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Etiology of asparagus replant-bound early decline

Wim J. Blok and Gerrit J. Bollen
Department of Phytopathology, Wageningen Agricultural University, P.O. Box 8025, 6700 EE Wageningen, The Netherlands

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Abstract

Asparagus replant-bound early decline (ARED) was characterized and its etiology was elucidated in experiments under greenhouse and field conditions. Selective soil treatments were used to differentiate between autotoxic compounds and soil-borne pathogens as causal agents. In greenhouse experiments, there were symptoms of ARED within 12–15 weeks. Asparagus plants grown in soil formerly used for asparagus (asparagus soil) showed brown lesions on primary and secondary roots, and many secondary roots had rotted. Root weights of plants grown in asparagus soil were lower than those of plants grown in fresh soil. *Fusarium oxysporum* f. sp. *asparagi* (Foa) was by far the most common species among the fungi isolated from roots with lesions. Under greenhouse and field conditions, there were similar symptoms, which indicates that the results obtained under greenhouse conditions are similar to those in the field. The vertical distribution of the ARED-causing factor(s) was studied in a greenhouse experiment in which plants were grown in soil from three layers: 0–30, 30–60, and 60–90 cm. For all four asparagus soils tested, there were ARED symptoms and similar disease severity in samples from all three depths. The causal factor persisted at least 11 years after soil was no longer used for asparagus. When asparagus soil was diluted with fresh soil to give mixtures with 100%, 80%, 50%, 20% and 0% asparagus soil, disease severity did not decrease with increasing dilution of the asparagus soil from 100% to 20%. Disease severity of all mixtures with asparagus soil was significantly higher than that for fresh soil. The results imply that ARED is caused by a pathogen colonizing the soil rather than inhibition by autotoxins released from residues of the preceding asparagus crop. This conclusion is supported by the results of greenhouse and outdoor experiments with heat and fungicide treatments of soil. ARED was nullified by heat treatments of 30 min at 55 or 60 °C but not 45 and 50 °C, eliminating autotoxins as an important cause of ARED because they are heat-stable. Foa is eliminated by a 30-min soil treatment at 55–60 °C but not 50 °C. Prochloraz, known for its toxicity to *F. oxysporum*, also nullified ARED. Disease severity level was related to the density of Foa in soil. The results provide conclusive evidence that *F. oxysporum* f. sp. *asparagi* is the main cause of ARED in the Netherlands, which largely removes the need to discriminate between early decline and replant-bound early decline, because Foa is the main cause of both diseases.

Introduction

In the Netherlands asparagus production is concentrated mainly in the southeast, where it is common to replant former asparagus land because the amount of fresh land (without a history of asparagus cropping) is limited. However, asparagus production on former asparagus land is less profitable than that on fresh land. Crop establishment generally is not affected, but after a few years of normal yields, growth and yield decline. The economic life of a crop on asparagus land averages only half that of a crop on fresh land. The symptoms of this phenomenon are similar to those described for asparagus early decline [Grogan and Kimble, 1959]. However, whereas early decline occurs on fresh soil as well as asparagus soil, problems in the Netherlands are confined to replant situations. Because of this difference and because the etiology of the phenomenon

was not clear for the Dutch situation, the phenomenon was distinguished tentatively from early decline and termed asparagus replant-bound early decline (ARED) [Blok and Bollen, 1995]. Replant-bound early decline is currently one of the main threats to asparagus production in the traditional asparagus-growing region of the Netherlands.

A number of factors have been associated with early decline of asparagus: increased infestation of the soil with soil-borne pathogenic fungi, mainly *Fusarium* spp. [Schofield, 1991], and direct or indirect effects of autotoxic compounds emanating from asparagus roots [Hartung, 1987; Wacker *et al.*, 1990; Peirce and Miller, 1993]. *F. oxysporum*, *F. moniliforme* and *F. proliferatum* are often mentioned as the major causal agents [Grogan and Kimble, 1959; Johnston *et al.*, 1979; Gindrat *et al.*, 1984; Elmer, 1990; Schreuder *et al.*, 1995]. However, studies in which the contribution of each of these factors to the etiology of the disease is estimated are lacking. For this reason and because it is unclear whether ARED is different from early decline, definitive conclusions about the etiology of the disease in the Netherlands could not be drawn.

In earlier studies, root residues of the preceding asparagus crop were shown to be a source of autotoxins, and the main soil-borne fungal pathogens in Dutch asparagus fields were identified [Blok and Bollen, 1993, 1995]. *Fusarium oxysporum* f. sp. *asparagi* (Foa) was the main pathogen because of its prevalence and virulence. To characterize ARED further and to estimate the relative contribution of various causal factors, a method was needed to reproduce the symptoms of the disease. In greenhouse experiments, Huiskamp and Poll [1990] and Poll and Huiskamp [1992, 1994] compared growth of asparagus plants in fresh and (former) asparagus soil from 15 locations. They found that plants grown in asparagus soil could be distinguished easily from those grown in fresh soil. The former had fewer secondary roots and had brown lesions, whereas the latter had healthy root systems with many secondary roots. Root weight of plants grown in asparagus soil was lower than that in fresh soil. The researchers demonstrated that ARED symptoms can be reproduced in greenhouse experiments within 9–15 weeks.

Our goal was to characterize ARED further and elucidate the etiology. We used soil samples from a limited number of locations of the asparagus-growing area in the Netherlands. We decided that this sample number would suffice because Poll and Huiskamp [1994; personal communication] tested a large number of samples from different locations in the Netherlands and found that the root systems were affected identically in all asparagus soils.

The vertical distribution of ARED was studied by comparing asparagus growth in fresh and asparagus soil collected from different depths. A possible dilution effect was studied in a series of mixtures of asparagus soil and fresh soil. A dilution effect suggests involvement of toxins or slow-growing organisms as major factors in ARED development. Since the autotoxins tolerate autoclaving [Yang, 1982; Blok and Bollen, 1993] and fungal pathogens are sensitive to heat, ARED soils were subjected to heat treatments to discriminate between these factors as causes of the disease. We attempted to characterize fungi involved in ARED further by treatment of soil with products with a different antifungal spectrum.

Materials and methods

Plant material and nutrition

As all Dutch asparagus cultivars suffer from ARED, the current cv. Gynlim was used throughout all experiments. Seeds were surface-disinfected in 2% sodium hypochlorite for 30 min and then in a suspension of benomyl in acetone (25 mg ml^{-1}) for 20–24 h, after which they were rinsed in acetone twice and tap water three times [Stephens and Elmer, 1988]. The seeds were sown in a commercial potting mixture (Trio-17, a decomposed sphagnum peat with some clay and marl; pH-KCl 5.4; organic-matter content 70%). After several weeks at 20–25 °C in the greenhouse, seedlings were uprooted, culled to a uniform size and planted in pots. Unless stated otherwise, every 10–14 days an application of 100 ml of a solution containing 1.67 g Nutriflora-T l^{-1} and 2.0 g calcium nitrate l^{-1} was made in each 4-l pot. This solution at pH 6.7 is used in soilless cultures as a complete nutrient solution. It contains the following elements (concentrations in mg l^{-1}): N, 344; P, 78; K, 560; Mg, 50; Ca, 340; S, 206; Fe, 1.2; Mn 2.2; B, 0.7; Zn, 0.5; Cu, 0.020; Mo, 0.116.

Disease rating

At the end of the experiments roots were washed free of soil carefully and rated for disease symptoms. Root dry weights (DWR) were determined after drying for 24 h at 105 °C. Disease symptoms on primary (DPR) and secondary roots (DSR) were rated combined (Expts 1 and 2) or separately (Expts 3 and 4) on a scale of 0–5 where 0 = no lesions, 1 = 0–5% of the root length

covered with lesions, 2 = 6–20%, 3 = 21–60%, 4 = 61–95%, and 5 = > 95% or dead plants. In many cases secondary roots were rotted completely and not retrieved when root systems were washed, which implies that the number of secondary roots is also a measure of disease severity. The loss of secondary roots (LSR) was rated on an arbitrary scale from 0 (abundant secondary roots, no loss) to 10 (no secondary roots present, total loss). Based on the ratings for DPR, DSR, and LSR, a disease index (DI) ranging from 0–10 was calculated. To equalize scales and give the three components the same weight, DI was calculated as DI = (2 × DPR + 2 × DSR + LSR) / 3.

Isolation from infested material

Root parts showing symptoms were washed free of adhering soil under running tap water and disinfested externally by washing, depending on the thickness of the root parts, in 1–2% sodium hypochlorite for 1–2 min and then three times in sterile distilled water. Small sections of secondary roots or tissue parts excised from the edge of a lesion on a primary root were plated onto potato dextrose agar or malt extract agar, both amended with oxytetracycline (50 μg ml^{-1}), or Komada's agar [Komada, 1975]. Fungi growing from these sections were subcultured on fresh PDA plates for further identification. *Fusarium* spp. were identified according to Nelson *et al.* [1983]. Other fungi were identified using the keys of Domsch *et al.* [1980]. Some of the *F. oxysporum* isolates were tested for pathogenicity to asparagus on plantlets grown aseptically in culture tubes containing Knop's agar, as described previously [Blok and Bollen, 1995].

Estimation of the population density of Fusarium oxysporum *f. sp.* asparagi *in soil*

Soils were assayed for *F. oxysporum* by placing 10.0-g samples (dry-weight equivalent) in Erlenmeyer flasks with 90 ml sterile distilled water. The flasks were shaken for 1 h on an orbital shaker, and the soil suspension then was diluted further by adding 10 ml of it to 90 ml sterile distilled water. Of each soil suspension, 0.5 ml was spread on each of four or five replicate plates with Komada's selective medium. After incubation for two weeks at 25 °C in the light, the number of colonies of *F. oxysporum* was counted and expressed as numbers of colony forming units (c.f.u.) per gram of dry soil. To determine the proportion of isolates of *F. oxysporum* that belonged to f. sp. *asparagi*, a number of isolates was selected randomly on the Komada plates and subcultured onto PDA slants. The pathogenicity of these isolates to asparagus was tested *in vitro* as described above.

Vertical distribution of ARED-causing factor(s) in soil (Expt 1)

To study the occurrence of the causal factor(s) of ARED at various depths, soil samples were collected from two different locations in the asparagus-growing region and tested in a bioassay. Soils from both locations were loamy sands with an organic-matter content of 2–3%. Location 1 was on the experimental farm Meterikse Veld at Horst, and location 2 was on a commercial farm at Castenray. A control field with fresh soil and two former asparagus fields were sampled at both locations. Five sites in each field were selected randomly. Six litres of soil was collected at each site from each of the following depths: 0–30, 30–60, and 60–90 cm. For each soil layer, the soil from the five sites was bulked and mixed well, creating a total of 18 samples (six fields × three layers) of 30 l each. For each sample the pH (KCl) and the amount of asparagus root residues were determined (Table 1). For the latter, a subsample of 6 l was sieved through a sieve with 5-mm mesh. Dry weight of root residues was determined after 24 h at 105 °C.

For each sample, six pots were filled with 4 l of soil, and each pot was planted with three 5-wk-old plants. The pots were placed in a greenhouse compartment at 20 ± 2 °C in a randomized complete block design with six blocks. The experiment was concluded after 22 weeks.

Effect of dilution of infested soil on disease severity (Expt 2)

A series of mixtures of asparagus soil and fresh soil was used to study the effect of dilution of infested soil on disease severity. On the experimental farm Meterikse Veld at Horst, soil was collected from the upper 25 cm of a field without an asparagus history (fresh soil) and from a field where asparagus production was terminated two years before (asparagus soil). In former experiments, soil from the latter field showed typical symptoms of ARED. Soil from both fields had similar soil type (loamy sand), pH (5.8), and organic-matter content (2.8%). Both soil samples were sieved through a screen with a 5-mm mesh to remove stones and soil clods. The root residues that remained were returned to the soil. The following mixtures of fresh and asparagus soil were made: 1:0, 4:1, 1:1, 1:4 and

Table 1. Data of soil samples used in the study of the occurrence of the causal factor in the soil profile (Expt 1)

Location	Field	Asparagus history	Soil layer (cm below soil surface)	Amount of asparagus root residues (g dw kg^{-1} dry soil)	pH (KCl)
1	1	None	0–30	0	5.7
			30–60	0	4.8
			60–90	0	4.5
1	2	Production field, terminated 2 y before	0–30	0.32	5.8
			30–60	0.26	5.1
			60–90	0.23	4.7
1	3	Production field, terminated 11 y before	0–30	0.03	6.0
			30–60	0.10	5.5
			60–90	0.04	4.8
2	4	None	0–30	0	5.3
			30–60	0	4.4
			60–90	0	4.6
2	5	Production field, terminated 2 y before	0–30	0.18	6.3
			30–60	0.16	6.1
			60–90	0.06	5.6
2	6	Production field, terminated 5 y before	0–30	0.39	5.6
			30–60	0.16	5.6
			60–90	0.08	5.9

0:1. For each mixture, eight pots were filled with 4 l of soil each. Every pot received three 9-wk-old plants. The experiment was set up as a randomized complete block design with eight blocks and carried out in a greenhouse at 20–24 °C. The experiment was concluded after 15 weeks.

Effects of selective heat treatments of soil in a greenhouse experiment (Expt 3)

In order to discriminate between the contribution of autotoxins and fungal pathogens as causal agents of ARED symptoms, soil was heated at different temperatures. For this experiment samples of fresh and asparagus soil were collected at the same locations as those for the dilution series (Expt 2). Sieved fresh and asparagus soil, with asparagus residues returned and mixed with the soil, were heated with aerated steam and kept for 30 min at 55, 60, or 65 °C (± 1.5 °C) or left untreated (control). The soil was pasteurized in a slightly modified version of the apparatus described by Aldrich and Nelson [1969]. After treatment the soil was stored in open plastic boxes, kept moist, and turned several times. After six weeks, seven 4-l pots for each soil type-treatment combination were filled. From each pot a soil sample of about 15 ml was collected with a sterile spoon. The samples were bulked for each soil type-treatment combination and assayed for *F. oxysporum*. For the untreated fresh and asparagus soil, 25 isolates of *F. oxysporum* were selected randomly from the soil dilution plates and tested for pathogenicity on asparagus *in vitro*. In each pot three 6-wk-old asparagus plants were planted. The pots were placed in a randomized complete block design with seven blocks. The experiment was carried out in a greenhouse at 22–25 °C. The experiment was finished after 14 weeks.

Effects of selective heat and fungicide treatments of soil in an outdoor pot experiment (Expt 4)

To identify the causal factor of ARED further and to check whether its symptoms could be reproduced under field conditions, the following experiment was conducted. Fresh and asparagus soil was collected from the upper 25 cm at Meterikse Veld. The fields were different from those in which soil was collected for Expts 1–3, but soil type, pH, and organic-matter content were similar. The asparagus soil came from a field where asparagus had been grown for 10 years and the crop was abandoned the year before. Both

soils were sieved through a screen with a 5-mm mesh. The asparagus root residues were returned and mixed through the soil. Details on treatments of soil are given in Table 2. Heat treatment of soil was made as in Expt 3. The fungicides were applied at the start of the experiment by mixing the products through soil in a concrete mixer. Later applications were given as soil drenches (100 ml per cylinder). Three samples each of the untreated, heat-treated, and irradiated soils were taken and assayed for *F. oxysporum*. Twenty randomly selected isolates of *F. oxysporum* from the untreated fresh and asparagus soil were tested for pathogenicity on asparagus *in vitro*. After treatment the soil was put in bags constructed from polypropylene cloth impenetrable to roots. Each bag was filled with 13 l of soil and placed in a pvc cylinder (height 50 cm, diameter 20 cm). The cylinders were sunk into the field to the soil surface. For each soil type-treatment combination, eight cylinders were filled. The cylinders were placed 125 × 80 cm apart in a randomized complete block design with eight blocks. In each cylinder two 15-wk-old asparagus plants were planted. The outer rows of cylinders were surrounded by border rows of pairs of plants planted in free soil. During the first eight weeks, plants were watered by sprinklers as needed. After eight weeks plants were watered by drip irrigation. Each cylinder was fertilized directly after planting and after 4, 8, and 12 weeks with 600 ml of a solution containing 1.67 g Nutriflora-T l^{-1} and 2.0 g calcium nitrate l^{-1}. After 15 weeks each cylinder received 1200 ml of the same solution. After 17 weeks needles started to yellow and plants were fertilized for the last time by spraying with a solution containing 10 ml of the compound leaf fertilizer Wuxal l^{-1}. The solution contained the following elements (concentrations in mg l^{-1}): N, 2070; Mg, 663; Fe, 13.8; Mn, 6.9; B, 2.8; Zn, 8.0; Cu, 2.8; Mo, 0.069. Plants of treatment 12 (no fertilization) received tap water instead of nutrient solution. After 24 weeks the shoots had completely died and the experiment was finished.

Statistical analysis

Data for DWR and DI were subjected to analysis of variance after a check for normality and homogeneity of variances. To make preplanned comparisons, linear contrasts were evaluated with normal F-tests. Comparisons that were not preplanned were evaluated with either *LSD* (pairwise comparisons of treatments) or Dunnett's multiple-comparison test (pairwise comparisons of treatments with the control) [Steel and Torrie, 1960]. Linear regression analysis was performed with data of all individual experimental units to study the relationship between DI and DWR. Adjusted R^2 values were calculated for the relationship between DWR and the individual components of the DI and compared with the adjusted R^2 value of the relationship between DWR and DI.

Results

Reproduction of ARED symptoms

There was a consistent difference between plants grown in fresh soil and those grown in asparagus soil. In the fresh soils, root systems were the normal pale cream with many secondary roots and low numbers of lesions. In the asparagus soils, however, although all plants survived during the experimental period, many of the secondary roots had rotted, giving the root systems a sparse appearance. The root system was brown because of the many smaller and larger lesions on the main as well as the secondary roots. Thus, ARED symptoms were reproduced easily in all experiments.

In all instances in which fungi were isolated from roots with symptoms, *F. oxysporum* predominated among the isolates, on the selective medium and the general media. *Phoma terrestris* was isolated from a few plants of the outdoor pot experiment. The fungus was found on reddish discoloured rootlets (which were encountered occasionally) and brown rootlets. Pathogenicity tests showed *Phoma terrestris* and almost all isolates of *F. oxysporum* to be pathogenic on asparagus. Other fungi, which were isolated occasionally, were not pathogenic.

ARED symptoms were indicative of a lower root weight. The shoot weight was influenced far less, and therefore these data are not presented.

Vertical distribution of ARED-causing factor(s) in soil (Expt 1)

ARED symptoms were present on roots from all asparagus soil samples, irrespective of depth. The DI values are given in Fig. 1. There were some lesions on roots in fresh soil. The numbers were highest for the 0 to 30- cm layer, and in the deepest layer, there were no lesions. These results were reflected in the DI values of the fresh soils, which were slightly higher for the upper soil layers than the deeper ones.

Table 2. Selective soil treatments applied in the outdoor pot experiment (Expt 4)

1.	Blank (untreated)
2.	Untreated and not fertilized
3.	Heat treatment for 30 min at 45 °C
4.	Heat treatment for 30 min at 50 °C
5.	Heat treatment for 30 min at 55 °C
6.	Heat treatment for 30 min at 60 °C
7.	Metalaxyl (Ridomil-5G, 5% granulate), one application of 3.2 mg a.i. kg^{-1} dry soil at the start of the experiment
8.	Quintozene (Luxan PCNB, 70% WP), one application of 16.0 mg a.i. kg^{-1} dry soil at the start of the experiment
9.	Benomyl (Benlate, 50% WP), applications at 0, 4, and 8 weeks of 54.0, 75.0 and 75.0 mg a.i. kg^{-1} dry soil, respectively
10.	Prochloraz (Sportak, 450 g l^{-1}), applications at 0, 4, and 8 weeks of 64.0, 30.0 and 30.0 mg a.i. kg^{-1} dry soil, respectively
11.	Captafol (Orthodifolatan-4F, 480 g l^{-1}), applications at 0, 4, and 8 weeks of 32.0, 20.0 and 20.0 mg a.i. kg^{-1} dry soil, respectively
12.	Irradiation (2.5 Mrad)

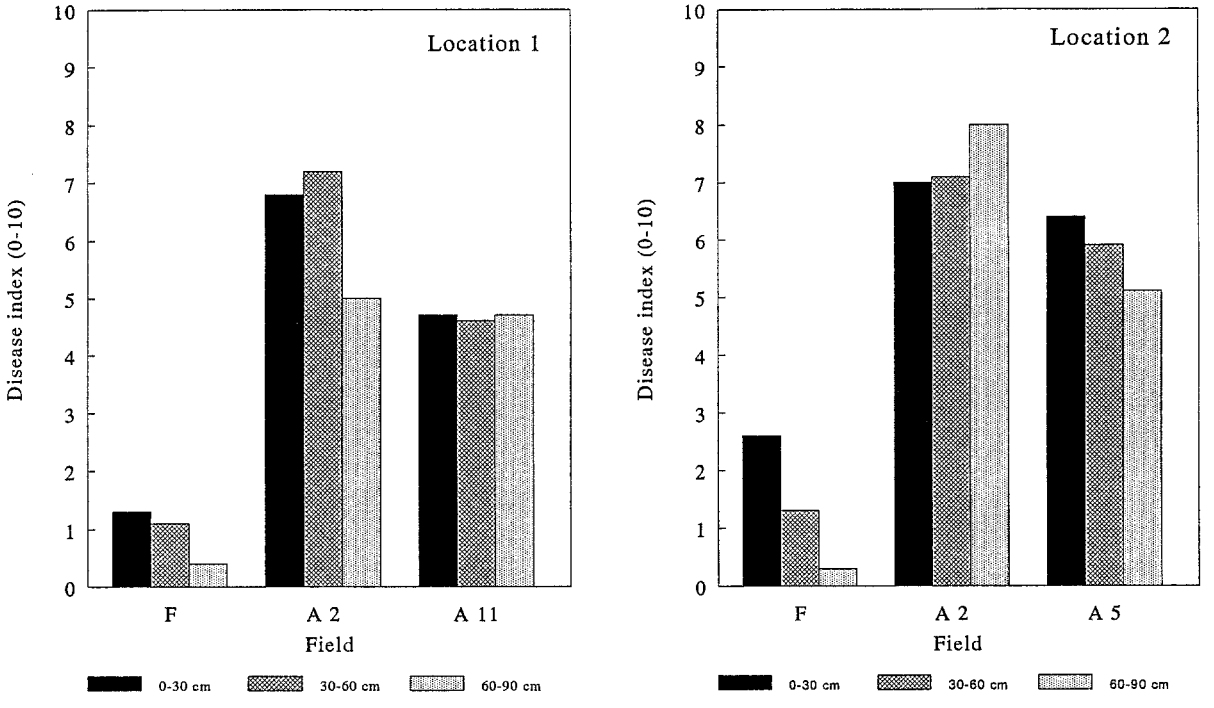

Fig. 1. Occurrence of ARED-causing factor(s) in the soil profile (Expt 1). Disease severity for location 1 (left) and location 2 (right). F = fresh soil; A = asparagus soil; the numbers indicate the number of years since asparagus production was stopped.

Effect of dilution of infested soil on disease severity (Expt 2)

There were typical ARED symptoms on plants in all four treatments with asparagus soil (asparagus treatments), whereas in fresh soil, few lesions were on the roots. For all asparagus treatments, the means for the DI were significantly higher, and for DWR significantly lower, than those for fresh soil (Table 3). Among the various asparagus treatments, the differences in DI were small and DWR were not significantly different. An indication of decreasing disease severity with decreasing rates of asparagus soil (dilution effect) was not found.

Table 3. Means for disease index and root dry weight of the soil dilution series experiment (Expt 2)

Treatment[1] A (%) : F (%)	Disease index[2] (0–10)	Root dry weight[2] (g pot^{-1})
100 : 0	4.9	7.79
80 : 20	6.0	7.30
50 : 50	5.7	7.79
20 : 80	4.8	6.36
0 : 100	2.5*	11.91*
LSD (Dunnett, $\alpha = 0.05$)	1.2	3.94

[1] A = asparagus soil; F = fresh soil.
[2] Means followed by an asterisk are significantly different from the nondiluted asparagus soil (100% A), according to Dunnett's test ($P \leq 0.05$).

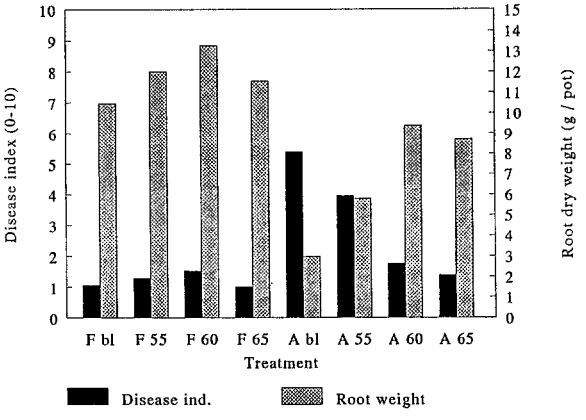

Fig. 2. Effect of heat treatments of soil on disease index and root dry weight (Expt 3). F = fresh soil; A = asparagus soil; bl = blank; 55 = 30 min at 55 °C; 60 = 30 min at 60 °C; 65 = 30 min at 65 °C. For significance levels of contrasts, see Table 4.

Table 4. Significance levels (P) from analysis of variance for disease index and root dry weight for plants grown in heat-treated soil (Expt 3)

Source	Disease index	Root dry weight
Soil	< 0.001	< 0.001
Treatment	< 0.001	0.009
Block	0.003	0.946
Soil * Treatment	< 0.001	0.287
Contrasts:		
F bl vs. A bl	< 0.001	< 0.001
F 55 vs. A 55	< 0.001	0.002
F 60 vs. A 60	0.492	0.045
F 65 vs. A 65	0.145	0.146

Table 5. Density of *Fusarium oxysporum* (Fo) in soil and fraction of isolates pathogenic to asparagus for the greenhouse experiment with heat treatments of soil (Expt 3)

Treatment	C.f.u. Fo g^{-1} soil		# pathogenic isolates / # isolates tested
	Mean[1]	S.D.	
F blank	212.0	± 61.5	1 / 23
F 55	20.0	± 12.0	
F 60	< 4		
F 65	< 4		
A blank	1029.3	± 348.3	6 / 24
A 55	98.7	± 10.0	
A 60	17.3	± 12.2	7 / 13
A 65	< 4		

[1] The lower limit of detection was 4 c.f.u. g^{-1} of soil; < 4 means that no colonies were detected on the plates.

Effects of selective heat treatments of soil in a greenhouse experiment (Expt 3)

Results for DI and DWR are given in Fig. 2, and significance levels for both variables are given in Table 4. Disease severity of plants grown in untreated asparagus soil was significantly higher than that of plants grown in fresh soil. After a heat treatment at 60 or 65 °C, disease severity in asparagus soil did not exceed that in fresh soil.

From each of the eight treatments, three soil samples were assayed for *F. oxysporum*. After the 55 °C treatment, the density of *F. oxysporum* had decreased to 10% of that in the untreated soil; after the 60 °C treatment, the fungus was no longer detectable in the fresh soil and had decreased to very low levels in the asparagus soil (Table 5).

Effects of selective heat and fungicide treatments of soil in an outdoor pot experiment (Expt 4)

DI values are given in Fig. 3. For DI and DWR significance levels for factors, interaction, and contrasts are given in Table 6. We conclude from the results that symptoms of ARED can be reproduced under field conditions. Plants grown in untreated asparagus soil were affected the same way as those grown under greenhouse conditions. ARED did not occur after application of prochloraz, heating at 55 or 60 °C, or irradiation of the soil. Captafol reduced the DI significantly (*LSD*, $\alpha = 0.05$).

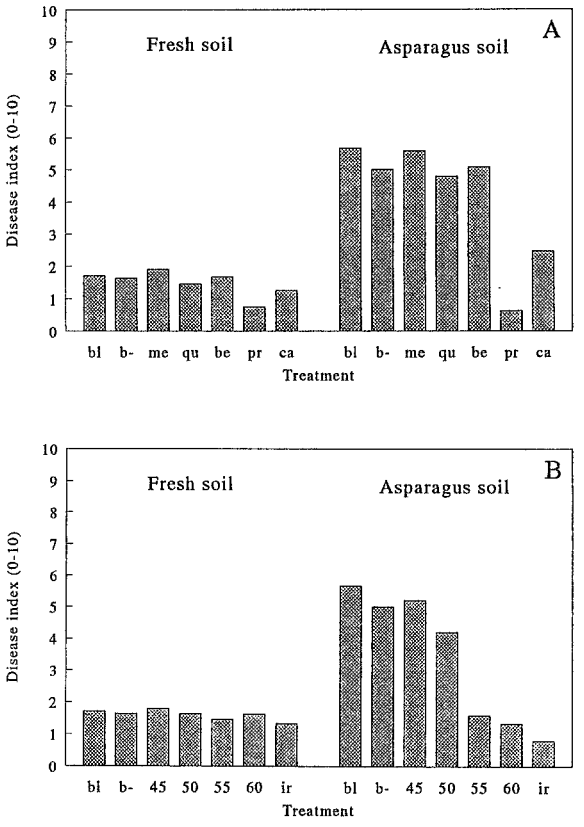

Fig. 3. Effect of fungicide, heat treatments, and irradiation on disease index (Expt 4). A: effects of fungicide treatments; B: effects of heat treatments and irradiation. bl = blank (untreated); b- = blank, nonfertilized; me = metalaxyl; qu = quintozene; be = benomyl; pr = prochloraz; ca = captafol; 45 = 30 min at 45 °C; 50 = 30 min at 50 °C; 55 = 30 min at 55 °C; 60 = 30 min at 60 °C; ir = irradiation. For significance levels of contrasts, see Table 6.

After the 50 °C treatment, the density of *F. oxysporum* had decreased considerably; after the 55 °C and 60 °C treatment, *F. oxysporum* was no longer detectable (Table 7).

Relationship between disease severity and root dry weight

For Expt 1 DWR for the fresh soil from location 1 were lower than expected. From information obtained afterward, we concluded that the most probable cause was herbicide residues. Therefore, data from this field were excluded from the regression analysis. For all four experiments, the linear regression of DWR on DI was highly significant ($P < 0.001$), with adjusted R^2 at 0.50 (n = 87), 0.34 (n = 35), 0.37 (n = 55), and 0.07 (n = 188) for Expts 1, 2, 3 and 4, respectively. The slopes of the regression lines for the three

Table 6. Significance levels (P) from analysis of variance for disease index and root dry weight data of the outdoor pot experiment with fungicide and heat treatments of soil (Expt 4). For a description of treatment codes, see Figure 3

Source	Disease index	Root dry weight
Soil	< 0.001	0.163
Treatment	< 0.001	< 0.001
Block	< 0.001	0.009
Soil * Treatment	< 0.001	0.014
Contrasts:		
F bl vs. A bl	< 0.001	0.017
F b- vs. A b-	< 0.001	0.555
F me vs. A me	< 0.001	0.134
F qu vs. A qu	< 0.001	0.408
F be vs. A be	< 0.001	0.167
F pr vs. A pr	0.647	0.407
F ca vs. A ca	< 0.001	0.023
F 45 vs. A 45	< 0.001	0.047
F 50 vs. A 50	< 0.001	0.280
F 55 vs. A 55	0.647	0.877
F 60 vs. A 60	0.260	0.037
F ir vs. A ir	0.046	0.709

Table 7. Density of *Fusarium oxysporum* (Fo) in soil and fraction of isolates pathogenic to asparagus for the untreated soil and the heated asparagus soil of the outdoor pot experiment (Expt 4)

Treatment	C.f.u. Fo g^{-1} soil		# isolates pathogenic / # isolates tested
	Mean[1]	S.D.	
F blank	687.0	± 20.7	0 / 20
A blank	773.3	± 21.2	4 / 20
A 45	1049.7	± 167.8	
A 50	114.3	± 54.6	
A 55	< 4		
A 60	< 4		

[1] The lower limit of detection was 4 c.f.u. g^{-1} of soil; < 4 means that no colonies were detected on the plates.

greenhouse experiments (Expts 1–3) were not significantly different at P = 0.05 and, therefore, data from these experiments were pooled and a linear regression line was fitted to the pooled data (Fig. 4d). The calculated regression lines for the three experiments also are shown separately (Fig. 4a, b, and c). The regression line obtained for the outdoor pot experiment (Expt 4)

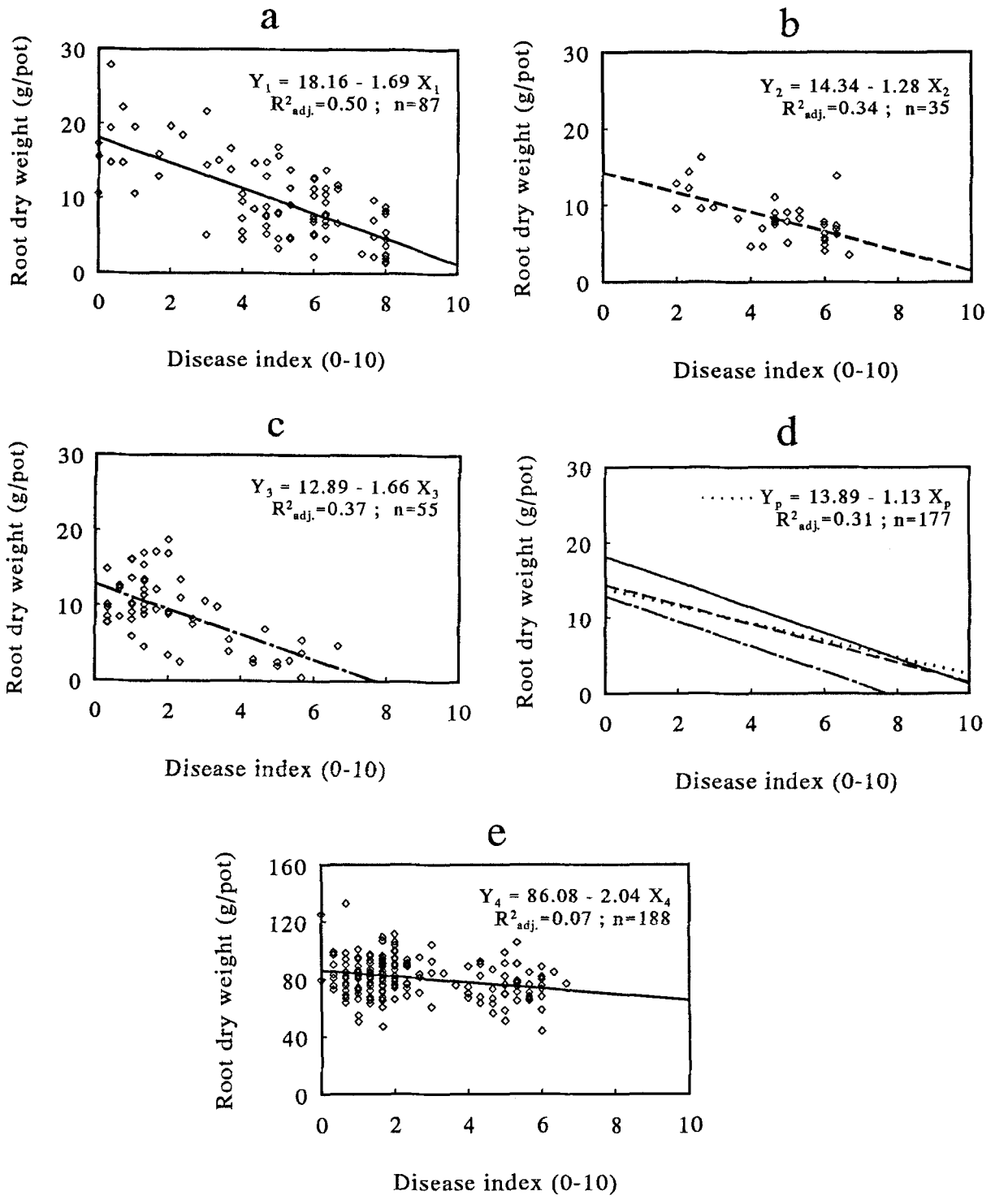

Fig. 4. Relationship between root dry weight and disease index. a, b, and c: scatter plots with linear regression lines for Expts 1, 2, and 3, respectively; d: regression lines for Expts 1, 2, and 3 and for the pooled data of these experiments (Y_p); e: scatter plot with linear regression line for Expt 4.

when DWR was regressed on DI was different from that for the greenhouse experiments (Fig. 4e), with an explained variance of only 7%. A main difference between the outdoor pot experiment and the greenhouse experiments is the higher overall level of DWR in the outdoor pot experiment.

For the relationship between DWR and DI or the single components of DI, generally DI explained variation in DWR best. Only for Expt 3 did the component LSR give a higher percentage explanation (57%) than the DI (38%).

Discussion

When asparagus soil was diluted with fresh soil, disease severity remained at the same level and there was no increase in root weight (Expt 2). These results provide evidence for involvement of a pathogen colonizing the soil and virtually exclude as an important ARED factor, direct growth inhibition by autotoxins of the preceding asparagus crop. This hypothesis is substantiated by the effects of selective heat treatments. Autotoxins of asparagus are very heat-stable, as was reported by Yang [1982]. The fact that ARED was nullified by heat treatments at 55–60 °C and treatment with prochloraz excludes autotoxins as a cause of ARED. In all experiments Foa was isolated from brown lesions, which corresponds with the results of an analysis of the most common soil-borne fungal pathogens of asparagus in the Netherlands, where Foa was the most prevalent and virulent species [Blok and Bollen, 1995]. All selective soil treatments that prevented brown lesions caused by Foa resulted in healthy root systems similar to those produced in fresh soil. The fact that ARED symptoms were reproduced under greenhouse and field conditions indicates that results obtained in the greenhouse are indicative of what occurs in the field. These arguments provide conclusive evidence that Foa is the main cause of ARED.

In a previous study we concluded that direct growth inhibition by autotoxins cannot be considered a major cause of ARED [Blok and Bollen, 1993]; adding enough sterilized asparagus roots to field soil to equal that found after termination of an asparagus crop did not result in a significant yield decrease. However, it is possible that autotoxic compounds are more persistent than root residues and that after decomposition of the roots, they remain bound to soil organic matter. In the present study asparagus soils were used, and autotoxins in root residues as well as those bound to organic matter could have affected the plants. The amount of asparagus root residues retrieved from the soils (Table 1) is similar to that found previously [Blok and Bollen, 1993].

We realize that assessments of nutrient deficiencies and deterioration of soil structure, two factors that are mentioned frequently as causes of replant diseases [Savory, 1966], were excluded from this study. Because of the meticulous asparagus-field preparation practised in the Netherlands, it is unlikely that these factors would be involved in the cause of ARED. It is common practice to plough the soil to a depth of 80 cm before asparagus is (re)planted to allow deep rooting. To restore soil fertility, one or two green manure crops are grown and soil is amended with large amounts of compost before planting. During crop growth organic and inorganic compound fertilizers are given each year.

The conclusion that Foa is the main cause of ARED implies that ARED and early decline are closely related phenomena. A difference that remains is that *F. moniliforme* and *F. proliferatum* are not involved in ARED [Blok and Bollen, 1995; this study] whereas these pathogens are reported to play a major role in early decline [Endo and Burkholder, 1971; Johnston *et al.*, 1979; Gindrat *et al.*, 1984; Elmer, 1990; Bousquet, 1993; Schreuder *et al.*, 1995]. Another difference is that ARED as it occurs in the Netherlands, is confined to replant situations whereas early decline occurs also in fields with a first asparagus crop [Grogan and Kimble, 1959]. That ARED is confined to replant situations is probably because *F. moniliforme* and *F. proliferatum* are not present in these situations. A second factor could be that in the Netherlands, asparagus is grown almost exclusively on fields that are very suitable for this crop. Suitable soil allows vigourous crop development, making problems with Foa less severe and economic life satisfactory. It is only when young plants are planted in heavily infested soil, as is the case in a replant situation, that problems become severe and economic life is significantly shortened. The conclusion that early decline and ARED are closely related, largely removes the need to maintain the term ARED; it can be substituted adequately by: asparagus crown and root rot caused by Foa.

The effect of heat treatments on the density of *F. oxysporum* was different in Expts 3 and 4. Whereas 10% of the Foa population survived the 55 °C treatment in Expt 3, the same treatment completely eliminated the population in Expt 4. An explanation might be a difference in metabolic activity of the soil microflora

caused by a difference in nutrient status of the soil. A high metabolic activity renders microorganisms more sensitive to heat [Bollen, 1974]. For both experiments the DI was related to the density of *F. oxysporum*. From the densities of *F. oxysporum* obtained from untreated asparagus soil and the fraction of isolates pathogenic to asparagus (Tables 5 and 7), the density of Foa in field soil one to two years after asparagus production is estimated at 150–250 c.f.u. g^{-1} dry soil. The Foa population in the root residues was not quantified in the assay.

It is remarkable that benomyl did not prevent lesions caused by Foa, which was also found in an experiment conducted under greenhouse conditions [unpublished data]. In the Netherlands, benomyl and the related compound carbendazim are used widely to disinfest crowns before planting. Its efficacy, based on the present results, is questionable. In the literature, successful and unsuccessful benomyl control of fusarium diseases have been reported. Successful control was obtained by Manning and Vardaro [1977], who used preplant crown soaks against Foa, and by Greenhalgh and Clarke [1985], who used soil drenches to prevent selectively fusarium root rot of subterranean clover. Smiley and Craven [1979], however, were not able to control fusarium with benomyl soil drenches in turfgrass.

The DI used to combine the three different parameters of disease severity is satisfactory because, for most experiments, the percentage variance explained in DWR is higher for the index than for single parameters. When young asparagus plants were tested under similar conditions in the greenhouse, the regression lines obtained when DWR was regressed on DI were similar, with percentage variance explained ranging from 34–50%. With an overall higher DWR in the outdoor pot experiment, the percentage was only 7%. In contrast to what generally was found in the greenhouse experiments, relative differences in root weight between fresh and asparagus soil for the outdoor pot experiment were slight. The difference may have been caused by the larger pot volume available in the outdoor pot experiment, which allowed plants to grow vigourously during the whole experimental period, delaying infection and colonization and extending the period that the roots actively took up water and nutrients. This explanation is consistent with several authors' opinion that fusarium foot and root rot is stress-related [Damicone and Manning, 1985; Nigh, 1990].

Phoma terrestris was isolated from a few plants grown under field conditions (Expt 4), which confirms our earlier finding [Blok and Bollen, 1995] that this warm-region pathogen is present in the Netherlands.

ARED has been reported for fields in which the asparagus crop was abandoned some 20 to 25 years previously [Boonen *et al.*, 1977]. High persistence of ARED was established also in Expt 1, in which ARED symptoms appeared on plants grown in soil from a field where asparagus production was terminated 11 years before. Poll and Huiskamp [1992] also detected ARED in samples from fields where asparagus production was terminated 20–25 years before.

Of the four asparagus soils used in Expt 1, ARED was detected in all three soil layers. There was no decrease in disease severity with increasing soil depth. The fact that ARED-causing organisms are found to at least one meter deep needs to be considered when control strategies are developed. Grogan and Kimble [1959] found Foa, which they considered a major cause of early decline, at a depth of 1.5 m.

The conclusion that Foa is the main cause of ARED in the Netherlands prompts us to concentrate our research on epidemiology and control methods for this pathogen.

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References

Aldrich RA and Nelson PE (1969) Equipment for aerated steam treatment of small quantities of soil and soil mixes. Plant Disease Reporter 53: 784–788

Blok WJ and Bollen GJ (1993) The role of autotoxins from root residues of the previous crop in the replant disease of asparagus. Netherlands Journal of Plant Pathology 99, Supplement 3: 29–40

Blok WJ and Bollen GJ (1995) Fungi on roots and stem bases of asparagus in the Netherlands: Species and pathogenicity. European Journal of Plant Pathology 101: 15–24

Bollen GJ (1974) Fungal recolonization of heat-treated glasshouse soils. Agro-Ecosystems 1: 139–155

Boonen P, Heutinck F and Kuenen A (1977) Asperge op de keper III. Problemen rond de bodemmoeheid (Asparagus in focus III. Soil sickness problems). Groenten en Fruit 32: 1181

Bousquet V (1993) Aspergeraies du Languedoc-Rousillon, et le puceron déclencha la fusariose. Phytoma – La Défense des végétaux no 446: 52–54

Damicone JP and Manning WJ (1985) Frequency and pathogenicity of *Fusarium* spp. isolated from first-year asparagus grown from transplants. Plant Disease 69: 413–416

Elmer WH (1990) *Fusarium proliferatum* as a causal agent in Fusarium crown and root rot of asparagus. Plant Disease 74: 938

Endo RM and Burkholder EC (1971) The association of *Fusarium moniliforme* with the crown rot complex of asparagus. Phytopathology 61: 891

Gindrat D, Varady C and Neury G (1984) Le dépérissement de l'asperge. Revue suisse de Viticulture, Arboriculture et Horticulture 16: 23–26

Greenhalgh FC and Clarke RG (1985) Use of fungicides to study significance and etiology of root rot of subterranean clover in dryland pastures of Victoria. In: Parker CA, Rovira AD, Moore KJ, Wong PTW and Kollmorgen JF (eds) Ecology and Management of Soilborne Plant Pathogens (pp. 234–236). American Phytopathological Society, St. Paul, USA

Grogan RG and Kimble KA (1959) The association of fusarium wilt with the asparagus decline and replant problem in California. Phytopathology 49: 122–125

Hartung AC (1987) Allelopathic Potential of Asparagus (*Asparagus officinalis* L.). Dissertation, Michigan State University, 142 pp

Huiskamp T and Poll JTK (1990) Development of a soil evaluation method for asparagus (*Asp. officinalis*) replanting. Acta Horticulturae 271: 267–268

Johnston SA, Springer JK and Lewis GD (1979) *Fusarium moniliforme* as a cause of stem and crown rot of asparagus and its association with asparagus decline. Phytopathology 69: 778–780

Komada H (1975) Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review of Plant Protection Research 8: 114–124

Manning WJ and Vardaro PM (1977) Soil fumigation and preplant fungicide crown soaks: effects on plant growth and fusarium incidence in newly planted asparagus. Plant Disease Reporter 61: 355–357

Nigh Jr EL (1990) Stress factors influencing *Fusarium* infection in asparagus. Acta Horticulturae nr 271: 315–322

Peirce LC and Miller HG (1993) Asparagus emergence in Fusarium-treated and sterile media following exposure of seeds or radicles to one or more cinnamic acids. Journal of the American Society for Horticultural Science 118: 23–29

Poll JTK and Huiskamp T (1992) The effect of the asparagus replant problem in time. Asparagus research newsletter 9 (no 2): 18–22

Poll JTK and Huiskamp T (1994) Ontwikkeling van een biotoets voor het aantonen van herinplantproblemen bij asperge (Development of a bio-assay to detect replant problems in asparagus, with summary in English). PAGV-report 185, PAGV, Lelystad, the Netherlands, pp. 75

Savory BM (1966) Specific Replant Diseases. Research review 1, Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 64

Schofield P (1991) Asparagus decline and replant problem in New Zealand. New Zealand Journal of Crop and Horticultural Science 19: 213–220

Schreuder W, Lamprecht SC, Marasas WFO and Calitz FJ (1995) Pathogenicity of three *Fusarium* species associated with asparagus decline in South Africa. Plant Disease 79: 177–181

Smiley RW and Craven MM (1979) *Fusarium* species in soil, thatch and crowns of *Poa pratensis* turfgrass treated with fungicides. Soil Biology and Biochemistry 11: 355–363

Steel RGD and Torrie JH (1960) Principles and Procedures in Statistics. McGraw-Hill, New York, pp. 481

Stephens CT and Elmer WH (1988) Use of an in vitro assay to evaluate sources of resistance in *Asparagus* spp. to Fusarium crown and root rot. Plant Disease 72: 334–337

Wacker TL, Safir GR and Stephens CT (1990) Effects of ferulic acid and *Glomus fasciculatum* and associated effects on phosphorus uptake and growth of asparagus (*Asparagus officinalis* L). Journal of Chemical Ecology 16: 901–910

Yang H-J (1982) Autotoxicity of *Asparagus officinalis* L. Journal of the American Society for Horticultural Science 107: 860–862