Interactions of *Pythium ultimum* with Germinating Resistant or Susceptible Pea Seeds

T. E. Stasz and G. E. Harman

Graduate research assistant and associate professor, respectively, Department of Seed and Vegetable Sciences, New York State Agricultural Experiment Station, Geneva 14456.

We thank G. Nash and E. P. Carney for laboratory and greenhouse assistance, J. Barnard for assistance with statistical analyses, and colleagues G. A. Marx, R. Baker, R. C. Seem, and H. C. Hoch for advice and for critical review of the manuscript.
Portion of PhD thesis submitted by the senior author to the Department of Plant Pathology, Cornell University, Ithaca, NY 14853.
Approved by the Director of the New York State Agricultural Experiment Station as Journal Series Paper 3221.
Accepted for publication 4 July 1979.

ABSTRACT

STASZ, T. E., and G. E. HARMAN. 1980. Interactions of *Pythium ultimum* with germinating resistant or susceptible pea seeds. Phytopathology 70:27-31.

Variously treated seeds of the susceptible pea line Dark Skin Perfection (DSP) and resistant accession, PI 257593, were planted in soil artificially infested with a range of densities of *Pythium ultimum* oospores. After 10 days, both the proportion of diseased plants and disease severity were measured. The resulting curves representing the proportion of diseased plants or disease severity versus inoculum density were similarly shaped for the two lines regardless of seed condition or soil fungistasis level. However, the curves differed markedly in position as reflected by ED_{50} values. The ED_{50} value for PI 257593 was greatly reduced by damaging the seed coats whereas that of DSP was only slightly reduced by similar treatment. Aging seeds prior to planting, which reduces seed vigor and increases exudation during germination, reduced the ED_{50} values of both lines. ED_{50} values also differed in two different soil mixtures that were tested, but the level of resistance of PI 257593 relative to that of DSP was not altered. In all cases, however, curves plotted for disease severity versus the proportion of diseased plants were similar in both shape and position. Levels of resistance or susceptibility as determined by disease severity are therefore due to differences in numbers of infections occurring at a given inoculum density; ie, are relative to the proportion of oospores which successfully infect the host, and not to differences in host-pathogen interactions following infection. Comparison of these data obtained by generation of proportion of diseased plants versus inoculum density curves suggest that only the oospores in the spermoplane are capable of infecting the host.

Additional key words: inoculum density, seed rot, seed aging, fungistasis.

Germinating seeds of pea (*Pisum sativum* L.) and of other crop plants are susceptible to seed rot and seedling blight caused by *Pythium ultimum* Trow. The pathogen is ubiquitous and can reduce stand establishment.

Resistance to *Pythium* seed rot is known (4,8,10), but at present is poorly understood. Analysis of disease intensity versus inoculum density (DI-ID) relationships allows interpretation of host-pathogen interactions (3,20). A comparison of host-pathogen interactions for resistant and susceptible hosts should allow insight into mechanisms of resistance.

In this study, we compared interactions of *P. ultimum* with resistant and susceptible pea lines by analyzing DI-ID relationships. Effects of seed aging, seed coat damage, and of two soils on disease incidence and severity were determined.

MATERIALS AND METHODS

Pathogen. Cultures of *P. ultimum* isolate 220, obtained from D. J. Hagedorn, University of Wisconsin-Madison, produce abundant oospores but few sporangia. The fungus was maintained on Difco corn meal agar and periodically grown on pimaricin-vancomycin agar (11) to avoid bacterial contamination. Disks (5 mm in diameter) cut from 2-day-old water agar cultures were seeded into petri dishes containing 15 ml of V8-cholesterol medium (1). After 1 wk, the culture medium was replaced with sterile distilled water. After an additional 2 wk, mycelial mats were rinsed in water and briefly comminuted in 20 ml of water per mat in a Waring Blendor. Hyphal debris was removed with a plastic screen with 0.6 mm^2 pores. Spores were collected by centrifugation at 2,500 *g* for 10 min, resuspended in water, and counted with a hemacytometer.

Soil preparation and infestation. Soil mixture A was prepared from flame-pasteurized Geneva potting soil (13) and sand (1:2, w/w). The mixture was autoclaved at 120 C for 2 hr to eliminate pathogens, amended with 1 g of 0-20-0 (N-P-K) superphosphate fertilizer per 5 kg, spread on a greenhouse bench for 1 wk, and stored at 16% moisture content in a plastic-lined bin for at least 3 wk prior to infestation. Experiments were performed in soil A unless otherwise indicated.

Soil mixture B was prepared from topsoil (freshly dug from under sod in June) and sand (1:2, w/w). The mixture was treated with aerated steam at 62 C for 1 hr and stored at 16% moisture content in polyethylene containers for at least 3 wk prior to infestation.

Soil fungistasis levels were determined by an agar disk method (6). Washed water agar disks were placed on soil and incubated in covered petri dishes at 25 C for 48 hr. Conidia of either *Alternaria alternata* (Fries) Keissler or *Fusarium solani* (Mart.) Appel & Wr. emend Snyd. & Hans. f. sp. *pisi* were collected from potato-dextrose agar colonies and washed by centrifugation. About 1,000 conidia were applied to each disk, which then was incubated at 25 C for 16 hr and the percent germination was calculated. In controls, glass slides prevented agar disks from contacting the soil.

Soil was partially dried, infested with up to 5×10^4 oospores per gram, readjusted to 16% moisture content (approximately −0.17 bar matric potential) and incubated at 25 C in covered polyethylene containers. The number of germinable oospores per gram of soil was determined at the beginning of an experiment by the surface soil dilution plate method (12) with modified pimaricin-vancomycin medium (11) as the selective medium. Incubation was at 18 C for 24 hr in the dark. The type of propagule giving rise to colonies was determined by microscopic observation of unwashed plates. Oospores also were observed after they had been buried in soil between Nuclepore filters (Nuclepore Corp., Pleasanton, CA 94566) (18).

0031-949X/80/01002705/$03.00/0

Hosts. Pea accession PI 257593, which is resistant to *P. ultimum* (8), was obtained from the Northeast Plant Introduction Station, Geneva, NY. Four healthy vigorous and productive plants were selected from a greenhouse planting and seeds from these plants were increased through two generations. The Asgrow Seed Co., Twin Falls, ID 83301, made the final seed increase of PI 257593 and also supplied seed of Dark Skin Perfection (DSP), which is susceptible to *P. ultimum*. Seeds were stored at 10 C and 20% relative humidity (RH).

Seeds were planted 2.5 cm deep in approximately 15 g of soil in plastic cups (one seed per cup) with lids and incubated at 16 C for 10 days in darkness. Unless otherwise noted, seeds were nicked to ensure uniform germination by removing 1–3 mm^2 sections of the seed coats distal to the embryonic axes. In some experiments, DSP and PI 257593 seeds were aged at 30 C and 92% RH (5) for 5 and 7 wk, respectively, after which 90% germinated. Germinability decreased rapidly if aging continued beyond those times.

Disease severity. The severity of seed and seedling disease was rated by evaluating six aspects of seedling development: seed germination, cotyledon integrity, taproot development and color, lateral root emergence, epicotyl elongation, and apical meristem survival. Each aspect was scored two points if the tissues were severely diseased or if normal development had not occurred, zero points if the tissues appeared healthy and normal development had occurred, or one point if tissues were moderately diseased or normal development was incomplete. The points were totalled to obtain a disease severity rating of from 0 (healthy) to 12 (maximum disease) and the ratings of 10 seedlings were averaged to obtain each data point. Log disease severity rating was regressed on log germinable oospore density per gram of soil to determine slope confidence intervals; regressions were reversed to determine inoculum densities required for 50% disease severity (ED_{50}) confidence intervals. Average disease severity ratings less than 1.2 or greater than 10.8 were excluded from regressions.

Proportion of diseased plants. The number of seedlings with a disease severity rating greater than zero per group of 10 seedlings determined the proportion of diseased plants. Log proportion of diseased plants was regressed on log germinable oospore density per gram of soil to determine slope confidence intervals; regressions were reversed to determine confidence intervals for ED_{50} values, inoculum densities required for one-half of the plants to be diseased. Proportions less than 0.1 or greater than 0.9 were excluded from regressions.

Average disease severity versus proportion of diseased plants. Average disease severity rating for a given inoculum density was regressed on proportion of diseased plants at that inoculum density, excluding proportions greater than 0.9.

Spermosphere versus spermoplane test. Proportions of diseased plants were corrected for multiple infections (20) and log corrected proportion of diseased plants was regressed on log germinable oospore density per gram of soil as suggested by Baker (3).

Statistical methods. Regression analyses were performed with a computerized Minitab program (14). Ninety-five percent confidence intervals were calculated for slope and ED_{50} values and a least significant difference criterion was applied (17).

RESULTS

Soil fungistasis. Soil mixture B reduced *F. solani* f. sp. *pisi* conidial germination on agar disks in contact with soil to 19% of the germination on control disks on glass slides whereas soil mixture A reduced germination to 86% of controls. Similarly, *Alternaria alternata* conidial germination on agar disks in contact with the same two soils was 44% and 61% of controls, respectively.

Oospores and infested soil. Oospores from cultures were thick-walled and failed to germinate on selective medium. Similarly, fewer than 0.05% of the oospores present gave rise to colonies on selective medium when soil was assayed immediately after infestation. However, 5% of the oospores in soil mixture A and 40% of those in soil mixture B gave rise to colonies on selective medium when infested soil was assayed after being incubated 3–6 wk.

When buried between Nuclepore filters in soil 3–6 wk, some oospores no longer had the thick inner walls, refringent bodies, and reserve globules which were evident in freshly harvested oospores. Oospores lacking thick walls had increased in size and resembled sporangia. Oospores between filters in soil did not germinate and some disintegrated after several months.

Disease severity versus inoculum density. All disease severity versus inoculum density curves were shaped similarly regardless of pea line, seed condition, or soil fungistasis level (Fig. 1). Slopes were nearly constant when the disease severity rating was less than six and decreased as disease severity increased. Log-log transformed curves were linear and their slopes did not differ significantly ($P = 0.05$) (T. E. Stasz and G. E. Harman, *unpublished*). However, transformed curves differed markedly in position ($P = 0.05$) as reflected by the germinable oospore density per gram of soil required to cause 50% disease; ie, a disease severity rating of six (ED_{50}) (Table 1). Seeds of PI 257593 were more resistant than those of comparably tested DSP, but ED_{50} values were affected by seed condition and soils. Nicking the seed coats reduced the ED_{50} value for PI257593 seeds more than sevenfold but only reduced that for DSP seeds twofold. Aging the seeds reduced the ED_{50} value for PI 257593 ninefold and of DSP 30-fold. ED_{50} values for nicked seeds of both pea lines were fivefold greater in soil B, than in soil A.

Proportion of diseased plants versus inoculum density. Regardless of pea line, seed condition, or soil fungistasis level DI-ID curves (not shown) were similarly shaped and closely resembled

TABLE 1. Density of germinable *Pythium ultimum* oospores which cause either pea seedling disease severity rating[a] to be 50% of the maximum or one half of the plants to be diseased in a resistant and a susceptible pea line as affected by seed aging, seed coat nicking, and soil fungistasis level

Pea line	Seed condition	Soil[c]	ED_{50} value (no. oospores per gram of soil) based on: Disease severity rating[c]	Proportion of diseased plants[d]
DSP[b]	Intact	A	11 A	4.0 A
	Nicked	A	6.0 B	3.5 A
	Nicked, aged	A	.20 C	.10 B
	Nicked	B	32 D	21 C
PI 257593	Intact	A	>2,500	...
	Nicked	A	350 E	200 D
	Nicked, aged	A	39 D	30 C
	Nicked	B	1,700 F	880 E

[a] Disease severity rating of 0 (healthy) to 12 (severe disease).
[b] Dark Skin Perfection.
[c] Soil A was prepared by autoclaving a mixture of potting soil and sand (1:2,w/w) and stored at least 4 wk prior to use. Soil B was prepared by treating a mixture of topsoil and sand (1:2,w/w) with aerated steam at 62 C for 1 hr and storing it a least 3 wk prior to use. More *Fusarium solani* f. sp. *pisi* and *Alternaria alternata* conidia germinated on washed agar disks in contact with soil A than did conidia on agar disks in contact with soil B.
[d] Values in a column followed by the same letter are not significantly different ($P = 0.05$).

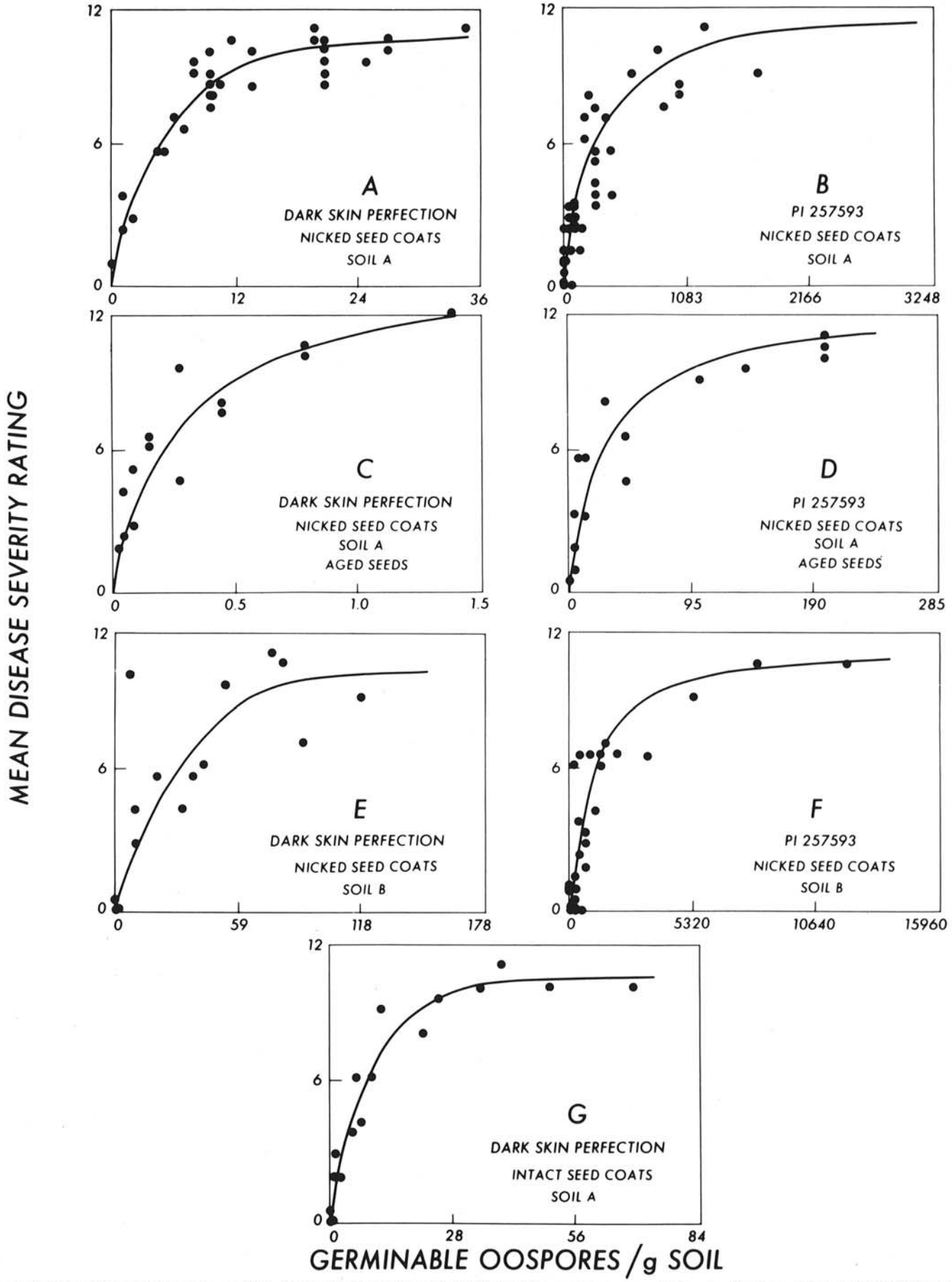

Fig. 1. Relationships between mean seedling disease severity rating and *Pythium ultimum* oospore population density for a resistant (PI 257593) and a susceptible (Dark Skin Perfection) pea line as affected by seed aging, seed coat nicking, and soil treatment. Soil A— a mixture of potting soil and sand (1:2,w/w), autoclaved 2 hr, and supplemented with 1 g superphosphate per 5 kg of mixture; Soil B— a mixture of topsoil and sand (1:2,w/w), aeration-steamed (62 C) for 1 hr. Both were stored 3 wk before being infested.

those of typical (3) disease severity-inoculum density curves (Fig. 1). However, positions of the curves, as reflected by inoculum densities required to cause disease of one half of the plants (ED_{50}), varied with pea line, seed conditions and soil (Table 1).

Disease severity versus proportion of diseased plants. Regresssion lines fitted to disease severity versus proportion of diseased plants data did not differ ($P = 0.05$) in slope or position regardless of pea line, seed condition, or soil (Fig. 2).

Spermosphere versus spermoplane test. Slopes of regression lines did not differ ($P = 0.05$) from 0.67 on a log-log basis for either DSP or nicked PI 257593 seeds (curves not shown).

DISCUSSION

DI-ID analyses require experimental conditions closely resembling field conditions for conclusions to be valid. In the present study, oospores were used as inoculum since either oospores or sporangia (or both) of *P. ultimum* function as overwintering structures and as primary inoculum (1,2,9). Since nutrients greatly influence spore behavior in soil (15,16,19), oospores were freed of most of the culture medium and mycelium before being added to soil. Oospore appearance in soil was consistent with that reported for oospores in natural soils (9). Autoclaving was a convenient means of eliminating native *Pythium* populations from soil but it increased the number of *F. solani* f. sp. *pisi* and *A. alternata* conidia that germinated in contact with soil and decreased the number of *Pythium* oospores required to cause a given level of disease, as compared with soil treated with aerated steam. However, the relative resistance of the two pea lines was not altered since the ratio of their ED_{50} values was the same in the two soils.

Disease severity is determined by the number of infections of the host by the pathogen and by the magnitude of disease induction in the host in response to these infections. However, the number of infections is related to the proportion of diseased plants (3,20). Therefore, disease severity-proportion of diseased plants curves are related to disease severity-number of infections curves. The former curves thus indicate how disease severity varies with numbers of infections.

In the present case, pea line, seed condition, and soil fungistasis level affected disease severity at a given inoculum density by altering the numbers of infections. This follows because disease severity versus proportion of diseased plants curves were similar in slope and position in all cases (Fig. 2), which indicated that disease severity-number of infections curves also were similar in slope and position in all cases. The analysis made by Horsfall and Dimond (7) also indicates that only quantitative aspects of infection, and not the biochemical pathways of disease, are affected by pea line, seed conditions, or soil since disease-inoculum density curves did not differ in slope. Since numbers of infections were similar when disease severities were similar, a comparison of ED_{50} values indicates the relative proportions of oospores that cause infections.

Thus, nicked PI 257593 seeds were more resistant than were nicked DSP seeds to *P. ultimum* because 58-fold more oospores were required to cause similar numbers of infections of the former. Since other conditions were comparable, this difference in oospore efficacy must have been due to differences in seed versus oospore interactions. Similarly, aging PI 257593 and DSP seeds increased the proportions of oospores causing infections nine-fold and 30-fold, respectively, perhaps because aging increased inoculum potential by increasing seed exudation during germination.

Analyses of DI-ID data in the present study by Baker's model (3) suggest that both PI 257593 and DSP are successfully infected only by *P. ultimum* oospores in the spermoplane since slopes do not differ from 0.67 on a log-log basis ($P = 0.05$) (3).

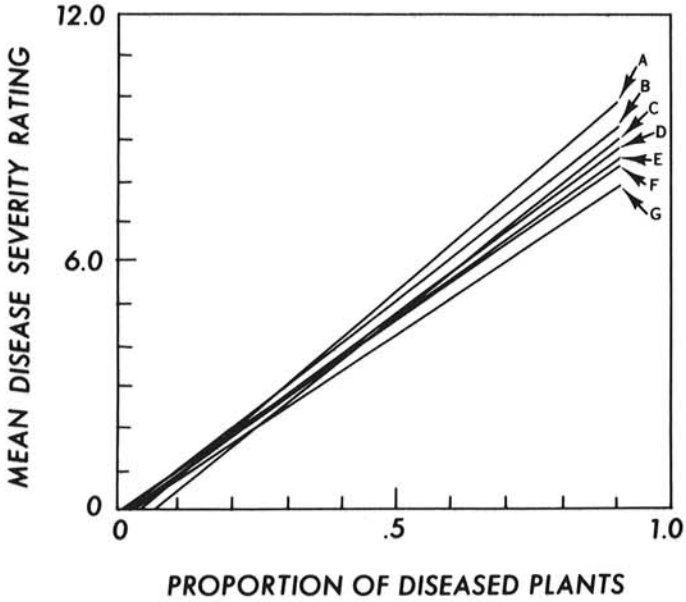

Fig. 2. Relationships between mean seedling disease severity rating and proportion of diseased plants for a resistant and a susceptible pea line as affected by seed condition and soil treatment. **A,** PI 257593 (resistant), nicked seed coats, soil mixture A, aged seeds; **B,** Dark Skin Perfection (susceptible), nicked seed coats, soil mixture B; **C,** Dark Skin Perfection, nicked seed coats, soil mixture A; aged seeds; **D,** Dark Skin Perfection, nicked seed coats, soil mixture A; **E,** PI 257593, nicked seed coats, soil mixture B; **F,** PI 257593, nicked seed coats, soil mixture A; and **G,** Dark Skin Perfection, intact seed coats, soil mixture A. Soil A— a mixture of potting soil and sand (1:2,w/w), autoclaved 2 hr, and supplemented with 1 g superphosphate per 5 kg of mixture; Soil B— a mixture of topsoil and sand (1:2, w/w), aeration-steamed (62 C) for 1 hr. Both were stored 3 wk before being infested.

LITERATURE CITED

1. AYERS, W. A., and R. D. LUMSDEN. 1975. Factors affecting production and germination of oospores of three *Pythium* species. Phytopathology 65:1094-1100.
2. BAINBRIDGE, A. 1966. The biology of *Pythium ultimum* Trow in an irrigated pea field. PhD Thesis, University of Adelaide, South Australia. 134 pp.
3. BAKER, R. 1971. Analyses involving inoculum density of soil-borne plant pathogens in epidemiology. Phytopathology 61:1280-1292.
4. EWING, E. E. 1959. Factors for resistance to pre-emergence damping off in pea (*Pisum sativum* L.) incited by *Pythium ultimum* Trow. PhD Thesis. Cornell University, Ithaca, NY. 118 pp.
5. HARMAN, G. E., and A. L. GRANETT. 1972. Deterioration of stored pea seed: changes in germination, membrane permeability, and ultrastructure resulting from infection by *Aspergillus ruber* and from aging. Physiol. Plant Path. 2:271-278.
6. HORA, T. S., R. BAKER, and G. J. GRIFFIN. 1977. Experimental evaluation of hypotheses explaining the nature of soil fungistasis. Phytopathology 67:373-379.
7. HORSFALL, J. G., and A. E. DIMOND. 1963. A perspective on inoculum potential. J. Indian Bot. Soc. 42A:46-57.
8. KRAFT, J. M. 1974. The influence of seedling exudates on the resistance of peas to Fusarium and Pythium root rot. Phytopathology 64:190-193.
9. LUMSDEN, R. D., and W. A. AYERS. 1975. Influence of soil environment on the germinability of constitutively dormant oospores of *Pythium ultimum*. Phytopathology 65:1101-1107.
10. McDONALD, W. C., and H. H. MARSHALL. 1961. Resistance to preemergence damping-off in garden peas. Can. Plant Dis. Survey 41:275-279.
11. MIRCETICH, S. M. 1971. The role of *Pythium* in feeder roots of diseased and symptomless peach trees and in orchard soils in peach tree decline. Phytopathology 61:357-360.
12. MIRCETICH, S. M., and J. M. KRAFT. 1973. Efficiency of various selective media in determining *Pythium* population in soil. Mycopathol. Mycol. Appl. 50:151-161.
13. NEWHALL, A. G., and W. T. SCHROEDER. 1951. New flash-flame soil pasteurizer. N.Y. Agric. Exp. Stn. Bull. 875. 19 pp.
14. RYAN, T. A. Jr., B. L. JOINER, and B. F. RYAN. 1976. Minitab Student Handbook. Duxbury Press, Wadworth Publishing Co., Inc., Belmont, CA. 341 pp.
15. SHORT, G. E. 1975. Determinants of pea seed and seedling rot. PhD

Thesis, Michigan State University, East Lansing, MI. 60 pp.
16. SINGH, R. S. 1965. Development of *Pythium ultimum* in soil in relation to presence and germination of seeds of different crops. Mycopathol. Mycol. Appl. 27:155-160.
17. SNEDECOR, G. W., and W. G. COCHRAN. 1967. Statistical Methods. 6th ed. Iowa State Univ. Press, Ames, IA. 593 pp.
18. SNEH, B. 1977. A method for observation and study of living fungal propagules incubated in soil. Soil Biol. Biochem. 9:65-66.
19. STANGHELLINI, M. E. 1974. Spore germination, growth and survival of *Pythium* in soil. Proc. Am. Phytopathol. Soc. 1:211-214.
20. Van der PLANK, J. E. 1975. Principles of Plant Infection. Academic Press, New York. 216 pp.