

Synergistic Interactions Between *Verticillium dahliae* and *Pratylenchus penetrans* in Potato Early Dying Disease

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ABSTRACT

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Field microplot studies were conducted on fumigated soil of two soil types over four seasons (1980-1983) to determine effects of *Verticillium dahliae* and *Pratylenchus penetrans* on the development of potato early dying disease. Tests with three population levels of *P. penetrans* and two of *V. dahliae*, alone and in all combinations, showed that disease occurred when both pathogens interacted synergistically at population levels that individually had little or no effect. Yield reductions of 25-50% often

occurred in the presence of both pathogens. Yield losses were, however, not always associated with foliar symptom development, but were most pronounced in seasons when high-temperature stress occurred during tuberization. The interaction of *P. penetrans* with *V. dahliae* in potato early dying is an important factor that must be considered in the development of disease forecasting and crop-loss predictive systems.

In areas of intensive potato (*Solanum tuberosum* L.) cultivation, yields often decline after fields have been in production for many years. Plants in these situations sometimes lose vigor in mid- to late-summer, mature, and die a few weeks early. This syndrome, called "potato early dying" (PED) or "early maturity wilt" is a serious problem in areas of long-term, intensive potato production (9,25,26,41). Because PED causes premature vine death during the period of greatest tuber development, yield reductions can be substantial (42,44).

Although PED is caused primarily by *Verticillium dahliae* Kleb. or *V. albo-atrum* Reinke & Berth. (4,25,26,34), other fungi (35,47), bacteria (39), and nematodes (14,21,22,24-26,45) have been implicated in the disease complex, especially *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans-Stekh. (6,8,11,19,23,25,31). The interaction of nematodes with soilborne wilt fungi is well established (10,26,36), and synergistic relationships between *Verticillium* and various nematode species have been documented on some host plants (5,13,18,30). The etiology of early dying in potato, however, has not been explored in detail. Evidence from field tests with nematicides and fungicides suggest nematode involvement (7,12,16,42,44), and correlations have been made with disease development and high field-population levels of various nematode species (21,24,45). Results of more controlled studies in greenhouses have been inconclusive (11,19,31).

Beginning in 1978, our studies in field microplots have provided evidence that PED can be caused by the interaction of *Pratylenchus* and *Verticillium* in which the two pathogens act synergistically to cause symptom development and yield reduction at population levels that individually cause little or no damage (27,28,43). This paper presents four years' data from two soil types that further

document this interaction and relate population levels of the two pathogens to potential yield losses.

MATERIALS AND METHODS

Field microplots were established each year from 1980 through 1983 in northeast Ohio on Wooster silt loam (65% silt, 20% fine sand, 15% clay, 2% organic matter, pH 5.8) at the Ohio Agricultural Research and Development Center (OARDC), Wooster, and in northcentral Ohio (Huron Co.) on Rifle peat, a partially decomposed organic soil derived from plant remains (15% silt, 1% fine sand, 9% clay, 75% organic matter, pH 5.4), at the OARDC Muck Crops Branch, Celeryville. At each location, plots were designed to test effects of *V. dahliae* and *P. penetrans* on development of early dying symptoms, plant growth, and yield in the *Verticillium* wilt-susceptible potato cultivar Superior. Treatments were: two population levels of *V. dahliae*, three levels of *P. penetrans*, all possible combinations of pathogen levels, and pathogen-free controls.

Inoculum production and soil infestation. *P. penetrans* was produced in infested alfalfa (*Medicago sativa* L. 'P545,' Pioneer Hi-Bred International Inc., Johnston, IA) callus from monoxenic cultures established by the authors on yeast extract sucrose agar (40). Serial transfers from stock cultures were made each August and November and incubated in the dark at 22 C to obtain sufficient populations to inoculate 3,000 culture tubes in February. Infested callus from these cultures was mixed with about 10 L of fumigated Rifle peat 3 days prior to planting of the microplots to allow migration of nematodes into the soil (29). Appropriate amounts of this infested soil were further diluted with fumigated soil taken from plot areas to obtain inoculum levels similar to those occurring naturally (8). Thorough mixing was accomplished in a 25-L twin-shell blender, as previously described (28). Actual nematode population levels in infested soils were determined by 24-hr Baerman pie pan extraction of two 100-cm soil samples from each 20-L batch that was mixed (Table 1).

Propagules of *V. dahliae* were produced in a mixture of silt loam soil, peat, and perlite (1:1:1, v/v) amended with dilute (1:10)

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Czapek's broth (about 100 ml/L of mix), autoclaved twice in 2-L flasks and inoculated as previously described (28). Flasks were incubated 4–6 wk in the dark at room temperature and inoculum was then removed, air-dried at room temperature for 1–2 wk, crushed gently by hand, and sieved through a 355- μ m sieve to remove large particles. The concentration of propagules of *V. dahliae* in the remaining inoculum was determined by dilution-plate assay on sodium polypectate agar (SPA) (28). Appropriate amounts were mixed with test soils in the twin-shell blender to obtain population levels of 10 and 100 propagules per 10 g of air-dried soil in the Rifle peat and 30 and 300 in the Wooster silt loam. These are similar to population levels found in naturally infested fields (4,16,34,46).

Plot establishment. Plot areas were fumigated each year in early May with 392 kg/ha of methyl bromide:chloropicrin (3:2) (Terr-O-Gas; Great Lakes Chemical Co., W. Lafayette, IN) on Wooster silt loam and 465 kg/ha on Rifle peat to eliminate variables due to other pathogens. The fumigant was injected at a depth of 20 cm under a 2.1-m wide continuous plastic tarp when soil temperature at injection depth had reached a minimum of 13 C. Tarps were removed 2–3 wk later after the fumigant had dissipated. Fumigated soil was collected and returned to the laboratory for controlled infestation as described above. Open-ended, unglazed, clay drain tiles (25 cm inside diameter \times 31 cm long) were then set upright at plow depth leaving about 5 cm exposed above the soil line (Fig. 1). Tiles were placed in rows on 50-cm centers in the middle of 2-m-wide fumigated strips of land.

One or two days after collection, fumigated soil infested with either or both pathogens was returned to the plot site in 35-L metal baskets. Infested and uninfested control soils were placed in appropriate tiles (about 8 L per tile) in a completely randomized design with 15 replicate tiles per treatment.

Pathogen-free potato seed pieces of the cultivar Superior for use in microplot tiles were generated in 1980 and 1981 as described previously (28). In 1982 and 1983, they were produced from rooted cuttings obtained from pathogen-free tissue cultures grown to maturity during the winter in steam-disinfested soil in 20-cm-diameter pots at 15–25 C in a greenhouse with an 18-hr photoperiod. Tubers from these plants were collected in February and stored in moist vermiculite at room temperature until they broke dormancy at about 90 days. Single-eye seed pieces were then cut with a 2.5-cm scoop from these tubers and presprouted for 2–3 wk in moist vermiculite. Single seed pieces selected for sprout uniformity of about 1 cm were planted in the center of each tile at a depth of 3–4 cm. Standard potato plant density (about 25 cm apart) was maintained by also planting seed potatoes between each tile. Guard rows 1 m on either side of each row of tiles were planted with seed pieces 25 cm apart to produce a natural plant canopy for the

tiled plants as the vines matured (Fig. 1). Planting and harvest dates for Wooster silt loam were: 13 June–9 September 1980, 11 June–9 September 1981, 3 June–2 September 1982, and 2 June–1 September 1983. Dates for Rifle peat were: 23 May–21 August 1980, 22 May–26 August 1981, 25 May–25 August 1982, and 26 May–30 August 1983. Standard fungicides and non-nematicidal insecticides were applied during each growing season to minimize variables caused by other pests.

Data collection and analysis. Visual foliage symptom ratings were made for each plant at onset of symptom development (60–70 days) and again at harvest (90 days), when maximal treatment differences occurred. A subjective rating scale was used in which 0 = no visible symptoms; 1 = some chlorosis, especially in older leaves; 2 = general chlorosis coupled with some necrosis and wilting; and 3 = severe wilting or death. In all studies, tiles were lifted by hand or with a tractor-mounted, hydraulic "tile puller" and the soil was removed. Fresh weights of foliage, roots, and tubers were determined for each plant, and soil and roots were collected for final nematode population determinations using a 24-hr Baerman pie pan extraction technique. Infection of plants with *V. dahliae* was determined from basal stem sections taken from each plant about 5 cm above the soil line and surface sterilized in 0.5% NaOCl for 30 sec. Small wafers of tissue were excised, placed on alcohol agar (33), and observed for development of conidia of *Verticillium*.

In 1981 and 1982, in an attempt to further quantify the amount of colonization of *V. dahliae* at harvest, the entire central stem of the plant from each microplot was stripped of leaves and air-dried 2–4 mo at room temperature. The upper and lower 10 cm of each stem were pulverized in a Wiley mill and placed on SPA using the methods of Davis et al (15).

Experimental design for both locations and all years was a completely randomized factorial with two factors, *V. dahliae* and *P. penetrans* population levels. After analysis of variance, contrasts of the means were used to evaluate the change in each variable (eg, yield) with increasing population levels of each pathogen.

A simple regression model was fit to the yield data in relation to the average temperature during July and August. Separate

TABLE 1. Initial population levels of *Pratylenchus penetrans* in microplot tiles at two Ohio locations, 1980–1983

Location	Initial population levels	Nematodes per 100 cc soil ^a			
		1980	1981	1982	1983
Wooster	1	9	8	38	3
	2	30	25	84	15
	3	106	143	245	30
	LSD ($P = 0.05$)	38	50	46	19
Celeryville	1	18	47	25	12
	2	56	137	92	15
	3	151	362	229	70
	LSD ($P = 0.05$)	38	83	47	32

^a Values are the means of 15 soil samples collected immediately after mixing and prior to placing of infested soil in tiles. Nematodes were extracted by a modified Baerman funnel technique at 20–23 C in 1980–1982. Because of a mechanical problem, samples were extracted at about 14 C in 1983 resulting in artificially low values.



Fig. 1. Clay-tile microplot system showing emerging potato plants. Note guard rows in upper photo and plants between each tile in the lower photo (arrows).

regressions were performed for the following treatments at each location: control, high *Verticillium*/no *Pratylenchus*, no *Verticillium*/high *Pratylenchus*, and high *Verticillium*/high *Pratylenchus*. The regression coefficients (slope and average yield) for the pathogen treatments were compared to the control with *t*-tests.

RESULTS

The effects of individual and combined population levels of *P. penetrans* and *V. dahliae* on potato cultivar Superior in field microplots are reported in Table 2. Each matrix reports the results of increasing populations of *V. dahliae* from left to right and increasing populations of *P. penetrans* populations from top to bottom.

In 1980 on Rifle peat at Celeryville, symptom development and top and root weight reductions were significantly ($P \leq 0.05$) related to increasing population levels of each pathogen individually and were accentuated in the presence of both. Patterns of yield reduction were similar, with >50% reduction at the highest combined levels and >25% in all combined levels. Effects of individual pathogens on yield were much less than combined-pathogen effects. On Wooster silt loam at Wooster, similar results were obtained, except that *P. penetrans* alone had no significant ($P \leq 0.05$) effect in any case. Yield reductions were substantial in that all combined population levels reduced yields >50%, as did the highest rate of *V. dahliae* alone. Total rainfall was above normal at both locations during June–August while average temperatures were below normal in June and above and near normal in July and August at Wooster and Celeryville, respectively (Table 3).

In 1981, excessive rainfall at Celeryville in June (Table 3) resulted in loss of a portion of the Rifle peat test due to flooding. Most patterns, however, were still evident. Increasing population levels of *V. dahliae* resulted in small, but significant ($P \leq 0.05$), reductions

only in root weight and yield. In the presence of both pathogens, however, large significant ($P \leq 0.05$) interactions occurred. Although yield effects were somewhat less than in 1980, the highest combined population levels caused a decline of >50% and those with one component at the highest level caused >25% declines. At Wooster, effects of most treatments were nonsignificant, in contrast to 1980, and yield reductions exceeded 25% in only two treatments. Temperatures and rainfall at Celeryville were variable throughout the summer, while at Wooster, it was cool and dry (Table 3).

In 1982, symptom development and effects on plant tops and roots again fell into the same patterns, with minimal effects from each pathogen alone but pronounced effects with combined population levels. Yield effects, however, were nonsignificant at both locations except at the highest combined levels. Temperatures and rainfall averaged below normal for summer months at both plot locations (Table 3).

In 1983, observed interactions were more nearly like those that occurred in 1980. At Celeryville, population levels of individual pathogens caused no significant ($P \leq 0.05$) effects on Rifle peat, but combined population levels resulted in significant ($P \leq 0.05$) increases in symptom expression and reductions in fresh weights. Yield reductions >25% occurred with all combined population levels. At Wooster, significant ($P \leq 0.05$) effects on symptom development and top growth were observed with *V. dahliae* alone on silt loam, but combined populations had significant ($P \leq 0.05$) effects on all parameters. Yield reductions exceeded 25% in all combined population levels with higher rates of *P. penetrans* and were >50% with the highest combinations. Temperatures were substantially above normal during July and August at both locations, and at Celeryville there were 15 consecutive days with maximum temperatures >32 C. Rainfall was below normal for the season (Table 3).

TABLE 2. Effects of combined population levels of *Verticillium dahliae* and *Pratylenchus penetrans* on symptom expression and plant production in potato grown in field microplots in silt loam or organic soils at two Ohio locations in four seasons.

Year of test	Wooster Silt Loam — Wooster, Ohio																			
	Symptom expression ^{a, b}				Fresh weight (g) per plant ^{a, c}															
					Tops				Roots				Tubers							
0	1	2 ^d	P ⁱ	0	1	2	P	0	1	2	P	0	1	2	P					
1980	0	0.8	1.3	2.3	0.00	0	412	339	149	0.00	0	2.9	3.2	2.0	0.66	0	320	291	114	0.05
	1	1.1	2.4	2.9	0.00	1	396	134	32	0.00	1	3.3	1.6	1.1	0.01	1	249	162	143	0.14
	2	1.0	2.7	3.0	0.00	2	417	54	20	0.00	2	3.9	1.0	0.8	0.00	2	325	107	77	0.00
	3	0.8	2.6	2.9	0.00	3	505	83	29	0.00	3	2.5	1.5	1.0	0.00	3	323	156	69	0.00
	P	0.80	0.00	0.00		P	0.21	0.00	0.00		P	0.79	0.00	0.00		P	0.75	0.02	0.14	
1981	0	1.8	1.4	1.9	0.67	0	288	353	278	0.85	0	7.7	6.2	4.8	0.00	0	740	572	564	0.02
	1	1.5	1.8	2.3	0.01	1	328	265	205	0.01	1	7.0	5.1	6.0	0.29	1	662	595	618	0.55
	2	1.8	1.5	2.4	0.06	2	289	309	231	0.22	2	7.6	6.1	6.5	0.12	2	742	628	617	0.07
	3	1.3	1.9	2.4	0.00	3	279	251	172	0.01	3	6.2	6.3	4.9	0.10	3	473	623	463	0.89
	P	0.13	0.24	0.20		P	0.57	0.11	0.07		P	0.09	0.57	0.83		P	0.00	0.66	0.12	
1982	0	0.3	1.4	1.6	0.00	0	360	404	262	0.07	0	3.7	2.9	2.8	0.12	0	412	709	555	0.15
	1	0.4	1.2	2.3	0.00	1	369	283	151	0.00	1	4.7	4.3	3.2	0.13	1	523	598	404	0.67
	2	0.9	1.5	3.0	0.00	2	307	297	88	0.00	2	3.6	4.0	2.3	0.07	2	518	581	476	0.56
	3	0.5	1.4	2.8	0.00	3	335	229	100	0.00	3	4.7	3.0	1.7	0.00	3	472	442	377	0.21
	P	0.22	0.68	0.00		P	0.66	0.01	0.00		P	0.57	0.92	0.02		P	0.51	0.03	0.05	
1983	0	0.9	0.8	1.9	0.01	0	285	329	146	0.01	0	3.0	3.6	2.9	0.89	0	454	463	438	1.00
	1	0.9	2.1	2.4	0.00	1	357	206	94	0.00	1	3.3	2.9	3.1	0.02	1	446	492	331	0.52
	2	0.8	1.3	2.6	0.00	2	347	233	83	0.00	2	4.1	2.5	1.8	0.00	2	410	310	286	0.04
	3	1.3	2.6	2.8	0.00	3	228	80	44	0.00	3	3.3	2.0	1.8	0.01	3	415	224	199	0.02
	P	0.29	0.00	0.03		P	0.28	0.00	0.01		P	0.58	0.00	0.01		P	0.53	0.00	0.00	

(continued)

Regression of all yield data from silt loam at Wooster against the average temperature in July and August of each year (Fig. 2A) showed no significant relationship in control plants or those inoculated with the highest levels of *Pratylenchus* (Table 4). In plants inoculated with the highest levels of *Verticillium*, there were significant ($P = 0.01$) temperature effects, but no significant yield losses. In the presence of both pathogens, however, higher summer temperatures resulted in significant ($P = 0.01$) yield losses (Fig. 2A, Table 4). At Celeryville, on Rifle peat, high temperatures resulted in significant yield declines with all treatments, although yield depressions were significantly ($P = 0.01$) increased in the presence of both pathogens (Fig. 2B, Table 4).

Each year, at harvest, basal stem colonization by *V. dahliae* was determined for plants from each microplot. In 1980 at Wooster, *V. dahliae* was isolated from 21% of the plants that were not in soil infested with *V. dahliae*, and from 82% of those that were. At Celeryville, results were 53 and 85%, respectively. Percentages of recovery of *V. dahliae* for succeeding years were as follows: 1981, Wooster, 39 and 79%, Celeryville, 79 and 74%, respectively; 1982, Wooster, 98 and 100%, Celeryville, 97 and 98%, respectively; 1983, Wooster, 51 and 75%, Celeryville, 92 and 89%, respectively. In 1982, isolations were also made from aerial portions of stems 5–10 cm below the apical meristems. Recovery rates of *V. dahliae* were nearly as high as those from basal stems. At Wooster, recovery was successful from 85% of plants that were not in soil infested with *V.*

dahliae and from 98% of those that were. At Celeryville, results were 73 and 87%, respectively.

In 1981 and 1982, a quantitative assay (15) was used in an attempt to relate colonization by *V. dahliae* at harvest to initial populations in infested soil. Although there was a trend towards increased numbers of propagules from stems grown in *V. dahliae*-infested soil, the method did not yield useful data.

DISCUSSION

Although there is yearly variability in the data in Table 2, trends can be seen that apply generally to both soil types. There was little or no response to increasing population levels of *P. penetrans* in the absence of *V. dahliae*. Increasing populations of *V. dahliae* alone caused some increase in symptom expression, but they did not usually affect yield. A strong synergistic effect occurred when both pathogens were combined, resulting in severe symptom expression, even with population levels that alone caused little or no damage. Effects of this synergism on yield were variable and were related to weather conditions. Significant yield reductions were seen on both soil types in 1980 and 1983. In both years, temperatures were above normal; however, 1980 was a wet season and 1983 was quite dry (Table 3). Temperatures and rainfall were below normal at Wooster in 1981 and 1982 and yield reductions were inconsequential. At Celeryville, yield reductions on Rifle peat were

TABLE 2 (continued). Effects of combined population levels of *Verticillium dahliae* and *Pratylenchus penetrans* on symptom expression and plant production in potato grown in field microplots in silt loam or organic soils at two Ohio locations in four seasons

Year of test	Symptom expression	Rifle Peat—Celeryville, Ohio																		
		Fresh weight (g) per plant																		
		Tops				Roots				Tubers										
0	1	2	P	0	1	2	P	0	1	2	P	0	1	2	P					
1980	0	0.8	1.1	2.1	0.00	0	520	603	308	0.01	0	9.8	8.3	6.0	0.00	0	746	724	644	0.14
	1	1.4	2.8	2.9	0.00	1	436	121	127	0.00	1	8.4	3.6	4.4	0.00	1	653	466	463	0.00
	2	1.9	2.9	2.9	0.00	2	330	139	121	0.01	2	6.1	4.1	3.8	0.01	2	623	414	480	0.04
	3	2.1	3.0	2.9	0.00	3	270	140	97	0.00	3	6.4	3.6	3.3	0.00	3	597	425	366	0.00
	P	0.00	0.00	0.00		P	0.00	0.00	0.00		P	0.00	0.00	0.00		P	0.03	0.00	0.00	
1981	0	1.6	1.8	1.9	0.62	0	392	269	310	0.68	0	12.6	10.8	7.2	0.00	0	821	798	668	0.04
	1	— ^b	1.6	3.0	—	1	— ^b	255	38	—	1	— ^b	8.6	2.3	—	1	— ^b	701	412	—
	2	—	2.0	2.7	—	2	—	228	114	—	2	—	7.2	4.5	—	2	—	618	454	—
	3	—	2.0	3.0	—	3	—	175	29	—	3	—	5.4	2.5	—	3	—	466	346	—
	P	—	0.62	0.00		P	—	0.03	0.00		P	—	0.00	0.00		P	—	0.00	0.00	
1982	0	0.5	0.8	1.1	0.05	0	659	625	452	0.05	0	7.4	—	—	—	0	821	941	848	0.77
	1	0.6	1.4	1.8	0.00	1	664	427	386	0.02	1	9.7	7.8	7.7	0.12	1	878	767	714	0.11
	2	0.5	0.7	1.9	0.00	2	646	759	337	0.02	2	8.6	11.1	7.0	0.32	2	871	824	550	0.00
	3	0.7	1.7	2.4	0.00	3	703	417	275	0.00	3	9.8	8.5	7.2	0.09	3	899	697	659	0.04
	P	0.52	0.09	0.00		P	0.72	0.50	0.08		P	0.15	0.69	0.68		P	0.51	0.05	0.01	
1983	0	0.7	1.2	1.0	0.65	0	348	256	301	0.56	0	7.3	6.2	6.2	0.58	0	413	345	350	0.67
	1	0.9	1.5	2.2	0.00	1	302	209	111	0.01	1	7.6	4.4	3.4	0.01	1	365	275	238	0.03
	2	1.0	2.3	2.5	0.00	2	391	95	82	0.00	2	7.8	3.5	2.7	0.00	2	453	240	269	0.01
	3	1.1	2.9	2.8	0.00	3	248	39	52	0.00	3	6.5	2.3	3.2	0.01	3	334	164	252	0.14
	P	0.25	0.00	0.00		P	0.61	0.00	0.00		P	0.67	0.00	0.00		P	0.52	0.00	0.10	

^a Each matrix reports the results of increasing *V. dahliae* populations from left to right and increasing *P. penetrans* populations from top to bottom.

^b Each value represents the average of 15 microplot plants evaluated visually 90 days after planting on a scale of 0=no visible symptoms; 1=some chlorosis, especially in older leaves; 2=general chlorosis coupled with some necrosis and wilting; 3=severe wilting or death.

^c Each value represents the average of 15 microplot plants at harvest 90 days after planting.

^d Population levels of *Verticillium dahliae* added to soil at planting: 0=none; 1=ca. 10 propagules per 10 g soil; 2=ca. 100 propagules per 10 g soil for Rifle Peat; or 1=ca. 30 propagules per 10 g soil; 2=ca. 300 propagules per 10 g soil for Wooster wilt loam.

^e Population levels of *Pratylenchus penetrans* added to soil at planting: 0=none; 1=low; 2=medium; 3=high (for exact figures see Table 1).

^f P denotes linear trend significance level, based on contrasts of the data means, for the change in a variable with increasing population levels of one pathogen at a constant population level of the other.

^g 1980 Rifle Peat data previously published in *Phytopathology* 72:640-644 in another format.

^h Data lost due to extreme weather conditions.

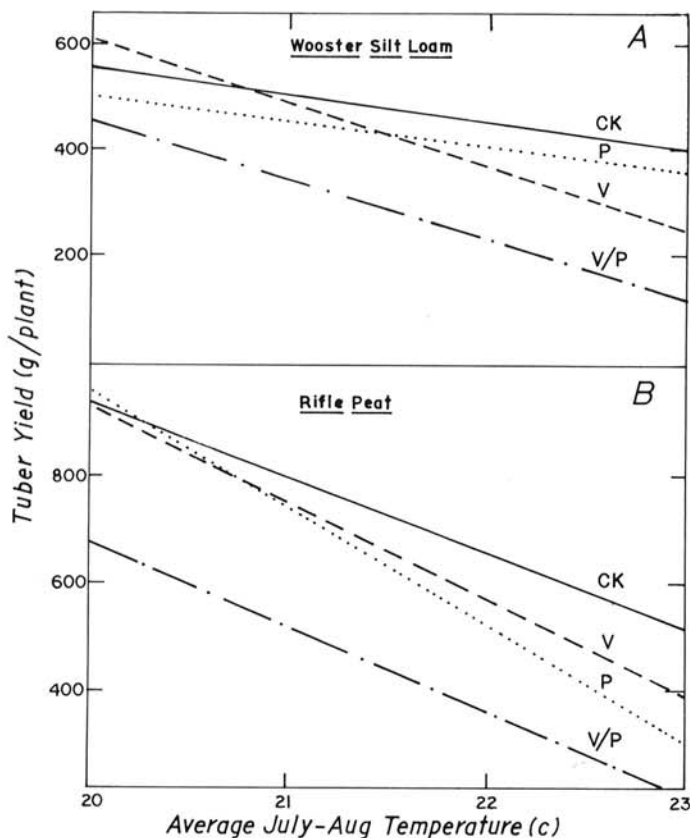


Fig. 2. Simple linear regression of tuber yield data from 1980 to 1983 on average temperature in July and August of each season **A**, on Wooster silt loam at Wooster, OH, and **B**, on Rifle peat at Celeryville, OH. Regression lines labeled as: CK = control plants grown in the absence of either pathogen initially; V = plants grown in soil infested with the highest tested levels of *Verticillium dahliae*; P = plants grown in soil infested with the highest tested levels of *Pratylenchus penetrans*; V/P = plants grown in soil infested with the highest tested levels of both pathogens.

TABLE 3. Summer rainfall (mm) and mean daily average temperature (C) at microplot locations in Wooster and Celeryville, OH, 1980-1983

	1980		1981		1982		1983	
	Amt	Dev ^a	Amt	Dev	Amt	Dev	Amt	Dev
Rainfall								
Wooster								
June	130	+31	135	+36	119	+20	58	-41
July	146	+44	84	-18	65	-37	111	+9
August	236	+145	54	-37	69	-22	38	-53
Total	512	+220	273	-19	253	-39	207	-85
Celeryville								
June	166	+68	240	+142	136	+38	55	-43
July	130	+32	83	-15	61	-37	38	-60
August	108	+17	94	+3	61	-30	66	-25
Total	404	+117	417	+130	258	-31	159	-128
Temperature								
Wooster								
June	18.2	-1.5	20.3	+0.6	17.3	-2.4	19.6	-0.1
July	22.4	+0.5	21.8	-0.1	21.4	-0.5	22.7	+0.8
August	22.7	+1.8	20.3	-0.6	18.5	-2.4	22.5	+1.5
Celeryville								
June	17.8	-2.3	21.0	+0.9	16.7	-3.4	19.7	-0.4
July	21.9	-0.1	23.4	+1.4	21.7	-0.3	23.0	+1.0
August	21.3	-0.6	20.3	-1.6	18.9	-3.0	22.7	+0.8

^aDeviation from long-term U.S. Weather Bureau averages.

moderate in 1981 with variable temperatures, and only minimal in 1982 with cooler temperatures.

Yield reductions due to potato early dying seem to be correlated with heat stress. Moisture stress appears to be less important, as seen in the comparison of 1980 with 1983 data at both locations (Tables 2 and 3). Regression analysis of the yield-loss/heat-stress relationship indicates a difference in temperature responses on the two soil types tested. On Wooster silt loam, heat stress did not affect tuber production in control or single-pathogen treatments, but it had a marked effect in the presence of both pathogens. This may explain the lack of substantial yield response in 1981 and 1982, even though symptom development was extensive. On Rifle peat, a significant temperature response occurred in all treatments that was greatly accentuated in the presence of both pathogens. This more general response may be related to physical characteristics of the soil but is also affected by the substantially higher yields obtained on organic soil, except in the excessively warm summer of 1983.

These observations may explain inconsistencies in yield response to PED noted in this study and by others (11,25,44). The response of potato to heat stress has been summarized by Ewing (17). An overall reduction in growth occurs whenever the crop is exposed to temperatures above the optimal mean of 17 C. Furthermore, under warm conditions, partitioning of photosynthates to tubers is reduced, which results in increased vine growth. Infection by PED pathogens also stresses the potato plant, but often not to the point of large yield reductions if other conditions are optimal. However, if both stresses are superimposed during the period of maximal tuber growth, the productive capacity of the plants is severely diminished.

Synergistic interactions between *Verticillium* and *Pratylenchus* have been documented for a number of hosts (13,18,30,32). With mint, Faulkner and Skotland (18) noted responses similar to those we have seen. They saw almost no effect on dry weight with *Pratylenchus* alone and only a limited effect with *V. dahliae*, but with both pathogens, significant reductions occurred. Krikun and Orion (25) in Israel, reported no yield reduction in potato with *P. thorneii* alone, but definite interactions occurred with *V. dahliae* (24). In eggplant, McKean and Mountain (30) reported *P. penetrans* to be nonpathogenic alone but synergistic with *V. albo-atrum*.

The effects of temperature on yield reductions due to *Verticillium* wilt have been noted in cotton (2) and tomato (1) in California. Recently, Nnodu and Harrison (34) in Colorado showed that more disease developed in potato at 29 than at 24 C and suggested that yield declines due to PED would be more common where potatoes mature in hot weather.

TABLE 4. Statistics for the simple linear regression analyses of tuber yield from 1980 to 1983 on average July and August temperatures at two locations in Ohio

Location	Treatment ^a	F	R ²	Significance levels of t-test comparisons with control	
				Slope	Mean yield
Celeryville	Ck	25.0**	0.32	—	—
	P	59.6**	0.60	0.05	NS
	V	50.4**	0.48	NS	NS
	V/P	32.2**	0.37	NS	0.01
Wooster	Ck	0.61 NS	0.01	—	—
	P	0.49 NS	0.001	NS	NS
	V	13.4**	0.20	0.05	NS
	V/P	43.6**	0.47	0.05	0.01

^aCk = control plants grown in uninfested soil; P = plants grown in soil infested with the highest tested levels of *Pratylenchus penetrans*; V = plants grown in soil infested with the highest tested levels of *Verticillium dahliae*; V/P = plants grown in soil infested with the highest tested levels of both pathogens.

The fact that we isolated *V. dahliae* from a large percentage of plants growing in both infested and uninfested soil and independent of nematode populations indicates that most plants in our studies were exposed to some levels of that pathogen. Quite possibly, as the season progressed, *V. dahliae* became dispersed at low levels and many plants became infected by the time of harvest. Ashworth (1) reported similar observations with tomatoes. Since our data show a clear relationship between disease development and initial population levels of *V. dahliae*, we feel that late-season infections probably were not significant. Furthermore, the fact that colonization by *Verticillium* was independent of nematode populations suggests that the interaction is more a result of additive stresses than of nematode wounding facilitating entry of *Verticillium* into the root as has been suggested (13,18).

Our data indicate that the presence of both pathogens is essential for maximal disease, and that disease development is a function of their initial population levels. Combined population levels of *V. dahliae* of about 10 microsclerotia per 10 g of soil and *P. penetrans* of 100–150 per 100 cc of soil often resulted in substantial yield reductions, especially under heat stress. Similar population levels of both pathogens commonly occur in potato soils in Ohio (8,46) and elsewhere (4,16,23,34,37). Although our studies were done in fumigated soil where other microorganisms were not present initially, we feel this synergism is a widespread natural occurrence. The magnitude of response, however, may vary with soil type or associated microbial populations.

Numerous attempts have been made to relate inoculum concentration of *Verticillium* to disease incidence in cotton (2,3,38), tomato (1,20) and potato (34,37). Although some authors claimed to find consistent relationships, others found difficulty comparing data from different locations or under different conditions. The fact that none of these studies reported associated populations of *Pratylenchus* may explain some of the variability, and also points out dangers in trying to simplify cause-and-effect relationships in complex soil-pathogen systems.

Early dying is the most significant disease limiting potato production in the United States. At present, effective control measures for PED are extremely limited and in most cases losses are undetermined. A thorough understanding of pathogenic relationships involved in this disease complex is essential in formulating future control strategies. Prediction of early dying by determining pre-plant soil populations of *Verticillium* is desirable, but obviously this must include determination of populations of *Pratylenchus* as well. Even when these relationships are well established, however, yield-loss predictions may not be precise due to environmental effects on disease development. The value of predictions, however, is to establish crop vulnerability and allow producers to make rational management decisions. In the case of PED, control efforts directed against the nematode component of the disease complex may prove to be the most effective.

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