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Fungi on roots and stem bases of asparagus in the Netherlands: species and pathogenicity

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Abstract

A survey was made to identify the most important soilborne fungal pathogens of asparagus crops in the Netherlands. Ten plants were selected from each of five fields with a young (1–4 y) first planting, five fields with an old (6–13 y) first planting and five fields with a young replanting. The analysis included fungi present in the stem base and the roots of plants with symptoms of foot and root rot or showing growth decline without specific disease symptoms. Isolates of each species were tested for pathogenicity to asparagus on aseptically grown plantlets on Knop's agar. Symptoms were caused by *Fusarium oxysporum*, *F. culmorum*, *Botrytis cinerea*, *Penicillium verrucosum* var. *cyclopium*, *Cylindrocarpon didymum*, *Phialophora malorum*, *Phoma terrestris* and *Acremonium strictum*. *F. oxysporum* was by far the most common species and was isolated from 80% of the plants. Not all of its isolates were pathogenic to asparagus. Symptoms were caused by 67%, 78% and 93% of the isolates obtained from young first plantings, old first plantings and replantings, respectively. *F. culmorum* was isolated from 31% of the plants. Two other notorious pathogens of asparagus, *F. moniliforme* and *F. proliferatum*, did not occur in our samples.

Species causing symptoms in the vitro test that were found on more than 5% of the plants were additionally tested for their pathogenicity in pot experiments. *F. oxysporum* f.sp. *asparagi* caused severe foot and root rot, significantly reduced root weights and killed most of the plants. *F. culmorum* caused lesions on the stem base often resulting in death of the plant. *P. terrestris*, a fungus only once reported as a pathogen of asparagus, caused an extensive root rot, mainly of secondary roots that became reddish. The fungus was isolated in only a few samples and is not to be regarded as an important pathogen in Dutch asparagus crops. *P. malorum* caused many small brown lesions on the stem base and incidentally also on the upper part of small main roots. This is the first report of its pathogenicity to asparagus. The fungus is one of the organisms inciting spear 'rust' and it reduced crop quality rather than crop yield. *P. verrucosum* var. *cyclopium* and *C. didymum* did not cause symptoms in pot experiments.

Because of its predominance on plants with foot and root rot and its high virulence, *F. oxysporum* sp. *asparagi* was considered to be the main soilborne pathogen of asparagus in the Netherlands.

Introduction

Asparagus (*Asparagus officinalis* L.) is one of the main vegetable crops grown in the southeastern part of the Netherlands. The asparagus produced is almost exclusively white asparagus. In this area, asparagus has been grown for several decades and many growers do not have fresh land available.

Therefore, replanting of former asparagus fields is common practice. Plantings on former asparagus land are, however, never as profitable as plantings on fresh land. Crop establishment occurs without problems, but after a few years with normal yields growth declines. An increasing number of plants suffers from early dying and, consequently, within a few years yields decrease, quantitatively and qualitatively, to a level at which maintaining the crop is no longer profitable. The economic life of a crop on a replanted field averages half that on fresh land. Initially, the phenomenon was termed 'asparagus replant disease' [Blok and Bollen, 1993]. However, this term can be misleading because it suggests problems in crop establishment. As the disease is expressed as an early decline rather than a failure of establishment, a more appropriate term is replant-bound early decline. The disease is currently a major factor impeding the increase of the area under asparagus in the southeastern part of the Netherlands.

Problems after replanting former asparagus land are reported from many regions [Grogan and Kimble, 1959; Young, 1984; Schofield, 1991]. In most cases, it is a matter of establishing a commercially profitable stand. In almost all reports on replant problems, the phenomenon is related to an early decline of the preceding asparagus crop. Early decline is attributed to two major factors. The first one is an increased infection by soilborne pathogens, mainly *F. oxysporum* f.sp. *asparagi* and *F. moniliforme*. The other one is growth inhibition by autotoxic compounds from roots and root residues of the standing crop [Schofield, 1991]. Most authors do not discriminate between early decline and replant problems. For the Dutch situation, it is relevant to distinguish between early decline and replant-bound early decline.

In order to develop methods to control replant-bound early decline, a project was started to identify the causal factors. Growth inhibition of asparagus by autotoxins from root residues of a previous asparagus crop was shown to be not a major factor in the etiology of replant-bound early decline [Blok and Bollen, 1993]. The aim of the present study is to identify the soilborne fungal pathogens in Dutch asparagus fields. We focused on fungal pathogens because several soilborne fungi are known to cause considerable losses in asparagus [Schofield, 1990], whereas nematode damage or symptoms of bacterial diseases were not yet observed. An analysis was made of fungi present in diseased stem bases and roots. Their pathogenicity was tested under *in vitro* conditions and in the greenhouse.

Materials and methods

Analysis of fungi present in stem base and root tissue

Collection of plant material. The area under asparagus production is mainly concentrated in the northern part of the province of Limburg. In this area, 15 representative fields were selected. On all fields white asparagus was produced. The size of the fields ranged between 0.5–1.5 ha. In september 1988, plant material was collected from five asparagus fields with an old (6–13 years) first planting, five fields with a young (1–4 years) first planting and five fields with a young replanting (Table 1). On each field, ten plants showing foot and root rot symptoms (yellowing, wilting or dying of stems) or growth decline without specific disease symptoms were selected. The plants were randomly selected from the whole field. From each of the plants a 10-cm piece from the subsoil part of two stems and a 4–8 cm piece of each of three storage roots were collected. The samples were stored in plastic bags at 4 °C. Isolations were made within two weeks.

Isolation of fungi. The stem and root pieces were thoroughly washed under running tap water. At both ends of each piece 1–2 cm was discarded to get rid of most of the micro-organisms which had invaded the tissue after collection. The remaining parts were externally disinfested in 2% sodium hypochlorite (20% household bleach) for 2 min and rinsed three times in sterile distilled water. Tissue pieces from the border zone between healthy and diseased tissue were plated onto two media. Mycophil agar (BBL, pH 4.7) with 50 μg ml^{-1} oxytetracycline was used to isolate non-oomycetous fungi and wateragar (Oxoid, 1.5%) with 100 μg ml^{-1} pimaricin was used to isolate oomycetous fungi. Fungi growing from the tissue pieces were subcultured to potato dextrose agar (PDA, Merck) slants, allowed to grow for one

Table 1. Description of the asparagus fields from which plant material was sampled. On all fields white asparagus was produced. Samples were taken in 1988

Field no.	Location	Asparagus history	Year of planting	Category[1]	Cultivar
1	Meterik	First planting	1975	1	Limbras-26
2	America	" "	1979	1	Limbras-26
3	Heythuysen	" "	1979	1	Lucullus
4	Helden	" "	1981	1	Venlim
5	Helden	" "	1982	1	Franklim
6	Castenray	" "	1984	2	Gynlim
7	Helden	" "	1985	2	Gynlim
8	Meterik	" "	1986	2	various
9	Grubbenvorst	" "	1987	2	Boonlim
10	Panningen	" "	1987	2	Gynlim
11	Grubbenvorst	Second planting	1982	3	Limbras-18
12	Grubbenvorst	" "	1983	3	Limbras-26
13	Castenray	" "	1984	3	Gynlim
14	Meterik	" "	1984	3	Boonlim
15	Helden	" "	1988	3	Boonlim

[1] Category 1: young (1–4 years), first planting; category 2: old (6–13 years), first planting; category 3: young (1–6 years), second planting.

week and stored at 4 °C for later identification and pathogenicity testing.

One of the species that were pathogenic to asparagus could initially not be identified because none of its isolates sporulated. Colony morphology and reddish discolouration of asparagus roots led us to the identification of the isolates as *Phoma terrestris* (syn. *Pyrenochaeta terrestris*). Fifteen isolates, including two well-sporulating reference strains of *P. terrestris* from *Calathea crocata* and rice (*Oryza sativa*) that were kindly provided by Mr. J. de Gruyter, Plant Protection Service, Wageningen, the Netherlands, were grown on five media. The media were: (1) malt extract agar (Oxoid), (2) oatmeal agar with sterilized lupin stem pieces, (3) oatmeal agar with sterilized grass leaves, (4) a basal mineral salts agar (3 g $NaNO_3$, 1 g $MgSo_4$. $7H_2O$, 20 g agar, 1 l dist. water) with sterilized chopped wheat straw sprinkled on the agar just before solidification [Watson, 1961], and (5) the same medium as (4) but with terile lupin stem pieces. The plates were incuated at 28 °C in the dark for one week and then laced under near-UV light.

For identification of *Fusarium* spp. the system f Nelson et al. [1983] was followed. Most of the ther fungi were identified using the keys of omsch et al. [1980].

In vitro *pathogenicity test*

The first step in the assessment of pathogenicity to asparagus was a test on plants grown under aseptic conditions in culture tubes according to Stephens and Elmer [1988] with a few modifications. Seeds of asparagus cv. Gynlim were surface-disinfested in 2% sodium hypochlorite for 30 min, rinsed in sterile distilled water, placed in an Erlenmeyer flask with 50 ml aceton plus 1.25 g benomyl (2.5 g Benlate 50 WP) and shaken in an orbital shaker at 20 °C for 20–24 h. To remove the fungicide, seeds were washed twice in aceton and three times in sterile distilled water. Subsequently, the seeds were placed under sterile conditions on moist filter paper in glass Petri dishes sealed with parafilm. After 5–7 days the seeds had germinated and single seeds with radicles of 1–5 mm were aseptically placed in culture tubes (height 150 mm, diam. 22 mm) containing 12 ml sterile Knop's solution plus 0.4% agar. The tubes were placed in a growth chamber at 20 °C and 16-h light period. After 10–20 days, the seedlings were inoculated by placing a PDA-plug with growing mycelium at the stem base of the seedling. Three seedlings were inoculated with each isolate. After an incubation period of 4–5 weeks in the growth chamber, the seedlings were examined for the presence of symptoms on roots or stem bases. An

isolate was rated as pathogenic when it caused lesions on one or more seedlings.

Fusarium oxysporum includes numerous formae speciales defined by their ability to cause disease symptoms on one or more host species. *Fusarium oxysporum* (Schlecht) emend. Snyd. & Hans. f.sp. *asparagi* Cohen is defined by its ability to cause disease symptoms on *Asparagus* spp. but not on many other plant species. To estimate the relative frequency of f.sp. *asparagi* within the population of *Fusarium oxysporum* isolates obtained, one isolate of *F. oxysporum* from each plant from which this species was obtained, was tested *in vitro* for pathogenicity on asparagus. When typical symptoms were caused, the isolate was regarded as a member of f.sp. *asparagi*. When *F. oxysporum* was isolated from roots and stems as well, an isolate obtained from the root was tested. For other fungal species isolated from more than 5% of the plants three isolates for each category of fields were included, so a total of nine isolates was tested. For the remaining species one isolate was tested.

Greenhouse pathogenicity test

Fungal isolates. Species which caused severe symptoms in the *in vitro* test and which were isolated from more than 5% of the plants were further tested for their pathogenicity in pot experiments under greenhouse conditions. The species were *F. oxysporum, F. culmorum, Phoma terrestris* (syn. *Pyrenochaeta terrestris*), *Phialophora malorum, Penicillium verrucosum* var. *cyclopium* and *Cylindrocarpon didymum.* Two isolates of each species were tested. An isolate of a *Rhizoctonia* species obtained from large, irregular, sunken lesions on harvested asparagus spears was included too. Of all isolates single-spore cultures were produced, except for *P. terrestris* and *Rhizoctonia* sp. of which hyphal-tip cultures were produced.

Plant material. Seeds of asparagus cv. Gynlim were surface-disinfested as described before and sown in flats with a commercial potting mixture (Trio-17; pH-KCl 5.4; org. matter content 63%). The plants were raised at 21–25 °C. After seven weeks they were carefully uprooted and the roots were washed free of soil. After selection for health and uniformity, the plants were used in the experiments.

Inoculum production and inoculation procedures. Pathogenicity was tested with two different procedures, one in which the roots of the plants were dipped in a suspension of spores or mycelial fragments (root-dip procedure) and one in which plants were planted in soil infested with a soil-meal inoculum (soil-infestation procedure).

For the root-dip procedure inoculum was produced in Erlenmeyer flasks with 100 ml malt extract broth (Oxoid). The flasks were incubated at 23 °C in an orbital shaker for 17 days. Conidial suspensions were prepared by filtering the cultures through glass wool. The suspensions were washed by centrifugation and adjusted to 1×10^6 microconidia ml^{-1}. Cultures of fungi that did not sporulate (*P. terrestris* and *Rhizoctonia* sp.) or showed poor sporulation (*C. didymum*) were chopped in a blender (Ultraturrax, 30 sec at 4000 rpm) and washed by centrifugation (30 min at 3400 g). Plants were inoculated by immersing their roots and stem bases in suspensions of conidia or mycelial fragments during 20 min. Thereafter, plants were planted in 1.5-l pots filled with autoclaved soil (loamy sand, pH-KCl 7.7, organic matter content 3.3%). For each treatment seven pots with two plants each were used. A treatment in which plant roots were immersed in tap water served as a control.

For the soil-infestation procedure, inoculum was produced in a soil-meal medium. Potting mixture (Trio-17) was amended with 15% (d.w./d.w.) oatmeal. Tap water was added to a pF-value of 1.8. Erlenmeyer flasks of 500 ml with 200 ml of the soil-oatmeal mixture were autoclaved for 30 min on two consecutive days. The flasks were inoculated with two PDA-plugs with growing mycelium and incubated at 25 °C for three weeks. Then the culture was mixed thoroughly with autoclaved soil at a rate of 0.75% (f.w./f.w.). The soil was the same as used for the root-dip procedure. For each treatment seven 1.5-l pots were filled and planted with two plants per pot. A treatment in which autoclaved soil-meal inoculum of one of the two isolates of *F. oxysporum* was mixed through soil, served as a control.

In both experiments, pots were placed on dishes and were watered carefully to prevent cross-infec-

tions. The pots were arranged in a randomised block design with seven blocks. The experiments were carried out in two separate greenhouse compartments at 20–25 °C. After 12 weeks observations were made on the presence of symptoms. When symptoms were present, pieces of tissue with typical symptoms were sampled. After external disinfestation (1 min in 2% sodium hypochlorite) the pieces were plated on PDA with 50 ppm vendarcine and outgrowing fungi were identified. Dry weights of roots and ferns were determined after 24 h drying at 105 °C.

Statistical analysis

Relative frequencies of isolation were calculated as fractions of plants from which a species was isolated. These fractions and the fraction of pathogenic isolates of *F. oxysporum*, were transformed (arcsin √fraction) and tested for normality and for homogeneity of variances between treatments before subjection to ANOVA. Significance of differences for isolation frequencies and for fraction pathogenic isolates was tested with the Studentized range test of Tukey. Root weight data obtained in the greenhouse tests were subjected to ANOVA without transformation. Significance of differences between the inoculated treatments and the noninoculated control treatment was tested with Dunnett's procedure [Steel and Torrie, 1980].

Results

Oomycetous fungi did not appear on the media. On Mycophil agar many fungi were isolated. The species and the isolation frequencies for the most common species are given in Tables 2, 3 and 4. *F. oxysporum* was the most common fungus followed by *F. culmorum*. Isolation frequencies for the six most common species were not significantly different for the three categories of fields except for *P. malorum*.

The two reference strains of *P. terrestris* readily formed pycnidia that appeared in large numbers on all five media. Four out of the 13 isolates from asparagus formed a few sporulating pycnidia on wheat straw (medium No 4) within 4 weeks. In this way, identification as *P. terrestris* was confirmed. After 4 months, an additional six isolates had formed pycnidia without conidia on wheat straw.

The species which caused symptoms in the *in vitro* test are mentioned in Table 5. Most species did not cause any disease symptoms in the *in vitro* test. The percentages of isolates of *F. oxysporum* that were pathogenic on asparagus were 67, 78 and 93% for category 1, 2 and 3, respectively. These percentages were not significantly different according to the Studentized range test of Tukey ($P \leq 0.05$).

In the greenhouse test symptoms were caused

Table 2. Frequency of isolation of fungi from stem bases or storage roots of asparagus plants showing symptoms of foot and root rot or showing growth decline. The species mentioned here were isolated from more than 10% of the total number of plants sampled (150)

Fungi	First planting, 1–4 y old (Category 1)		First planting, 6–13 y old (Category 2)		Second planting, 1–6 y old (Category 3)	
	# Fields[1]	Isol.[2] freq.	# Fields	Isol. freq.	# Fields	Isol. freq.
Fusarium oxysporum	5	0.78 a[3]	5	0.82 a	5	0.80 a
F. culmorum	4	0.38 ab	4	0.33 bc	4	0.45 ab
Botrytis cinerea	4	0.35 b	4	0.30 bc	4	0.18 b
Penicillium verrucosum var. *cyclopium*	4	0.20 b	3	0.50 b	3	0.27 b
Cylindrocarpon didymum	1	0.40 b	4	0.18 c	4	0.28 b
Phialophora malorum	4	0.33 b x	5	0.16 c y	3	0.13 b y

[1] Number of fields from which the species were isolated (maximum is 5).
[2] Isolation frequency = Mean fraction of plants from which the fungus was obtained (this mean was calculated with the data of those fields only from which the fungus was isolated).
[3] Means without a letter in common are significantly different at $P \leq 0.05$ according to Studentized range test of Tukey. Letters a,b and c are used for comparisons within columns, letters x and y are used for comparisons within rows, but only for *P. malorum* as frequencies for the other species did not differ significantly between categories of fields.

Table 3. Frequency of isolation from root and stem base tissue for the six most common fungi. For both substrate types, the fraction of the total number of plants (n = 150) from which the fungus was isolated, is given

	Root	Stem
Fusarium oxysporum	0.56	0.61
F. culmorum	0.11	0.16
Botrytis cinerea	0.15	0.07
P. verrucosum var. *cyclopium*	0.13	0.13
Cylindrocarpon didymum	0.14	0.01
Phialophora malorum	0.08	0.11

Table 4. Fungi isolated from stem base or storage root tissue of asparagus plants showing symptoms of foot and root rot or showing growth decline. The species listed here were isolated from less than 10% of the total number of plants sampled (150)

Acremonium strictum	*Paecilomyces marquandi*
Alternaria alternata	*Penicillium* sp. II
A. tenuissima	*Penicillium* sp. III
Aureobasidium pullulans	*Phoma leveillei*
Cylindrocarpon destructans	*P. terrestris*
C. olidum	*Phoma* sp.
Exophiala sp.	*Rhizoctonia solani*
Fusarium equiseti	*Sesquicillium candelabrum*
Geotrichum sp.	*Trichoderma harzianum*
Gliocladium catenulatum	*Trichosporiella cerebriformis*
G. roseum	*Ulocladium atrum*
G. virens	*Verticillium tricorpus*
Humicola fuscoatra	*Volutella ciliata*
Mucor racemosus	*Zygorrhynchus moelleri*
M. hiemalis	Sterile mycelia

by *F. oxysporum*, *F. culmorum*, *P. malorum* and *P. terrestris*. These fungi could be re-isolated from diseased tissue. *C. didymum*, *Penicillium* sp. and *Rhizoctonia* sp. did not cause any disease symptom.

F. oxysporum caused brown lesions on roots and stem bases, resulting in dying off of stems and roots, and significantly lower root weights (Table 6). When stem bases were heavily attacked in an early stage, the plants died. The attack was more severe with the soil-infestation procedure than with the root-dip procedure.

F. culmorum caused mainly stem base lesions resulting in stem death. Inoculation by the root-dip procedure resulted in a heavy attack of the stem base in an early stage and death of most plantlets. With the soil-infestation procedure, the lesions on the stems developed less rapidly and all plants, except one, survived, but root weights were significantly lower than those of the control plants (Table 6). On most plants, a few brown lesions were formed on the roots.

P. malorum caused many small, elongated, brown lesions on the stem base. Root lesions were formed only incidentally on the upper part of small main roots (Fig. 1).

P. terrestris caused a root rot, mainly of the

Table 5. Fungi causing disease symptoms on asparagus plantlets in the pathogenicity test *in vitro*

Fungal species	# Isolates tested	# Pathogenic isolates	Description of symptoms
F. oxysporum	120	95	Many brown oval lesions on roots, sometimes also on stem bases. Most plantlets died.
F. culmorum	9	9	Most plantlets died rapidly, with or without formation of brown oval lesions on roots and stem bases.
B. cinerea	9	6	A small number of lesions on the stem base. Sometimes small root lesions were formed. Plantlets were not killed.
P. verrucosum var. *cyclopium*	9	9	Rot of upper part of main root. Browning of root system. A few plantlets died.
C. didymum	9	7	Browning of roots and inhibition of root growth. No lesions formed, no plantlets died.
P. malorum	9	9	Whole root system and stem base covered with small, elongated brown lesions. Secondary root growth inhibited. Plantlets were not killed.
P. terrestris	3	3	Strong inhibition of root growth. Many elongated brown lesions formed on roots and stem bases. Large parts of the root system were discoloured red.
A. strictum	1	1	Many elongated brown lesions on the roots. None of the plantlets died.

Table 6. Survival and root dry weight of asparagus plants inoculated with seven soil fungi using two procedures of inoculation

Inoculum	Isolate	Soil infestation		Root dipping	
		Surviving plants[1]	Root dry weight[2]	Surviving plants[1]	Root dry weight[2]
None		2.0	9.3	2.0	8.7
F. oxysporum	CWB 1	1.4	1.0 *	2.0	3.5 *
	CWB 6	1.9	2.0 *	1.6	3.4 *
F. culmorum	CWB 500	2.0	4.3 *	0.1	0.9 *
	CWB 501	1.9	4.2 *	0.9	1.6 *
P. verrucosum	CWB 515	2.0	9.7	2.0	10.1
var. *cyclopium*	CWB 516	2.0	10.3	2.0	10.1
C. didymum	CWB 510	2.0	7.6	2.0	11.0
	CWB 511	2.0	9.4	2.0	9.8
P. malorum	CWB 505	2.0	9.7	2.0	10.9
	CWB 506	2.0	9.8	2.0	10.1
P. terrestris	CWB 520	2.0	7.4 *	2.0	8.4
	CWB 521	2.0	6.9 *	2.0	7.2
Rhizoctonia sp.	CWB 530	2.0	10.1	2.0	8.7
LSD (Dunnett, P = 0.05)			1.7		3.0

[1] Mean number of surviving plants per pot (per pot two plants were planted).
[2] Mean total root dry weight per pot (g). Means followed by an asterisk differ significantly from the control according to the test of Dunnett ($P \leq 0.05$).

secondary roots. The main roots showed brown to redbrown irregular, superficial lesions and the secondary roots became reddish (Fig. 1). The discolouration of the roots was far more extensive with the soil-infestation procedure than with the root-dip procedure.

Discussion

Among the many fungi isolated from diseased stem base and root tissue, only eight species were pathogenic to asparagus. Apparently, most of the isolates had colonized the diseased tissue without causing symptoms.

Two *Fusarium* species which are notorious pathogens of asparagus, *F. moniliforme* and *F. proliferatum*, were not isolated from the plants in our survey. *F. moniliforme* is associated with asparagus decline in many regions [Johnston et al., 1979; Fantino, 1990]. *F. proliferatum* was recently described as a causal agent of crown and root rot of asparagus [Elmer, 1990]. The latter species was often isolated from carnations in Dutch greenhouses [Aloi and Baayen, 1993]. The temperature requirements of *F. proliferatum* may explain why it was not isolated from asparagus fields. Another explanation can be that isolates of *F. proliferatum* from carnation are not pathogenic to asparagus. *F. proliferatum* was isolated only once from asparagus by Mrs. Veenbaas-Rijks of the Plant Protection Service, Wageningen, the Netherlands. This isolate was not pathogenic to asparagus in our tests. *F. moniliforme* and *F. proliferatum* had not been recorded in an earlier survey for fungi on stem bases of plants with foot rot symptoms in the Netherlands [Van Bakel and Kerstens, 1970].

Among the eight pathogens, *F. oxysporum* f.sp. *asparagi* was by far the most important one because of its high frequency of isolation and its virulence. This applied to plantings on fresh soil as well as to replantings. *F. oxysporum* f.sp. *asparagi* was reported worldwide as the cause of foot and root rot and as one of the main factors in asparagus decline [Schofield, 1991]. *F. culmorum* is known as the cause of foot rot and is also mentioned in relation to asparagus decline in European countries [Weise, 1939; Gindrat et al., 1984; Fantino, 1990]. Both pathogens have earlier been reported from asparagus crops in the Netherlands [Van Bakel and Kerstens, 1970; Van Bakel and Krom-Kerstens, 1974]. In the greenhouse experi-

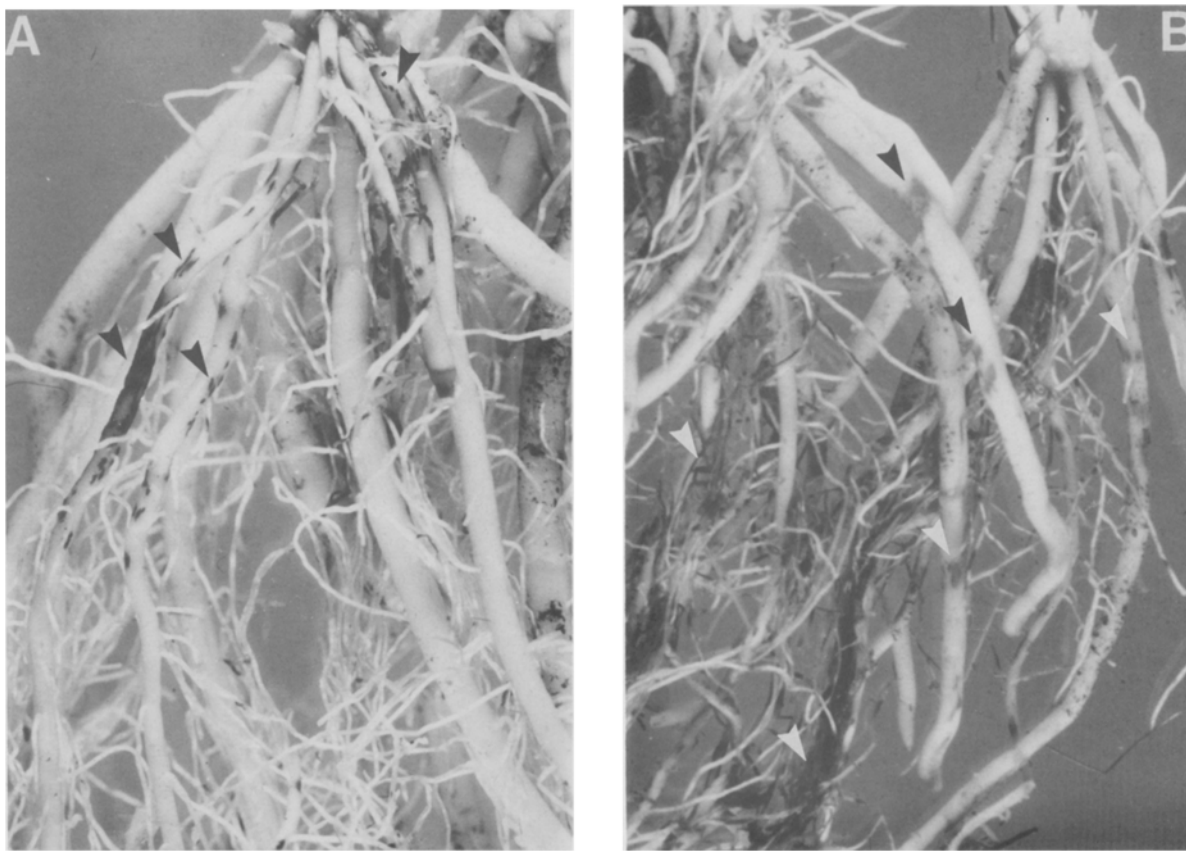

Fig. 1. Symptoms on the roots of asparagus plants caused by *Phialophora malorum* (A) and *Phoma terrestris* (B). *P. malorum* caused similar lesions on the stem base. Arrows show specific symptoms (see text).

ments, *F. oxysporum* f.sp. *asparagi* attacked both roots and stem bases, whereas *F. culmorum* attacked mainly the stems and only rarely caused lesions on the roots. This corresponds with the descriptions of the symptoms in the literature [Weise, 1939]. Its presence in roots and stem bases in similar frequencies (Table 3) is worth mentioning.

The plantlets used for the pathogenicity tests had only thin stems. *F. culmorum*, being a parasite on the stem base, rapidly killed the plantlets. Therefore, these tests probably overestimate its pathogenicity. Under field conditions normally only one to a few stems per plant are attacked by *F. culmorum* and damage remains limited. On the other hand, *F. oxysporum* f.sp. *asparagi* attacks both stems and roots which weakens the plant more strongly and can lead to plant death, especially under stress conditions.

P. terrestris is well-known as the cause of pink root of *Allium* spp. [Punithalingam and Holliday, 1973] and root rot of maize in warmer regions [Rouhani et al., 1979; Campbell et al., 1991]. From its low frequency of isolation it is concluded that it is of minor importance as a pathogen under the cool conditions of the Netherlands. Its pathogenicity was clearly demonstrated in the greenhouse test (20–25 °C), where it caused a severe root rot. The typical reddish discolouration of the secondary roots was also observed in plants grown in former asparagus soil. From these roots the fungus could readily be isolated. The low frequency of its isolation in our analysis is most probably due to its high temperature requirements, the optimum temperature for growth and disease expression being at 28 °C [Punithalingam and Holliday, 1973]. The fungus was only rarely recorded on asparagus. Grogan and Kimble [1959]

tested 20 isolates of *P. terrestris* on asparagus and found that all of them induced reddish discolourations of roots. The fungus was also isolated from asparagus roots by Messiaen and Lafon [1970], but these authors did not provide data on its pathogenicity. The low number of records suggests that the fungus is not widespread in asparagus-growing regions. It might be that it is overlooked on isolation plates, as it is easily overgrown because of its low growth rate. Moreover, the fungus is not easily recognized because of poor sporulation of most isolates.

The pathogenicity of *P. malorum* was clearly demonstrated in the greenhouse test. The fungus is known as a causal agent of storage rot of apple and pear [Sugar and Spotts, 1992]. To the best of our knowledge, this is the first account of its pathogenicity to asparagus. The numerous small lesions on the stem bases and, to a far less extent, on the roots did not result in lower root weights. In the field, significant growth inhibition by this pathogen is not to be expected but the lesions on the spears severely reduce the quality of the product. In asparagus production, spears covered with brown, small lesions are often harvested and the disease is called 'rust' or 'physiological rust' [Blanchard and Faure, 1988]. *P. malorum* was, together with other fungi, e.g. *Fusarium oxysporum* and *C. didymum*, isolated from 'rusty' spears.

P. verrucosum var. *cyclopium*, *C. didymum* and *A. strictum* caused symptoms under *in vitro* conditions only and were, therefore, not regarded as true pathogens. *P. verrucosum* var. *cyclopium* is a synonym of *P. martensii* [Samson et al., 1976]. The latter species was described by Menzies [1955] as the cause of crown rot of injured asparagus seedlings. This symptom did not appear in our inoculation experiments, which is in line with Menzies' observation that inoculation was only followed by infection after wounding the plants. In previous trials we isolated the fungus from rotten primary roots of asparagus seedlings. Another variety of the same species, var. *corymbiferum*, was mentioned by Gordon-Lennox and Gindrat [1987] as being highly pathogenic to aseptically grown plantlets. *C. didymum* was reported by Brayford [1987] as a weak pathogen of asparagus but details on symptoms were not given.

Notable is the high frequency of isolation of *B. cinerea*. This fungus infects shoot tissue and, if not controlled, can cause considerable losses by inciting premature shoot dying. In our samples it was frequently isolated from stem bases and roots.

In conclusion, *F. oxysporum* f.sp. *asparagi* is the most important soilborne pathogen of asparagus in the Netherlands because of its predominance on plants with foot and root rot symptoms and its high virulence. *F. culmorum* is less frequently isolated and under field conditions less virulent than *F. oxysporum* f.sp. *asparagi*. The other pathogenic species do not cause significant growth inhibition. They can, however, cause a decrease in quality of harvested spears. The role of soilborne pathogens in the cause of replant-bound early decline will be dealt with in a next publication.

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