Soil Suppressiveness towards *Meloidogyne*, *Verticillium* or *Pythium* in Greenhouse Horticulture

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Abstract

Soils originating from fourteen greenhouse horticultural companies were assessed for the level of suppressiveness of three major pathogens of vegetables and flowers, namely the root knot nematode *Meloidogyne incognita*, *Pythium aphanidermatum* and *Verticillium dahliae*. As controls, three well-documented soils in terms of disease suppressiveness and coarse sand were included for each pathogen.

Soils were distributed over three treatments with forty replicates each in a random block design under standardized conditions. Each soil was treated with γ-radiation in order to assess the contribution of abiotic properties to disease suppressiveness. Non-sterilized soils served as a measure of abiotic- and biotic factors, and soil without the addition of pathogens served as a control to determine background contamination of indigenous pathogens.

Soils were acclimatized for one week. Afterwards, irrigation was started and pathogens were inoculated. Survival stages of *V. dahliae* or *P. aphanidermatum* were added as pure suspensions, i.e., microsclerotia or oospores, respectively. For *Meloidogyne*, second stadium larvae (J2) were used. Seedlings were planted for *M. incognita*, while for *P. aphanidermatum* and *V. dahliae*, seeds were used.

A large variation in the level of suppressiveness was observed. Soil suppressiveness towards *Meloidogyne*, *Verticillium* or *Pythium* was not correlated. This means that suppressiveness is pathogen dependent. However, soil structure contributes to a large extent to the level of suppressiveness as exemplified by the survival rate of *Meloidogyne* and *Pythium* in sandy soils.

With this dataset we aim to unravel the underlying mechanisms and investigate whether suppressiveness levels can be increased and used in an integrated soil management system.

INTRODUCTION

Outbreaks of soil-borne plant diseases in horticulture are generally determined by presence and density of pathogen propagules, host plant resistance and soil characteristics. However, presence of pathogen propagules does not per se result in crop damage. This is described by the phenomenon of soil suppressiveness (Baker and Cook, 1974). If soil suppressiveness could be stimulated, it would represent a sustainable means for the control of plant pathogens.

Examples of mechanisms involved in suppressiveness are competition for nutrients such as iron, carbon or root exudates, natural enemies, physical protection of roots by, e.g., endophytes and induced systemic resistance.

Suppressive soils have been described for many soil-borne pathogens (Weller et al., 2002). Although extensive progress has been made in documenting mechanisms of suppression, few studies have attempted to investigate the competence of soils when exposed to different pathogens. The latter is important since the level and mechanism of suppressiveness may be dependent on the identity of the pathogen.

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In order to investigate the interaction between suppressiveness and pathogen identity, a selection of pathogens was made based on differences in survival strategy, life history traits and phylogeny. Three species were selected, *Meloidogyne incognita* (Nematoda), *Pythium aphanidermatum* (Heterokontophyta) and *Verticillium dahliae* (Ascomycota).

The nematodes (Nematoda) are one of the most diverse of all animals (Holterman et al., 2006). Over 28,000 species have been described, of which over 16,000 are parasitic. An example is the root-knot nematode (*Meloidogyne* spp.). They are one of the most economically damaging genera of plant-parasitic nematodes on horticultural and field crops, as they cause approximately 5% of global crop loss (Sasser et al., 1983).

Oömycota or oömycetes form a distinct group of fungus-like eukaryotic microorganisms. Many oömycetes are aggressive plant pathogens. *Pythium* damping off is a very common problem in greenhouses, where the organism kills newly emerged seedlings.

The sac fungi (Ascomycota) are the largest phylum of Fungi, with over 64,000 species. *Verticillium dahliae* and *V. albo-atrum* cause a wilt disease in more than 400 eudicot plant species.

The objective of this research was to investigate variation of soil suppressiveness in greenhouse horticulture and its dependence on pathogen identity and soil structure. Future research will aim to unravel the underlying mechanisms and investigate whether suppressiveness levels can be increased and used in an integrated soil management system.

MATERIALS AND METHODS

Sampling

Experiments were performed using topsoil (0 to 30 cm) collected from eighteen soils of which seven originated from greenhouses of organically grown tomato, seven from conventionally grown *Chrysanthemum*, three from an experimental field and a negative control of coarse sand with no horticultural history (Table 1). An experimental field (Wageningen UR, Lisse, The Netherlands) was used from 2006 onwards and represents a well-documented history of increased suppressiveness to *Meloidogyne hapla* and *Pythium ultimum* with an organic matter level of 0.8, 1.8 and 2.1% (G. van Os, unpubl. data).

Soils were sampled in October 2009 and sieved (<3 mm) within 7 days after sampling. Sample sites had not been subjected to occasional treatments with crop protection agents and were visually selected based on absence of diseases in crops. Roughly one-third of each soil was sterilized with 25 kGy γ-irradiation; Gammaster, Ede, The Netherlands, and soils were subsequently stored at 12°C in dark. Soils were stored seven weeks prior to use in the *Pythium* and *Verticillium* bio-assays. For *Meloidogyne*, the majority of soils were stored for seven weeks. However, soils 12st, 10kl, 14we, 13wi and 15tu were again collected from the original source two weeks prior to the start of the experiment. These soils proved difficult to sieve owing to a high moisture content and a large amount of soil aggregates and this resulted in a low yield, i.e., not sufficient for all three experiments.

Bio-Assays

For each pathogen, a fully controlled greenhouse compartment of 144 m^2 was used with sunscreens closed and a light regime of 10,000 lux during 9:13 (D:N) with 70% RV. Compartment temperature was held at 30°C for *P. aphanidermatum* and 25°C for *V. dahliae* and *M. incognita*. For each pathogen, a randomized block design was used with two blocks. For each treatment per soil, 40 pots (TEKU TO 13D) of 800 ml were divided over two blocks. Each block consisted of four replicates, each with five pseudo-replicates placed in a tray. Trays of 30.5 x 40.5 cm contained a wetting filter covered with a black anti-algae plastic sheet. Each pot contained a 20 µM filter (Nedfilter B.V. The

Netherlands) at the bottom.

Each day, trays were irrigated automatically with drip nozzles so that pots were quickly water-saturated once a day. As plant size increased, pots were watered twice daily. The irrigation water contained a standard nutrient mix with EC 2.2 mS cm^{-1}, pH 5.5 (van der Gaag and Wever, 2005). Pots were covered with a transparent plastic film of 20 µM for one week prior to the addition of pathogens to prevent desiccation.

Three treatments were used: a non-treated control, a treatment with pathogen, and a sterilization of soil with pathogen addition. Per tray, soils of five pots were pooled, mixed with fungal inocula and redistributed over the pots. After four days, two week old *Capsicum annuum* L. 'Ferrari" (Enza seeds, The Netherlands) seedlings were planted for the *M. Incognita* treatment*,* while for the *P. aphanidermatum* and *V. dahliae* treatments*,* one *Cucumis sativus* L. 'Aramon' (Rijk Zwaan, The Netherlands) or three *C. annuum* L. 'Ferrari' seeds, respectively, were added to each pot. All plant material was organically propagated. After two weeks, excess plantlets in *Verticillium* pots were randomly removed, leaving one plantlet per pot. The experimental time frame was seven weeks for *P. aphanidermatum* and *M. incognita* and five months for *V. dahliae*. In the *Verticillium* experiment, only soils originating from conventional *Chrysanthemum* greenhouses, the experimental field and a control consisting of coarse sand were used.

In the *Pythium* experiment, some plants showed symptoms of *Phytophtera* spp. (Heterokontophyta) infestation, or seeds failed to emerge as a result of a poor nutrient status of sandy soils at the beginning of the experiment. These bio-assays were subsequently left out of the analyses. In addition, in the *Pythium* and *Verticillium* experiments, some data on bio-assays were missing due to procedural mistakes and were not used in the analyses. The final numbers of bio-assays per experiment that were used in the analyses are presented in Tables 2–4.

1. *Pythium aphanidermatum*. An isolate of *P. aphanidermatum* was grown on V8-medium for three weeks at room temperature, according to a modified protocol of van der Gaag and Wever (2005). Mycelium was gently rinsed with tap water, blended for 20 s, filtered through cheese cloth and the number of oospores was determined. Each pot was inoculated with 4.4×10^4 spores. Viability of oospores was checked on CMA agar. After seven weeks, plants with typical *P. aphanidermatum* symptoms were scored as present (1) or absent (0).

2. *Meloidogyne incognita*. *Meloidogyne incognita* J2 was obtained from HZPC Holland B.V., The Netherlands. The purity of the culture regarding other root-knot nematodes was assessed with genus specific markers by Blgg AgroXpertus (Wageningen, The Netherlands). The culture contained 99% *M. incognita* and 1% *M. hapla*. The viability of the larvae was assessed by eye, using a dissecting microscope. For each bio-assay, 8000 J2 larvae were inoculated on top of the soil in the middle of the pot. After seven weeks, the root-knot index (RKI) was determined on a scale from 0 to 10, 0 being no damage and 10 being the entire root covered with root knots. Roots of five plants per tray were pooled, cut into one cm pieces and mixed. Subsequently, 50 g of roots were incubated in the dark at 20°C for four weeks in a mistifier (Seinhorst, 1950). Following this treatment, juveniles (J2) were counted per 10 ml subsample and expressed as numbers per gram of roots.

3. *Verticillium dahliae*. An isolate of *V. dahliae* was grown on SSN agar for two months at RT, according to a modified protocol of Termorshuizen et al. (2006). Mycelium with microsclerotia (MS) was homogenized with an omnimixer for 20 s, filtered over a mesh screen of 50 µM and gently washed with tap water. Microsclerotia of 53–200 µM were collected and their viability was checked on MSEA agar. Each pot was inoculated with 5×10^4 MS. After five months, a 1 mm slice of each stem base was placed on MSEA agar and incubated at room temperature. After three weeks *V. dahliae* was scored as present (1) or absent (0).

Statistics

Data from *P. aphanidermatum* and *V. dahliae* were analyzed with a generalized linear mixed model (GLMM). For *M. incognita* RKI and J2 g^{-1} roots, a restricted maximum likelihood (REML) and GLMM were used, respectively. Tukey-Kramer tests were used in all pair-wise comparisons. All analyses were performed using GenStat 12.1.

RESULTS AND DISCUSSION

The results obtained with bio-assays may represent a variety of underlying mechanisms. They may be attributed to, e.g., competition for nutrients, direct action of antagonists and enhanced systemic response of host plants.

The results on sterilized soils have to be discussed with caution. Troelstra et al. (2001) showed an effect of γ-irradiation on the availability of nutrients for host plants in bio-assays, especially when sandy soils are used. When non-sterilized soils are more conducive to pathogens than sterilized soils, it can be attributed to a synergistic action of organisms, such as between *Pratylenchus* spp. and *Verticillium dahliae* (Powelson and Rowe, 1993) or an effect of increased nutrient availability on plant tolerance. When sterilized soils are more conducive to pathogens, this can be attributed to, e.g., an elimination of antagonists *sensu lato*. The latter is observed in the experiment with *Pythium* in sharp contrast to the experiment with *Meloidogyne* or *Verticillium*.

Pythium aphanidermatum

Soils did not show significant background contamination with *Pythium* spp. (Table 2). Although *Chrysanthemum* is considered a good host for *Pythium* and therefore background contamination of soils with *Pythium* was expected, the number of diseased plants in the bio-assays did not differ from those in soils originating from organically grown tomato.

When corrected for background contamination, soils such as 6ve and 11gr showed a significant level of suppression, while coarse sand, 3jo and 15top2.1 were conducive to pythium growth (Table 2). Since these soils are predominantly sandy, this may imply that soil structure may be a major component of soil suppressiveness towards *P. aphanidermatum*, where sandy soils may be more conducive compared with sandy clay and clay.

Meloidogyne incognita

In contrast to *Pythium,* the bio-assays not inoculated with *M. incognita,* revealed a significant background contamination (Table 3) in, e.g., soils 6ve and 8en.

The RKI and J2 were correlated in the non-sterilized soils (Pearson's r=0.62**, n=18) as well as in the sterilized soils (Pearson's r=0.50*, n=18). The variation of J2 is much larger than RKI and this resulted in a higher discriminative power when soils were compared. In general, the organic greenhouses performed better in terms of suppressive soil. This may be expected since organic soils have a long history of exposure to *Meloidogyne* and this may have resulted in a build up of suppression.

Verticillium dahliae

Only coarse sand exhibited a minor background contamination of *V. dahliae* (Table 4). Probably, it originated from the inoculated pots since it had no horticultural history.

About half of the soils showed a lower level of plant infestation in the sterilized soils. This may be attributed to the aforementioned effect of sterilization on nutrient availability and plant tolerance or the elimination of pathogens that act synergistically with *Verticillium*.

CONCLUSIONS

The methodology is robust, since results of *Meloidogyne* and *Pythium* in soils originating from the experimental field correspond significantly to previous results

obtained by fellow scientists at Wageningen UR institute of Applied Plant Research (PPO-BBF; G. van Os, unpubl. data). Interestingly, the enhanced suppression of *Meloidogyne* spp. and *Pythium* spp. with an increase of organic matter in soils 11top0.8, 13top1.8 and 15top2.1, respectively, did not depend on plant-, nor on pathogen identity, since previous results were obtained with bio-assays that included *Meloidogyne hapla* on *Lactuca sativa* L. 'Brighton' and *Pythium ultimum* on *Hyacinthus orientalis* L. 'Pink Pearl'.

A similar statement can be made with regard to pathogen identity and soil texture. Both *Meloidogyne* and *Pythium* seem to respond in a similar fashion, namely, "clay poor sand" was conducive for all pathogens when compared with other soils.

However, levels of suppressiveness of the soils, when exposed to *Pythium*, *Meloidogyne* or *Verticillium* were not correlated. This implies that suppressiveness is at least partly pathogen dependent.

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Tables

Table 1. Overview of characteristics of the eighteen soils used in the experiment.

Soil codes	Classification based on clay	Classification based on OM
	Reference	
sand	sand	moderate humose
	Organic tomato	
2vo	light clay	very humose
3jo	clayey sand	moderate humose
4di	heavy sandy clay	very humose
5oo	very light sandy clay	rich in humus
6ve	very late sandy clay	very humose
7ko	moderate light sandy clay	moderate humose
8en	clayey sand	very humose
	Chrysanthemum	
10kl	heavy sandy clay	rich in humus
11gr	very light sandy clay	very humose
12st	heavy sandy clay	very humose
13wi	light clay	peaty clay
14we	light clay	rich in humus
15tu	light clay	rich in humus
16hu	moderate light sandy clay	rich in humus
	Experimental field	
11top0.8	clay poor sand	poor in humus
13top1.8	clay poor sand	moderate humose
15top2.1	clay poor sand	moderate humose

Table 2. Fraction (back transformed means) of plants per treatment with *Pythium aphanidermatum*.

Soils	Control		*Pythium*		Corrected *Pythium*[3]	Sterilized, *Pythium*	
	Fraction	n[2]	Fraction	n	Fraction	Fraction	n
sand	0.00a[1]	40	0.58d	12	0.58c	0.76g	25
2vo	0.00a	37	0.06abc	33	0.06ab	0.14a	37
3jo	0.07a	38	0.24cd	21	0.22b	0.67fg	36
4di	0.03a	40	0.08abc	38	0.08ab	0.24abcd	37
5oo	0.00a	40	0.10abc	39	0.10ab	0.10a	39
6ve	0.03a	40	0.03a	38	0.03a	0.21abc	39
7ko	0.00a	40	0.05abc	40	0.05ab	0.21abc	39
8en	0.00a	40	0.08abc	39	0.08ab	0.25abcd	40
10kl	0.00a	31	0.07abc	29	0.07ab	0.14a	37
11gr	0.00a	39	0.03ab	34	0.03a	0.50efg	34
12st	0.03a	34	0.11abc	38	0.10ab	0.19abc	37
13wi	0.09a	31	0.10abc	30	0.09ab	0.26abcd	39
14we	0.00a	26	0.14abc	28	0.14ab	0.34bcde	38
15tu	0.06a	29	0.15abc	33	0.13ab	0.16ab	38
16hu	0.00a	25	0.03abc	29	0.03ab	0.33bcde	39
11top0.8	0.00a	21	0.15abc	26	0.15ab	0.46def	37
13top1.8	0.00a	39	0.08abc	26	0.08ab	0.40cde	25
15top2.1	0.00a	24	0.20bc	30	0.20b	0.72g	28

[1]Letters indicate significant subgroups at 0.05 level as determined by pair-wise comparison (Tukey-Kramer test).
[2]n = number of bio-assays divided over two blocks.
[3]Results are corrected for contamination observed in control pots.

Table 3. Fraction (back transformed means) of plants per treatment with *Meloidogyne incognita*.

Soils	Control[1]		*Meloidogyne*[1]		Corrected *Meloidogyne*[1,3,4]		Sterilized, *Meloidogyne*[1]	
	RKI	$J2 \cdot g^{-1}$	RKI	$J2 \cdot g^{-1}$	RKI	$J2 \cdot g^{-1}$	RKI	$J2 \cdot g^{-1}$
sand	0.0	1.1ab	3.1bcde	1509.6h	3.1	1508.5	1.7a	501.3abcde
2vo	0.0a[2]	2.0ab	2.3a	61.9ab	2.2	59.9	2.4bc	104.0a
3jo	0.2a	8.3bc	3.1bcde	88.8abcd	2.9	80.5	2.4ab	325.7abcd
4di	0.0	0.4a	3.0abcd	196.3abcdefg	2.9	196.0	2.3ab	629.5abcde
5oo	0.2ab	0.6a	3.2bcde	405.2bcdefgh	3.0	404.6	2.4abc	112.1ab
6ve	0.9bc	29.9c	2.8abc	144.3abcde	1.9	114.4	3.3d	622.2abcde
7ko	0.2a	5.8bc	2.7ab	169.1abcdef	2.5	163.3	2.8bcd	275.9abcd
8en	0.9c	4.4bc	3.2bcde	38.9a	2.2	34.5	3.2d	960.9cde
10kl	0.1a	1.4ab	3.6def	741.1efgh	3.4	739.7	3.3d	433.0abcde
11gr	0.3ab	6.7bc	3.1bcde	498.8defgh	2.9	492.1	3.4d	908.8cde
12st	0.0	2.0ab	3.2bcde	72.6abc	3.2	70.6	2.7bcd	133.3abc
13wi	0.0a	1.4ab	2.7ab	1556.2h	2.6	1554.8	3.0bcd	813.2cde
14we	0.0	0.6a	3.1bcde	2535.3h	3.1	2534.8	3.1cd	1165.1de
15tu	0.0	1.0ab	3.4bcde	583.1defgh	3.4	582.2	3.2d	749.7bcde
16hu	0.2	2.4ab	3.8ef	1112.5fgh	3.6	1110.1	3.1cd	1133.1de
11top0.8	0.0	1.7ab	4.2f	2223.9h	4.2	2222.1	3.4d	2685.2e
13top1.8	0.1a	1.8ab	3.0abcd	1296.8gh	2.9	1295.0	2.8bcd	1726.3de
15top2.1	0.0	0.4a	3.4cde	478.4cdefgh	3.4	478.0	2.8bcd	1446.3de

[1]For each treatment, 40 bio-assays divided over two blocks were used.
[2]Letters indicate significant subgroups at 0.05 level as determined by pairwise comparison (Tukey-Kramer test).
[3]Significant subgroups are identical to uncorrected numbers.
[4]Results are corrected for contamination observed in control pots.

Table 4. Fraction (back transformed means) of plants per treatment with *Verticillium dahliae*.

Soils	Control		*Verticillium*		Corrected *Verticillium*[3]	Sterilized, *Verticillium*	
	Fraction	*n*[1]	Fraction	*n*	Fraction	Fraction	*n*
sand	0.02	39	0.42b[2]	39	0.44b	0.30b	40
10kl	0.00	38	0.11ab	38	0.11ab	0.05ab	40
11gr	0.00	39	0.05a	39	0.05a	0.00ab	40
12sta	0.00	31	0.06a	34	0.06a	0.13ab	40
13wi	0.00	36	0.02a	40	0.02a	0.23ab	40
14we	0.00	39	0.13ab	39	0.13ab	0.18ab	40
15tu	0.00	40	0.07a	40	0.07a	0.08ab	39
16hu	0.00	39	0.10ab	38	0.10ab	0.03a	40
11top0.8	0.00	36	0.00a	36	0.00a	0.08ab	40
13top1.8	0.00	40	0.07a	39	0.07a	0.13ab	39
15top2.1	0.00	39	0.18ab	39	0.18ab	0.08ab	40

[1]*n* = total number of bio-assays divided over two blocks.
[2]Letters indicate significant subgroups at the 0.05 level as determined by pair-wise comparison (Tukey-Kramer test).
[3]Results are corrected for contamination observed in control pots.