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NEW USSR DATA ON SUGAR-BEET-ROOT DISEASE /TIP ROT/

D. L. Tverskoy, Cand Agr Sci
All-Union Sci Res Inst of Beet Culture

/Tables and figures referred to are appended./

In some of the rayons of the old beet-growing zone (Chernigov and Sumy oblasts and the rayons of Kiev, Poltava, and Kursk oblasts which border on them) the sugar-beet crop each year suffers great damage from root disease. In certain years acute infection by root disease is nearly universal. Obviously the field management measures for control of root disease were not quite effective and therefore it was urgent that root disease, first of all its ecology, be studied in great detail.

In our investigations in 1938, new facts became evident quite promptly which indicated the possibility of infection not only of weakened but also strongly developed seedlings. One of the most instructive cases was reported in 1939 at the Chernigov station by phytopathologist M. K. Fomyuk. The most affected sugar-beet seedlings were found in soil having 60-80% of total moisture-holding capacity, at atmospheric temperatures of 21-27%, in other words in conditions relatively favorable for plants, which was confirmed by the heavy weight of the seedlings. Although we did not understand this fact at the time, we were prompted to prolong the study of root disease so that we might explain it later.

As a first step, we considered it necessary to determine the causal agents of the disease and to determine the role of each of them in their contact with normally developing seedlings.

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Causal Agents of Root Disease and Their Propagation

The first analyses of diseased seedlings taken directly from sugar-beet plantings at the "Sukhanovo" Sovkhoz in Moscow Oblast indicated that a series of microorganisms are found in diseased seedlings and that the fungus Pythium de Baryanum Hesse is particularly widespread. Since this fungus was found in nearly every diseased seedling, there was no doubt that it was responsible for the development of the disease (Tverskoy and Zhukova, 1948).

It should be mentioned that in the USSR, P. de Baryanum Hesse was reported in sugar beets only once before, in 1907, by the phytopathologist Trzhebinskiy. It is possible that even earlier, in 1890, this fungus was encountered by Karlson, which is confirmed by descriptions and sketches. However, up to very recent times, there were no reports of this fungus being found in the analysis of diseased seedlings. On the contrary, some investigators explicitly reported finding in diseased seedlings such fungi as Fusarium, Phoma betae, Penicillium, and others, while the subject fungus was not detected.

Since we constantly found P. de Baryanum in diseased sugar-beet seedlings in Moscow Oblast, it was decided to establish its propagation in the sugar beets of other beet-growing regions. In 1947, soil samples were taken from Voronezh, Kursk, Kiev, and Moscow oblasts and from Krasnodar Kray. The samples were taken at a depth of not more than 10 cm under sugar beets, wheat, and grass (clover and lucerne). In each soil sample were planted sugar-beet-seed clusters which had been treated with preparation 1 of the Scientific Institute of Fertilizers and Insectofungicides (NIUFI-1) and carefully washed in running water. To check the appearance of the sugar beets, they were subsequently gathered and analyzed as far as growth on nutrient media is concerned. In all cases the moisture of the soil was maintained at 70% of total moisture-holding capacity.

In the results of these investigations, approximately the same microorganisms were found as in the previous analyses of diseased seedlings from sugar-beet crops of Moscow Oblast (the analyses of diseased seedlings were made by Aspirant K. P. Zhukova and B. S. Navsuts, junior scientific coworker of the laboratory). One of the most widespread causal agents was here again found to be P. de Baryanum. The highest percentage of infected plants and the plants most strongly infected by fungi were found in the soil samples taken from Kursk Oblast (in particular from the Deryuginsk Beet Sovkhoz) and those least infected among the samples taken from the Voronezh Experiment Station. At the same time, fungi of the species Fusarium were found in large amounts in the diseased seedlings, but they were nearly always accompanied by fungi of the species Pythium. In connection with this, it can be surmised that the development of the Fusarium fungi is closely connected with the previous infection by other relatively more aggressive fungi or follows the weakening of the seedlings under the influence of unfavorable soil conditions.

Other fungi, Alternaria, Verticillium, Rhizoctonia, etc., were also found, but in smaller amounts. The Ph. betae are not included, as their presence in the diseased seedlings in our investigations can be attributed rather to incomplete disinfection of the seed clusters than to their presence in the soil. In general, the degree of propagation of the basic causal agents of root disease in the infected sugar-beet seedlings from various previous crops for an average of all the tested samples is as shown in Table 1.

In 1948, the investigation of the infestation of the soil was considerably expanded. Thirty-five new soil samples were taken from Khar'kov, Vinnitsa, Chernigov, Kursk, Voronezh, Penza, and Moscow oblasts, and also from Alma-Ata Oblast, Kazakh SSR, and Tashkent Oblast, Uzbek SSR. A somewhat different method was employed for examining the soil and diseased seedlings, primarily with the aim of checking a larger field of moisture-loving fungi. As is seen in Table 2, the mycological analysis of the diseased seedlings in this case indicates that one of the basic causal agents of root disease in nearly all the soil samples was found to be P. de Baryanum Hesse. Besides this, a completely new causal agent, Aphanomyces cochlioides Drechs, was found in the course of this investigation. As far as known, this fungus up to now had not been reported in the beet-growing regions, with the exception of the solitary mention in 1907 by the phytopathologist Trzhebinskiy, who stated that fungi were detected on two occasions. As seen in Table 2, the Aphanomyces cochlioides were found in nearly all the soils with the exception of the samples from Central Asia and from Penza Oblast, and in quantities exceeding the P. de Baryanum.

Among the other fungi found in the analysis of the diseased seedlings, nearly all were representatives of the species Rizoctonia and Fusarium, and were particularly widespread in the soils from Central Asia. In all, there were fewer Alternaria fungi in the diseased seedlings, and fewer Ph. betae, the fundamental source of which appears to be not the soil but the infected seed. The P. de Baryanum were most highly represented in the soils taken from under grass, the A. cochlioides in the soil from under beets and wheat, the Rhizoctonia from under lucerne, and the Fusarium from under lucerne, clover, and wheat (see Table 3).

Thus our investigations indicated that in diseased sugar-beet seedlings planted in the soils of the various beet-growing regions, there were found a fairly large number of the same varieties of fungi, relatively stable in their ratios. However, Pythium de Baryanum, Aphanomyces cochlioides, and Fusarium must be considered to be the most widespread among them. All the remaining fungi were more weakly represented, with the exception of some obviously having narrow local importance (for instance, the Rhizoctonia in Central Asia).

Parasitic Activity of the Causal Agents of Root Disease

It was interesting to trace in what measure the fungi which we had found appeared to be actually aggressive on contact with normally developing sugar-beet seedlings. To clear up this problem, a few tests were set up in containers holding soil which had been artifically infested with each of the investigated fungi. The required amount of fertilizer was introduced into the soil and the moisture was maintained at 70% of total moisture-holding capacity. Table 4 shows the results of one of those tests which was set up in a greenhouse during the summer and under very favorable temperature conditions.

As shown in Table 4, the greatest infection of the seedlings for relatively normal conditions of development is produced by P. de Baryanum, A. Cochlioides, R. Aderholdi, R. Solani, and Ph. betae. All the other fungi caused only very insignificant infection of the plants. Thus it is clarified that the microorganisms which have a part in root disease are totally distinct in their parasitic activity. Under certain soil conditions, some of the parasitic fungi are able to infect normally developed seedlings.

Obviously we could not leave out the important characteristic of activity of the causal agents of root disease given by the degree of infection of the seedlings in various phases of their development. Some of the conducted tests indicated that in artificial infection by fungi of seed clusters of plants in the phase of forking and of those in the stage of the first pair of leaves, P. de Baryanum principally attacks the young seedlings in the soil and plants in the forking phase. Ph. betae appears to be most aggressive in the infection of seedlings in the phase of forking and that of the first pair of leaves, in other words, in that period when the young plants are converting to self-supported nutrition and bleaching of the root begins. Fungi of the genus Rhizoctonia develop almost equally on young seedlings and on more mature plants (two or three pairs of leaves). However, the degree of destruction of the seedlings decreases as their development progresses. A. cochlioides, when there is sufficient moisture in the soil, strongly attacks plants in the forking phase and in later periods of development, so that we must report the highest degree of development of this fungus on plants having three and even four pairs of permanent leaves.

From these investigations it follows that in natural conditions when there are suitable causal agents in the soil, root disease can develop over a rather long time. Such a case is rarely observed, mainly in years with protracted high moisture content of the soil in the spring.

In the enumerated tests, we were able also to trace the course of the disease in some detail. It is necessary to cite the characteristic symptoms of the disease caused by the more aggressive parasites, since up to now the diagnosis of root disease of sugar-beet seedlings has not been covered.

External Symptoms of Diseased Sugar-Beet Seedlings

A. cochlioides affects only the part of the plant above ground, from the root neck upward. Therefore, it is distributed on the petiole and cotyledon. At the onset of the disease, the infected tissue has a characteristic watery gray-green color. Later, the infected part turns brown, becomes thinner, and finally is converted into a dark-brown or black fiber. Due to the infection, in the basic cortical parts of the plant, the leaflets remain turgescent and dark green for a long time. P. de Baryanum in contrast to the preceding fungus affects the underground portions of the plant and rarely appears above the soil. Only on very young plants is the disease able to go so far as to take in the whole seedling. In the beginning, the disease is characterized by the appearance of a shiny, as if watery, light-brown rot, which in time withers and turns dark brown, and almost black.

The development of the disease starts in the soil and evidently at a very early stage in the development of the plant, which is why many plants do not come above to the surface. The diseased plants which do appear on the surface show symptoms of withering and have yellow-green (lemon) colored leaves. Ph. betae affects only the underground part of the plant, predominately at the place where the radicle and pedicle come out of the seed. In the first stage of the disease, on the affected seedlings there appear small dark-brown or black marks which are difficult to detect and which then spread, blend together, and encircle the seedling. Later the infected part advances above or below the initial locus of the disease, darkens, and assumes the carbon-like color of the ensuing dry rot. At high temperatures, the infected plants wither as if from a lack of water. R. Aderholdi develops on the underground part of the plant in the form of reddish-brown lesions, some of which go so far as to completely encircle the root. Badly diseased plants turn yellow and wither.

R. solani, induces relatively less intensive lesions, with a golden brown color on the underground part of the plant. It rarely kills the plant. Fusarium infects mostly the small radicles and the lower part of the main root, developing a dry rot with a dark-brown or yellow-brown color.

Effect of Temperature and Moisture Content on the Development of the Causal Agents of Root Disease in the Soil

It is very important to clarify to what degree the development of the subject fungi is connected with the moisture content and temperature of the soil. Tests were set up in plant containers with artificially infested soil the moisture content of which was maintained at 40, 60, and 80% of total moisture-holding capacity. To prevent dustiness, the soil was first mixed with uncontaminated calcined sand in the proportion of one part of sand to three parts of soil. The tests were conducted in a greenhouse during the summer at a temperature most favorable for growth of the seedlings. The number of dead plants was checked each day. On the thirtieth day, when all the sugar-beet seedlings had developed their first pair of leaves, a check was made, the results of which are recorded in Table 5. As the table shows, the fungi which we tested, with the exception of

P. de Baryanum and A. cochlioides, infected the greatest number of seedlings in the soil with 40% moisture content. Under these conditions the seedlings fell prey not only to the more aggressive fungi, but also to the less active microorganisms such as B. cinerea, Fusarium, and A. tenuis.

Reduction of the amount of moisture in the soil evidently weakens the plants and they become vulnerable to the attacks of even the less active fungi. From this we can conclude that where the seedlings have considerably deteriorated almost to the point of destruction, it is possible for them to be infested by the very harmless saprophytic microorganisms.

With increased moisture in the soil, the parasitic activity of subject fungi drops and the aggressiveness of P. de Baryanum and A. cochlioides is increased. In our tests, due to the mixing of the soil with sand in containers and the resulting high moisture content, the physical conditions were completely normal. Consequently, the infection of the seedlings in this series of containers must be considered as highly representative of the characteristics of the parasitic activity of the fungi.

It is clearly apparent from the test that root disease can infect both the weak and strongly developed sugar-beet seedlings. However, chiefly the semiparasitic and even saprophytic fungi attack the weak plants, whereas only some of the most aggressive parasitic fungi develop in the healthy seedlings. From among the group of fungi chiefly attacking the weakened seedlings, the most important in the beet-growing regions is Ph. betae. However, it does not cause serious damage to the sugar-beet crop. According to the available data on Ph. betae, it is not preserved in soil when crop rotation is applied, and the seriousness of the infection of seedlings by Ph. betae is completely dependent on the degree it has infected the seed. Therefore, treatment of the seed by a fungicide can serve as a very effective method of controlling this causal agent of root disease. The measures for controlling the other less active causal agents of root disease can basically be reduced to maintaining normal moisture content in the soil, which increases the strength of the plants and their resistance to infection.

From the group of more active fungi infecting mostly the healthy seedlings, P. de Baryanum and A. cochlioides are particularly widespread among sugar beets. These fungi are permanent constituents of the soil and must be considered the most dangerous causal agents of root disease. Since large-scale development of the subject fungi is invariably connected with an increase in soil moisture, the control of these fungi must involve the elimination of excess moisture, in addition to other measures.

According to available data, one of the causal agents of root disease, P. de Baryanum, despite its need for moisture, is pronouncedly aerobic and therefore develops mostly in the upper layers of the soil. We arranged a little test with dried soil. The test was set up in wooden boxes of completely sterile soil, artificially infested with P. de Baryanum. The moisture content of the soil was maintained at around 60% of the maximum moisture-holding capacity. The soil in one box was loosened every 2 days, starting when the seedlings emerged; in the other, it was loosened only twice, once when sprouts emerged and the second time 5 days later. The test was concluded when the plants reached the completely forked stage; then the data arranged in Table 6 were compiled.

In the box where the soil was repeatedly loosened, 44.1% of the plants were found to be diseased, and 27.1% of them died. In the other box where the soil was only loosened twice, 91.4% of the plants became infected, and 58.6% died. Thus this short test indicated that for control of moisture-loving fungi such as P. de Baryanum, repeated loosening of the soil was of value. The loosening of the soil, in the first place, resulted in the drying out of the upper soil layer, which destroyed the fungi in the mycelia stage when they are very sensitive to lack of moisture. Secondly, the frequent aeration of the soil accelerated the development of the young plants, which shortened the period of greatest susceptibility to infection.

The data arrived at here is conformed to closely by investigations made under field conditions. In tests conducted in 1936 at the Kiev (Mironovskiy) Experiment Station of the All-Union Scientific Research Institute of Beet Culture, it was found that when the soil was loosened up with a rye harrow 4 - 5 days after sowing, 24.2% of the seedlings were infected with root disease; when it was hoed "blind," using market plants, 30.5% were infected; and when it was hoed after the seedlings emerged, 41.7% were infected.

In other cases, hoeing at the time of the first marking of the rows resulted in 22% diseased seedlings, hoeing on the second day after this produced 29% diseased seedlings, on the fifth day 31%, and on the tenth day 47%.

In these tests, there was evidenced not only the importance of loosening the soil, but also of the time at which it is carried out. The greatest reduction in the development of root disease was found when the loosening was done prior to the emergence of the seedlings or at the time when they broke through the surface of the soil. These results do not differ from those produced in our box tests. Early loosening here produced the same results, the drying out of the soil. As is known, the soil is moister during the period of seed germination. Timely loosening dries the soil in the upper levels but does not change the amount of moisture in the lower layers, which leads decidedly to reduction of the activity of the moisture-loving fungi. Loosening of the soil after rainfalls achieves the same results, and the sooner this operation is done the more effective it is. Loosening of moist soil is very valuable for other reasons as well. Timely loosening prevents crusting of the soil.

Tests were also made on soils from "Sukhanovo" Sovkhoz in Moscow Oblast, "Pobeditel'" Kolkhoz in Rakityansk Rayon, Kursk Oblast; and on soils from the Nemarchansk State Selection Station. In these experiments, increasing the moisture content of the soils up to 60-80% of the total moisture-holding capacity also always sharply increased the infection of seedlings by root disease. Analysis of the diseased seedlings invariably indicated the presence of the fungi P. de Baryanum and A. cochlioides.

We checked the effect of temperature on the development of microorganisms in the sugar beet seedlings in a polythermostat [incubator]. Small clay dishes of completely sterile soil were infested with subject fungi and placed in chambers with the temperature gradients shown in Table 7. Dishes with sterile noninfested soil were used as controls. In each dish were planted 25 seed clusters of uniform size, each of which had been disinfected in a solution of NIUIF-1 and carefully washed in sterile water. The moisture of the soil was maintained at 70% of total moisture-holding capacity. The test was repeated four times for accuracy. In the course of the test, the appearance of the seedlings and the number of dead plants were recorded. The test was extended until all the seedlings emerged, which action took place at different times in each chamber. The data collected in this test are arranged in Table 7.

First of all, the tested microorganisms reacted differently on the sugar-beet seedlings. As in the previous tests, the first four fungi were found to be the most aggressive. The others caused very mild disease in the seedlings, even in conditions which were not favorable for the development of the plants (faint illumination). The effect of the temperature was shown to be such that the P. de Baryanum and R. Aderholdi infected the greatest number of seedlings at from 21-25° C, the Ph. betae and R. solani at 21° C, the B. cinerea at 25° C, and the A. tenuis at 32° C. The Fusarium fungi produced only a very low percent of infection at all temperatures.

The infection of the seedlings at different temperatures very naturally conforms to the intensity of growth of the fungi at the same temperatures on nutrient mediums in a Petri dish.

Previously, we had succeeded in determining the dependence of A. cochlioides on temperature. As compared to P. de Baryanum, it has a somewhat higher optimal temperature of growth, in relation to which the maximum degree of infection of seedlings by it dropped to 29-30° C.

The dependence of the growth and activity of fungi on temperature are shown graphically in Figure 1, using as an example one of the most widespread causal agents of root disease, P. de Baryanum.

The number of seedlings remaining in the infested soil in comparison to the sterile soil control is decreased gradually by the rise in temperature. The number of seedlings killed were found to be; at 9° C - 23.0%, at 12.5° C - 50.0%, at 16.5° C - 64.3%, at 21° C - 80.0%, at 25° C - 100%, and at 32° C - 83.4%.

In Figure 1, it is obvious that the maximum number of killed seedlings and the greatest growth of fungi were found at 25° C. At 32° C the growth of the fungus is somewhat depressed, which is directly reflected in the reduction of the number of killed seedlings. The relation we found, namely, that of a direct connection between the energy of development of the fungi and the energy of growth of the plant, demonstrates that fungi P. de Baryanum has a clearly expressed parasitic nature when it affects sugar-beet seedlings. It appears to be a well adapted parasite developing best in conditions which are the most favorable for the beets. This biological feature of the fungus makes it very difficult to eliminate from sugar beets. Along with this, the intimate connection of the fungus with the plants in the root contradicts the previous hypothesis that healthy sugar-beet seedlings could not be infected by soil fungi (Murav'yev, 1939). What must be taken into consideration is not only the necessity to produce strongly developed seedlings but also the equally important requirement of creating in the soil conditions which inhibit the development and accumulation of parasitic fungi.

At the same time, we studied the effect of temperature on the degree of infection of seedlings in naturally infested soils with the same moisture content. Sterile uninfested soil was used as a control. The data derived in this test concurs almost completely with the results of the previous test for P. de Baryanum. As is seen in Table 8, the largest number of seedlings remaining alive when all the seedlings had emerged was at the point when the temperature was 12.6° C; the smallest number was at the point when the temperature was 20.7 and 26.5° C.

Damage Done by Root Disease

In our tests made in the conditions of Moscow Oblast corresponding to severe infection of sugar-beet seedlings by root disease, the productivity of the roots was reduced as much as 80% and the total yield of the harvest as much as 40%. On the average, for the past 3 years the infection by root disease reduced the sugar-beet yield around 30%. On the basis of the data in our possession we can surmise that in regions where there is constant and severe infection of sugar beets by root disease, the loss due to this disease is around 25-30%.

In 1948, we did some tests which indicated that the harmful effect of root disease is due to the disruption of the carbohydrate metabolism by reason of severe infections of the cortical parts through which the circulation of assimilated substances occurs. This disruption is shown in delayed drawing off of sugars at night and the slow accumulation of them in the daytime.

In one of these tests the amount of sugar in infected seedlings during the daylight hours was 40% less than that in the healthy plants. In time the disruption of the carbohydrate metabolism leads to a sharp inhibition of the growth of the plant. The plants are inhibited in passing through their stages of growth and have lower weights than the healthy seedlings (see Table 10).

Tests for studying the effect of root disease on the thinning out of a crop of seedlings after emergence were made at "Sukhanovo" Sovkhoz in Moscow Oblast on sugar-beet crops 94% infected with root disease. It was found that after emergence approximately 70% of diseased seedlings remained in the field. Furthermore, about 25% of the diseased seedlings died in the first 2 months; the remainder survived, but their yield was lowered. In one of these tests it was established that in a case of 73% infection of the field after emergence, the sugar-beet yield was reduced more than 38%. Thus one important side of the destructive activity of root disease was made apparent, which up to the present had not been paid the attention due to it and which had been frequently ascribed to other factors (principally vermin).

Susceptibility of Crops Rotated With Beets to the Basic Causal Agents of Root Disease

Since the majority of root disease causal agents are typical soil parasitic fungi, it was very important to determine in what degree they are able to infect other crops which are rotated with sugar beets and thus be preserved in the soil. Special tests were set up in which some crops were planted in artificially infested soil. The tests were conducted in a greenhouse in broad clay dishes and a quadruple check was used. The moisture content was kept at 70% of the maximum moisture-holding capacity. Dishes with sterile soil in which the same crops were planted were used as controls. After 30 days a complete record was made, the results of which are arranged in Table 11.

As can be seen in the table, A. cochlioides infected only the sugar beets. All the other crops were shown to be completely resistant to this parasite. The two other fungi were unusually severe as regards the infection caused by the fungi in beets, peas, clover, lucerne, and exparsette. Vetch and sunflowers were considerably less affected and the grain cultures - wheat and rye - were almost completely unaffected.

Thus it has been shown that in beet-crop rotation there are crops which are susceptible to the basic causal agents of root disease. Due to these crops, the causal agents are preserved in the soil. In addition, their preservation can be due to some weeds (pigweed and others). Data can also be cited on the infection of sugar beets by root disease from previous crops. This data, taken from a report by K. P. Zhukova, an aspirant of the laboratory, is shown in Table 12.

It can be seen from the table that the largest number of sugar beets infected by root disease occurred when the sugar beets were planted after beets and the smallest number when they followed grain crops. Very high infection of the seedlings occurred when the sugar beets followed clover, potatoes, peas, and also wheat when the crop preceding wheat was also sugar beets.

The above test was conducted in a greenhouse in special boxes and therefore the length of time any of the crops were cultivated was not observed here, which was particularly important in relation to the clover, the long cultivation of which promotes the accumulation in the soil of valuable saprophytic flora which aid in the control of parasitic fungi. The data produced here ought to be checked in crop rotation under field conditions. None the less, even now we can assume that, to reduce the preservation of parasitic fungi in the soil, it would be necessary first of all to introduce grassland crop rotation with the greatest possible use of a grass mixture containing those plants of the grass family which our tests showed not to be infected by the active causal agents of root disease and thus facilitate ridding the soil of them.

Beet sowing should be done after an uninfected previous crop (chiefly after winter grains, which were planted on pure fertilized fallow, or on spring grain after grass), where beets have not been grown for 2-3 years. In addition, it is necessary to carefully control the weeds on all crop-rotation fields.

Treating Sugar Beet Seeds With a Fungicide to Control Root Disease

Karlson had shown in 1890 that treatment of seed clusters with a 2% solution of blue vitriol cut in half the infection by root disease. Carbolic acid (2%) showed a milder effect, but did not affect the germination of the seed. In 1905, treatment of the seed by a fungicide was studied by the phytopathologist Trzhebinskiy. After a great number of chiefly laboratory tests, he came to the conclusion that the best fungicides for use against root disease were a 2% solution of blue vitriol and an 0.5% solution of mercuric chloride. A more detailed study of fungicides was made from 1925 to 1929 by Grushevoy at the Mironov Experimental Selection Station. A great number of different fungicides, containing mercury, copper, arsenic, and other poisonous ingredients, were tested. The tests showed that the fungicides reduced the infection by root disease only slightly, but that they commonly impeded the germination of the seed. The mercury preparations were somewhat better than the other substances tested. From the results, it was concluded that treatment of the seed clusters by fungicides as a measure for controlling root disease is of little effect and therefore it was not recommended in practice (3).

After the tests at the Mironov Experimental Station, the further study of treatment of the seed by fungicides was almost completely discontinued, because the principal causes of root disease were considered to be different types of unfavorable soil factors, the elimination of which would reduce the development of the disease. A minor role was relegated to microorganisms, so that the treatment of the seed by fungicides was considered to be without purpose, which was a premature judgment. Then it was found that one of the most widespread causal agents of root disease, Ph. betae, was carried by the seeds. The necessity of treating the seeds with fungicides became obvious, if only for the reason that the seeds had to be protected against this particular fungus. Nevertheless, research on new fungicides capable not only of destroying the infection in the seeds but also possibly capable of protecting the young sprouts from microorganisms of the soil was indicated.

A decision was made to restudy the treatment of seeds with fungicides. N. V. Perkel', a co-worker of our laboratory, tested a large selection of contemporary fungicides. He found that the ones which were most toxic for the microorganisms and which did not adversely affect the germination of the seeds, were the preparation NIUIF-1 in the dilution 1:300 and "Granozan" in the amount of 5 grams per kilogram of seed. These fungicides almost completely free the seed clusters of Ph. betae and offer considerable protection to the seedlings against infection in the soil.

These mercury fungicides were tested under practical conditions at the "Sukhanova" Sovkhoz of Moscow Oblast and the "Pyatiletka" Kolkhoz in Rakityansk Rayon of Kursk Oblast. The results are shown in Table 13. As shown in the table, the treatment of the beet seeds by the fungicides, in particular by "Granozan," reduced the infection of the crop by root disease, increased the density of root spacing, and as a final result, produced an increase in the sugar-beet yield. In addition to this, it was found that the fungicides did not protect the seedlings in the field in the same degree as in the tests using the fungicides against each causal agent of the disease individually. In the crops treated with fungicide, it was found that at

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first the fungicides promoted dense sprouting and relatively less infection by root disease, but gradually the number of diseased plants increased, reaching a considerable figure at the end of the test.

Special tests were set up to uncover the reasons for the poor protection which the fungicides gave to the seedlings after the seedlings emerged from the soil surface. We had noted the poor effectiveness of the fungicides under field conditions in 1946, when we thought that it appeared to be connected with the growth of A. cochlioides on the sugar-beet seedlings. As soon as this fungus was found in infected sugar-beet seedlings and a pure culture of it isolated, we quickly proceeded to investigate the effect which fungicides have on it in comparison to the effect the fungicides have on P. de Baryanum. The test was conducted in a greenhouse with the average temperature no lower than 18° C, and the soil was artificially infested with A. cochlioides in one series and P. de Baryanum in the other. The seed used was treated with "Granozan" in the amount of 5 grams per kilogram of seed. During the whole series of tests, observations were made on the development of the disease, and the number of diseased plants was recorded daily. The results of the tests are shown in Figure 2. It is clearly seen that treating the seed with "Granozan" protects the seedlings from P. de Baryanum unusually well. In this section of the test, the treatment by fungicide effectively promoted a high degree of sprouting of the seedlings and their number remained more or less constant up to the end of the investigation. In the other section of the investigation, the subject treatment had almost no effect on A. cochlioides. In this case, the treated and untreated seed produced almost an identical number of sprouts, but they quickly started to die and were all dead by the end of the test. Thus it is shown that fungicides, in particular "Granozan," protect the seedlings only from P. de Baryanum and do not protect them at all from A. cochlioides, and that this is obviously what takes place when fungicides are used in practice.

Surmising that in the given case the difference in the effect of the fungicide was directly connected with peculiarities in the development of these fungi, we set up a test for determining the dynamics of infection of the sugar-beet seedlings in soil artificially infested with P. de Baryanum and A. cochlioides.

As can be seen in Figure 3, the development of the subject fungi is completely different in character. P. de Baryanum develops chiefly in the early stages of seedling growth and usually leaves most of the plants on the fourteenth day after planting. On the contrary, A. cochlioides attacks the seedlings in the later phases of their growth, so that the greatest number of seedlings were destroyed by it in the second half of the test.

Quantitatively this difference was expressed in about the following degree. If the first fungus on the fourteenth day has destroyed 46 seedlings and in the remaining time only 14 plants, then the second fungus destroys only 16 plants in the first 14 days but kills 56 in the remaining 7 days. Thus the first fungus is adapted to the earlier growth stages of the seedling while they are still germinating in the soil, and the second fungus is adapted to the later stages and infects them on the surface of the soil. Apparently the fungicide is adsorbed into the soil and prevents infection of the above-ground part of the young plant, either to a slight extent or not at all. In view of this, the treatment of seed with a fungicide, despite its undeniable advantages, will be of limited value until such time as a method can be found for protecting the shoots from attack by the parasitic fungus A. cochlioides. In this connection, a total disinfection of the soil in rows seems to promise the greatest degree of success.

In conclusion, it should be mentioned that much of the data given here obviously ought to be carefully checked in practice.

[redacted]

Conclusions

It was established in our tests that, contrary to previous opinion, root disease infects the strongly developed sugar-beet seedlings as well as the weaker ones. In both cases, the disease is caused by microorganisms, but semi-parasitic and even saprophytic fungi attack the weaker seedlings, whereas only the very active parasites attack the healthier ones. Thus, contrary to previous opinion, the fungi in some cases represent directly the determining factor in the development of root disease.

The main causal agents in large-scale developments of root disease are the parasitic soil fungi P. de Baryanum and A. cochlioides. Ph. betae is of secondary importance. This fungus is propagated chiefly from the seeds, from which it can be eliminated by treatment with fungicides. All the other fungi found in diseased seedlings are weakly represented (R. Aderholdi) or they are typical semiparasites or even saprophytes (Fusarium, Alternaria, Penicillium). These fungi are able to develop only as secondary causal agents (more frequently after Pythium) or else they attack only the weak plants purely as a result of faulty field management.

Typical parasitic soil fungi cause the greatest infection of seedlings when the moisture content of the soil is high (60% or higher) and the temperature is relatively high (16^{o} and above). Ph. betae, on the other hand, infects the seedlings to the greatest degree when there is a lack of moisture, i.e., when the seedlings are somewhat weakened. These data enable us to provide an explanation for large-scale outbursts of root disease and also to check the regions where it is most widespread.

The data compiled in this article permit us to assume that the control of root disease should be reduced to the use of suitable methods of field management and some special measures. The field management methods must not only provide for the normal development of the plants, but also create conditions in the soil which inhibit the development and preservation of parasitic fungi. Among these measures the following are recommended at present:

1. Introduction of grassland crop rotation, using a grass mixture of leguminous and grass family plants which promote the greatest reduction of parasitic fungi in the soil.

2. Drainage of wet soil ground water which occurs nearby.

3. Planting the beets subsequently to uninfectable previous crops, preferably after winter grain crops which had been planted in pure fertilized fallow or after spring grain crops which had been planted subsequently to grass, and replanting beets on an area only after an interruption of 2-3 years.

4. Treating the seed before planting with "Granozan" in the amount of 5 grams per kilogram of seed or with the NIUFI-1 preparation in the dilution 1:300, using the moist type of treatment and letting the seed lie for 2 hours.

5. Loosening the soil over the crop with a light weight harrow (nail type) before the emergence of the seedlings and promptly hoeing after they emerge. In case of rains, the hoeing should be repeated to prevent caking and drying out of the upper layer of soil.

6. Digging the beets out promptly and requiring the collection and removal of all vegetative litter to reduce the preservation of infection in the soil.

7. Careful control of weeds, since it is known that the root disease causal agents infect many weeds as well as sugar beets. Control of weeds should be carried out in all crop rotation fields, especially fields left in fallow prior to winter crops, after which sugar beets are planted.

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[Tables and figures follow.]

Table 1. Propagation of Fungi as Affected by Previous Crop

Previous Crop	No of Diseased Seedlings (%)	Degree of Occurrence of Fungi (%)		
		P. de Baryanum	Fusarium	Ph. betae
Beets	60.9	41.7	47.6	13.6
Wheat	46.4	34.6	60.6	15.2
Grass	51.3	29.3	55.7	16.5
Average	51.1	35.2	55.3	15.1

Table 2. Mycological Analysis of Diseased Beet Seedlings

Place From Which Soil Sample was Taken	Preceding Crop	Quantity of Diseased Seedlings (%)	Seedlings Killed (%) Before Emergence	Seedlings Killed (%) After Emergence	Microorganisms (%) Phythium	Microorganisms (%) Aphanomyces	Microorganisms (%) Rhizoctonia	Microorganisms (%) Fusarium
Khar'kov Oblast								
Khar'kov State Selection Station	Beets	95.8	7.5	85.8	50.6	71.6	--	10.0
	Wheat	21.6	15.7	0.0	0.0	0.0	0.0	0.0
	Lucerne	67.1	2.3	20.8	65.2	10.4	9.0	31.4
Vinnitsa Oblast								
Nemerchansk State Selection Station	Beets	100	9.6	90.4	55.4	83.9	--	--
	Wheat	20.9	7.0	0.9	35.0	58.0	--	20.0
	Clover	65.8	12.8	27.2	51.1	24.8	11.6	19.3
"Chervonyy Zhovten" Kolkhoz	Beets	39.0	10.0	7.0	28.5	28.5	14.2	71.4
Vladovo-Lyulenetsk Selection Station	Beets	100	17.0	83.0	35.7	84.2	3.4	9.2
	Wheat	19.0	12.0	2.0	26.8	38.3	--	40.9
	Clover	30.0	14.0	6.0	44.3	10.5	11.2	34.0
Chernigov Oblast								
Nosovskiy ROSKh [Regional Department of Ministry of Agriculture?]	Beets	100	16.0	84.0	54.2	72.0	8.5	12.8
Kursk Oblast								
L'govsk Selection Station	Beets	88.0	7.0	42.0	31.0	55.0	13.7	20.6
"Strana Sovetov" Kolkhoz	Beets	57.0	0.0	36.0	32.5	67.5	6.8	18.7
"Pobeditel" Kolkhoz	Beets	100.	28.0	72.0	30.6	71.0	--.	13.1

Table 2 (Contd)

Place From Which Soil Sample was Taken	Preceding Crop	Quantity of Diseased Seedlings (%)	Seedlings Killed (%) Before Emergence	Seedlings Killed (%) After Emergence	Microorganisms (%) Phythium	Microorganisms (%) Aphanomyces	Microorganisms (%) Rhizoctonia	Microorganisms (%) Fusarium
Voronezh Oblast								
Voronezh Experiment Station	Beets	20.0	7.5	5.9	83.3	16.5	--	30.2
	Clover	11.9	0.0	0.7	--*	--	--	--*
	Lucerne	25.3	12.7	0.9	70.0	--	--	100
Ramonsk Selection Station	Beets	100	0.0	100	37.8	75.8	1.4	--
	Wheat	63.1	4.0	25.0	29.0	60.0	9.4	47.3
	Clover	19.0	14.0	3.0	40.0	8.0	--	60.0
Penzena Oblast								
Sosedsk ROSKh	Beets	100	2.0	7.0	15.7	--	21.0	78.9
Moscow Oblast								
"Sukhanovo" Sovkhoz	Beets	96.3	0.8	88.6	42.3	73.6	8.0	28.0
	Wheat	93.0	4.5	77.0	33.8	53.0	--	20.9
	Clover	59.4	27.0	19.0	31.4	27.1	14.2	30.7
	Potatoes	95.4	0.1	85.9	25.4	67.2	14.5	24.5
	Beets	95.3	0.0	5.6	48.0	62.9	16.0	25.3
Alma-Ata Oblast								
"Passvet" Kolkhoz	Beets	12.0	0.0	0.0	--*	--	--*	--*
"Pervoye Maya", Kol-khoz, Brigade No 3	Beets	13.0	0.0	4.0	15.8	--	20.0	80.0
Tashkent Oblast								
Ak-Kavak Agricultural Experiment Station	Cotton	11.0	2.0	4.0	--*	--	--*	--*
	Wheat	17.0	9.0	3.0	--	--	13.7	86.3
	Lucerne	35.0	19.0	16.0	9.8	--	40.2	50.0
Average		57.3	10.3	42.1	38.8	52.2	13.4	38.5

* Small amount of fungi, percentage not calculated.

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Table 3. Occurrence of Fungi in Diseased Seedlings

Preceding Crop	No of Diseased Seedlings (%)	Propagation of Fungi (%)			
		Pythium	Aphanomyc	Rhizoctonia	Fusarium
Beets	73.5	38.4	65.8	12.7	33.6
Wheat	39.5	31.1	52.3	11.3	43.2
Clover	37.2	41.7	17.6	12.3	36.3
Lucerne	42.4	48.3	10.4	25.5	60.4
Average	43.1	39.9	36.5	15.6	43.4

Table 4. Percentage of Diseased Seedlings in Soil Infested by Various Fungi

Causal Agents	Percent of Seedlings		
	Healthy	Medium and Intensely Diseased	Killed
Control	100	0.0	0.0
P. de Baryanum	26.4	27.0	36.6
A. cochlioides	10.6	20.2	59.0
R. Aderholdi	18.8	26.4	41.6
R. solani	56.2	13.4	9.8
Ph. betae	27.3	38.5	5.2
A. tenuis	92.3	0.0	0.0
L. cinerea	97.5	0.0	0.0
Fusarium strain No 1	84.8	4.2	0.0

Table 5. Effect of Moisture Content of Soil on Infection of Seedlings by Causal Agents of Root Disease

Moisture Content	40%			60%			80%		
	Percent of Seedlings			Percent of Seedlings			Percent of Seedlings		
Causal Agents	Healthy	Medium and Severely Diseased	Killed	Healthy	Medium and Severely Diseased	Killed	Healthy	Medium and Severely Diseased	Killed
Control	100	--	--	100	--	--	10.0	--	--
P. de Baryanum	70.0	14.0	9.4	30.5	19.4	38.0	1.2	15.7	78.4
A. cochlioides	72.5	14.5	8.2	27.3	16.2	50.0	0.0	17.0	88.0
R. Aderholdi	3.7	61.0	35.6	13.5	29.7	37.4	11.7	21.3	52.4
R. solani	24.8	47.0	5.7	41.5	44.7	0.0	70.0	11.7	1.9
Ph. betae	27.7	53.7	10.4	38.6	34.1	8.0	69.4	12.1	1.5
B. cinerea	69.9	5.8	10.9	87.6	0.6	3.8	95.8	1.6	0.0
Fusarium strain No 71	67.0	8.0	3.7	86.8	1.4	0.0	97.5	0.8	0.0
A. tenuis	66.4	2.8	0.0	81.7	4.1	0.0	94.0	0.7	0.0

Table 6. Effect of Loosening the Soil on Infection of Beet Seedlings by the Fungus P. de Baryanum

Variables of the Test	Diseased Seedlings (%)	Diseased Seedlings (%) Mild	Avg	Severe	Killed Seedlings (%)
Loosened twice	8.6	15.5	6.8	10.5	58.6
Loosened many times	55.9	5.1	5.1	6.8	27.1

Table 7. Effect of Temperature on the Infection of Seedlings by Causal Agents of Root Disease

Type of Fungus	Percent of Seedlings for Temperatures (°C)											
	32°		25°		21°		16.5°		12.5°		9°	
	Diseased	Killed	Diseased	Killed	Diseased	Killed	Diseased	Killed	Diseased	Killed	Diseased	Killed
P. de Baryanum	100	83.3	100	100	100	80.0	95.2	64.3	60.2	50.8	-23.0	23.0
R. Aderholdi	100	100	100	100	100	79.5	95.2	83.7	75.8	60.8	10.1	3.2
R. solani	34.6	0.0	73.2	44.1	82.2	20.0	54.8	4.6	38.7	6.2	8.2	3.1
Ph. betae	62.9	8.1	75.6	58.5	78.7	36.1	51.2	24.0	18.1	0.0	8.3	0.0
B. cinerea	11.1	0.0	24.1	8.2	0.0	0.0	9.5	9.5	5.1	3.2	3.8	1.6
Fusarium strain No 71	5.5	5.5	7.3	7.3	8.8	12.2	12.4	0.0	7.5	0.0	0.0	0.0
A. tenuis	26.2	0.0	13.7	2.8	2.2	2.2	0.0	0.0	5.0	0.0	0.0	0.0

Table 8. Effect of Temperature on Development of Root Disease in Naturally Infested Soil

Temp (°C)	Quantity of Seedlings Completely Emerged in Percent of Control	No of Killed Seedlings	
		Before Emergence	After Emergence
30.7	52.9	4.7	42.4
26.5	30.0	9.0	61.0
20.7	27.7	24.1	48.2
17.2	36.2	19.0	44.0
12.6	72.3	3.3	24.4

Table 10. Effect of Root Disease on Seedlings

Condition of Seedling	No of Seedlings in the Following Phases (%)				Avg Wt of One Plant in Percent of Control
	1st Pr of Leaves	2d Pr of Leaves	3d Pr of Leaves	4th Pr of Leaves	
Healthy	0.0	11.4	34.3	54.3	100
Slightly infected	5.4	24.3	40.5	29.8	78.5
Infected to an average degree	6.9	44.8	48.3	0.0	53.2
Severely infected	15.4	76.9	7.7	0.0	23.1

Table 11. Infection of Various Crops by Causal Agents of Root Disease

	Percent of Seedlings								
	Aphanomyces			Pythium			Rhizoctonia		
Crops	Healthy	Degree of Infection: Avg and Severe	Killed	Healthy	Degree of Infection: Avg and Severe	Killed	Healthy	Degree of Infection: Avg and Severe	Killed
Wheat	100	0.0	0.0	93.7	0.0	0.0	12.5	6.2	0.0
Barley	100	0.0	0.0	97.3	0.0	0.0	100	0.0	0.0
Beets	0.0	5.8	94.2	1.2	3.5	95.3	0.0	5.1	83.0
Peas	100	0.0	0.0	0.0	8.3	65.7	0.0	58.3	41.7
Vetch	100	0.0	0.0	86.8	0.0	7.9	26.3	0.0	0.0
Clover	100	0.0	0.0	1.1	0.0	96.6	0.0	0.0	93.2
Lucerne	100	0.0	0.0	1.1	3.3	87.8	0.0	0.0	95.0
Esparsette	100	0.0	0.0	3.5	43.4	32.1	0.0	18.7	81.3
Sunflowers	100	0.0	0.0	87.5	0.0	10.0	70.0	10.0	2.5

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Table 12. Infection of Sugar Beets by Root Disease After Various Preceding Crops

Preceding Crops	Healthy Seedlings (%)	Infected Seedlings (%)			Killed Seedlings (%)
		Mild	Average	Severe	
Wheat, fallow, rye	66.1	31.0	0.0	0.0	2.8
Wheat, wheat, wheat	74.6	22.0	0.0	0.0	3.4
Wheat, oats, peas	68.1	9.5	5.7	0.0	16.7
Wheat, rye, clover	38.6	16.8	12.0	0.0	32.5
Wheat, wheat, potatoes	25.9	14.8	11.9	11.1	36.0
Wheat, spring wheat, peas	28.9	14.0	11.7	15.5	39.8
Wheat, beets, wheat	15.3	5.8	11.5	17.4	50.0
Wheat, barley, beets	0.0	0.0	0.0	7.2	92.8

Table 13. Results of Using Mercury Fungicides in Practice

Place Tested, Name of Chemical, and Year of Test	Percent of Reduction of Infection by Root Disease	Increase (%)		
		Density of Sprouts	Density of Planted Roots	Yield of Roots
"Sukhanovo", Sovkhoz Moscow Oblast. Granozan in doses of 5 gr per kilogram of seed (1947)	36.2	57.3	30.0	15.0*
Same (1948)	43.4	44.2	29.4	--
NIUIF-1, in 1:300 dilution (1948)	21.4	16.0	20.2	--
"Pyatiletka" Kolkhoz Rakityanskiy Rayon, Kursk Oblast. Granozan in doses of 5 gr per kilogram of seed (1948)	51.0	41.0	29.0	16.0*

*Data by N. V. Perkel'

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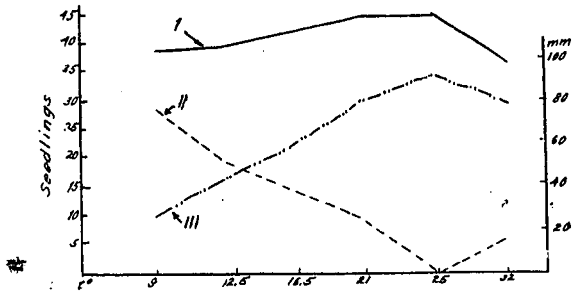

Figure 1. Quantity of Seedlings Related to the Total Number of Seeds Sprouted in Sterile Soil and Soil Artificially Infested With P. de Baryanum. I - sterile soil, II - infested, III - growth of fungi in a Petri dish

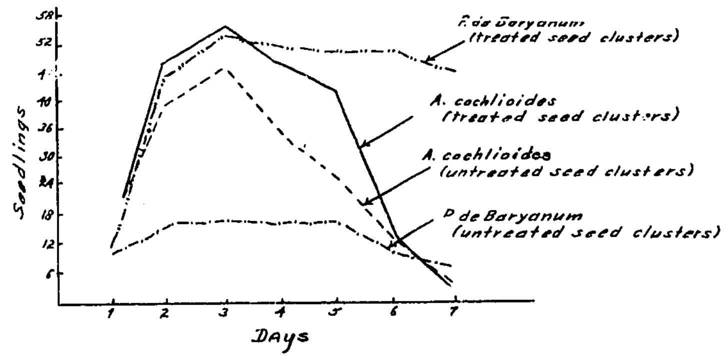

Figure 2. Dynamics of the Emergence and Destruction of Sugar-Beet Seedlings for the Planting in Naturally Infested Soil of Seed Treated With a Fungicide and That Which Has Not Been Treated

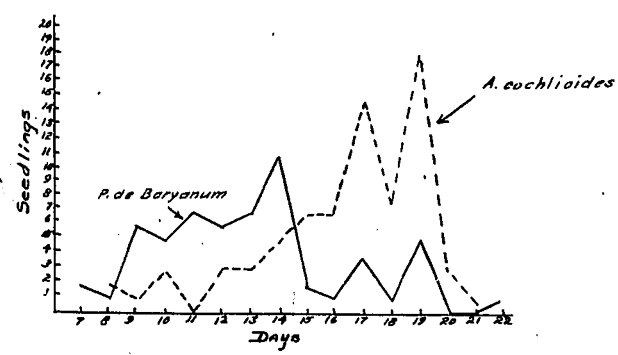

Figure 3. Dynamics of the Dying of Seedlings in Soil Artificially Infested With P. de Baryanum and A. cochlioides

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