European Journal of Plant Pathology **101**: 679–685, 1995.
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Effect of plant roots on the germination of microsclerotia of *Verticillium dahliae*

II. Quantitative analysis of the luring effect of crops

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Accepted 5 April 1995

Key words: germination, microsclerotia, root density, root exudates, *Verticillium dahliae*

Abstract

Induction of germination of microsclerotia by exudates from plant roots may be important for the control of *V. dahliae*. Laboratory experiments with root observation boxes were carried out to assess the influence of root tips of seven crop species and cultivars on the germination of microsclerotia of *Verticillium dahliae* in soil under controlled conditions. The root density of crops was measured in a field experiment. The results of the laboratory experiments and the field experiment were combined to estimate the total effect of crops on the population of microsclerotia in the field. Germination of microsclerotia was stimulated by all crops compared to a control without a crop. Among crops, roots of potato cvs Element and Astarte had a larger stimulation effect on microsclerotia than that of potato 'Ostara', pea, flax, sugar beet or onion. The number of hyphae per microsclerotium decreased with distance from the root surface regardless of the crop species or cultivar. Differences in root densities, in the affected root zones and in the stimulation effect on germination of microsclerotia caused large differences among crops in the effect on the population of microsclerotia in the soil. However, growing a crop with the special purpose to reduce the level of *V. dahliae* inoculum in the soil is an inefficient control measure, because only a small part of the total soil volume is affected by roots and the number of hyphae per microscleroium affected is too low.

Abbreviations: MS = microsclerotia, microsclerotium.

Introduction

Root exudates of crops can stimulate the germination of microsclerotia (MS) of *Verticillium dahliae* Kleb. in the soil [Schreiber and Green, 1969; Mol and Van Riessen, 1995]. However, only a very small portion of the hyphae will successfully infect the root. Microsclerotia of *V. dahliae* can germinate more than once, but eventually become exhausted [Farley *et al.*, 1971; Ben-Yephet and Pinkas, 1977]. Crops could affect the soil population of MS through stimulation of MS germination by its roots. Schreiber and Green [1969] found differences between the effects root exudates of tomato and wheat on MS germination. Using root observation boxes, Mol and Van Riessen [1995] showed similar differences among five crops.

In crops with a high root density and without subsequent proliferation of *V. dahliae* in the root system, the effect of the roots on the MS population may be relevant to reduce the MS density in the soil. Benson and Ashworth [1976] did not find differences in inoculum density between soil adhering to the root system of plants and soil without roots. This suggests that the negative effect of roots on the existing MS population was negligible. Knowledge of effects of roots of crops on the population of MS is important to understand the value of crops to reduce the MS density in the soil.

In two laboratory experiments, the influence of root tips of seven crop species and cultivars has been quantified using the method of Mol and Van Riessen [1995]. In a field experiment, the root density of the crops was measured. The results of these experiments and the results of Mol and Van Riessen [1995] are used to calculate the effect of the crops on the MS population.

Materials and methods

Root observation boxes were constructed and prepared according to Mol and Van Riessen [1995]. In these boxes roots grow along a transparent wall, coated with an agar film containing individual MS. Microsclerotia of *V. dahliae* were taken from potato stems (cv. Bintje) from a commercial field of the Department of Agronomy in the autumn of 1990. The MS were scraped off the stems and wet-sieved through a 125 μm mesh and a 38 μm mesh sieve. The residue on the 38 μm sieve was collected. The boxes were filled with unsterilized moistened sandy soil (pH: 5.5, 2.7% organic matter).

Forty boxes were placed in a growth chamber at 22/15 °C day/night with a 14 h thermo- and photophase. After a pre-incubation period of 7 days, two 10-day-old seedlings or two pre-rooted potato sprouts were planted per box. Seven crops were grown in five replications: potato (*Solanum tuberosum* cvs Element (a cultivar sensitive to *V. dahliae* [Scholte and s'Jacob, 1990]), Ostara and Astarte), pea (*Pisum sativum* cv. Finale), sugar beet (*Beta vulgaris* cv. Univers), onion (*Allium cepa* cv. Jumbo), and flax (*Linum usitatissimum* cv. Viking). Five boxes without a crop were used as controls. Two weeks after planting, the roots had reached the bottom of the box and observations on effects of roots on germination of MS were started. Germination was quantified using the method as described by Mol and Van Riessen [1995]: counting the number of hyphae originating from a MS and measuring the distance between a germinated or a non-germinated MS and the root surface with a binocular microscope (magnification 100×). The experiment was completely repeated with a different observer (Experiments 1 and 2).

Root density of field crops

Root densities of crops were measured in a field trial on a sandy soil (pH: 5.4, 3.7% organic matter) (Experiment 3). On May 7 ten crops were planted or sown on plots of 4 × 4 m^2: potato cvs Element, Mirka, Ostara and Astarte, spring barley (*Hordeum vulgare* cv. Prisma), field bean (*Vicia faba* vc. Victor), pea cv. Finale, sugar beet cv. Univers, onion cv. Jumbo, and flax cv. Viking. The distance between the rows was 50 cm for potato, field bean and sugar beet, and 25 cm for the other crops. Weeds were removed by hand.

Root samples were taken at the time that a maximum root density was expected. For barley, field bean, pea, and flax this was at the onset of flowering, for onion at the onset of bulbing, for potato when tuber bulking started, and for sugar beet in the first half of August, several weeks after closure of the canopy. Except for potato, samples were taken between plants in the row, and between the rows. Per plot eight cores were taken. for potato the sampling scheme was based on Vos and Groenwold [1986]: one data point was the average of two bore holes in the row, two halfway the slope of the potato hill, and two in the furrow between the rows. In Exp. 3 the bore holes halfway the slope of the potato hill were skipped. Two duplicates were taken per potato plot, each of four subsamples.

Samples were taken at three depths 0–10 cm, 10–20 cm, and 20–30 cm, using an auger with a diameter of 7 cm and a volume of 179 ml. Root samples were washed and the roots were stored in a 15% ethanol solution until analysis. The root length was estimated by the line-intersection method described by Newman [1966], as modified by Tennant [1975], using a 2 cm mesh grid.

Data analysis and calculations

Based on the observations of Fitzell *et al.* [1980] and Gerik and Huisman [1988] the induction of MS to germinate by the root system of a plant is assumed to diffuse from the root tip. From the results of Expts 1 and 2, the effect per root tip was calculated for each crop tested. The results of Expts 1 and 2 were combined, because both experiments gave similar results. Qualitative linear regression was used to fit the number of hyphae per MS to the distance of the MS from the root surface. The intercepts and the slopes of the different crop species and cultivars were compared. Before the regression was calculated, MS were grouped into five distance classes: 0, 0.01–0.25, 0.26–0.50, 0.51–0.75, and 0.76–1.00 mm. For the analysis the median was taken to represent each class.

Root lengths of crops of Exp. 3 were calculated per 10 cm soil layer from the means of eight cores per plot.

Root lengths for the soil layer 0–30 cm were calculated by adding up the means of the three layers.

For the calculation of the effect of roots on the MS population in the soil, the data of Expts 1, 2, and the results of laboratory experiments of Mol and Van Riessen [1995] were combined with the root density data collected in Exp. 3. The measured values and the mean slopes of Expts 1 and 2 differed from those observed by Mol and Van Riessen [1995] (see Results). To arrive at a valid comparison of crops from current and previous experiments, results had to be adjusted. Results of potato cv. Element and the control treatment were used as references, because they were present in all experiments. In a first set of calculations the mean numbers of hyphae.MS^{-1} as published by Mol and Van Riessen [1995] were reduced to the values of Expts 1 and 2, and the slope of Expts 1 and 2 was used to calculate the variables. In a second set of calculations the mean numbers of hyphae.MS^{-1} from Expts 1 and 2 were increased to the values as published by Mol and Van Riessen [1995], and the slope calculated by them was used for the calculations.

The radius of the rhizosphere (R) of a single root of each crop was defined as the distance from the root at which the influence of the root on MS germination became nil (i.e. was decreased to the level of the control treatment; c_0). The linear regression line was used to estimate the radius of the rhizosphere (for formulas and calculations see Appendix A). For each crop species and cultivar the percentage of the soil volume affected by the roots (V) was calculated from the radius of the rhizosphere and the root density determined in Exp. 3 using the formula for a cylinder. Because the effect is measured at the root tip, the thickness of the root is considered to be negligible. The mean effect of plant roots on the MS population in the soil ($\overline{E}$) was calculated by:

$$\overline{E} = c_1 + \frac{2}{3} c_2 . R$$

The formula is the result of the integration of the linear relationship of the effect from the root (c_1 = intercept, c_2 = slope) and the area of a circle over the radius of the rhizosphere (R) (Fig. 1, Appendix A).

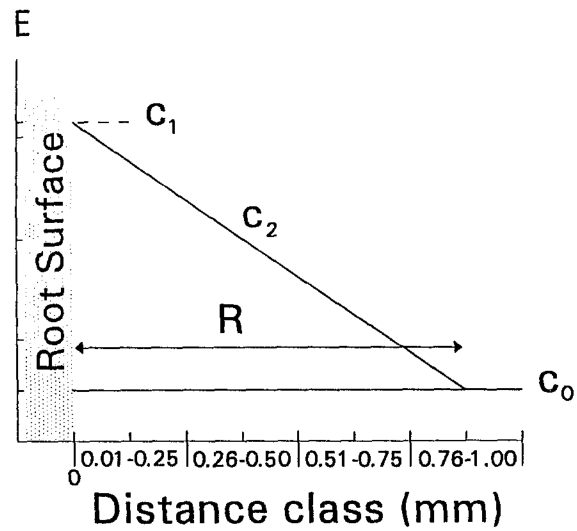

Fig. 1. Schematic representation of the meaning of the parameters used in and obtained form the linear regression.

Results

The highest levels of MS germination were found for potato cvs Element and Astarte, followed by potato cv. Ostara and the other crop species (Table 1). All crops showed a significantly higher germination than the control. Differences in the number of hyphae per germinated MS were small between crops. The number of hyphae per germinated MS in the control was significantly lower than for most crops. Small differences among crops were found in the number of hyphae per germinated MS. Therefore, the number of hyphae per MS showed similar differences among crops as the MS germination. The susceptible potato cv. Element had the highest values, and all crops showed significantly higher values than the control treatment.

The differences in root density between crops were comparable for the different soil layers (Table 2). The root density to a depth of 30 cm was the highest for barley and flax. In a decreasing order, the sequence for the other plant crops was pea, sugar beet, field bean, potato cvs Mirka, Astarte, Element, and Ostara, and onion, the value of onion being only 25% of the one of barley. Differences among potato cultivars were large. The root density of 'Mirka' was almost double the one of 'Ostara'.

Linear regression showed that the number of hyphae per MS decreased significantly ($P < 0.01$) with increasing distance form the root tip. The intercepts of the lines for the crops were significantly different ($P < 0.05$), but the slopes of the regression lines did not differ significantly. The average slope had a value of -0.49 hypha $MS^{-1}mm^{-1}$.

Expts 1 and 2 differed from the experiments of Mol and Van Riessen [1995] in the effect of roots on the

Table 1. Mean effects of roots on the germination of microsclerotia (MS) within a radius of 1 mm around the root tip in Experiments 1 and 2

Crop	MS germinated/ tip (%)[a]	Number of hyphae/ (germinated MS)[a]	Number of hyphae/ (MS)[a,b]
Potato cv. Element	33.3 a	1.18 ab	0.40 a
Potato cv. Astarte	29.3 a	1.21 ab	0.36 a
Potato cv. Ostara	18.4 b	1.25 a	0.23 b
Pea	23.1 b	1.25 a	0.29 b
Flax	19.5 b	1.28 a	0.25 b
Sugar Beet	20.9 b	1.28 a	0.27 b
Onion	19.2 b	1.26 a	0.25 b
Control	9.3 c	1.12 b	0.10 c

[a] Different letters indicate significant differences between the treatments based on LSD values ($P = 0.05$) of the combined data of Expts 1 and 2.
[b] Number of hyphae/MS = (MS germinated/tip (%) × Number of hyphae/germinated MS)/100.

Table 2. Root densities ($cm.cm^{-3}$ of 10 crops in various soil layers. Experiment 3

	Soil layer Depth in the soil (cm)*			
	0–10	10–20	20–30	0–30
Potato cv.Element	1.49 c	1.47 cd	1.67 c	1.54 cd
Potato cv. Astarte	2.31 b	1.93 c	1.65 cd	1.96 c
Potato cv. Ostara	1.36 c	1.13 d	1.09 de	1.19 d
Potato cv. Mirka	2.65 b	1.90 c	2.01 c	2.19 bc
Field Bean	2.60 b	2.50 b	1.51 cd	2.20 bc
Barley	4.34 a	3.89 a	4.38 a	4.20 a
Pea	2.53 b	2.75 b	2.61 bc	2.63 b
Flax	4.89 a	3.49 a	2.79 b	3.72 a
Sugar beet	3.04 b	2.38 bc	1.83 c	2.42 bc
Onion	1.00 c	1.24 d	0.80 e	1.01 d

* Different letters indicate significant differences between the treatments based on LSD values ($P = 0.05$).

number of hyphae per MS. For the control and potato cv. Element the effect in Expts 1 and 2 was 0.58 and 0.44 times the effect found by Mol and Van Riessen [1995], respectively. For the calculation based on the observed number of hyphae per MS of the current experiments, the mean effects of potato cv. Mirka, field bean, and barley obtained in the latter experiments were multiplied by 0.51 (the average of 0.58 and 0.44) (Table 3A). For the calculation based on the values measured by Mol and Van Riessen [1995] the observed numbers of $hyphae.MS^{-1}$ of the current experiments were divided by 0.51 (Table 3B).

Differences in the mean number of hyphae per MS between crops led to comparable differences in the intercepts, the affected root zones, and the mean numbers of hyphae per MS in the soil volume (Table 3). Because of the mathematical integration of the linear decrease of the effect from the root and the affected volume, differences among the crops were smaller when the mean number of hyphae per Ms in the affected soil volume was calculated. Differences in both root densities and in the affected root zones caused large differences in the affected soil volume between crop species and cultivars. Differences were largest when the MS were artificially produced. Potato cvs Astarte and Element, pea and flax had the highest proportions of the soil volume affected, followed by field bean, sugar beet, potato cv. Mirka, barley, with onion and potato cv. Ostara lagging far behind.

Based on the observations of the current experiments, the effect of plant roots on the soil population of MS was highest for potato cv. Element followed by cv. Astarte, flax, pea, field bean, barley, sugar beet, potato cvs Mirka and Ostara, and onion. Relative to the control, potato cv. Element had an additional effect of 10% whereas cv. Ostara and onion had an additional effect of only 2%. When the values were adjusted to the values observed by Mol and Van Riessen [1995] the order of the relative differences was comparable with the former calculation, but absolute differences among crops were larger.

Table 3. Effects of roots on the soil population of microsclerotia (MS) of *V. dahliae*, obtained after adjusting the level to the values of Expts 1 and 2 (**A**), and adjusted to the level of the values measured by Mol and Van Riessen [1995] (**B**). Transformations are explained in the text

Crop	Number of hyphae/MS	Intercept of (number of hyphase/MS)[b]	Affected root zone (mm)[c]	Affected soil volume (%)[d]	Mean number of hyphae in affected volume/MS[e]	Effect on the soil population/(MS)[f]	Relative differences
A (adjusted to the level of the values of Expts 1 and 2)							
Potato 'Element'	0.40	0.63	1.07	5.6	0.28	0.113	110
Potato 'Astarte'	0.36	0.57	0.96	5.7	0.26	0.112	109
Potato 'Ostara'	0.23	0.43	0.67	1.7	0.21	0.105	102
Potato 'Mirka'[a]	0.27	0.50	0.81	4.5	0.24	0.109	106
Field Bean[a]	0.29	0.52	0.85	5.0	0.24	0.110	107
Barley[a]	0.21	0.44	0.70	6.4	0.22	0.110	107
Pea	0.29	0.52	0.84	5.8	0.24	0.111	108
Flax	0.25	0.49	0.79	7.3	0.23	0.112	109
Sugar Beet	0.27	0.51	0.83	5.2	0.24	0.110	107
Onion	0.25	0.49	0.77	1.9	0.23	0.105	102
Control	0.10	–	–	–	–	0.103	100
B (adjusted to the level of the values measured by Mol and Van Riessen [1995])							
Potato 'Element'	0.69	0.78	2.37	27.2	0.41	0.282	121
Potato 'Astarte'	0.70	0.79	2.42	36.2	0.42	0.300	129
Potato 'Ostara'	0.46	0.55	1.36	6.9	0.34	0.239	103
Potato 'Mirka'[a]	0.52	0.59	1.53	16.1	0.35	0.251	108
Field Bean[a]	0.56	0.64	1.77	21.6	0.37	0.261	113
Barley[a]	0.41	0.48	1.09	15.6	0.32	0.245	106
Pea	0.57	0.66	1.85	28.5	0.38	0.273	118
Flax	0.49	0.58	1.53	27.3	0.35	0.264	114
Sugar Beet	0.52	0.61	1.65	20.8	0.36	0.258	111
Onion	0.48	0.58	1.49	7.1	0.35	0.240	104
Control	0.23	–	–	–	–	0.232	100

[a] Original data are obtained from Mol and Van Riessen [1995]. The transformations are explained in the text.
[b] Result of a linear regression. There was a significant decrease over the distance ($P < 0.01$). Intercepts of the different crops differed significantly ($P < 0.05$), but slopes did not (**A**: slope is -0.49, and **B**: slope is -0.23 hyphae MS^{-1} mm^{-1}).
[c] The affected root zone is the distance from the root to the point at which the regression line obtained with a linear regression reaches the level of the control. See text for further explanation.
[d] Calculated with the formula of a cylinder with the affected root zone (radius) and the root length (Table 2) as measures.
[e] Obtained from integration of the volume around the root within the active distance with the decrease of the number of hyphae from the linear regression.
[f] The mean number of hyphae in the affected soil volume combined with the effect of the control in the volume not affected by the root.

Discussion

In the current experiments the effects on germination of MS were lower and the absolute differences between crops were smaller than the differences and levels measured by Mol and Van Riessen [1995]. However, the trends in both sets of experiments were the same and the relative differences between effects of crops were also the same in all experiments. The lower germination levels of Ms in the current experiments may be ascribed to the lower vitality or to latency of the MS. The MS used in Expts 1 and 2 were collected from field stems and were older than the MS used by Mol and Van Riessen. Older MS have a lower germination level [Ben-Yephet and Pinkas, 1977]. The soil used was different between the sets of experiments and may also have affected the level of germination, e.g. by different levels of soil fungistasis.

It is unclear whether the conditions in the root observation boxes are representative for field conditions. the calculated values are to a large extent influenced by the value of the affected root zone. The levels of the slopes of the lines and the mean numbers of hyphae per MS from Expts 1 and 2 are considered the ones most representative for a field situation, because the MS used in these experiments were produced in the field. The MS used by Mol and Van Riessen were produced artificially in the laboratory. Although much larger than calculated from Expts 1 and 2, the effect calculated with the artificially produced MS is still too small to contribute significantly to the control of the MS population in the soil. The main reasons for the small effects are the low number of hyphae per MS when a MS is stimulated to germinate by a root and the small percentage of the soil volume affected by the roots. Even when the root density is comparatively high, the soil volume influenced is a limiting factor. Because of this, the elimination of MS by germination will be insignificant and growing a crop with the special objective to control *V. dahliae* will be of little value. Nevertheless, the relative differences between crops show that one crop (potato cv. Astarte) can have a nine-fold effect on germination compared to another crop (onion or potato cv. Ostara). Probably, differences among crops may change with the composition of the MS population in the soil. An influence of the MS source on germination of the MS has been observed for various crops [Zilberstein *et al.*, 1983; Krikun and Bernier, 1987].

The results of Expts 1 and 2 show the situation at a well defined age of the plant. During plant growth and development the germination of MS could change as a result of changes in plant properties and root environment. However, Huisman [1988a, 1988b] showed that the colonization of roots with *V. dahliae* was fairly constant over the season under different environmental conditions.

Gilligan [1979] and Ferriss [1981] calculated the radius at which roots influence organisms in the soil based on the probability of infection of the root and the inoculum density in the soil. Their proposed equations can be used for modeling colony incidence on plant roots but are not sufficient to calculate the effect of plant roots on the population of MS in the soil. For that purpose, properties of the MS population in the soil as calculated in this paper and the source of the MS [Zilberstein *et al.*, 1983] have to be included. Moreover, knowledge of the distance at which a hypha of *V. dahliae* can reach and infect the cortex of a root is necessary to calculate the inoculum reducing effect of plant roots.

Expts 1, 2 and 3 show that potato cultivars differ in their effect on the germination of MS and also in root length. The intercepts as shown in Table 3 indicate the level of germination at the root surface. At that place the hyphae will have the highest chance to colonise the root. So, the intercept could give an indication of the maximum chance of a plant root to be colonised by *V. dahliae*. In combination with the root length the maximum probability of infection could be calculated. Because a longer root passes along a higher number of MS the differences in root density could explain differences among potato cultivars in the chance to become infected. For other crops only one cultivar was included in the experiments. One should be aware of possible differences among cultivars of the crops in the germination of MS and in root length.

Effects as shown in Table 3 depend to a large extent on the level of the spontaneous germination in the control treatment and the vitality and latency of the MS population in the soil. If the spontaneous germination without the influence of a plant is lower the effect of roots on the MS population will become more important. Therefore, verification of the results in the field is needed, but is very difficult because no techniques for observation are available yet.

Acknowledgements

I thank Mr H. W. van Riessen and Dr M. Schütz for their participation in Expts 1 and 2, Mr L. Haalstra for his participation in Expt 3, and Dr K. Scholte, Professor P. C. Struik and Dr J. Vos for critically reading the manuscript.

Appendix A

Formulas and parameters

$E = c_1 + c_2.r$	E = number of hyphae.MS^{-1};
$R = \frac{c_0 - c_1}{c_2}$	$\overline{E}$ = mean number of hyphae.MS^{-1} in the affected root zone;
$V = \pi.R^2.L.100$	c_0 = measured number of hyphae.MS^{-1} in the control;
$S = \pi.r^2$	c_1 = intercept obtained from linear regression (Hyphae.MS^{-1});
$\overline{E} = \frac{1}{S}\int E.dS$	c_2 = slope of the regression line (hyphae.MS^{-1}.mm^{-1});
$= \frac{1}{S}\int_0^R . \int_0^{2\pi} E.r.dr.d\varphi$	r = distance from the root surface (mm);

$$= \frac{2\pi}{\pi.R^2} \cdot \int_0^R E.r.dr$$
$$= \frac{2}{r^2} \cdot \int_0^R (c_1 + c_2.r).r.dr$$
$$= \frac{2}{r^2} \cdot \int_0^R (r.c_1 + r^2.c_2).dr$$
$$= \frac{2}{r^2} . [\tfrac{1}{2}.c_1.r^2 + \tfrac{1}{3}.c_2 r^3]_0^R$$
$$= c_1 + \tfrac{2}{3}.c_2.R$$

R = radius of the rhizosphere (mm);
V = affected soil volume (%);
L = root density (mm.mm^{-3});
S = area of a circle (mm^2).

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