MEDEDELING 58

footrot in asparagus caused by fusarium oxysporum f. sp. asparagi

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top wilting in asparagus

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PROEFSTATION VOOR DE GROENTETEELT IN DE VOLLEGROND IN NEDERLAND

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Footrot in asparagus caused by *Fusarium oxysporum* f. sp. *asparagi*

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Abstract

In the asparagus crop at least four soil-borne diseases can be distinguished. Footrot is one which appears to be caused by *Fusarium oxysporum* f. sp. *asparagi* and is characterized by brown oval lesions on the lower parts of stems. A method is described for testing for pathogenicity the species of *Fusarium* and other fungi isolated from diseased plants. A negative correlation was found between the number of *F. oxysporum* f. sp. *asparagi* isolates and the 'G-value' which provides an indication of the development of an asparagus crop.

Introduction

The area, where most of the asparagus crop (*Asparagus officinalis* L.) is grown is situated in the two southern provinces of the Netherlands, 74 % of the total area of about 3400 ha being located in Limburg. Although the crop is a profitable one, the area under asparagus decreased from 5000 ha in 1963 to the present value of 3400 ha. Soil sickness and footrot are among the causes of this decrease. Because of soil sickness, soils suitable for growing asparagus cannot be used again; footrot is held responsible for an abnormally fast decline in plant growth and stem thickness. Farmers consider practically every deviation of the normal growth footrot, therefore it was necessary to make an inventory of the various symptoms that occur in asparagus plants. On these observations the following four soil-borne diseases could be easily separated:

1. Yellowing of stems, combined with light red-coloured lesions on the green parts of the stem.

On the stems brown lesions which later become light red-coloured are visible mostly at soil level. Infected stems become soft and show a reddish discolouration when cut longitudinally. The part of the stem above the lesion turns yellow and dies off. These symptoms are associated with *Fusarium culmorum* (W.G. Sm.) Sacc. (Weise, 1939).

2. Decline of plant growth combined with small lesions on the stem base. We call these symptoms footrot.

On the base of the stem and on the fleshy roots brown oval-shaped lesions are visible (Fig. 1). Sometimes the plant shows a severe decline. This kind of symptoms is ascribed to *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen f. sp. *asparagi* Cohen by Cohen and Heald (1941).

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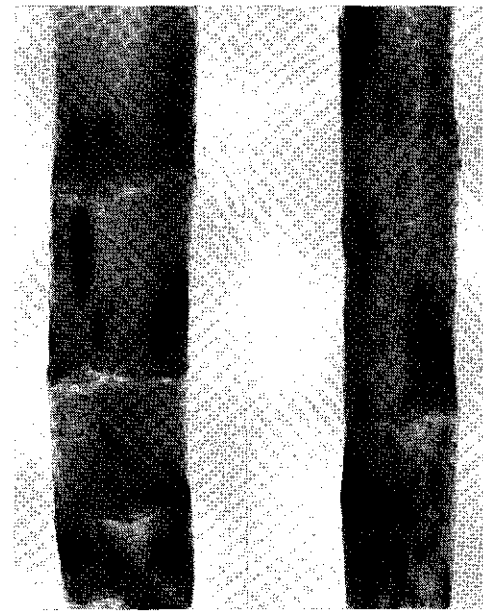

Fig. 1. Footrot symptoms in asparagus.
Fig. 1. Voetziektesymptomen in asperge.

3. Wilting and dying off of young shoots.

In summer newly developed shoots show a severe wilting and mostly die off. These symptoms seemed to be due to unfavourable water conditions in the soil.

4. A strong decline in plant growth when fields are replanted with asparagus.

This decline appears after replanting asparagus on a field which had asparagus before and is called soil sickness. The time elapsing between the two plantings is unimportant. The first symptoms consisting of a strong reduction in growth combined with a decrease in stem diameter usually appear in the third year after replanting. The root system is poorly developed and has many dead secondary roots. The reduction in growth is often so strong that continued culture of the crop is no longer economic. The cause of this so-called soil sickness is not yet known, but depth of rooting, exhaustion of minor elements of the soil, soil structure, and fungi (*Fusarium* spp.) or other factors may be implicated.

A few fungi other than *Fusarium oxysporum* f. sp. *asparagi* are held responsible for rootrot or footrot diseases in asparagus. These fungi are *Phytophthora* sp. (Ark and Barrett, 1938), *Fusarium culmorum* (Weise, 1939), *Rhizoctonia violacea* (Berville and Lavy, 1962, Molot et Simone, 1965), *Rhizoctonia* sp. and *Zopfia* sp. (Wiemer, 1940), *Penicillium martensii* (Menzies, 1955) and a *Sclerotium* sp. (Suzui and Abumiya, 1963). However, none of these fungi caused the typical footrot symptoms described above. In 1941, Cohen and Heald described a disease with symptoms typical of footrot. The causal organism appeared to be *Fusarium oxysporum*. According to the taxonomy of Snyder and Hansen (1940) the fungus was called *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen f. sp. *asparagi* Cohen (1946). The relation between this fungus and footrot of asparagus has also been suggested by van der Vliet (1955), Kempenaers (1961), Grogan and Kimble (1959), van den Broeck (1963), van Assche and van den Broeck (1964) and Lewis and Shoemaker (1964). It is not always clear, however if a separation has been made between infection by *Fusarium oxysporum* f. sp.

asparagi and *F. culmorum*. The purpose of our investigations was to attempt to gain a better insight into the footrot complex, to identify the fungi involved and to test these fungi for pathogenicity to asparagus.

Material and methods

From 1966 to 1968 stem samples were taken from some fields in the autumn. As the plants in the various plots varied in appearance, it was necessary to measure these differences, since there might be a correlation between plant development and footrot. At the time of sampling it was expressed in a figure by taking the average of the squares of the diameters of stems being over 10 mm (a); the percentage of stems with diameters over 10 mm (b); and the percentage of dead plants (c). These figures were obtained from plants, growing in 5-meter rows in plots in which open places marked dead plants. From every field two plots were measured. The figures a and b were added and c was subtracted from the total. This led to figures ranging from 125 (from plants in a very bad condition) to about 400 (from very well developed plants). This figure was called the 'G-value'.

For the isolation of fungi, six mature green stems per plot were used. These stems were thoroughly washed and the basal 6 cm was taken. The stems were put in a mixer with 200 ml of water. The macerated material was passed through a nylon sieve, mesh width 1 mm. The fluid was diluted with tap water (1:1000) and 0,5 ml was then placed in a Petri dish with 10 ml Martin's agar. The fungi which developed were subcultured on potato-dextrose agar and identified.

Tests for pathogenicity to asparagus were carried out in Erlenmeyer flaska with seedlings grown under aseptic conditions. Erlenmeyer flasks of 500 ml were filled with 250 ml perlite, sterilized and subsequently moistened with about 150 ml Knop's solution. Five sterile germinated asparagus seeds were placed in each Erlenmeyer flask. The flasks were closed with a cork and placed between TL tubes (65W 57RS), daylength 12 hours. When the seedlings were about 10 cm high, usually after about one week, the plants were inoculated with mycelium of the fungal isolate to be tested for pathogenicity by placing mycelium against the stem of the seedling. Only one isolate was tested in each Erlenmeyer flaks. The inoculated plants were examined after three to four weeks and from the plants which showed typical brown oval-shaped lesions on the stem, re-isolations were made. Sometimes an isolate was tested in a second experiment.

Results

In 1966 samples were taken from 20 asparagus fields on which footrot symptoms occured. In this year isolations were made only from stems with footrot symptoms. The isolated *Fusarium* spp. were identified according to the system of Snyder and Hansen. Most isolations were referred to the species *Fusarium oxysporum*. From the 200 *Fusarium* spp. isolates examined 196 were *Fusarium oxysporum*, 1 belonged to *F. culmorum* while 3 other *Fusarium* isolates were not identified. In the pathogenicity tests only 30 % of the tested 196 isolates of *Fusarium* were pathogenic and produced footrot symptoms as on the stems of the plants in the field. After re-isolation from the test plant all the organisms appeared to belong to the species *Fusarium oxysporum*

again. These *Fusarium* isolates (still 196) produced after testing for pathogenicity again footrot symptoms, like on the testplants in the first test and on the stems of the plants in the field. We have interpreted these isolates as *Fusarium oxysporum* f. sp. *asparagi*.

To study the influence of other fungi that could be isolated from asparagus stems, samples were taken from 80 fields in 1967 and most of the fungi in these samples were isolated and identified. Of 150 isolations of *Fusarium* spp. 146 belonged to *Fusarium* oxysporum, 2 to *F. solani* and 2 unidentified species. In the pathogenicity tests with the *Fusarium* spp. and with ten representatives of each fungus isolated, it appeared again that only 30 % of the isolates of *Fusarium oxysporum* were pathogenic but none of the other isolates caused a disease.

When the material was sampled, the impression was obtained that a negative correlation existed between the number of *Fusarium* spp. and the G–value of a crop. So in 1968 isolates were made from stems samples from crops with different G-values, but all growing on the same type of sandy soil, a so-called 'vorstvaag' soil near Grubbenvorst. From 56 fields samples were taken, fungi were isolated and identified. The results are also given in Table 1. From the genus *Fusarium* 316 isolates belonged to *F. oxysporum*, 5 to *F. solani* and 1 unidentified species. In the pathogenicity test, again

Table 1. Fungi isolated from asparagus stems

Alternaria sp.	*M. spinosus* van Tieghem
Areobasidium pullulans (de Bary) Arnaud	*Paecilomyces fumoso-roseus* (Wizw) Brown & Smith
Aureobasidium sp.	*P. victoriae* (Szilvinyi) Brown & Smith
Cephalosporium acremonium Corda	*Penicillium brevicompactum* Dierckx
C. asperum E. March.	*P. corylophilum* Dierckx
Cephalosporium sp.	*P. frequentans* Westling
Chaetomium olivaceum Cooke & Ellis	*P. funiculosum* Thom.
Chrysosporium pannorum (Link) Hughes	*P. notatum* Westling
Cladosporium cladosporioides (Fres.) de Vries	*P. waksmanii* Zaleski
C. herbarum (Pers.) Link ex Fr.	*Penicillium* sp.
Fusarium oxysporum Schlecht. emend. Snyder & Hansen	*Phialophora* sp.
F. solani (Mart.) Appel & Woll. emend. Snyder & Hansen	*Phoma* sp.
Gliocladium catenulatum Gilman & Abbott	*Pyrenochaeta decipiens* March.
Gliomastix sp.	*Rhizopus nigricans* Ehrenb.
Humicola fuscoatra Traaen	*Trichoderma hamatum* (Bon.) Bain.
Margarinomyces sp.	*T. polysporum* (Link ex Pers.) Rifai
Monilia grisea Daszewska	*T. viride* Pers. ex Fr.
Mucor fragilis Bain.	*Verticillium albo-atrum* Reinke & Berthold
M. racemosus Fres.	*Verticillium* sp.
	Volutella roseola Cooke

Tabel 1. Schimmels geïsoleerd uit aspergestengels

only 30 % of the *Fusarium oxysporum* isolates appeared to be pathogenic. The relation between the number of pathogenic and non-pathogenic isolates of *Fusarium oxysporum* and the G-value of a field is shown in Table 2. From this table it is clear, that *Fusarium oxysporum* f. sp. *asparagi* occurs more frequently in fields with a G-value lower than 250 than in fields with a high G-value. It is also clear that when sampled at random in the area where asparagus is grown, about 30 % of the *Fusarium oxysporum* isolates is pathogenic.

Table 2. Relation between the G-value and the occurrence of *Fusarium oxysporum* f. sp. *asparagi*

G-value	Number of fields sampled	Number of *Fusarium oxysporum* isolates	Number of *Fusarium oxysporum* f. sp. *asparagi* isolates	Percentage of *F. oxysporum* f. sp. *asparagi* among the total isolates
250	40	237	90	26
250–300	11	69	14	5
300	5	4	0	0
Total	56	310	104	29

Tabel 2. Verband tussen de G-waarde en het voorkomen van Fusarium oxysporum f. sp. asparagi

Discussion

In the asparagus area at least four different soil-borne diseases can be distinguished, all causing stunting, yellowing and sometimes dying off of the asparagus plants. One of these, which is called footrot, appears to be due to infection by *Fusarium oxysporum* f. sp. *asparagi*.

From all the samples taken, *F. oxysporum* was shown to be widespread in the area where asparagus is grown. Of the *F. oxysporum* isolates about 30 % belongs to the pathogenic form *asparagi*. Other fungi, also isolated from asparagus stems, appeared to be non-pathogenic to asparagus seedlings.

Penicillium martensii was not isolated, although Menzies (1955) mentions this fungus as a causal organism of a rot of asparagus rootstocks in Australia.

In order to test all the fungi isolated, use was made of a rather simple method under standardized conditions. However, other relationships may exist between plant and parasite under field conditions.

A negative correlation was found between the number of isolates of *Fusarium oxysporum* f. sp. *asparagi* and the G-value of an asparagus crop. This G-value was arbitrarily chosen, but it was calculated from measurements which indicated the success of the asparagus crop. Furthermore, these measurements could be made very easily in the field, and the results obtained appeared to be very satisfactory. It has not, however, been proved that infection with *Fusarium oxysporum* f. sp. *asparagi* is the only cause of a low G-value. Other factors which may be involved are mineral nutrition, minor elements and infection by the asparagus miner (*Ophiomiya simplex* (Loew.) Spencer). By more detailed study of these factors in relation to the disease a clearer picture may be obtained.

Acknowledgments

The authors are indebted to Dr K. Verhoeff I.P.O., Wageningen for his very helpful suggestions in preparing the manuscript and to Prof. J. Colhoun, Dept. of Cryptogamic Botany, Univ. of Manchester, for critical reading it and for correcting the English text.

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Top wilting in asparagus.

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Abstract

Wilting and dying off of young shoots of the 'second flush' of asparagus are typical symptoms of one of the soil-borne diseases of this crop. Fungi could not be isolated from these shoots. A positive correlation was found between an increase of the pF-value of the soil and the occurrence of 'top-wilting'.

Introduction

One of the soil-borne diseases in asparagus (*Asparagus officinalis* L.) is characterized by wilting and dying off of young shoots. This disorder was earlier described as top wilting (van Bakel and Kerstens, 1970). Symptoms can be observed in young shoots which appear in July and August, the so-called 'second flush'. The first symptoms are wilting of the tips of the spear and the young laterals, followed in a few hours by dying off of these tips, which then turn black, while the stem tissue shrivels (Fig. 1). Sometimes a shoot recovers but the presence of a typical S curve in the mature stem indicates that wilting had occurred earlier (Fig. 2). It also can happen that only a few laterals grow out. However, usually the shoots die off and remain as skeletons on the field. This top wilting is most serious in young, vigorous growing asparagus crops, especially in the first three years after planting. Very often the dead stems are later on infected by *Botrytis cinerea*, by *Fusarium culmorum* or by both.

In the literature little is known about the cause of this disease. Van de Vliet (1955) and Kempenaers (1961) ascribe it to infection by a form of *Fusarium oxysporum*. De Leeuw (1965) considers shortage of boron the cause of the disorder. Experiments to test this possibility were carried out in co-operation with Dr. Ir. K. W. Smilde of the Institute for Soil Fertility at Groningen. No correlation between Boron and top wilting could be observed (in press). From glasshouse irrigation experiments, Ellison (1956) concludes that top wilting ('dieback') is caused by a lack of water in a critical period in the development of the plants. The purpose of our investigations was to get a better understanding of this phenomenon and of its significance in the whole complex of soil-borne diseases of asparagus.

Material and methods

In summer, samples of stems showing top wilting symptoms were taken from 20

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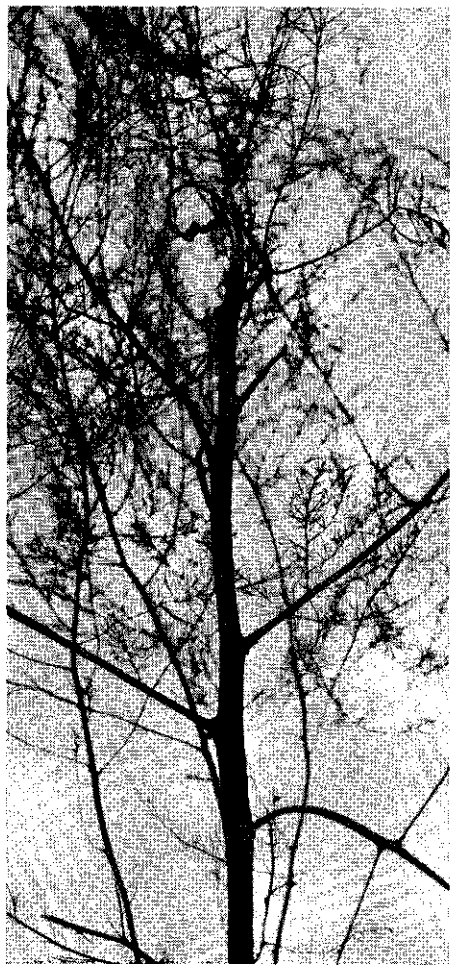

Fig. 1. Top wilting
Fig. 1. Topverwelking

Fig. 2. Mature stem recovered from topwilting
Fig. 2. Uitgegroeide aspergestengel, die zich hersteld heeft van topverwelking

different fields. Although the stems did not show any visible signs of infection by fungi, isolations were made as described earlier (van Bakel and Kerstens, 1970). Special attention was paid to the occurrence of *Fusarium oxysporum* f. sp. *asparagi* Cohen, the causal organism of foot rot in asparagus. Since asparagus is grown in sandy soils, it could very well be, as suggested by Ellison (1956), that shortage of water plays an important role in top wilting. Therefore, during the growing period soil samples were taken form four asparagus fields in 1967, 1968 and 1969. These samples were taken at intervals of 14 days at various depths between 25 and 60 cm below soil level, as this is the region where most of the roots are present (Franken and Roorda van Eysinga, 1958). At a given depth, four identical cylinders of soil per sample were taken. Soil samples were dried at 105 °C for two days, the water content was deter-

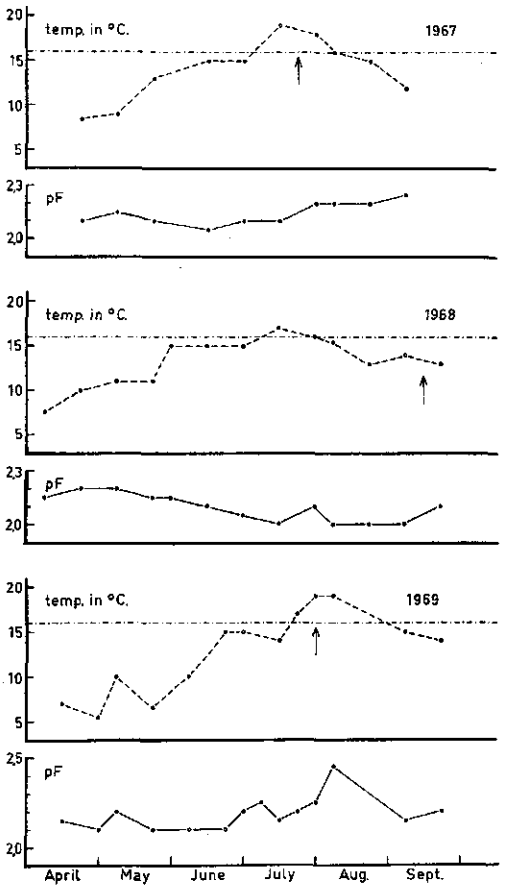

Fig. 3. Various data about measurement of temperature and determination of pF value. In the area above the broken line shoots of the second flush start growing. The occurrence of top wilting is indicated by an arrow.

Fig. 3. Gegevens omtrent de temperatuurmetingen en pF-bepalingen. In het gedeelte boven de streeplijn beginnen de stengels van het tweede schot te groeien. Het optreden van top-verwelking is aangegeven met een pijl.

mined by weighing the samples before and after drying. With a standard pF curve of this soil, obtained from the Soil Survey Institute at Wageningen, the figures for the water contents were converted into pF values. Besides the water content of the soil, the temperature plays an important role in the growth of the spears (Keuls and Post, 1956). So soil temperatures were measured 25 cm below soil level, at the position of the rootstocks.

Results

The various figures for soil temperatures and pF values of the soil are given in Fig. 3.

From field observations it appeared, that young shoots from the second flush start growing at a soil temperature of about 16 °C. Subsequently, in 1967 and 1969 young shoots appeared in the first and in the third week of July, respectively. Top wilting occurred 10 to 14 days later, as indicated by arrows in Fig. 3. In these two years top wilting symptoms developed at a time of increasing pF values. In 1968 soil temperatures remained low, due to very unfavourable wheather conditions. Young shoots of the second flush were present in the second week of September. Although top wilting

was much less than in 1967 and 1969, some symptoms developed in the second and third weeks of September. At that time a slight increase in the pF value was measured.

No fungi were isolated from stems showing symptoms of top wilting, neither from the top-wilted parts, nor from the basal portion.

Discussion

As no fungi were isolated from stems showing symptoms of top wilting and in view of the results shown in Fig. 3, it is evident that a strong correlation exists between the water content of the soil and the appearance of top wilting in young shoots in summer. This might mean, that this dying off of young shoots is due to water shortage at the time of full growth of these parts of the plant as was suggested by Ellison (1956). At that time weather conditions can be such that the plants are losing much water by transpiration. Such weather conditions existed in 1967 and 1969 and to a smaller extent in 1968. When the soil is comparatively dry, the water uptake cannot be sufficient, resulting in a wilting of the weaker parts of the plant i.e. the young, fast growing shoots. In the whole complex of soil-borne diseases, top wilting seems to be the only recognised so far that is not caused by fungi, but might be due to shortage of water at a critical period of plant growth.

Acknowledgments

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Samenvatting

Topverwelking in asperge

Een van de ziekten van asperge is topverwelking, waarbij de stengeltoppen van jonge scheuten vrij plotseling verwelken, en vervolgens de gehele stengel afsterft. Dit komt vooral voor in jonge stengels van het zogenoemde tweede schot in juli en augustus. Er werd een positieve correlatie gevonden tussen het optreden van dit verschijnsel en het oplopen van de pF curve (Fig. 3). Schimmels konden uit juist verwelkte stengels niet geïsoleerd worden.

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