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Effects of soil fumigation and flooding on suppression of *Pythium* root rot in ornamental bulb culture

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Abstract

The effects of flooding and soil fumigation with *cis*-dichloropropene or methylisothiocyanate (MIT) on disease suppression against *Pythium* spp. were tested in pot and field experiments in sandy soil. Disease suppression was reduced by both flooding and fumigation treatments, resulting in severe infection in *Iris* and *Crocus* and reduction of bulb yields. It is demonstrated that the disease suppression has a biological nature, and that disease severity is more related to effects of the soil treatments on the soil microflora than to the initial inoculum density of *Pythium*. After flooding, disease suppression was restored within the experimental period of two years, whereas after fumigation, disease suppression was only partially restored. The effect of repeated fumigation in two consecutive years on the disease suppression was less severe than the effect of a single fumigation treatment prior to cultivation of a susceptible crop.

Introduction

Root rot caused by fungi of the genus *Pythium* (Pringsh.) is an important disease in bulb cultivation on sandy soils in the Netherlands. Several *Pythium* species are capable of causing root infection in *Iris*, *Crocus*, *Hyacinthus* and *Tulipa* (Van der Plaats-Niterink, 1981; Saaltink, 1969). Infection leads to retarded growth, wilting, yellowing, and finally premature die-off of the crop, caused by insufficient water uptake (Saaltink, 1969; Moore, 1979). In order to control *Pythium* root rot and several other soilborne diseases and weeds, soil fumigation and flooding are frequently applied. These control measures are performed at fallow in the summer period. There are many references to the control of *Pythium* spp. by fumigants and resulting yield increases (Goring, 1962; Kreutzer, 1961; Wilhelm, 1966). Since 1950, broad-spectrum soil sterilants, including 1,3-dichloropropene and sodium-N-methyldithiocarbamate (metam-Na) have been recommended for *Pythium* control in flower bulb culture (Weststeijn and De Rooij, 1974). Under practical conditions, however, the effects of fumigants against *Pythium* spp. were inconsistent (Koster and De Rooij, 1980). The efficacy of soil fumigation depends on tolerance of target organisms and escape, for example in deeper soil layers. The occurrence of severe damage caused by *Pythium* after a biocidal treatment is usually explained by inadequate application techniques, high temperatures during or shortly after application of the fumigant, or by reintroduction of the fungus with planting material. Nevertheless, inconsistent effects also occur under optimal conditions for soil fumigation combined with the use of disinfected planting material. Flooding is applied to control pathogens other than *Pythium* (Muller, 1987; Van Zaayen et al., 1986). As a result of flooding, marked qualitative and quantitative microbiological changes take place in soil

(Gochenaur, 1981; Mitchell and Alexander, 1962) and many species die, illustrated by the effective control of several pathogens (Muller, 1987; Van Zaayen et al., 1986). Growers are frequently confronted with enhanced damage by *Pythium* after flooding. Shokes and McCarter (1979) detected *Pythium* spp. in irrigation water sources in Georgia. Gill (1970) demonstrated that pathogenic species of *Pythium* persisted in irrigation ponds and mentioned numerous similar studies that reinforce this point. It must be concluded that *Pythium* spp. can survive in water sources used for flooding as well as in floodwater itself, in the water–soil interface of flooded fields, and in floating or submerged plant debris (Strandberg, 1987).

Both fumigation and flooding are known to also eliminate non-target organisms. Much research has been done on this subject (Bollen, 1979; Domsch et al., 1983; Martin et al., 1957; Mitchell and Alexander, 1962; Powlson, 1975). It is well known that *Pythium* spp. are opportunists whose activity is significantly influenced by other organisms of the soil microflora (Hendrix and Campbell, 1973; Chen et al., 1988). Plant pathogenic *Pythium* spp. are suppressed through a mechanism known as 'general suppression', based on the numbers of nonspecific microorganisms competing with the saprophytic activities of *Pythium* spp. (Chen et al., 1988; Mandelbaum and Hadar, 1990; Hoitink et al., 1996). In soils with high propagule densities of other primary colonizing fungi and, hence, greater competition, *Pythium* spp. have low saprophytic activity and subsequently caused less disease (Bouhot and Joannes, 1979).

Considering the fact that *Pythium* spp. can survive or be (re-)introduced after fumigation or flooding, while many other microorganisms will be reduced or eliminated, the question was raised whether these cultural practices could have an adverse effect on the occurrence of *Pythium* root rot in flower bulb culture on sandy soil. The aim of this research was to study the effect of fumigation and flooding on the population density of *Pythium* and on disease suppression against the fungus as reflected by disease severity and yield in *Iris* and *Crocus*.

Materials and methods

Soil and plant material

Field experiments were performed in sandy soil, with a low content of organic matter (1–1.5%) and a pH 7, at the Bulb Research Centre in Lisse, The Netherlands. For pot experiments, soil was collected from the field, pasteurized (2 h at $\geq 70\,^{\circ}$C) to eliminate native pathogens and left to be recolonized by microorganisms in open air for 6 months prior to use. This soil is referred to as untreated or non-sterilized soil. Soil sterilization for pot experiments was performed by autoclaving (90 min 121°C, twice with an interval of 48 h).

Plant material used for pot experiments were bulbs of Dutch *Iris xyphium* 'White van Vliet' (size 7–8 cm circumference) and corms of *Crocus ancyrensis* 'Golden Bunch' (size 6–7 cm). For field experiments, *Iris xyphium* 'White van Vliet' (size 5–6 cm), *Crocus vernus* 'Flower Record' (size 5–6 cm) and *Narcissus pseudonarcissus* cv. 'Carlton' (size 14 cm) were used. All bulbs were disinfected by submerging in a solution of 0.4% formaldehyde (1% formalin, 40% a.i.) for 15 min prior to planting. In field experiments, crops were planted in October and treated according to standard cultivation practice for bulb production. For pot experiments, bulbs and corms were planted in 1-l pots (5 per pot, 5 pots per soil treatment, unless stated otherwise). Pots were placed in randomized blocks in a climate chamber at 18 °C for 8 weeks for *Iris*, and 9 weeks at 9 °C followed by 3 weeks at 18 °C for *Crocus*, according to standard practice for flower production.

Pythium *inoculum and population density assessment in pot experiments*

For experiments with *Iris*, soil was artificially infested with a 3-week-old sand-oatmeal culture of *P. macrosporum* Vaartaja and Van der Plaats-Niterink sp. nov. (isolate P60) to a content of 5% (v/v) and for experiments with *Crocus* with a sand-oatmeal culture of *P. irregulare* Buisman (isolate I1683) to a content of 2% (v/v).

Soil samples (30 g) were oven-dried at 105 °C for 12 h and reweighed to determine soil moisture contents. The remainder of the samples was stored at 5 °C until further processing the next day. *Pythium* population density (PD) was determined by a modified method, adapted from the soil-drop-method of Stanghellini and Hancock (1970). Dilutions of 1 : 1 and 1 : 5 (g dry soil adjusted for moisture content, ml^{-1} sterile 0.2% water agar at 35 °C) were used for *P. macrosporum* and *P. irregulare* respectively, based on anticipated *Pythium* population densities. Soil dilutions were thoroughly mixed for 30 s on a Vortex tube mixer. One ml aliquots of each mixture were removed

with an electronic digital pipette (Rainin EDP2) and dispensed in 40 drops of 10 µl on the surface of 4 Petri dishes (9 cm diameter, 10 drops per dish) containing a selective medium of 2% corn meal agar (Oxoid) with 25 µg ml^{-1} pimaricin (Merck) and 1 µg ml^{-1} terramycin (oxytetracyclin HCl, Pfizer). Dishes were incubated at room temperatue for 48 h and the number of droplets with *Pythium*-outgrowth was counted. The percentage positive droplets per replicate was determined.

Fumigation experiments

Experiment 1. For pot experiments, fumigation was performed with *cis*-dichloropropene (DCP) 0.08 ml l^{-1} soil (Nematrap 1160 g l^{-1}, Cyanamid Agro, Breda, The Netherlands) or with methylisothiocyanate (MIT) 0.13 ml l^{-1} soil (Trapex 40% a.i., Schering, Boxtel, The Netherlands), the active breakdown product of metam-Na. It was assumed that the efficacy of MIT was equivalent to metam-Na. Soil (5 l) was fumigated with MIT or DCP in closed, double plastic bags (0.1 mm thick), incubated at 20 °C for 5 weeks and allowed to evaporate for one week. The effect of soil fumigation on disease suppression against *Pythium* was determined in sterilized and non-sterilized soil. This soil was fumigated with MIT or DCP or not fumigated and subsequently infested with *Pythium* or not infested and planted with *Iris* or *Crocus*. At the end of the growing period, soil samples of infested treatments were taken from each pot to determine *Pythium* PD and to assess disease severity. Means per treatment were calculated. The experiment was performed 3 times with *Iris* and twice with *Crocus*. Each experiment was considered as a replicate.

Experiment 2. In order to determine the effect of fumigation on bulb yield caused by reduction of disease suppression and to investigate whether these effects exceed one growing season, a two-year field experiment was performed from 1995 to 1997 and repeated from 1996 to 1998 on a field plot with no or little natural *Pythium* infestation. Soil was injected by a professional injection machine at 18 cm depth with metam-Na 160 l ha^{-1} (AAMonam 510 g l^{-1}, AgrEvo, Haren, The Netherlands) in August according to standard application and covered with cellulose pulp (30 t ha^{-1}) in order to prevent evaporation. It was found that there was no effect of fumigation on *Crocus* yield induced by an infestation potentially present in the soil at the beginning of the experiment. Fumigation treatments were performed in the first and the second year (Table 1). In year 1, soil was fumigated or not fumigated and *Narcissus* was cultivated as a non-susceptible fore-crop. In year 2, naturally infested soil was collected from a severely infested field where the disease had been observed in *Crocus* during the preceding season. Six weeks after the fumigation treatment in August (year 2), plots were infested by mixing naturally infested soil (3 l m^{-2}) through the upper 30 cm. In non-infested plots furalaxyl 15 kg ha^{-1} (Fongarid 25 Wp 25% a.i., Ciba-Geigy Agro B.V., Roosendaal, the Netherlands), a fungicide with specific action against Oomycetes (Tomlin, 1994), was mixed through the soil to prevent infestation by cross-infection from infested plots. Treated field plots were 2 × 2.25 m. *Crocus* corms were planted (160 m^{-2}) on 1 × 1.25 m within the treated plots and yields were determined. Due to the impracticability to apply fumigation treatments to small units, the concept of a split plot design was used for designing the experimental layout. Each of 3 blocks contained 4 subplots, and subplots were divided into 2 units. Thus, for establishing treatment effects, 2 levels of variation were involved. Levels of fumigation in year 1 and year 2 were allocated to 4 subplots per block, and levels of *Pythium* infestation to units within subplots. Within each subplot, the infested treatment was performed twice and the non-infested treatment once, resulting in 3 units per subplot. Summarizing, each block consisted of $4 \times (2 + 1) = 12$ units. In order to perform disease assessments on *Crocus* roots without disturbing the field plots and without damaging the roots by harvest procedures, a system using pipes as plant containers was used as described in Van Os

Table 1. Summary of soil treatments in experiment 2. Soil was fumigated or not with metam-Na in 2 successive years and, subsequently, infested or not with *Pythium* spp. prior to planting

	Year 1	Year 2	
	Fumigation	Fumigation	Infestation
1	−	−	−
2	−	−	+
3	−	+	−
4	−	+	+
5	+	−	−
6	+	−	+
7	+	+	−
8	+	+	+

et al. (1998). Soil (5 l) was taken from the upper 30 cm soil layer of all plots from infested treatments and from one non-infested treatment (control, without fumigation). This soil was transferred into plastic pipes (1 pipe for each plot) and buried in the field. *Crocus* corms were planted in the pipes and roots were assessed for disease severity in April. The whole experiment was performed twice.

Flooding experiments

Experiment 3. A pot experiment was performed with *Iris* and *Crocus* to determine the effect of flooding on *P. macrosporum* and *P. irregulare* respectively. Flooding treatments were performed in plastic polyvinyl chloride (PVC) pipes according to Asjes et al. (1996). Pipes (length 70.0 cm; diameter 10.0 cm), covered with fine nylon mesh at the bottom, were filled with 5 l soil, leaving 10 cm for a water layer on top, and placed in wider and slightly shorter pipes (length 60.7 cm; diameter 12.0 cm). These outer-pipes were closed with a watertight PVC lid at the bottom. Pipes were flooded with water for 8 weeks and drip irrigation was placed in inner-pipes to provide a continuous supply of water. A constant water percolation of 5.5 mm day^{-1} through the soil was established by a height difference of 3 mm between inner- and outer-pipes. In flooded bulb fields percolation varies between 0.5 and 6.0 mm day^{-1}, depending on soil profile. Flooding was performed at 18 °C, which resembles soil temperature under field conditions in August in the Netherlands. Pipes were filled with either sterilized soil or non-sterilized soil, infested or not infested, and subsequently flooded or not flooded, in 4 replicates per treatment. After the water was drained, soil contents of each pipe were divided over 3 pots. Soil samples of infested treatments were taken from each pot to determine *Pythium* PD and pots were planted with *Iris* or *Crocus* and disease severity assessed. Means of 3 pots per pipe were used in statistical analysis. The experiments were performed twice with each crop.

Experiment 4. In order to determine the effect of flooding on yield reduction caused by *Pythium* a field experiment was performed with *Iris*. Flooding was performed in buried polyester containers (1.40 m long, 0.85 m wide, 0.85 m deep). Containers were filled with untreated field soil with a natural infestation of *Pythium* spp. Each container was equipped with an individual drainage system. Flooding was established by blocking the drainage system and filling the container with water up to 5 cm above soil surface. Flooding was performed for 8 weeks in August and September. At the end of the flooding period, water was drained to a level of 60 cm below the soil surface, resembling standard ground water level in ornamental bulb culture on sandy soils. In non-flooded containers this water level was maintained constantly. Prior to planting, soil in all containers was tilled. Soil was flooded or not flooded and furalaxyl 20 kg ha^{-1} was added in control treatments to inactivate all *Pythium* spp. (*in vitro* tests revealed no resistance of *Pythium* spp. to furalaxyl). Bulbs were planted in 4 replicates per treatment (300 bulbs per container) and yields were determined.

Experiment 5. A two-year experiment was performed to examine whether effects of flooding exceed one growing season. Soil was flooded or not flooded and subsequently planted with *Iris* or with *Narcissus* (85 bulbs per container) as a non-susceptible fore-crop for the second year. For each crop, 4 replicates per treatment were planted. Following *Narcissus* cultivation in the first year, *Iris* bulbs were planted the second year. Bulb yields were determined. The experiment was performed twice.

Effect of inoculum density on infection

Experiment 6. A pot experiment was carried out to determine the effect of soil treatments on disease suppression against *P. macrosporum* at different inoculum densities. A dilution series of inoculum (10.0%, 5.0%, 1.0%, 0.5%, 0.0% v/v) was applied in untreated, fumigated (MIT), flooded and sterilized soil. *Iris* bulbs were planted (4 soil treatments $\times$ 5 inoculum levels $\times$ 5 pots $=$ 100 pots in total) and disease severity was assessed.

Reintroduction of microflora in treated soil

Experiment 7. A pot experiment was performed to determine whether effects of soil fumigation (MIT), flooding and sterilization on disease suppression against *P. macrosporum* could be reversed by applying the original soil microflora from untreated soil. Treated soil was supplemented with 0.0%, 1.0%, or 5.0% (v/v) of untreated soil one week prior to infestation and planting of *Iris*. In addition, 3 control treatments were included: untreated soil, sterilized soil supplemented

with 5% (v/v) sterilized soil, and a non-infested control treatment (untreated soil). At the end of the growing period soil samples were taken from each pot to determine *Pythium* PD, and disease severity was assessed.

Disease severity and yield assessments

For disease severity assessment, roots were washed with tap water at the end of the growing period, and root-rot ratings of infested treatments were related to the healthy root systems of non-infested control treatments. Roots were visually assessed for root-rot severity using a disease index ranging from 0 to 5, where 0 = no root rot, 1 = 1–20%, 2 = 21–40%, 3 = 41–60%, 4 = 61–80%, and 5 = >80% root rot, i.e., relative loss of healthy root mass induced by infection (Van Os et al., 1998). Root rot was determined for each plant individually and a mean root-rot index per pot was calculated. In field experiments, *Crocus* corms and *Iris* bulbs were harvested at the end of the growing season, in June and August respectively, and yields (bulb weight g plot^{-1}) were determined.

Statistical analysis

Data of *Pythium* PD were analysed as binomial proportions using a generalized linear model (GLM) and a logit link in order to determine soil-treatment effects. Root-rot ratings were converted to percentages and a binomial regression using a GLM was performed. In experiments 6 and 7, inoculum densities and quota of untreated soil respectively were log-transformed in order to achieve linearity on the linear scale. Bulb yields were analysed using analysis of variance (ANOVA). Data from repeated experiments were analysed together. All calculations were performed using the statistical programming language Genstat 5 (Genstat 5 Committee, 1993; Goedhart and Thissen, 1992). T-tests were used to determine all pair-wise differences of means at significance level $P < 0.05$.

Results

In non-infested treatments no pathogenic *Pythium* was observed, i.e. no root infection in *Iris* or *Crocus*, nor outgrowth on corn meal agar in the population density assessments.

Fumigation experiments

Experiment 1. In sterilized soil, both *Iris* and *Crocus* were severely infected, and inoculum densities of *P. macrosporum* and *P. irregulare* after crop cultivation were high, compared to non-sterilized soil (Table 2), indicating lack of disease suppression. Fumigation with MIT or DCP had no effect on disease development in sterilized soil. In non-sterilized soil, with a natural microbial population, infestation resulted in a mild infection of *Iris* and *Crocus*. After fumigation, both tested *Pythium* species caused significantly more infection, up to a level as high as in sterilized soil, indicating a reduction of the natural disease suppression which occurred in untreated soil. *Pythium* population densities after crop cultivation correlated with disease

Table 2. Effect of fumigation on disease suppression (pot experiment). Disease severity (% infection) in *Iris xyphium* 'White van Vliet' and *Crocus ancyrensis* 'Golden Bunch' and population densities (% droplets with *Pythium*−outgrowth) of *P. macrosporum* (PD_m) and *P. irregulare* (PD_i) after cultivation of respectively *Iris* and *Crocus* in soil sterilized or not, fumigated with MIT, DCP or not, and infested before planting

Soil treatment		*Iris*		*Crocus*	
Sterilized	Fumigation	% infection	PD_m	% infection	PD_i
+	−	76 a[1]	62 a	66 a	62 a
+	MIT	77 a	57 a	59 a	58 a
+	DCP	77 a	58 a	71 a	66 a
−	−	11 c	7 c	27 b	34 b
−	MIT	62 ab	27 b	55 a	58 a
−	DCP	53 b	17 bc	52 a	55 a

[1]Treatment means within columns followed by the same letter do not differ significantly ($P < 0.05$, Student's two-tailed t-test).

severity, showing similar tendencies and significant effects as a result of soil treatments.

Experiment 2. In non-infested treatments, fumigation with metam-Na had no effect on *Crocus* yields (Table 3). Apparently, no pathogens were present in the soil which were influenced by the fumigation treatment. Infestation of non-fumigated soil resulted in a considerable yield reduction caused by *Pythium*. In soil, fumigated 6 weeks prior to infestation and planting, a further yield reduction occurred compared to non-fumigated soil, indicating reduction of disease suppression as a result of the fumigation treatment. Fumigation one year prior to infestation and planting also resulted in a further yield reduction compared to the non-fumigated treatment. This indicates that disease suppression was still reduced to a certain extent one year after the treatment, although a significant recovery was observed compared to fumigation 6 weeks prior to infestation and planting. Repeated fumigation (in years 1 and 2) resulted in further yield reduction compared to a single fumigation in year 1. However, the yield reduction was less than after a single fumigation in year 2. Root infection was enhanced in all fumigated treatments (Table 3). However, no significant differences were found between different fumigation applications because of large variation between replicates.

Table 3. Effect of fumigation on disease suppression (field experiment). Relative yield of *Crocus vernus* 'Flower Record' in soil fumigated or not with metam-Na in growing season 1 and/or 2, prior to infestation or not with *Pythium* and planting in growing season 2; and disease severity (% infection) in *Crocus* in infested treatments (growing season 2)

Fumigation		Relative yield		% infection
1	2	− *Pythium*	+ *Pythium*	
−	−	[1]100 a[2]	89 b	12 a
−	+	98 a	76 e	28 b
+	−	101 a	84 c	22 b
+	+	101 a	80 d	26 b

[1]Bulb yield of this treatment is standardized to 100%.
[2]Treatment means followed by the same letter do not differ significantly ($P < 0.05$, Student's two-tailed *t*-test).

Flooding experiments

Experiment 3. In non-flooded treatments, PDs of both *Pythium* species were significantly higher in sterilized soil than in non-sterilized soil (Table 4). This was also evident in the percentage of root infection which was higher in sterilized soil than in non-sterilized soil for both crops, indicating the presence of a certain level of natural disease suppression in non-sterilized soil. Flooding reduced PDs in sterilized soil and this resulted in less infection in *Crocus*, but not in *Iris*. In non-sterilized soil, flooding had no effect on *Pythium* PDs, but the treatment induced a significant increase of infection in both crops, indicating reduction of the natural disease suppression.

Experiment 4. Addition of furalaxyl to non-flooded soil had no effect on the bulb yield (Table 5). Apparently, no significant damage was caused by the natural *Pythium* infestation in non-flooded soil. Flooding resulted in a yield increase when *Pythium* was inhibited

Table 4. Effect of flooding on *Pythium* and disease suppression (pot experiment). Population densities (% droplets with *Pythium*-outgrowth) of *P. macrosporum* (PD_m) and *P. irregulare* (PD_i) and disease severity (% infection) in respectively *Iris xyphium* 'White van Vliet' and *Crocus ancyrensis* 'Golden Bunch' in sterilized (+) and non-sterilized (−) soil after flooding (+) or not (−)

Soil treatment		*Iris*		*Crocus*	
Sterilized	Flooding	PD_m	% infection	PD_i	% infection
+	−	48 a[1]	83 a	48 a	89 a
+	+	23 b	78 a	28 b	73 b
−	−	3 c	17 c	27 b	37 c
−	+	2 c	45 b	32 b	79 b

[1]Treatment means within columns followed by the same letter do not differ significantly ($P < 0.05$, Student's two-tailed *t*-test).

Table 5. Effect of flooding on *Pythium* and disease suppression (field experiment). Relative yield of *Iris* 'White van Vliet' in naturally infested soil after flooding (+) or not (−), and with (+) or without (−) addition of furalaxyl prior to planting

Flooding	Furalaxyl	
	−	+
−	[1]100 b[2]	102 b
+	90 c	115 a

[1]Bulb yield of this treatment is standardized to 100%.
[2]Treatment means followed by the same letter do not differ significantly ($P < 0.05$, Student's two-tailed *t*-test).

by furalaxyl, indicating suppression of other pathogens present in the soil. This beneficial effect of flooding may also be expected to occur in the flooded treatment without furalaxyl. However, without furalaxyl the beneficial effect of flooding was negated and, moreover, an additional yield loss occurred. Since *Pythium* caused no significant yield reduction in non-flooded soil, it can be concluded that flooding reduced disease suppression, resulting in a yield loss of 25% caused by enhanced root infection by *Pythium*.

Experiment 5. Flooding of infested soil followed by cultivation of *Iris* resulted in a significant 15% yield reduction compared to the non-flooded treatment. Flooding had no effect on *Narcissus* yields. When *Iris* was cultivated succeeding *Narcissus*, one year after the flooding treatment, no significant effects of flooding were observed. Apparently, disease suppression against *Pythium* was restored.

Effect of inoculum density on infection

Experiment 6. Disease severity in *Iris* was significantly enhanced at higher infestation levels (Figure 1). Independent of inoculum density, soil treatments had a major effect on disease severity. Percentage of infection was lowest in untreated soil and progressively increased in flooded, fumigated and sterilized soil.

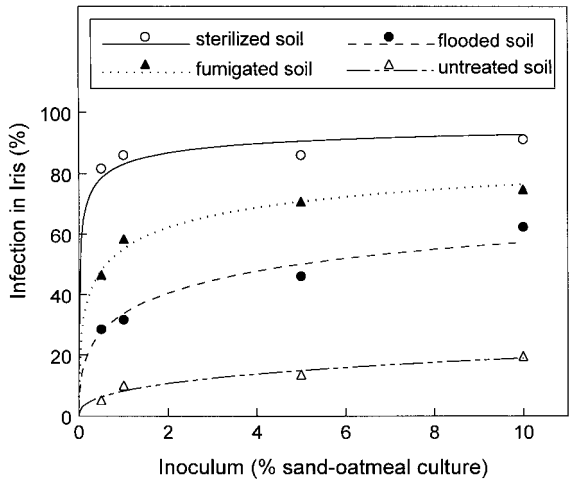

Figure 1. Effect of inoculum density on infection. Observed means (symbols) and GLM-predicted curves (lines) for disease severity (% infection) in *Iris xyphium* 'White van Vliet' in sterilized, fumigated, flooded and untreated soil after infestation with a series of inoculum densities of *Pythium macrosporum* (% sand-oatmeal culture) prior to planting.

Reintroduction of microflora in treated soil

Experiment 7. Again, progressive disease development occurred in flooded, fumigated and sterilized soil. Disease severity was significantly reduced with increasing amount of untreated soil applied to treated soil (Figure 2), implying re-establishment of disease suppression. Addition of 5% sterilized soil to sterilized soil had no significant effect on disease development (85% infection, data not shown), indicating the essential role of the soil microflora in suppression of *P. macrosporum*. In neither soil treatment, the disease suppression was completely restored compared to 12% infection which was found in untreated soil (data not shown). PD of *P. macrosporum* after crop cultivation (data not shown) correlated with disease severity, with similar tendencies and significant effects as a result of soil treatments.

Discussion

The more effective a biocidal soil treatment, the smaller the population of survivors and the slower the recolonization (Powlson, 1975). This repopulation varies considerably according to the compound or method used and may lead to a typical microflora (Welvaert, 1974). The organisms which survive the treatment or

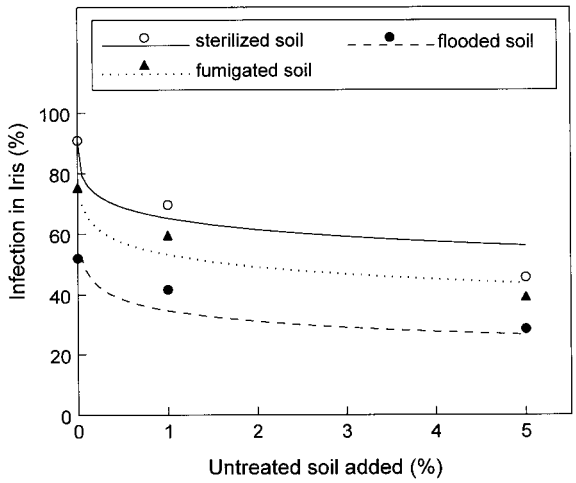

Figure 2. Reintroduction of microflora in treated soil. Observed means (symbols) and GLM-predicted curves (lines) for disease severity (% infection) in *Iris xyphium* 'White van Vliet' in sterilized, fumigated and flooded soil supplemented with 0%, 1% and 5% of untreated soil one week prior to infestation with *Pythium macrosporum* and planting.

those which become re-established first reach very high numbers in a less competitive environment (Martin, 1972). Often one species becomes dominant. Of the fungi, species of *Trichoderma*, *Penicillium* spp., *Mucor* and several others usually predominate, owing to their short response time and their fast growth rate (Powlson, 1975; Welvaert, 1974; Warcup, 1952). Unfortunately, *Pythium* spp. are also rapid colonisers of (partially) sterilized soil (Kreutzer, 1960).

In our experiments on the effects of fumigation and flooding on the disease suppression against *Pythium*, results of pot experiments were confirmed by the outcome of field experiments. Untreated soil had a level of disease suppression against *Pythium*, which was reduced after soil fumigation or flooding. In the absence of the soil microflora (sterilized soil) no disease suppression was observed. It is concluded that disease suppression has a biological nature. This was confirmed by the partial restoration of disease suppression by reintroduction of the original microflora in sterilized, fumigated and flooded soil. The experimental period may have been too short for full recovery of disease suppression, since different species of the original microflora, including possible antagonists, become re-established at various periods after the treatment and may or may not reach the quantities found in the original soil (Martin, 1972). Bouhot (1979) stated that for prediction of disease, ecological conditions favourable for the saprophytic growth of *Pythium* are more important than the initial inoculum density. This is confirmed by our experiments, in which differences in levels of disease suppression between soil treatments were independent of inoculum densities of *P. macrosporum* (Figure 2) and levels of disease were related less to the inoculum density of the pathogen than to soil treatments.

Diseases that are increased by control measures are called iatrogenic diseases (Griffiths, 1981). Whereas stimulation of antagonism can lead to integrated and indirect disease control, conversely, inhibition of antagonism can result in a change of the dominant pathogens (Bollen, 1979). The presented results imply that *Pythium* root rot can occur as an iatrogenic disease in bulb crops after both soil fumigation or flooding. This may occur particularly if a susceptible crop is grown immediately after treatment, thereby providing abundant susceptible roots for the pathogen. Under these circumstances, *Pythium* is more likely to become one of the dominant species in the soil. An adequate delay between soil treatment and cultivation of a susceptible crop might provide a greater advantage to other species as colonists of the soil.

The control of *Pythium* by flooding has been reported. Strandberg (1984) showed that soil populations of *Pythium* spp., that cause cavity spot on carrots and other vegetables, rapidly decreased during a 4–6 week flooding period, and increased slowly in time after draining and cultivation of the fields. Populations remained low long enough (30–90 days) to allow the production of a carrot crop under relatively low *Pythium* population levels. These findings do not correspond with the results from our experiments, where reduction of *P. macrosporum* and *P. irregulare* by flooding was not observed in non-sterilized soil, and, moreover, an enhanced infection and yield reduction occurred after draining and planting of a susceptible crop. *Pythium* species responsible for cavity spot are *P. violae* Chester and Hickman (Montfort and Rouxel, 1988) and *P. sulcatum* Pratt and Mitchell (Van der Plaats-Niterink, 1981). These species may respond in a different way to flooding compared with *P. macrosporum*, *P. irregulare* and the *Pythium* spp. studied in our experiments. Since the most susceptible period for *Iris* during cultivation in the field is from early spring till the end of the growing season (Van Os et al., 1998), it is possible that enhanced *Pythium* activity lasted at least during that period, i.e. 10 months. In the following growing season, one year after the

flooding treatment, no effect on disease development was observed in *Iris*. Therefore, it is concluded that disease suppression was restored before spring in the second year after flooding.

For fumigation, contradictory experiences exist for the overall effects on *Pythium* in flower bulb culture. Fumigation often results in a yield increase. However, in some cases fumigation seems to have adverse effects. Similarly, in the literature on pesticide effects on the microflora, contradictory results have also been obtained (Kreutzer, 1965). The fungal species that becomes dominant after a biocidal treatment will be determined by a combination of many factors, such as the type of treatment, the chemical and physical properties of the soil, the relative abundance of different species in the original soil, whether the soil is reinoculated either by chance or deliberately, and the nature of the cultivated crop after the treatment. Because the dominant species often differ from soil to soil, it is understandable that for pesticides with selective actions, contradictory results are obtained (Bollen, 1979; Powlson, 1975). Our field experiments showed that disease suppression against *Pythiumin* was still reduced one year after fumigation, although partial restoration had occurred. Since the susceptible period for *Pythiumin Crocus* is within two months after planting (Van Os et al., 1998), it is concluded that recovery of disease suppression takes longer than one year and two months after fumigation with metam-Na. Surprisingly, the damage caused by *Pythium* after repeated fumigation in two successive years was less severe than after a single application prior to cultivation of *Crocus*. Theoretically, this phenomenon could be due to adaptation and selection of tolerant species, which, after the first fumigation, recolonized the soil and would also survive a repeated treatment. The impact would therefore be less radical the second time. If this hypothesis is correct, repeated fumigation in consecutive years will result in disease suppression at a lower level than in untreated soil, but higher than shortly after a single fumigation treatment. Different periods for recovery of the fungal microflora after soil fumigation are reported (Martin, 1972; Reber, 1967; Welvaert, 1974). Verhagen et al. (1996) found recovery of the microflora within three years after application of metam-Na and requiring more than three years after application of DCP.

Fumigation and flooding have different modes of action for disease control and different species of the soil microflora are killed as a result of these measures. Effects on microbial biomass, activity and diversity are subjects for further research. With respect to disease suppression, it is relevant to differentiate reversible influences from persistent influences. Eventually, disease suppression seemed to recover completely after flooding. Gochenaur (1981) reported an eventual reconstruction of the microbial community both quantitatively and qualitatively within as short a period as three or four months after flooding. Apparently, the effects of flooding are reversible. For fumigation on the other hand, no complete recovery of disease suppression was observed within the experimental period. Persistent effects cannot be excluded. In order to find a way to restore disease suppression and accelerate the recovery after fumigation and flooding, possibilities for (re-) introduction of specific antagonists or an over-abundance of competitive microorganisms could be an interesting option.

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