Single-Site Root Inoculations on Eggplant with Microsclerotia of *Verticillium dahliae*

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For many soilborne plant pathogens, disease results from multiple root infections. Studying the infection dynamics of single or multiple propagules of these pathogens applied at one site of the root system may be the basis for understanding the development of disease caused by multiple root infections. The effect of single-site inoculations of roots of eggplant seedlings with microsclerotia of the wilt-causing fungus *Verticillium dahliae*, was studied. Experiments were conducted using specially designed pots which enabled the incorporation and removal of inoculum in the soil. Inoculations were carried out by placing microsclerotia, firmly embedded in small sections of polypropylene screen filter, directly below the growing tip of the main root of young eggplant seedlings. Three to 4 days after inoculation, the root had grown over the screen filter, and the filter was removed. Root platings showed high infection levels at the inoculation site, but also several (discrete) root infections were noted some distance above and below the site of inoculation. Exposure of the root to the lowest number of microsclerotia (26/inoculation site) was sufficient to lead to up to 65% root infections. Number of plants with root infections declined over time, ranging from a maximum of 65-100% 2–4 wk after inoculation, to 10–29% at 6–7 wk after inoculation. Apparently, *V. dahliae* died in nonsystemic infections after some time.
KEY WORDS: Inoculum potential; root infection; *Solanum melongena* L.; eggplant; *Verticillium dahliae.*

INTRODUCTION

Verticillium dahliae Kleb. causes a vascular wilt disease in many dicotyledonous plant species (*e.g.* cotton, eggplant, potato, olive) (4,32). The pathogen survives host-free seasons in soil in the form of highly persistent microsclerotia. These microsclerotia remain inactive until root exudates from a closely situated root induce their germination (21,33). Root infection occurs only if microsclerotia are very close to, or in direct contact with, the roots (17,18). Infection may take place near the root tip (2,9,11,30) or at sites where the root is wounded (29). Effective methods to control the disease are not available. Cultivars that are resistant or tolerant to *V. dahliae* are lacking for most crops. Therefore, avoidance of *V. dahliae* must rely on growing plants in fields with low or no infestation. In general, Verticillium wilt incidence and severity increase as inoculum density of *V. dahliae* in soil increases (14,28,31,36). However, the minimum number of microsclerotia in soil required to cause wilt may vary with the crop (14,15) and with the *V. dahliae* pathotypes

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(1). Also, the threshold level suffers from unreliable detection assays (34) and insufficient understanding of the relationship between inoculum density and disease incidence (35).

For many soilborne plant pathogens including *V. dahliae*, a single plant may be exposed to several hundred root infections during one growing season (11,23). These root infections are not likely to contribute equally to the severity of wilting of the shoot, since the effects of infections may depend on their location on the root system and the age of the plant. Therefore, the relationship between inoculum density and disease incidence of *V. dahliae* may be understood better if the effects of single infections are known.

The aim of this work was to investigate the effect of single-site inoculations of *V. dahliae* on the infection of the root and the shoot system of eggplant seedlings. It is part of a study in which we attempt to explain conidial densities in stem tissue as a function of single and multiple root infections. Initially we planned to perform inoculation experiments with single microsclerotia. However, we found it more practical to start with inoculations applying a number of microsclerotia at one site of the root system, since most root infections of *V. dahliae* fail to penetrate deeper than the epidermal or cortical layers of the root (2,9,29) and it has been estimated that only 0.02% of cortical infections lead to shoot infection (20). In the present study, seedlings were inoculated by placing many microsclerotia embedded in a small polypropylene screen filter just in front of a root tip for 3–4 days.

MATERIALS AND METHODS

Growing conditions

PVC pots (20 $\times$ 8.2 $\times$ 3.2 cm; height $\times$ length $\times$ width), with a polypropylene 20-μm screen attached at the bottom, were used. For manipulation of the inoculum, a 5 $\times$ 10 cm hole was made in the middle of one of the large sides of each pot and covered with a transparent polyethylene plastic strip.

Seeds of eggplant (*Solanum melongena* L.) of the susceptible cv. 'Black Beauty' (7,8) were surface-disinfested in 25% sodium hypochlorite (available chlorine 1%) for 30 min, then washed twice in sterile water, and germinated in petri dishes on sterile wet filter paper at 22°C in the dark. Pregerminated seeds were planted in pots (one per pot), each of which was filled with 720 g of non-sterilized sandy soil (10% water, -6.2 kPa, 7.0 pH-KCl, 0.3% organic matter content) which had been sieved previously through a screen of 4.8-mm pore size. Experiments were performed in a growth chamber at 20°C with a light intensity of 29 W m^{-2} for 16 h day^{-1} and a relative air humidity of 80%. The pots were maintained in an inclined position to force the root system to grow over the plastic strip. After concluding the inoculation (*i.e.*, after 7–8 days), pots were maintained in a vertical position for the rest of the experiment. After correcting for weight loss, watering was done with Hoagland's solution that was added to the bottom of the pots.

Preparation of inoculum

Small potato stem pieces which were naturally infected, or rye (*Secale cereale* L.) seeds artificially infected by *V. dahliae* (22), were ground using a mortar and pestle and subsequently mixed thoroughly twice in tap water for 30 sec and sieved through nested 106- and 20-μm pore size sieves. The microsclerotia remaining on the 20-μm sieve were suspended in tap water and filtered through a polypropylene 45-μm screen filter (Millipore,

Etten-Leur, the Netherlands) using a vacuum pump to attach the microsclerotia tightly to the screen filter. The filtering procedure was repeated three times, after which the filter was thoroughly washed with running tap water for 1 min, to leave only microsclerotia which were strongly attached to it. Subsequently, the screen filter was divided into squares of 3 $\times$ 3 mm (Exp. 1) or rectangles of 3 $\times$ 6 mm (Exps. 2 and 3). The concentration of part of the suspension was adjusted to 25 microsclerotia per 0.4 ml, and the germinability of the microsclerotia was determined by plating ten drops of 0.4 ml each on ethanol agar (EA) (25) amended with 50 ppm oxytetracycline and incubating the petri dishes at 22°C in the dark for 14 days. Average number $\pm$ S.D. of germinable microsclerotia was 71 $\pm$ 16 per screen filter of 9 mm^2 for Exp. 1; 563 $\pm$ 100 and 1072 $\pm$ 322 per screen filter of 18 mm^2 for Exp. 2 at low and high inoculum density, respectively; and 140 $\pm$ 37 per screen filter of 18 mm^2 for Exp. 3. These numbers represent an average of 7.9, 31.3, 59.6 and 7.8 microsclerotia mm^{-2} screen filter, respectively.

Inoculation

Four days after planting, the screen filter containing the microsclerotia was fixed with adhesive tape against the inner side of the plastic strip that covered the hole at one side of the pot, just in front of the main root tip. In the case of the 3 $\times$ 6 mm screen filter, fixation was done vertically. At the time of inoculation, no secondary roots (root branches from the main root) were present. When the main root of the eggplant seedling had passed through the screen filter, the inoculum was removed (3–4 days after inoculation). Plants were harvested at 3, 4, 5, 6 and 7 wk after inoculation for Exp. 1; at 2, 3, 4, 5 and 6 wk after inoculation for Exp. 2; and at 2 and 3 wk after inoculation for Exp. 3.

Experimental design

Experiments were set up as a randomized complete block design with 17 (Exp. 1) or ten (Exps. 2 and 3) replications per treatment and harvest time. The control, consisting of attachment of the screen filter without microsclerotia, was harvested only at the final harvest date for each experiment (control 1). An additional control was carried out in Exps. 2 and 3, where a non-inoculated plant was grown in the same pot and at a distance of 3 cm from the inoculated plant (control 2). Four days after inoculation, the screen filter as well as the inoculated seedling were removed from the pot, and the non-inoculated plant was harvested 6 and 3 wk after inoculation for Exps. 2 and 3, respectively. This second control was included in order to exclude the possibility that microsclerotia dispersed through soil would be able to cause infection.

Evaluation of the harvested plants

The population density of *V. dahliae* on the root system and the presence and density of the pathogen in the shoot were determined. Plants were dug out of the pots, placed on a 250-μm pore size sieve and washed gently with tap water to remove soil. Stems were cut just above the soil level using a surface disinfested knife. Height and fresh weight of the stem as well as fresh weight of the root system were determined at the last harvest for Exps. 1 and 2.

The root system of the plants was washed with tap water in a root washing box for approximately 20 min, after which roots were washed twice in sterile water and allowed to dry on sterile filter paper in a flow cabinet. The dry roots from each plant were plated

in a 13.5-cm-diam petri dish containing EA, and the root length was measured using the line-intersection method (27). After incubation for 14 days at 22–24°C in the dark, the number of colonies of *V. dahliae* that developed on the roots was counted using a dissecting microscope (magnification 6-50×) and expressed as the number of colonies per meter of root. Observations were made separately for main and secondary roots, and also for areas above and below the inoculation site on each type of root. The inoculation site was defined as the part of the main root 1 cm above and 1 cm below the first point of the root that was attached to the inoculum, at the time of inoculation.

To assess the colonization of *V. dahliae* on the stem, two methods were applied. For each plant, after removal of the leaves, the first 3 cm of stem above the soil level were surface-disinfested with 25% sodium hypochlorite (available chlorine 1%) for 30 sec followed by washing twice in sterile water. Then, small pieces were cut at both sides of the stem segment, plated in petri dishes on EA and incubated at 22–24°C in the dark. Also, to determine the conidial density per gram of fresh weight of the stem piece, the remaining stem piece was cut to a length of 2 cm, weighed, and macerated in 0.2 ml of a sterile solution of 0.1 M $MgSO_4.7H_2O$ in an Eppendorf tube; the suspension obtained was pipetted onto a 8.5-cm-diam petri dish containing EA (16). The Eppendorf tube was washed using another 0.2 ml of the solution, which amount was also pipetted onto the same petri dish. Finally, the suspension in the petri dish was spread over the medium using a sterile glass rod. The plates were incubated as described above and examined for growth of *V. dahliae* colonies after 7 days.

RESULTS

Root length increased with time, mainly due to the increase in length of the secondary roots (Table 1). At the time of inoculation, no secondary roots were present, but from the first harvest onwards, secondary roots had grown both from the main root which existed at the time of inoculation and from the main root which developed subsequently. Effects of *V. dahliae* on the root length or fresh weight were not detected, nor were any disease symptoms or effects of *V. dahliae* on shoot growth.

Plants with root infections were present within 3 wk (Exp. 1) and 2 wk (Exps. 2 and 3) after inoculation, varying from 30% in Exp. 3 to 80–100% in Exp. 2 (Table 2). Maximum incidence of infected plants occurred in the early harvests: in wk 4 for Exp. 1 (64.7%), in wk 2 and 4 for Exp. 2 at the low inoculum density (80%), and in wk 2 for Exp. 2 at the high inoculum density (100%). After these harvests, root infection percentages declined sharply to 10–29.4% in the final harvests (Exps. 1 and 2).

The number of root infections per infected plant was rather low, ranging from 1.5 to 3.5 in Exp. 1, 1.0 to 6.0 in Exp. 2, and 1.0 to 1.7 in Exp. 3 (Table 2). In 75% and 43.8% of all infected plants of Exps. 1 and 2, respectively, root infections were found at sites on the root other than the inoculation site, on both the main and secondary roots (Table 2). For the main root, infections were observed both above and below the inoculation site. For the secondary roots, infections were observed on roots originating either above or below the inoculation point. The average distance of these root infections from the inoculation site was larger for the area below the inoculation site than for that above the inoculation site (Table 3), because the latter area constituted only a small part of the main root, where only relatively few secondary roots were attached (Table 1). Root infections other than those at the inoculation site occurred not only close to the inoculation site but also quite far away:

TABLE 1. Root lengths of eggplant seedlings harvested at various times after inoculation (Exp. 1[z])

Inoculum (microsclerotia mm^{-2} screen filter)	Plants sampled (no.)	Time after inoculation (wk)	Root length (m)[y]						
			Total	Main root			Secondary roots		
				Total	Above IS[x]	Below IS[w]	Total	Above IS	Below IS
7.9	17	3	3.9±0.9	0.18±0.04	0.06±0.01	0.12±0.04	3.7±0.8	2.0±1.0	1.6±0.5
		4	6.3±2.7	0.20±0.07	0.06±0.01	0.14±0.07	6.1±2.7	3.0±1.4	3.1±1.5
		5	7.3±2.8	0.24±0.06	0.06±0.01	0.19±0.05	7.0±2.8	3.2±2.3	3.8±1.3
		6	11.3±3.3	0.27±0.05	0.05±0.01	0.22±0.06	11.1±3.3	4.7±2.6	6.4±2.0
		7	11.5±2.1	0.27±0.05	0.06±0.01	0.21±0.05	11.2±2.1	5.0±1.7	6.2±1.7
0	12	7	12.2±2.8	0.26±0.08	–[v]	–	11.9±2.8	–	–

[z] Root growth results from Exps. 2 and 3 were similar.
[y] Mean ± standard deviation.
[x] The root tissue above the inoculation site (IS), *i.e.*, already present at the time of inoculation.
[w] The root tissue below the inoculation site (IS), *i.e.*, not present at the time of inoculation.
[v] Not determined.

TABLE 2. Effect of single-site root inoculations with *Verticillium dahliae* on the infection with time of shoot and root tissue of eggplant seedlings

Inoculum (microsclerotia mm^{-2} screen filter)	Plants sampled (no.)	Time after inoculation (wk)	Plants with infected roots (%)	Plants with infected shoots (%)		Colonies on the roots (no./infected plant)	Colonies (%) in infected plants							
							Main root				Secondary roots			
				Plating on EA[z]	Plating on EA[y]		Total	Above IS[x]	IS[w]	Below IS[v]	Total	Above IS	IS[w, u]	Below IS
Exp. 1														
7.9	17	3	35.3	5.9	0	1.5	33.3	0	100.0	0	66.7	66.7	0	33.3
		4	64.7	0	0	3.5	7.9	0	33.3	66.7	92.1	28.6	20.0	51.4
		5	29.4	0	0	1.0	60.0	0	100.0	0	40.0	50.0	50.0	0
		6	29.4	0	0	3.0	26.7	25.0	75.0	0	73.3	63.6	0	36.4
		7	29.4	0	0	2.4	41.7	20.0	60.0	20.0	58.3	57.1	14.3	28.6
		Avg.	37.6	1.2	0	2.3	33.9	9.0	73.7	17.3	66.1	53.2	16.9	29.9
0, Control 1[t]	12	7	0	0	0	0	0	–	–	–	0	–	–	–
Exp. 2														
31.3	10	2	80	0	0	3.1	60.0	0	80.0	20.0	40.0	10.0	40.0	50.0
		3	40	0	0	1.3	100.0	0	100.0	0	0	–	–	–
		4	80	0	0	1.4	63.6	0	100.0	0	36.4	50.0	25.0	25.0
		5	50	0	0	1.0	80.0	0	100.0	0	20.0	0	0	100.0
		6	10	0	0	1.0	100.0	100.0	0	0	0	–	–	–
		Avg.	52	0	0	1.6	80.7	20.0	76.0	4.0	19.3	20.0	21.7	58.3
59.6	10	2	100	0	0	5.1	54.9	17.9	60.7	21.4	45.1	43.5	47.8	8.7
		3	50	0	0	2.8	35.7	0	100.0	0	64.3	44.4	55.6	0
		4	50	0	0	1.8	77.8	0	100.0	0	22.2	50.0	50.0	0
		5	10	0	0	6.0	66.7	0	75.0	25.0	33.3	0	0	100.0
		6	10	0	0	1.0	0	–	–	–	100.0	100.0	0	0
		Avg.	44	0	0	3.3	47.0	4.5	83.9	11.6	53.0	47.6	30.7	21.7
0, Control 1	10	6	10	0	0	1.0	100.0	0	100.0[s]	0	0	–	–	–
0, Control 2[r]	10	6	0	0	0	0	0	–	–	–	0	–	–	–
Exp. 3														
7.8	10	2	30	0	0	1.7	100	0	100	0	0	–	–	–
		3	40	0	0	1.0	100	0	100	0	0	–	–	–
		Avg.	35	0	0	1.4	100	0	100	0	0	–	–	–
0, Control 1	10	2	0	0	0	0	0	–	–	–	0	–	–	–
		3	0	0	0	0	0	–	–	–	0	–	–	–
0, Control 2	10	2	0	0	0	0	0	–	–	–	0	–	–	–
		3	0	0	0	0	0	–	–	–	0	–	–	–

[z] Plating of surface-disinfested stem segments on ethanol agar (EA). [y] Plating of squeezed stem sap from surface-disinfested stem segments on ethanol agar (EA). [x] The root tissue above the inoculation site (IS), *i.e.*, already present at the time of inoculation. [w] Inoculation site (IS); defined as the part of the main root from 1 cm above to 1 cm below the first point of the root that, at the time of inoculation, was attached to the inoculum. [v] The root tissue below the inoculation site (IS), *i.e.*, not present at the time of inoculation. [u] All secondary roots arising from the main root within the area of the inoculation site were also considered to belong to the inoculation site. [t] Control, consisting of inoculation of plants with screen filters free of microsclerotia. [s] *V. dahliae* was isolated from the site where the screen filter, free of microsclerotia, was attached to the root. [r] Control with a second, non-inoculated plant in the pot, 3 cm distant from the inoculated plant.

TABLE 3. Locations of the *Verticillium dahliae* infections on main and secondary roots outside the inoculation site (IS) relative to the IS

Inoculum (microsclerotia mm^{-2} screen filter)	Time after inoculation (wk)	Plants with infections on main root				Plants with infections on secondary roots					
		Above IS[z]		Below IS[y]		Above IS			Below IS		
						Location[v]			Location		
		No. IP[x]	Distance[w] (cm)	No. IP	Distance (cm)	No. IP	Type of distance	Distance (cm)	No. IP	Type of distance	Distance (cm)
Exp. 1											
7.9	3	0	–	0	–	2[u]	m	2.8(2.5-3.0)	1	m	4.0(-)
						4	s	3.0(2.0-3.5)	2	s	2.3(1.0-3.5)
	4	0	–	2	13.0(8.0-18.0)	4	m	3.9(3.5-5.0)	11	m	4.7(1.5-8.0)
						4	s	4.0(2.0-5.0)	11	s	6.5(2.0-16.0)
	5	0	–	0	–	1	m	2.5(-)	0	m	–
						1	s	3.0(-)	0	s	–
	6	1	1.5(-)[t]	0	–	6	m	3.4(2.5-6.0)	3	m	7.8(3.5-11.0)
						6	s	4.3(2.0-11.0)	3	s	4.3(2.0-9.0)
	7	1	1.5(-)	0	–	4	m	2.8(2.5-3.5)	2	m	6.3(1.5-11.0)
						4	s	5.1(2.0-13.0)	2	s	8.5(6.0-11.0)
Avg.[s]			1.5(-)		13.0(8.0-18.0)		m	3.1(2.5-6.0)		m	5.7(1.5-11.0)
							s	3.9(2.0-13.0)		s	5.4(1.0-16.0)
Exp. 2											
31.3	2	0	–	3	5.0(1.0-10.0)	1	m	3.0(-)	5	m	4.2(1.0-9.0)
						1	s	1.0(-)	5	s	1.5(0.5-3.)
	3	0	–	0	–	0	m	–	0	m	–
						0	s	–	0	s	–
	4	0	–	0	–	2	m	3.0(2.0-4.0)	1	m	4.0(-)
						2	s	3.5(3.0-4.0)	1	s	4.0(-)
	5	0	–	0	–	0	m	–	1	m	2.0(-)
						0	s	–	1	s	6.0(-)
	6	1	1.0(-)	0	–	0	m	–	0	m	–
						0	s	–	0	s	–
Avg.			1.0(-)		5.0(1.0-10.0)		m	3.0(2.0-4.0)		m	3.4(1.0-9.0)
							s	2.3(1.0-4.0)		s	3.8(0.5-6.0)
59.6	2	5	3.8(2.0-6.0)	6	5.8(2.5-8.0)	10	m	2.6(1.0-5.0)	2	m	3.0(2.0-4.0)
						10	s	4.1(1.5-8.0)	2	s	1.8(1.5-2.0)
	3	0	–	0	–	4	m	1.5(1.0-3.0)	0	m	–
						4	s	2.8(1.0-4.0)	0	s	–
	4	0	–	0	–	1	m	4.0(-)	0	m	–
						1	s	3.5(-)	0	s	–
	5	0	–	1	3.0(-)	0	m	–	1	m	3(-)
						0	s	–	1	s	2(-)
	6	0	–	0	–	1	m	3.5(-)	0	m	–
						1	s	7.0(-)	0	s	–
Avg.			3.8(2.0-6.0)		4.4(2.5-8.0)		m	2.9(1.0-5.0)		m	3.0(2.0-4.0)
							s	4.4(1.0-8.0)		s	1.9(1.5-2.0)

[z] The root tissue above the inoculation site (IS), *i.e.*, already present at the time of inoculation. [y] The root tissue below the inoculation site (IS), *i.e.*, not present at the time of inoculation. [x] Number of infected plants. [w] Mean (minimum - maximum) distance of root infections from the inoculation site. [v] The location of root infections on secondary roots is expressed as a function of two distances: 'm', distance of the attachment site of the secondary root to the main root from the inoculation site; and 's', distance of the infection on the secondary root from the attachment site of the secondary root to the main root. [u] Numbers of infected plants for 'm' and 's' are not always the same, since in 'm' the distance of the attachment site of the secondary root to the main root from the inoculation site could not be determined if the secondary root had broken off from the primary root. [t] Minimum and maximum distances are not indicated when only one infected plant was observed. [s] The absolute minima and maxima, respectively, are given in parentheses.

up to 18 and 10 cm on the main root for Exps. 1 and 2, respectively, and on secondary roots up to 11 and 9 cm from the site of attachment to the main root for Exps. 1 and 2, respectively. On the other hand, in Exp. 3, where only 30–40% of the plants showed root infections, all of them were observed at the inoculation site only (Table 2).

Only in Exp. 1 at the first harvest, 3 wk after inoculation, was the shoot of one plant found to be infected by plating stem pieces on EA, whereas the plating of stem sap on EA gave negative results. Control plants that were exposed to a screen filter without inoculum were not infected except in one case, in Exp. 2, where one root showed infection at the inoculation site (Table 2). Apparently, a contaminated screen filter had been employed. No other control plant was infected (Table 2).

DISCUSSION

Few attempts have been made to study the behavior of single propagules of root-infecting fungi, probably due to technical difficulties in delivering the inoculum to the root without unacceptable disturbance of the soil or root wounding. Single-site inoculations were carried out near the root tip, the region considered to be the most susceptible part of an unwounded root system. Bowers *et al.* (2) found that infection of potato roots by *V. dahliae* occurred in the region of the root tip and the zone of elongation, regardless of the presence of root-lesion nematodes.

In our experiment we applied very high inoculum densities of *V. dahliae* at a single site on the root of eggplant seedlings. Root infections were observed very frequently, indicating the infectivity and viability of the inoculum and the susceptibility of the seedling's root system. Following Garrett (10), an increase in inoculum density results in an increased inoculum potential which may give rise to higher probability of obtaining infection of the shoot. However, only one shoot infection was found in one harvest, out of a total of 205 inoculations. This observation is in agreement with the estimation of Huisman and Gerik (20) that only 0.02% of root infections lead to systemic infection of the shoot. Inoculation experiments using the same sources of eggplant and microsclerotia have previously identified a significant correlation between inoculum density of *V. dahliae* in soil and population density of conidia in stem sap, and between population density on/in roots and in stem sap (26). Apparently, the range of inoculum potential of *V. dahliae* at one site on the root, as used in our experiments, was not enough to increase the probability of shoot infection. Thus, overall our results are in agreement with those of other authors in that a large number of infections of the roots must take place for disease development (9,11,20,23,29).

It has been reported that microsclerotia farther than 100–400 μm (20,26) from the root are not able to cause root infection. Thus, only microsclerotia on that area of the screen filter over which the root was growing, would have been able to infect the root. Since the root was growing almost straight over the polypropylene screen, this area is approximated at most by the product of the length of the polypropylene screen (3 mm in Exp. 1; 6 mm in Exps. 2 and 3) with the sum of the diameter of the root tip (0.3 mm) and twice the distance across which the pathogen can grow to induce infection (0.8 mm) (12; displacement model), equaling $3 \times 1.1 = 3.3$ mm^2 for Exp. 1 and $6 \times 1.1 = 6.6$ mm^2 for Exps. 2 and 3. Thus, the amount of inoculum to which the root was exposed approximated 26, 207–393 and 51 microsclerotia for Exps. 1, 2 and 3, respectively. For the two experiments (no. 1 and no. 3) with low inoculum densities, the high incidence of root

infection, 65% and 40%, respectively, suggests that single propagules probably are able to infect the root.

Root infections were not limited to the inoculation site but occurred also in other parts of the root system. Thus, *V. dahliae* was present in tissue that already existed at the time of inoculation but was also present in tissue that was forming at that time, in both primary and secondary roots. All colonies growing from the plated roots were disjunct from each other, indicating limited growth of *V. dahliae* in the root cortex. Most colonies of *V. dahliae* appear to be restricted to superficial infection sites in the root cortex (5,19). This suggests that in our experiments the presence of *V. dahliae* in areas of the root system other than those close to the inoculation site cannot be explained by growth through the cortex from the infection site. On the other hand, cortical colonization occurring as a result of vascular infection and further hyphal growth from the vascular tissues into the cortex (2,29) would not explain the presence of colonies in those parts of the root system which were formed after the inoculation was done, since the xylem sap stream is upwards. Therefore, root infections at sites other than the inoculation site should have arisen from inoculum already existing in soil, although at a level below the detection threshold, or from the screen filter inoculum. Nevertheless, the possibility of infections from existing inoculum in soil was taken into account by including specific controls (control 1). No infections occurred in these controls. The possibility that some microsclerotia remained in the soil upon removal of the polypropylene screen cannot be excluded. Great care was taken that microsclerotia were strongly fixed in the screen and the soil was watered to avoid shrinkage of the microsclerotia during the inoculation period of 3–4 days. In addition, given the fact that microsclerotia must be very close to the root to cause infection, the probability of infection of some 'lost' microsclerotia would be quite small. A more likely explanation for the discrete colonies at locations other than the inoculation site may be that microsclerotia are able to sporulate in soil within 2–3 days after incubation under favorable conditions (6). Lacy and Horner (23), Menzies and Griebel (24) and Green and Papavizas (13) noticed population increases after incorporation of microsclerotia of *V. dahliae* in soil and attributed this to sporulation of microsclerotia in soil. Thirty to 50 conidia per microsclerotium have been reported in soil (6). In addition, the formation of viable secondary microsclerotia from germinated microsclerotia has been described (3). Soil conditions, such as soil humidity, available nutrients and soil fungistasis, may play a decisive role. On the other hand, Huisman (19) observed that the density of root colonies was proportional to the density of microsclerotia in soil. Therefore, it seems likely that sporulation of microsclerotia did not play a significant role in his experiments; this may have been due to the sporulation capacity of the inoculum used and to the prevailing soil conditions.

In Exps. 1 and 2, the number of root infections declined consistently with time. This is in agreement with Lacy and Horner (23), who found significantly declining densities of nonsystemic colonies of the mint isolate of *V. dahliae* on the roots of wilt-immune wheat and corn and resistant tomato plants. Apparently, colonies that fail to gain access to the vascular system do not contribute to maintenance of the pathogen population in soil.

From our observations we conclude that root infection by *V. dahliae* may be caused by few microsclerotia, if not by one. Infection of the vascular tissue was not improved by exposing roots to a range in number of microsclerotia, indicating that host defense mechanisms act efficiently even at high inoculum densities. Apparently, these inoculum

potentials are sufficient to cause root infection but, in general, insufficient to induce shoot infection.

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