

Comparative Virulence of *Aphanomyces euteiches* f. sp. *phaseoli* and *Pythium ultimum* on *Phaseolus vulgaris* at Naturally Occurring Inoculum Levels

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ABSTRACT

Pfender, W. F., and Hagedorn, D. J. 1982. Comparative virulence of *Aphanomyces euteiches* f. sp. *phaseoli* and *Pythium ultimum* on *Phaseolus vulgaris* at naturally occurring inoculum levels. *Phytopathology* 72:1200-1204.

Aphanomyces euteiches f. sp. *phaseoli* was found to occur commonly in the sandy soil of Wisconsin's major snap bean production area. In greenhouse testing of soil inoculum levels typical for the area, this pathogen caused more damage to beans than did *Pythium ultimum* at 24 and 28 C. The two pathogens were equally damaging to beans at 20 C, and at 16 C *P.*

ultimum caused more severe disease than did *A. euteiches* f. sp. *phaseoli*. When plants were infected by both pathogens simultaneously, disease severity was similar at all temperatures tested. Plants were seldom killed by either pathogen alone, but 30-40% of the plants grown at 20 or 24 C were killed if simultaneously infected by both pathogens.

Root rot is a major limiting factor in snap bean production in Wisconsin. This is true particularly in central Wisconsin, where over half of the state's snap bean crop is grown under irrigation on very sandy, well-drained soil. Bean root rot in this area has been attributed to *Fusarium solani* (Mart.) Appel & Wr. (13) and to species of *Pythium* (5,10). However, Reeleder (10) more recently found *F. solani* to be unimportant as a pathogen of beans in central Wisconsin. He further determined that root rot severity in production fields was correlated with soil populations of *P. ultimum* Trow, but not with populations of other *Pythium* species. We recently described a bean root and hypocotyl pathogen, *Aphanomyces euteiches* Drechs. f. sp. *phaseoli* Pfend. & Hag., which was recovered from diseased beans in central Wisconsin (7).

The study reported here was undertaken to assess the importance of *A. euteiches* f. sp. *phaseoli*, relative to *P. ultimum*, in inciting bean root rot in central Wisconsin. A preliminary report (6) has been made.

MATERIALS AND METHODS

To determine whether *A. euteiches* f. sp. *phaseoli* occurs commonly enough to be a hazard to the Wisconsin bean crop, isolations were first attempted from snap beans collected in several commercial fields where root rot was prevalent. Since it was sometimes impossible to isolate any organism from upper portions of hypocotyl lesions on these plants, an additional assay for the presence of the pathogen was conducted by testing soils collected from 15 bean fields, whose root rot history was unknown, in central Wisconsin. For this test, several captan-treated seeds of snap beans (*Phaseolus vulgaris* L. 'Early Gallatin') were planted in 10-cm-diameter pots containing the soils and grown in a greenhouse at 24 C. Pots were watered lightly until plants emerged, then watered to

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saturation daily. Ten to 20 days after planting, when brown streaking appeared on the hypocotyl above the soil level, diseased plants were removed from the pots. Hypocotyls were surface disinfested in 0.5% sodium hypochlorite for 30 sec, dipped in 2% sodium thiosulfate for 30 sec, rinsed in sterile distilled water, and placed on Bacto water agar. Fungi having the mycelial appearance of *Aphanomyces* were recovered from the plates, identified to species, and tested for forma specialis identity by determining growth rate at 32 C and pathogenicity to beans and peas (7).

The amount of damage caused by *Aphanomyces* and *Pythium* was compared by growing beans in pasteurized soil (heated with aerated steam at 60 C for 30 min and stored 3 wk before use) that had been reinfested with oospores of one or both fungi at inoculum levels typical of fields in central Wisconsin. Inoculum levels in field soil and reinfested soil were determined by soil dilution plating on PVP medium (12) for *Pythium*, and by a most probable number bioassay for *Aphanomyces*. We modified a previously described most probable number assay (8) by using beans as the test plant and conducting the test at 28 C.

Inoculum of *Aphanomyces* and *Pythium* consisted of oospores grown in oatmeal broth (11) and V-8 cholesterol broth (1), respectively. In both cases, 20-ml quantities of medium in 150-ml prescription bottles were inoculated with agar plugs of mycelium, and the bottles were incubated on their sides at 20 C in the dark for 3 wk. To collect oospores from a 3-wk-old culture, the mycelial mat was washed and comminuted with water in a blender at high speed for 1.5 min. The resulting suspension was washed by centrifugation, resuspended, and further dispersed by using a glass tissue grinder. We infested soil by mixing it while spraying a spore suspension into it with an atomizer. Separate batches of soil were infested with each pathogen, inoculum of each fungus consisting of a mixture of two isolates. Infested soil was moistened to 14% (w/w) water content, stored in closed plastic bags for 3 wk, then air-dried to 2% water content and assayed for inoculum concentration. Inoculum level was adjusted by mixing with noninfested pasteurized soil.

The soil used in these tests, collected from central Wisconsin, is classified as Plainfield Sand (91.3% sand, 4.3% silt, and 4.4% clay). Its soil moisture characteristics, determined by using a Haines apparatus (4), are as follows: soil water contents (wt water/wt oven-dry soil) of 21.5, 15.0, 7.5, 6.5, 5.5, and 5.0% are associated with matric water potentials of 0, -30, -60, -90, -130, and -180 mb, respectively.

Infested soil (1,425 g) was packed into 14-cm-diameter pots at a bulk density of 1.6 g/cc, which is a representative value for this soil in the field (3). Seven captan-treated seeds of snap bean cultivar Early Gallatin (thinned to four seedlings after emergence) were planted in each pot. Pots were watered with 50 ml of water every other day until plants emerged. Thereafter, the soil was brought to 16% water by weight (approximately -25 mb matric water potential) whenever the soil moisture dropped to 5% (w/w) (approximately -180 mb). Preliminary tests showed that water would distribute evenly throughout the volume of the soil soon after it was watered to 16% soil moisture.

In the first experiment, plants were grown in a greenhouse where air temperature ranged from 22 to 26 C and soil temperatures ranged from 22 to 28 C. Based on Reeleder's (10) data for field populations of *P. ultimum* (0-165 propagules per gram of soil [ppg]), and our findings with regard to inoculum levels of *A. euteiches* f. sp. *phaseoli* in field soil (0-3.6 infective propagules per gram [ippg]), we chose two inoculum levels of each pathogen for testing. We used *Pythium* at 30 or 150 ppg to represent low and high levels of field inoculum. Because preliminary tests showed *A. euteiches* f. sp. *phaseoli* to be extremely damaging to beans at 4 ippg in greenhouse tests, precluding observation of interactions with *Pythium*, inoculum levels of 0.1 and 1.5 ippg were chosen to represent low and moderate field inoculum levels of *Aphanomyces*. Treatments in the first experiment consisted of all combinations of pathogens and inoculum levels, including a noninfested check.

In the second experiment, only the higher inoculum density of each pathogen was used, but for each pathogen treatment some of the plants were grown at each of four temperatures: 16, 20, 24, and

28 C. These experiments were conducted in growth chambers with a 12-hr photoperiod and light intensity of 2.2×10^4 lux (fluorescent plus incandescent). Soil temperatures were within ± 1 C of air temperatures.

All plants at a given temperature were harvested when the first trifoliolates of the check plants at that temperature were approximately two-thirds expanded. Thus, plants were grown for 21, 24, 30, and 40 days at 28, 24, 20, and 16 C, respectively. At harvest, both the hypocotyl and the root system of each plant were rated for disease severity on a scale of 0 (healthy) to 4 (maximum severity). Hypocotyl ratings were based on size of necrotic lesion and firmness of tissue; root ratings were based on size, discoloration, and deterioration of the root system. Dry weight of roots and shoot were determined after drying for 48 hr at 90 C.

There were three or four replicate pots per treatment in each experiment; all experiments were repeated once. Analysis of variance was performed on the data with percentage values converted to the angular transformation. Disease severity ratings were found to be approximately normally distributed (ratings for check plants were not included) and, therefore, were also examined by analysis of variance.

RESULTS

In isolations from field-grown beans showing root and hypocotyl rot, *Pythium* was recovered from 60% of the plants, *Aphanomyces* from 30%, *Fusarium* from 8%, and *Rhizoctonia* from 6%. Ninety percent of the isolates recovered from roots were *Pythium*, whereas 60% of the isolates recovered from the upper edge of hypocotyl lesions were *Aphanomyces*.

Beans planted in soil taken from each of 15 bean fields developed symptoms of root and hypocotyl rot. *Aphanomyces* was recovered from hypocotyl lesions on these plants. In 14 cases, pathogenicity and growth rate tests indicated that the fungus was *A. euteiches* f. sp. *phaseoli*. In one case, the fungus was found to be *A. euteiches* f. sp. *pisi*. Inoculum levels of *A. euteiches* f. sp. *phaseoli*, determined for five of the soils in which this pathogen was found, ranged from 1.3 to 3.6 ippg.

In the greenhouse test conducted at 22-26 C, disease symptoms incited by *P. ultimum* alone were less severe than those incited by *A. euteiches* f. sp. *phaseoli* alone (Fig. 1). Stunting and wilting were

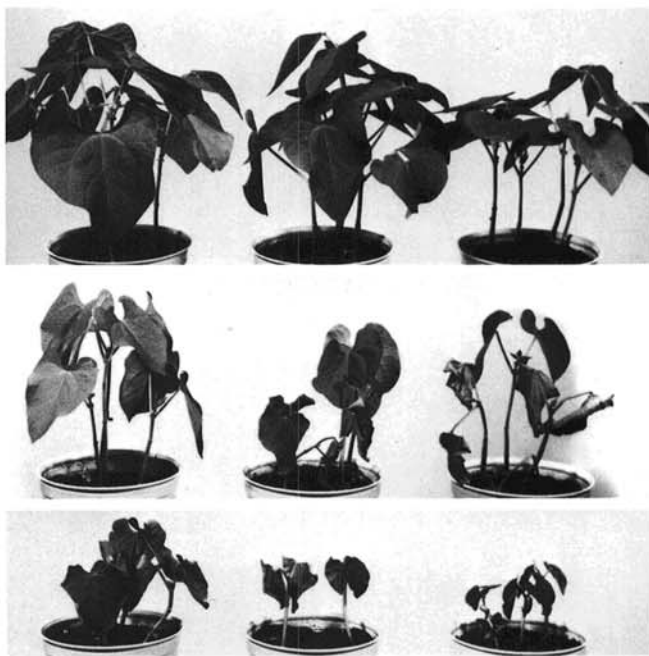
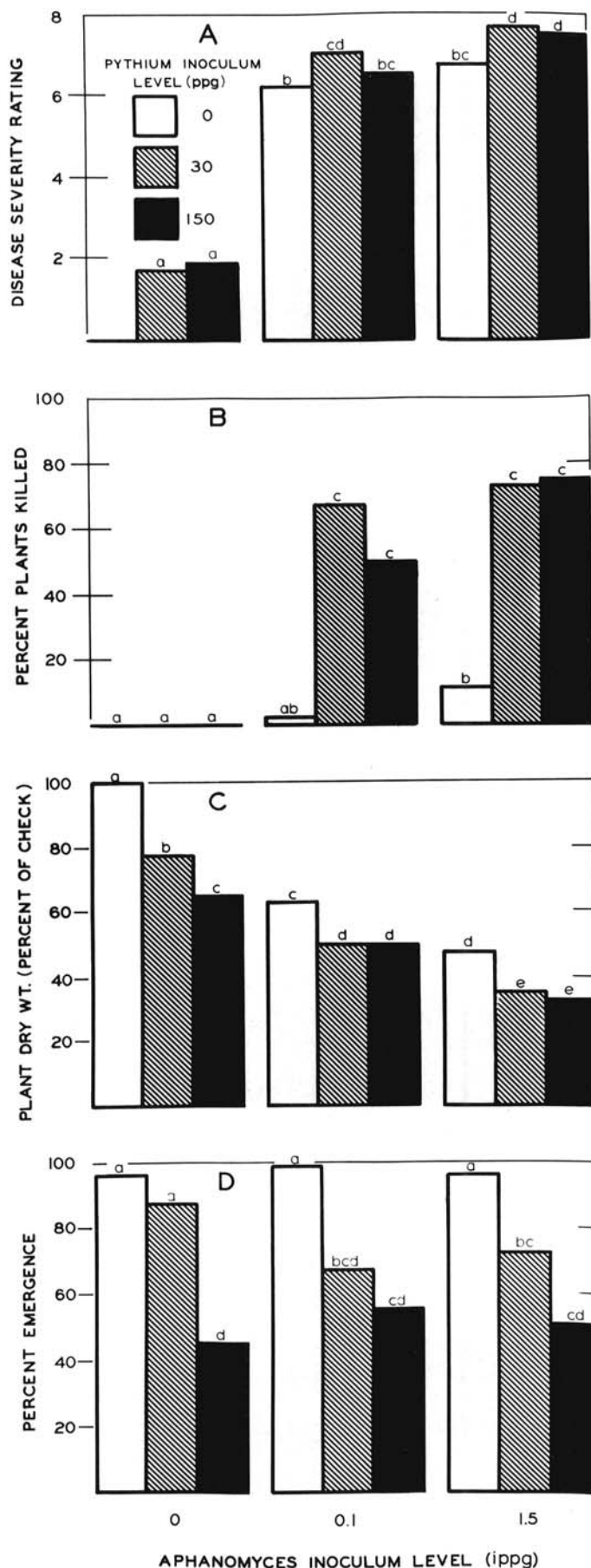


Fig. 1. Beans grown for 3 wk at 22-26 C in soil containing oospores of *Aphanomyces euteiches* f. sp. *phaseoli* and/or *Pythium ultimum* at the following inoculum levels: Left to right in each row, *Pythium* at 0, 30, or 150 propagules per gram; top to bottom in each column, *Aphanomyces* at 0, 0.1, or 1.5 infective propagules per gram.



most severe in plants exposed to both pathogens. Disease severity ratings (Fig. 2A) indicate that *Aphanomyces* caused greater damage than *Pythium*. At the higher inoculum level of *Aphanomyces*, symptom severity was increased if *Pythium* was also present. No plants were killed by *Pythium* alone in this test, and few were killed by *Aphanomyces* alone. However, mortality was high in plants infected by both pathogens simultaneously (Fig. 2B). A highly significant interaction term in a factorial analysis of variance for the data indicated that this increased mortality was a synergistic effect of *Pythium* and *Aphanomyces*. *Pythium* acting alone decreased plant weight significantly in comparison with the noninfected checks (Fig. 2C). *Aphanomyces* alone at the low inoculum level decreased plant weights to the same extent as did *Pythium* alone at a high inoculum level, and a high inoculum level of *Aphanomyces* further decreased plant weights. Weight of plants infected by both pathogens was less than that of plants infected by either pathogen alone at a given inoculum level, but data analysis did not indicate a synergistic effect. *Pythium* reduced emergence of beans, whereas *Aphanomyces* did not (Fig. 2D).

Data for the second experiment, in which plants were exposed to the pathogens singly or in combination at each of four temperatures, are shown in Table 1. Emergence near 100% was observed at all temperatures in soil containing *Aphanomyces* alone, but emergence was reduced at 16 and 20 C in soil containing *Pythium*, whether alone or in combination with *Aphanomyces*. As indicated by plant dry weights, *Pythium* reduced plant growth more than *Aphanomyces* at 16 C, whereas the reverse was observed at 24 and 28 C; at 20 C the two pathogens had equivalent effects on growth. Simultaneous infection by both pathogens caused greater growth reduction than either pathogen alone at 20 C, but did not cause significantly more damage than infection by *Aphanomyces* alone at 24 and 28 C, or by *Pythium* alone at 16 C. In all cases, root weights were more severely affected than shoot weights. Symptom severity readings reflected the same effects as did plant weights. Damage caused by *Aphanomyces* alone was severe at all temperatures, though less severe at 16 and 20 C, whereas damage caused by *Pythium* alone was severe only at 16 and 20 C. *Pythium* primarily caused feeder root necrosis; the taproot usually remaining firm and only slightly discolored. At cool temperatures, this feeder root damage was severe enough to cause substantial stunting of the plants (Fig. 3). *Aphanomyces* caused necrosis of both feeder and taproots at all temperatures. This necrosis usually extended up the hypocotyl as well. Hypocotyl necrosis was most severe in plants infected by both pathogens simultaneously; at 20 and 24 C, this combined attack often resulted in necrosis that extended to the growing point of the plant and caused death (Fig. 3B and C, Table 1).

Factorial analysis of the data for main effect of pathogens indicated that, averaged across all temperatures tested, severity of disease was least for plants exposed to *Pythium* alone, and increased successively for plants exposed to *Aphanomyces* alone and both pathogens together. These averages reflect the fact that damage caused by *Pythium* alone decreases as temperature increases, in contrast with the substantial damage caused by *Aphanomyces* at all temperatures; the additive effect of both organisms acting together is also indicated. Due to the counteracting influences of temperature on the damage caused by

Fig. 2. Bean root rot severity at 24 ± 2 C as influenced by soil inoculum levels of *Aphanomyces euteiches* f. sp. *phaseoli* and *Pythium ultimum*. *Aphanomyces* inoculum was determined by a most probable number bioassay, and is expressed as infective propagules per gram of soil (ippg). Inoculum level of *Pythium* was determined by soil dilution plating on selective medium and is expressed as propagules per gram (ppg). Within a figure, bars headed by the same letter do not differ, $\alpha = 0.05$, as determined by Duncan's new multiple range test. A, Symptom severity, ranging from 0 (healthy) to 8 (maximum severity), obtained by summing scores (0-4) for root and hypocotyl. B, Percentage of emerged plants killed at the end of the 3-wk period. C, Dry weight of plants expressed as a percentage of weight of check plants. D, Percentage of seeds planted that emerged (hypocotyl crook straightened) successfully.

TABLE 1. Severity of bean root and hypocotyl rot incited by *Pythium ultimum* and/or *Aphanomyces euteiches* f. sp. *phaseoli*, at four temperatures

Temperature (C)	Pathogen ^x	Emergence (%)	Plant dry weight % of check			Symptom severity ^y	Plants killed (%)
			Root	Shoot	Total		
16	<i>Pythium</i>	66 cd ^z	21 ab ^z	57 b ^z	45 ab ^z	4.8 c ^z	0 a ^z
	<i>Aphanomyces</i>	98 a	38 d	77 c	64 c	5.0 c	0 a
	<i>Pythium</i> + <i>Aphanomyces</i>	43 d	11 a	49 ab	37 ab	5.9 de	1 ab
20	<i>Pythium</i>	61 cd	34 cd	55 ab	48 b	4.0 b	4 ab
	<i>Aphanomyces</i>	99 a	28 bc	51 ab	45 ab	5.4 c	0 a
	<i>Pythium</i> + <i>Aphanomyces</i>	63 cd	13 a	35 a	29 a	6.9 g	31 c
24	<i>Pythium</i>	100 a	70 e	89 cd	82 d	2.0 a	0 a
	<i>Aphanomyces</i>	96 ab	24 bc	52 ab	43 ab	6.2 ef	0 a
	<i>Pythium</i> + <i>Aphanomyces</i>	98 a	18 ab	48 ab	38 ab	6.6 fg	44 c
28	<i>Pythium</i>	84 bc	69 e	94 d	83 d	2.2 a	0 a
	<i>Aphanomyces</i>	98 a	28 bc	48 ab	41 ab	6.2 ef	0 a
	<i>Pythium</i> + <i>Aphanomyces</i>	95 ab	20 ab	48 ab	39 ab	6.7 fg	7 b

^xBeans were grown in pasteurized soil reinfested with *P. ultimum* and/or *A. euteiches* f. sp. *phaseoli* at 150 propagules per gram and 1.5 infective propagules per gram, respectively.

^ySymptom severity ranges from 0 (healthy) to 8 (maximum severity). Values were obtained by summing scores (0–4) for root and hypocotyl.

^zAll values are average of two experiments, three or four replicate pots of each pathogen × temperature treatment per experiment, four plants per pot. Values within a column followed by the same letter do not differ significantly according to Duncan's new multiple range test, $\alpha = 0.05$.

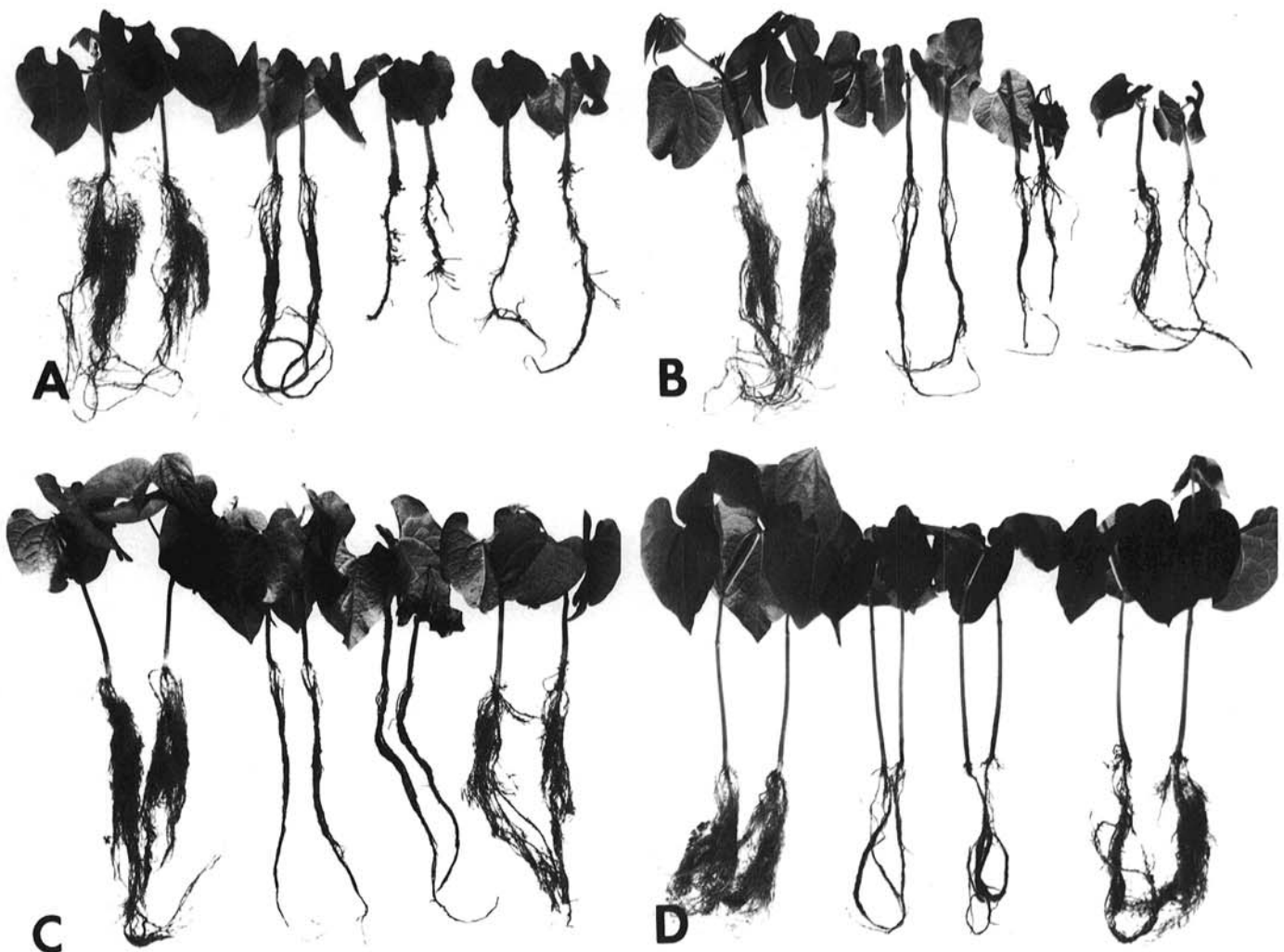


Fig. 3. Beans grown at: A, 16 C; B, 20 C; C, 24 C; or D, 28 C in soil containing (left to right) no inoculum, *Aphanomyces euteiches* f. sp. *phaseoli*, *Aphanomyces* plus *Pythium*, or *Pythium ultimum*.

the two organisms acting separately, the main effect of temperatures on disease was relatively slight. Symptom severity, plant weights, and percentage of plants killed indicate that, averaged across causal organisms, 20 C is the most favorable temperature for root rot development. Emergence was lowest at low temperatures.

DISCUSSION

We found *A. euteiches* f. sp. *phaseoli* in 14 of 15 bean fields we tested in Wisconsin's major snap bean production area. Therefore, this pathogen is potentially a problem to bean growers in the area. At soil inoculum levels representative of field soil, this fungus caused at least as much damage to beans in growth chamber tests as did *P. ultimum* (also at inoculum levels common for that pathogen in field soil), except at 16 C. *A. euteiches* f. sp. *phaseoli* is apparently the major pathogen inciting bean root rot in central Wisconsin in warm soil, and shares this role with *P. ultimum* in cool soil. When both pathogens are present, disease is severe at 16–28 C.

In central Wisconsin, snap beans are planted as early as 15 May and as late as 15 July. Soil temperatures in mid-May generally range from 10 to 20 C, and those in mid-July from 24 to 30 C. Although beans planted late may escape severe *Pythium ultimum* damage by virtue of growing in warm soil, they may be subject to severe damage from *Aphanomyces* and vice versa. Furthermore, we found in numerous fields that a diseased bean plant is commonly infected with both pathogens. Because severity of disease induced by both pathogens acting in concert is similar at all temperatures, time of planting is not likely to provide a control measure for bean root rot in central Wisconsin.

Our finding that bean root rot incited by *P. ultimum* is most severe at low temperatures is in agreement with the data of Pieczarka and Abawi (9). They grew beans in soil containing 500 ppg of the fungus and recorded plants weighing 27 and 78% as much as noninfected plants when grown at 15 and 27 C, respectively. Our results differ from those of Hoch et al (5), however. They found disease caused by *P. ultimum* to be about equally severe at 16 and 28 C. Their method of soil infestation (incorporation of half of a petri plate culture of fungus in a 10-cm pot of soil) may have produced too high an inoculum level to distinguish temperature effects of *P. ultimum*. They also found disease in naturally infested soil from central Wisconsin to be slight at 16 C and higher at 20, 24, and 28 C. This observation may have been due to the undetected presence of *A. euteiches* f. sp. *phaseoli*; it may also have been due to the activity of *Pythium aphanidermatum*, a fungus with a high optimum temperature, which they recovered from infected beans. We did not test *P. aphanidermatum* under our conditions and, therefore, cannot say how its virulence on bean would compare with those of *P. ultimum* and *A. euteiches* f. sp. *phaseoli*. Indeed, the interactions of "high-temperature" *Pythium* species with *Aphanomyces* is unknown and potentially significant. However, Hoch et al (5) did not report how commonly they found *P. aphanidermatum*, in comparison with *P.*

ultimum, in isolations from beans. Reeleder (10), working with bean root rot in the area four years later, found that *P. ultimum* was the most frequently isolated species of *Pythium*, and that *P. aphanidermatum* was very rarely recovered from naturally infected beans.

Severe root rot on very sandy, well-drained soil is unusual because root rots are generally associated with poorly drained soil. Irrigation frequency may contribute to this anomaly. Plainfield sand has a very low water storage capacity (2). Matric water potential below approximately –200 mb may result in plant water stress due to this low water-holding capacity and the low hydraulic conductivity of the soil. It is very difficult to achieve matric potentials above –30 mb in this soil, so growers irrigate frequently, and the matric water potential of the soil may remain between –30 mb and –200 mb during a large part of the growing season. Thus, although well drained and seldom saturated, the soil may be chronically wet.

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