THE RHIZOCTONIA- AND OLPIDIUM DISEASE OF CAULIFLOWER SEEDLINGS.

INTRODUCTION.

In the neighbourhood of Utrecht a peculiar disease among cauliflowers attracted attention in May 1925. After transplanting in the field many of the seedlings did not grow, their root system was poorly developed and some of them could be pulled out of the soil very easily. However most of the plants had recovered in June and at the end the profits resulting from the sale of the vegetable did not suffer much from the disease among the seedlings.

§ 1. MACROSCOPICAL AND MICROSCOPICAL STUDY OF THE DISEASED SEEDLINGS.

Between the healthy lateral roots of the seedlings a number of yellowish brown discoloured ones were observed, especially at the base of the main root. Microscopical examination of the brown root portions showed the presence of sporangia and resting spores of Olpidium. In May and June 13 diseased plants were collected: 11 of them contained Olpidium, whereas on the roots of 5 specimens Rhizoctonia was found.

§ 2. INOCULATIONS.

The cauliflower-variety "Haagsche vroege" was used in all experiments.

1. *Inoculation with infected soil from Utrecht.*

By transplanting healthy seedlings in pots with sterilised and untreated infected soil, the controls developed more vigorously than the others. The microscopical study of the roots showed that Olpidium was present in all cases and that Rhizoctonia often accompanied it.

Therefore it was necessary to experiment with both fungi separately.

GRATZ (4) described a cabbage-disease in America, the so-called "wire stem", caused by *Corticium vagum* B. & C., with similar symptoms as observed among the cauliflower seedlings in Utrecht.

WORONIN (12) attributed the "damping off"-disease of cabbage seedlings to *Chytridium Brassicae* Wor.

MATHILDE BENSAUDE (1) mentioned the presence of Olpidium in the roots of cabbage seedlings, which developed quite normally. Her opinion is, that the fungus corresponds very closely to the description of *Olpidium brassicae* (Wor.) Dangeard given by WORONIN.

According to TOMSA (8) the "damping off" of cruciferous seedlings was caused by *Olpidium brassicae* Woron.

2. *Inoculation with Rhizoctonia.*

a. The seed of cauliflower was sown in soil contaminated before with two different strains of cauliflower-Rhizoctonia. After some days only a few plants appeared above the surface of the soil. Most of them were killed by the fungus immediately after germination (Table I).

b. The three remaining plants of the sowing pan, contaminated with strain K 9, were transplanted at the end of September in pots with sterilized soil.

c. Seedlings developed from seed, sown Aug. 12, 1925 in sterilized soil, were inoculated during transplanting.

Experiments *b* and *c* gave the same results: the plants did not grow, the leaves were limp, some of the plants were badly attacked at their foot, others resembled the diseased cauliflowers in Utrecht (Pl. VI, fig. 1).

Several cross-inoculations with the Rhizoctonia-strains from cauliflower and potato on both hosts showed that the strains were physiologically distinct from each other (Table II, Pl. VI, fig. 2). GRATZ (4) mentioned the same results. However the Dutch cauliflower-Rhizoctonia produced a number of small sclerotia on the tubers of inoculated potato plants, whereas the American cabbage strain failed to do so.

3. *Inoculation with Olpidium.*

As Olpidium is an obligate parasite, it was necessary to use living plants for media in the pure cultures of this fungus.

With regard to the inoculations two methods were practised. At the outset the contamination occurred in the following way:

Cauliflower seeds disinfected with mercuric chloride 2 $^0/_{00}$ germinated quickly in a Petri dish with Knop's solution agar. As soon as the plants had developed the cleaned roots of a diseased cauliflower from Utrecht were put into a small quantity of water. After 5—10 minutes the zoospores of Olpidium emerged. The water was filtered through a piece of lawn and examined under the microscope. On a rather dark back-ground the zoospores could be easily observed with a weak enlargement. When a sufficient number of zoospores were present, each root of the sterile seedlings in the Petri dish received a drop of the filtrate. After some days the plants contained a great deal of sporangia, other fungi being quite absent. Two or three of these inoculated plants were placed in a little pot with sterilized soil. In order to cultivate a large quantity of Olpidium, the number of plants was increased by sowing disinfected seeds all around them. The pots were abundantly watered. The produced zoospores might in this way frequently contaminate the healthy root parts.

As soon as the experiments demanded inoculation material the seedlings were removed from some pots, cleaned and put into a dish with water. The filtrate of the zoospore-suspension was added to the plants which had to be inoculated.

At intervals new Olpidium cultures were prepared.

In the later experiments another more practical method was applied:

Some vigorously-developed cauliflower plants, each of them growing in a pot with sterilized soil, were artificially infected with Olpidium. The rootsystem acted as a zoospore-reservoir. The pot was cleaned on the outside, placed on a sterile Petri dish and abundantly watered. When percolated for some time, the water in the dish contained many zoospores. In this way a large quantity of infection-material could be

obtained. Occasionally a drop of the water was examined under the microscope in order to see, if a sufficient number of zoospores was still present. After some days new sporangia had developed and it was possible to dwaw again from those plants.

Several inoculation experiments taught that an attack of the roots with Olpidium caused a slight delay in the growth of cauliflower seedlings. The roots of infected plants had a more yellowish colour than those of the controls.

The plants figured on Pl. VI, fig. 3 were inoculated from the day, they appeared above the surface of the soil. The contaminated seedlings and their controls always received the same quantity of water. After three weeks they were transplanted and a month later the examination of the seedlings began. They were divided into ten groups and tied up into bunches. Pl. VI, fig. 3 shows the less vigorous growth of the infected cauliflowers. The differences in weight are indicated on p. 223.

Table III contains the results of a comparison of the measurements of hypocotyl, stem and leaves of 59 infected plants with their checks.

It was shown experimentally that humidity of the soil supports in a large degree the infection with Olpidium.

The months of March, April and May in the year 1925 were characterized by a great number of rainy days. Besides, the experiments with the infected soil from Utrecht taught that water did not pass very quickly through it. Both agents will result into a bad attack of Olpidium. Therefore it is quite possible that the slighter cases of the disease among the cauliflower seedlings in Utrecht may be caused by Olpidium.

"Damping off" of the seedlings as WORONIN (12) mentioned in his description of the cabbage disease caused by *Chytridium Brassicae* was never observed in any experiment. There was much more resemblance with the case studied by MATHILDE BENSAUDE (1). When she was occupied in Wisconsin with a root disease of tomato and tobacco, she observed Olpidium in the roots. Sowing cabbage in the contaminated soil, the plants developed quite normally, still the roots were infected by Olpidium.

The fungus Olpidium mentioned both by MATHILDE BENSAUDE and by the writer corresponded morphologically in many respects to the description of *Olpidium brassicae* (Wor.) Dang. given by WORONIN. However WORONIN observed the fungus in the hypocotyl cells and the writer never did so. WORONIN described globular sporangia with one neck, whereas MATHILDE BENSAUDE and writer observed both globular and elongated sporangia. The most elongated ones bore two to six necks.

DE WILDEMAN (10 and 11) gave a description of *Olpidium Borzii*, since 1896 called *Olpidium radicicolum*, which was found in the roots of *Brassica oleracea* and *Capsella bursa-pastoris*. This Olpidium species had elongated sporangia with one to several necks.

It will be necessary to make a pure culture of the cabbage Olpidium originating from one zoospore, in order to observe, if the globular and elongated sporangia belong to the same species.

§ 3. THE PRESENCE OF OLPIDIUM IN CABBAGE AND IN OTHER PLANTS.

Olpidium was observed in the roots of cauliflower and cabbage in two different localities and in those of "green cabbage" on the infected ground in Utrecht.

The examination of the following plants: *Urtica urens* L., *Solanum nigrum* L., *Euphorbia* spec., *Stellaria media* Cyrillo, *Lamium purpureum* L., *Polygonum Persicaria* L., *Chenopodium album* L. and beet, growing on the fore-mentioned ground gave only positive results for Chenopodium and beet. Olpidium could sporadically be seen in the roots of these two Chenopodiaceae.

Inoculation experiments showed that tobacco was not attacked by the cabbage Olpidium. This fungus appears therefore to be specialized in a rather large degree.

PREISSECKER [see SORAUER (7)] and PETERS & SCHWARTZ (6) attributed the so-called "Gelbsucht", often connected with "damping off", to *Olpidium nicotianae*, probably a variety of *Olpidium brassicae*. PREISSECKER observed Olpidium also in the roots of *Chenopodium album*, *Portulaca oleracea* and cabbage seedlings.

MATHILDE BENSAUDE (1) mentioned the occurrence of Olpidium in the roots of tobacco and cabbage, the attacked plants developed quite normally.

§ 4. THE WAY IN WHICH OLPIDIUM PENETRATES INTO THE INTERIOR OF CAULIFLOWER SEEDLINGS.

The preliminary observation that the zoospores of Olpidium penetrated the root hairs of cauliflower (Pl. VII, fig. 1 and 2) confirmed the supposition of CHUPP (2): "The organism (Olpidium) evidently enters by way of the root hairs." CHUPP observed Olpidium when he was studying *Plasmodiophora brassicae* Wor. He mentioned the presence of 2—12 strange nuclei in root hairs and epidermal cells, from 3—4 μ in diameter, smaller than the nuclei of the host cells. According to CHUPP, they appeared to be entire swarmspores containing no visible cytoplasm.

For an exact study of the way in which Olpidium enters its host, it was necessary to determine beforehand the temperature optimum of the infection. It was experimentally shown that it lay between 15 and 20° C.

In order to get a culture of Olpidium as pure as possible, the fungus was cultivated in sterile cauliflower seedlings growing in tubes with pumice and Knop's solution. The first inoculation occurred by adding a zoospore-suspension to the seedlings in six tubes. After 5 days two plants of one of the tubes were cleaned in sterile water and placed in a little water on the bottom of a tube. Microscopical examination showed in a short time the presence of many zoospores. The suspension was transferred to tubes with healthy seedlings. After having been exposed to a temperature of 20° C. in the incubator for some days, the tubes were placed on a light place in the room. In this way transferring occurred twelve times.

The study of the mode of infection began December 17, 1925. Three seedlings of culture no. 6 were cleaned in sterile water. Each of the roots was put into a drop of sterile water on a slide. After several minutes the zoospores started swimming about. On December 14 disinfected cauliflower seed had been spread on humid filterpaper in a Petri dish. Three of the developed sterile seedlings were placed in the drops one on a slide. In the humid atmosphere of Petri dishes, the covers of which were coated with watered filterpaper, the slides

were exposed to the temperature of 20° C. in the incubator, respectively at 9.50, 10.10 and 10.20 a.m.

After an hour some zoospores were still moving. They measured 3 μ in diameter. After removing the diseased root portion from slide no. 1 at 11.30 a.m., a cover glass was put on the root of the sterile seedling. Microscopic examination showed that a great deal of the zoospores had come to rest on the surface of the root hairs. In order to prevent a drying up of the object, sterile water was added carefully at the edge of the cover glass. The slide stayed under the microscope till 1.50 P. M. One of the root hairs surrounded by seven zoospores was minutely examined. At two o'clock two of the zoospores penetrated into the interior of the root hair leaving the empty spore-shells behind. Apparently the zoospores had been surrounded with a wall after they had come to rest. Half an hour later the plasma of Olpidium at the inside of the root hair-wall changed in shape and suddenly at 2.45 it was carried as a globular body of 3 μ in diameter by the streaming plasma of the host cell. It moved in different directions, till at last on its way to the base of the root hair, it disappeared in the epidermal cell. At that moment it was three o'clock.

Therefore in one hour Olpidium succeeded in penetrating through the root hair wall and entering the epidermal cell.

As Miss CURTIS (3) used the term prosorus for the first stages of *Synchytrium endobioticum* (Schilb.) Perc., I propose the name prosporangium for the stages between the beginning of transport of the zoospore-plasma by the root hair plasma and the formation of zoospores.

Meanwhile other zoospores had succeeded in entering and the same observations were made. One of the prosporangia, moving on at 4.13 P. M., floated nearly straight on to the base of the root hair and arrived in the epidermal cell already after seven minutes.

At five o'clock the slide was put back in the dish and incubator.

The 2—12 strange nuclei from 3—4 μ in diameter, in root hairs and epidermal cells, mentioned by CHUPP (2) will have been prosporangia of Olpidium.

Microscopic examination of the experiment seedlings showed after 24 hours prosporangia from 8—13 μ, after $\pm$ 48 hours prosporangia from 17 μ (Fig. 1), prosporangia from 20—27 μ with necks and hyaline plasma (Fig. 2) and oblong sporangia with zoospores (Fig. 3).

Exactly in the same way the experiment was made with zoospores produced by Olpidium present in the roots of cauliflower pot plants. The mode of infection of one zoospore was studied in detail. The contamination of sterile seedlings occurred in the morning of December 29, 1925. Microscopical examination began about half past two in the afternoon. One of the root hairs, surrounded by a great deal of swarmspores, was drawn with the camera lucida at 2.30 P. M. (Pl. VII, fig. 3a). At 2.43 the plasma of the root hair showed a protuberance just under one of the zoospores (the second from the left side). This zoospore emptied its contents in ten minutes through an extraordinarily narrow channel in the centre of the plasma-process within the root hair (Pl. VII, fig. 3b, c, d, e). After five minutes the prosporangium had rounded off (Pl. VII, fig. 3 f), at three o'clock it was lying a little aside (Pl. VII, fig. 3g) and at 3.02 it had become quite free and was transported by the root hair-plasma.

The time between the first indication of the penetration and the

loosening of the prosporangium was in the above-mentioned case 19 minutes.

The slide was placed back into the dish and incubator and examined again the following day. At 9.30 a.m. the prosporangia measured 10 μ, at 3.40 P.M. the same bodies were 17 μ (Pl. VII, fig. 4). The following morning at ten o'clock sporangia from 20—23 μ in diameter with necks and zoospores were observed. Soon the zoospores emerged and penetrated the young root hairs after some time. So a second generation had developed in 44 hours.

In order to study if Olpidium was able to enter the epidermal cells directly, sterile cauliflower seedlings from a Petri dish with moist filterpaper were put into drops of a zoospore-suspension at the bottom of a Petri dish, the cover of which was coated with humid filter paper. The plants were exposed to a temperature of 20° C. in the incubator. Three days later the microscopic examination of the epidermal tissue at the base of root and hypocotyl showed, that only the cells bearing root hairs were infected, the adjacent cells without root hairs had no sporangia at all (Fig. 5).

The same results were obtained from the experiment with cauliflower seedlings in tubes with pumice and Knop's solution. The contamination with a zoospore-suspension occurred in such a way that the surface of the water reached about halfway the hypocotyl.

It may be concluded that Olpidium penetrates only through the root hairs of its host.

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VERKLARING DER PLATEN.

Plaat VI.

Fig. 1. Grondinfectieproef met Rhizoctonia, geïsoleerd uit bloemkool. Twee maanden oude bloemkoolplanten werden 19 Oct. 1925 in potten met besmette aarde gezet.
Voorste rij links: aarde geïnfecteerd met stam K 12.
Achterste rij links: aarde geïnfecteerd met stam K 9.
Beide rijen rechts: contrôle.
Gephotographeerd 30 Oct. 1925.

Fig. 2. Bloemkoolkiemplantjes, gezaaid 14 Nov. '25, werden den 27en November geïnfecteerd met:
den kool-Rhizoctonia-stam K 12 in den voorsten bak links: alle plantjes omgevallen;
den kool-Rhizoctonia-stam K 9 in den achtersten bak links: vele plantjes omgevallen;
den aardappel-Rhizoctonia-stam in de beide bakken rechts: geen enkel plantje omgevallen.
Gephotographeerd 2 Dec. 1925.

Fig. 3. *a*. 5 partijen ± 8 weken oude bloemkoolplanten, vanaf de eerste ontwikkeling geïnfecteerd met Olpidium.
b. De bijbehoorende contrôle-partijen.
Gephotographeerd 16 Juni 1925.

Plaat VII.

Fig. 1. Het binnendringen van de zoösporen van Olpidium in de wortelharen van een bloemkoolplantje. × 100.
Gephotographeerd 3 Juni 1925.

Fig. 2. Een binnendringende zoöspore sterker vergroot. × 650.

Fig. 3. Binnendringende zoösporen van Olpidium in een wortelhaar van een bloemkoolplantje. × 750.
Geteekend met teekenprisma 29 Dec. 1925.
a. Toestand om 2.30 n.m.
b. „ „ 2.43 „
c. „ „ 2.44 „
d. „ „ 2.47 „
e. „ „ 2.53 „
f. „ „ 2.58 „
g. „ „ 3.— „ : het prosporangium bijna vrij.

Fig. 4. Epidermiscel van hetzelfde worteltje als in fig. 3 met prosporangiën. × 350.
Geteekend met teekenprisma 30 Dec. 1925, 3.40 n.m.

DESCRIPTION OF PLATES.

PLATE VI.

Fig. 1. Soil inoculations with Rhizoctonia isolated from cauliflower. Two-month-old cauliflower seedlings were transplanted in pots with contaminated soil October 19, 1925.
First row at the left: soil inoculated with strain K 12.
Second row at the left: soil inoculated with strain K 9.
Both rows at the right hand: controls.
Photographed October 30, 1925.

Fig. 2. Cauliflower seedlings, sown November 14, 1925, were inoculated November 27 with:
the cauliflower-Rhizoctonia strain K 12 in the first pan at the left: all plants showing "damping off";
the cauliflower-Rhizoctonia strain K 9 in the second pan at the left: many plants showing "damping off".
the potato-Rhizoctonia strain in both pans at the right: no plants showing "damping off".
Photographed December 2, 1925.

Fig. 3. *a*. 5 bunches of ± 8-week-old cauliflower seedlings artificially inoculated with Olpidium from the first moment they developed their cotyledons.
b. The corresponding control-bunches.
Photographed June 16, 1925.

PLATE VII.

Fig. 1. The penetration of the zoospores of Olpidium into the interior of the root hairs of a cauliflower seedling. × 100.
Photographed June 3, 1925.

Fig. 2. A penetrating zoospore. × 650.

Fig. 3. Penetrating zoospores of Olpidium into the interior of a root hair of a cauliflower seedling. × 750.
Drawn with camera lucida on December 29, 1925.
a. Stage at 2.30 P.M
b. „ „ 2.43 „
c. „ „ 2.44 „
d. „ „ 2.47 „
e. „ „ 2.53 „
f. „ „ 2.58 „
g. „ „ 3.— „ : prosporangium nearly loosened.

Fig. 4. Epidermal cell of the same root as in fig. 3 with prosporangia. × 350.
Drawn with camera lucida on December 30, 1925, at 3.40 P.M.

Fig. 1.

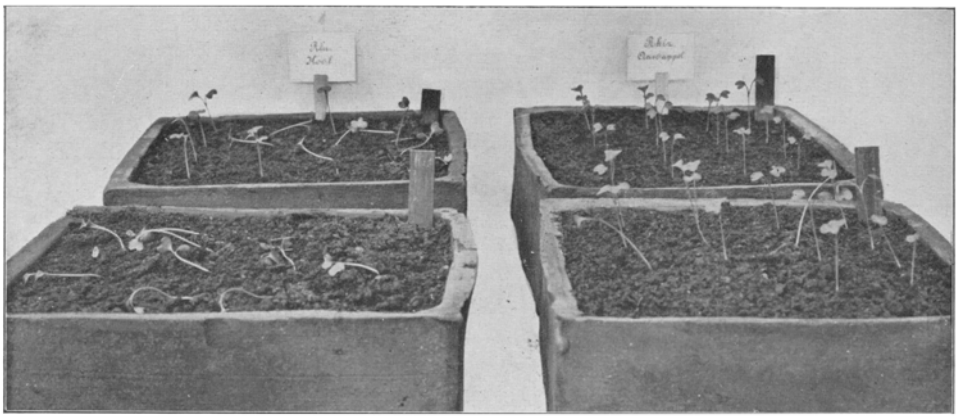


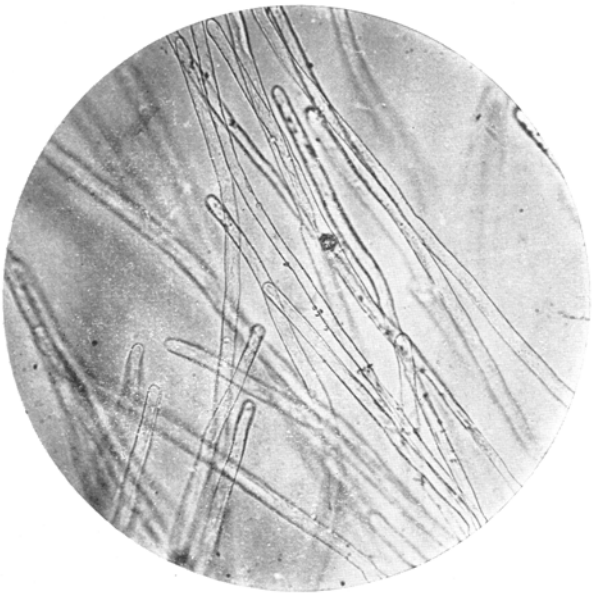
Fig. 2.

a

b

Fig. 3.

Photo's van J. v. d. Peppel Bzn en J. Boekhorst.



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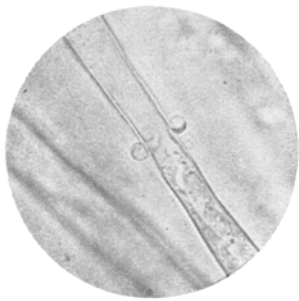

Fig. 2.

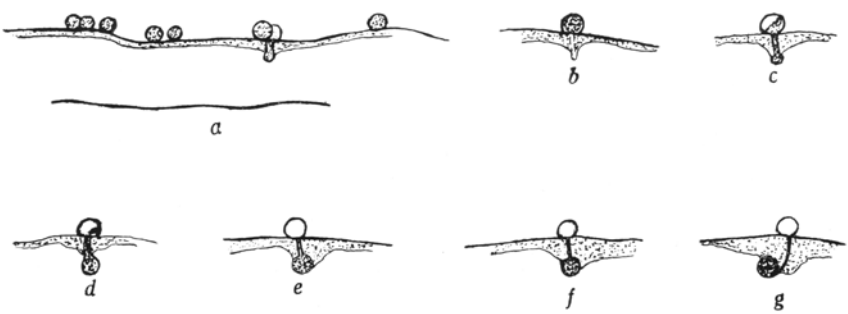

Fig. 3.

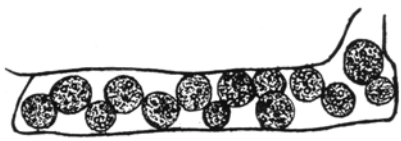

Fig. 4.