Relationship Between Soil Inoculum Density of *Verticillium dahliae* and Systemic Colonization of Potato Stems in Commercial Fields Over Time

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ABSTRACT

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The purpose of this study was to relate soil inoculum density of *Verticillium dahliae* to systemic stem colonization by the fungus over time. Within four 64-ha commercial potato fields 100 small plots were located on a grid covering most of each field. The soil inoculum density of *V. dahliae* was determined for each plot. Percent potato stems colonized by *V. dahliae*, *Colletotrichum coccodes*, and *Erwinia carotovora* and root colonization by *Pratylenchus penetrans* was determined at four times during the season by destructively sampling plants within individual plots on the grid. A linear logit model adequately fitted soil inoculum density of *V. dahliae* vs. stem colonization data albeit with a large random error term for the model. Data were grouped into inoculum level classes according to soil inoculum density as follows: 0 (fungus not detected), 1–5, 6–10, 11–25, and >25 ppg. The proportion of stems colonized increased with increasing soil inoculum level of *V. dahliae* at any given sampling time. This was also true when stem colonization by *V. dahliae* was measured by percentage of vascular bundles colonized. A stem had a greater probability of being infected at its base than at its top for each sampling time and for any given soil inoculum level. The proportion of stems infected by *V. dahliae* increased over time. The rate of disease progress was similar regardless of inoculum density except when no *V. dahliae* was detected in the soil, in which case the rate was close to zero. Incidence of stem infection by soft-rot *E. carotovora* increased over time to nearly 100% by the last sampling time. There was no evidence that *E. carotovora* influenced the dose-response relationship between *V. dahliae* and proportion of stems infected. Similarly, there was no evidence that *C. coccodes* influenced the relationship between soil inoculum levels of *V. dahliae* and colonization of potato stems. Root infection by *P. penetrans* significantly increased the probability of stem infection by *V. dahliae* in some cases.

Additional key words: dose-response function, potato early dying.

Potato cultivars grown widely in the United States are susceptible to *Verticillium dahliae* Kleb., the principal causal agent of the potato early dying syndrome (4,23). Yield losses of up to 50% have been reported (15,20). Two control measures are available for fields where severe potato early dying may occur: rotation to nonhost crops for long periods of time to decrease soil inoculum densities of the fungus, and soil fumigation, for example with metam-sodium (3,20). Either alternative imposes high costs on the grower and may not be economically justified. Information on the relationship between soil inoculum densities of *V. dahliae* and subsequent disease development and yield losses would provide a useful tool for the evaluation of the efficacy of either control method and to facilitate the growers' management decisions.

Direct relationships have been established between soil inoculum density of *V. dahliae* and disease incidence, severity, and yield losses on cotton (1,21). A few attempts, reviewed by Nnodu and Harrison (16), have been made to establish such relationships for the potato crop. A high level of variability was observed in these studies (16,24). *V. dahliae* was isolated from 100% of the stems regardless of detection in the soil, and increasing soil inoculum densities of the fungus were associated with increasing severity of symptoms or yield losses in some cases but not all (16). Among the reasons that may account for this variability, the most frequently invoked was the heterogeneity of environmental parameters, particularly soil type and air temperature (16,26). Effect of the environment on disease expression is known to be of great importance for Verticillium wilt diseases (11,23,24,27). It has also been shown that cultural practices such as irrigation and fertility management can influence potato early dying severity caused by *V. dahliae*, which may account for poor relationships between soil inoculum density and disease when data are collected from different farms (7).

A major portion of Wisconsin potato production is concentrated in the center of the state on a flat, intensively irrigated area of sandy loam soils (13). The predominant cultivar is Russet Burbank. Based on the results obtained by Nnodu and Harrison (16) and Spaulding (26), it seemed possible, under the relatively homogeneous environmental and agronomic conditions of potato production in central Wisconsin, to establish a significant relationship between disease and soil inoculum density of *V. dahliae*. The present study was initiated to relate soil inoculum density of *V. dahliae* in commercial potato fields to the probability that the main stems of a plant would become systemically colonized by the fungus.

Several pathogens, including *Colletotrichum coccodes* (Wallr.) Hughes, *Erwinia carotovora* (Jones) Bergey et al, and *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans-Stakh. have been studied in association with the potato early dying syndrome (7,10,12–14,18,22,24,25). However, it is not known to what extent the presence of any of these pathogens affects the probability that a plant will become systemically infected by *V. dahliae*. To address this question, the incidence of plant infection by these pathogens was monitored in the second year of this study.

MATERIALS AND METHODS

Site selection and sampling. Four commercial potato fields with a history of Verticillium wilt were selected in the Central Sands area of Wisconsin. These fields consisted of a loamy sand soil (sandy, mixed, mesic, Typic Udipsamment) and were flat, circular (about 800 m in diameter), and irrigated, with center pivot systems. Research was conducted in field A in 1982 and in fields B, C, and D in 1983. A wedge of field B, covering one-twelfth of the total area of the field, had been fumigated in October 1980 with metam-sodium

(468 L/ha). A similar treatment was applied to the eastern half of field C in October 1982. The fields were planted in late April-early May with different seed lots of the potato cultivar Russet Burbank. The rows were 90 cm apart, and seed pieces were 30 cm apart in the rows. The cultural practices applied to these fields during the growing season were comparable, although not identical (Table 1).

Ten locations were randomly chosen in field A in 1982. A "study area" (4.5 × 4.5 m) was delimited at each location and divided into 25 contiguous square quadrats, 90 cm on a side, each encompassing a segment of row with three plants. From the center of each quadrat, one soil core was removed in early June to a depth of 30 cm with a standard soil-sampling tube, 2.5 cm in diameter. Each soil sample was placed in a polyethylene bag labeled with the study area number and quadrat coordinates and transported to the laboratory to be assayed for *V. dahliae*. In each study area, one main stem was removed from one plant of each quadrat in early August 1982. The sampled stems were cut at the soil level, defoliated, labeled with the study area number and the quadrat coordinates, and transported to the laboratory. The 250 stems were stored at 4 C until assayed for *V. dahliae* (within 3 days).

One hundred individual square quadrats, 90 cm on a side (Fig. 1), were delimited in each of fields B, C, and D in 1983. These quadrats were located 54 m apart, on a regular grid pattern approximately centered on the irrigation pivot. In addition, three (6.3 × 6.3 m) study areas were delimited in each field, and each was divided into 49 contiguous square quadrats, 90 cm on a side; these will be referred to as study area quadrats, to distinguish them from the individual quadrats described above. One study area was located in the fumigated and two in the nonfumigated portion of each of fields B and C. Based on 1982 data, the sampling procedures for 1983 were modified to increase the volume of soil and the number of stems sampled in each quadrat. Soil samples were taken from each quadrat in early June 1983, shortly after emergence. Each sample consisted of nine 2.5- × 30-cm soil cores removed from locations shown in Figure 1, and bulked. Samples were kept separate and labeled as indicated for the 1982 season. Plant samples were collected from each of fields B, C, and D on 7 and 21 July, and 4 and 18 August 1983. These sampling dates will be referred to as T1, T2, T3, and T4, respectively. Each individual quadrat was sampled once during the season, at a sampling date assigned at random at the beginning of the season. Twenty-five of the 100 individual quadrats were sampled in each field at each sampling date. Sampling consisted of cutting at the soil surface the main stems from each of the three plants present in each quadrat. Each stem was labeled, defoliated, and brought to the laboratory as in 1982. The root systems of the three plants in each quadrat were hand dug and placed in a single polyethylene bag. The quadrats in the 6.3- × 6.3-m study areas were sampled at times T2 and T3 for field D, T2 for field C, and T3 for field B. Samples in each study area quadrat consisted of one main stem from each of the three plants present in these quadrats. Stem and root samples were kept at 4 C until assayed. Plant samples were processed within 7 days of the sampling date.

Soil and plant assays. The soil samples were air dried and assayed for *V. dahliae* with a dilution plating technique as described previously (15). One 10-g subsample was suspended in 100 ml of water and five 1-ml aliquots were plated onto an NPX-

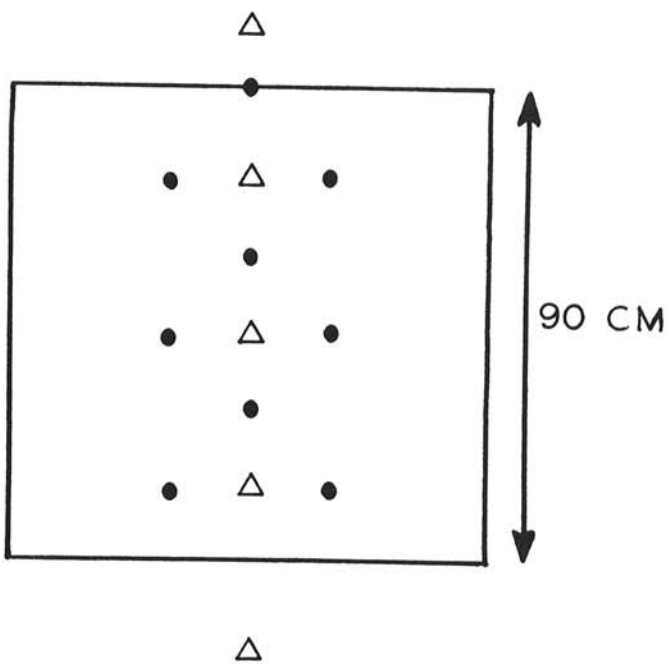

Fig. 1. Sampling scheme within the 1983 quadrats. Potato plants are represented by triangles (△) and locations of soil cores by circles (•).

TABLE 1. Main cultural practices applied in 1983 to three commercial potato fields of central Wisconsin

Type and time of practice	Implement used or product applied		
	Field B	Field C	Field D
Tillage			
Preplant	Disk + plow	Plow	Plow
Postemergence	Hilling	Hilling	Hilling
Fertilization[a]			
Before planting		0-0-62: 670 kg	lime: 2,240 kg 0-0-62: 500 kg $CaSO_4$: 1,120 kg
At planting	5-20-20: 670 kg	8-16-24: 900 kg	7-14-28: 670 kg
At emergence	34-0-0: 340 kg	34-0-0: 420 kg	34-0-0: 450 kg
Mid-June	34-0-0: 340 kg	34-0-0: 420 kg	30 kg equ. N[b]
Early July			30 kg equ. N[b]
Mid-July			30 kg equ. N[b]
Herbicide[c]	Linuron + alachlor	Linuron + alachlor	Metribuzin
Nematicide[d]	Aldicarb	Aldicarb	Aldicarb
Insecticide[e]		Fenvalerate	Permethrin
Fungicide[f]	Triphenyltinhydroxide	Triphenyltinhydroxide	Maneb + chlorothalonil

[a] The type of fertilizer indicates the equivalent N-P-K. The doses are for 1 ha.
[b] Applied through the irrigation system.
[c] Postemergence.
[d] Applied early May at 2.24 kg a.i./ha.
[e] Applied twice in field C (late June and early July) and once in field D (early August) to control Colorado potato beetle.
[f] Applied at weekly intervals from mid-June to vinekill.

pectate medium selective for *V. dahliae* (5), for each soil sample assayed in 1982. To increase the precision of the assay, three 10-g subsamples were taken from each sample assayed in 1983 and two 1-ml aliquots were plated for each subsample. All the plates were incubated at 20–22 C for at least 2 wk and microsclerotial colonies of *V. dahliae* were counted using a dissecting microscope (magnification 15×).

A 10-cm section was taken from the base of each stem in 1982, surface sterilized for 1 min in 0.5% NaOCl, and rinsed for 1 min in sterile distilled water. A cross section, approximately 2 mm thick, was cut from the center of the surface sterilized portion and plated on modified Menzies-Griebel medium (10). After a 14-day incubation at 20–22 C, each stem section was examined under the dissecting microscope, and the number of main vascular bundles infected by *V. dahliae* was recorded.

Two 10-cm sections, one at the base and one at the top of each stem, were taken in 1983 and assayed for *V. dahliae* as described above. Presence of *C. coccodes* growing from the stem sections was recorded. The stems were assayed for soft-rot Erwinia by streaking a drop of sap, squeezed from the surface sterilized base portion of each stem, onto Crystal Violet Pectate medium (6). After 24- and 48-hr incubations at 22 C, the plates were examined and the presence or absence of soft-rot Erwinia was recorded. To assess the root population density of *P. penetrans*, the roots were washed under tap water to remove soil and organic debris and blotted dry with paper towels. A 1-g root sample was placed in a 125-ml flask with 50 ml of a 100 ppm streptomycin solution and incubated for 7 days on a reciprocating shaker (13). Root debris was removed by passing the solution through a 150-mm-mesh sieve, after which samples were stored at 4 C until the nematodes were counted.

RESULTS

Soil inoculum densities. Density of soil inoculum of *V. dahliae* in the quadrats within study areas ranged from 0 (fungus not detected in the assay) to 16 propagules per gram (ppg) of soil in 1982 and 0–64 ppg in 1983 (Table 2), with averages between 1.9 and 4.9 ppg in 1982 and 0.2 and 17.7 ppg in 1983. Few study areas had quadrats with inoculum densities greater than 20 ppg. The lowest average inoculum densities were observed in study areas B1 and C1, which were established in the fumigated part of fields B and C. The inoculum densities of *V. dahliae* in these study areas were not greater than 5 ppg, and the fungus was detected in the soil of fewer than 20% of the quadrats (Table 2).

In the 1983 individual quadrats, inoculum levels ranged from 0 to 80 ppg with field averages ranging from 3.7 to 10.5 ppg (Table 2). Although the inoculum densities averaged across fields B or D were comparable, the two fields differed in the frequency at which various inoculum levels occurred. The fungus was detected in only 40% of the individual quadrats of field D, but a few quadrats had relatively high inoculum levels. In field B, 79% of the quadrats had detectable levels of *V. dahliae*, and in no quadrat was the level greater than 25 ppg. The fumigated portions of fields B and C had significantly lower ($P < 0.05$) inoculum levels of *V. dahliae* than the

TABLE 2. Soil inoculum levels (propagules per gram, ppg) of *Verticillium dahliae* by frequency in the study areas and the individual quadrats established in four commercial potato fields

Field or study area	Range of inoculum densities (ppg)	Frequency of soil inoculum level class[a]							Average (ppg)
		0[b]	1–5	6–10	11–20	21–45	>45	Total	
Study areas[c]									
A1	0–10	12	10	3	0	0	0	25	1.92
A2	0–14	8	14	2	1	0	0	25	2.24
A3	0–14	8	13	2	2	0	0	25	2.72
A4	0–12	8	13	3	1	0	0	25	2.72
A5	0–10	10	10	5	0	0	0	25	2.72
A6	0–10	11	9	5	0	0	0	25	2.72
A7	0–14	6	14	4	1	0	0	25	3.28
A8	0–12	11	5	8	1	0	0	25	3.44
A9	0–16	8	8	7	2	0	0	25	4.08
A10	0–16	6	8	9	2	0	0	25	4.88
B1	0–3	40	9	0	0	0	0	49	0.34
B2	0–7	33	14	2	0	0	0	49	1.02
B3	5–45	0	1	11	21	16	0	49	17.65
C1	0–3	45	4	0	0	0	0	49	0.17
C2	0–45	4	15	9	14	7	0	49	10.85
C3	0–64	1	10	6	18	11	3	49	17.31
D1	0–7	39	9	1	0	0	0	49	0.51
D2	0–5	39	10	0	0	0	0	49	0.58
D3	0–14	31	14	3	1	0	0	49	1.46
Individual quadrats									
Field B[d]									
F	0–2	8	1	0	0	0	0	9	0.19
NF	0–25	21	45	13	9	3	0	91	4.82
All	0–25	29	46	13	9	3	0	100	4.40
Field C[d]									
F	0–12	41	5	3	1	0	0	50	0.97
NF	0–80	1	1	13	19	15	1	50	19.97
All	0–80	42	6	16	20	15	1	100	10.47
Field D									
Whole field	0–58	60	20	6	11	2	1	100	3.72

[a] Number of quadrats in each inoculum level class.
[b] Fungus not detected in the soil assay.
[c] The study areas are lettered after the fields in which they were established.
[d] Part of this field was fumigated with metam-sodium. The data are presented for the individual quadrats located in the fumigated (F) and the nonfumigated (NF) portions of the field, and for all quadrats (All).

rest of the fields. The fungus was detected in only 11% (field B) and 18% (field D) of the quadrats located in these areas (Table 2).

Relationship between stem colonization and soil inoculum density in the study areas. The incidence of stem colonization, estimated in each study area by the proportion of sampled stems infected by *V. dahliae* at 100 ± 10 days after planting, was related to the average soil inoculum level of the fungus (Fig. 2). Results from the sampling of the study areas of fields C and D at time T2 were not included in this comparison as the number of days after planting was less than 90. The incidence of stem colonization remained lower than 20% for plants from study areas with average soil inoculum levels of *V. dahliae* below 2 ppg and sharply increased to 70–100% when inoculum levels were greater than 2 ppg.

A significant ($P < 0.05$) positive correlation was found between the degree of stem infection, assessed by the number of main vascular bundles infected by *V. dahliae* at the base of the stems, and soil inoculum levels of the fungus for most 1983 study areas (Table 3). However, the correlation coefficients were not significant for the 1982 study areas, in which only one stem and one soil core were assayed in each quadrat.

Relationship between stem colonization and soil inoculum density in the individual quadrats. The proportion of stems and vascular bundles infected by *V. dahliae* at each sampling time was computed for each inoculum density determined by the soil assay. Data from all quadrats with the same inoculum density were pooled. The response (proportion of stems or of vascular bundles infected at the base or at the top of the stems) was then related to the dose (soil inoculum level of *V. dahliae*) for the data from each sampling time in each field (Fig. 3). To describe the dose-response relationships and facilitate their comparison between fields and over time, probit and linear logit models (8) were fit to each data set. The linear logit model gave a better fit to the data overall. The model was expressed as $\text{Log}[p/(1-p)] = \alpha_0 + \alpha_1 ID_1$, where p is the probability a main stem was infected if it was sampled from a plant growing on soil with an inoculum density ID (in ppg) of *V. dahliae*. The computations for the fitting procedures were done with the computer program GLIM (2). GLIM includes procedures for handling proportional data with values of 0 or 1 as well as estimates of proportions based on different numbers of observations (2). The fit of each model was evaluated with a Pearson type chi-square goodness-of-fit test (9). The model fit the data well for field B (Table 4). For fields C and D, significant lack of fit ($P < 0.05$) was observed for sampling times T1 and T2.

A low incidence of stem infection was observed in fields B and D in quadrats where the pathogen was not detected in the soil (soil inoculum density lower than 1.7 ppg, the detection limit of the assay). In field C, however, with the exception of one stem at sampling time T3, the fungus was never isolated from stem samples in quadrats where the soil inoculum level was lower than 2 ppg.

At sampling times T3 and T4, increases in soil inoculum levels from 0 (fungus not detected in the soil assay) to 5 ppg (fields B and D) or from 4 to 7 ppg (field C) coincided with a sharp increase in incidence of stem infection (base of stem) from nearly 0 (field D) or 10–30 (fields B and D) to nearly 100%. For inoculum levels greater than these values, the fungus was isolated from the base of nearly 100% of the stems examined (Fig. 3A, C, and E). This phenomenon was reflected by the high values of coefficients α_1 in the logit models (Table 4).

For all three fields the data points were widely scattered around the fitted curves (Fig. 3). The variability of the response to increasing doses of soil inoculum of the pathogen was greater for fields D and C than for field B. It was also greater for sampling times T1 and T2 than for T3 and T4 and greater for infection at the top of the stems than at the base for all three fields. The apparent variability may have resulted from the fact that the probability of stem infection corresponding to each nonzero soil inoculum level of the pathogen was estimated from the examination of an average of only 18 stems for each sampling time in each field.

To increase the accuracy of the estimates of the probability of stem infection, the number of observations used in the computation of each estimate was increased by grouping the data into five inoculum level classes according to soil inoculum density: 0 (fungus not detected in the soil), 1–5 ppg, 6–10 ppg, 11–25 ppg, and greater than 25 ppg. The resulting dose-response plots (Fig. 4) confirmed the trends detectable on the initial graphs of Figure 3. The probability that a stem would be infected tended to increase with increasing soil inoculum levels of the pathogen at any given sampling time, and with time for a given level of soil inoculum. The few exceptions to this trend corresponded to proportions of stems infected that were estimated from the examination of only a few stems (20 or less).

A stem had a greater probability to be infected at its base than at its top for each sampling time and for any given soil inoculum level (Figs. 3 and 4). However, in approximately 1% of the stems sampled from each field at each sampling time, the fungus was isolated from the top but not from the base of the stem.

The degree of stem infection by *V. dahliae*, measured by the percentage of main vascular bundles infected at the base or at the top of the sampled stems, also tended to increase over time, and with increasing soil inoculum levels of the fungus (Fig. 5). Similarly, a higher proportion of vascular bundles was found infected at the base than at the top of the stems, regardless of sampling time and soil inoculum level.

TABLE 3. Correlation between soil inoculum densities of *Verticillium dahliae* and the proportion of vascular bundles infected in the study areas at 100 ± 10 days after planting

1982 data[a]		1983 data[b]	
Study area	Correlation[c]	Study area	Correlation[c]
A1	−0.19	B1	0.37 *
A2	−0.02	B2	0.88 *
A3	0.12	B3	0.02
A4	−0.03	C1	0.79 *
A5	0.41	C2	0.59 *
A6	−0.20	C3	0.77 *
A7	0.09	D1	0.27
A8	−0.12	D2	0.37 *
A9	−0.21	D3	0.00[d]
A10	0.19		

[a] Each study area consisted of 25 quadrats. One soil core and one stem were assayed in each quadrat.

[b] Each study area consisted of 49 quadrats. In each quadrat, nine soil cores were bulked into a soil sample and assayed, and three stems were assayed.

[c] An asterisk indicates that the correlation coefficient is significantly different from zero at $P = 0.05$.

[d] No stems were infected by the fungus in the study area.

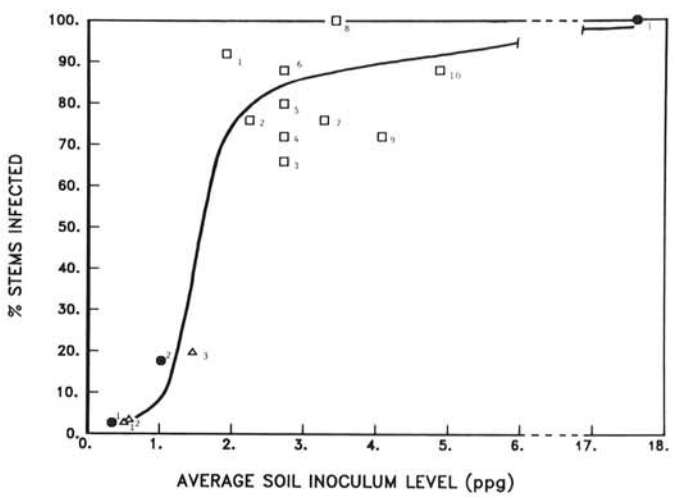

Fig. 2. Relationship between incidence of stem infection by *Verticillium dahliae* at 100 ± 10 days after planting and average soil inoculum level in the study areas of fields A (□), B (•), and D (Δ). The number near each data point refers to the identification number of each study area as described in Table 3. The curve was fit visually to the data.

Relationship between disease progress and soil inoculum level. The proportion of stems infected by *V. dahliae* at their base and at their top increased over time in all three fields during the 1983 growing season (Table 5). By the last sampling date, 45–70% of the stems sampled in the fields were infected at their base, and the fungus had reached the top of 20–44% of the stems.

To account for the dose-response relationships examined earlier, the progress of disease was studied separately for stems from quadrats with various soil inoculum levels of the pathogen. Four inoculum levels were distinguished, based on the results presented earlier: 0 ppg (fungus not detected in the soil), 1–5 ppg, 5–10 ppg, and greater than 10 ppg. To obtain maximum accuracy, the estimates of incidence of stem infection and proportion of vascular bundles infected were computed with the pooled data from all three fields, including the data from the study areas. These parameters were plotted against time, measured as the number of days after planting (Fig. 6). As field D was planted 15 days after field B, the data from sampling time T1 in field D were not pooled with the data from T1 in fields B and C. The data from T1 in fields B and C were pooled with those from T2 in field D. Similarly, the

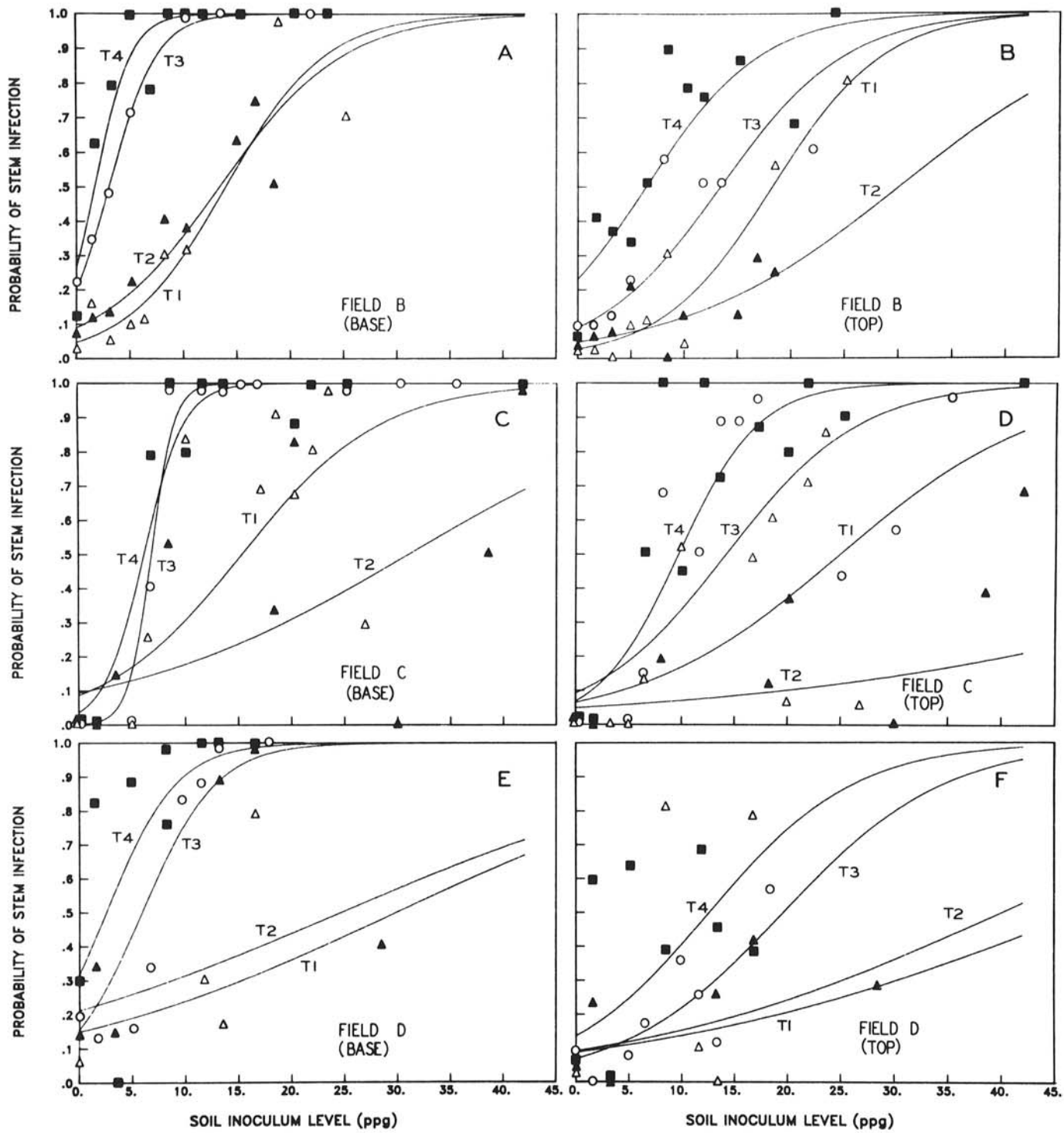

Fig. 3. Relationship between the probability of isolating *Verticillium dahliae* from main stems of potato plants (estimated by the proportion of infected stems) and the inoculum level of the fungus in the volume of soil explored by the root system of the plants. The relationship was quantified in three commercial fields at four times, T1 (Δ), T2 (▲), T3 (o), and T4 (■), during the 1983 growing season, as described in Table 4. The base (A, C, E) and the top (B, D, F) of each stem were assayed. The fitted curves are those of the linear logit model described in Table 4. A soil inoculum level of 0 ppg indicates that the fungus was not detected in the soil assay.

data from T2 and T3 in fields B and C were pooled with those from T3 and T4, respectively, in field D. Finally, the data from T4 in fields B and C were pooled. An average number of days after planting was associated with each data set to obtain the plots presented in Figure 6. The percent plant infection over time was different for each level of soil inoculum. Disease incidence and the degree of stem colonization remained very low in quadrats where the fungus was not detected with the soil assay. For the three nonzero levels of soil inoculum the curves were nearly parallel, suggesting a similar rate of disease progress between 75 and 120 days after planting.

Interactions with other pathogens. The incidence of stem infection by soft-rot Erwinia increased over time in 1983 to nearly 100% by sampling time T3, in all three fields (Table 5). Percent plant infection was as high in field D, where a massive amount of calcium salts (lime and $CaSO_4$) had been applied, as in the two others, in which no calcium had been added (Table 1). The incidence of stem colonization by *C. coccodes* in the 1983 growing season was higher at the base than at the top of the stems and increased over time in all three fields (Table 5). Only 15% of the stems were colonized at their base in field C by sampling time T4, compared with 43% in field B. In field D colonization of stems by

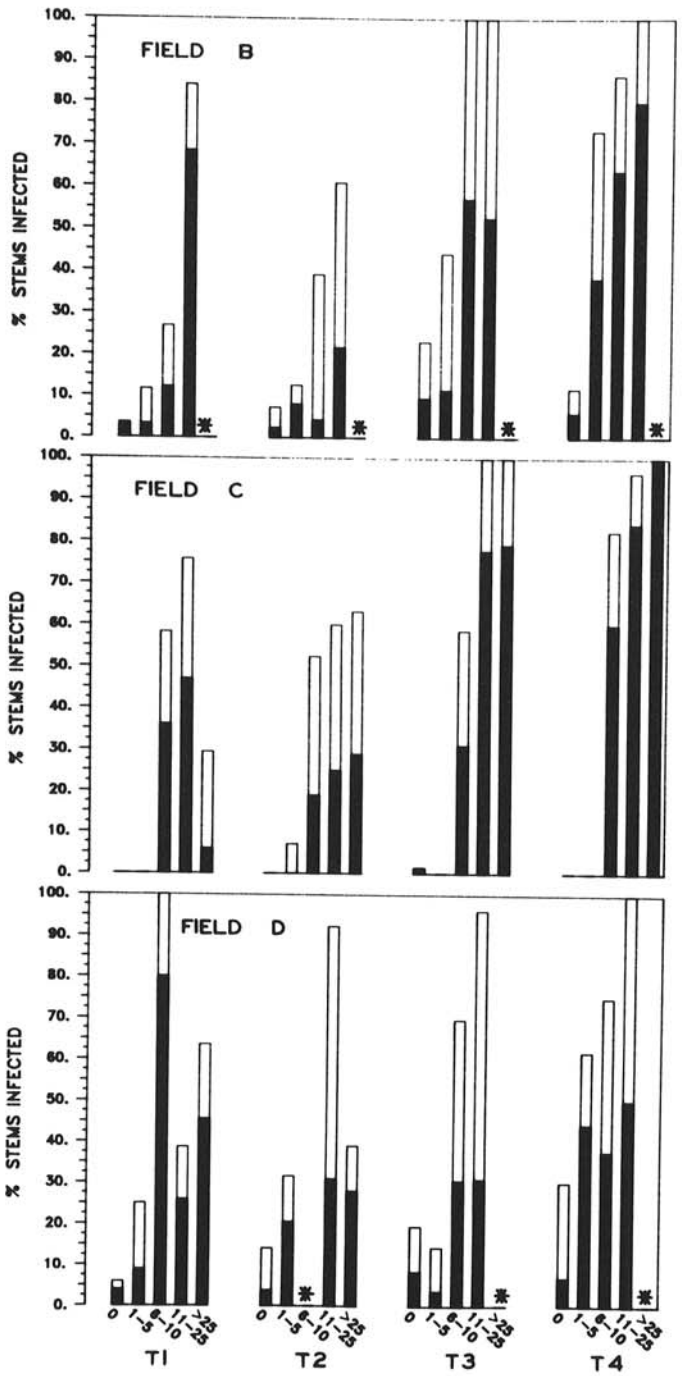

Fig. 4. Percentage of potato stems infected by *Verticillium dahliae* at their base (□) or at their top (■), from plants growing on soil with inoculum levels of 0 (fungus not detected in the soil assay), 1–5 ppg, 6–10 ppg, 11–25 ppg, and greater than 25 ppg, in three commercial potato fields. Stems were examined at four times (T1–T4, as described in Table 4) during the 1983 growing season. A star indicates that no data were available for a particular class of inoculum levels.

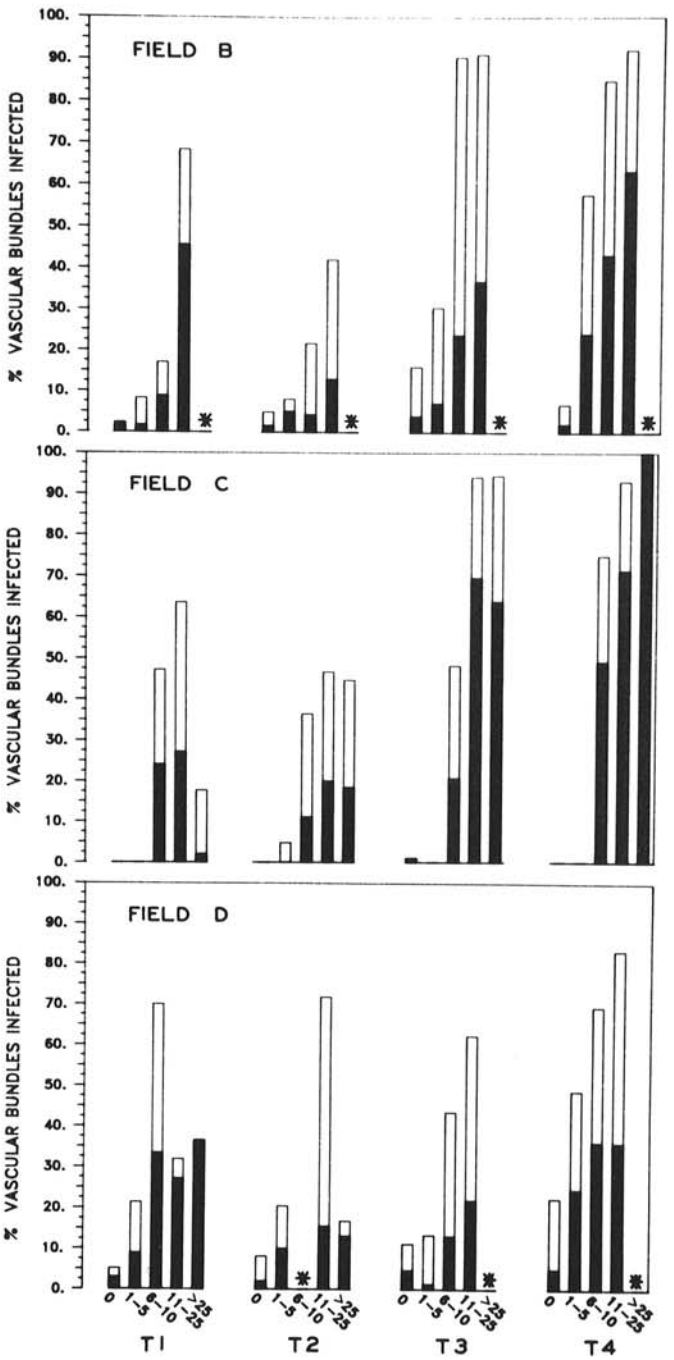

Fig. 5. Percentage of main vascular bundles infected by *Verticillium dahliae* at their base (□) or at their top (■), from plants growing on soil with inoculum levels of 0 (fungus not detected in the soil assay), 1–5 ppg, 6–10 ppg, 11–25 ppg, and greater than 25 ppg, in three commercial potato fields. Stems were examined at four times (T1–T4, as described in Table 4) during the 1983 growing season. A star indicates that no data were available for a particular class of inoculum levels.

C. coccodes progressed very rapidly, resulting in nearly 100% of the stems being colonized at their base by sampling time T2. The level of root colonization by *P. penetrans* increased over time in all three fields (Table 6). However, the concentration of nematodes per gram of roots remained generally low, and the pathogen was detected in only 37% of the quadrats sampled.

To test the hypothesis that the relationship between the incidence of stem infection by *V. dahliae* and the soil inoculum level of the pathogen might be influenced by interactions with one or several of these other pathogens, the logit model described earlier was expanded to include terms for each of these pathogens. The model with all such terms was expressed as $\text{Log}[p/(1-p)] = \alpha_0$

TABLE 4. Fit of the linear logit model $\text{Log}[p/(1-p)] = \alpha_0 + \alpha_1 ID$ relating the probability p of stem infection by *Verticillium dahliae* to the soil inoculum density ID of the fungus[a]

Field	Sampling time[c]	Base of the stem assayed[b]			Top of the stem assayed[b]		
		α_0 + SE	α_1 + SE	$p(\chi^2>X^2)$	α_0 + SE	α_1 + SE	$p(\chi^2>X^2)$
B	T1	−2.997 + 0.300	0.215 + 0.033	0.41	−3.548 + 0.366	0.194 + 0.031	0.57
	T2	−2.313 + 0.282	0.173 + 0.033	0.98	−2.983 + 0.386	0.099 + 0.040	0.65
	T3	−1.378 + 0.260	0.450 + 0.089	>0.99	−2.309 + 0.281	0.172 + 0.040	0.84
	T4	−1.022 + 0.302	0.592 + 0.104	0.85	−1.201 + 0.231	0.187 + 0.037	0.47
C	T1	−2.355 + 0.304	0.154 + 0.020	<0.01	−2.661 + 0.344	0.107 + 0.020	<0.01
	T2	−2.259 + 0.283	0.073 + 0.012	<0.01	−2.956 + 0.363	0.039 + 0.089	<0.01
	T3	−6.303 + 1.691	0.925 + 0.248	0.52	−2.280 + 0.322	0.161 + 0.025	0.74
	T4	−3.270 + 0.521	0.534 + 0.080	0.77	−2.605 + 0.359	0.269 + 0.036	<0.01
D	T1	−1.733 + 0.182	0.058 + 0.014	<0.01	−2.329 + 0.225	0.049 + 0.011	<0.01
	T2	−1.307 + 0.194	0.053 + 0.018	<0.01	−2.283 + 0.271	0.057 + 0.020	0.01
	T3	−1.683 + 0.240	0.287 + 0.043	0.81	−2.595 + 0.330	0.132 + 0.035	0.06
	T4	−0.763 + 0.176	0.304 + 0.057	0.12	−1.848 + 0.230	0.146 + 0.032	0.06

[a]Stem samples were taken from three commercial potato fields at four times during the 1983 growing season and assayed at their base and at their top.
[b]SE = standard error of coefficient a. The fit of the model was evaluated by a chi-square goodness-of-fit test. P values below 0.05 indicate a significant lack-of-fit of the model at the 5% significance level.
[c]Dates of sampling were 7 and 21 July, and 4 and 18 August 1983 for T1-T4, respectively.

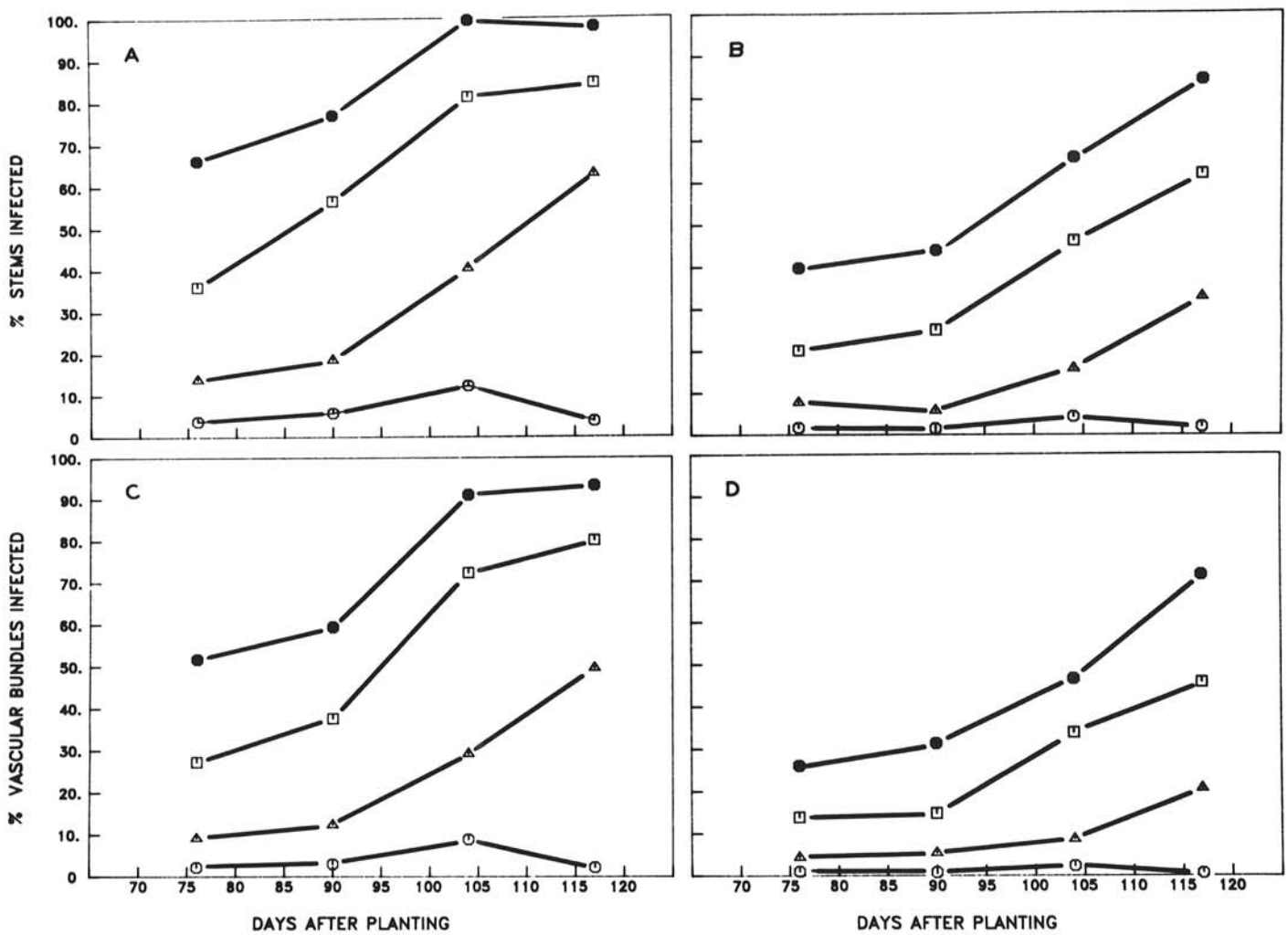

Fig. 6. Relationship between disease progress and soil inoculum level of *Verticillium dahliae* in three commercial potato fields (pooled data) during the 1983 growing season. Disease was expressed as a percentage of stems infected at their base A or at their top B, or as a percentage of main vascular bundles infected at the base C or at the top D of the stems. The soil inoculum levels were: o = pathogen not detected in soil, Δ = 1–5 ppg, □ = 6–10 ppg, and • = greater than 10 ppg.

$+ \alpha_1 ID + \alpha_2 PP + \alpha_3 E + \alpha_4 CC$, where, as before, p was the probability for a main stem to be infected by *V. dahliae* if it was sampled from a plant growing on soil with an inoculum density ID (expressed in ppg) of the fungus. The term PP was the concentration of *P. penetrans*, expressed as a number of nematodes per gram of root, in the root sample from the quadrat where the stem was taken. The terms E and CC took the value 1 if the stem was colonized by soft-rot Erwinia or *C. coccodes*, respectively, and 0 otherwise. The computations of the model fitting were performed, as before, with the computer program GLIM. For each data set, the new terms were added to the original model, $\text{Log}[p/(1-p)] = \alpha_0 + \alpha_1 ID$, in a step-wise fashion. A chi-square test was performed to estimate whether a significant improvement in fit ($P < 0.05$) was obtained by adding these terms one at a time to the original model (8). The step-wise fitting procedure was continued until no significant improvement over the best model of the previous step occurred. Table 7 shows the best logit models fitted with this procedure for the data from each field at each sampling time. Addition to the original model of terms representing the effect of other pathogens provided a significant improvement of the fit for fewer than half of the data sets, most of which were associated with field D. The term PP was present in seven of those models, mostly associated with positive values of the coefficient α_2. This indicated that for a given soil inoculum level of the fungus, the probability of stem infection by *V. dahliae* was higher if *P. penetrans* was present in the root system of the plants than otherwise. The term E was present in four models, twice associated with a positive α_3 and twice with a negative α_3. This suggested that the probability of stem infection by *V. dahliae* was higher in some cases and lower in others, if the stem was also colonized by soft-rot Erwinia. The term CC was present in three models, twice associated with negative values and once with a positive value of coefficient α_4. Logit models not containing ID but only PP, E, and/or CC were not significant.

DISCUSSION

This study demonstrated that in four commercial potato fields of central Wisconsin, increasing soil inoculum levels of *V. dahliae* were associated with an increase in the probability of isolating the fungus from the vascular system at the base or at the top of the main stems of Russet Burbank potatoes.

TABLE 5. Incidence of stem infection (in percent) by *Verticillium dahliae* (VD), soft-rot Erwinia (E), and *Colletotrichum coccodes* (CC) in three commercial potato fields at four times during the 1983 growing season

Pathogen[b]		Field B[a]				Field C[a]				Field D[a]			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
VD	Base	14.4	20.2	46.1	70.2	34.1	25.8	49.4	50.3	20.1	26.0	35.7	47.6
	Top	9.5	8.1	16.8	44.4	19.0	9.7	36.4	41.9	12.4	15.6	13.0	21.4
E	Base	26.1	53.0	95.3	78.0	42.2	81.7	92.6	94.8	17.0	70.8	96.2	94.7
CC	Base	5.3	35.9	35.6	43.4	0.9	4.3	9.7	15.2	15.1	96.9	100.0	97.6
	Top	2.3	11.6	18.3	13.7	0.0	0.5	6.3	5.2	3.1	74.0	69.7	85.4

[a] Dates of sampling were 7 and 21 July, and 4 and 18 August 1983 for T1-T4, respectively.
[b] Stems were assayed for infection by the pathogens at their base (Base) and at their top (Top).

TABLE 6. Root colonization by *Pratylenchus penetrans* in three commercial potato fields at four times during the 1983 growing season

Nematodes/ gram root	Field B[a]				Field C[a]				Field D[a]			
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
0	20[b]	19	19	11	16	16	14	12	24	13	14	10
1–10	5	5	6	11	6	7	6	6	0	11	8	9
11–100	0	1	0	3	3	2	4	7	1	1	3	5
>100	0	0	0	0	0	0	1	0	0	0	0	1
Total	25	25	25	25	25	25	25	25	25	25	25	25

[a] Dates of sampling were 7 and 21 July, and 4 and 18 August 1983 for T1-T4, respectively.
[b] Numbers of quadrats in class of root population of *P. penetrans*.

TABLE 7. Best linear logit model relating the probability of stem infection by *Verticillium dahliae* to the soil inoculum level (ID) of the fungus

Field	Sampling time[a]	Base of the stem assayed	Top of the stem assayed
B	T1	$-3.45 + 0.22$ ID $+ 1.19$ E[b]	$-3.55 + 0.19$ ID
	T2	$-2.31 + 0.17$ ID	$-2.98 + 0.099$ ID
	T3	$-1.38 + 0.26$ ID	$-2.31 + 0.17$ ID
	T4	$-1.02 + 0.59$ ID	$-1.20 + 0.19$ ID
C	T1	$-2.36 + 0.15$ ID	$-2.81 + 0.10$ ID $+ 0.04$ PP
	T2	$-2.31 + 0.11$ ID $- 0.18$ PP	$-2.96 + 0.04$ ID
	T3	$-6.30 + 0.93$ ID	$-2.28 + 0.16$ ID
	T4	$-3.27 + 0.53$ ID	$-2.61 + 0.27$ ID
D	T1	$-1.90 + 0.07$ ID $+ 0.22$ PP $- 1.74$ CC	$-2.49 + 0.05$ ID $+ 0.09$ PP
	T2	$-0.85 + 0.06$ ID $+ 0.03$ PP $- 1.23$ E $- 1.94$ CC	$-3.92 + 0.04$ ID $+ 1.97$ CC
	T3	$-0.21 + 0.29$ ID $- 2.00$ E	$-2.60 + 0.13$ ID
	T4	$-0.29 + 0.31$ ID $+ 0.02$ PP $+ 1.44$ E	$-2.28 + 0.17$ ID $+ 0.01$ PP

[a] Dates of sampling were 7 and 21 July, and 4 and 18 August 1983 for T1-T4, respectively.
[b] The original model of Table 5 was expanded with terms representing the effect of other pathogens: *Pratylenchus penetrans* (PP), soft-rot Erwinia (E), and *Colletotrichum coccodes* (CC).

The relationship between the dose (soil inoculum level in early June) and the response (incidence of stem infection by *V. dahliae* at four times during the 1983 growing season) was quantified in three fields. A logit model adequately fitted the data from sampling times T3 and T4 in all three fields and those from sampling times T1 and T2 in one of the fields. In all three fields, the data points were widely scattered around the fitted model. One possible reason for this variability was that for each nonzero level of soil inoculum, only a few stems (18 on the average) were available in the samples for the estimation of disease incidence and degree of stem colonization in each field and each sampling time. A greater scatter of data points observed for field D than for fields C and B coincided with a lower number of stems examined from each class of soil inoculum level. The proportion of quadrats where the fungus was not detected in the soil was greater in field D (60%) than in field C (42%) and in field B (29%), leaving only approximately 42, 58, and 68% of the stems sampled for the estimation of disease incidence at various nonzero soil inoculum levels in fields D, C, and B, respectively. Other possible reasons for this apparent variability in the response to increasing doses of soil inoculum of *V. dahliae* include the variability associated with the stem assay and with the soil assay, differences in the physical and biological microenvironment of the root systems and in the physiology of individual plants, and differences in the aggressiveness of various strains of *V. dahliae*. The occurrence of strains of this fungus with various degrees of aggressiveness is well documented for cotton fields (19,23,24) and has been demonstrated in potato fields (16,17). Although wide differences in pathotypes are usually not expected to be found within individual cotton fields (19), no information is available for potato fields.

Based on the results of previous studies on the relationship between disease and soil inoculum levels of *V. dahliae* for various crops, a high level of variability in the dose-response relationship was not unexpected (15,25). A unique aspect of the present study is the consistency of the results found in three different fields, despite the slight differences in cultural practices applied to each field (Table 1). In all three fields, the probability of isolating *V. dahliae* from the base of the stems at sampling times T3 and T4 increased sharply from nearly 0 to nearly 100% for soil inoculum levels increasing from 0 to 5–7 ppg. In all three fields, the fungus could be isolated at sampling times T3 and T4 from nearly 100% of the stems from quadrats where the soil inoculum level was greater than 5–7 ppg. The average monthly temperatures for June, July, and August were 3.9, 1.7, and 3.9 C higher, respectively, in 1983 then 1982. Although the climatic conditions during the 1982 and 1983 growing seasons were very different, the results from 1983 were consistent with the high incidence of stem infection found in the study areas of field A in 1982 at a date corresponding to sampling date T3 of 1983 (100 ±10 DAP).

Nnodu and Harrison (16) reported that 100% incidence of stem infection by *V. dahliae* was observed at 10 wk after plant emergence in 1975, in 30 Colorado fields with a range of soil inoculum levels from 0 to 67 ppg and planted to potato varieties Norgold and Norchip. In the present study, the fungus was never isolated from the stems in quadrats of field C where the fungus was not detected in the soil. In the two other fields, an incidence of stem infection of up to 23 (field B, sampling time T3) and 30% (field D, sampling time T4) was observed in quadrats where the fungus was not detected in the soil. This result could be attributed in part to the variability of the soil assay and that of the plant response. It also suggests that soil inoculum levels below the detection limit of the soil assay (1.7 ppg) were sufficient to result in low disease incidence in fields B and D by the end of the season. The differences between field C and fields B and D may be related to the fumigation of half of field C, where 41 of the 42 quadrats where *V. dahliae* was not detected in the soil were located. Failing to detect the fungus in the soil of fumigated quadrats could reflect the actual absence of the fungus, whereas in nonfumigated portions of the fields, a low but nonzero inoculum level of the fungus may have been present even if the fungus was not detected by the soil assay.

Increasing levels of root colonization by *P. penetrans* tended to be associated with a greater probability of observing stem infection by *V. dahliae*. This phenomenon was statistically significant for only six of the 24 data sets examined (Table 7) and contrasted with previous reports of greatly increased disease severity in plants infected by both pathogens (13). This may be related to the low levels of root colonization observed in this study (Table 6), compared to levels reported by others (11–13), and the fact that aldicarb was applied in the three fields studied (Table 1). The results on the effect of soft-rot Erwinia were inconclusive. Presence of soft-rot Erwinia was associated with both a significantly increased probability of stem infection by *V. dahliae*, for two of the 24 data sets examined, and a decreased probability for two others. The effect of the bacteria might be masked by the very rapid progress of the epidemic resulting in nearly 100% incidence of stem infection by *Erwinia* sp. in all three fields by sampling time T3. The results of the present study and observations for other years and other fields (21) suggest that a high incidence of stem infection by soft-rot Erwinia at the end of the growing season may be a general phenomenon in potato fields of central Wisconsin, regardless of the year and location. Presence or absence of *C. coccodes* appeared to have virtually no effect on the dose-response relationship. For two data sets, however, stems infected by *C. coccodes* were found less likely to be infected by *V. dahliae* than stems not infected by this fungus. Similar trends have been reported by Goodel et al (9).

Although it was found in this study that soil inoculum densities above 5–7 ppg were associated with a near certain infection of potato stems by *V. dahliae* by the end of the growing season, it is not known whether this would directly translate into significant yield loss. Further research is needed to investigate whether the time at which a stem becomes systemically infected during the growing season is related to subsequent symptom expression and significant yield loss.

LITERATURE CITED

1. Ashworth, L. J., Jr., McCutcheon, O. D., and George, A. G. 1972. *Verticillium albo-atrum*: The quantitative relationship between inoculum density and infection of cotton. Phytopathology 62:901-903.
2. Baker, R. J., and Nelder, J. A. 1978. The GLIM system: Generalized Linear Interactive Modeling. Royal Statistical Society, Numerical Algorithms Group, Oxford, England. 160 pp.
3. Ben-Yephet, Y., Siti, E., and Frank, Z. 1983. Control of *Verticillium dahliae* by metam-sodium in loessial soil and effect on potato tuber yields. Plant Dis. 67:1223-1225.
4. Busch, L. V. 1966. Susceptibility of potato varieties to Ontario isolates of *Verticillium albo-atrum*. Am. Potato J. 43:439-442.
5. Butterfield, E. J., and DeVay, J.E. 1977. Reassessment of soil assays for *Verticillium dahliae*. Phytopathology 67:1073-1078.
6. Cuppels, D., and Kelman, A. 1974. Evaluation of selective media for isolation of soft-rot bacteria from soil and plant tissue. Phytopathology 64:468-475.
7. Davis, J. R., and Everson, D. O. 1986. Relation of *Verticillium dahliae* in soil and plant tissue, irrigation method, and N-fertility to Verticillium wilt of potato. Phytopathology 76:730-736.
8. Davis, J. R., and Howard, M. N. 1976. Presence of *Colletotrichum atramentarium* in Idaho and relation to Verticillium wilt (*Verticillium dahliae*). (Abstr.) Am. Potato J. 53:397-398.
9. Fienberg, S. E. 1980. The Analysis of Cross-Classified Categorical Data. M. I. T. Press, Cambridge, Massachusetts. 198 pp.
10. Goodell, J. J., Powelson, M. L., and Allen, T. C. 1982. Interrelations between potato virus X, *Verticillium dahliae*, and *Colletotrichum atramentarium* in potato. Phytopathology 72:631-634.
11. Green, R. J., Jr., and Papavizas, G. C. 1968. The effect of carbon source, carbon to nitrogen ratios, and organic amendments on survival of propagules of *Verticillium albo-atrum* in soil. Phytopathology 58:567-570.
12. Kotcon, J. B. 1983. Root deterioration in the potato early dying syndrome. Ph.D. thesis. University of Wisconsin-Madison.
13. Kotcon, J. B., Rouse, D. I., and Mitchell, J. E. 1985. Interactions of Verticillium dahliae, *Colletotrichum coccodes*, *Rhizoctonia solani*, and *Pratylenchus penetrans* in the early dying syndrome of Russet Burbank potatoes. Phytopathology 75:68-74.
14. Martin, M. J., Riedel, R. M., and Rowe, R. C. 1982. *Verticillium dahliae* and *Pratylenchus penetrans*: Interactions in the early dying complex of potato in Ohio. Phytopathology 72:640-644.
15. Nicot, P. C., and Rouse, D. I. 1987. Precision and bias of three

quantitative soil assays for *Verticillium dahliae*. Phytopathology 77:875-881.
16. Nnodu, E. C., and Harrison, M. D. 1979. The relationship between *Verticillium albo-atrum* inoculum density and potato yield. Am. Potato J. 56:11-25.
17. Okeke, G. C. 1978. Diversity and characterization of *Verticillium* spp. associated with early dying of potatoes in Wisconsin. M. S. thesis. University of Wisconsin-Madison. 96 pp.
18. Otazu, V., Gudmestad, N. C., and Zink, R. T. 1978. The role of *Colletotrichum atramentarium* in the potato wilt complex in North Dakota. Plant Dis. Rep. 62:847-851.
19. Powelson, M. L. 1979. Verticillium wilt of potatoes in irrigated sands: The Oregon experience. Oreg. Agric. Exp. Stn. Tech. Paper 5106. 6 pp.
20. Powelson, M. L. 1981. Soil fumigation evaluation for control of Verticillium wilt and black dot of potatoes, 1979. Fungic. Nematic. Tests 36:71.
21. Pullman, G. S., and DeVay, J. E. 1982. Epidemiology of Verticillium wilt of cotton: A relationship between inoculum density and disease progression. Phytopathology 72:549-554.
22. Rahimian, M. L. 1982. The colonization of potato stems by microorganisms in relation to the "Early Dying" disease of potato. Ph.D. thesis. University of Wisconsin-Madison. 120 pp.
23. Robinson, D. B., Larson, R. H., and Walker, J. C. 1957. Verticillium wilt of potato in relation to symptoms, epidemiology, and variability of the pathogen. Wis. Agric. Exp. Stn. Res. Bull. 202. 48 pp.
24. Schnathorst, W. C. 1981. Life cycle and epidemiology of *Verticillium*. Pages 81-111 in: Fungal Wilt Diseases of Plants. M. E. Mace, A. A. Bell, and C. H. Beckman, eds. Academic Press, New York, 640 pp.
25. Schnathorst, W. C., and Mathre, D. E. 1966. Host range and differentiation of a severe form of *Verticillium albo-atrum* in cotton. Phytopathology 56:1155-1161.
26. Spaulding, W. C. 1970. The effects of inoculum density on Verticillium wilt of potato. M. S. thesis. Colorado State University. 72 pp.
27. Talboys, P. W. 1970. The effect of temperature and rainfall on the incidence of wilt (*Verticillium albo-atrum*) in hops. Ann. Appl. Biol. 66:51-58.