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THE SAPROPHYTIC LIFE OF PHYTOPHTHORA IN THE SOIL

BY

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INTRODUCTION.

My researches into the nature of *Phytophthora syringae* KLEB. brought me to the same conclusion as KLEBAHN (1904), who is of opinion that this fungus persists in the soil. This was the beginning of my experiments on saprophytic life in the soil not only of *Phytophthora syringae* but also of two other species of Phytophthora, namely *Phytophthora erythroseptica* PETH. and *Phytophthora infestans* (MONT.) DE BY. Before entering into the details of my work, I will first try to show that the presence of the fungus in the soil is not an exception in this genus, on the contrary it seems to be of quite general occurrence. Many authors think that the fungus remains in the soil in some kind of resting form; but of some species oospores have never been found in nature, their existence is therefore often only supposed, which supposition is, however, sometimes supported by finding oospores in artificial cultures. This has also been the great problem of *Phytophthora infestans*. As oospores of this species have never been found under natural conditions, most people have thought it impossible for the soil to play a part in the life-history of this fungus. The possibility of a real saprophytic life of a species of Phytophthora, has only been thought of in a few cases and some investigators have even tried to cultivate the fungus on sterilized soil but without any satisfactory result.

LITERATURE.

In order to prove that the soil is an important factor in the life of the genus Phytophthora, I will mention all those species whose life-histories are more or less fully known and consider our knowledge about the way in which they overwinter and about the presence of their oospores in nature. Species as *Phyt. allii*, *Phyt. Melongenae*, *Phyt. thalictri* of which chiefly only taxonomic characters are known, will not be considered.

Phytophthora Fagi HART.

HARTIG (1880) on working at this species, which causes the damping-off of beechseedlings, made several experiments to show that the soil harbours this fungus. Infected soil from a nursery was put into water and with this water healthy seedlings were sprayed. The seedlings became all diseased and after ten days no living plant was left. As, however, no control was taken, the result may not be quite convincing. After some years a similar experiment was made, but this time with a control trial. Infected soil was put into flower-pots, which was kept indoors without being watered the whole winter and in spring beechseedlings were cultivated in them, all of which died. On the other hand seedlings grown at the same time in non-infected soil, remained healthy. In this way HARTIG was able to prove that this fungus can remain alive in the soil. Oospores are found in great quantity and HARTIG supposes them to be the cause of the overwintering. Sowing beechseeds in infected soil in which no beechseedlings had been grown for 4 years, the disease occurred again. HARTIG came to the conclusion that oospores will keep alive for at least 4 years and perhaps still longer. However, the fact that young plants sown in July in soil in which diseased plants had been grown in spring became infected, proved that oospores must also be able to germinate even after an interval of some weeks. If we accept this theory, then we must infer that the presence of young beechseedlings must be the cause of the germination of the oospores, as it is impossible to think that temperature and moisture will not have been favourable for the growth of the fungus during 4 years. The explanation that the fungus lives in the soil as a saprophyte, seems to me to be more satisfactory, as, in this case, it will be quite easy to explain why the seedlings are infected as well after a few weeks as after 4 years. During this saprophytic life new oospores might be formed as well; these are questions which could easily be solved by experiments in the laboratory.

Phytophthora cactorum (LEBERT *and* COHN) SCHRÖT.

It is generally accepted that this fungus occurs in soil and overwinters in the form of oospores. It infects apples and pears, especially those hanging on the lower branches as well as fallen fruits OSTERWALDER (1906) noticed that the places where the fruits were infected, were just those where they touched the soil. In America this fungus does much harm to the culture of *Panax quinquefolium* L. (ginseng). A field once infected cannot be used again for ginseng for some years, for even after the land had been lying fallow for two years the seedlings cultivated on it were badly attacked by the disease, (ROSENBAUM, 1915). CLINTON (1920), in 1919 in Connecticut, found that *Phytophthora cactorum* caused root rot by several kinds of plants (corn, peas, Lathyrus). Now that year was a very moist one, and he explains the many cases of root rot by supposing a general occurrence of this fungus in the soil. As far as I know no special experiments on the question of whether this fungus can live in soil have ever been made.

Phytophthora nicotianae VAN BREDA DE HAAN.

This species of Phytophthora does much harm to the tobacco-culture in the East-Indies. VAN BREDA DE HAAN (1896) proved that the soil was the source of infection and that a field once infected was capable of producing the disease after 3 years, also no tobacco had been cultivated during that time. HJ. JENSEN (1913*a*) has proved the same for a period of 2 years by experiments in Java. HJ. JENSEN (1917) also analysed soil samples for the presence of *Phytophthora nicotianae.* A certain quantity of soil was put on the lower side of tobacco-leaves, which were kept moist on bricks placed in water. After 2 or 3 days the soil was washed off and the number of spots, developed after using 1000 cc. of soil, determined. He proved by this method that Phytophthora was present in soil samples of several estates where diseased tobacco was found. According to D'ANGREMOND (1920) the samples used by JENSEN, were derived from ground, already treated with manure. He found that the manure is the chief source of infection for the fields. On some of his test plots, however, it was suspected, that infection from contaminated soil had occurred. The strong development of the fungus on the organic manure does not exclude its living in soil as a saprophyte under certain conditions. This was investigated by HJ. JENSEN (1917) by means of the method mentioned above. Infected soil was kept dry during 6 months after wich time its infectionpower proved to be very small. The soil was moistened and after 4 days its infectionpower had multiplied more than a 100 times. The soil was again allowed to desiccate, analysing this soil gave no result. The process of alternately moistening and desiccating was then twice applied and four days after the last moistening the analysis number was 469. JENSEN comes to the conclusion from this experiment that the fungus has increased in the soil as a saprophyte. In order to prove this fact in still another way, he tried to cultivate *Phytophthora nicotianae* in soil in Petridishes, but without any satisfactory result.

Oospores of *Phytophthora nicotianae* are not known for certain. VAN BREDA DE HAAN thought he had found them on seedlings in Buitenzorg but he was not able to demonstrate their presence in Deli. JENSEN (1913*b*) doubts if VAN BREDA DE HAAN really has seen oospores of Phytophthora, he himself was able to find sometimes oospores in old tobaccostems but he believes them to belong to some other Phycomycete and not to *Phytophthora nicotianae.* Neither ROSENBAUM (1917), nor PETHYBRIDGE (1913), nor RUTGERS (1917) could find oospores on artificial cultures, they only found chlamydospores; LODEWYKS (1909), however, believed he had seen oospores on agar medium. As the experiments of JENSEN make it highly probably that the fungus can live as a saprophyte from the soilcontents, the occurrence of oospores is not wanted for explaining the persistence of the fungus in the soil.

Phytophthora syringae KLEB.

In some years much harm is done by this fungus in the nurseries, where lilacs are forced. KLEBAHN (1909) thought that the infection took place from the soil, in as much as the disease showed a preference for attacking the lower branches of lilacs in pots, placed in a sloping position in a nursery. He tried to prove this by experiments. A flower-pot was cut in two, and the halves bound together again round a branch of lilac. The pot was filled with soil mixed with infected bark. From two plants treated in this way one showed the disease on one branch. It was proved by this experiment that the disease can be transmitted through the soil. In the same nursery the majority of the plants were so arranged in autumn that the branches were quite free in the air, while a small number of plants were arranged so that their buds and branches touched the soil. Next spring the buds of the second lot were diseased, while the first lot remained healthy. Oospores occur in nature in the infected buds, as well as in the infected bark. They can easily fall on the ground when, for instance, the dried buds are broken off. KLEBAHN writes: „So musz eine Verbreitung der Oösporen zustande kommen und so wäre wohl denkbar, dass auch das Mycel in Erdboden Gelegenheit zu saprophytische Ernährung fände." So KLEBAHN himself believes it possible for the fungus to live in soil as a saprophyte.

Phytophthora terristria SHERB.

The very name of this Phytophthora is an indication that it lives in soil. It causes the buckeyerot of tomatoes. It is very remarkable that SHERBAKOFF (1917) on describing this species, writes: „this fungus should be classed with members of the genus Phytophthora in spite of its soil habitat". Oospores developed in old cultures on artificial media, he does not, however, mention if they occur in nature.

Phytophthora cryptogea PETH. *and* LAFF.

PETHYBRIDGE and LAFFERTY (1919) proved by experiments that this Phytophthora lives in the soil and infects from the soil young tomato and some other plants. Tomatoseedlings, grown in beech-leafmould mixed with loam, which was used in a nursery in Ireland, were attacked by the disease, while other seedlings, grown in the same but sterilized soil mixture, remained healthy. In the dead tissues of the hostplant oospores were found, but they did not quite resemble the oospores formed in artificial cultures, so it is not proved that they belong to *Phytophthora cryptogea*. PETHYBRIDGE and LAFFERTY only *suppose* the oospores to be necessary for overwintering. They mention that in artificial cultures conidia were not formed often, only in some cases, e.g. on sterile soil, but no particulars of these cultures on sterile soil are given.

BEWLEY (1920) found that seedlings of tomatoes sown in soil gathered from different nurseries, were attacked by two species of Phytophthora, which he supposes to be *Phytophthora cryptogea* and *Phytophthora terristria.* He proved therefore once more that the two last mentioned Phytophthora's are present in the soil.

Phytophthora erythroseptica PETH.

On land on which potatoes are cultivated year after year in succession the number of tubers infected from „Pink rot" is increasing gradually, which proves that the disease is contracted from the soil.

PETHYBRIDGE (1914) confirmed this by his experiments. Healthy potatotubers were planted in pots, filled with contaminated, with non-contaminated and with sterilized soil. One plant grown, in the contaminated soil, was attacked by the fungus, the other plants all remained healthy. The experiment was also made in the field. A small plot was inoculated with contaminated soil and infected tubers, while another similar small plot was not thus treated but kept as control. Ten healthy tubers were planted in each plot. The three plants which germinated on the contaminated plot showed the characteristic symptoms of the disease, while all the eight plants, grown on the control plot, kept healthy.

Oospores were found in infected underground portions of the stalk and once in an infected nearly entirely rotten tuber. PETHYBRIDGE supposes that the soil becomes contaminated with the oospores through decaying of the infected portions of the plant.

PETHYBRIDGE (1913) tried to cultivate the fungus on sterilized bog-soil in Petridishes. The fungus showed poor growth and the presence of abundant oospores. In spite of this positive result, he does not accept the theory that the soilcontents can be used as a nutritive medium by the fungus. He supposes that the growth takes place at the expense of the wortgelatine or of the pieces of infected potato which are put into the Petridish along with the fungus. Through my own experiments it will be shown later that the supposition of PETHRYBRIDGE was not a right one.

Phytophthora arecae (COLEM) PETH.

This fungus attacks chiefly the fruits of areca-palms, which drop off soon after they become infected. COLEMAN (1910) is inclined to consider the soil as source of infection because he observed from fallen trees in infected gardens that especially those bunches which were close to or touched the ground, became diseased. In cultivated gardens the disease was more severe than in neglected ones. An explanation for this would be that the near approaching of the trees and the presence of leafmould mixed with stable manure in the cultivated gardens favour the development of the fungus. To investigate this point, he removed soil and leaf-mould from an infected tree, kept them in a dry place for two or three weeks, and then placed them in a beaker of distilled water. Healthy arecanuts placed in this water, became diseased, hence the fungus can remain alive in the soil during 3 weeks. COLEMAN attempted to cultivate the fungus on sterilized leaf-mould or soil, but had little success. Only when the soil or leaf-mould had been soaked with malt extract, the growth was such that it was noticeable to the naked eye. It is to be regretted that no investigations were made in any other way to establish real growth. If we let the determination of the growth depend only on what can be seen, we are apt to make mistakes. It is only when much air mycelium is formed that the fungus shows itself distinctly on the soil, but the hyphae can easily be present between the particles constituting the soil, without being visible to the naked eye. Through his poor results with the cultivation of the fungus on sterilized soil, Coleman feels himself „reluctlantly forced to give up the soil and leaf-mould theory for the present." These words seem to me to indicate that the progress of the disease gives him strong support in the supposition that the soil plays a part in the life-history of the fungus. A second reason why he gives up his theory is that no oospores have been found in nature. They develop in the laboratory on inoculated parts of the plant but were never discovered in diseased plant tissues under natural conditions.

Phytophthora parasitica Dastur.

This Phytophthora was described by Dastur (1913) from *Ricinus communis*. He made experiments to prove that it can live in the soil. Soil from a land where 50 or 60 % of the Ricinus seedlings were infected, was put into flowerpots, and in each pot 10 seeds were sown. On the 6th day one of the seedlings showed symptoms of the disease and then gradually most of the seedlings died. When all the seedlings had died off, two of the pots were put in the laboratory, and kept moist. After a month, 10 seeds were sown in one of the pots. After 6 days, 8 of the 10 seedlings were infected. In the second pot, 10 seeds were sown after 2 months and all the plants which developed from these seeds remained healthy. The same was the case with the plants in the pots used as control. Dastur concludes, that *Phytophthora parasitica* retains its vitality in the soil only during one month. As the last experiment was only made with one flowerpot, it does not seem to me desirable to make such a conclusion from negative results. As it is proved that the fungus can live in the soil during one month, it is highly probably that under favourable conditions, it also will keep alive during a longer period, and it seems to me quite reasonable to accept the theory that it lives in the soil as a saprophyte. In nature oospores are never found, only resting bodies were discovered in old leaves. On artificial media oospores as well as chlamydospores developed.

From these nine Phytophthora species mentioned above, it is proved that they are present in the soil. Some species will now be considered, whose presence in the soil is not yet established, but whose life-history may possibly be affected by the soil when we consider that the way of overwintering has not been definitely ascertained in all cases. As we know now that so many species of Phytophthora can live in the soil, we are apt to suppose that in the case of the remaining species, we may look to the same possibilities for the solving of the overwintering problem or at any rate of the question whether a temporary life of these species in the soil may occur.

Phytophthora Phaseoli THAXTER.

This species occurs on lima beans (*Phaseolus lunatus*). CLINTON (1906) believes that the fungus is carried over from one year to another in the seeds, but found that from infected seeds only healthy plants were developed. In infected seeds oospores were found and CLINTON supposes that the soil gets contaminated with oospores by the decaying of the seeds and therefore advises as one of the preventive measures rotation of the crop. Though the real way of overwintering is not yet established, we observe that the soil is again thought of as playing some part in the progress of the disease.

Phytophthora colocasiae RAC.

BUTLER and KULKARNI (1913) mention that the way of persistence of this fungus is the same as that of *Phytophthora infestans*. The corms of the Colocasia are compared with the tubers of the potato and it is supposed that the mycelium persists in these corms. They add, however, that the persistence of the mycelium in the potatotuber is a problem not yet quite solved, and go on to say: „the fungus is likely to disappear from the soil, unless reintroduced in infected corms". This last sentence makes it appear that they have observed that contaminated soil has some influence on the infection of the plants.

Neither oospores, nor chlamydospores were ever found in nature, though both developed in artificial cultures.

Phytophthora infestans (MONT) DE BY.

Though this Phytophthora belongs to one of the first known and most studied fungi, the problem of its overwintering is not yet solved. The theory of DE BARY (1861, 1863, 1876) that the mycelium hibernates in the potatotuber, grows from the tuber into the young sprouts and thus develops further, was supported by J. L. JENSEN (1887) and later by MELHUS (1915). The theory, however, had also many opponents, while it was proved by experiments that healthy potato plants develop from blighted tubers (KÜHN 1871, PRINGSHEIM 1876, SMORAWSKI 1890, HECKE 1898, CLINTON 1906, PETHYBRIDGE 1911, 1911—1916 [1]), LÖHNIS 1922). These facts will not be treated more extensively nor will other theories about the hibernation of *Phytophthora infestans* be considered. For the purpose here aimed at, it is only of importance to consider the literature dealing with the possibility of a saprophytic life of the fungus in the soil.

According to ERIKSSON (1916) and MELHUS (1915), KÜHN (1870) brought forward in 1870 the theory that the fungus of the potatoblight is carried over from one year to another in infected soil and from here infects the tubers. I do not believe that this is what KÜHN meant. He found, in contradiction with the prevailing opinion, that conidia develop on blighted tubers in the ground, and therefore supposes that other tubers can be infected by these underground conidia without visible foliage-disease. It is very difficult to be sure that all the foliage of a whole potatofield is really healthy — there may be some small spots which can only be observed by very minute observation. KÜHN supposes that some small spots must be present on the foliage; the conidia from these spots, falling on the ground, will be able to infect a few tubers and now the disease can spread underground through the conidia developing on these tubers to others. This spreading of the disease underground meant by KÜHN is not a saprophytic life of the fungus on the soilcontents. According to me BREFELD (1883) was really the first to suppose that a saprophytic life of *Phytophthora infestans* in the soil could be possible. His theory was based on purely theoretical principles. Observing that the facts did not quite agree with the theory of DE BARY, he thought that it might be possible for the fungus to live in the soil, because it developed abundantly on an artificial medium: „die Phytophthora infestans wuchs in künstlicher Ernährung wie Unkraut". HECKE (1898) though not finding evidence through his experiments for the theory of BREFELD, does not reject it as a whole.

Next to these purely theoretical suppositions we must also mention some facts which give some more real evidence of the

1) PETHYBRIDGE (1921) now' adays seems to accept the theory of DE BARY. The facts, on which his changed opinion is based, are not mentioned.

saprophytic life of the fungus in the soil. Clinton (1906) observed that the blight first appeared on the leaves which had been or were still in contact with the soil, while at the same time the other parts of the plants showed no sign of the disease. He considers that from these leaves the blight gradually spreads over the field by means of secondary infection. He writes: „this disease comes by contact of the leaves with the ground at the critical wet periods of July and August when the germs of the blight are probably first generally available in the soil for infection, and that this is the usual method of primary infections in the fields". According to this opinion he tried to cultivate the fungus in pure culture on sterilized manure, on sterilized earth, on a sterilized mixture of these and on a sterilized mixture of these with other ingredients. The growth made was none or very slight, therefore Clinton does not accept the possibility of the growth of the fungus in the soil, but he is more inclined to believe that the fungus is present in the soil in some form e.g. as oospores.

Still more minute observations of the first infection on the field were made by Brooks (1919), who with a number of collaborators daily inspected in 1918 each individual plant of some small plots. On June 25th on one of the plots the first symptoms of blight were observed on one plant. These first symptoms were some discoloured areas on one of the stems 1 a $2\frac{1}{2}$ c.M. above soil level. After the disease had spread over the plant to some extent, the petioles of the lower leaves being also attacked, the plant was dug up on July 7th. It appeared that three tubers were diseased, but the underground stems were quite healthy. On July 8th seven other plants on the same plot were found to be affected in the same way as the plant first mentioned. From these seven, two plants were dug up, which showed discoloration of parts of the stem just above and immediately below soil level, but the tubers were quite sound. After July 14th 17 other plants showed discoloration of the basal parts of the stem and lower petioles, and then on July 22nd the first typical blight spots showed on the leaves. On these spots conidia were produced and this was the beginning of the spread of the epidemic. The small garden plots in the neighbourhood of the carefully examined one, showed no symptoms of blight before July 24th, therefore the source of infection must have existed within the plot itself. However carefully searched for, no diseased shoots were observed arising from the parent tubers. On a second plot, which was as minutely examined as the above mentioned, the first symptoms of the disease were the same viz. discoloured areas of the stems and of the lower petioles. Of great importance is the fact that the plants which were attacked first, grew all on a part of the plot on which in the previous autumn many blighted tubers were thrown away when sorted. In vain careful search was made for diseased shoots arising from those last year's blighted tubers or from parent tubers. BROOKS concludes from his observations: „The facts observed are capable of interpretation either by infection *from the soil*, through the agency of some form of resting body or by infection from blighted shoots growing upwards from diseased sets. The latter were not found in spite of careful search, although it is known that they have occasionally functioned under experimental conditions."

Apart from these observations in the field, which form some proof for the existence of the fungus in the soil, we must also mention some facts relating directly to the growth of the mycelium on soil. JONES, GIDDINGS and LUTMAN (1912) write: „In some places the mycelium was ramifying through the interstices of the soil at least one-half an inch from the surface of the decaying tuber, although doubtless nourished by it", and MELHUS (1915) mentions: „The mycelium grew out from it into the soil for a distance of about 1 c.M. This is not a usual occurrence and happens only when conditions are very favorable for the growth of the fungus. As light decrease in the moisture content of the soil and the fungus is no longer in evidence, nor does it return if the original moisture condition is restored". Both authors have observed that the fungus can live in the soil under favourable conditions but neither of them believe that this life can be maintained long. Real proofs on which they base their opinion, are not given and probably their prejudice against the idea of a saprophytic life of Phytophthora has prevented them from investigating this matter more precisely. They have proved, however, that the mycelium does not restrict itself to the potatoplant but can appear outside it.

J. L. JENSEN (1887) proved through experiments that soil mixed with conidia is still capable of infecting living potatopieces after 4 days. He supposes that the conidia keep their vitality in moist soil during that period and draws attention to the danger of infection of the tubers at digging time from the soil. MURPHY (1921) made experiments in the field on the same subject and proved, that: „Soil contaminated by means of spores, shed from the leaves continues capable of inducing blight in freshly dug tubers which are brought into contact with it over a period of at least ten days and probably longer".

In my opinion this possibility of infection from the soil 10 days and longer after the removing of the foliage could be explained by a saprophytic life of the fungus in the soil.

The accounts given on infection of the tubers, while the foliage remained healthy must also be mentioned. (Kühn 1870, Horne 1914, Schander 1918, Löhnis 1922). These cases might also be explained by a persistence of the fungus in the soil, but we must be careful with these facts for as is said above, it is not an easy task to determine whether the foliage is perfectly healthy, without even a single little diseased spot on it.

Experiments have been made to investigate the persistence of *Phytophthora infestans* in the soil. Stewart (1913) cultivated plants in soil contaminated with diseased tubers and foliage, but all these plants remained healthy. Stewart says himself that these negative results really do not prove that *Phytophthora infestans* does not persist in the soil. For the success of such an experiment many factors are wanted and we know still very little of the influence which each factor exercises. Only after repeated experiments made under as many different conditions as possible, may we draw conclusions from negative results. According to Melhus Darnell—Smith has also made some experiments on the persistence of the fungus in the soil and also with negative results. The article of Darnell—Smith was, however, not available for me. Experiments on the same subject made in a field where in the previous year blighted potatoes had grown are mentioned by Pethybridge (report 1912) and he too had no success. Miss Löhnis (1922) planted healthy seed tubers after which they were strown over with contaminated soil. The result was that the plants were blighted a day later than the control plants. On the other side we have, however, the opinion established by practice that the disease is worst on fields where potatoes are grown in succession year after year. It would be worth while, to establish this point by accurate observations during many years.

Oospores of *Phytophthora infestans* have never been found in nature but Clinton (1911) and Pethybridge and Murphy (1913) succeeded in cultivating them on artificial media.

Phytophthora Faberi Maubl.

This tropical species does much harm to the culture of Cocoa and Hevea. The fruits as well as the stem of both trees are attacked. It is generally accepted that diseased fruits, which are lying on the ground or remain hanging on the trees are the chief source of the spreading of the disease. Still some facts are mentioned, which might give some evidence of the presence of the fungus in the soil. DEMANDT (1918) observed that the trees first attacked, were those along the road and he supposes that the soil, which the natives spread with their feet is the cause of infection. Next to this in importance as a factor is water. After a flooding of the river 80 % of the trees were attacked just above soil level. DEMANDT thinks that infection by means of the water and the soil will be brought about through the presence of conidia. Infection from the ground can be as easily explained from diseased fruits lying on the ground as from the fungus living as a saprophyte in the soil. Experiments to investigate this last supposition have never been made. Oospores of *Phytophthora Faberi* are not known, they do not develop in artificial cultures, but chlamydospores are easily formed.

There is still one Phytophthora left in the life-history of which, the soil probably plays no part at all viz.

Phytophthora Meadii MC RAE.

This Phytophthora also infects Hevea's. According to Mc RAE (1918) it persists in the branches at the junction of the dead and living tissues. Probably only a very small number of the partially died-back branches can actually reproduce the disease in the succeeding wet season. The greatest number of diseased branches are attacked by secondary fungi which overgrow Phytophthora. During the dry weather the mycelium was also found in the fruitstalks, which remained on the tree and at their points of junction. In nature oospores are not found frequently and then only on the fruits and only in small numbers.

THE CULTIVATION ON STERILIZED SOIL.

The growth on soil was investigated with three species of the genus Phytophthora viz.

Phytophthora syringae KLEB. isolated in February 1920 from diseased branches of lilacs received from Aalsmeer.

Phytophthora erythroseptica PETH. received from the „Centraalbureau voor schimmelculturen" at present at Baarn.

Phytophthora infestans (MONT) DE BY, isolated in September 1920 from affected foliage of potatoplants at Wageningen.

In the beginning the experiments were only made with *Phytophthora syringae*, therefore soil from lilac-nurseries at Aalsmeer was used. The soil at Aalsmeer is a dark bog-soil (baggeraarde), consisting entirely of organic matter. Afterwards other types of soil were used as well viz. leafmould, as used in nurseries, consisting also nearly entirely of organic matter and clay and sand, taken from fields at Wageningen. As these fields are already in cultivation for many years, they will contain some organic matter, derived from the manure put on them.

These different soils were put, just as they are found in nature, in test-tubes and sterilized in the autoclave. If the soil does not retain the necessary humidity, some sterilized water is added afterwards. By this method the soil retains better its natural composition of lumps and airholes as when it is moistened before sterilization.

Parts of a pure culture of the fungus were transferred to a tube with soil, a piece of the medium taken along with it. Very shortly afterwards the mycelium grows from this piece on the surrounding soil. This soil, containing the fungus, was again transferred to another tube with soil and now it is certain that the fungus lives entirely from the soil-contents. After a pure culture of the fungus on soil was thus obtained, transferring on fresh tubes with soil, was done regularly. It was proved that all the three species of Phytophthora, above mentioned, can keep alive on soil, that the quantity of their mycelium increases, showing, that these species of Phytophthora can use the soilcontents as food and live in the soil as saprophytes. The external appearance and the behaviour of the fungi on the soil, however, is not the same for the three species, each species will therefore be treated apart.

Phytophthora syringae forms white airmycelium on the top of the soil in the tube if the necessary humidity is present. This airmycelium is clearly visible to the naked eye and forms a thick, white cottony layer if the culture is some months old. If the soil in the tube is too moist, it may happen that the fungus is not to be seen, still it may be present, showing itself as soon as the moisture diminishes. The fungus grows best on bog-soil and leaf-mould, on clay and sand the growth is less, still the fungus keeps alive. Cultures of *Phytophthora syringae* are present, which have had no other food for two years than sterilized bog-soil. That the fungus has kept its normal vitality during that time was proved by transferring on other media and by inoculations on lilacs. Inoculations were made by putting contaminated soil in a cut of the bark of the lilacbranches, the wound being covered afterwards. In winter they could also be made by putting contaminated soil between the budscales.

If the inoculation succeed, the symptoms, discoloration and dying of the buds, show after a few weeks. In spring 1922 many inoculations did not give satisfactory results on account of the bad weather, the cold and especially the drought were extremely unfavourable for the experiments. But of those which did succeed, one was done with a culture that had lived on bog-soil for two years, while two other cultures that had lived on bog-soil for 1½ years were also able to infect the plants. By the experiments made in the winter of 1920—'21, no difference was to be observed between the results of the inoculations made with cultures on bog-soil or with those on other media. On leaf-mould and sand, the fungus is still living after one year and 9 months and on clay after 6 months. The fungus of the cultures on these three soils developed again quite normally, when they were transferred on other media and also inoculation experiments on lilacs with cultures that had lived on these three soils for some months, gave good results. It is proved therefore, that living on the 4 different soils does not affect the pathogenicity of the fungus. Moisture is necessary for the development of the fungus but having once developed well, it can stand desiccating of the soil to a rather large extent. The fungus of old cultures on soil, which was rather desiccated, was still alive, which was proved when they were transferred on other media, this was e.g. the case with several one-year old cultures on bog-soil to which no water was added during that time.

Phytophthora erythroseptica forms on soil in tubes more airmycelium than *Phytophthora syringae*, which happens however also on artificial media. It behaves differently in growth on the different kinds of soil, as it develops very well on clay, even better than on the other soils. I have at this moment cultures of *Phytophthora erythroseptica* which are still living and which have had no other food than either clay for 1½ years, or bog-soil, leafmould, or sand for 1 year and 9 months respectively. Potato-tubers inoculated with cultures on the different soils, showed after a week the characteristic symptoms of the *pinkrot*. The long stay on the soil had not diminished the pathogenic power of the fungus. For inoculation potatotubers were treated with corrosive sublimate, then wedge-shaped pieces were cut out as sterile as possible, and the contaminated soil was put in the hole and the potatopiece replaced. After inoculation the tubers were kept in a glass vessel. *Phytophthora erythroseptica* can also resist the desiccation of the soil rather well, which was proved by the fact that one year-old cultures on the 4 different soils developed quite normally after being transferred on fresh media.

After the satisfactory results obtained by the cultivation of two species of Phytophthora as a saprophyte on soil, experiments were made to investigate if this could also be done with **Phytophthora infestans.** This species is not so easily cultivated in pure culture as the other two. The growth of the mycelium of this fungus is rather slow and even by apparently the same treatment often quite unequal. Therefore the pure cultures on soil were not made in quite the same way as those of the other species. A piece of a medium with pure culture was transferred to a tube with sterilized bog-soil and the mycelium soon spread over the surrounding soil. After 4 weeks the transferred piece of agar was taken out of the tube, leaving the mycelium on the soil. This kept growing and after some time when the culture had developed quite distinctly, the bog-soil with the fungus was transferred on all the other soils. It was proved that *Phytophthora infestans* could live on the different soils, using the soilcontents as food. In the tubes clearly visible airmycelium is formed, developing especially in the airholes towards the lower parts of the tube, while the other two species formed their airmycelium mostly at the top of the soil. In some tubes with bog-soil and clay the airmycelium was so abundant, that a white, cottony mass was obtained (fig. 1, 2). The fungus grows best on clay and bog-soil, less on leafmould and rather badly on sand. I have not as yet been able to transfer the cultures on sand. However, the quantity of the fungus on sand did increase and the myceliumthreads could easily be seen growing over the sand. After having lived on the sandcontents for 4 months, the fungus was still able to infect living potatopieces, so it had not lost its pathogenicity. The cultures on the other soils could be transferred. The result is that there exist now cultures of *Phytophthora infestans* having had no other food than the contents of clay for 11 months, of leaf-mould for 9 months and of bog-soil for 8 months (the original culture on bog-soil was lost). The fungus, after living on these soils for the times mentioned, was still able to infect living potatopieces, which proved that it had not lost its pathogenic power. In summer, experiments were made to investigate if *Phytophthora infestans*, grown on soil, could infect the foliage of potatoplants. Contaminated soil was put round the stem and on the leaves of potatoplants and kept in moist condition. A 5½ months-old culture on bog-soil and a 4 months-old culture on leaf-mould were used for these inoculations. Both leaves and stems were affected and developed typical Phytophthora-conidia in moist atmosphere.

We may therefore suppose that in nature contaminated soil will also be able to infect the foliage of potatoplants under favourable weather conditions. Whether *Phytophthora infestans*, once developed well, can stand the desiccation of the soil, could not yet be established. Six months-old cultures on bog-soil, the soil of which was already rather dry, proved, however, to be still alive, also a 7 months-old culture on clay, but the soil of the last named was still a little bit moist.

Soil was inoculated with *Phytophthora infestans* also according to two other methods, resembling more what really happens in nature. The fungus was cultivated on raw, sterile potato-pieces, which after sufficient growth, were covered with sterilized soil. The myceliumthreads grew through the soil till they reached the surface (fig. 3). Parts of the upper layer of the soil, containing the fungus could be transferred to other tubes with soil, on which the fungus developed further. In nature the myceliumthreads can grow under favourable conditions from the diseased tubers in the surrounding soil, develop further as a saprophyte and reach the surface of the ground. Diseased potatotubers are therefore able to infect the neighbouring soil.

The third method used, tried to imitate what happens if the fungus from the diseased foliage is brought on the ground by rain. Sterile water was poured over cultures of *Phytophthora infestans* on raw, sterile potatopieces in the fruiting stage. This water, containing a great number of conidia and also some myceliumthreads, was poured over sterilized soil in tubes. The fungus was thus spread over all the soil in the tube, which in some cases could be distinctly seen. On several lumps in the tube conidiophores appeared. In some tubes with clay, the development of the fungus was very luxurious, the mycelium forming a white, dense, cottony mass. In nature conidia can be brought into the soil by rain and there develop further. If under favourable conditions the fungus increases vigorously in the soil as a saprophyte, forming also conidiophores, the spreading of the fungus will be very extensive. The interpretation of DE BARY (1861) that the tubers in the soil are infected by the conidia brought to them by the water, need not now be maintained. The fungus, once it starts growing in the soil as a saprophyte, can reach the tuber and infection can take place without any current of water. The result is the same but through this explanation tuber-infection is not so dependent on water as was thought to be the case and we can better understand how under certain circumstances strong tuberinfection may occur together with a weak infection of the foliage.

Except in culture tubes the different fungi were also cultivated in Petridishes and in glass-vessels, half filled with soil. The drawback of both is that sterility cannot be maintained as easily as in tubes, but the conditions of the soil will be more equal to those in nature. When the fungi were cultivated in dishes, they were often not be seen with the naked eye, because mostly no airmycelium was formed. By inoculationexperiments as well as by the microscope it could, however, be determined that the organism was really present. It grows between the soilparticles and increases in the dishes as well as in the tubes. The apparent absence of growth on soil in dishes has perhaps been the reason why some investigators have had no satisfactory results with their experiments on the cultivation of Phytophthora on soil. PETHYBRIDGE (1913) with *Phytophthora erythroseptica* as well as HJ. JENSEN (1917) with *Phytophthora nicotianae*, both used Petridishes. It could be easily established again by cultivation in glass-vessels that the mycelium of *Phytophthora infestans* is able to grow from inoculated raw potatopieces in the surrounding soil. In the moist atmosphere of the glass-vessel the mycelium developed in the soil very distinctly, also after the potatopiece had been removed.

The microscopic image of the different species is not alike. **Phytophthora syringae** forms on soil in Petridishes many conidia, appearing on all sides of the lump of soil and owing to the short conidiophores giving the impression that the conidia are directly attached on the soil (fig. 6, 7). If such a lump of soil is put in water under a cover glass zoospores are formed after a short time. In the same way, rain under natural conditions can cause the spreading of the fungus in the soil to a large extent. The germination of the conidia cannot only occur by means of zoospores, but also by means of myceliumthreads which may again produce new conidia. In tubes with old cultures, whole chains of repeatedly formed conidia could be seen and sometimes also abnormalities of the mycelium. At first, oospores were looked for in vain, but afterwards they were found on all the different soils in tubes, only in small number and in some tubes more than in others .They were distinctly to be seen after boiling the soil in KOH. (fig. 8).

Contrary to *Phytophthora syringae*, **Phytophthora erythroseptica** forms in soil many oospores, which appear even as the conidia of *Phytophthora syringae*, on all sides of the lump of soil. Only a single conidie could be seen,but after putting the soil in water under a coverglass many conidia were formed (fig. 4, 5). PETHYBRIDGE (1913) also found that the conidia of

Phytophthora erythroseptica are only formed in watery solutions. So *Phytophthora syringae* produces already conidia in a moist atmosphere, while *erythroseptica* really wants staying in water for their formation. In nature this condition will occur be it only through the waterdrops between the soilparticles.

As already mentioned **Phytophthora infestans** produces in soil a great number of conidia with longer conidiophores than those of *Phytophthora syringae*. The conidia may germinate either by means of zoospores or myceliumthreads.

OVERWINTERING IN THE SOIL.

Also it has now been proved that the three Phytophthora species can live in the soil as saprophytes, the problem of their overwintering in the soil still remains. The overwinteringsproblem of *Phytophthora syringae* is not of so great importance as that of the other two for the simple reason that this fungus attacks the plant in autumn, while the first symptoms are generally to be seen in January. In some years however e.g. in the last two, the disease does not occur, while the weather has not been favourable for the spreading of the fungus during the period that the lilacs are susceptible to infection. If, however, the fungus can keep alive in the soil during the winters of those years, the sudden general outbreak of the disease in other years can be explained.

In order to investigate whether the different organisms can stand low temperatures, as well as changes of temperature, several tubes with cultures on soil and other media were put out of doors during the winter of 1920—'21. The tubes were placed on the floor of an open bicycle shed, protected against sun and rain. The temperature was registered daily by means of a maximum- and minimum-thermometer. In the winter of 1921—'22 the experiments were repeated on a larger scale. A greater number of tubes was now placed in a cupboard, shut with a grated door and situated at the north-east corner of the building, where in winter the sun never shines. Next to the tubes was placed a self-registering thermometer. The changes of temperature, which take place in the soil under natural conditions can never coincide with those of the tubes in the cupboard, and the difference in temperature is further increased by them being placed at some distance from the ground, this difference, however, will be so small, that it need not to be taken into account here.

The winter of 1920—'21 was mild. The temperature was only during one week permanently below 0° C., while the minimum-temperature in that week was about — 9° C. Moreover on about 20 nights there was frost. On the contrary the winter of 1921—'22 was for our country very severe. Table I shows the periods in which the temperature was permanently below 0° C. and which occurred between the middle of October 1921 and 1 March 1922. As seen from this table, this was the case during 28 days and moreover on 15 nights night frosts were recorded. The minimum temperature in this winter was — 12° C.

TABLE I.

RECORDS OF TEMP. BELOW 0° C. REGISTERED BETWEEN OCT. 15TH 1921 AND MARCH 1ST 1922.

Periods of more than 24 hours during which temp. was permanently below 0° C.	Minimum temp. in that period.	Dates on which temp. was below 0° C. only during the night·
		Nov. 9, 10, 11, 24, 25, 26
27 Nov. 1921— 6 Dec. 1921	-12° C.	Dec. 13, 26—Jan. 5
6 Jan. 1922— 8 Jan. 1922	- 7,5° C.	Jan. 13, 14
15 Jan. 1922—16 Jan. 1922	- 4,5° C.	Jan. 19, 20
23 Jan. 1922—28 Jan. 1922	-12° C.	Jan. 29
4 Febr. 1922—13 Febr. 1922	-10,5° C.	Febr. 15
Total 28 days below 0° C.	Min. -12° C.	Total 16 nights temp. below 0° C.

In table II the results are recorded of the overwintering of **Phytophthora syringae** in 1920—'21. During the winter some tubes were transferred at various dates with a view to investigating if the fungus was still alive, which always happened to be the case. After the transfer, the tubes were again placed out of doors. By transferring in April or at a still later date it appeared that the fungus in all the tubes was still alive. Table III gives the results obtained in winter of 1921—'22. It appears that the fungus can stand the low temperatures of a severe winter, as well as the successive periods of frost. The theory may therefore be accepted that also in nature *Phytophthora syringae* can remain alive in the soil during severe winters.

The effect on the fungus of sudden large changes of temperature was also investigated. In December, when the temperature was — 7° C. some tubes were brought into the laboratory; from some of them the fungus was transferred at once with

TABLE II.

OVERWINTERING OF PHYTOPHTHORA SYRINGAE 1920—1921.

Medium.	Presence of oospores.	Number of tubes placed out of doors in Oct. and Nov. 1920.	Number of tubes of which the fungus was still alive at the time of transferring in April 1921 or at a still later date.	Number of tubes of which the fungus did not develop any more after transferring.
Carrots	oospores	6	6	0
Lilac-leaves and lilac-stems	oospores	2	2	0
Malt-agar ...	no oospores	2	2	0
Potato-agar .	no oospores	2	2	0
Malt-gelatine	no oospores	1	1	0
Potato-gelatine	no oospores	1	1	0
Bog-soil	a few oospores	6	6	0

a bit of the frozen medium, while the others were allowed small changes of temperature till ordinary roomtemperature was reached after a period of 3 days. On nearly all the pieces transferred directly from the low temperature, growth was to be observed as early as the following day, while on the pieces of the cultures of which the temperature had been raised gradually, growth was to be seen, partly the next day, partly the second day after transferring. The number of tubes used was too small to draw general conclusions, the experiment, however, shows that a change of —7° C. to about 18° C. has no bad effect on *Phytophthora syringae*, on the contrary the results rather indicate that the sudden change of temperature is apt to stimulize the growth of the fungus.

Another important point is the question whether the mycelium can stand the low temperatures or whether the fungus can only keep alive by means of oospores. Table III shows that on some of the media used for cultures which had been out of doors during the winter and were still alive in the spring no oospores were found. Unfortunately some cultures were transferred from media on which oospores developed. In the beginning it was thought that no oospores were produced on them, but afterwards it was found that this really was the case. Therefore the possibility exists that in the transferred piece some oospores may have

TABLE III.

OVERWINTERING OF PHYTOPHTHORA SYRINGAE 1921—1922.

Medium.	Presence of oospores	Number of tubes, placed out of doors in October 1921.	Number of tubes of which the fungus was still alive at the time of transferring in Febr. 1922.	Number of tubes of which the fungus was alive at the time of transferring after March 15th 1922.	Number of tubes of which the fungus did not develop any more after transferring.
Carrots	oospores	14		13	1
Cherry-agar	oospores	5		5	0
Prune-agar.	oospores	5		5	0
[1]) Ground Quaker-oats-agar.......	oospores	2		2	0
[1]) Oat-extract-agar	oospores	2		2	0
Cornmeal-agar....	oospores	7		7	0
Carrot-agar	no oospores	2		1	1
Potato-agar	no oospores	4		2	2
Potato-gelatine ..	no oospores	3		3	0
Malt-gelatine.....	no oospores	3		3	0
Beefbouillon-gelatine..........	no oospores	2		2	0
[2]) Sugar-gelatine..	no oospores	2	(Dec.) 1 (Jan.) 1		
[3]) Water-gelatine.	no oospores	2	(Jan.) 1	1	0
Bog-soil	a few oospores	24	5	19	0
Leafmould........	a few oospores	3	1	2	0
Clay.............	a few oospores	3	1	2	0
Sand.............	a few oospores	3	1	2	0

1) According the PETHYBRIDGE and MURPHY (1913). ROSENBAUM (1917) did not find oospores on oat-agar, he used oat-juice-agar according to CLINTON which is prepared in another way than those used by me. Neither did ROSENBAUM find oospores on corn-meal-agar, He used corn-meal-agar according to SHEAR and WOOD, while the one used here was prepared in the same way as ground Quaker-oats-agar, the difference lies largely in the last one not being filtered,

2) 4% sugar and 12% gelatine.

3) ± 12% gelatine in water.

been present. However, it is certain, that the fungus was transferred to some of the tubes from media on which no oospore have ever been found as yet. The number of tubes, on which no oospores were present, may therefore not be quite reliable, the fact remains, however, that the mycelium itself is not killed by the low temperatures. A second proof for this statement was got in January when pieces of a sugar gelatine culture and a water gelatine culture at a temperature of — 7° C., were transferred to moist chambers kept at 18° C. The transparent pieces of gelatine in the moist chambers could be carefully examined under the microscope; although no oospores nor abnormalities of the mycelium could be found, nevertheless the mycelium developed further and the growth of the fungus could easily be followed.

Attention must also be drawn to the presence of abnormalities of the mycelium on media, on which no oospores occur, and which are specially found in old cultures. These abnormalities are enlargements of the hyphae of different degree and form. Sometimes they resemble conidia, while others give more the impression of oogonia. This is especially the case when these enlargements are globular and possess a thickened wall. From the globular parts a great number of new myceliumthreads may develop. Intermediate stages between all these abnormalities and the ordinary mycelium can be found. Real oogonia and antheridia are, however, never to be seen nor oospores. This is easily proved by treatment with chlor-zinc-iodide or still better with concentrated sulphuric acid. In this last case the wall of the oospore remains clearly visible, while the wall of the abnormalities is dissolved. On oat-agar abnormalities and real oospores both were found next to each other. These abnormalities are probably the first stage of forming of oogonia or conidia, which, however, never reach maturity. In the table the presence of oospores is only stated when true oospores have been observed.

Of the 24 cultures on bog-soil, 7 were more than one year old, 5 of them had also been staying out of doors during the previous winter, in October some sterile water was added to them, the other two were put out of doors in dry condition. After transferring in spring the fungus of all 7 was still alive. *Phytophthora syringae* can therefore endure both cold and drought on soil.

Inoculations on lilacs were made in the beginning of February before the last period of frost, with 8 cultures on soil, which had been out of doors during the winter. In April two branches showed the symptoms of the disease. The unfavourable weather may be the reason why the number of successful inoculations was so small. On March 14th, new inoculations were made with 5 cultures on soil which had been out of doors during the winter, and this time all succeeded. It is therefore proved that the fungus does not lose its pathogenicity either by staying in the soil nor by enduring the cold.

In the winter of 1920—'21 4 tubes with cultures of **Phytophthora erythroseptica** on bog-soil were put out of doors. One tube was contaminated but in the other three, the fungus was still alive in the spring. Table IV gives the results obtained in the winter of 1921—'22; ¼ of all the cultures died, a much larger number than was the case with *Phytophthora syringae*. The fungus kept better alive in cultures on soil than on the other media. The drawback of agarmedia is that they change by freezing, they lose water and their original condition is not restored by thawing. *Phytophthora erythroseptica* was also not affected by changes of temperature of — 7° C. to about 18° C.

Table IV would seem to indicate that only the oospores and not the mycelium of *Phytophthora erythroseptica* can stand low temperatures. The number of tubes used, on which no oospores were produced, was, however, too small to obtain certainty, while several cultures with oospores died as well.

Of the 9 cultures on bog-soil placed out of doors in the autumn of 1921, 3 had been out of doors during the previous winter. Some sterile water was added to them. Two of these cultures were still alive in spring, the third one is the only culture on bog-soil which died. Of the cultures on other soils, 4 were more than a year old, when transferred and no water was ever added to them. One of these cultures on clay died, the other kept alive. *Phytophthora erythroseptica* can therefore withstand in soil both drought and cold. The pathogenicity of the fungus was not lost during the winter. In February and March inoculations were made on healthy potatotubers with two cultures of each soil. The typical pinkrot developed and *Phytophthora erythroseptica* was reisolated from these tubers. The fungus can therefore overwinter in the soil without losing its vitality.

Two tubes with cultures of **Phytophthora infestans** on raw sterile potatopieces were placed out of doors on December 1st 1920. They were again brought indoors on December 22nd, in the meantime there had been frost for a week with a minimum of about — 9° C. The potatopieces were quite black and the fungus was transferred to fresh ones. After 9 days, development of the mycelium was distinctly to be seen, so *Phytophthora*

TABLE IV.

OVERWINTERING OF PHYTOPHTHORA ERYTHROSEPTICA 1921—1922.

Medium.	Presence of *oospores.*	Number of tubes placed out of doors in October 1921.	Number of tubes of which the fungus was still alive at the time of transferring in Febr. 1922.	Number of tubes of which the fungus was still alive at the time of transferring in March 1922.	Number of tubes of which the fungus did not develop any more after transferring.
[1]) Carrots........	many oospores	4		3	1
Cherry-agar	many oospores	2		2	0
Prune-agar	a few oospores	2			2
Oat-extract-agar..	many oospores	2		2	0
Cornmeal-agar	many oospores	2		1	1
Malt-gelatine	a few *oospores*	3	(Dec.) 1	1	1
Potato-gelatine ...	no oospores	2			2
Sugar-gelatine....	no oospores	2			2
Bog-soil	many oospores	9	1	7	1
Leafmould	many oospores	4	1	3	0
Clay	many oospores	7	4	2	1
Sand	many oospores	5	1	4	0

1) PETHYBRIDGE (1913) mentions that on sterilized carrot no oospores occur, in my cultures they developed on carrots in great quantity.

infestans was able to stand a temperature of about — 9° C. Also Matruchot and Molliard (1903) mention that *Phytophthora infestans* kept alive with a temperature of — 5° a — 10° on artificial media. On January 15th 1921, cultures on soil, ground Quaker-oats-agar and oat-extract-agar were placed out of doors. Severe frost did not occur after that date, only on 8 nights the temperature was below 0° C. with a minimum of about — 3° C. In spring the fungus was still alive on the soil but it had died on both agars. In October 1921 several cultures of *Phytophthora infestans* on different media were again placed out of doors viz. 2 on raw sterile potatopieces, 2 on ground Quaker-oats-agar, 8 on bog-soil, 7 on clay, 6 on sand and 5 on leafmould. Not one of these cultures remained alive, even in December no growth could be obtained after transferring. Probably all cultures died through the severe frost in the beginning of December. So the results of the experiments in the two winters are quite opposite. Similar experiments during many winters will be needed before we can establish the fact whether there exists any possibility, of *Phytophthora infestans* being able to overwinter in the soil; perhaps this only happens in mild winters and is excluded in severe ones.

The experiments of Bartram (1916) show that another Phytophthoraspecies can also stand low temperatures. Cultures of *Phytophthora omnivora* on limabean agar were still alive after passing the winter in a corncrib. The minimumtemperature reached was — 29° C. Bartram does not mention if oospores were present, but it is highly probable that they are formed on the medium used by him (Rosenbaum 1917).

THE CULTIVATION ON NON-STERILIZED SOIL.

All experiments described were done with sterilized soil. The fungus will probably keep alive as well on non-sterilized as on sterilized soil, still it was thought desirable to investigate this point. The difficulty of these experiments is that the presence of the fungus can only be established by inoculation experiments. The organism will also be present in a smaller quantity than in the cultures on sterilized soil, therefore the percentage of successful inoculations will often be very small.

In the spring of 1921 inoculations on lilacs succeeded with 2 cultures of *Phytophthora syringae* on non-sterilized bog-soil in Petridishes. One dish was inoculated in October with a pure culture on carrots, the other with a pure culture on bog-soil. This last dish had been out of doors during the winter, the other was kept in the laboratory. The experiments were repeated in the winter of 1920—'21, but that year all the inoculations failed due for large part to the unfavourable weather, so no results could be obtained.

It was much easier to make experiments with *Phytophthora erythroseptica* on non-sterilized soil, while the inoculations of this fungus on the potatotubers can be done indoors, independent of the weather. About the end of September 1921, 8 Petridishes with non-sterilized soil, 2 with each type of soil, were placed out of doors, after being inoculated with a pure culture of *Phytophthora erythroseptica* either on carrots or on malt gelatine. About the end of February inoculations were made on healthy potatotubers with soil from these Petridishes, directly after they had been brought indoors. The inoculations did not succeed. After the dishes were kept at a temperature of about 18° C. for a week, a number of tubers were again inoculated with soil from each dish. The result was now that the soil from each dish had infected at least one but often more potatotubers. After cutting they showed distinctly the symptoms of pinkrot and moreover *Phytophthora erythroseptica* was reisolated from some of the diseased tubers. *Phytophthora erythroseptica* can therefore keep alive as well on non-sterilized as on sterilized soil. When cultures of erythroseptica were transferred during the winter directly after coming from outside, growth was never to be observed before the 2nd day after transferring. The first failure of the experiment must therefore be attributed to the woundcork formed by the potato before the fungus could attack it. According to PETHYBRIDGE (1914), the fungus cannot penetrate through the skin of the tuber and infection will take place in nature either through the rhizomes or through wounds. The experiment described above proves that woundcork can also prevent the penetrating of the fungus. After it had remained at a higher temperature for a week, it had started to develop strongly and could penetrate before woundcork was formed.

CONCLUSIONS.

The genus *Phytophthora* is not such an obligate parasite as thought formerly. The experiments described, proved that *Phytophthora syringae*, as well as *P. erythroseptica* and *P. infestans* can live as saprophytes in different soils viz. in bog-soil, in leafmould, in clay and in sand. They can resist the desiccating of the soil to a rather large extent and *Phytophthora syringae* and *P. erythroseptica* can stand in the soil the low temperatures of winter without losing their vitality. The pathogenicity of none of the three species is diminished by living in the soil. The literature on the subject would lead us to expect that not only these three, but that most species of Phytophthora can develop as saprophytes in the soil. The fact that of some species no oospores have ever as yet been found in nature, does not prevent their living in the soil as a saprophyte. It is only not certain if they can overwinter in the soil. As it was, however, proved that the mycelium of *Phytophthora syringae* can stand the low temperatures of winter, the possibility remains that species, forming no oospores, can overwinter in the soil as well. This must be established for each species separately as the ability of the mycelium to resist cold is a specific character. The important problem of the tropical species, of which no oospores are known in nature, will be the resistance of the mycelium against soil-desiccation. The possibility remains, however, for all species that oospores are really formed in nature, be it only very few, but that they have not as yet been found.

The occurring of Phytophthoraspecies in the soil as saprophytes, makes it impossible to control these diseases by means of rotation of the crop. By this method the fungi are not killed especially if they can also keep alive in the soil during winter. In literature several examples of diseases caused by Phytophthora, are to be found, where rotation of the crop did not exterminate the fungus. *Phytophthora fagi* was still present in the soil after 4 years (HARTIG 1880), *Phytophthora nicotianae* after 3 years (VAN BREDA DE HAAN, 1896) and *Phytophthora cactorum* after 2 years (ROSENBAUM, 1915). Controlmeasures must be looked for which prevent the attack of the fungus on the plants, either by spraying with fungicides or by cultivating resistent varieties. The sudden epidemic outbreak of some Phytophthora-diseases as well as the extraordinary strong influence of the weather conditions on these diseases can easily be explained. The weather conditions, being favourable for the development of the fungus, will cause a strong increase of the organism in the soil, with the result that on an extensive area an enormous number of spores will be present for the further spreading of the disease.

It may be accepted that the soil of many lilac nurseries at Aalsmeer will be contaminated with **Phytophthora syringae,** as has already been supposed by SCHOEVERS (1913). The bog-soil of Aalsmeer proved to be a very good medium for the fungus. My experiments, which will be published later more extensively, showed that the lilacs are only susceptible to the disease during a certain period of the year. If the weather conditions favouring the development of the fungus coincide with the susceptible period of the lilac, the disease will become epidemic, while in years when this is not the case, the disease does not occur at all or only a few separate cases are to be found. The investigations on the period of general infection and on the probable measures of controlling the disease are not yet finished.

Phytophthora erythroseptica is in our country rather rare. Attention must however be paid to the possible increase of diseased tubers with a view to ascertaining if the fungus is spreading. Once an infectioncentrum is established, it will not easily be annihilated.

To the question whether **Phytophthora infestans** can overwinter in the soil no answer could be obtained. Perhaps this may be possible in mild winters and not in severe ones. According to Pethybridge (1921), there are some indications that oospores of *Phytophthora infestans* may be found in nature. The establishment of this fact will be of great importance for the problem of overwintering. But the ability to live in the soil as a saprophyte will also influence the life of the fungus in other periods than winter. This is already proved by the experiments of Murphy (1921) on the danger of infection by digging of the tubers, also after removal of the blighted foliage, for this fact can be easily explained by a saprophytic life of the fungus in the soil. In a blight year the weather conditions prevailing before and during the digging of the tubers will have influence on the number of tubers attacked, a fact which may possibly be taken into account in practice.

The possibility that the fungus overwinters in the soil, does not prevent blighted seedpotatoes also playing some part in the development of the disease. As already mentioned before many investigators came to the conclusion that from diseased tubers none or healthy plants developed. The blighted seedpotato can, however, be dangerous in a different way, by infecting the surrounding soil. My experiments proved that from diseased potatopieces the mycelium can grow in the surrounding soil. This way of indirect infection by means of blighted seedpotatoes was already thought of before. J. L. Jensen (1887) observed that 2 sprouts, originating from blighted tubers, and having not yet reached the surface of the soil, were attacked by the disease half-way. The fungus had therefore not entered the sprouts from the parent tuber but according to Jensen they were infected by conidia from the soil. He thinks that these conidia are formed in the neighbouring, diseased parts of the tubers and thus explains their presence in the soil. As we know now that the fungus can live in the soil as a saprophyte, this infection of healthy sprouts from diseased tubers in the neighbourhood can be explained otherwise. Jensen thinks it more likely that the sprouts, infected during their passage through the soil, will be the source of spreading of the disease, than that the sprouts diseased by entrance of the mycelium from the parent tuber, as these last ones die before reaching the surface of the ground. He says: „Nous regardons comme probable que les pousses qui sont infectées ainsi, pendant qu'elle se dirigent vers la surface de la terre, sont celles qui ont le plus de chance de percer et de former les foyers de dissémination de la maladie sur les feuilles''. Pethybridge (1921) also believes that under certain circumstances, the foliage of the potato-plant may be affected in such a way. He writes: „The possibility is not to be excluded that ordinary spores produced on mycelium growing out into the surrounding soil from the surface of a blighted seed tuber or „set'' may, in certain cases, cause infection of the plant derived from that tuber''. Such an infection depends of course very much on the weather and is only possible if the moisture and the constitution of the soil both favour the development of the fungus.

The fact that blight attacks potatoplants more heavily on clay than on sandy soils may have something to do with the ability of the fungus to be cultivated better on clay than on sand. It may also be possible that manure by increasing the organic substances of the soil may influence the attack of *Phytophthora infestans.*

May, 1922.

HET LEVEN VAN PHYTOPHTHORA IN DE AARDE ALS SAPROPHIET.

KORT OVERZICHT.

Door mijne onderzoekingen over *Phytophthora syringae* KLEB. kwam ik tot dezelfde gevolgtrekkingen als KLEBAHN (1909) n.l. dat deze schimmel vermoedelijk in den grond overblijft en van hieruit steeds weer opnieuw de seringen infecteert. Dit leidde tot een nader onderzoek betreffende de mogelijkheid van en de wijze waarop deze schimmel in de aarde zou kunnen blijven leven en wel werd dit niet alleen nagegaan van deze Phytophthora-soort, doch ook werd het onderzoek uitgebreid over twee andere soorten, n.l. over *Phytophthora erythroseptica* PETH., die het roodrot van de aardappelknollen veroorzaakt en over de economisch meest belangrijke, de welbekende *Phytophthora infestans* (MONT.) DE BY. Indien wij in de literatuur nagaan in hoeverre het van het geheele geslacht *Phytophthora* bekend is, of het verblijf in de aarde een rol in het leven speelt, dan valt ons al spoedig op, dat bij de meeste soorten dit het geval is. Van 9 soorten werd reeds vastgesteld, dat zij in de aarde voorkomen, terwijl van enkele andere de levensgeschiedenis nog niet volkomen bekend is, zoodat bij deze de mogelijkheid niet is uitgesloten, dat ook zij in de aarde kunnen verblijven. De meeste auteurs beschouwen dit leven in den grond slechts als een voortbestaan in een of anderen rusttoestand. Van sommige soorten echter, waarvan blijkt, dat de aarde de besmettingsbron is, werden in de natuur nooit oösporen gevonden, men veronderstelt dan dikwijls hun bestaan, om het verblijf in de aarde te kunnen verklaren, terwijl deze meening steun vindt in 't ontstaan der oosporen in sommige gevallen op kunstmatige voedingsbodems in het laboratorium. Dit is ook altijd het groote vraagstuk geweest bij de *Phytophthora infestans*. Daar van deze de oösporen, niettegenstaande herhaalde pogingen, in de natuur nooit gevonden zijn, hebben velen de mogelijkheid, dat bij deze schimmel de aarde een rol in het leven zou spelen voor uitgesloten gehouden. Slechts enkele auteurs hebben de meening geuit, dat een saprophitisch leven van de aarddeeltjes bij Phytophthora-soorten mogelijk zou zijn, sommige hebben getracht deze schimmels op steriele aarde te kweeken, tot nu toe heeft men hier echter geen resultaten mee bereikt.

Door mijne proeven werd bewezen, dat zoowel *Phytophthora syringae* als *P. erythroseptica* en *P. infestans* zich gemakkelijk lieten kweeken op gesteriliseerde aarde in buisjes of in schalen. Het mycelium vermeerderde zich, zoodat werkelijk vaststaat, dat de schimmels de bestanddeelen van de aarde als voedsel kunnen gebruiken, dat zij dus in de aarde kunnen leven als saprophiet. De schimmels werden gekweekt op vier verschillende grondsoorten, n.l. op Aalsmeersche baggeraarde, bladaarde, klei en zand. Het gedrag van de schimmels op de verschillende grondsoorten was niet voor alle drie soorten gelijk, zoo ontwikkelde *Phytophthora syringae* zich het beste op baggeraarde en bladaarde, groeide *Phytophthora erythroseptica* buitengewoon goed op klei, terwijl *Phytophthora infestans* zich het gemakkelijkst liet kweeken op baggeraarde en klei en daarentegen zeer moeilijk op zand. Hoewel het van zelf spreekt, dat voor goede ontwikkeling van de schimmels vocht noodig is, zoo bleek toch ook, dat, als zij eenmaal goed gegroeid zijn, de schimmels een vrij sterke uitdroging van de aarde kunnen verdragen. Door infectieproeven op levende plantendeelen werd van elke soort bewezen, dat zelfs door een langdurig verblijf in de aarde het infectievermogen onveranderd blijft.

Bekijkt men de op aarde gekweekte schimmels onder het mikroskoop zoo blijkt, dat zoowel *Phytophthora syringae* als *P. infestans* in vochtige aarde vele conidiën vormen, terwijl deze bij *P. erythroseptica* zich pas ontwikkelen, nadat de aarde met de schimmel gedurende eenigen tijd in water gestaan heeft. Daar deze toestand ook in de natuur voor kan komen, zoo is dus bij alle drie soorten verdere verspreiding van de schimmel in de aarde door middel van sporen mogelijk, hetgeen vooral zal plaats hebben door regen. Behalve conidiën werden bij *Phytophthora syringae* en *P. erythroseptica* in de aarde ook oösporen gevormd, bij de laatste in grooten getale bij de eerste slechts enkele.

Gedurende twee jaar werd nagegaan of de schimmels in staat zijn in de aarde te overwinteren. De eerste winter 1920—'21 was een zeer zachte, de tweede 1921—'22 daarentegen was voor ons land buitengewoon streng (zie tabel I). *Phytophthora syringae* en *P. erythroseptica* konden beide de lage temperaturen (minimum —12° C.), zoowel als de afwisselende perioden van koude, in de aarde zeer goed verdragen, terwijl door infectieproeven bewezen werd, dat, niettegenstaande de doorgestane koude, het infectievermogen volkomen behouden bleef. Beide schimmels konden ook plotselinge, groote verschillen van temperatuur, n.l. een overbrenging van —7° C. tot ± 18° C. zonder eenig nadeel verdragen. Tevens werd aangetoond, dat het mycelium van *Phytophthora syringae* zelf in staat is de lage wintertemperaturen te weerstaan en voor deze soort dus de aanwezigheid van oosporen niet noodzakelijk is om het overwinteren van de schimmel mogelijk te maken. Over het overwinteringsvraagstuk van *Phytophthora infestans* kon nog geen zekerheid verkregen worden; in den eersten zachten winter had de schimmel wel een temperatuur van ± —9° C. verdragen, doch niet in aarde, daar de culturen op aarde toen nog niet aanwezig waren, terwijl in den strengen winter de schimmel niet in het leven bleef. Dergelijke proeven zullen nog gedurende meerdere winters herhaald moeten worden om uit te maken of er eenige mogelijkheid bestaat, dat *Phytophthora infestans* in de aarde kan overwinteren, zij het wellicht dat dit alleen in zachte winters kan plaats hebben, doch in strenge winters uitgesloten is.

Nu het vaststaat, dat de drie onderzochte Phytophthora-soorten als saprophiet in de aarde kunnen leven, is het waarschijnlijk, ook in verband met de literatuur, dat dit bij de meeste soorten van het geslacht Phytophthora het geval is. Het feit, dat van sommige soorten tot nog toe geen oösporen in de natuur gevonden zijn, sluit niet uit, dat deze soorten als saprophiet in de aarde kunnen leven. Het is alleen niet zeker of zij in de aarde kunnen overwinteren. Daar het echter bleek, dat het mycelium van *Phytophthora syringae* de lage wintertemperaturen kan verdragen, blijft de mogelijkheid bestaan, dat de soorten, waarbij geen oösporen gevormd worden, 's winters in de aarde kunnen overblijven. Dit zou voor iedere soort afzonderlijk vastgesteld moeten worden, daar het vermogen van het mycelium om de koude te weerstaan een eigenschap van de soort is. Voor de tropische soorten, waarvan tot nog toe in de natuur geen oösporen gevonden zijn, is de vraag in hoeverre het mycelium in de aarde uitdroging kan verdragen, van groot belang. Ook blijft voor alle soorten de mogelijkheid bestaan, dat de oösporen, zij het sporadisch, in de natuur gevormd worden en slechts nog niet door ons gevonden zijn.

Het voorkomen van Phytophthora-soorten als saprophiet in de aarde is oorzaak, dat wisselbouw niet als bestrijdingsmiddel in aanmerking komt. Op deze wijze toch worden de schimmels niet gedood. Men zal dus naar voorbehoedsmiddelen moeten omzien, die aantasting van de planten door de schimmel voorkomen, hetzij door besproeien met fungiciden, hetzij door het kweeken van onvatbare soorten.

Het plotseling hevig optreden van enkele Phytophthoraziekten en de buitengewoon sterke invloed, die de weersgesteldheid hierop heeft, is zeer goed te verklaren. Is de weersgesteldheid gunstig voor de ontwikkeling van de schimmel dan zal een sterke vermeerdering van deze in de aarde plaats hebben, waardoor over een groot oppervlak een onnoemlijk aantal sporen voor verdere verspreiding van de ziekte aanwezig zullen zijn.

Als vaststaand mag wel aangenomen worden, dat de grond van vele seringenkweekerijen te Aalsmeer met **Phytophthora syringae** besmet is, hetgeen ook reeds door Schoevers (1913) verondersteld werd. De Aalsmeersche baggeraarde bleek een uitstekende voedingsbodem voor de schimmel te zijn. Door mijn later nog te publiceeren proeven werd vastgesteld, dat de seringen slechts gedurende een bepaalde periode van het jaar vatbaar zijn voor infectie. Indien de voor de ontwikkeling van de schimmel gunstige weersgesteldheid samenvalt met de infecteerbare periode van de sering, zal de ziekte hevig optreden, is dit niet het geval dan komt de ziekte niet of slechts sporadisch voor. Het zeer sterke verschil in het optreden van de ziekte in de verschillende jaren is hierdoor te verklaren. Het onderzoek over het tijdstip van algemeene infectie en over de daar eventueel uit af te leiden bestrijdingsmiddelen is nog niet afgesloten.

Phytophthora erythroseptica komt in ons land tot nog toe slechts zelden voor. Opgelet zal dienen te worden of het aantal ziektegevallen toeneemt, vooral met het oog op de vraag of de schimmel zich sterker gaat verspreiden, daar een eenmaal gevormde infectiehaard zeer moeilijk te vernietigen zal zijn.

Zooals reeds gemeld kon geen positief antwoord verkregen worden op de vraag of **Phytophthora infestans** in de aarde kan overwinteren. Volgens Pethybridge (1921) zijn er aanduidingen, dat de oösporen van *Phytophthora infestans* ook in de natuur gevormd kunnen worden; zekerheid hieromtrent zal voor het overwinteringsvraagstuk van groot belang zijn. Doch niet alleen voor het overwinteringsvraagstuk, ook voor de andere tijdperken van het leven van de schimmel zal het vermogen, om zich als saprophiet in de aarde te kunnen ontwikkelen, van beteekenis zijn. Hierop wijzen reeds de belangrijke proeven van Murphy (1921) over het besmettingsgevaar bij het rooien, dat ook aanwezig is nadat het zieke loof verwijderd is, daar dit besmettingsgevaar zeer gemakkelijk door een saprophitisch leven van de schimmel in de aarde verklaard kan worden. In een Phytophthora-jaar zal dus ook de weersgesteldheid, die vóór en tijdens het rooien heerscht, invloed uit kunnen oefenen op het aantal knollen, dat aangetast wordt, waarmee in de praktijk zoo mogelijk rekening gehouden kan worden.

De mogelijkheid, dat de schimmel in de aarde overwintert, sluit niet uit, dat ook zieke poters een rol kunnen spelen bij het ontstaan der ziekte. Vele onderzoekers kwamen door hun proeven tot de gevolgtrekking, dat uit zieke knollen geen of gezonde aardappelplanten zich ontwikkelen. De zieke poter kan echter nog op een andere wijze gevaarlijk zijn n.l. door de omgevende aarde te besmetten. Dat uit zieke aardappelstukjes het mycelium zich in de omgevende aarde kan ontwikkelen, werd door mijne proeven bewezen. Onnoodig is het erop te wijzen, dat een dergelijke infectie zeer afhankelijk zal zijn van de weersgesteldheid en alleen mogelijk is, als behalve de vochtigheidstoestand ook de samenstelling van de aarde de ontwikkeling van de schimmel begunstigt.

De opvatting van De Bary (1861) dat de aardappelknollen in den grond geïnfecteerd worden door de conidiën van *Phytophthora infestans*, die door het water bij deze gebracht worden, behoeft niet gehandhaafd te worden. De schimmel, eenmaal in de aarde groeiende als saprophiet kan den knol bereiken en infecteeren, zonder dat er eenige strooming in het water plaats vindt. Het resultaat blijft hetzelfde, doch de knolinfectie is door deze verklaring minder afhankelijk van het water. De onder sommige omstandigheden plaats hebbende sterke knolinfectie bij zwakke loofinfectie is nu ook beter verklaarbaar.

Het feit, dat de *aardappelziekte* sterker optreedt bij aardappelplanten op klei dan op zandgronden zou in verband kunnen staan met de eigenschap, dat de schimmel zich als saprophiet veel gemakkelijker op klei dan op zand liet kweeken. Ook zou het mogelijk zijn, dat natuurlijke meststoffen door het vermeerderen van organische bestanddeelen in de aarde invloed uitoefenen op een sterker optreden van *Phytophthora infestans*.

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EXPLANATION OF FIGURES.

VERKLARING DER FIGUREN.

Fig. 1. Pure culture of *Phytophthora infestans* on bog-soil aged 1½ months.

Reincultuur van *Phytophthora infestans* op baggeraarde. 1½ maand oud.

Fig. 2. Pure culture of *Phytophthora infestans* on clay, aged 3 months.

Reincultuur van *Phytophthora infestans* op klei, 3 maanden oud.

Fig. 3. Pure culture of *Phytophthora infestans* on raw, sterile potatopieces, covered with sterilized bog-soil. The mycelium has grown through the soil and reached the surface.

Reincultuur van *Phytophthora infestans* op rauwe, steriele aardappelstukjes, bedekt door steriele baggeraarde. Het mycelium is door de aarde heen gegroeid en heeft de oppervlakte bereikt.

Fig. 4. Pure culture of *Phytophthora erythroseptica* on bog-soil in Petridish. Oöspores are formed in large quantity and are to be seen between the lumps of the soil. × 150.

Reincultuur van *Phytophthora erythroseptica* op baggeraarde in Petrischaal. Oösporen ontwikkelen zich overvloedig en zijn tusschen de aardkluitjes te zien. Vergr. 150 ×.

Fig. 5. Pure culture of *Phytophthora erythroseptica* on bog-soil in Petridish. Lumps of soil after remaining for some days in water under a cover-glass when conidia are formed. × 150.

Reincultuur van *Phytophthora erythroseptica* op baggeraarde in Petrischaal. Kluitjes aarde na een verblijf van eenige dagen in water onder een dekglas, waarna conidiënvorming plaats vindt. Vergr. 150 ×.

Fig. 6. Pure culture of *Phytophthora syringae* on bog-soil in Petridish. Conidia are to be seen in great quantity on the side of the lump of soil. × 50.

Reincultuur van *Phytophthora syringae* op baggeraarde in Petrischaal. Conidiën komen in grooten getale aan den kant van het kluitje aarde te voorschijn. Vergr. 50 ×.

Fig. 7. The same lump as in fig. 6, more highly magnified. × 170.

Hetzelfde kluitje als in fig. 6 sterker vergroot. Vergr. 170 ×.

Fig. 8. Pure culture of *Phytophthora syringae* on bog-soil in a tube. Oospores are visible after boiling in K O H. × 170.

Reincultuur van *Phytophthora syringae* op baggeraarde in een buisje. Oösporen, duidelijk zichtbaar na behandeling in kokende KOH. Vergr. 170 ×.

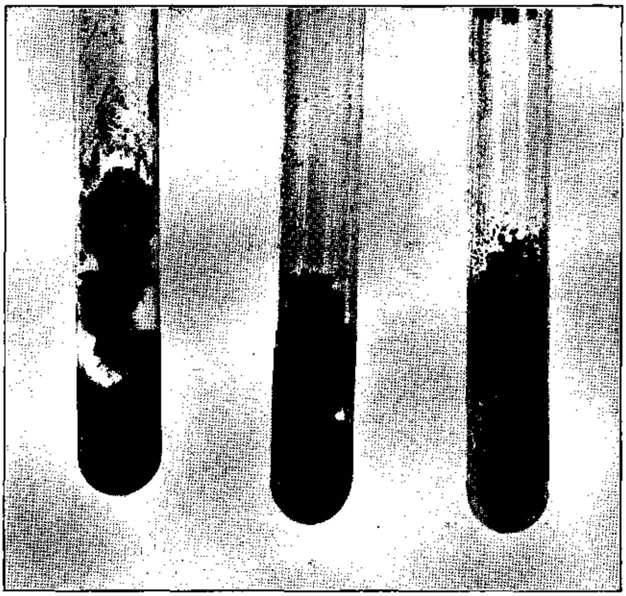


Fig. 1. Fig. 2. Fig. 3.

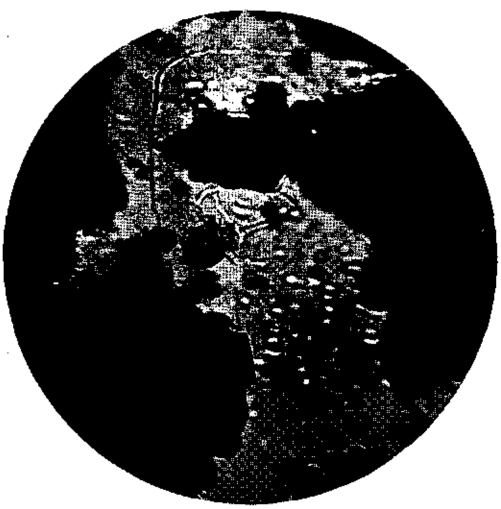


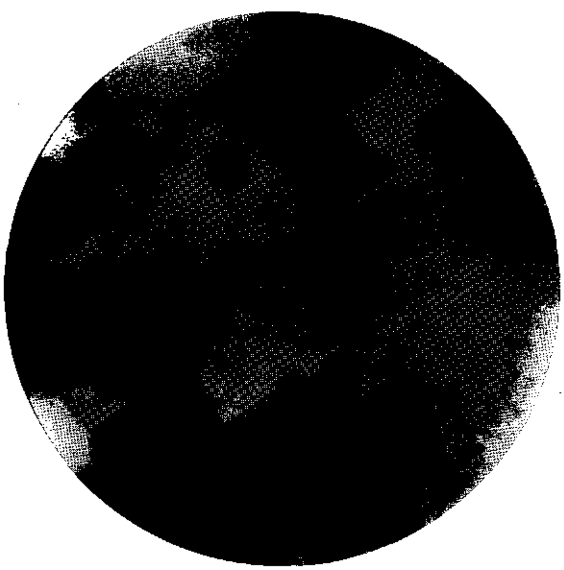
Fig. 4.

Fig. 5.

J. Boekhorst, phot.

Fig. 6.

Fig. 7.



J. Boekhorst, phot.

Fig. 8.