form a germ tube only at complete saturation of air with moisture (100%) (Stok, 1931).

On the infection process of P. triticina light has a rather inhibiting effect since it has been disclosed in Hart's and Forbes' (1935) experiments, that the number of successful infections in darkness was 4.5% higher than in light.

Preliminary moistening of spores at optimum and minimum temperatures increases the germination capacity of spores, while similar moistening at a maximum temperature suppresses germination. Moistening with subsequent drying considerably influences germination, decreasing it; a similar effect is produced by lengthening the period of drying. Drying at high temperatures results in a rapid loss of germination capacity by uredo-spores (according to N. S. Novotel'nikova, 1935).

3. Puccinia glumarum Erikss. et Henn.

"Following data are determined for P. glumarum (as a result of Shtrede's /Strode?/ research, 1933).

The temperature minimum is hardly above 0° (as for P. dispersa), but lower than for the other rust species on cereals.

The temperature optimum is 11°, though a 100% germination of its uredo-spores is observed also at higher degrees within the 20° limits, but here the germination time is considerably longer as compared with lower temperatures.

The temperature maximum is 25° which is lower as compared with the maximum temperature for the other rust species which develop on cereals.

The rapidity of germination is influenced substantially by the age of the uredo-spores themselves; thus for 2 - 3 day old spores at a 19 - 20° temperature, the percentage of germinated spores reaches 40 - 50 already after 12 hours, while the 8 - 9 day old spores, during the same time period reach only a 5% germination. But then such difference in uredo-spores' behavior is observed only at high germination temperatures, at lower temperatures (16° and lower) the spores of various ages behave similarly.

[p. 81]

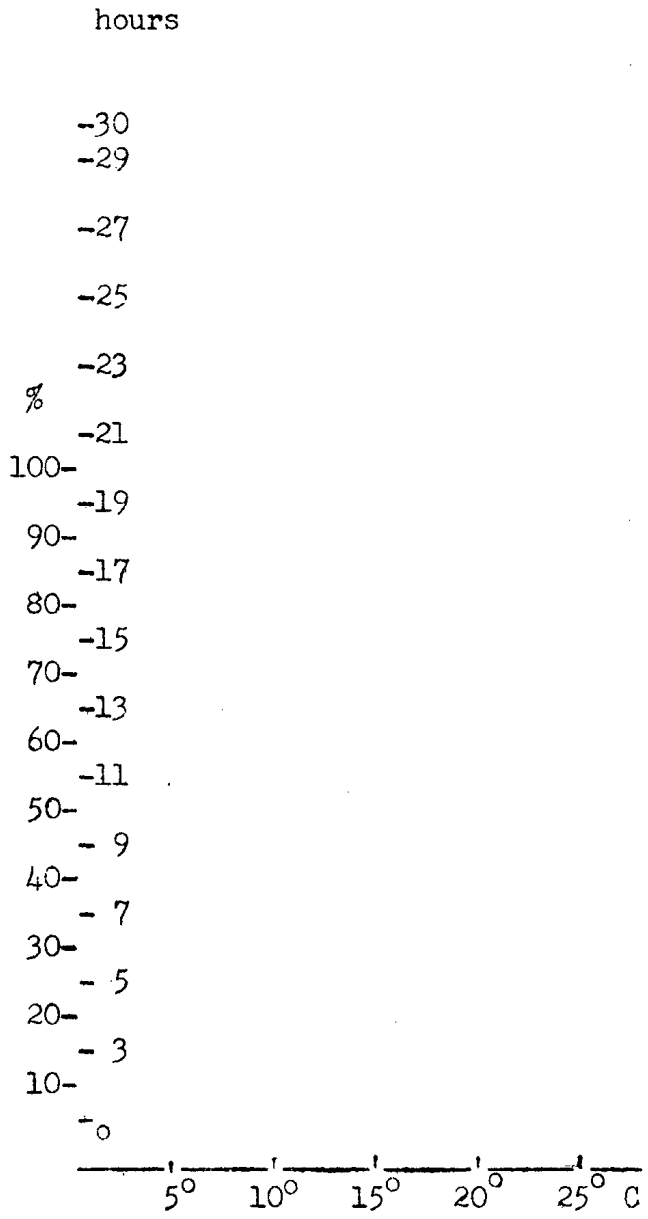


Fig. 23. Diagram of germination of uredo-spores of Puccinia glumarum at various temperatures (according to Shtrede).

Light inhibits noticeably the germination of uredo-spores of this species, which is seen from the following comparison: /p. 81/

Change in temperature during the first 6 hours			Number of germinated spores in %	
At the beginning	At the end	Further	In light	In darkness
13°	20°	13°	After 6 hours -- 50	80
			After 9 hours -- 76	99
			After 12 hours-- 80	99
			After 24 hours-- 99	99
12°	17°	—	After 6 hours -- 26	99
			After 9 hours -- 40	99
			After 12 hours-- 55	99
			After 24 hours-- 99	99

The germination capacity of uredo-spores of P. glumarum depends (according to Rider and Bever data, 1931) to a considerable degree on conditions of the environment. And their behavior differs little from that of uredo-spores of other rust species, which can be seen from the following figures: /p. 82/

Relative air humidity in %	Air temperature in °C	Duration of maintaining by uredo-spores of their viability in days
49	7.2 -- 10.0	88
49	25.0 -- 25.5	42
25	9.0 -- 12.7	63
25	23.3	18

The authors point out that under the conditions of the province of Alberta (49 - 55° of N. latitude), the yellow rust does not hibernate in the form of uredo-mycelium; only as an exception was its hibernation observed locally twice, therefore it is assumed that the source of infection is in states farther south -- Washington, Idaho and possibly, Montana.

Uredo-spores of this species which were just formed, maintain their viability when kept at + 5° and with relative air humidity of 50% for three months, some spores germinated even after 128 days; optimum temperature for germination is 10 - 12° (Newton, Johnson and Brown, 1933).

According to Diukome's (1925) indications, uredo-spores of P. glumarum and P. triticina can preserve viability for 220 days (under laboratory conditions).

Studying P. glumarum, Wilhelm (1934) noticed that decrease in amount of light hinders the manifestation of the complete type of infection, but does not affect the relative susceptibility of individual varieties. Bever (1934) found that there is an inverse relation between the duration of light periods (expositions in his experiments) and the time of appearance of spore-bearing on barley, which is seen from the following comparison: [p. 82]

Character and periods of light in hours	Duration of incubation period in days	Types of infection
6	20	4
8	16	4
10	13	4
12	9 - 11	4
15	12	0
Uninterrupted light	12	0
Day of natural duration	21	4

As it appears from the table, the duration of light exposure does not influence the type of infection (not counting the cases which deviate from the norm), but reflects on the duration of the incubation period.

4. Puccinia coronifera Kleb.

Uredo-spores of crown rust germinate only in presence of drop-liquid water in form of even very thin film. On the other hand, their emerging under a thick layer (even of a drop) of water, influences unfavorably the germination process. From here a practical conclusion -- it should be preferable to use in the process of experimental work on rust instead of the spraying method the method of dusting the plant with spores but with a previous moistening of the plant surface.

The minimum temperature for germination of uredo-spores of this species is 1° (or about that), optimum -- 20° and maximum -- near 30°.

Some substances applied for gluing of the cover glass to the Van Tieghm ring, definitely stimulate the germination of uredo-spores of this species, which coincides completely with Arthur's (1927) data; thus vaseline produced an increase of 23%, kerosene ("parafine oil", may be just parafine oil?) -- of 70%. The cause remained unexplained (Mel'hus and Durrell, 1919).

Relation between the speed of spore germination of crown rust and the temperature of the environment (according to Marland and Kupriianova, 1935)
[p. 83]

Teleuto-spores

Duration of observations	Temperature in °C				
	5	15	20	25	31
12 hours	1.7	33.4	20.5	7.5	0
18 hours	3.0	46.9	57.6	16.7	0
24 hours	10.2	70.5	81.8	47.3	0

Basidia-spores

Duration of observations	Temperature in °C				
	6.5	12.4	22.0	26.5	31.0
0.5 hours	0	0.0	6.6	0.0	0
1 hour	0	10.7	20.0	21.7	0
3 hours	7	32.0	30.0	28.1	0
20 hours	25	42.8	40.0	28.1	0
24 hours	25	50.0	50.0	28.1	0

Uredo-spores

Duration of observations	Temperature in °C							
	3.5	5.5	10.0	15.2	20.0	24.0	30.3	35.0
3 hours . . .	0	18.7	6.6	29	87	50.0	50.0	0
6 hours . . .	0	40.0	40.0	55	94	79.6	67.7	0
24 hours . . .	2	53.7	65.0	71	96	84.3	75.0	1.7

The figures in the tables express the number of germinated spores in percents. All these spores, according to the mentioned authors, can germinate also without drops of water when the air humidity is 100%.

The viability of uredo-spores of crown rust of oats is not very high; thus in Mel'hus and Durrell's (1919) experiments, when they were preserved during 55 days at -15°, their germination was determined as being 20%.

The uredo-spores of P. coronifera (of oats) possess approximately the same degree of viability as the uredo-spores of P. graminis. In particular, when they were stored at -34°, -27° and up to $+5^{\circ}$ temperatures, they lost their viability on the 22nd day, but being covered with dry leaves and snow (a layer 30 cm. thick), they retained their viability during 44 days.

According to Kherner's (1921) experiments, light affects them unfavorably: in the light at a -2° up to $+31^{\circ}$ temperature they perish during 23 days, and in darkness -- with all the other conditions being similar -- they stand storing up to 79 days.

Cardinal points for germination of uredo-spores of this species are 7; 18; 32° (Kherner, 1921).

Duration of incubation period in relation to temperature of the environment for uredo-spores as well as aecidia-spores of crown rust.
(according to Marland and Kupriianova)
[p. 84]

24 hours fluctuations of temperature (in °C)

Minimum	Mean	Maximum	Incubation period
14.6	19.3	25.1	7 days
7.2	10.7	14.3	14 days
Constant		18 - 20	6 - 7 days
Constant	25 - 36		9 days
Constant	Above 36		No infection takes place

Speed of shoot penetration into the leaf tissue at various temperatures (aecidia- and uredo-spores).
[p. 84]

Duration of observations	Temperature in °C					
	11	12	17.5 - 18.0	20.0 - 22.8	28 - 30	33
2 hours	—	—	—	—	—	—
3 hours	—	—	—	+	—	—
4 hours	—	—	—	+	+	—
5 hours	+	+	+	+	+	—
6 hours	+	+	+	+	+	—
24 hours	+	+	+	+	+	—

It is seen from the table that the infection of oats sprouts i.e.. penetration of the germ tube into the leaf tissue takes place within 11 to 30° temperatures after 5 hours.

At an optimum temperature (20.0 - 22.8°) the penetration takes place even after 3 hours.

In 1936 the methods of this experiment were altered. At the end of the indicated periods the plants were taken out of the chambers of the poly-incubator, water drops with the spores were dried out with filter paper and then the plants were transferred into the hot house, where they were kept until the appearance of pustules.

The results of the experiments are compiled in the table. [p. 847]

Speed of infection of oats sprouts at various temperatures
(aecidia- and uredo-spores)

Observation time	Temperature in °C						
	4.5 - 5.5	6	7 - 12	14 - 16	17 - 27	30.0 - 31.4	33
3 hours . . .	—	—	—	—	—	—	—
4 hours . . .	—	—	—	—	—	—	—
5 hours . . .	—	—	—	—	+	—	—
6 hours . . .	—	—	—	+	+	—	—
8 hours . . .	—	—	—	+	+	—	—
10 hours . . .	—	—	+	+	+	—	—
12 hours . . .	—	—	+	+	+	+	—
18 hours . . .	—	—	+	+	+	+	—
20 hours . . .	—	+	+	+	+	+	—
24 hours . . .	+	+	+	+	+	+	—

It is seen from the table that the infection of oats takes place within the 14 to 27° temperatures, after 5 hours. At temperatures below 14° and above 27° the infection sets in after a longer period of time (10 - 12 hours and more).

The reported data indicate that the completion of infection of oats with aecidio- and uredo-spores (i.e. that which results in rust manifestation) requires longer time than that needed only for penetration of germ tubes into the tissue. Thus penetration of shoots is possible after only three hours, and complete infection, which later produces pustules, is possible at the same temperatures only after 5 hours. There are still more differences between the temperature limits within which germination of spores and complete infection are possible. Thus germination of uredospores at an optimum temperature attains noticeable intensity already after 1½ hours and a completion of infection is possible at the same temperature only after 5 - 6 hours. In order to make a prognosis it is of course necessary to be guided only by the speed of infection (Marland, 1936).

When light is lacking, the development of P. coronifera on the plant is inhibited, but the first moments of infection proceed just as successfully as with light (Meins, 1917).

5. Puccinia anomala Rostr.

Dependency of the germination of uredo-spores of P. anomala on temperature
(according to Brown, 1929)
[p. 85]

	Temperature in °C						
	5	11	14	15	17	20	23
Germination of Uredo-spores in %...	7.2	41.7	77.8	80.4	84.9	64.5	27.5

In conclusion, we cite some information characterizing the conditions of spore germination of rust fungi in the form of compiled tables.

Temperature norms for rust fungi (in °C)

	Minimum	Optimum	Maximum	Supermaximum
1. Puccinia graminis Pers.				
1. For germination:				
Basidio-spores . . .	8-10	17-21	35	
Aecidio-spores . . .	5	15-20	22	
Uredo-spores	2-3	21-23	Above 30 (32)	
Teleuto-spores . . .	5	22	25	
2. For infection:				
By basidio-spores . .		17-18	26	
By aecidio-spores. .				
By uredo-spores. . .	10	25-30	30	
3. To over-winter:				
With uredo-spores. .	-6, -10	+5	--	
With teleuto-spores.	-35		--	
2. Puccinia triticina Erikss.				
1. For germination:				
Basidio-spores . . .				
Aecidio-spores . . .				
Uredo-spores	2, 5 (7)	22-25	A little above 30	
Teleuto-spores.		12,5-25,0		

	Minimum	Optimum	Maximum	Supermaximum
2. For infection:				
By basidio-spores. .				
By aecidio-spores. .				
By uredo-spores . . .	15	25	30	43
3. To over-winter:				
With uredo-spores. .	—6, —10	2-5		
With teleuto-spores..				
3. Puccinia glumarum Erikss. et Henn.				
1. For germination:				
Basidio-spores. . .				
Aecidio-spores. . .				
Uredo-spores. . .	A little above 0	11	22-23	29-30
Teleuto-spores. .				
2. For infection:				
By basidio-spores..				
By aecidio-spores..				
By uredo-spores...				
3. To over-winter:				
With uredo-spores..				
With teleuto-spores.				
4. Puccinia dispersa Erikss. et Henn.				
1. For germination:				
Basidio-spores. . .				
Aecidio-spores. . .				
Uredo-spores . . .	A little above 0°	9,0-22,5	A little above 30	
Teleuto-spores. . .				
2. For infection:				
By basidio-spores..				
By aecidio-spores..				
By uredo-spores....	About 6	About 22		
3. To over-winter:				
With uredo-spores..				
With teleuto-spores..				

	Minimum	Optimum	Maximum	Supermaximum
5. Puccinia coronifera Kleb.				
1. For germination:				
Basidio-spores. . .	3-4	12-22	31	
Aecidio-spores. . .				
Uredo-spores. . . .	1-3(5)	15-25	35	About 43
Teleuto-spores. . .	4	15-20	31.5	
2. For infection:				
By basidio-spores.				
By aecidio-spores..				
By uredo-spores....	13 or below	20		
3. To over-winter:				
With uredo-spores..				
With teleutospores.				
6. Puccinia anomala Rostr.				
1. For germination:				
Basidio-spores . . .				
Aecidio-spores . . .				
Uredo-spores		15-17		
Teleuto-spores . . .				
2. For infection:				
By basidio-spores. .				
By aecidio-spores. .				
By uredo-spores... .	8	22-25	About 32	
3. To over-winter:				
With uredo-spores, .				
With teleuto-spores.				

Optimal temperatures in °C and pH of culture media at the moment of the germination of uredo-spores of the basic rust varieties.

Rust varieties	By number of spores germinated	By length of shoots	Rust varieties	Optimal pH
P. graminis...	11.0-25.0	20	P. graminis. . .	3.5-7.3
P. triticina..	12.5-25.0	10-15	P. triticina . .	4.5-6.5
P. coronifera.	14.0-25.5	15-20	P. coronifera. .	4.5-7.4
P. dispersa...	9.0-22.5	10	P. dispersa. . .	1.3-7.6

The comparative data on temperatures best suited for the germination of uredo-spores of rust varieties, developing on cereals, indicates as follows: the wider range of temperatures, which permits equally good germination, is observed simultaneously for three varieties -- P. graminis tritici, P. triticina and P. dispersa -- a range of 5 to 25°. A somewhat smaller one -- 5 to 20°-- has been established for two varieties -- P. coronifera and P. glumarum f. tritici. Finally, a range of similar extent, but with a somewhat different importance of the limit /predel'nykh/ temperatures, is known for P. anomala -- from 10 to 25°-- which on the basis of these data can be looked upon as a more thermophyllic form of rust (according to Wil'khel'm, 1931).

Chapter VII, pp. 87-92

Characteristics of rust manifestation in relation to exterior conditions.

General survey of conditions on which depends the character of rust development in field surroundings. -- Usual time of manifectation.-- Importance of temperature. -- Importance of soil composition and of properties of fertilizers being introduced. -- Role of individual elements-- nitrogen, potassium, phosphorus. -- Influence of soil humidity. -- Influence of air humidity. -- Influence of air composition. -- Influence of light. -- Significance of age of the plant. -- Relation between the degree of rust manifestation and the distance from sources of primary infection. -- Significance of sowing dates and other agro-technical methods. -- Relation between the degree of rust affection and the presence of other parasites. -- Parasitic fungi on rust (parasites of second order).

Specific characteristics of rust species and their considerable plasticity reflect in a high degree on the manifestation and course of the disease and thus it is absolutely impossible to speak of any general disease picture even having in mind only one species of parasite. Not infrequently the most usual rust species as P. triticina, present in their development under different conditions a far different picture, which fact frequently influences [negatively] possibilities of diagnostics according to exterior symptoms. Among the basic factors which influence the exterior manifestation of pheno-type, the following should be considered: 1) species peculiarities of host plants (for rust species which specialize on several cereal species), 2) their varietal properties, 3) phase of plant development in connection with "stageness" [stage?] of development (winter and spring types of cereals), 4) climate and weather characteristics as well as single components of the latter.

And if to this should be added, -- as a variable interesting for us -- the date of rust appearance, then the presented list would have to be lengthened by including in it such circumstances as latitude of the locality, relief, micro-relief, dates of sowing, fertilization.

However the subject of this chapter should not be the indicators of morphological changeability of the fungus as such, but only the evaluation of exterior conditions which affect the time, amount and degree of rust manifestation.

In cases when the biological independence of individual rust biotics will be manifest to a degree when it could reflect on the character of manifestation, it will be later on pointed out (depending on the presence of material). First of all a few words on normal dates of manifestation of individual rust species in the field.

Earliest can be found species which under the given condition are capable of overwintering in uredo-stage. Therefore when the cereals come out from under the snow disclosed on them can be P. dispersa on rye, P. triticina and P. glumarum on wheat. Their further development continues with varying speed, depending on exterior conditions which will be discussed in this chapter. But usually the manifestation of rust which depends on the latter's development in the given year makes itself noticeable for these rust species at following dates:

P. glumarum	March - April
P. triticina and P. dispersa. .	June, sometimes May
P. graminis and P. coronifera .	End of June, July
P. anomala	End of July, sometimes earlier, for hibernating uredo-spores on winter barley -- May

Further are presented dates of most intensive development:

P. glumarum	May - June
P. triticina and P. dispersa. .	June - July
P. graminis and P. coronifera .	July, August, for the latter-- frequently up to the middle of September.
P. anomala	July

I. Importance of temperature.

There is no factor of exterior surroundings which could be compared with temperature in the degree of its importance. The importance of humidity -- a factor of indisputable importance which to a high degree regulates the rust development -- has to be put in second place; this is due to the fact that the humidity can further the process of infection and in this respect no one will argue its importance, but by that its role is limited inasmuch as during the subsequent rust development inside the plant tissues as well as during spore-formation, the humidity is of almost no importance. And the development of rust is regulated in first place only by temperature.

Thus if the final result should be considered as a measure of importance of these two factors, i.e. the degree of affection of the cereals, then the humidity and the temperature could be considered as being to a certain degree equivalent, but if a more accurate analysis of phenomena should be undertaken, with an evaluation of importance of all the stages of rust development, then the effect of temperature as a factor which influences the rust development uninterruptedly during the entire development of the parasite and not only during one short moment as it is known of humidity, -- should be placed in the foreground.

The most favorable rust development is observed, of course, within the range of temperatures which we designate as optimum. Deviation from the optimum in either direction brings about a decline in the development of the parasite, prolonging the periods of all its development stages, starting with the moment of incubation. Thus marking the very obvious manifestation of the effect of lower (as well as higher) temperatures we ascribe quite justly to temperature, as a factor in rust manifestation, the regulating role, referring it to the category of limiting factors. It should be understood under this, that however favorable for the development of the fungus should be all the other conditions, its development will be inhibited or even suspended if the temperature persists within a range not corresponding with the optimum temperature for the development of the given fungus species.

Fig. 24 [p. 89] Reaction of sprouts of the Mindum variety to inoculation with Puccinia graminis f. tritici at various temperatures (According to Johnson).

Fig. 25 [p. 89] Effect of temperature on manifestation of Puccinia glumarum on the leaf of Strubes Neuzucht 3186 wheat at +° = 5-10°, 11-15° and 20° (biotype Schlanstedt). (According to Gassner and Straib).

Strictly speaking the temperature optimum for various development stages of one parasite species can be of varying importance, but in practice the germination of spores, formation of mycelium, its growth and spore-bearing take place within about the same narrow margins. But since in the process of inoculation participate two organisms -- the parasite and its feeding plant -- it is necessary to pay attention also to the properties of the other side /?party?/, marking the temperature which furthers the greatest susceptibility of the plant.

In field surroundings these single moments are not always strictly distinguishable, so that in regard to these conditions it is necessary to speak of optimum (correspondingly -- minimum and maximum) temperature zones. Among the optimums known for certain rust species are the following:

For P. graminis . . . . . 18 - 20°
For P. triticina. . . . . 15 - 22°
For P. glumarum . . . . . 10 - 15°

As to temperature zones within which an increased or decreased susceptibility of a plant is observed, such information exists not for all rust species in connection with corresponding cereals. Best known in this respect is the yellow rust P. glumarum. According to Newton, Johnson and Brown (1933), at a 26° temperature all the wheat varieties can be considered resistant, at 14° the majority of varieties disclose susceptibility Khern reports on similar relations of cereals to P. graminis f. tritici, biotypes 11 and 27.

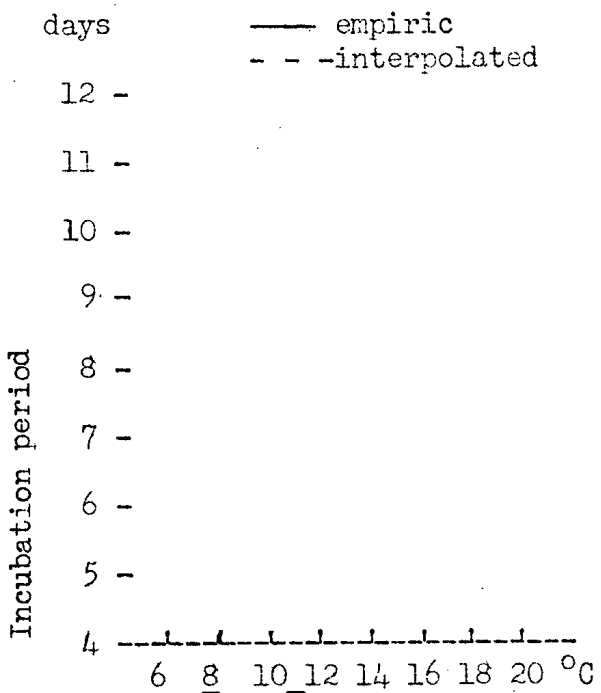


Fig. 26 /p. 90/ Relation between the length of the incubation period and the minimum temperatures for Puccinia triticina (According to N. A. Naumova).

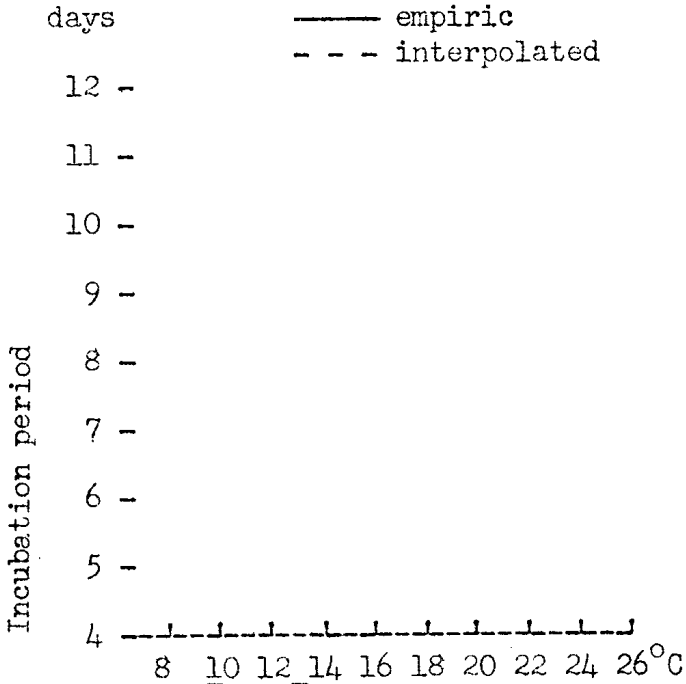


Fig. 27 /p. 90/ Relation between the length of the incubation period and the mean temperatures for Puccinia triticina (According to N. A. Naumova).

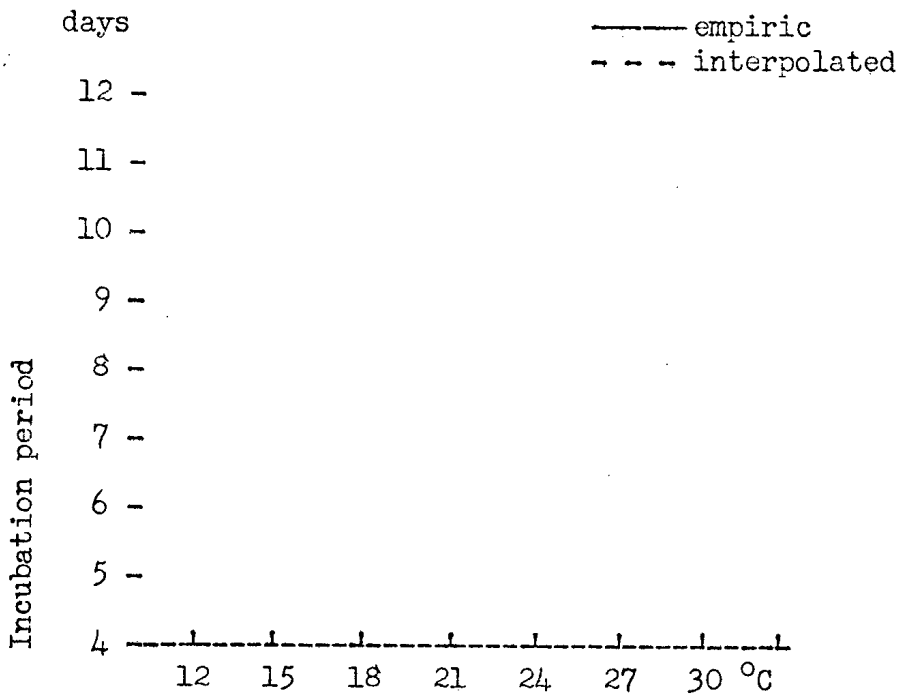


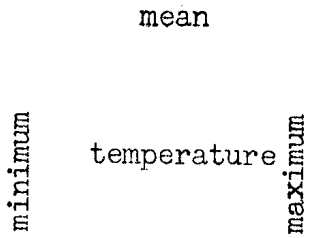
Fig. 28 /p. 907 Relation between the length of the incubation period and the maximum temperature for Puccinia triticina (According to N. A. Naumova).

In regard to P. coronifera some oats varieties are susceptible to biotype 7 at 25°, very resistant to it at 21° and practically immune at 14° (Peturson, 1930). The Zhoanett Strein variety also greatly changes the character of its reaction to P. graminis avenae, being resistant to biotype 4 at 14° and fully susceptible to it at 24° (Gordon, 1930).

In regard to yellow rust of wheat, Gassner and Straib (1934) proved that the high temperature furthers the development of the plant's resistance. But not all the varieties behave similarly, because together with varieties in which the acquired resistance degree borders with immunity when the temperature rises, there are also such, in which the point of "critical temperature" lies very high and which due to it can even at high temperature possess all the intermediate degrees between resistance and susceptibility. All this leads to the fact, that in selecting suitable varieties it is necessary to pay attention not to resistance in general, but to "summer resistance", possession of which is a positive feature of some varieties.

Concrete importance of temperatures favorable to spore germination, penetration of mycelium, spore-bearing of individual species -- are presented in corresponding chapters (Chapters VI and X).

Fig. 29 /p. 917 Effect of temperature on behavior of Puccinia triticina: 1 - 4 -- on Malakhov variety at 20.8° -- 12 days, at 12° -- 16 days, at 5.3° -- 49 days, at 5.3° -- 60 days; 5 - 7 -- on Rumker's Sommerdickkopf variety -- at 20.8° -- 12 days, at 12° -- 16 days, at 5.3° -- 29 days. (According to Gassner and Shtraib).



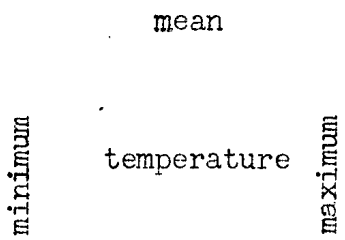


Fig. 30 [p. 92] N. A. Naumova's nomogram for Puccinia triticina.

Fig. 31 [p. 92] N. A. Naumova's nomogram for Puccinia glumarum.

II. Importance of soil composition and of properties of fertilizers being introduced.

It is quite natural that the problems of plants' resistance to rust should be studied parallel with the evaluating of the effect of exterior conditions some of which have an influence on the plant, others can influence the rust organism, but most frequently have a complex effect changing the state of the plant and thus affecting the parasite. Among similar problems there is one, the importance of which is accepted without reservations by all -- it is the role of nutrient substances in the metabolism of the plant-host. Most closely connected with this problem is the problem of importance of fertilizers being introduced into the soil. General opinion in regard to that is apparently established as a result of observations over the manifestation of infection when some fertilizers or others were introduced and it summarizes that nitrogen substances (speaking most generally) increase the degree of susceptibility; potassium has a contrary action, furthering the manifestation of resistance; the role of phosphorus is not always clear, it seems to be capable of increasing or decreasing the degree of resistance (respectively -- of susceptibility) in relation to other conditions.

Chapter XI, pp. 225 - 231

Conditions for originating of epiphytotics of rust.

("Epiphytotiology" of rust)

General deliberations on rust epiphytotics. -- Role of conditions which further rust development and role of stores /? accumulations ?/ of the infection element (local or remote). -- Hibernation of rust in the uredo- and teleuto-stages; conditions necessary for it. -- Biological significance of individual rust development stages, -- Hibernation in the uredo-stage, -- Puccinia graminis. -- Deliberations on the impossibility of its hibernation in form of uredo-spores or uredo-mycelium in the majority of European countries. -- Conditions of East Siberia and peculiar behavior of rust. -- Information on hibernation procedures in other areas. -- Analogous reports from non-European countries, -- P. triticina and P. dispersa. -- Hibernation in form of uredo-spores and uredo-mycelium. -- Peculiarities in hibernation of the feeding plants themselves. -- The role of adjoining fields and of self-seeding. Conditions for hibernation of this species /? type ?/ in different parts of the USSR. -- P. glumarum. -- Possibility of hibernation in the uredo-stage. -- P. coronifera. -- Lack of possibility to hibernate in the uredo-stage. -- P. anomala. -- Originating of primary infection when intermediate hosts are lacking and where it is impossible for rust to hibernate locally. -- Role of various sources of infection -- specialized forms and biotypes. -- Import of rust spores by air currents. -- General information. -- Height of spores' elevation and conditions furthering it. -- Theoretical information on rust fungi as "anemokhornyi" /anemophilous ?/ organisms. -- Physical and biological borderlines for spreading. -- Regularities in distribution of rust spores through the air. -- Deliberations on the power of the infection source. -- Typical case of air migration. -- Settling of spores. -- Dynamics of air distribution of spores. -- Degree of probability for settling of spores on a suitable plant. -- Regularities in spreading of rust spores through the air. -- Concrete examples. -- Hypotheses on air migration of rust species for different areas in the USSR and for other countries. -- Rust years. -- Possibility of importing the spores with the seeding material. -- Role of uredo-spores as a mechanical admixture and the role of uredo-pustules which originate on the grain. -- Difficulties in contamination of cereals with uredo-spores from seeds. -- Importance of this circumstance in eternal changes of local micro-flora. -- Examples of introduction of rust species in this manner. -- Imported rust species. -- Prognosis in regard to rust.-- Long-term and short-term prognosis.

I. General deliberations on rust epiphytotics.

The most severe rust epiphytotics as those of 1932 which spread in almost all the countries of the temperate zone of the northern hemisphere, as well as those of almost equal force but which spread over smaller areas, as in our country in 1933, -- were accompanied with a considerable decrease in yields of one or several cereals and therefore attracted the attention of very wide circles. And it is characteristic that their appearance was always unexpected not only for wide masses of agricultural population but also for specialists - phytopathologists. This leads to a quite definite conclusion that we do not possess yet the necessary elements for forecasting severe (or rather -- any kind) rust epiphytotics. In reality the situation in regard to this problem is even worse, because we not only are not able to make a prognosis for the future but very often cannot explain satisfactorily the phenomena in the past, and in particular, -- not always are the causes of flare-ups of rust development in a certain place at a certain time clear to ourselves. It is probably due to the fact that the problem of epiphytotics is too little advanced in phytopathology, while there is a large analogous section in medicine -- epidemiology.

Underlying are particular historical reasons connected with the general course of development of phytopathology on which there is no possibility to expand here. There is no doubt that should sucha section exist, its central object would be rust of cereals. Thus the conclusion: taking into consideration the great practical significance of the possibility of making a prognosis of rust development and its spreading, it is better to start late than never to gather facts on the character of origin of epiphytotics and on the ways of their development so that the obtained generalization could become the foundation of this new chapter of phytopathology.

The necessity of starting such independent section is dictated from the theoretical point of view by the fact that there actually are not yet data needed for a prognosis. It would be erroneous to think that for the explanation of causes of rust manifestation in some degree or other it is enough to know the development cycle of the given species and to know how to take into consideration the meaning of exterior conditions; simple comparing of one and the other does not lead us yet to a complete notion of the causes which brought about the given form of rust manifestation. There is no doubt that the knowledge of the development cycle and the mastering of the course of meteorological elements can serve as decisive moments in the solving of the problem, but that is not all: ontogenesis of parasite supplemented by meteorology does not give yet in the sum a science of epiphytotics, the latter is a derivative of one and the other but placed on a higher level. The correctness of this is especially easily demonstrated in regard to insufficiency of the development cycle; it is determined by way of studying the individual and therefore there can be mention of development cycle in regard to the individual only; applied to a mass of individuals the notion of development cycle becomes less definite, because under natural conditions,

having to do with an endless number of individuals, there is no possibility of proving the passing by each individual through the standard development cycle.

Thus, in seeing a contaminated field, who could trace by which way each given uredo-spore developed? Is it an element in one of the links of uredo-generations which develop locally, or did it hibernate under the given conditions, finally, was it not imported from far away by air currents? If it is of local origin, what is its age, did it originate immediately after the inoculation of the cereal or was it formed in subsequent uredo-generations? If the latter is correct then the assumption of gradual accumulation of the infection element with the creation of an infection nucleus obtains a weighty reinforcement.

It is hard to consider it possible that the development process of two uredo-spores of one species picked at random are absolutely different. Therefore the difficulty in determining the course of primary infection of cereals, and in the latter is the key to the study of epiphytotics.

One cannot think of a more complex and intricate problem than the deciphering of ways of origin of field contamination with rust. It can really be compared with solving of equations with several unknowns, since usually all the elements in the presentation of the problem itself -- original source of infections, degree of its remoteness, ways of distribution of the infection element, the role of the local infection at that time, in form of aecidio-spores or uredo-spores, -- all this, for each example being analysed, is in majority of cases not definite but only hypothetical.

However the state of the problem is not entirely hopeless. If there are not always direct data, indirect considerations can be always drawn. Thus in judging the moments which determine epiphytotics usually these two facts are mentioned: abundance of local or imported infection element and especially favorable meteorological conditions for rust development. Actually one of these conditions is sufficient for originating of epiphytotics and for explanation of this phenomenon, but to which condition should the leading role be ascribed? The abundance of the infection element itself must have at a certain time also been conditioned by a similar effect of favorable conditions on rust, which caused an intensive accumulation of the contagious element. To aid in this and similar cases comes an indirect consideration, that if the leading role belongs to a favorable combination of meteorological factors and to the abundance of rust spores, then it [contagious element] should spread over the majority of rust species, causing general rust epiphytotics and not only epiphytotics on one plant species caused by one rust species.

The practice teaches that this is how it really occurs and that in years of intensive development of one rust, the curve of intensiveness of affection rises also for other species belonging to one ecological group. Therefore it is much simpler to suppose that the leading role belongs nevertheless to exterior factors (weather etc.) which affect all the species which are characterized by the same ecological indicators, almost equally, -- and not to the excess of the infection element, which would hardly be excessive for all the rust species during the same years.

Judgement on the role of local infection can be passed according to the increase in the affection degree of fields in areas where importation of rust is usually lacking. Among those the Voronezh oblast' can be mentioned, where there is apparently no importation of uredospores of P. coronifera from outside, at the same time, according to Gorlenko's data (1934) it follows, that in recording rust on August 10, the amount of affected plants was determined as 4.8% and 5 days later it increased more than 5 times and reached 25.3%.

The problem appears to us in such a form, that for a correct clarification of the process of originating of epiphytotics it is necessary to obtain beforehand a precise answer to following questions: 1) what is the source of primary infection and what is the course of this infection? 2) what is the source of secondary infection and what is the course of this infection?

Both questions can lead to the determining of local and distant sources of the infection element and in the second case it will be necessary to determine the ways of importation of the parasite (its introduction).

Among the conclusions which can be drawn from all the preceding, is the possibility of determining the course of distribution of the infection element and of substantiating the prognosis of its further development.

II. Over-wintering of rust in the uredo- and teleuto-stage; conditions necessary for it.

Usual notions of rust are always connected with a statement, that the uredo stage is a "summer", propagative stage, while the teleuto-spores have to be considered as a hibernating stage. If this is true as a basic scheme,[1] it is not always correct in details: many single cases are observed which deviate considerably from it. In any case, besides the teleuto-stage the uredo-stage also frequently participates in the hibernation of the fungus and that not as an exception but as a rule (for some species).

Thus examining the participation of the uredo-stage in the hibernation process it is necessary to foresee, that a successful hibernation can be explained differently, namely: 1) hibernation of uredo-spores which originated in the summer or before the fall, 2) hibernation of uredo-mycelium, established in affected organs since the fall and capable to wake up to life in the spring, to start new uredo-spores, and, finally, 3) possibility to originate several successive generations of uredo-spores with each newly formed generation serving as an infection source for the next one. There can, of course, be a mixed case, namely 4) when, with the capacity of uredo-spores to hibernate, the mycelium preserves its viability, and the case 5) when, beside them, the teleuto-spores hibernate as well.

In solving the problem of the role of hibernating uredo-spores it is necessary to consider not only the uredo-spores which are formed on winter crops but also those which can be formed on the grain within

---

1. Actually for the entire group of rust fungi as a whole, the problem is much more complex, because there are numerous other hibernation cases which give evidence that the adjustment process to exterior conditions followed different roads. All the known cases used as examples can be presented in the following table:

Only III stage		Only II stage		II + III stages	I stage	
spores	mycelium	spores	mycelium	spores and mycelium	spores	mycelium
Many species of rust fungi on cereals: *P. graminis* *P. coronifera* *P. anomala* and majority of species on wild-growing plants.	Genus Gymnosporangium	Rarely	Rarely	*P.glumarum* *P.triticina* *P.dispersa* *Melampsorella caryophyllacearum*	Puccinastrum	Genus Cromartium /? not clear/

the limits of pericarp layers, or which can get into the tuft [?] and be preserved there among its hairs.

Much attention was paid to this problem by English and French researchers at the beginning of the second decade of our century (Pritchard, Bovery and others). But exact tests led always to negative results in the majority of rust species (P. coronifera (Kherner, 1921), P. triticina).

Among possible explanations can be given that of young sprouts being in general considered, even in the most susceptible varieties, as quite resistant to rust contamination, also, that if uredo-spores introduced into the soil with the grain do not cause infection immediately -- chances with the elapsing of time will decrease and not increase; thus the possibility of their importation on the green parts of plants is gradually falling off.

Thus a case is observed here contrary to that which occurs with smut, when contamination of seeds with spores always causes contamination of the cereal. In connection with this should be mentioned that cases were recorded of importation of P. dispersa and other rust species with seeds.

Since a survey of conditions necessary for hibernation of teleuto-spores of the basic rust species is given in chapters VI and X, it should be sufficient here to present facts and notions in regard to hibernation of rust fungi in uredo-stage, for all the variants of this case, as well as for the mixed case no. 5, when both uredo- and teleuto-stage hibernate.

1. Puccinia graminis Pers.

Over-wintering in the uredo-spore stage.

In order to pass a judgement on the possibility of such hibernation and its role, it is necessary if possible to proceed from data on life duration of uredo-spores. It was experimentally established by A. A. Iachevskii (1909) that the life span for uredo-spores of stem rust of summer origin does not exceed 2 weeks (it is possible that the period is longer for the fall ones).

Apparently other possibilities have to be sought for this.

By the way, research by Erikson in Sweden (1896 and earlier), by Gassner and Pishel (1935) in Germany, by Nielsen (1875) in Denmark, by Rusakov in our country -- lead quite definitely to the statement of impossibility to hibernate for this species in the form of uredo-spores in either of the North-European countries. Detailed observations in bio-ecology of uredo-spores of this species make it possible to note that in the fall there are extremely unfavorable conditions

for preservation of this stage; the spores are rapidly washed off by lasting and intensive fall rains, they enter the soil and perish there not being able to cause an infection even if there was a possibility for it provided by other circumstances. Probably in areas of East Siberia with its peculiar conditions similar occurrences take place. In any case the problem requires further profound attention.

Possibility of over-wintering of the uredo-mycelium

The resistance of this stage of the given species is well known from direct observations by many researchers-- there is not even any need to quote them-- and it is extremely insignificant in regard to low temperatures; therefore the statement that the mycelium of this species, in countries well investigated in this respect (Europe, with possible exception of the extreme South-West, U.S.A., Canada), does not hibernate-- is absolutely indisputable.

The problem of the possibility of P. graminis over-wintering in the II stage (uredo-spores or uredo-mycelium) is solved positively on the basis of precise observations and experiments only for a number of countries with a mild climate. Such is the state of the matter in Brazil-- according to data by Gassner (1916), in Texas, Mexico and Mississippi basin-- according to data by Stakman (1931), in Australia-- according to data by Vatergus (1929) etc. The question is how likely it is to expect a manifestation of a similar capacity of this rust species to hibernate under conditions of Europe, in particular on the USSR territory? Since this problem is not solved experimentally even for the southern-most points of our territory, the majority of indirect data impel us to accept fully Prof. A. A. Iachevskii's and L. F. Rusakov's conclusions on the impossibility of hibernation of P. graminis in form of uredo-spores, as well as in the stage of uredo-mycelium. At least there are no such facts which would demand the opposite. It will be appropriate to mention here that for a number of rust species (P. triticina, P. glumarum, P. dispersa) the capacity of preservation from year to year in the II stage has been proved quite accurately.

An indirect but very important confirmation of such a statement on the impossibility of hibernation of the given species in uredo-stage (spores or mycelium) for any point in the European USSR are the general hibernation laws of this species in the uredo-spore stage established by Gassner. It appears that not even all the subtropical countries are able to provide all needed conditions for a successful uninterrupted hibernation of this species and it is possible only for countries north of the 35° of northern latitude, but as a regular phenomenon such hibernation takes place only south of the 30 - 25° parallel (Gassner, 1916).

Quite distinct stands our East Siberia with its peculiar severe climate. From Bryzgalova's data (report in the section for mycology and phytopathology of the National Botanical Society on December 7,1935)

follows that the given rust species under conditions of East Siberia does not originate teleuto-spores (on Agropyrum) and goes under the snow as powerfully formed heaps of uredo-spores. According to the East Siberian STAZRA /Station for plant protection?/ the first appearance of linear /stem/ rust on rye occurs at the end of July - August; at this time rye is in the stage of wax ripeness. There is a basis to assume that this rust species transfers under local conditions from Agropyrum repens because its appearance occurs most frequently on borders of fields which are adjacent to "mezhniki" /intermediate spaces?/ overgrown with couch grass contaminated with linear /stem/ rust. Here, according to V. A. Bryzgalova's observations, P. graminis never forms teleuto-stages on rye and goes to hibernate on it and on couch grass in uredo stage.

It is evident that taking all these indications as a basis, the entire eastern part of the Asiatic continent, the Far East and East Siberia are under particular conditions in regard to rust: the climatic conditions are not favorable either for /secondary/ maturing of teleuto-spores or for their germination in the spring; in East Siberia the teleuto-spores of linear rust are not even initiated. All this leads to the following: basidia do not develop, aecidia are not initiated and the role of the intermediate hosts (at least of barberry) is brought down to an extremely slight importance. On the other hand where the teleuto-spores are formed (as, for example, the teleuto-spores of P. triticina in the Amur oblast'), they are initiated extremely early-- starting with the end of June (Rusakov, 1925). It is necessary to compare with this also the peculiar specialization in East Siberia of the brown rust of wheat (P. triticina) in aecidial stage which affects here and nowhere else the Isopyrum (Leptopyrum) fumarioides.

Under the conditions of the sub-taiga belt of Siberia, according to Smirnova's data (1935), the main source of rust contamination for grain cereals is the flora of wild-growing perennial grasses; thus it has been proved through experiments that P. graminis f. tritici found on couch grass (Agropyrum repens) was capable of contaminating all the species of grain crops; but especially readily contaminated with this material were winter rye and winter wheat. The same can be said also about P. phlei-pratensis obtained from timothy and which is also capable to contaminate all species of cereals, but most readily-- winter rye and barley. The third species-- P. gibberosa Lagerh. (??) from Festuca pratensis was capable to contaminate winter rye and winter and spring wheat. On the other hand, according to data by the same author, which, in our opinion, emphatically require to be checked, the aecidia-spores of P. persistens taken from Thalictrum simplex contaminate the couch grass; thus taking into consideration the possibility of passing of rust from couch grass to rye and wheat, it has to be admitted that T. simplex is a kind of an intermediate host for wheat and rye leaf rust. Further Puccina triticina is capable, -- as the results of works of the same study indicate,-- of contaminating rye, which serves as a reason to assume the presence here of a specific particular biotype of P. triticina.

Due to all that, according to Smirnova's data, an assumption can be made on the capacity of rust species which parasitize cereals, of over-wintering as teleuto-spores on wild cereals producing in the spring aecidia on crowfoot species.

There are reports that in the Tsimlianskaia stanitsa /village/ (Rostov oblast'), since barberry is absent there, an important role in rust (linear) hibernation has to be played by the uredo-stage. No data are given for it, but it is immediately mentioned: importation of inoculum is possible also by winds (Popova, report 1935). Apparently there is a complete confusion about this problem. Such information does not contribute anything to solving of the problem on a local scale and does not further the clarification of general regularities.

Speaking for the possibility of hibernation of rust in uredo-stage, or, possibly, for the importation with air currents, are the facts mentioned by many researchers, that the uredo-spore stage can be disclosed earlier than the beginning of distribution of aecidia-spores (Murashkinskii and Kleimenov, (1912) -- observations during 1911 and 1912; Rusakov and Shitikova (1927) and most complete observations /?/).

As to the information on the possibility of local hibernation of uredo-spores of linear /stem/ rust in other countries, both European and non-European, the basic data comes down to the following.

Among areas (where it is known) besides Australia (a certain doubt exists) in tropical parts of North and South America -- California can be also mentioned. Due to a mild climate, the uredo-stage of P. graminis exists there the year round. On the contrary, linear /stem/ rust does not hibernate either in form of uredo-spores or in that of uredo-mycelium in following countries and areas: the entire European USSR, the entire West Europe with possible exception of some single southern-most areas in Italy (according to Montemartini, 1914), where P. graminis hibernates as uredo-spores on respective meadow grasses.

In regard to Portugal a question arises whether P. graminis can hibernate there, especially in its southern provinces. This question is raised in Bensod's work (1929 - 1930) on rust of cereals, but it is not answered. Neither does it hibernate in uredo-stage in Canada, Oklahoma, Kansas, Missouri, Kentucky, Nebraska(Stakman, 1918, also Meki, 1931), in Minnesota (Kherner, 1921). Due to the fact that barberry is of little consequence in the matter of spring regeneration and distribution of rust in West Canada and the uredo-mycelium and uredo-spores do not hibernate there, the source of primary infection for the fields (P. graminis) is probably beyond the borders of Canada, in the U.S.A. In Canada it appears first of all in the South and then spreads north and west (Beilei /Bailey?/, 1923).

Goriachenkova, E. V.

Ferment chesnoka, obrazuiushchii allicin (alliinaza) - proteid fosfopiridoksalia.

Garlic enzyme producing allicin (alliinase) - proteid of phosphopyridoxal.

Doklady Akademii Nauk SSSR. 87(3) :457-460. November 21, 1952. 511 P44A

(In Russian).

GARLIC ENZYME PRODUCING ALLICIN (ALLIINASE)- PROTEID OF PHOSPHOPYRIDOXAL

(Submitted by Academician A. I. Oparin, September 26, 1952)

470

B. P. Tokin (1) deserves credit for the discovery, in the paste obtained from the cloves of garlic, of the volatile antibacterial substance (phytoncide) and for its adaptation to clinical practice. Caballito and his collaborators (2) isolated the antibacterial active element of garlic in pure form and named it allicin. They established that this substance (with the characteristic odor of garlic) represents oxide of diallyldisulfide and is formed, by fermentation, from a more complex molecule on injuring the cloves of garlic.

The mechanism of the formation of allicin and the nature of its predecessor were explained by Stoll and Seebeck (3). These authors isolated from garlic a peculiar sulfur-containing aminoacid, (4) S-allyl-L-cysteinesulfoxide, which they named alliin. Under the influence of a specific enzyme, allinase, pulverizing the cloves of a garlic brings about a quick splitting up of alliin [coupled] with the formation of allicin, ammonia and pyroracemic acid; the transformation occurs as follows (3):

$$CH_2=CH-CH_2-\overset{\overset{O}{\|}}{S}-CH_2-CH(NH_2)-COOH \longrightarrow$$

alliin

$$\longrightarrow CH_2=\overset{\overset{O}{\|}}{S}H-CH_2-SH+CH_2=C(NH_2)-COOH; \qquad (1)$$

allilsulfonic acid — a-aminoacrilic acid

$$CH_2=C(NH_2)-COOH \xrightarrow{+H_2O} NH_3+CH_3-CO-COOH; \qquad (2)$$

$$2\left[CH_2=CH-CH_2-\overset{\overset{O}{\|}}{S}H\right] \xrightarrow{-H_2O} CH_2=CH-CH_2-\overset{\overset{O}{\|}}{S}-S-CH_2-CH=CH_2 \qquad (3)$$

allicin

Allinase catalyzes a reaction (1); transformations (2) and (3) proceed spontaneously at great speed.

In the B-position. There is a series of enzyme reactions in which the a-aminoacids with less sharp polar substitutes in the B-position, namely, tryptophane, cysteine, various thioethers of cysteine, serine and theronine, are subject to splitting in the overall scheme (4) in accordance with the above reactions (1÷2) (6):

$$XCH_2\text{-}CH(NH_2)\text{-}COOH \rightarrow XH + CH_2{=}C(NH_2)\text{-}COOH \rightarrow$$

$$+H_2O \rightarrow NH_3 + CH_3\text{-}CO\text{-}COOH.$$

In recent years it has been established that enzymes accomplishing the indicated transformations namely, tryptophanaze (7), cysteinedesulfhydrase (8) B-thionase (9,10), Deaminases of serine (11,12) and threonine (12), are proteids of phosphopyridoxal.

A. E. Braunshtein and M. M. Shemiakin (6), who developed the general transformation theory of amino acids catalysed by pyridoxal enzymes, have pointed out the probable, pyridoxal-proteidin nature of alliinase.

In the present investigation we cite experimental evidence confirming this hypothesis. It appears probable that alliin is formed in the plant by the oxidation of S-allyl-L-cysteine (desoxyalliin) and that the latter is synthesized by the condensation of allylsulfide with serine (or, possibly, with cysteine), assisted by the phosphopyridoxal enzyme (see mechanism of the synthesis of cystathionine (9) and other B-substituted a-aminoacids (6)).

EXPERIMENTAL PART

Experiments were conducted with the synthetic preparation of alliin containing besides the inherent (+)S-allyl-L-cysteinesulfoxide, its (-)S-diastereoisomer; we synthesized the preparation through oxidation with hydrogen peroide of L-desoxyalliin obtained from allylbromide and L-cysteine (4); both diastereoisomers are split by alliinase, with the splitting up of the (+)S-isomer proceeding at great speed.

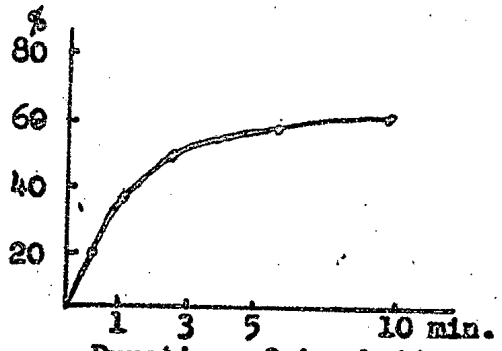


Fig. 1. Fission of alliin by alliinase

To prepare the solution of a partly purified alliinase (5) the cloves of garlic were quickly pulverized while being cooled in a mortar with quartz sand and 4 volumes of distilled water. The paste obtained was heated for 20 min. at 37° [C] and centrifuged. After the centrifuging, a 10% solution of CH_3COOH at pH 5.5 was added to the supernatant. The precipitate suspended in a phosphate buffer (M/15, pH 6.4) was used in experiments in the capacity of an alliinase preparation.

Certain experiments utilized partly purified extracts of alliinase inactivated ("apoenzyme" [apoferment]) by various means: 1) by dialysis against 0.01 M acetate buffer pH 5.0; 2) by ultraviolet radiation at a distance of 15 cm from a mercury quartz lamp for 60-90 min. and 3) by "aging" the enzyme through storage under toluene at 1-3° [C] (up to 14 days).

The activity of alliinase was tested as follows. Test specimens containing 0.5 ml [milliliter] of enzyme extract, 10 mg [milligram] of alliin and 2 ml of phosphate buffer (M/15, pH 6.4) common in a total volume of 5 ml. were incubated for 10 min. at 37° [C]. In trichloracetic filtrates of the experimental specimens, the increment of ammonia was determined by the Convey-Byrne [Konvei-Birn] method and, in some experiments, the formation of pyroracemic acid, by the direct colorimetric determination of 2.4-dinitrophenylhydrazones of keto-acids (13)

Phosphopyridoxal (PP [FP]) was added in the form of pure Mg-salt (14) synthesized from pyridoxine by A. E. Braunshtein and R. M. Azarkh. Its actvity per unit weight is approximately 2-1/2 - 3 times higher than the activity of the preparation of PP [FP] Ba-salt utilized in previous work of our laboratory.

The splitting of the alliin preparation by alliinase, which we obtained in the is depicted graphically on fig. 1. The results cited show that the process of alliin splitting proceeds very rapidly; in 10 min. of incubation the decrease in the substrate reach 60-70%. Proceeding from this premise, all experimental specimens were incubated for 10 min.

Table 1.

Influence of Chemical agents upon the Activity of Alliinase.

Molar Concentration of poison	Hydroxylamine: Formation of N-NH3 in mg/g	Hydroxylamine: Inhibition in %	Phenylhydrazine: Formation N-NH3 in mg/g	Phenylhydrazine: Inhibition in %	Semicarbazide: Formation N-NH3 in mg/g	Semicarbazide: Inhibition in %
Control	3.39	--	3.39	--	3.39	--
2.10-3	0	100	0.10	97.2	0.42	87/6
10-3	0.16	95.2	0.19	94.4	0.42	87.6
10-4	0.16	95.2	2.42	28.7	2.06	39.3

As it is shown in table 1, the chemical agents obstructing the carbonyl group (hydroxylamine, phenylhydrazine and semicarbazide) inhibit the action of alliinase in concentration 10-3 M almost completely, with the greatest inhibition being caused by hydroxylamine.

In partly purified alliinase extracts the increment of ammonia nitrogen.

]

owing to the splitting of alliin, constitutes on the average 4.5 mg per gram of the initial garlic cloves (table 2). The numbers cited on table 2 show that the activity of alliinase decreases gradually in proportion to the length of storage at 1-3°C (for example, by 35-40% in 9 days and by 73% in 12 days), and, likewise, during dialysis against an acid buffer solution (by 42% in 20 hours and 75% in 48 hours.). Adding to such alliinase extracts ("apoenzymes") phosphopyridoxal in the amount of 20 y/5 ml reduces enzyme activity almost to its original level.

Table 2.

Activation by phosphopyridoxal of alliinase inactivated by means of "aging", dialysis or ultraviolet radiation (formation of $N\text{-}NH_3$ in mg per gram of garlic)

Activity after storage				Activity after dialysis				Activity after ultraviolet radiation			
Initial Activity	Duration of storage in days	without additions	with PP[FP] (20 y)	Initial activity	Duration of dialysis in hours	without additions	with PP [FP] (20 y)	Initial activity	Duration of radiation in hours	without additions	with PP [FP] (20 y)
4.20	7	3.06	4.40	4.20	8	4.07	--	4.60	1	0.37	1.27
4.65	8	2.79	4.65	4.20	20	2.44	3.84	4.70	1	1.08	2.39
4.40	9	3.46	4.40	3.60	26	1.34	3.19	4.70	1.5	0.30	1.21
4.10	9	2.65	4.07	4.68	48	1.28	2.25				
4.65	12	1.24	3.12								

Ultraviolet radiation of a freshly purified alliinase solution for a period of 60-90 min. reduces considerably (by 70-90%) the cleavage of alliin. On adding PP [FP] to such extracts, the activity of alliinase becomes partly reduced without reaching the initial level. An analogous phenomenon was observed in experiments in which the alliinase extract used had been dialyzed for 48 hours or stored 12 days, which, obviously, can be explained by the partially destroyed albuminoid part of the enzyme.

In Fig. 2 a curve is shown demonstrating the dependency of alliin splitting on the amount of PP[FP] added to alliinase extract with a low activity (on the 9th day of storage at 1-3°[C]). The rates of alliin cleavage are shown in micromoles N - NH_3(1) and of pyroracemic acid (2) in the specimen; with a concentration of PP[FP] 5y/5 ml a maximum activity is reached.

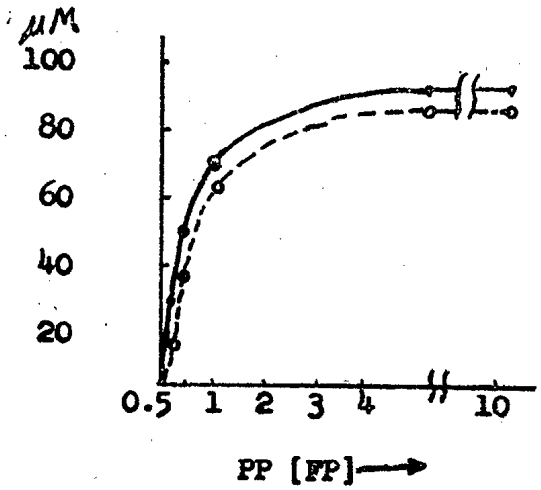


Fig. 2 Cleavage of alliin by allinase (stored 9 days) in relation to the added amount of PP [FP]. 1--N--NH_3; 2--keto acids (PP[FP]-- in γ per specimen of 5 ml dimensions; N--NH_3 and keto acid-- in μM per gr of garlic)

CONCLUSIONS. The alliinase of garlic possesses a high senitivity to enzyme toxins inhibiting the carbonyl group. The activity of the "apoenzyme" of alliinase obtained through dialysis, "aging" or ultraviolet radiation is reduced on adding synthetic PP [FP]. These data show that the enzyme of garlic forming allicin is a proteid of phosphopyridoxal.

Received
September 6, 1952

REFERENCES CITED.

[1] B. P. Tokin, Phytoncides, M., 1948. [2]C. Cavzallito et al., Jour. Am. Chem. Soc., 66, 1952 (1844); 67, 1032 (1945). [3]A. Stoll, E. Seebeck, Adv. Enzymol., 11, 377 (1951). [5] A. Stoll, E. Seebeck, ibid, 32, 197 (1949). [6] A. Braunshtein, M. Shemiakin, DAN [Reports Academy of Sciences] 85, No. 5 (1952). [7] W. Wood et al., Jour. Biol. Chem., 170, 313 (1947). [8]A. Braunshtein, R. Azarkh, DAN, 71, 93 (1950); R. Azarkh, V. Gladkova, DAN, 85, No.1 (1952). [9]A. Braunshtein, E. Goriachenkova, DAN, 74, 429 (1950); E. Gorianchenkova, DAN, 85, No. 3 (1952). [10]F. Blinkley et al., Jour. Biol. Chem., 194, 109 (1952). [11]C. Yanofsky, ibid., 194, 279 (1952). [12]J. Reissig, Arch. Biochem. Biophys., 36, 234 (1952). [13]A. Braunshtein, R. Azarkh, DAN, 85, No. 2 (1952). [14]M. Viscontini et al, Helv. Chim. Acta, 34, 1834, 2198 (1951)

Trans. 471
(In Part)
By:
R. Adelman

Beilin, I. G.

Epifitii rzhavchin na pshenitse za poslednie gody na severnom Kavkaze i faktory, sposobstvovavshie ikh vozniknoveniiu i rasvitiiu

[Recent Wheat rust epidemics in North Caucasus and factors favouring their outbreak and development].

Izvestiia Akademiia Nauk SSSR, no. 5-6, 1938, pp. 995-1016 (In Russian)
(Trans. in part - p. 996)

In the year 1933 there was an outbreak of epiphyte /epifitiia/ of P. graminis. In the central and southern parts of the Azovo-Blacksea Territory and in the North-Caucasus Territory it reduced the yield by almost half and, according to Kuzmichev's data, in separate kolkhozes, for example, those of the Georgievsk Region, the crop dropped to 1.2 c per he. In 1935 there was a recurrence of epiphyte of P. triticina, in 1936 - of P. graminis and in the South /there was/ even P. glumarum. In the Azov-Black Sea coastal territory, especially in the Ordzhonikidzevsk Region /oblast'/ (within the new borders), the development of epiphyte of P. glumarum was intensive and it determined that year the quality and quantity of crop. Here are several illustrations of the role of P. glumarum in the year 1937.

In the Mozdok Region, the grain from an area of approximately 20 thousand he of the standard variety Kooperatorka, non-resistant to this type of rust, was so fine and sickly that seed grain for this entire area had to be imported from other regions. In the Georgievsk Region in a number of kolkhozes the yield dropped as low as 4-6 c per he, while the yield of the more resistant Stavropol'ka, grown on the same kind of soil and during the same planting period was twice as large. In the Tersk Region, Kooperatorka yielded an average 6-8 c per he, yet Stavropol'ka yielded 17 c. Table 3 shows the influence yellow rust exerted that year on the quality and quantity of wheat crops on one of the test sectors - Essentuki - under foot-hill conditions, where the 1937 wheat crops had been infected only by this rust.

Trans. 471
(In Part)
p. 996
By:
A. Antik

EXCERPT FROM :

BEILIN, I.G. Epiphytes of rust on wheat in recent years in North Caucasus and contributing factors for their formation and development.

Trans. 472
(In full)
By:
A. Antik

Smirnova, V. A.

O prigodnosti durmana (Datura stromonium) dlia opredeleniia titra virusa tabachnoi mozaiki

[Usefulness of Datura stramonium for determining the titre of tobacco mosaic virus].

Mikrobiologiia 22:714-718. Nov./Dec. 1953
448.3 M582 (In Russian)

Institut Mikrobiologii Akademii Nauk SSSR

It is hardly possible to overestimate the significance of plant-indicators in virological practice. All the works on plants' viruses connected with the determination of the titer of the virus, its activity, speed of accumulation etc., are conducted with the help of plant-indicators. An accessible and sensitive indicator speeds up the work and increases its reliability.

Holmes in 1929 was the first to take Nicotiana glutinosa (1) for a quantitative calculation of the virus titer of tobacco mosaic (TMV). Now it is the most widely used indicator for TMV here in the USSR as well as abroad.

472

N. glutinosa is a southern plant, it is quite difficult to grow it under our conditions. This plant easily decreases in sensitivity to TMV in aging as well as with a slight worsening of the growing conditions. One N. glutinosa plant produces 5-6 leaves suitable for work.

Some varieties of kidney beans produce a necrotic reaction to TMV, but since only the first two leaves are sensitive, for extensive work with this object a large area is needed and continous additional sowings.

The hybrid N. glutinosa x N. tabacum is used as an indicator; it is characterized by the capacity inherited from N. glutinosa to produce local lesions without a general infection, but it also has a series of shortcomings: too large leaves, necessity to breed with the help of seedlings etc.

There are works (Gendron (2), Manil (3), Pfankuch (4) etc.) in which Jimson weed (Datura stromonium) was used for the determination of the TMV titer, but as an indicator this plant enjoys little popularity.

Trans. 472
(In full)
By: A. Antik

The Jimson weed is a seed spread in the South of the USSR and found in the Moscow oblast'; it reproduces here excellently through self-sowing, producing shoots early in the spring; it goes under the snow with leaves not fallen off and even not yellowed; it is extremely tolerant regarding cultivation and resistant to low temperature. A bush of Jimson weed produces a large number of uniform leaves and speedily regenerates them after they are picked off.

All these properties did not cause any doubt in its advantage as compared with N. glutinosa, but in order to use it with certainty as an indicator it was necessary to check its reactive properties which we did by comparing it with N. glutinosa.

Part of the tests were conducted especially with this purpose, part were conducted in passing, i.e.; in current experiments where Nicotina glutinosa served as a basic indicator, Datura stromonium was taken for comparison.

Usually in virological pracitce the inoculation of leaves is carried out on the plant itself. In our laboratory, according to Ryzhkov's suggestion, we are working with isolated leaves, which simplifies the methods considerably.

After inoculation, we place the cut leaves into a moist chamber, usually a desiccator, suspending them in rows and seeing to it that they do not touch each other. This method had one more advantage that the moist chamber can be easily placed at a temperature optimum for the development of lesions.

In the experiments being described, the leaves of N. glutinosa and Datura stromonium being compared were always placed together in one desiccator.

(begin p. 715)

A preliminary experiment for checking of the sinsitivity of the indicators being tested, was conducted as follows; leaves of N. glutionsa and Jimson weed approximately equal in size were inoculated with juice of the diseased tobacco in various dilutions (table 1).

Table 1 (p. 715)

Number of necroses on whole leaves inoculated with sap of diseased tobacco at various dilutions (N. glutinosa / D. stromonium)

Trans. 472
(In full)
By: A. Antik

Date	Solutions	Total number of necroses	Number of leaves	Mean number of necroses per 1 leaf
26.VI 1951 r.	1:100	110/522	6/6	18/87
	1:1000	59/217	5/5	12/43
	1:10000	22/40	5/5	4/8

Remark: In table 1 as in all the following tables in the numerator are given the numbers of local lesions on N. glutinosa and in the denominator- on Jimson weed

In all three dilutions the Jimson weed appeared to be considerably more sensitive than the N. glutinosa. It is necessary to point out, that the maturing of local lesions on Jimson weed starts several hours later than on N. glutinosa. Having in mind to characterize the Jimson weed in its sensitivity to TMV under conditions related to those of open ground, we conducted tests during the hottest period of the summer and in the fall at low termperatures, on plants grown in a green house.

In the middle of July the temperature in the green house reached 40° during the day. The test was conducted on 15. VII. (July 15). After the inoculation the picked leaves were kept in a moist chamber in the laboratory where the maximum temperature was 35° and it was never below 24°.

(begin p. 716)

In the test of July 15 (table 2) the Jimson weed produced in all three dilutions about 3 times fewer local lesions than N. glutinosa but the latter formed a small number of them as well.

Table 2 (p. 715)

Number of necroses produced by N. glutinosa / D. stromonium during the hot period of summer and in the fall.

Trans. 472
(In full)
By: A. Antik

Date	Dilution of sap on half leaves		Mean number of necroses on a leaf		Number of leaves in the test
	left	right	left half	right half	
15.VII 1951 r.	1:500	1:750	7 / 2,5	6 / 2,5	18 / 17
	1:500	1:1000	13 / 3,5	6 / 2	16 / 16
24.IX 1951 r.		1:1	4,0 / 200	3,2 / 170	10 / 10
		1:2	3,7 / 210	2,4 / 165	10 / 10

We conducted the second test at the end of September when the temperature at night went down to 4°, During the maturing of necroses the desiccator with inoculated leaves was at a 18-20° room temperature. Counting on low sensitivity of indicators, the sap of the diseased tobacco was taken without dilution. in ratio 1:1 and 1:2.

Under the conditions of the given experiment N. glutinosa formed 2-4 necroses per half leaf, and Jimson weed - up to 200, i.e. 50 times more. In tables 3 and 4 are presented in a different connection large amounts of lesions produced by thorn apple growing in the green house in October.

In table 3 are compiled the results of a series of tests in which the sensitivity of indicators to various concentrations of TMV was checked. Dilutions were taken from 1:100 to 1:500000.

Table 3 (p. 716)

Number of lesions on N. glutinosa / D. strominum with various dilutions of sap of diseased tobacco.

Trans. 472
(In full)
By: A. Antik

Date	Dilution of sap on half leaves		Mean number of necroses per half leaf		Number of leaves in the test
	left	right	left	right	
1952 r. 26.VII	1:100	1:120	7/23	11/18	18/23
	1:100	1:150	2,8/15	3,3/9	21/23
1951 r. 29.IX	1:100	1:500	3,3/190	2/140	10/10
	1:500	1:750	0,6/156	0,7/96	10/10
9.X	1:1000	1:1200	2/5,6	1,5/5,4	4/12
	1:1000	1:1500	0,6/6	1/3,5	5/14
10.X	1:1000	1:10000	0/14	0/2,5	3/9
	1:1000	1:100000	3,3/10,5	0,3/0,8	3/10
	1:1000	1:500000	0,6/14,3	0/0,1	6/8

In all cases the Jimson weed appeared to be more sensitive. Som etimes it formed amounts of necroses tens of times exceeding those of N.glutinosa In almost all our tests N. glutinosa reacts very weakly to the sap of diseased tobacco in a 1:1000 dilution, while the Jimson weed responds quite clearly at a 1:10000 dilution and only the 1:500000 ml concentration cannot be detected anymore.

The experiment of July 26 (table 3) is interesting. Here the thorn apple on 23 leaves produced ratios which in numbers of lesions are very close to the actual (begin p. 717) dilution of the sap. Namely: in comparing the sap diluted 1:100 and 1:120. The ratios of numbers of necroses on leaf halves of Jimson weed are 1:1.3; when the dilution of sap is 1:100 and 1:150 the ratio of the number of necroses is 1:1.6.

N. glutinosa did not show such regularity in this experiment. The high sensitivity of Jimson weed appeared to be particularly valuable in selecting plants carrying hidden TMV.

Trans. 472
(In full)
By: A. Antik

In order to check the sensitivity of indicator's leaves, the left halves were inoculated with the sap of a clearly diseased tobacco and the right halves - with the sap of the plant being tested (table 4).

Table 4(p. 717)

Checking of TMV content in plants being tested.

Date	No. of plants being tested	Number of necroses on leaf halves of Nicotiana glutinosa from sap of		Datura stromonium from sap of	
		clearly diseased tobacco	plant being tested	clearly diseased tobacco	plant being tested
1952 r. 9.IX	1	97	0	91	29
		72	0	270	37
		67	8	2000	176
31.VII	2	15	0	110	17
		11	0		
		45	4		
22.IX	3	0	0	172	140
		2	1	300	150
				500	50
22.IX	4	0	0	140	45
		10	0	130	7
				120	9

It is seen from table 4 that N. glutinosa producing large numbers of lesions on left halves (control), forms a negligible number of lesions from the sap of plants being tested and more frequently does not form them at all.

On the basis of the lack of lesions on N. glutinosa we should consider such plants as healthy, though in reality they are diseased as it is indicated by necroses on Jimson weed.

Summarizing the results of our experimental data we note that Jimson weed is a very sensitive indicator for TMV, a better one than the generally accepted N. glutinosa. Accessibility of the seed material, easy growing of Jimson weed from seeds in the open ground make the extensive use of it as an indicator for TMV possible not only in special virological laboratories but also for workers who have no facilities to cultivate in greenhouses such specific plants as N. glutinosa or the hybrid N. glutinosa x N. tabacum

Trans. 472
(In full)
By: A. Antik

Conclusions.

Under our conditions the Jimson weed as an indicator for the virus of tobacco mosaic excels in many characteristics the classical test-object- N. glutinosa.

1. Under equal conditions it produces a larger number of lesions per area unit than the N. glutinosa, i.e. it is more sensitive than the latter.

(begin p. 718)

2. Lesions develop well on Jimson weed leaves growing at low temperature.

3. The growing period of Jimson weed is longer, foliation richer, regeneration of leaves considerably faster than that of N. glutinosa.

4. Unlike the latter, the Jimson weed is very easy to cultivate.

We recommend using the thorn apple (Datura stromonium) in virological practice as an indicator for TMV.

Received: 31.XII. 1952

Institute of Microbiology
AN SSSR Moscow

Bibliography

1. Ryzhkov, V.L. Fundamentals of the study of virus diseases of plants. 1944.

Trans. 473
(In full)
By:
A. Antik

Konikova, A. S., Kritsman, M. G.,
Iakobson, L. M.

Obrazovanie aminoazota iz ammiaka i a-ketokislot suspenziiami B. subtilis

[Formation of amino nitrogen from ammonia and A-keto acids by a suspension of B. subtilis].

Biokhimiia 13(1):39-41, 1948
385 B523

(In Russian)

Formation of Amino Nitrogen from Ammonia and -keto Acids by Suspensions of B. subtilis

Synthesis of amino acids from ammonia and α-keto acids in bacteria has not been studied much.

Numerous attempts to disclose formation of amino acids in various species of microbes led mostly to negative results (1, 2, 3). Only Adler (4) and his coworkers succeeded to observe a fermentation system in B. coli which catalyzes the synthesis of glutamic acid from α-ketoglutaric acid and ammonia. Besides that, according to an oral report, Obel (5) observed in some species of Clostridium a synthesis of alanine from ammonia and pyroracemic acid. More in detail was studied in bacteria the peculiar process of formation of aspartic acid under the influence of aspartase enzyme from unsaturated aliphatic acid--fumaric and ammonia. As it was indicated (6, 7) it is characteristic to many species of facultative anaerobes. Our studies of the synthesis of amino acid in B. subtilis from various -keto acids and ammonia demonstrated that the given species of microbes synthesizes energetically the amino nitrogen from ammonia and -keto acids of aliphatic and aromatic series.

In the present report are given the data of experiments on amination with suspensions of B. subtilis of pyroracemic, oxalacetic, α-ketoglutaric and phenyl-pyroracemic acids. The results of these studies are presented in tables 1 and 2.

Experimental part

The experiments were conducted with thick suspensions of B. subtilis prepared from a 24 hour agar culture of these bacteria.

Table 1 [p. 397]

Synthesis of amino-nitrogen from α-keto acids and ammonia.

Contents of samples: bacterial suspensions (100 mg. of dry weight).
Substrata: α-keto acids 0.1 M. ammonium carbonate 0.02 M. bicarbonate buffer pH = 7.4.
Volume of test mixture: 6 ml.
Incubation time: 3 hours.
Temperature: 38°.

No. of experiment	Added α-keto acids	NH_2 - N . mg. in test sample		Difference	
		with ammonia	ammonia + + α-keto acid	in mg. per test sample	in M/g of dry weight
1	Pyroracemic acid	0.34	0.50	0.16	116
6	Oxalacetic acid	0.25	0.25	0	0
8	α-keto-glutaric acid	0.74	1.34	0.64	430
12	Phenyl-pyroracemic acid	0.26	0.54	0.28	200

For study were taken experimental or non-washed off bacteria suspended in bicarbonate buffer pH - 7.3 - 7.5. Definite amounts of bacteria corresponding to 100 mg. of dry weight were placed into small flasks which contained as substrata 0.05 - 0.1 M solutions of α-keto acid being tested and 0.05 M ammonium carbonate. The small flasks were filled with a gaseous mixture consisting of 95% of oxygen and 5% of carbonic acid. As a control served samples without added α-keto acids. The volume of the test mixture was 6 ml. The incubation was carried out in a water bath with agitation, during 3 hours at 37-38°. After the incubation definite amounts of the test mixture were taken for determination of the total nitrogen, after which all the test samples were precipitated by trichloroacetic acid. In the trichloroacetic filtrate the ammonia was determined by the Конюх method; the amino nitrogen--by the nitrite method of Van-Slaik and the residual nitrogen by the micro-Kjeldahl method.

The data in table 1 show that of the 4 α-keto acids which were studied, only three underwent amination. A maximum synthesis of amino-nitrogen was noted in tests with α-keto-glutaric acid, slightly weaker aminates the pyroracemic and the phenyl-pyroracemic acid. Practically complete absence of amino-nitrogen formation was observed in tests with oxalacetic acid.

We already mentioned that part of the experiments were conducted with washed bacteria (bacteria were washed twice in 100 ml. of physiological solution). The results of these experiments are given in table 2.

Table 2 [p. 40]

Synthesis of amino-nitrogen from α-keto-glutaric, pyroracemic acids and ammonia in washed suspensions of B. subtilis (content of test samples--see table 1).

No. of experiment	Added keto acids	NH_2 - N in mg. with ammonium	NH_2 - N in mg. ammonium + keto acid	Difference in mg. per test sample	Difference in mg/g of dry weight
1	Pyroracemic acid	0.24	0.34	0.10	71
3	α-keto-glutaric acid	0.29	0.42	0.13	93

The data of this table indicate, that as a result of a repeat washing of bacterial suspension of B. subtilis with a comparatively small volume of physiological solution, a noticeable decrease in amination activity of α-keto acids takes place.

Table 3 [p. 40]

Nitrogen fractions of B. subtilis suspension

No. of experiment	Contents of test samples	Total nitrogen in mg. per sample	Residual nitrogen in mg. per test sample	Albumen nitrogen in mg. per sample (calculated)	Remarks
1	Suspension	4.4	2.2	2.2	
2	Suspension and ammonium	8.1	5.6	2.5	
3	Suspension + ammonium + pyroracemic acid	8.1	5.7	2.4	
4	Suspension + ammonium + phenylpyroracemic acid	8.7	6.0	2.7	
5	Suspension	-	1.54	-	Suspension
6	Suspension + ammonium	-	4.3	-	diluted
7	Suspension + ammonium + pyroracemic acid	-	4.1	-	1:4

In the experiments presented in table 1, determinations of nitrogen fractions of the B. subtilis suspension were carried out after the incubation, because under the conditions of our experiments the possibility of an increase of amino-nitrogen at the expense of an autolytic decomposition of albumens of bacterial suspension was not excluded. In table 3 are given the determination data of the total, residual and albumen nitrogen.

It is seen from the presented figures, that with a 3-hour incubation there is practically no noticeable change in the amount of residual nitrogen under the influence of α-keto acids, which provides a basis to consider the disclosed increase in amino-nitrogen in the above mentioned tests as a result of amination of keto acids, and not as an effect of autolysis of albumens of the suspension.

Conclusions

1. The suspension of B. subtilis is capable of synthesizing amino-nitrogen from ammonium and pyroracemic, phenyl-pyroracemic and α-keto-glutaric acids.

2. With greatest intensiveness B. subtilis synthesizes amino-nitrogen from the α-keto-glutaric acid and ammonia.

Received: 13. V, 1947

Laboratory of chemistry
of nitrogen exchange of
the Institute for biological
and medical chemistry at the
AMN SSSR, Moscow.

Bibliography

2. Konikova, A. S., Dobbert, N. N.; Biokhimiia, 12, 79, 1947.

3. Vyshepan, E. D., Doklad Konfer, In-ta biolog. i medits. khimii AMN SSSR.

Trans. 474
(In full)
By:
A. Antik

Fridliand, I. B.

Vliianie tuksinov Bac. perfringens na dykhanie tkanei zhivotnykh
[Effect of Toxins of Bac. Pereringens on Respiration of Animal Tissues].

Biokhimiia 14(5):449-451, Sept./Oct. 1949. (In Russian)
385 B523

EFFECT OF TOXINS OF BAC. PERFRINGENS ON RESPIRATION OF ANIMAL TISSUES

The problem of mechanism of action of toxins secreted by Bac. perfringens which are one of the principal agents of gaseous gangrene in a human, advanced considerably after it had been proved that the so-called a-toxin of this microbe represents a "letsitinaza" (lecithinase) which breaks down specifically the lecithin with a formation of phosphoryl-choline and diglyceride (3).

By that the a-toxin of Bac. perfringens differs from the "lecithinase" which is in the snake and bee poison and which splits from lecithin one molecule of aliphatic acid and of the usual "lecithinase" which splits from lecithin two molecules of aliphatic acids. Inasmuch as a-toxin is a basic toxin which is secreted by Bac. perfringens into the surrounding media, it could be assumed that the first moment in the mechanism of its action is the destruction of lecithin which is contained in the membrane of cells and the subsequent disturbance in their function. However there are almost no works in the literature on how the metabolism processes change in tissues under the influence of the given toxin. We found only one work by Wooldridge who established that the a-toxin after being added to normally breathing homogenates of tissues, decreases considerably the oxidation of succinic acid (4). Whether there is a change in the oxidation of succinic acid and of other substrata in animal tissue affected by toxin, author did not investigate.

474

In carrying out the undertaken research, the purpose of which was the study of effects of Bac. perfringens toxins on metabolism processes in affected animals, we studied first of all the oxidizing processes in various organs. In the present work are exposed data which we obtained on respiration changes of organs of guinea pigs which were inoculated with a Bac. perfringens culture or obtained a deadly dose of toxin.

Trans. 474
(In full)
By:
A. Antik

PROCESS OF EXPERIMENTATION

The experiments were conducted on 287-440g. guinea pigs which were given in the inner side of the rear right limb intramuscularly by the Chertkosa method(2), a 0.2ml solution of 1:100 Bac. perfringens suspension together with a 0.1ml of a 50% solution of $CaCl_2$. The indicated dose of culture caused regularly the destruction of guinea pigs on the third day after inoculation. In a series of experiments into the guinea pigs was introduced dry toxin of the SR_{12} strain, which contained basically a-toxin, a little O-toxin and hyaluronidase(1). For inoculation of guinea pigs a toxin dose (2mg) was applied which caused destruction of the animals on the third day.

19-26 hours after the inoculation, when a definite swelling was noticeable in the guinea pigs, they were decapitated and then pieces of various tissues were taken from them in order to study the intensiveness of respiration. Pieces of muscles were taken from the locus of infection as well as from areas located far from this site. Muscles and brain were ground to paste; from other tissues sections were prepared in the usual manner. Weighed portions of 100-200mg were placed into Warburg containers with "Ringer"-phosphate prepared according to Krebs(pH 7.4). The amount of O_2 being absorbed was determined in the Warburg apparatus during 1-2, and in some cases, 3-4 hours in a row after which the Q_{O2} was calculated (microliters O_2 during 1 hour per 1mg of dry tissue).

The obtained data are shown in table 1. In consideration of economy of space the figures for individual tests could not be given, therefore mean values are presented with indication (in parentheses) of the fluctuation range.

Trans. 474
By: A. Antik

TABLE 1.

Absorption of O_2 by organs of guinea pigs
The figures express the Qo_2 Values

Muscles			Heart	Brain	Liver	Kidneys
Healthy Animals						
1,74 (1,08-3,27)			3,01 (2,59-4,45)	5,01 (3,67-6,23)	4,19 (3,30-5,37)	9,08 (6,11-11,35)
Inoculated with Bac. perfringens						
From the nucleus of affection	Above the Nucleus	Opposite Limb				
0,39 (0,00-0,83)	0,87 (0,54-1,08)	1,02 (0,47-1,69)	3,31 (2,62-4,28)	5,09 (3,76-6,34)	4,33 (3,39-5,81)	8,40 (6,45-10,84)
Inoculated with the SR_{12} toxin						
0,46 (0,00-0,81)	0,92 (0,64-1,43)	1,14 (0,60-1,50)	3,15 (2,19-4,05)	5,08 (3,40-6,03)	4,01 (3,62-5,08)	8,56 (7,36-10,86)

Examining the data of table 1, we can see that the use of oxygen by muscles of sick guinea pigs is considerably lower than by muscles of normal animals. Especially sharply expressed is the decrease in respiration of muscles in the nucleus of affection (75%), less considerable is the decrease in muscles above the nucleus (48-52%) and still less - in the muscles of the opposite limb (35-40%). We did not succeed in establishing the difference between the respiration of guinea pigs inoculated with culture or those which received toxin. The respiration of the heart muscle and of the tissues of liver, brain and kidneys is in fact not disturbed.

In order to establish, the oxidation of what substances in the muscles is disturbed, we conducted a series of experiments with addition of various respiration substrata to muscles, liver, kidneys, heart muscle and brain. As a substrate we used succinic, fumaric, malic and pyroracemic acids which were added to a phosphate buffer taking into consideration that the final concentration of these substrata should equal M/20.

The results of these experiments are presented in tables 2 and 3.

It is seen from tables 2 and 3, that disturbance in oxidation of succinic acid is observed in muscles of the nucleus of affection, where $Qo_2 = 2.75$ and in muscles above the nucleus of affection $Qo_2 = 6.56$; in the corresponding muscles of healthy animals the $Qo_2 = 9.81$. In other examined organs we did not discover any

disturbance in oxidation of succinic acid. In muscles of inoculated animals the oxidation of fumaric and malic acids is also disturbed.

In order to clarify the problem whether the decrease in respiration in muscles of affected animals is connected with disturbance in the cytochrome system, we added, in a series of experiments, para-phenylene-diamene. It was disclosed in all these experiments, that the respiration intensiveness of muscles of affected animals is similar to that of normal animals, which indicates that the cytochrome system does not suffer at all.

TABLE 2.

Influence of various substrata on absorption of O_2 by organs of healthy guinea pigs

Substrate	Muscles		Heart		Brain		Liver		Kidneys	
	Without substrate	With substrate	Without substrate	With substrate	Without substrate	With substrate	Without substrate	With substrate	Without substrate	With substrate
Succinic acid (mean data)	1,86	9,81	2,80	14,0	5,35	11,99	4,71	19,63	9,19	17,4
Fumaric acid	2,33	3,45	2,61	3,54	-	-	-	-	-	-
Malic acid	1,24	2,27	2,41	2,76	-	-	-	-	-	-
Pyroracemic acid	2,18	2,26	-	-	4,22	6,84	-	-	-	-
Para-phenylene-diamene	2,53	9,51	4,45	21,14	-	-	-	-	-	-

TABLE 3.

Influence of various substrata on absorption of O_2 by organs of sick guinea pigs

Substrate	Muscles from nucleus		Muscles above the nucleus		Heart		Brain		Liver		Kidneys	
	Without substrate	With substrate	Without substrate	With substrate	Without substrate	With substrate	Without substrate	With substrate	Without substrate	With substrate	Without substrate	With substrate
Succinic acid (mean data)	0,34	2,75	0,97	6,56	3,01	17,46	4,61	13,41	4,33	19,59	8,40	20,34
Fumaric acid	(0,32	0,47	-	-	3,30	4,79	5,03	5,68	-	-	-	-
	(0,36	0,44	0,74	0,84	2,97	3,13	-	-	-	-	-	-
	(0,52	0,60	0,60	1,56	-	-	-	-	-	-	-	-
Malic acid	(-	-	0,54	0,72	1,71	3,37	5,56	7,98	-	-	-	-
	(-	-	0,83	0,90	3,48	5,09	5,88	8,12	5,21	9,48	-	-
Pyroracemic acid	(0,23	0,32	1,34	1,42	-	-	5,47	6,83	-	-	-	-
	(0,19	0,31	0,85	0,90	2,78	3,0	5,28	8,22	-	-	-	-
Para-phenylene-diamene	0,83	7,26	1,62	8,91	4,28	18,03	-	-	-	-	-	-

CONCLUSIONS

Respiration in the muscles of guinea pigs inoculated with suspension of bacteria Bac. perfringens or with the toxin of strain SR_{12} decreases drastically. Noticeable changes in oxygen absorption in the heart muscle, liver, kidneys, brain of sick animals are not observed. Succinic acid added as a respiration substrate increases the respiration of affected muscles; however it never reaches values observed in muscles of normal animals. O_2 absorption by muscles of sick guinea pigs after addition of fumaric and malic acids is also below normal. Intoxication or infection with the SR_{12} strain of Bac. perfringens has no inhibiting effect on the function of the iron-containing enzyme systems of the muscles.

Received: 27.I.1949

Dept. of biological chemistry of the 2d
Moscow State Medical Institute im I. V. Stalin
and the anaerobic dept. of the Central State
Scientific Control Institute im. Tarasevich

Bibliography

1. Chertkova, F. A. Zhurnal Mikrobiologii, epidamiologii i immunobiologii, No. 5, 1946
2. Chertkova, F. A. Same, No. 12, 1946

Trans. 475
(In Part)
By: A. Antik
pp. 148-153;
272; 276-278

Naumov, N. A.

Bolezni sel'skokhoziaistvennykh rastenii

[Diseases of Agricultural Plants].
Moscow, 1952, ed. 2. 664 p.

Translated in part. pp. 148-153;
272; 276-278

(In Russian)

Prognosis of diseases. P 148-153

Significance of prognosis

Due to the fact that the development of plant diseases is not anything completely permanent for one area during a number of years and that the intensiveness of their manifestation can fluctuate within a very wide range from hardly noticeable cases and up to mass epiphytotics--it is very important, for a number of reasons, to have guiding information on the expected degree of appearance of one or another disease.

Such forecast of an appearance or development of a disease, especially the possibility of determining ahead of time its mass flair-up,--is called prognosis. Therefore in the plant protection field this term has a different meaning than in medicine, where it is applied in the sense of a possibility to foresee the outcome of a disease (and that for the particular individual).

It is quite evident that the necessity in disease prognosis for agricultural plants is felt especially sharply in our country where the disease control is conducted according to plan. If smoothness in any work is based on the possibility to take into account the course of occurences, then the smoothness in disease control has to be based on the possibility to prepare ahead of time the farm forces in order to prevent epiphytotics. There are in the socialistic order more possibilities than anywhere else for determination of a prognosis, because the entire matter of plant protection, including the system of disease records, form one whole (unit), which at the same time is only a division of the general national-economy plan.

Trans. 475
(In part)
By: A. Antik

Dealing with a planned distribution of crops in a territory, having the opportunity of observing them as often as necessary and then by distributing the observation points according to a certain system--we shall obtain the complete possibility to change from the usual retrospective review of (widely) spread diseases to a perspective foresight of their spreading.

How close we are from the set goal and how much we can say about the prognosis theory being developed and practically tested, will be demonstrated further on, here should be mentioned that such wide (extensive?) approach to the problem of prognosis was outlined for the first time already in 1930.

The importance of prognosis in agricultural life of the country consists in the fact, that the possibility of using it simplifies considerably the methods of disease control, makes the latter less expensive and increases its effectiveness. Having the information on a possibility of appearance of epiphytotics, the farm can prepare ahead of time the needed equipment and provide suitable material, take care of live force, mark ahead of time the dates of some measures or others (spraying or dusting etc.). avoid useless expenses which are inevitable in "blind" work and, finally, control the incoming danger more completely and thoroughly.

Various types of prognosis.

Disease prognosis of agricultural plants can be of different amplitude in time as well as in space. A goal can be set to foresee an appearance of diseases in a given area within the borders of a small territory for a certain time ahead and this time period does not step outside the limits of the usual dates of disease appearance in the given area. Such prognosis is usually called a short-range prognosis. But sometimes it is necessary to foresee a disease appearance an indefinitely long time ahead; this is called a long-range prognosis.

As to the substantiating of prognosis, it is not difficult to see that at the basis of our conclusions on dates and possible degrees of disease appearance are the following data.

1. Biological information on the causal agent: peculiarities of each stage, their order, duration of development etc.

2. Data on disease ecology: information on the direction and the degree of effect on the paresite and the diseased plant of the weather factors and other conditions of the surroundings.

3. Weather prognosis for the given area. Since the time of manifestation of disease and its degree are depending closely on weather conditions (this is confirmed almost without exceptions), the weather forecast and, in particular, timely information on the beginning of a rain period are frequently estimated as a forecast of a flair-up of epiphytotics.

Trans. 475
(In Part)
By: A. Antik

For solving of the problem of a short-range prognosis in first approximation, the data of many years on each disease can be used, which represents a record of time and conditions of appearance of one or another disease. This is a valuable auxiliary material which, if skillfully compared with information on the development cycle of the causal agent and its ecological requirements, leads us directly to a sufficiently precise solving of the problem. Of course the necessity in weather prognosis is not in the least eliminated by this fact, and in order to set a long-range prognosis there should be information covering a rather considerable time period ahead.

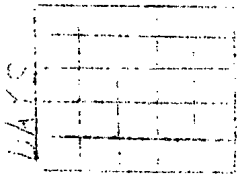
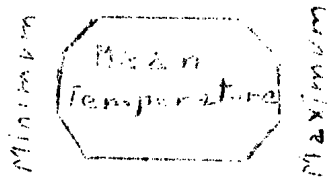


Fig. 65 (p. 150) Curve of incubation periods for Plasmopara Viticola

Since it was long ago noted that some diseases are disclosed each year at about the same time, the prognosis in its simplest form, frequently not yet realized, was built exclusively on calendar dates. Later on, as cases of deviation in development of vegetation were accumulating, in particular, in disease development due to such a simple and at the same time unbending hard scheme, attempts were made to have the forecast possibilities more accurate. For this purpose a tendency was starting to connect the dates of disease manifestation with plant phenology (relation between the stages of the disease process and the stages of plant growth), on the other hand the necessity became emphatically obvious to take in strict consideration the phenomena taking place in the surroundings, mainly in the field of short-term fluctuations of temperatur and humidity--two factors the importance of which reflects most rapidly and quite directly on the speed and intensiveness of disease manifestation. Thus the first "phenological calendars" were originated for spraying of vineyards and orchards.

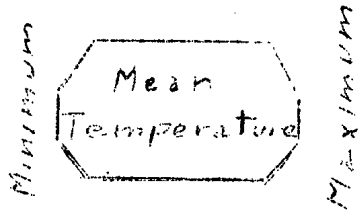
Penetration of the ecological moment led to the originating of "incubation calendars" which are based on the use of the noticed relation between the duration of the incubation period and conditions in the surroundings. The most complete and thorough expression of this is the incubation curve for Plasmopara Viticola (chapter 40) drawn in 1922; as a final result, knowing at what temperature the germination of conidia takes place, what conditions are necessary for the development of the infection and for the appearance of a new generation of conidia-bearers and observing the course of the weather during a short time interval, it is possible on the basis of this curve, to foresee with a rather great accuracy, the critical moments and to recommend a more suitable date for spraying. It not only provides the highest guaranty for protection of vineyards against mildew attacks, but eliminates as well the necessity in frequent unsystematic, repeat sprayings which in turn causes superfluous waste of time and material.

Besides mildew there are several other diseases in regard to which the problem of prognosis can be considered solved more or less satisfactorily; they are-the potato disease (Phytophthora infestans), scab mange of the apple tree (Fusicladium dendriticum), fungus (Erisyphe oidium), (acc. to Callaham) and three species of rust.



incubation period

Fig. 66 (p. 151) Nomogram by N.A. Naumova for the causal agent of brown rust of wheat-Pu cinia triticina



incubation period

Fig. 67 (p. 151) Nomogram by N.A. Naumova for the causal agent of yellow rust of cereals-Puccinia glumarum

At the present time a more accurate method is established for determination of periods of Phytophthora appearence, it is based on observations of differences in combinations of 24 hour temperatures of the air (minimum, mean and maximum). A special nomogram (N.A. Naumova, 1935) is built for the finding of probable incubation periods; its detailed use is explained in the chapter on potato diseases.

Similarly built are corresponding nomograms for determination of duration of incubation periods of the intra-tissue development of the yellow and brown rust of wheat, on the basis of comparing the minimum, mean and maximum temperatures(N.A. Naumova, 1935, 1936, 1937, see chapter 18). Finally, a very precise formula for determination of duration of the incubation period of Puccinia triticina, P. graminis f. tritici and P. dispersa in relation to temperature was evolved by K.M. Stepanov (VT2B, 1949) as follows:

$$t(T-K) = C,$$

where: t-duration of incubation period
T-observed temperature (mean of Mean 24 hour temperatures during the period).
K-lower termal limit
C-sum of effective temperatures (i.e. possible for development.

From here is determined the speed of development period of the parasite during 24 hours at given temperature conditions:

$$\frac{1}{t} = \frac{T-k}{C}$$

Summing up in succession, beginning with the inoculation day, these 24 hour development indexes, a prognosis of the next disease flair-up can be obtained, which has to come to realization when this sum reaches the value of one (?unit?)

As an example can be quoted a concrete case of calculation of duration of uredo-stage development (before the appearance of uredo-pustules) of Puccinia species (according to K.M. Stepanov, 1940).

For sub-optimum temperatures:

Puccinia triticina $t = \frac{85}{T - 1.9}$

P. dispersa $t = \frac{94}{T - 1.9}$

P. graminis f. tritici $t = \frac{125}{T - 2}$

Duration of the incubation period of grape mildew, before the appearance of spots (according to Shatskii, 1939).

For temperatures from t 8 to + 25°:

$$t_1 = \frac{60.7}{T-8}$$

For temperatures from + 25 to + 32.8°:

$$t_2 = \frac{27.6}{32.8 - T}$$

Signalization service.

Successfull solving of the problem of prognosis of some diseases or others can serve as a basis for organization of the so-called "spraying service" or "signalization service". Understood under these names is one of the forms of phytopathological service, when a special organization for plant protection, which is analoguous to record taking service, conducts systematical observations on plant diseases and at needed moments "signalizes" (on) the necessity of carryihg out one or another measure.

In our country such service of signalization and prognosis of appearance and movemeht of pests and diseases of agricultural crops was introduced on March 1, 1940 on the basis of an NK2 SSSR decree of February 22, 1940. It (the service) has the following structure. In the main administration of agro-technique and mechanization, at the department for control of pests and diseases of agricultural crops MSKh SSSR, a special sector of prognosis service is organized; there are analoguous sectors in the MSKh of republics

and in the krai (oblast') administrations of agriculture. In the rayons are distributed observations points for signalization and prognoses which serve 5-6 kol khoys and their correspondence net (system?) These points conduct recording of the species and quantitative composition of diseases (and pests), systematical observations on their development dynamics and their movement, as well as determine the destruction degree caused by them to corresponding crops, carry out control examinations, check the quality of the control measures against them, consult (?give consultations?) kolkhoys, MTS and rayon departments of agriculture, inform regularly the higher land organs of appearance and course of diseases (and pests).

To correspondents of the signalization and prognosis service is conferred the direct observation the appearance and spreading of diseases (and pests) and the signalization to the kolkhoy (and simultaneously to the observation point) about it in order to apply control measures.

Trans. 475
(In Part)
By: A. Antik

p. 272.

Among the biological peculiarities of the given species it is also necessary to point out, that the circle (? group ?) of recorded cereals being affected by linear (?) rust (Puccinia graminis) comprises about 300 species.

As to aecidial hosts, various barberry species manifest also various degrees of susceptibility. Among the highly susceptible ones we shall name besides the usual Berberis vulgaris also the B. amurensis, B. sibirica, and among the very resistant (up to almost completely immune ones)-B.Thunbergii, B. Potaninii etc. The Mahonia aquifolium (Odostemon aquifolium) shrub, which we cultivate in gardens, especially in the South, has a completely resistant foliage and susceptible fruits and therefore cannot be of particular importance as a transmitter.

Fig. 81 (p. 272) Linear (?) rust
of cereals (Puccinia graminis)
Teleuto-pustules on oats.

Apparently the resistance of barberry species is determined by characteristics of the epidermis (thickness, resistance to pricks).

Ecological peculiarities of the parasite deserve great attention, mainly in regard to regularities of its development.

Viability of the uredo-spore stage of linear rust has a very wide temperature range: with an optimum at 20° and a maximum at 31°, the formation of uredopustules can take place even at a 0-1° temperature, morphologically quite normally, though slower.

The temperature has an extremely great influence on the duration of the incubation period of the disease; thus the appearance of uredo-pustules at 10° is inhibited for 7-15 days, as compared with occurences at optimum conditions (about 20°), and at 0-1° the duration of incubation is about 70 days. But not all the bio-types can be spore-bearing at such low temperature.

The infection process is regulated by temperature conditions much more than by all the other conditions, in particular, by humidity, which is also of great importance at the moment of infection. As to the subsequent development and intensiveness of spore-bearing, the humidity degree at this point has no influence and the decisive role belongs to temperature.

As to the importance of temperature in the preservation by uredo-spores of their viability-the low temperatures of about 5° are optimum temperatures uredo-spores readily stand temperatures below zero; thus they can stay 40-45 days at -29° and even -40° temperatures.

P 276-278

Trans. 475
(In Part)
By: A. Antik

Crown Rust of Oats (Causal Agent - Puccinia coronifera).

This rust variety got its name from the occurence of a series of pustules at the top of the teleutospores. This variety is spread rather widely but, in contrast to the preceeding one, it possesses a more limited specialization, since oats is the only cultural cereal it infects. Detailed data concerning the group of plants which act as its hosts during its aecidial stage as well as during the uredospore and teleutospore stages are not as yet available. Until recently it was generally assumed that Puccinia coronifera was leading a parasitic existence on the cathartic buckthorn (Rhamnus cathartica and Rh. Pallasii) during its aecidial stage, but during its other stages - on oats, yet it has been admitted that along with this variety there existed a closely (resembling), morphologically scarcely distinguishable P. coronata with aecidia on the fragile buckthorn (Rh. frangula) and, while in other stages, on many wild and meadow grasses. Investigations of late years have disclosed that the question of specialization and even distinction of these two varieties calls for a revision, which, in part, has already been accomplished by Soviet scientists under European conditions (M.K. KHokhriakov, 1939, published in 1941).

Maintaining the opinion of a distinction between both varieties referred to, and, likewise, adhering to the same nomenclature, we cite a list of species of the host plants for each of these varieties separately.

Host plants of Puccinia coronifera

1. In aecidial stage - Rhamnus cathartica, Rh. Pallasii, Rh. dahurica and some other varieties
2. In stage of uredo- and teleutospores: Agropyrum, Alopecurus, Calamagrostis, Arrhenatherum, Avena, Bromus, Dactylis, Festuca, Glyceria, Holcus, Lolium, (Secale)

Host plants of Puccinia Coronata

1. In aecidial stage - Rhamnus frangula and some other varieties
2. In stage of uredo- and teleutospores Agropyrum, Agrostis, Bromus, Calamagrotis, Dactylis, Festuca, Holcus, Poa, Phalaris, Scolochloa

The symptoms of the infection are most characteristic and in the uredostage they partially, remind one of linea rust (Puccinia graminis) by the large pustules which here, however, do not merge in the direction of the lobes (v dolevom napravlenii) and are found chiefly on the surface of the leaf. Their coloring is bright orange, epidermic fissures are here very noticable; uredospore pustules occur less frequently on the stem. Even more typical is the infection of oats during the teleutospore stage: teleutopustules develop and remain beneath the epidermis and take no chance of emerging on its surface. They are black and shiny; at first they form into a circle or ellipsoid around the uredopustules; later, as their number increases, such accuracy of their arrangement becomes obscure.

For crown rust of oats, developing on the spring form of the cereal, the absence of infection on sprouts is characteristic. The first symptoms of infection are usually discovered very late, after the formation of the spike or, even more often, at the moment the grain is ripening.

The ecological characteristics of the parasite, in general, remind one very much of those of P. graminis, with temperatures essential for the spreading of uredospores being the same, and with the same relation to moisture in environment.

The span of the incubation period may vary from 7 to 14 days depending on temperature, infection does not occur if it is above 36° C these data pertain to uredospores as well as to aecidiospores).

With respect to its properties while in the teleutospore stage, P. coronifere reminds one very much of linea stem rust. The teleutospores also require a definite period of dormancy, and for ripening they require all the influences which are generally observed in nature, i.e., they must experience the effect of frost, thawing, drying, soaking etc.

The optimal range of temperature needed for the manifestation of this rust variety on oats in the field is 18 - 21°C.

The uredospore stage, similar to that in linear rust, is immaterial as regards the overwintering of rust: the sole means needed for this is - preservation in the teleutospore stage, with the subsequent development of aecidia on buckthorn varieties. The extent to which the presence of the latter affects the condition of oat crops is evidenced by the fact that the drop in stem infection is concomitant with the degree to which the aecidial source of infection is eliminated.

The development of crown rust in the summer proceeds in the form of a series of consecutive generations of uredospores each of which requires 8-9 days for its completion under favorable conditions, however, considering the late date of the manifestation of this rust, it has to be concluded that the number of such generations is very insignificant.

With reference to the influence of agrotechnical factors, it has been observed that there is an almost complete conformity with the facts established for linear stem rust.

Data on the difference in infection of oat varieties by crown rust are cited on table 13.

Control measures for this rust variety are identical with those worked out for the control of linear stem rust, with the corresponding changes relating to the alternate host and the characteristics of the plant itself as spring crop.

Translated from Zhurnal Mikrobiologiyi, Epidemiologiyi and Immunobiologiyi, 1941, 10/1, 67-71.

THE NATURE OF THE TOXIN OF BACILLUS BOTULINUS

By

A.K. Rodopulo

Microbiological Laboratory, All-Union Research Institute of Refrigeration Industry.

The botulinus toxin is an extremely strong poison which, in small doses, is capable of killing healthy animals.

Since the time when the botulinus toxin was discovered by van Ermengem, numerous experiments have been made on the extraction of the pure toxin and the study of its nature. These experiments, however, have not met with success, since the majority of those working with this toxin have been unable to purify it of substances associated with it.

Briner and Kemper attempted to extract the toxin by precipitation of proteins with sodium sulfate, but these experiments gave a negative result because of large losses of the toxin. E. and F. Sommer produced dry preparations of the toxin by means of adsorption on alumina hydrate and on kaolin, but these preparations contained ensymes of the triptase type.

Later Schuebel succeeded in extracting the toxin by way of dialysis, but the dialysate showed the biuret reaction. Schuebel's lack of success was due to his use of an ultrafiltrate which apparently contained soluble proteins capable of passing through an ultrafilter.

Dozier, Wagner, and Meyer suppose that the toxin is liberated as a result of autolysis of the bacterial cell. On the basis of their experimental findings, they arrived at the conclusion that in the period of initial multiplication on an alimentary medium (the

first 15 to 20 hours of growth), no toxin is liberated, because there are no cells dying off.

In Meyer's opinion, the carrier of the toxin is a protein (nucleoprotein) with an isoelectric point at pH 4.4. All the dry toxins produced in this author's laboratory showed the biuret reaction for protein. Weinberg, and also Meyer's associates Levinson and Bronfenbrenner, consider it possible that the molecule of the toxin consists of two groups, namely, a toxophoric group, the carrier of toxic principle, and a haptophoric group, which binds the antitoxin. The former is not very stable; the latter is comparatively stable and may persist unaltered for a long time.

In the research here described, we attempted to obtain botulinus toxin in dry form and to study its properties. In addition, our intention was to establish the relationship between products of the decomposition of carbohydrate substances and the process of toxin-formation in B.botulinus; likewise to discover whether this toxin was an endotoxin or an exotoxin.

For growing bacillus botulinus cultures and for the production of the toxin, we employed green peas with 20% glucose.

We preferred to work with a vegetable medium because with this type of culture medium we always obtained a stronger toxin than with the usual meat-peptone broth. Working with a vegetable medium is also more convenient, since vegetable proteins have been better studied. For our research, we took B. botulinus Type A (American strain 180).

The method of extracting the toxin was as follows. 100 ml of the culture were vacuum-distilled at a temperature below 40°C so that the toxin would not be destroyed in the process of separating the volatile substances. The residue, after distillation, was brought back to its original volume with physiological solution and tested for toxicity.

Here we found that if the titer of the toxin before distillation was 0.00001 ml, then after distillation it would be considerably increased and would go up to 0.0000001 ml. This increase in the titer may be explained as due to cells of B. botu-

linus being destroyed during the distillation, with the result that toxin is liberated into the surrounding medium. This was confirmed in the next experiment, in which the culture, before distillation, was filtered through a double-layered collodion bag to remove bacterial cells. Upon vacuum-distillation of this ultrafiltrate, at a temperature less than 40°C, no increase in the toxin titer was observed.

This is grounds for concluding that the toxin of the B. bacillus is an endotoxin, which is released to the surrounding medium when bacterial cells are destroyed.

In our tests, we always found the toxin to be already present after a 15-hour incubation; this disagrees with the findings of Dozier, Wagner and Meyer, quoted above. The table shows the lethal dose of the toxin, for mice, in a culture on meat-peptone broth, at the end of 15 hours, and then at the end of each successive 24 hours.

Duration of growth (hrs)	Lethal dose of toxin (ml)	No. of mice
15	0.0001	2
40	0.00001	2
64	0.000001	2
88	0.000001	2
112	0.000001	2
136	0.000001	2
160	0.0000001	2
182	0.0000001	2
208	0.0000001	2
232	0.0000001	2
258	0.000001	2

These data are evidence that toxin is already present in the culture after 15 hours of bacterial growth, and in quite large amounts. Of course, the toxin titer continues to rise as the culture grows and ages; only on the tenth or eleventh day does it begin to fall. It is therefore impossible to agree with the opinion of the above-mentioned authors, namely that in a young culture there are no bacterial cells dying.

According to the findings of Topley and Wilson the development of bacteria in an alimentary medium takes place in four phases. The first phase lasts 2-3 hours, during which time the multiplication proceeds very slowly. In the second phase, the multiplication of the bacteria

proceeds in a geometric progression, and this period lasts 5-6 hours. Then, in the third phase, there begins a period of throttling-down, in which the bacteria gradually lose their initial speed of multiplication. This latter period lasts from the 8th or 9th hour to the 12th or 13th hour from the start of the incubation. Finally there occurs the fourth phase, in which the bacteria gradually decrease in numbers and enter upon a period of mass mortality.

According to this, the period in which the bacteria begin to die off en masse will begin 12 or 14 hours from the start of incubation, which is completely in accord with our findings on the formation of toxin in a culture of B. botulinus after 15 hours of incubation.

For the extraction of the pure toxin, we employed two methods.

The first method is based on the extraction of the albumin and globulin fractions of the proteins by salting-out with ammonium sulfate and sodium chloride. The washed protein is then placed in the dialyser (a double-layered collodion bag) and dialysed. The water is changed every two days and the amounts of toxin, ammonium sulfate and sodium chloride in this water are determined. The amount of ammonium chloride is found by titration with $Ba(OH)_2$ solution, and the amount of sodium chloride by titration with silver nitrate.

The second portion of the dialysate did not contain any ammonium sulfate or sodium chloride, but there was toxin in it. The ammonium sulfate and sodium chloride, as crystalloids, had gone with the first portion of the water. The toxin on the other hand dialysed slowly, and thus could be detected after the ammonium sulfate and sodium chloride had been removed.

The toxin thus extracted did not show the biuret reaction for protein; it was neutralized by antibotulinus serum. In addition to the biuret reaction, we also carried out the xanthoprotein reaction and Milon and Pauli's reaction to test for protein.

However, all these tests were negative. The dialysates obtained were toxic, killing mice at a concentration of 0.0001 ml.

50 ml of the dialysate were evaporated in a vacuum-dryer at room temperature. 5 mg of the dried toxin thus obtained were dissolved in 5 ml of physiological solution and tested for toxicity. The toxin in the dry form had not suffered any alteration.

The original material had a titer of 0.0001 ml. After evaporation and re-dissolving in physiological solution, the toxin titer was 0.0001 ml, that is, ten times greater, which corresponds to the decreased volume.

We attempted to precipitate proteins along with the toxin by using alcohol and acetone and then subjecting the residue to dialysis, since alcohol and acetone can easily be removed, but it was found that both the alcohol and acetone partially destroyed the toxin and, in consequence, the dialysate obtained had a very low titer (0.5 ml).

In the second method, 500 ml of the culture were subjected to ultrafiltration in a double collodion bag to rid it of protein. The toxin in the ultrafiltrate was precipitated with trichloracetic acid; after centrifuging and washing with sulfuric ether, we would obtain, from the trichloracetic acid, 50 mg of the toxin. The ultrafiltrate showed the biuret reaction for protein, but the toxin precipitated from this ultrafiltrate, after being dissolved in water, would not give this reaction. It seems that soluble proteins go through the double collodion bag and are not precipitated by trichloracetic acid.

Testing of the toxin here obtained showed that it too would preserve its original titer.

Toxin obtained by both these methods had the same titer, which demonstrates that the two preparations are identical.

From all the above, we may conclude that B. botulinus toxin is a specific isolable substance not bound to a protein.

In the culture, the toxin is mechanically bound to a protein molecule, and after dialysis through a collodion bag, it passes into the dialysate. All findings controvert the old notion of its protein nature. The toxin produced by us does not dissolve in acetone, ether, or alcohol, but is partially destroyed by acetone and alcohol; it dissolves with difficulty in water, and loses its toxicity when acted upon by dilute alkali.

CONCLUSIONS

1) Our findings establish that the toxin of the B. botulinus is not of a protein nature. The toxin is mechanically bound to protein substances, upon dialysis of which it passes into the dialysate.

2) The toxin of B. botulinus is an endotoxin, and is released upon destruction of the bacterial cell.

3) Botulinus toxin is insoluble in acetone, ether and alcohol; it is soluble with difficulty in water, and is neutralized by a weak alkali.

REFERENCES

(1) KRESTOVNIKOVA. Zh. Mikrob., Epid. i Immun., vol. 18, 5, 1937.

(2) KRESTOVNIKOVA, RIAKHIN and PETROVA. ibid. vol. 19, 1, 1937.

(3) MEYER. Botulism. Translation from German; editor Prof. Nikitinski, 1935.

(4) Boivin, Compt. rend Soc. Biol. t. CXXVI, p. 218, 1937.

(5) Bronfenbrenner and Schlesinger, Proc. Soc. exp. Biol. a Med. vol. XVII, 1919, 1920.

(6) Sommer and Sommer F., J. Infect. Dis. Vol. XIIII, p. 496, 1928.

(7) Wagner and Meyer, J. Bact., Balt. Vol. X, p. 321, 1925.

Kalina, G.T. and B.A. Fixman
Obrazovanie kristallov v kulturakh Bakterii
(Formation of Crystals in Cultures of Bacteria)
Mikrobiologiya XXI, no. 5, pp. 528-539, Sept. - Oct. 1952
(In Russian)

"It is generally recognized that
no science can develop successfully
without differences of opinion, without
freedom of criticism."

Joseph Stalin

In March 1950 there appeared a book by G.M. Boshyan entitled "The Nature of Viruses and Microbes" (1). In this book the author made statements (set for theses) which were interesting insofar as their contents were concerned but with little experimental basis. Among such statements we should mention first of all the hypothesis dealing with crystallization of bacterial and virus albumin. This hypothesis boils down briefly to the following: microbes and viruses, being in close genetic relationship, may pass into each other; and both of them under certain conditions are converted into crystals -- formations "which are highly stable against all possible physical, thermic and chemical reactions". With this he also made another assumption, namely in the stage of crystals microbes can resist influences which were always regarded as sufficient for destruction not only of vegetative forms but even of spores, namely, treatment in an autoclave, the action of high concentrations of disinfecting substances, prolonged action of light, drying etc.

These two postulates of Boshyan, namely, the capacity for crystallization and the special stability of microbes in this condition, are the only ones of all the theses advanced in the book which may be ascribed to the author himself. All the rest, namely, the presence among the microbes of filtrable forms, the probable genetic connection of viruses with the so-called microparasites, the role of the filtrable forms in the support of non-sterile immunity, were known even before Boshyan and in certain cases may be considered as long established (the filtrable forms); while others have been subjects of lively discussion over a period of ten years (the genetic relationship between viruses and microparasites).

Of all the statements of Boshyan the one with the weakest basis is that concerning the crystallization of microbic albumin, from the crystals of which one can according to Boshyan, under certain conditions, again regenerate bacterial forms. Unfortunately, Boshyan gives very few points of support for checking his factual material. Excepting the microphotography, abundantly shown in his book, there is little information which would give us a precise idea of the conditions under which crystals are formed, the generation of them in the pure form, the separation from accompanying vegetative forms, and the conditions under which a conversion of crystals into bacterial forms may take place. Boshyan does not even state whether or not he made any chemical investigation of the crystals, and whether or not one may regard them as organic substances. There are only some general phrases stating that microbes are converted into

477

crystals, that the latter may be "regenerated" to give bacterial forms again and that they are extremely stable.

The question of the formation of crystals in bacterial culture is not new. In 1907 Mandelbaum (7), in describing a variant microbe of enteric fever isolated by him, noted the formation of crystals in an agar medium in the immediate vicinity of colonies of microbes. In 1912, he gives a fuller description of the crystals (8), and he notes the role of the alkalinity of the medium in their formation. Mandelbaum discovered crystals in the typical typhosus bacteria and in the intestinal bacilli in those cases when these microbes were cultivated on a simple nutritive agar. Crystals did not always appear, and Mandelbaum assumed that for their formation the water with which the nutritive medium is prepared has some significance.

In 1925, Zholkevich (2) described the law of the formation of crystals discovered by her with a chromomicrobe. The crystals were disposed in the immediate vicinity of the microbic cells and had the color of the pigment -- a circumstance which impelled the author to assume that the crystals are formed by the pigment.

In 1925 Preiss (10) noted the formation of crystals in an agar medium on which there grew microbes attacked by a bacteriophage. This phenomenom was confirmed in 1926 by Manninger (9) in the study of a spontaneous bacteriophage in cultures of intestinal bacilli.

In 1927, the question was studied somewhat more in detail by Jeney (6), who not only described the crystals but also showed micro-photograms, executed, unfortunately very imperfectly. And this author did not determine the chemical composition of the crystals.

As in the case of Jeney so in the case of Zholkevich, the crystal formation was such a regular phenomenon that the authors gave the following names to the microbes described by them Bac. cloacae szegediensis crystalliformans - Jeney and Bac. crystallino violaceum -- Zholkevich.

In the cultures on liquid nutritive media, the crystals were discovered in 1927 by Muddleson and Winter (5). In the semi-liquid agar prepared with liver bouillon (infusion of beef liver with tap water), pH 8 6.6, the causative agents of brucellosis gave the formation of crystals as soon as the reaction of the medium reached to 7.2 -- 7.4.

The carefully washed crystals were subjected to chemical analysis and showed an ammonium salt of magnesium phosphate.

For the removal of the surplus alkaline substance in the developing bacterial culture (which could exert an unfavorable influence upon the production of toxin) Berthelot and Amoureux (3) proposed to add to the bouillon some alkaline phosphates and phosphate of magnesium. With the microbes generated in the process of the exchange of substance the ammonia combined with these salts and was precipitated in the form of crystals of ammonium magnesium-phosphate on the bottom of the flask or test tube.

From the data of Berthelot and Amoureux we can understand the importance of the water with which one prepares the nutritive medium. The water which was employed by Mandelbaum and Huddleson and Winter, apparently, contained phosphate and magnesium salts, as a result of which there also was a formation of crystals due to the combining of the ammonia generated by the microbes.

The contribution of Boshyan was that he determined that all microbes can form crystals. However, Boshyan in contrast to the authors listed and whom he does not mention, set forth the hypothesis of crystals as a form of existence of living material, an hypothesis which, in his book, is not reinforced by any convincing experimental data.

Our task was to solve the problem as to whether crystals are salts forming as a result of the mutual action of products of excahnge of substances with the chemical components of the medium, or crystals which really represent an organic substance, crystallizing albumin, as Boshyan supposes, because living substance, even passing into the crystalline state, can be only albumin. The non-albuminous substance cannot give rise to new life, without passing through very complicated stages of conversion into albuminous substances. As we know, Engels pointed out that life is a form of existance of albuminous bodies and no other substances, except albumin, cannot be alive. The salts of phosphoric acid cannot be the bearers of living characteristics and this is an axiom requiring no proof.

First of all it was necessary to establish that crystals are really formed regularly both in liquid and in solid nutritive media, and then show that these crystals are identical with the crystals mentioned by Boshyan and, finally, to determine the chemical nature of the crystals. It is in this sequence that we shall explain our own experiments.

THE EXPERIMENTAL PART

Even before beginning the systematic study of crystal we discovered that in the sowing of a filtrate of an old bouillon culture of dysenteric bacillus with a fresh homologic strain crystals were precipitated after 8 to 10 days. Along with the formation of crystals in the filtrates of the old culture we observed many times with repeated sowings the formation of crystals in the old bouillon cultures without filtration and subsequent additional sowing. In these cases the crystals also appeared on the 8th to 10the day of cultivation. Hence, by the beginning of the systematic study of the nature of the crystals we had at our disposal data indicating a regulsr formation of crystals in test tubes with old cultures on slant agar or in agar dishes sown with various microbes, kept for some time at room temperatures. All this facilitated to a certain degree the beginning of the study of the essential character of the phenomenon of crystal formation in cultures of bacteria.

We studied cultures of the following kinds of microbes: intestinal bacillis, bacteria of enteric fever, paratyphosus A, paratyphosus B, mouse typhus, the dysentery type of Sonne, Shmitz-Stutzer, Flexner, and Oxford strains of staphylococcus and aureus, ordinary protea bacilli. We observed the formation of crystals in liquid media in test tubes and flasks of different sizes, in agar dishes and on slant agar.

The appearance of crystals in liquid cultures of different kinds of microbes was very regular in character. Together with this we noticed certain variations in the periods of appearance of the crystals in the cultures of different kinds of microbes. With a simulatneous sowing of several flasks with bouillon cultures of different kinds crystals appeared in cultures of staphylococci after 6 days; in the culture of intestinal bacilli and ordinary protes bacilli; they appeared after 7 days in cultures of the bacteria of enteric fever, paratyphoid B, mouse typhus, dysentery type of Flexner they appeared after 9 days; in the dysenteric bacteria of the Sonne and Schmitz-Stutzer type they appeared after 10 days and the culture of bacteria paratyphosus A, they appeared after 11 days (figures 1,2,3,4,5,6,7.)

One should note that the crystal formation in a culture of one and the same species of microbes but in bouillons of different series may also occur at different periods. For example, crystal formation in a culture of mouse typhus took place in one instance as early as the third day of cultivation,

As to the quantity of the crystals formed we will say that we did not observe any great variations among the different cultures. As arule, however, crystals were formed in small quantities: in order to obtain one gram of crystals it is necessary to have approximately 1-2 liters of bouillon culture. The larger the quantity of sown bouillon, the smaller the relative quantity of crystals formed. the greatest relative quantity of crystals is formed in test tubes with 2-3 ml of medium.

All the crystals forming in liquid media generally cling to the walls of the vessels. Subsequently, they increase, grow coarser, acquire their characteristics rhombohedral shape and gather on the bottom of the test tube or flask. In the end we may discover crystals which are of exactly the same shape, both sticking to the walls of the vessel and gathering at the bottom. Under the ordinary microscope the crystal are clearly and sharply contoured and shine in the dark background; they possess a clearly expressed double refraction. When heated, they lose their water of crystallization and become opaque and white. When heated for a long time at 70 degrees and also with boiling in an autoclave the crystals not only turn white but also stick together toform conglomerates.

The washed and dried crystals formed in the cultures of bacteria of paratyphoid A, paratyphoid B, mouse typhus, intestinal bacilli and staphylococci and also one sample of crystals from a solid medium were subjected to crystallographic study (the investigation was made by N.A. Domaratsky of the Chair of Petrography of the Geological Faculty of the Chernovitsky State University) and as a result one found that all the six samples were absolutely identical. Their physical constants were as follows:

1) Index of greatest refraction (Ng) - 1,501; 2) index of minimum refracti (Np) -- 1.495; 3) double refraction -- 0.006; 4) character of zones -- negative; 5) crystals biaxial 6) character of the crystals -- positive; 7) syngony (symmetry) of the crystal -- rhomboidal (figure 8) In the crystals one observed a large quantity of inclusions.

In the agar cultures the formatioh of crystals is observed both on slant agar and in the dishes with agar media. In the old cultures on the slant agar the crystals are formed after a certain drying of the medium, either in the form of small needle-like formations, sown along the course of the streak, or in the form of arborescent conglomerates, as if growing from the surface of the ag into its depth. In the dishes the crystal formation takes place more quickly and sometimes as early as 3 or 4 days after the sowing, especially if the dish is kept at room temperature, conglomerates will appear. The study of these crystals under great magnification showed that they consist of exactly the same elements as those formed in bouillon cultures (figure 9). The identity is confirmed also by the fact that the formation of crystals takes place both in bouillon and in the nutritive agar prepared in the same series of broth. And on the other hand these series of "meat water" (broth) which did not show any formation of crystals in the liquid bouillon do not produce them in solid media. The crystals also showed to be identical crystallographically.

The book of Boshyan shows numerous photographs of conglomerates of crystals obtained from solid media. Unfortunately, there are hardly any photographs of the separate crystals forming in liquid media. A cardful comparison of the crystalline conglomerates obtained by us with the photographs in the book of Boshyan show them to be completely identical, at any rate in external appearance. A large part of the crystals photographed by us were obtained from

cultures of dysenteric microbes on solid media. In external appearance they were identical with the crystals obtained by Boshyan both from dysenteric cultures (figures 10 and 11) and from the most varied cultures and bacterial preparations of nondysenteric origin. Thus, the conglomerates of crystals in the culture of the causative agent of hog cholera (figure 12) showed a great resemblance to the crystals obtained by us from the cultures of dysentery bacilli (Figure 13) and to the crystals of typhus cultures in the work of Mandelbaum. The crystals of the causative agent of fowl pest given in the work of Boshyan on the basis of arrangement did not differ from the crystals of the dysenteric bacteria obtained by us (figure 14). And, finally, the crystals of B. aerogenes showed to be just like the conglomerate of crystals of the dysentery bacillus (figure 15).

Inasmuch as in various series of nutritive media crystal formation differed in rate and intensity and in certain series of media the crystals were not formed at all, we set ourselves the task of making an investigation for the purpose of determining the conditions exercising an influence upon this porcess. In view of the fact that the study of these conditions could be carried out most con veniently in liquid media, we then made use of the latter in further experiments.

As a result of these investigations we ascertained that the formation of crystals depends on two factors -- the composition of the nutritive midium and the exchange of substances of the microbic culture. The meat peptone agar and the meat peptone bouillon made from the dry preparations of the "TsIEM" * in distilled water give no formation of crystals. These same media, but prepared with tap water, give a very small formation of crystals. In media prepared from a fresh infusion of meat, both with distilled water and with tap water, we observe the formation of crystals, and in the case of tap water the process of crystal formation is much accelerated. In peptone water, which is a medium relatively poor nutritive substances (a solution of 1% of peptone and 0.5% of table salt in distilled water), crystals were not formed a single time with sowings of dysenteric bacteria.

An important factor in the process of crystal formation was the purity of the medium: the presence of a precipitate, deposited by the alkalinization of the medium (especially abundant when the medium is prepared with tap water) and consisting of phosphates insoluble in water, slows up the formation of crystals. In the absence of a precipitate or its removal by repeated filtration and repeated sterilization, the crystal formation takes place quickest and goes on most intensively.

Crystal formation does not take place in suspensions of microbic bodies in distilled water or physiological solutions, that is, under conditions such that the exchange of the substances of the microbes is absent or very insignificant. The stopping of the exchange of substances, for example, by heating, prevents crystal formation. A culture placed under unfavorable conditions (the presence in the medium of unfavorable factors) does not form crystals.

The temperature factor does not have great importance. At first, for the development of the culture, it is necessary to place it in a thermostat. After this the crystal formation takes place somewhat more intensively if the culture is kept at room temperature. If, however, we introduce into the medium a considerable quantity of sown material, the crystals form quickly even when the test tube with the sowm medium is kept at room temperature from the very beginning.

There is no doubt about the influence of the alkalinity of the medium.

*Trans. note: Possibly Central Institute of Experimental Microbiology

Crystallization always occurs in a medium having a weakly alkaline reaction. In media with carbohydrates, decomposed by the given form of microbe, crystals are not formed, as a result of the acid reaction of the medium.

In a mixed culture of two antagonists -- intestinal bacilli and B. prodigiosus the crystals do not appear, whereas in the control test tubes, in which these microbes are cultivated separately, there is intensive crystal formation. On the contrary, if the antagonism is absent, the crystals appear in the mixed cultures more quickly than in the control test tubes with clean cultures of the same microbes (dysenteric bacilli and staphylococci). Consequently, the mutual braking of the exchange of substances with the microbes-antagonists slows up the crystal formation and, vice versa, the symbiotic relationship stimulates this process.

In phagolysates, in which, after the passing of a certain time, we observe a secondary growth of regenerating filtrable forms, we observe a formation of crystals having the same intensity as in the case of bouillon cultures. But even in transparent phagefiltrates crystals are formed very slowly and gradually, and in scant quantities. We shall give below the proposed explanation of this phenomenon.

In the study of crystal formations in agar dishes, we observed the appearance of crystals not only in direct contact with microbic colonies but also on sectors of the medium free of growth. Usually, crystals are first formed in the medium in colonies, and after this the whole dish, regardless the place of the sowing of the microbic culture, becomes covered with crystals, just like the first (figure 16). This phenomenon, by the way, may also be observed in the photographs of Boshyan.

The crystals also appear rather quickly and intensively at those places from which, after a 24-hour stay of the dish in the thermostat, we removed the coating of microbic substance. And, finally, the crystals are observed in "virgin" spots forming when we put on the microbic coating a drop of bacteriophage. All these facts, which indicate the absence of a direct connection of crystals with microbic cells, constitute telling arguments against the hypothesis of Boshyan.

DISCUSSION OF THE RESULTS

The experimental materials obtained show without a doubt that the crystal formation in bacterial cultures is an entirely regular phenomenon. In solid media, just as in liquids, we can observe the formation of crystals, and this process is certainly related to the vital activity of the microbic population. All the forms of microbes studied by us possess the capacity, in a definite period of development of the culture, to form crystals. In this part we can fully confirm the data of Boshyan.

However, the crystal formation is more a function of the nutritive medium than of the species of the microbe. As crystallographic investigations showed, all the microbic species form completely identical crystals, whereas not all the series of nutritive media are equally suited for obtaining crystals. The absence of the formation of crystals in media prepared from the dry powder of the "Is IEM" in distilled water and the appearance of crystals in bouillon prepared from fresh meat and especially in tap water show a close connection between crystal formation and certain ingredients to be found both in beef and in tap water. Crystals are not formed under conditions rendering impossible a more or less intensive exchange

of substances of the microbic population, for example, in suspensions of microbes in a psychological solution, tap water or distilled water. This is an absolute proof of the dependence of crystal formation on the process of exchange of the substances of the culture. A stoppage of the exchange of substances (heating at 70 degrees for one hour) blocks the formation of crystals. The weakly alkaline reaction of the medium makes possible the formation of crystals, while an acid reaction prevents this process completely.

Consequently, crystals in microbic cultures are formed under conditions favoring to the maximum the intensification of the processes of exchange of substances and in the presence of a definite composition of the nutritive media.

According to Boshyan, "The basic form of the conservation of the species in the struggle for existance is the ability of the bacterial cells to pass into a crystalline form". And the conservation of the species becomes necessary when the microbe comes under conditions threatening its existence, that is, unfavorable conditions. It was natural to expect, starting from the conception of Boshyan, that crystals first of all and in maximum quantities will be formed under conditions making possible a more or less intensive dying out of vegetative forms, their autolysis: a suspension of culture in a physiological solution, distilled water, stay in a medium with an acid reaction etc. However, it is precisely under these conditions, which are unfavorable for the culture, that crystal formation is not observed.

The results obtained by us with the mixed culture are particularly significant. The microbes-antagonists, braking the processes of mutual exchange, do not form crystals in mixed cultures but form them well in clean cultures. On the contrary microbes-symbionts produce crystals more intensively in mixed cultures than in each culture separately. Starting from the conception of Boshyan, it was natural to expect exactly the reverse: in mixed cultures of microbes-antagonists both "associates" were placed in a more favorable condition than when they were developed in clean cultures. Consequently, it is precisely in mixed cultures of these microbes that one should expect an earlier formation of crystals.

Hence, a purely biological study of the regularity of crystal formation leads to conclusions which are directly contradictory to the conclusions of Boshyan. In addition to this, the physical qualities of the crystals and their crystallographic constants argue against the hypothesis of Boshyan. The crystals certainly undergo changes, even with cautious heating up to 70 degrees, to say nothing of treatment in the autoclave. But, of course, according to Boshyan, the crystals should be "highly stable in the presence of all kinds of physical, thermic and chemical influences". The crystals of various species of microbes are exactly identical, something which contradicts the claim of Boshyan that the crystals are a special form of existence of albumin, specific for each microbic species. Even the preliminary investigation of the chemical nature of crystals allowed us to suspect that we were dealing with inorganic compounds, or at any rate not with albuminous substances. Even the weak solutions of inorganic and organic acids dissolve the crystals quickly without a residue. Albumins have the reverse characteristic -- coagulation in acids and dissolution in alkaline. Such behavior in the presence of acids explains the absence of crystals in media with an acid reaction. Even if all necessary conditions are present in such media for the formation of crystals, the latter, upon forming, are immediately dissolved.

The spectral analysis showed the presence in the crystals of cations of magnesium (investigation carried out by Makarenko of the Chair of Experimental Physics of the Physic-Mathematics Faculty of the Chernovitsky State University). In the chemical analysis of the crystals (investigation carried out by L.N. Zamansky and P.Y. Silver of the Chair of Medical Chemistry of the Chernovitsky Medical Institute), both in the case of those obtained from liquid media and those extracted from solid media and carefully washed (precisely those crystals which have a surprising resemblance to the crystals of Boshyan), we discovered the presence of anions of phosphorous, ammonia and cations of magnesium. The quantitative analysis of several specimens showed the average percentage of phosphorous to be 15.35; magnesium -- 10.34, which brings them close to the hexahydrate of phosphate of ammonium-magnesium. The absence, in the mixture, of crystals of carbon, sulphur, pentide compounds and amino acid indicates that the crystals, without doubt, are not of albuminous origin, but are inorganic salts of phosphoric acid, that is, what was discovered in the cultures of bacteri of Huddleson and Winters and also Berthelot and Amouret as early as 1927.

In the light of these data we can understand the importance of nutritive media for crystal formation. The crystals can form only in a medium in which there are the necessary ingredients for the synthesis of ammunium-magnesium phosphate, that is, phosphates and the compounds of magnesium. We are convinced that in media prepared from fresh meat and tap water these compounds are present in sufficient quantity (investigation carried out by Shulman of the Chair of Pharmacology of the Chernovitsky Medical Institute). The alkalinization of the medium causes the dropping out of an abundant amorphous precipitate which is, as chemical analysis has shown the ammonium-magnesium salt of phosphoric acid (investigation carried out by L.N. Zamansky and I.Y. Siven).

The importance of the intensity of the exchange of substances becomes understandable if we consider the mechanism of the formation of crystals in the culture. In the process of the exchange of substance and the breaking up of the ammonia compounds under the influence of the vital activity of the microbic population there is a release of ammonia, as the final product of the breakup of the peptone and semi-pentides. Ammonia binds the salts of magnesium and phosphates that are in the medium forming a double salt of phosphoric acid. The alkalinization of the medium drives out the ammonia from the ammonium compounds, and, consequently, leads to a process which in principle is identical. However, for the formation of a crystalline salt and not an amorphous salt, the quantity of ammonia forming in the medium, and, probably, the continuity of its formation are certainly of significance. We carried out the artificial formation of crystals in a sterile medium. If to the sterile bouillon we add a small quantity of aqua ammonia, there is deposited immediately an abundant amorphous precipitate. If we moisten the plug of the test tube with this aqua ammonia, we have a precipitate of fine needle-like crystals forming somewhat slower, beginning with the upper layers of the liquid. If, however, we place a small test tube with bouillon in a wider tube and moisten the cotton stopper of the latter with aqua ammonia, that is, render still more difficult the access of the ammonia to the medium, there drops out in the precipitate along with needle-like crystals, the typical rhomboidal crystals (17), exactly like the crystals forming in cultures of bacteria and identical externally with the crystals of the causative agent of infectious anemia described by Boshyan.

Hence, the crystals formed by us artificially, without the participation of microbes, do not differ from the crystals which, according to Boshyan, crystallize from microbic living albumin.

We observed that by putting the crystals in fresh culture of a homological or heterological microbe and also in a fresh filtrate of such culture the crystals were dissolved. This may be explained by the presence of acid products in the young culture, forming as a result of the decomposition of an insignificant quantities of glucose, present constantly in the meat peptonic bouillon. Fresh cultures in peptone water, suspensions of microbes in distilled water or a physiological solution do not produce such phenomena.

From the point of view of the mechanism of the formation of crystals, which we have set forth, we can fully explain the fact of the formation of crystals in solid media in sectors free of microbic sowing or outside the microbic colonies. The ammonia forming as a result of the exchange of substances not only diffuses in the medium, as a result of which crystals are formed in submerged microbic growth (submerged formation), but it is also liberated in a closed air space as in a test tube. While being adsorbed by the medium, the ammonia forms a compound with the salts of magnesium and phosphates which are in the medium, as a result of which crystals are formed. This may also explain the precipitation of crystals in the virgin spots, which have formed under the influence of bacteriophages or in places from which one took the microbic coating. In these cases the gaseous ammonia is able to come in direct contact with the surface of the medium, a contact which was prevented up until then by the layer of microbic bodies which covered the medium. The next test confirms our assumption as to the influence of the gaseous ammonia. If in a meat peptone agar, in a Petri dish, we cut the central canal which separates the surface of the agar into two completely isolated halves, and we made an abundant sowing of microbic culture on one half and leave the other half free, crystals are formed both in the mass of the agar of the first (or sown half) (in larger quantities) and in the mass of the remaining sterile agar of the unsown half.

The most interesting is the fact of the formation of crystals, slowed up to be sure and not abundant, in the "sterile" phagofiltrates, that is, in filtrates lysated by the bacteriophage of the cultures. If kept at room temperature for one and a half to two months, in a transparent liquid, in the absence of any vegetative forms in it, crystals will be precipitated, at first small and in scant quantities, then gradually and very slowly they increase in quantities, then gradually and very slowly they increase in quantity. If we follow Boshyan, this fact may be treated as the crystallization of the filtrable forms which are in the phagofiltrate. However, the chemical analysis (investigation made by L.N. Zamansky and P.Y. Siver). even in this case showed the presence of ammonia, magnesium and phosphorous in these crystals. Consequently, even here the crystals are the result of certain processes of exchange taking place in the phagofiltrate. It is possible that the formation of crystals in the phagofiltrates indicates the presence of an exchange of substances in the filtrable form; however we must made further study in order to render a final decision on this question. At any rate, neither in sterile bouillon or in phagofiltrate heated up to 100 degrees, kept under the same conditions as the native filtrate, any crystals were formed. Hence, one cannot consider the influence evaporation of the medium or the formation in the medium of chemical substances making possible the dropping our to crystals, before the lysis of the culture.

Boshyan says -- also without giving any documentary or scientifically grounded material -- that he obtained the regeneration of microbes from crystals. Unless there has been a gross error in methods, this fact merits attention. There is no doubt that from a double phosphorous salt it is impossible to obtain a live microbe. Consequently, it is necessary to seek some other explanation for the statement (data) of Boshyan. In this connection an hypothesis meriting consideration is that of Muromtsev to the effect that crystals may contain in themselves, in the form of inclusions, some filtrable forms, and it is possible, let us add, also vegetative forms of microbes. The crystals surrounding these inclusions, acting as an armor, make them more stable in the presence of all kinds of harmful influences, more so to chemical influences, probably than to physical. For this reason we should recall the interesting observations of Brussoff (4) pertaining to microorganisms, isolated by him from the water of hot springs. Brussoff showed that these microbes, in the process of the exchange of substances, calcium carbonate which is deposited around the microbic cell. Gradually, the crystals of calcium carbonate enclose the microbes, so to speak, in a calcareous envelope, and upon the dissolution of this envelope by acid we can release the viable microbe. It is possible that we may have the same process in the formation of ammonium-magnesium salts of phosphoric acid, all the more so as in the crystallographic investigation of the latter one discovered in them numerous inclusions of an unknown nature. Attempts at regeneration of the filtrable forms from the crystals, both native and those dissolved by weak acids from a subsequent neutralization, undertaken by us, has not yet given any positive results, but we do not exclude such a possibility and we shall continue the investigation in this direction.

CONCLUSIONS

We shall not deny the possibility, in principle, of crystallization of living substance. The crystallization of plant viruses, first described by Ivanovsky, and later experimentally proved by Stanley, left no doubt as to the possibility of the existence of life in such a crystalline form. The extracellular existance of life, either in the filtrable form of microbes or "dokletochnoi zhnizvri" (precellular) life?, proved by the brilliant investigations of O.B. Lepeshinsky, leaves no doubt that it is possible to have a stage of crystallization of living substance. O.P. Lepeshinsky described the formation of crystalline elements from which there were formed new cellular elements. Some day and by some one (we firmly believe that it will be a Soviet scientist) it will be proved that there is crystallization of living microbic albumin, capable of subsequent regeneration. But we do not think that Boshyan, who did not study the crystals described by him either chemically or crystallographically, gave in his work any proof of the crystallization of living microbic albumin. There is no doubt as to resemblance of the crystalline conglomerates and separate crystals studied by us. However, these crystals, according to what we find, are formed in bacterial cultures as a result of the processes of exchange of substances and have nothing in common with crystallization of living microbic albumin.

Chernovitsky Medical Institute

Received 22-IV-1951

BIBLOGRAPHY

1. Beshyan, G.M. O Prirode virusov i mikrobov, Moscow 1949.
(The Nature of Viruses and Microbes)
2. Zholkevich, A.Y., O Novoi Khromogennoi bakterii -- Bacterium crystallino-violaceum.
Mikro biol. Zhurn., 1, 213, 1925. (New Chromogenic Bacteria).
3. Berthelot A. et Amoureux C. Sur l'auto-elimination de l'ammoniaque dans les cultures microbiennes. Bull. soc. chim. biol., 9, 932, 1927.
4. Brussoff A. Ueber ein Kalkspeicherndes Bakterium und die von ihm gebildeten Kristalle. Arch Mikr. 4, 171, 1933.
5. Huddleson G. a Winter O. Magnesium-ammonium-phosphate crystals in aerobic culture of Brucella abortus and Brucella melitensis . J. Infect. Diseases 40, 469, 1927.
6. Jeney A. Bakteriophage Ersheinungen an einem Wasserbakterium B. cloacae zegediensis crystalliformans Ctbl Bakt 1, or, 102, 263, 1927.
7. Mandelbaum M. Munch. med. Wochschr, N. 36, 1907.
8. Mandelbaum M. Ueber das Bacterium metatyphi. Ctbl. Bakt. 1, or. 63, 46, 1912
9. Manninger R. Beitrag zur Kenntnis der Bakteriophagie. Ctbl. Bakt., 1 Or., 99, 203, 1926.
10. Preiss H. Die Bakteriophagie. Jena, 1925.

Figure 1. Crystals in a bouillon culture of staphylococcus aureus

Figure 2. Crystals in bouillon culture of intestinal bacilli.

Figure 3. Crystals in bouillon culture of "ordinary" protea.

Figure 4. Crystals in bouillon culture of paratyphoid B. Bacteria

Figure 5. Crystals in bouillon culture of bacteria of mouse typhus.

Figure 6. Crystals in bouillon culture of dysenteric bacteria of the Flexner type.

Figure 7. Crystals in bouillon culture of paratyphoid A bacteria

Figure 8. High magnification of the crystals in bouillon cultures: staphylococcus a-aureus; b-intestinal bacilli; c-enteric fever bacteria.

Figure 9. Crystal formation in agar culture of dysenteric bacteria of the Flexner type.

Figure 10. Crystals of dysenteric bacteria of the Flexner type.

Figure 11. Crystals in agar culture of dysenteric bacteria of the Flexner type.

Figure 12. Stage of crystallization of the causative agent of hog cholera.

Figure 13. Crystals in agar culture of dysenteric bacteria of Flexner type.

Figure 14. Crystals in agar culture of dysenteric bacteria of Flexner type.

Figure 15. Crystals in agar culture of dysenteric bacteria of Flexner type.

Figure 16. Formation of crystals outside the microbic colony (the center of crystallization lies outside the colony).

Figure 17. Crystals of ammonium-magnesium salts of phosphoric acid, formed as a result of the action of ammonia on sterile bouillon.

Trans. 478
(In full)
By:
A. Antik

Ritus, I. G.

Rastenievodstvo

[Plant Industry]

Plant Industry, pp. 40-41; 117-118, 1952. 64 R51

(In Russian)

3. Economical-botanical peculiarities of rye and varieties most widely spread in the USSR.

Many varieties belong to the rye species Secale cereale. Among them the "Vulgare" variety is cultivated extensively; it is characterized by a white-owned spike with a non-brittle /fragile/ spike stem /rachis/ and a semi-open or open grain. Winter and spring (iaritsa) rye are cultivated. Winter rye predominates in the fields due to its higher and relatively resistant yield capacity. It uses up more completely the moisture of the fall and winter precipitation, is more resistant to drouth, ripens earlier than the spring rye. "Iaritsa" is cultivated in the USSR in areas where winter rye frequently perishes mainly due to severe frosts--in Buriat-Mongolia, in the Trans-Baikal, on the Sakhalin.

Rye is a typical cross-pollinator, therefore rye varieties are populations (mixtures of individual races). Already Ch. Darwin proved that self-pollinating plants produce a weak progeny which is less viable than the cross-pollinating ones. Academician T. D. Lysenko established that repeated pollination of various rye varieties by other varieties does not lead to their loss of valuable properties and characteristics and that their cultivation together is useful. This statement by Academician T. D. Lysenko is at the present time the basis of the method of inter-varietal free pollination for breeding of new varieties. Repeated pollination of rye varieties by other varieties increases the yield capacity. However, with a background of low agro-technique, especially under unfavorable conditions, for example--heavy rains or lodging during the flowering stage--part of the flowers might remain unpollinated. This leads to the so-called "cherezzernitsa" /partially filled ears/ in the spike and not infrequently to considerable reduction in the grain harvesting. Therefore a complex of methods of high agro-technique which increase rye's resistance to lodging, as well as artificial

completion of pollination, can be of importance for a decrease in only a partial filling of ears.

In many selective rye varieties the grains are protruded considerably from the hulls and in a number of local varieties they are deep in the spikelets. Therefore certain selective varieties have frequently a stronger tendency for lodging than the local ones. Some local varieties are more resistant to diseases than selective ones. Thus, for example, the Karsavaiskaia local rye (Udmurt ASSR) is resistant to affection by "Sclerotinia" and during the varietal tests in 1938 produced a higher yield than the Viatka variety. The Tobol'sk local rye produced 4.8 centners per hectare higher yield than the Viatka and the Razdolinskaia local rye of the Kursk oblast' had a 2 centners per hectare higher yield than the Lisitsyna variety. These examples demonstrate that (begin page 41) many local varieties can be of great significance for production and also serve as a good material for selection. Besides, it is necessary to use extensively the natural diversity of the weed-field /volunteer/ rye, which, according to its economic and biological properties, is a most valuable population for selection.

For cultivation are used selective and local varieties of winter and spring rye. In local varieties the grain is usually small (1000 grain weight not more than 20 grams) and in selective ones--larger (1000 grain weight up to 28-30 grams).

The following valuable rye varieties should be pointed out from among those distrubuted according to areas and cultivated extensively in the USSR: a) winter rye: Viatka, Lisitsyna, Saratovskaia

1. Eliseevskaia, Bezenchukskaia-yellow grain, Veselopodslianskaia, Kharkovskaia Kazanskaia, Avangard, Voronezhskaia SkhI, Viatka Moskovskaia, Novozykovskaia M-4, Polesskaia, Tarashchanskaia.

2. Omka, Dolinskaia, Manychskaia, Volzhanka, Zhitkinskaia, Partizanskaia lokal etc.:

b.) Spring rye--Onokhoiskaia etc.

4. Characteristics of growth and development of rye in connection with conditions of growth.

Characteristics of growth, development and requirements of rye to growth conditions are manifest basically in the following. Winter rye is characterized first of all by its less exacting demands upon conditions of climate and soil than, for example, those of winter wheat. It is frost resistant; according to Academician V. P. Mosolov's data it can stand temperature up to -37^0 and according to Academician D. N. Prianishnikov's data, it can endure frosts up to -25^0 even during snowless winters. The high frost resistance of rye varieties of USSR selection, furthered the spreading of their plantings in the south-eastern and eastern areas. It is also more drouth resistant than winter wheat. In winter rye the root system developes fast and intensely already in the fall which furthers its greater resistance to spring drouth. Fast and

intensive development of the root system, a more complete hardening of rye plants than of wheat in the fall during a prolonged vernalization stage, and a fast accumulation of carbohydrates during the fall period-further its frost resistance.

In comparison with wheat, winter rye is relatively less demanding with respect to soils. Fast and vigorous development of its root system which reaches towards the end of the tillering stage 1m. and during the heading stage-1.5m in depth, as well as a higher assimilating capacity of the roots, give the rye the capacity to use the nutrient substances of the deep soil layers. Therefore it succeeds not only in podzol soils but also in sandy loam and even sand and marshy soils after they have been prepared. However, rye is very responsive to increased fertility of soil even in the black-earth belt of the USSR

begin page 117

2. Areas of cultivation, yield capacity and perspectives of further development of corn cultivation in the USSR.

The basic concentrations of corn plantings in our country are located in the Ukraine SSR and in the North Caucasus--up to 80% of the entire area of corn plantings in the USSR. Considerable areas are occupied by corn in the Georgia SSR--up to 15% of the entire plantings of this crop in the Soviet Union. Smaller areas of corn fields are distributed in the Moldavia SSR, the Crimean and Voronezh oblasts, the Kirgiz, Kazakh and Azerbauzhan SSR, in the Uzbek and Tadzhik SSR, in the Far East, in the Kursk, Chkalov oblasts etc. (also the Volga Region)

It is necessary to point out that in our country during the Stalin five-year plans, even before the Great Fatherland war, not only has corn production increased more than three times in comparison with the pre-revolutionary period, but, on the basis of achievements of Michurin's agro-biology, this crop was pushed forward into new areas--Volga region, Kursk and Voronezh oblasts, southern areas of West Siberia, areas of the Poles'e in the Ukranian and Belorussian SSR.

Finally should be noted also the great achievements in higher yields. Thus if in 1909-1913 the mean yield of corn in our country was 10.3 centners, then already in 1933-1937 it increased up to 11.6 centners and in 1937-up to 13.8 centners per hectare as a mean for the USSR. But in the basic areas of corn planting the mean yield was above 20 centners per hectare. Thus, for example, in 1940 the mean average corn yield in the Ukrainian SSR was 20.5 centners per hectare. Certain oblasts, rayons and kolkhozs succeeded in obtaining even higher yields. For example, in 1940, the following mean yield of corn was obtained in a number of oblasts in the Ukrainian SSR: in Kamenets-Podol'skaia oblast'-21 centners, in the Vinnitsa and Kirosograd oblasts-21.1, in the Dnepropetrovsk oblast'-21.6, in the Poltava oblast'-24.7 centners per hectare. In the Rybnitskii rayon /begin p. 118/ of the Moldavia SSR in 1940, a mean

yield of corn for the rayon was 36 centners per hectare. In 1949 the Kolkhozs of the Tsarichanskii and Magdalinovskii rayons, Dnepropetrovsk oblast', as well as of the Kotovskii rayon, Odessa oblast' obtained in large areas mean yields of 40-50 centners per hectare. The Kolkhozs of the Likhovskii raion, Dnepropetrovsk oblast' gathered the same year, as a mean of the raion, 54 centners per hectare.

Leading Kolkhozs were obtaining very high yields. Thus, for example, im the Kolkhoz , in Voroshilov, Shirokovskii raion, Dnepropetrovsk oblast', in 1939, in a 60 hectare area, the gathered grain yield was 30 centners per hectare, and in 1940, in a 105 hectare area--40.4 centners per hectare; im the Kolkhoz in Chkalov, Novo-Moskovskii raion, in 1939, in a 160 hectare area a yield of 80 centners per hectare was obtained. Masters of high yields grow up to 150-200 centners of grain per hectare. For example, the Hero of Socialistic Labor, M.E. Osernyi, in the Kolkhoz "Red partisan", Likhovskii raion, Dnepropetrovsk oblast', obtained from the entire area of the "link" 175 centners per hectare and in an individual section--223.3 centners per hectare; brigade leader of the Kolkhoz im. Stalin, Gal'skii raion, Ackhazskaia ASSR, D.D. Rigvava gathered in 1947, in a 6 hectare area on the average 136.6 centners per hectare and in a one hectare plot--210 centners.

The above-mentioned and other examples of high and record high yield capacity of corn indicate that it is one of the highest yielding grain crops, and that not only individual front line workers but also front line kolkhozs and raions assimilated the complex of methods of high agro-technique, skillful application of which provided such yields.

The many-sided and very important significance of corn, especially in regard to fodder, industrial raw material and agro-technique, as well as the achievements in obtaining of high yields--determine the necessity of further development of this crop in the USSR in the direction of expanding planted areas as well as in increasing its yield capacity in all the corn-sowing areas.

Contemporary achievements of the USSR in the field of mechanization of sowing by using the square-hill /check row/ sowing machine S Sh-6 /S Sh-might stand for the U.S.A. ?/, the inter-row lengthwise and crosswise cultivation with specific tractor cultivators and harvesting with combines-allow to cut considerably the input of work in cultivating corn and thus determine the possibilities of a further expansion of the area of its plantings and of an increase in yield capacity. Use in large areas of mass sowings of hybrid corn seeds, will in turn, result in higher yields and gross harvesting of grain of this crop in the USSR.

Trans. 479
(Summary)
By:
A. Antik

Gomoliako, N. I.

K anatomicheskoi kharakteristike gribnykh porazhenii zerna khlebnykh zlakov

[On anatomical characteristics of fungal diseases of cereal grain].

Mikrobiologichnii Zhurnal vol. 15, no. 2, pp. 72-80, 1953. 448.3 K54

(In Ukrainian)

Report II

Summary

In this report is disclosed the character of infection of grain of corn by Fungi: Helminthosporium zeicola Stout., Nigrospora oryzae Petch., Alternaria tenuis Nees., Botrytis cinerea Pers., Cladosporium zeae Lobik, Cl. griseo-olivaceum Pidopl. et Deniak, Cl. Transchelii Pidopl. et Deniak, as well as of grain of other cereals by fungi: Cladosporium herbarum Link., Gonatobotrys flava Bonord., Sclerotium sp., Typhula pusilla Pers.

The material for study was selected while inspecting the grain and it shows infection under natural conditions.

The disease character of corn grain [?] inffected by Helminthosporium zeicola coincides fully with that previously described for other cereals by Helminthosporium sativum P. K. et B., namely, the mycelium of the fungus is always found in the tissue of the embryo where it spreads at first without noticeable harm to tissue cells. Side by side with diseased cells are absolutely normal ones with a nucleus and nucleolus. Dissolving of starch and of cell walls is not observed either in embryo cells or in the endosperm when they are penetrated by mycelium.

The mycelium of Nigrospora ozyzae Petch. is found in tissues of the scar ["rubchik"] and of the walls of the grain. In most cases the embryo in diseased grain is dead. There is reason to assume that the destruction of the embryo took place during the early stages of its

According to Callaham "zernovka" means "weevil." Seems to me that it is rather the ear full of grain, since "zerno" is "grain." I'll mark it with [?] each time.

development, as a result of isolation of the cob from the core by the affected tissue of the scar /"rubchik"/ and therefore--due to difficulty in water supply. There is no basis to consider a toxic effect on cells of mycelium secretions probable, inasmuch as next to the mycelium, absolutely healthy cells can be observed.

Alternaria tenuis Nees. in corn grain affect only the tissues of the wall and the scar /"rubchik"/. The embryo tissue is healthy, which provides a basis to assume that the infection of the grain takes place during the stage after a completed formation of the embryo.

The fungi of the genus Cladosporium involve, in corn and other cereals, only the surface tissues of the wall and do not enter either the endosperm or the embryo. On corn grain 3 species were found: Cladosporium zeae Lobik, Cl. griseo-olivaceum Pidopl. et Deniak, Cl. Transchelii Pidopl. et Deniak. On grain of other cereals--Cladosporium herbarum Link.

Mycelium Botrytis cinerea Pers. was found only in the endosperm of corn grain where it penetrates densely the tissue and completely dissolves the starch due to which empty spaces are formed.

According to data in the literature, Gonatobotrys flava Bonord. develops on rotted plant remains, however it is frequently found on germinating grain of various cereals with the exception of corn. Its mycelium is usually found in the capsule of the grain, from which it penetrates into the embryo and the endosperm, dissolving in the latter the starch and the wall of the cells. In the embryo tissue the mycelium spreads at first without noticeable harm but soon fills with a dense interweaving the cavities of the cells and their protoplasm shrinks around the nucleus.

Sometimes sclerotia were found on rye grain in the exterior layers of the hull. This infection had a local character and no spreading of mycelium from sclerotia was observed. Attempts to grow and cultivate these sclerotia in agar were not successful and therefore it was not possible to determine the fungus. There is no basis for identification of these sclerotia with those of Curvularia pallescens Boedijn, inasmuch as this fungus was not once discovered on the grain during analyses.

In analyzing, a disease of oats grain with Typhula pusilla (Pers.) was discovered. Spreading of mycelium was observed only in hull tissue and the tissues of grain proper remained free of mycelium.

Trans. 480
(Abstracts)
By:
A. Antik

480

Marland, A. G.

Prodolzhitel'nost' razvitiia uredostadii koronchatoi rzhavchiny ovsa (Puccinia coronifera Kleb.) V zavisimosti ot temperatury i otnositel'noi vlashnosti vozdukha

[Duration of development of the uredo-stage of crown rust of oats (Puccinia coronifera Kleb.) in relation to temperature and relative air humidity.

Vestnik Zashchity Rastenii, 1941, no. 1, pp. 100-105. 421 P942

[In Russian]

Duration of the development of the uredo-stage of crown rust of oats (Puccinia coronifera Kleb.) in relation to temperature and relative air humidity.

Among the rust fungi affecting cereals, the crown rust of oats (Puccinia coronifera Kleb), being very destructive, deserves serious attention.

The crown rust of oats is of various intensiveness depending on different weather conditions. According to Gorlenko's observations (1935) in the Voronezh oblast, in 1931 and 1934 it broke out weakly, and in 1932 and 1933, it developed to a rather high degree. This can be explained by exceptional meterological conditions of the given years. Thus, 1934 was characterized by a complete lack of precipitation in the spring, while in 1933 during this period there was an extremely abundant precipitation. Gorlenko concludes that an amount of precipitation not deviating from the many-year average (for the Voronezh oblast') in May and June, favors considerable[1] development of crown rust on oats; deviation from the average towards increase causes a flare-up of rust and on the contrary, deviation towards decrease acts depressingly on rust development.

Grushevoi (1930) reports, that the mean 24-hour temperature of 18.0° and the minimum temperature of 13.5 during the dispersion of aecidia-spores, further the appearance of rust on oats; slight amount of rains in June inhibit further spreading of rust. Shechenko (1931) comes to analogous conclusions. The authors of the above-mentioned works pertaining to the effect of weather conditions on rust development base their conclusions exclusively on observations.

---

[1] Evidently, average development. A.M.

There are few experimental data on this problem, in particular, in regard to influence of temperature on the duration of development of the uredo-stage.

N. A. Naumova established (1934, 1935, 1937) the relation between the duration of the incubation period of Phytophthora infestans, Puccinia triticina and Puccinia glumarum and the 24-hour intensity of air temperature, and this induced us to find out similar relation for Puccinia coronifera. In connection with this, we conducted special research in the ecological laboratory of the V I Z R (All-Union Inst. for Plant Protection). The work was carried out in 1936-1937 in the greenhouse. We assume that the data which we obtained will lead us closer to the understanding of regularities in the development of crown rust of oats.

In order to make a correct prognosis of the expected epidemic and to carry out corresponsing measures, it is first of all necessary to know the biology of the fungus, conditions of its development and the development of the plant host itself. Of course the study of the environmental factors alone, which condition the disease development, does not give a clue to the clarification of epidemics of rust as a whole and to prognosis of the latter. Nevertheless, the regularities being established can serve as a certain basis for making a prognosis and present a basis for individual control measures.

The literature on the influence of environment factors on the development of crown rust of oats is not manifold as compared with the material pertaining to other rust species.

Research was carried out on the effect of temperature on susceptibility of oats to various forms of Puccinia coronata Cda fungus (Peturson, 1930). It was established that some varieties of oats are susceptible to certain rust types at some temperatures (21-25°C), but resistant at others (14°C). While working with physiological races of crown rust of oats, Murphy (1932) established that the reaction of varieties is to a considerable degree determined by temperature. Thus at a high temperature (24-26°C) a susceptible reaction is observed and at lower (15-18°C) usually a resistant reaction. According to Johnson and Newton (1937) data, at a temperature higher than the optimum, less viable spores of the stem leaf and crown rust develop than at temperatures below the optimum. Peturson (1930) and Murphy (1935) demonstrated that with the increase of temperature, decreases the resistance of oats to P. coronata. Frenzel (1930) attributes little significance to temperature. According to Straib's (1937) data, of all the factors, the temperature is of greatest influence on the susceptibility and resistance of oats. On the whole, according to Straib's opinion, the data of American works has been confirmed: At 10-20° the

resistance changes relatively little, and at 23-25° a great increase in susceptibility is observed.

Great importance in development of crown rust of oats is attributed to humidity. For infection, it is necessary to keep the plants for various periods of time in an atmosphere saturated with water vapor, the time depending on temperature. After the penetration of the fungus hypha of crown rust into the tissue of the plant host, the humidity acquires a secondary importance as compared with temperature (Gassner and Appel, 1927). A 100% humidity is necessary for infection. Thus at a 93% humidity, when artificial inoculation with Puccinia coronifera Kleb was applied, the plant affection was 6%, and at 75-80% humidity, no infection took place (Fromme, 1913). According to T. Hemmi and T. Abe's (1933) data, a 100% air humidity is necessary for germination of uredo-spores of crown rust and a direct contact of spores with water; at a 95% relative air humidity, there was no germination.

There are almost no data on the problem of duration of the incubation period of crown rust in relation to temperature, with the exception of single indications by Mains (1916), Fromme (1913), Gassner and Appel (1927) and others. According to Main's investigations, the incubation period for P. coronata at 20° is 9 days, at 15° - 13-15 days, and at 13° - 15 days; still lower temperatures inhibit rust development. Fromme indicates that the incubation period of P. coronifera at 20-30° was 6-7 days, at 14.5 -21°- 8 days. According to Gassner and Appel (1927), the duration of the incubation period at 10° is 19 days, at 15° - 11.1 days and at 20° - 7-8 days. They consider 15° to be the best temperature for the development of P. coronifera. Straib (1937) indicates 18° as being an optimum temperature for the development of rust of oats at which the incubation period is 12-14 days. Frenzel (1930) mentions that under optimum temperature conditions, the incubation period continues for 8-9 days.

The information given here is fragmental, and we assume that the following data on our experiments will be of certain interest.

Experiments in inoculation of oats with aecidia-and uredo-spores were conducted during sprouting and flowering stages. Their aim-- to establish whether there is a relation between development periods in uredo-stage of P. coronifera and[1] temperature and relative air humidity under natural intensities. Accordingly, the experiments were conducted in the green house. Temperature and humidity were recorded by automatic recorders which were installed in the

[1] We use the term development period in uredo-stage as designating the time interval between the germination of spores and the appearing of uredo-pustules (Stepanov, 1940)

meteorological booth on a rack with test plants. 42 series of tests were conducted on inoculation with aecidia-spores and 82 series on inoculation with uredo-spores during the sprouting stage of oats. The "Zolotoi dozhd" ("golden rain") variety of oats was taken for the experiments; it was grown in Vagner containers (pots), 25 plants in each.

Oats leaves were rubbed with a light finger pressure. Then the plants were sprayed with an aecidia-and uredo-spore susponsion taken from 5-6 day-old pustules and were covered with a bell glass which was lined inside with wet filter paper. After 24 hours, the bell glasses were removed and from this moment on, daily observations were conducted of the dates of rust appearance on plants with recordings of temperature and relative air humidity.

As a result of the experiments, a definite relation between the duration of the development period of the uredo-stage and the temperature was noted. With the increase of the mean 24-hour temperature, the period shortened, and vice versa — with the decrease in temperature, the development period became longer. Unfortunately, the experiments were conducted within relatively narrow temperature ranges, and there are not enough data for drawing a nomogram or a diagram.

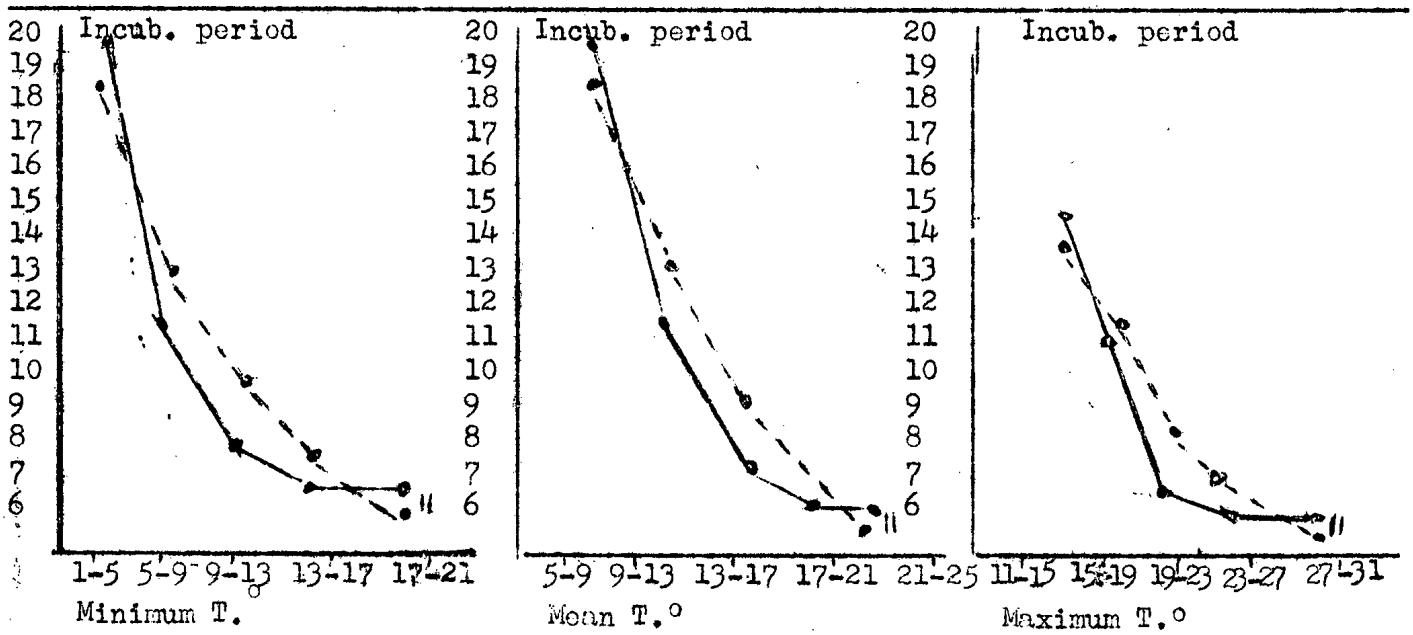


Fig. 1 Curves of relation between the development period of uredo-stage and the temperature: I - empiric, II - interpolated.

It can be assumed on the basis of obtained data that with mean temperatures within the 17-24° range, the development period of the uredo-stage is 6-7 days. As to the relative air humidity, in spite

of its wide amplitudes, no connection between this factor and the development duration of the uredo-stage was established in our experiments.

We had considerably more possibilities for conducting such experiments with uredo-spores. Keeping up a nursery of the latter, we could start experiments in the greenhouse early in the spring and continue them into the fall, covering thus a rather wide temperature range.

The effect of temperature on the duration of the development period of the uredo-stage can be seen from the curves in Fig. 1, from which it is evident that the duration of the period decreases to 6 days when the temperature rises (minimum temperature up to 13-17°, mean 24-hour temperature to 17-21°, and maximum - up to 19-27°), and in case of lower temperature (minimum up to 1-5° and mean up to 5-9°). The duration of the rust development period takes up to 20 days.

Our data was treated by methods of statistical analysis of a complex of characteristics according to an observation scheme based on the so-called "theory of lesser selection" by Fisher-Student (Pomorskii, 1938). The results of the treatment of experimental data are presented in Table I.

Table 1. (p. 103)

Connection between development periods of uredo-stage of P. coronifera with temperature and air humidity.

COEFFICIENTS AND DEGREE OF PROBABILITY OF CONNECTION							
Number of Experiments	Minimum temperatures	Mean 24 hour temperature	Maximum Temperatures	3 elements of temp. (min., mean, max.	Minimum of relative humidity	Mean of relative humidity	Maximum of relative humidity
82	W=0,88 θ=84,35	W=0,19 θ=122,02	W=0,80 θ=45,08	W= 0,93 θ=46,75	W=0,03 θ=1,19	W=0,08 θ=1,69	θ=0,07

It is seen from the table that high coefficients of connections (W) were obtained for all three elements of the 24-hour temperature. And according to methods exposed by Prof. Iu. L. Pomorskii, the connection is statistically proven with a probability exceeding 0.999[1].

As to the effect of air humidity on the development period of the uredo-stage, it has not been proved statistically.

Thus, in regard to causal agents of other diseases as well, for

[1]The probability degree of connection θ three times underscored, indicates the presence of a connection statistically proved with a probability exceeding 0.999, θ with double underscoring speaks of a connection statistically proved with a probability exceeding 0.99 and θ with one underscoring - of a connection proved with a probability exceeding 0.95 (in our experiments, there were none of the latter two cases.) Finally, in cases where θ is not underscored, we dealt with an unproven connection (as, for example, in our case it took place in regard to air humidity).

example Phytophthora infestans, P. tritici, P. glumarum (Naumova), their development period in the plant depends mainly on the 24-hour temperature intensity and does not depend, at least not within the limits which were in our experiments, on the relative air humidity.

In accordance with Prof. Iu. L. Pomorskii's direction, a diagram, with the subsequent presentation of determined connections in the form of a nomograph, was applied for further treatment of material on inoculation during the uredo-stage (Fig. 2).

The developed nomogram makes it possible to determine according to the three elements of the 24-hour air temperature (minimum, mean, and maximum) the development period of the fungus and to make accordingly a prognosis of the next rust appearance.

On the other hand the nomogram allows one to establish the number of parasite generations during a growing period which is important for distribution in various areas and for long-range prognosis.

It should be mentioned that there was a certain difference up to ± 1-2 days, between the actual duration of the development periods of the uredo-stage in our experiments and the theoretical duration calculated according to the nomogram.

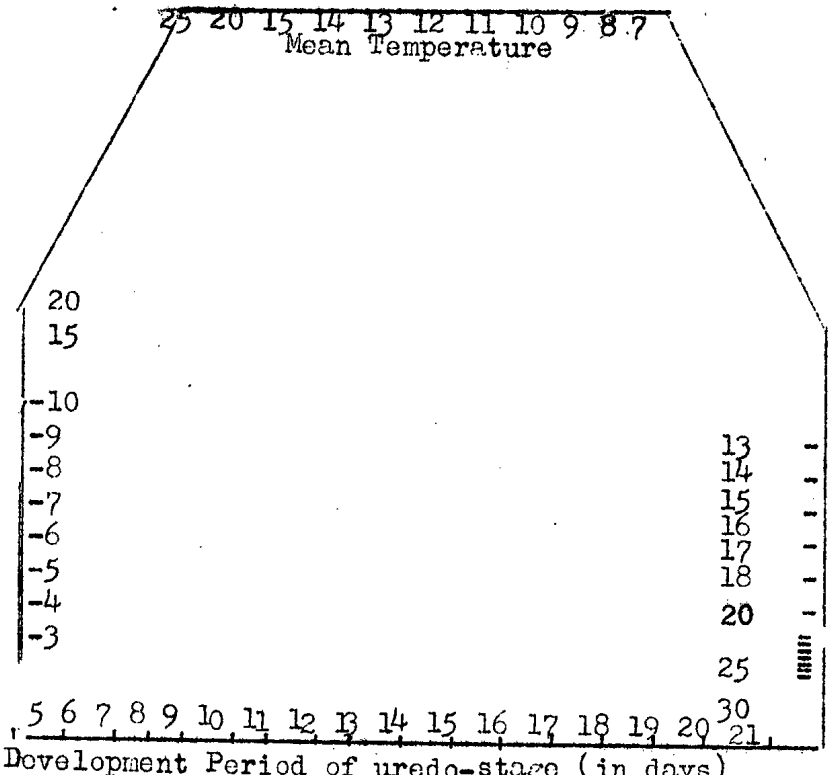


Fig. 2 Nomogram for the establishment of development duration of the of the uredo-stage of Puccinia coronifera.

It is hard to compare our data with the data of the above mentioned authors (Mains, Fromme, Gassner and Appel): they present development periods of crown rust mainly for hot-house conditions, with little changing temperatures, while our experiments were conducted under natural, quite extensive temperature fluctuations. Besides that, the data of these researchers are fragmental.

It is of interest to compare among them the development periods of the uredo-stage when oats are inoculated with aecidia-and uredo-spores. We compiled for this purpose a table (table 2) in which are simultaneously presented for the same mean 24 hours temperatures, development periods which were observed in our experiments.

Table 2 (p.104)

Duration Of development periods of uredo-spores Picoronifera when inoculation with aecidia-and urido-spores took place.

TEMPERATURE	Duration of development periods of uredo-stage			
	When inoculated with aecidia-spores		When inoculated with uredo-spores	
	Number of repeats	Mean duration of period in days	Number of repeats	Mean duration of period in days
17,1-18,0°	5	7,0	7	6,7
18,1-19,0	5	6,6	4	6,0
19,1-20,0	12	6,4	6	6,3
20,1-21,0	8	6,4	6	5,8
21,1-22,0	4	6,5	3	6,0
23,1-24,0	2	6,0	2	6,0

Thus for 17-24° temperatures almost no difference was noticeable in the duration of development periods, whether inoculated with uredo or aecidia-spores. On the other hand, as we already established (Marland, 1938), no difference between the aecidia-and uredo-spores is observed in regard to infection speed. This gives us the opportunity to assume, that it is possible to use within certain limits the nomogram also for calculations of duration of the development periods when inoculation with aecidia-spores took place. Of course, the aecidia-spores and uredo-spores are not equivalent and it can be assumed that at extreme temperatures there might be a difference between them. Therefore the use of the given nomogram for aecidia-spores presents only a first rough approximation.

Our obtained data exposed in the present work are of importance not only for a short-range prognosis; in our opinion they should be a basis for the development of indexes of distribution of crown rust of

oats in different areas, of long-range prognosis of flare-ups of its epidemics, etc. All this is a task for further research.

BIBLIOGRAPHY

Gorlenko, M. V., 1935, Causes of mass affection of oats by crown rust (Puccinia coronifera Kleb.) in 1933, in the Voronesh oblast' Bot. zhurnal SSSR.V.20, no.5

Grushevoi, S.E., 1930, To the problem of conditions of development and harmfulness of crown rust of oats (Puccinia coronifera Kleb.). From works of "fitop. sektsii Mironovskoi op. selekts. st."

Marland, A. G., 1938, Speed of infection inoculation with uredospores of Puccinia coronifera Kleb. Zashchita rastenii, no. 17

Naumova, N. A., 1934, Regularities in development of Phytophthora and brown rust of wheat in connection with meteorological factors. Short report in scient.-US. works of VIZR for 1934

Naumova, N. A., 1935, Effects of temperature and air humidity on the incubation period of Puccinia triticina. Zashch.rast.no.5

Naumova, N. A., 1936, Natural fluctuations of temperature and duration of the incubation period of Puccinia glumarum f.tritici. Zashch. rastenii no. 12

Pomorskii, Iu. L., 1938, Statistical analysis of complex of characteristics. VIZR

Stepanov, K. M., 1940, Effect of heat on the development of the uredostage of the stem rust of wheat and the brown rust of rye (Puccinia graminis tritici and P. dispersa), Izv. Kursov. prikl.zool.i fitopatol, no. X

Shevchenko, V.M., 1931, Affection of oats and rye with Puccinia coronifera Kleb. and of winter wheat and rye with P.trit.Erikss. et Henn, Tr. Bilotserk. sort ivnichoi, p. 62.