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Root rot of hyacinths caused by species of Pythium

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Abstract

The root rot widely seen in hyacinth was found to be caused by *Pythium* spp. instead of by *Fusarium culmorum*. Of six *Pythium* species isolated, three, included *P. ultimum* and *P. violae*, were investigated in glasshouse experiments and their pathogenicity demonstrated. In these experiments plants were successfully grown in containers in which an aqueous mist was maintained, or in water cultures. In experimental plots on infected soil, Dexon, a fungicide selective for Pythiaceae, distinctly reduced the number of dead plants and increased yield and bulb size, thus confirming the role of *Pythium* in causing root rot. Practical application of Dexon deserves further attention.

Introduction

Carruthers (1902) observed *Pythium* in diseased roots of hyacinth and considered this fungus to be the possible cause of the symptoms. Root rot in hyacinths was also reported by Ritzema Bos (1906). He did not succeed in isolating a parasite from diseased roots, but the fact that the diseased patches in the field enlarged over a period of a few years led him to conclude that the cause was probably a parasite. Gerretsen et al. (1927) sought a relationship between the chemical composition of the soil and the occurrence of the disease. They too came to the conclusion that a parasite, which they were unable to identify, must be responsible, an important indication in this respect being the effect of a soil treatment with formalin. From the results of inoculation experiments with material isolated from a sample of diseased hyacinth roots, Feekes (1931) concluded that *Fusarium culmorum* could induce the symptoms of the disease. For the last years the role of *Fusarium culmorum* as causal agent of the widespread root damage has been questioned, because this fungus was seldom found in the roots.

The control method applied by Dutch hyacinth growers before 1920 consisted of spading to a depth of 75 cm. The method proposed by Gerretsen et al. (1927) for disinfecting the soil with formalin was so much simpler that it was soon widely adopted. In this method the soil of the bed in which bulbs are to be planted is shovelled aside. Formalin is then poured on the subsoil of the bed and raked in, after which the bulbs are planted and the displaced soil is spread over them. This method is still applied, forty years later, but on soils intensively used for hyacinth cultivation it often no longer provides adequate control. In such cases, a few weeks before planting the soil is treated with chlorobromopropene (CBP) or sodium N-methyl dithiocarbamate

(methamsodium or Vapam), but in most cases it is still necessary to apply formalin during planting. The importance of the disease is indicated by the fact that on half the total area suitable for hyacinths (450 ha), it would be impossible to raise a paying crop if no control were applied. The total costs of the control methods used are estimated to be a half million guilders per year, and in spite of this expenditure considerable damage still occurs. From 1963 to 1967, the cause of this root rot was investigated in the "Laboratorium voor Bloembollenonderzoek' (Flower Bulb Research Centre).

Symptoms and course of the disease

Plants with affected roots show retarded growth, and on sunny days their leaves become less turgid. In a more advanced stage the leaf tissue dies, starting at the tip. This often occurs several weeks before the end of the growth period. As a consequence, in many fields the presence of the disease becomes evident as areas of yellowed or dead plants. These areas may be small and limited in number, but in other cases a large part of the plot may be affected.

Many of the roots of plants with withering leaves no longer show the normal white colour; they first become glassy and then soon turn grey and limp (Fig. 1).

The time of appearance of the root symptoms was followed over a period of three years. After it had been ascertained that the disease is not yet observable in the winter months of December and January, a number of plants were dug up for evaluation each week, starting on February 1st (the soil temperature as an average of many years is at this moment 3 °C at 10 cm depth). In 1966, a glass plate measuring 1 × 0.80 m was

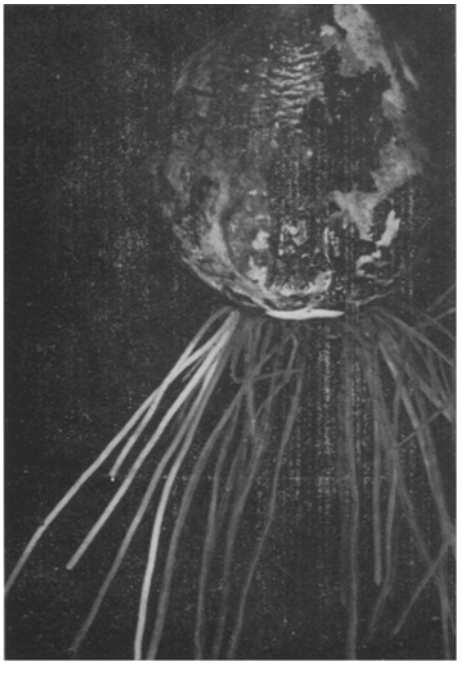

Fig. 1. Bulb with diseased roots. At the left, some white healthy roots (roots broken off).

Fig. 1. Bol met aangetaste wortels, links enkele witte, gezonde, wortels (wortels afgebroken).

placed vertically in the soil on one side of a pit to permit observation of root development (Fig. 2). To minimize temperature influences, the pit was filled with straw and covered with a board. On the other side of the glass, bulbs were planted. Both the regular sampling of the experimental plots and the observations of the roots behind the glass showed that the first symptoms of disease appeared about the time at which

Fig. 2. The observation pit.

Fig. 2. De waarnemingskuil.

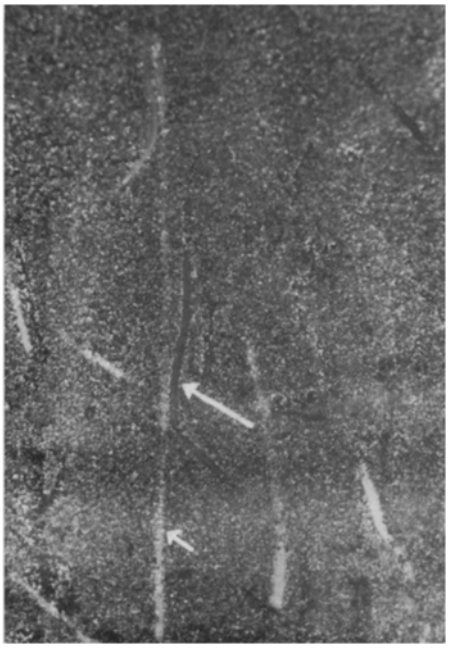

Fig. 3. White healthy roots (short arrow) and greyish water soaked roots (long arrow).

Fig. 3. Witte gezonde wortels (korte pijl) en grijsachtige waterige wortels (lange pijl).

blossoming reaches its peak (end of April, average soil temperature 8 °C) (Fig. 3). Thereafter the disease developed rapidly, the damage being manifested in reduced growth of the bulb, the quality in other respects remaining unaffected.

Material and methods

For all the experiments, the susceptible cultivar 'Pink Pearl' was used.

All of the experimental plots were located in parts of fields where the disease had been observed fairly regularly distributed in a hyacinth crop during the preceding season.

In the sampling of plots, a distinctly affected area was always chosen for the removal of a number of diseased but not yet completely dead plants to be examined for the presence of parasitic fungi. For the microscopical investigation, the roots were stained with cotton blue in lactophenol. In addition, diseased roots of the sampled plants were placed on a CMA medium containing the antibiotics pimaricin, penicillin, and polymycin B, according to Eckert and Tsao (1962); this medium is highly selective with respect to Phycomycetes.

For several infection experiments, use was made of Wisconsin tanks. Bulbs were also placed on containers in which a constant aqueous mist could be maintained (Fig. 4). Under these conditions, the plants developed excellent roots which could be easily handled for inoculation experiments. Equally good results were obtained with a modification of the apparatus described by de Stigter (1969), which is shown in Fig. 7 and is based on a drip system.

Inoculation experiments were performed in various ways. In the soil, about 5 cm under the base of the bulb, a layer of wheat and rice heavily infested with *Pythium* spp. was applied. In the containers with mist, inoculum on glass wool was spread on a plastic-coated grid about 35 cm below the base of the bulb shortly before the roots

Fig. 4. Container for aqueous mist technique.

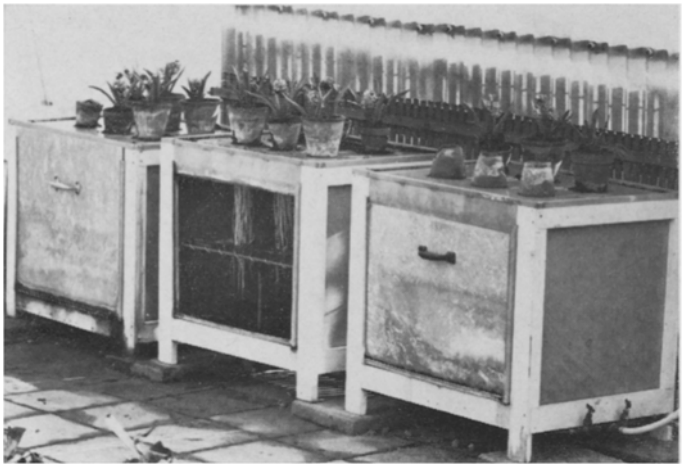

Fig. 4. Kist voor het vernevelen van water.

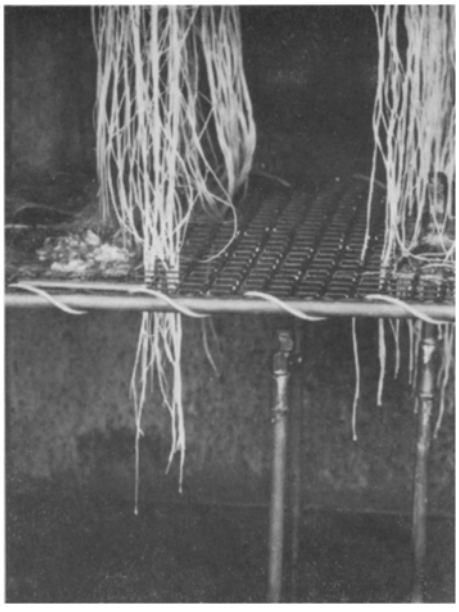

Fig. 5. Root growth in aqueous mist culture. The inoculum is placed on the grid. Note cessation of growth and disease of roots after touching the inoculum.

Fig. 5. Wortelgroei in de waternevel. Het inoculum wordt op geplastificeerd gaas geplaatst. De wortelgroei stopt en de wortels worden aangetast nu ze in aanraking komen met het inoculum.

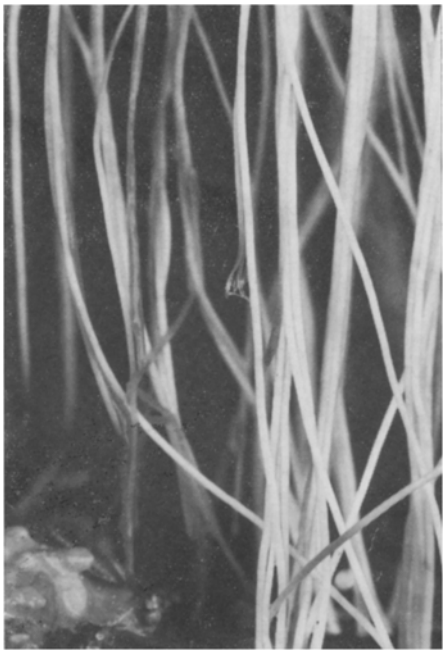

Fig. 6. Enlarged detail of Fig. 5.

Fig. 6. Vergroot detail van Fig. 5.

reached the grid (Fig. 4, 5 and 6). In the modified de Stigter apparatus (which will be referred to here as the drip culture), a piece of agar covered with fungus was placed close to a root tip on the nylon cloth (Fig. 8).

In field experiments, the selective fungicide Dexon (dimethylaminobenzenediazosodiumsulfonate, Bayer), which acts only on Pythiaceae and does not influence the growth of, for example *Fusarium* spp., *Trichoderma* spp., and Mucorales (Leach et al., 1960; Alconero and Hagedorn, 1968), was used to study the role of *Pythium* in the genesis of root rot. The fungicide used contained 70 % active ingredient.

The determination of the Pythiaceae isolated was done at the Centraalbureau voor Schimmelcultures (C.B.S.) at Baarn.

Fig. 7. The de Stigter apparatus, in which the water flows across a nylon cloth (A) covered by a sheet of black plastic (B); plastic sheet rolled up. Note pieces of sunflower stem carrying inoculum.

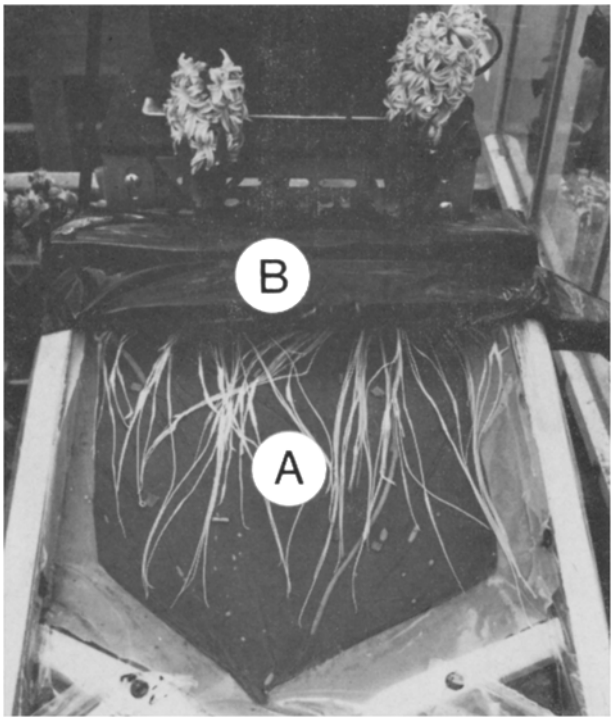

Fig. 7. Het apparaat volgens de Stigter waarin water over nylondoek (A) vloeit onder een dek van zwart plastic-folie (B; opgerold). Stukjes zonnebloemstengel waarop inoculum groeit liggen tussen de wortels.

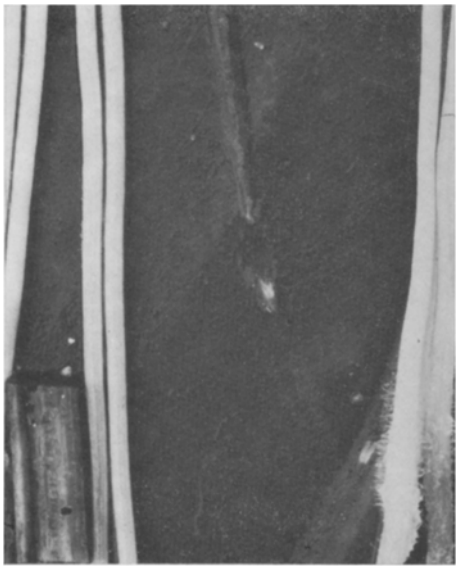

Fig. 8. Enlarged detail of Fig. 7, showing progressive disease in the vicinity of inoculum.

Fig. 8. Vergroot detail van Fig. 7. In de nabijheid van het inoculum heeft de ziekte zich ontwikkeld.

Results of sampling

From 1963 to 1965, *Pythium* spp. were isolated from diseased plants grown on 45 different fields. The isolates from 11 plots were identified. The following species were found: *P. ultimum* Trow (4 times), *P. paroecandrum* Drechsler (3 times), *P. irregulare* Buisman (7 times), *P. violae* Chesters and Hickman (8 times), *P. intermedium* de Bary (6 times), and isolates unidentifiable owing to poor spore formation (5 times). Of these *Pythium* species, *P. intermedium* (van der Plaats-Niterink, 1968) *P. irregulare* (Matthews, 1931), and *P. paroecandrum* (Rangaswami, 1962) are known as parasites of various plants. *P. ultimum* is a parasite of tulip (Grouet, 1961), crocus (Tomlinson, 1952), and many other plants (Middleton, 1943; Rangaswami, 1962). *P. violae* (Chesters and Hickman, 1944) is known only as a parasite of violets.

Microscopical examination of diseased roots of the samples always showed mycelium, conidia, and oogonia of *Pythium*. The most frequently seen were oogonia strongly resembling those of *P. violae* or *P. ultimum* (Fig. 9). These oogonia occur so consistently that their presence may be considered a characteristic of the disease.
Fusarium spores or mycelium with septa were never found in diseased roots except in the latest stage of disease development.

Inoculation experiments

In two experiments, marked differences were found between the results on soil inoculated with *Pythium* or *Fusarium* spp. and non-inoculated soil. After inoculation of the soil with a *Pythium* isolate (S36) that has not yet been identified owing to the absence of reproductive organs (in cultures on different media) and with a culture of *P. ultimum*, many of the roots became severely infected. They showed the characteristic picture of root rot: grey decayed roots easily broken when the plants are pulled up, so that they remain in the soil. After inoculation of the soil with an unidentified *Fusarium* isolate and with *Fusarium culmorum* (W.G. Sm.) Sacc. obtained from the C.B.S. at Baarn, a high percentage of the roots became brown and thinner than normal roots.

Fig. 9. Oogonia (resembling those of *P. violae* and *P. ultimum*) frequently seen in diseased roots.

Fig. 9. Oögonia (gelijkend op die van P. violae en P. ultimum) zoals ze vaak in aangetaste wortels worden aangetroffen.

At soil temperatures of 10°, 17° and 24 °C in Wisconsin tanks, *P. ultimum* gave no infection of the roots at 10 °C, but at 17 °C, 50% and at 24 °C, 60% of the roots became attacked.

Other inoculation experiments repeatedly gave the difficulty that the bulbs grown on non-inoculated soil (unsterilized dune sand) showed a similar amount of dead roots. For hyacinths planted in boxes, the condition of the roots is apparently easily affected by certain factors. This unfavourable influence cannot be abolished by disinfection of the bulbs, because such treatments might be expected to have an unfavourable influence on the results of the experiments.

Good reproducibility was obtained for the inoculation experiments using the large containers. The inoculum was applied to the grid in these experiments, just before the roots reached it. A few days after contact with the inoculum the roots became glassy, after which the presence of *Pythium* in these roots could be demonstrated. Roots that were not exposed to the inoculum maintained healthy growth (Fig. 5 and 6).

Inoculation with both *Fusarium* cultures also caused disease, but not the typical picture of root rot. In this case the infection spread more slowly and, as in the experiments in boxes, the roots became brown. For the inoculation experiments in the large containers, two cultures of *Pythium ultimum* were used as well as one culture of *Pythium* S36. In various experiments, all three of these cultures gave the characteristic picture of the disease. In two experiments with *P. violae* root rot symptoms were also obtained. *P. sylvaticum* isolated from crocus (van der Plaats-Niterink, 1968) did not affect hyacinth roots. In the drip cultures, *P. ultimum* and *Pythium* S36 rapidly led to disease symptoms in the roots (Fig. 7 and 8).

Thus *Pythium ultimum* easily infected the roots of hyacinth and the unidentified *Pythium* S36 caused similar root rot symptoms. *P. violae* was also found to be pathogenic. The pathogenicity of the other *Pythium* species has not yet been tested.

Field experiments

As mentioned in the introduction, root rot of hyacinths is controlled in the field by disinfection of the soil with formalin, CBP, or metham-sodium. These chemicals have a lethal effect on many soil organisms. Dexon, on the other hand, kills *Pythium* selectively. To demonstrate indirectly that *Pythium* spp. cause the root rot, seven experimental plots were maintained for three successive years in fields in which severe infection with root rot had been observed during the preceding season. In all these cases it was found that the disease could be controlled with Dexon. When more than 10 g 70% Dexon per m^2 was milled into the soil just before planting, a fairly marked phytotoxic effect was observed. When 10 g was milled into the soil, a limited phytotoxic effect was observed, but with this treatment the control obtained was almost the same as that given by metham-sodium (Table 1). When Dexon was applied after the development of roots, it appeared the plants could tolerate higher quantities without signs of phytoxicity. Applied one month after planting 20 g/m^2 showed no phytotoxic effect. In another experiment even 25 g/m^2 was not phytotoxic in this way. In the 1966–1967 season the effect of spring treatment was tried on one experimental plot and it appeared that this gave improved results (Table 1). This application of 10 g Dexon per m^2 in shallow furrows between the rows in February (treatment 5) gave results equalling those obtained with 20 g raked in in the autumn (treatment 4), 10 g

Table 1. The effect of treatment with dimethylaminobenzenediazo-sodiumsulfonate 70% active ingredient (Dexon) on infection with root rot. Per treatment 4 plots of 5 m² arranged according to a Youndon square; per plot 250 bulbs.

Treatment	Yield (kg)	Number of dead plants on 28 June	Number of bulbs, size 15 cm or larger
1 Dexon, 10 g per m² milled in + 5 g in shallow furrows (see 5); applied 17 Oct.	68,9	14	843
2 Dexon, 10 g raked in ± 1 month after planting, before application of winter cover; 14 Dec.	66.0	156	716
3 Dexon, 15 g raked in ± 1 month after planting, before appl. of winter cover; 14 Dec.	66,1	75	815
4 Dexon, 20 g raked in ± 1 month after planting before appl. of winter cover; 14 Dec.	69.0	51	828
5 Dexon, 10 g in shallow furrows between plant rows; applied in Febr.	69.8	13	842
6 Untreated	60.1	302	542
7 Metham-sodium 100 ml/m² + 40% formalin 140 ml/m²; applied 6 Sept.	68.0	112	806
95% confidence interval	6.2	153	-

Table 1. Het effect van een behandeling met dimethylaminobenzeendiazo-natrium sulfonaat 70% actieve stof (Dexon) op de aantasting door wortelrot. Per behandeling 4 veldjes van 5 m², gerangschikt volgens een Youndon square; per veldje 250 bollen.

milled in + 5 g per m² raked in in the autumn (treatment 1), or 100 ml metham-sodium (6 weeks before planting) + 140 ml 40% formalin per m² given in the autumn (Fig. 10). In this experiment the worst affected plants did not show the severe wilting symptoms followed by death until the latter half of the month of July. The numbers of dead plants show tendencies not reflected in the yield because of the late development of the symptoms. The size of the harvested bulb, i.e. the growth per bulb, is just as important as the total yield. The bulbs planted in this experimental plot were considered to show adequate growth if they reached a circumference of 15 cm (size 15). From the yield calculations it is evident that all the treatments gave good control of root rot, although after application of 10 or 15 g Dexon/m² one month after planting (treatment 2 and 3) the differences from the control (treatment 6) were no longer significant.

In the autumn, immediately after the raking in of Dexon, the cover of dry reed customarily used for frost protection was spread to prevent the degradation of Dexon due to exposure to light (Hills and Leach, 1962).

For the practical aspects of the use of Dexon it is important to know whether lower doses would also provide an adequate control of root rot especially when the inoculum potential is lower than was the case in the heavily infected experimental plots. Lower doses might be expected to give good results judging from some results recently obtained with Dexon milled in for the control of root rot in iris.

Fig. 10. Two plots of an experiment on field control. Right: soil treated with metham-sodium. Left: untreated.

Fig. 10. Twee veldjes van een bestrijdingsproefveld. Rechts: de grond behandeld met metam-natrium. Links: onbehandeld.

Discussion

It may be concluded from the results of the present investigation that *Pythium* spp. and not *Fusarium culmorum* (Feekes, 1931) cause the root rot occurring generally in hyacinths. This is demonstrated by the results of inoculation experiments in artificially infected soil, in so-called containers with mist and, indirectly, in the field, by the application of the selective control substance Dexon.

Difficulties experienced with infested soil have led many investigators to study the pathogenicity of *Pythium* spp. in plants grown on other media. In many cases it has been demonstrated that various species of *Pythium* can cause disease symptoms in one kind of plant (Halpin and Hansen, 1958; Middleton, 1952; Moore and Buddin, 1937; Rangaswami, 1962; Vaartaja, 1937; Vanterpool 1938).

As compared with the laboratory methods reported in the literature, the experimental use of containers with mist seems completely acceptable for hyacinth. It is important that under these conditions *P. sylvaticum* does not cause infection. Pathogenic effects were given by *P. ultimum*, *P. violae*, and one unidentified *Pythium* isolate. *P. ultimum* is important because it is considered to be a highly pathogenic organism for many plants (Rangaswami, 1962). *P. violae* has so far only been mentioned as a parasite of violets and it is therefore noteworthy that this organism was so frequently isolated from hyacinth in the present study.

It should also be mentioned in this connection that in fields in which hyacinths became seriously diseased, iris and crocus remained healthy. In fields in which crocus

or iris were infected by *Pythium*, hyacinth did not show root rot. *Pythium* species were repeatedly isolated from the rotting roots of iris and crocus, but never *P. violae*. This suggests the possibility that this species determines the specificity of the root rot complex for hyacinth.

In the continuation of this investigation, three other isolated species recently identified for the first time require evaluation with respect to pathogenicity. An attempt must also be made to develop methods to find out whether one species is always solely responsible for the infection in the field, or whether prevailling conditions determine which species will affect the roots. Many investigators have found that it is very difficult to decide which *Pythium* species of a series of isolated species is the most pathogenic under natural conditions. The result is that for each kind of plant various investigators repeatedly indicated different *Pythium* species as the cause of root rot (Rangaswami, 1962).

The results of control experiments with Dexon have provided a valuable confirmation of the importance of *Pythium* spp. in root rot of hyacinth. Dexon deserves attention, because it is simple to apply and its selective effect may even continue beyond the year of application. It is quite possible that the cost of root rot control could be reduced by the use of Dexon.

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Samenvatting

Wortelrot van hyacint veroorzaakt door Pythium-soorten

Aangetaste hyacinteplanten hebben grijze, glazige wortels die later slap worden (Fig. 1). Van dergelijke planten is de groei geremd, terwijl op zonnige dagen hun bladeren slap hangen. In een vergevorderd stadium van de aantasting sterft het bladweefsel af. In de hyacintevelden wordt de ziekte na de bloei zichtbaar door het ontstaan van plekken met dode planten. Deze plekken kunnen klein zijn en beperkt in aantal, ze kunnen ook een groot deel van het veld beslaan.

Een aantasting van de wortels werd nooit voor de bloei waargenomen. De waarnemingen werden voor een deel uitgevoerd in een observatiekuil (Fig. 2 en 3).

Uit een groot aantal monsters aangetaste wortels verzameld op plekken met zieke planten werden altijd *Pythium*-soorten gekweekt. Van 11 monsters werd nagegaan welke soorten dit waren. Hiervan werden *Pythium ultimum*, *P. violae* en een nog niet geïdentificeerde *Pythium*-isolatie in kasproeven getoetst en pathogeen bevonden. Drie *Pythium*-soorten werden nog niet getoetst.

P. ultimum is bekend als een pathogeen organisme voor allerlei planten. *P. violae* is tot nu toe alleen genoemd als parasiet van het viooltje, het is daarom opmerkelijk dat deze schimmel pathogeen voor hyacint bleek te zijn en vaak uit aangetaste wortels werd gekweekt.

Bij het onderzoek werd met succes gebruik gemaakt van wortels van planten die wa-

ren gekweekt op kisten waarin door sproeiers water werd verneveld (Fig 4, 5 en 6) of in een watercultuur volgens de Stigter (Fig. 7 en 8).

Een toepassing van het selectief tegen Pythiaceae werkende fungicide "Dexon" gaf op 7 willekeurig gekozen proefvelden op besmette grond een duidelijk effect (Tabel 1).

Dit vormt een bevestiging voor de betekenis van *Pythium* bij het ontstaan van dit ziektebeeld. Het middel Dexon verdient voor praktische toepassing om verschillende redenen de aandacht. Uit het onderzoek moet worden geconcludeerd dat *Fusarium culmorum* niet de oorzaak van dit wortelrot is, zoals op grond van een onderzoek door Feekes (1931) algemeen werd aangenomen.

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