Relationship of Seedling Age to Development of Pythium ultimum on Roots of Antirrhinum majus

H. M. Mellano, D. E. Munnecke, and R. M. Endo

Former Research Assistant, Professor, and Associate Professor, respectively, Department of Plant Pathology, University of California, Riverside 92502.

Based on a portion of the Ph.D. thesis submitted by the senior author in partial fulfillment of the requirements in the Department of Plant Pathology, University of California, Riverside.

Accepted for publication 14 January 1970.

ABSTRACT

Snapdragon (*Antirrhinum majus*) seedlings 15 days old or younger (susceptible) infected with *Pythium ultimum* were killed within 6 days because colonization of host tissue was rapid and unrestricted. However, 25-day-old (tolerant) plants developed tolerance to infection and host colonization that persisted for the rest of their life cycle. Infection of tolerant plants resulted in wilting and stunting intensified by high temp. Increased fertilization intensified root rot in seedling stages. Penetration and infection of roots of susceptible and tolerant plants were similar, but colonization of host tissues was different. On susceptible plants, appressoria arising from mycelia were formed commonly in the regions of elongation and maturation of roots, occasionally on the root cap or in the older regions of maturation, but never in the meristematic region. Several cells were invaded within 4-8 hr after appressoria formed; subsequent intracellular and intercellular colonization by mycelia was rapid and complete. Growth into meristematic areas appeared to be dependent upon death of the tissues. On tolerant plants, appressoria were formed abundantly on young tertiary and quaternary roots but sparingly on thickened primary and secondary roots. Colonization of the cortex of tertiary and quaternary roots was rapid but mycelial growth in the cortex of primary and secondary roots was greatly restricted. Oospores formed in infected tissue of both young and old roots 4-6 days after inoculation. The difference in response of tolerant and susceptible plants to infection could not be related to lignification of host cells. These observations indicated that tolerance to infection of snapdragon by *P. ultimum* was due to physiological rather than anatomical characteristics of the host tissues. Phytopathology 60:935-942.

Resistance of older plants to *Pythium* spp. has been reported frequently. In general, seedlings are killed but older plants are not, since infections are usually restricted to the root tips. Because of this, the effects of *Pythium* spp. on older plants have received little attention, even though *Pythium* infections resulting in yield reductions of wheat (20), root rot of muskmelon (13), stunting of *Poinsettia* (19), and reduced growth of corn (10) are ample proof that *Pythium* spp. injure plants past the seedling stage.

The nature of resistance to *Pythium* spp. shown by older plants has not been studied, but resistance mechanisms based on (i) differences in host exudates; (ii) differences in cell walls of the host; and (iii) production of toxic compounds by the host have been reported.

Flentje (4) showed that exudates from germinating susceptible pea seeds were better substrates for mycelial growth of *Pythium* than exudates from resistant seeds. Less sucrose was exuded from resistant varieties as compared to susceptible varieties. Presumably, exudates could influence susceptibility or resistance.

More mechanical pressure was necessary to puncture cell walls of tubers of resistant potato varieties than those of susceptible ones (8). Photomicrographs indicated that cell wall penetration by *P. debaryanum* was mechanical, and that walls of susceptible varieties were more easily penetrated than those of resistant ones.

The production of toxic compounds by the host may be important in resistance, since a necrotic fleck reaction similar to the hypersensitive reaction, has been reported in safflower plants resistant to *Pythium* (12). Hawker et al. (7) reported *Pythium* as a mycorrhizal fungus on *Allium ursinum*, and related its parasitism and symbiotic behavior to the presence of an unidentified inhibitor extracted from the host. Mycorrhiza formed only on mature plants; when seedlings were inoculated, almost 100% damping-off occurred. In mature *Allium* the fungus was found in the root, but not in the bulb. When sterilized root sap was added to growth medium, linear growth of *Pythium* was inhibited.

Chemical differences in cell walls may be important in resistance. English & Albersheim (3) reported that *Colletotrichum lindemuthianum* cleaved high levels of α-D-galactose from extracted cell walls of susceptible 5-day-old bean hypocotyls, but only low levels from resistant 18-day-old hypocotyls. They suggested that host cell walls may be involved in determining the amounts of a polysaccharide-degrading enzyme produced by a pathogen.

Inoculations of snapdragon (*Antirrhinum majus* L.) plants to determine the effects of infection by *Pythium ultimum* Trow revealed an abrupt change from extreme susceptibility, wherein seedlings died, to tolerance in plants several weeks old. Tolerant plants became infected, but colonization was restricted and plants survived. The basis of this ontogenetic resistance was investigated; the results are presented in this and the following paper (17). Portions of this work have been reported (15, 16).

METHODS.—*Fungus culture.*—A hyphal-tip culture of *P. ultimum* (isolate 420) isolated from roots of wilted *A. majus* by D. E. Munnecke in 1960 was used. The fungus, when subcultured weekly on potato-dextrose agar (PDA), remained stable, and no changes in pathogenicity or cultural characters were observed during these experiments.

Plant growing conditions.—*A. majus* (Burpee's Sentinel, var. Majorette) was used. Aseptic seedlings were

produced in vitro as follows: the seeds were washed for 24 hr in running tap water, soaked in 0.5% NaOCl for 10 min, transferred to 70% ethanol for 1 min, rinsed in sterile deionized water, and germinated at 22-27 C for 48 hr. After germination, the seeds were transferred to petri plates containing filter paper soaked with sterile 0.1 M Hoagland's solution (9) and placed in a growth chamber set at 22 C. They were illuminated for 12 hr daily with 1,000 ft-c fluorescent light. The fertilizer solution was changed every 2 days.

Plants were also grown in a growth chamber or in a smog-free greenhouse in a U.C.-type soil mix (1). The mix consisted of equal parts by volume of fine sand and sphagnum peat moss. Fertilizer incorporated per m^3 at mixing consisted of: KNO_3, 149 g; K_2HPO_4, 149 g; single superphosphate, 1.75 kg; dolomite lime, 2.02 kg. The soil mix and most containers were steam-sterilized. Heat-labile containers were disinfested by soaking in 0.5% NaOCl for at least 24 hr. Seedling plants were produced by planting seeds in 100-ml plastic pots and allowing four plants to develop/pot. Mature plants were grown in large containers and fertilized weekly with Hoagland's complete fertilizer solution.

Inoculation.—Seedlings growing in 100-ml plastic pots were inoculated by a modification of Halpin & Hanson's technique (6). A 1-cm diam glass rod was inserted vertically in the middle of the pot equidistant from the four plants when the plants were 8-12 days old. The glass rods were removed and two plugs of inoculum (5-mm diam) taken from the edge of a vigorously growing culture of *P. ultimum* on PDA were placed in each hole. The plugs were covered with soil, and the plants were watered.

Seedlings grown in vitro were inoculated by placing discs of inoculum on glass rods so that the discs were slightly above the surface of the nutrient solution and within 1 cm of roots. This was done to prevent an abnormal root tip development that occurred when the inoculum discs touched the nutrient solution.

In one experiment, inoculum was produced in Erlenmeyer flasks containing 400 ml vermiculite and 200 ml Czapek's solution. The medium was sterilized in an autoclave for 45 min at 121 C. The flasks were inoculated with discs of *P. ultimum* taken from week-old cultures produced on PDA and incubated for 28 days on the laboratory bench (22-27 C). Soil was infested by mixing 3% of this inoculum in soil on a w/w basis.

Amount of inoculum in the soil.—The amount of *P. ultimum* inoculum in the soil was determined using a modification of Richardson & Munnecke's technique (18). Infested soil was collected and mixed with uninfested soil for 5 min with a twin-shell blender to make concentrations of infested soil of approximately 16, 4, 1, and 0.3%. The next day the soil was put into 5.6 × 5.6 cm "jiffy" pots (Jiffy-pot Ltd., Gronud, Norway). Four pots tacked to a board with five Little Marvel pea seeds planted in each pot constituted one unit. There were three units for each dilution of each soil tested. The pots were placed in the growth chamber and watered daily with deionized water. Emergence counts were taken 9 days after planting, and the per cent damping-off was calculated.

Histochemical test.—Lignification of the cell walls of root tissue was examined using Jensen's modification (11) of Johansen & Sieglel's phloroglucinol test. Roots of plants grown in the growth chamber were harvested at 5-day intervals from 10 to 30 days after seeding, fixed in Nawashins' solution, dehydrated in tertiary-butyl alcohol, and embedded in paraffin. Mounted 15-μ sections were hydrated, stained with 20% HCl saturated with phloroglucinol, and observed immediately.

RESULTS.—*The effect of age, fertilization, and temp on the resistance of A. majus plants to resistance to infection and disease development by P. ultimum.*—Seeds were planted at 5-day intervals, and half the pots were fertilized daily with 0.25 M Hoagland's solution. Half the fertilized and half the unfertilized seedlings from each age group (Table 1) were inoculated with *P. ultimum*. All seedlings 15 days old or younger died within 6 days, whereas most 20-day-old and all 25- and 30-day-old plants survived (Table 1, Fig. 1). The fungus was isolated from samples of inoculated plants from each age group. In the surviving plants, root rot was more severe on plants inoculated at 20 days than on plants inoculated at 25 or 30 days after planting. In the tolerant plants, the fungus infection was restricted to the root tips of tertiary and quaternary roots and to small necrotic lesions formed on the cortical tissue of primary and secondary roots. These lesions appeared to result from hypersensitive reactions, but a detailed examination was not made.

Fertilization caused an unusual effect. In the absence of *P. ultimum*, the tops of the fertilized plants were much larger than those of unfertilized plants, but the root system was smaller (Fig. 2). In the presence of root rot, stunting of the top was always more severe in fertilized plants than in unfertilized plants (Fig. 2, 3). The experiment was repeated several times with the same results. Following one of these experiments, the amount of inoculum in the soil from fertilized and unfertilized plants inoculated at 10, 20, and 30 days of age was estimated (Table 2). There was more inoculum in the fertilized soils, and there appeared to be more inoculum when older plants were inoculated.

To determine whether the plants lost tolerance with age, plants were grown for over 4 months in the greenhouse. In one experiment, 20 unfertilized, 30-day-old plants were inoculated and transplanted to 4-inch clay pots 16 days later. Twenty noninoculated 46-day-old plants were transplanted to 4-inch clay pots as controls. One week later the plants were transplanted to 6-inch clay pots and fertilized with bloodmeal. All noninoculated and some inoculated plants had formed

TABLE 1. Effect of age of seedlings of *Antirrhinum majus* on susceptibility to damping-off caused by *P. ultimum*

Age when inoculated, (days)	No. from 12 surviving inoculation	
	After 6 days	After 20 days
10	0	0
15	0	0
20	10	10
25	12	12
30	12	12

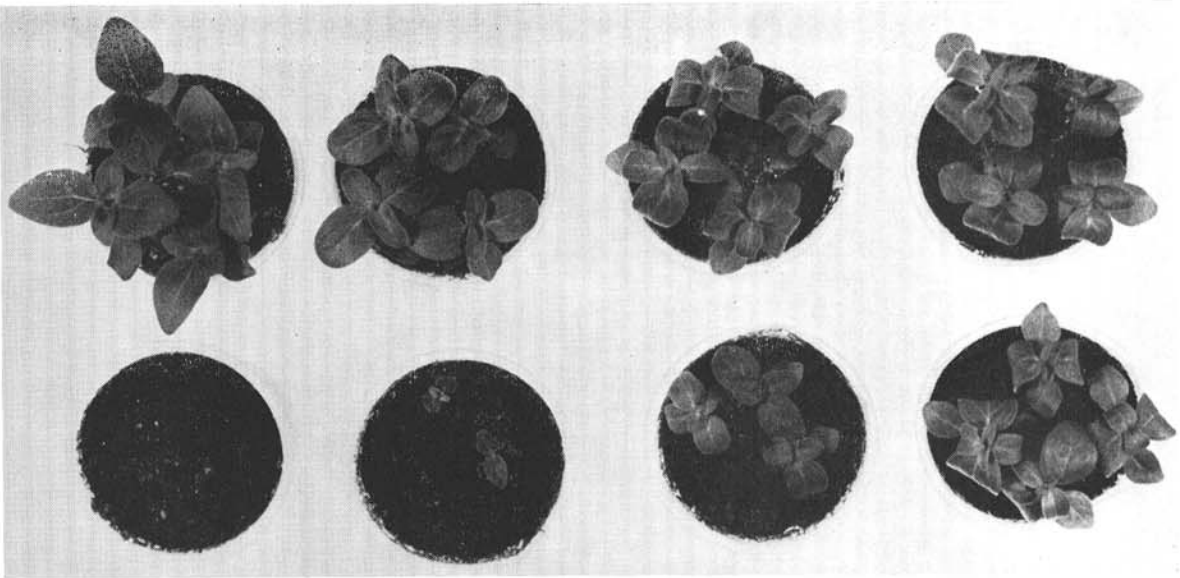

Fig. 1. Effect of age on response of *Antirrhinum majus* seedlings to infection by *Pythium ultimum* 16 days after inoculation. Top row was not inoculated; bottom row was inoculated. Left to right: 10, 15, 20, 25 days after seeding.

flower buds when they were 60 days old. The inoculated plants wilted during a period of hot summer days, but a few days later they recovered. The inoculated plants were either severely or slightly affected. Plants with severe symptoms were stunted, the upper leaves were chlorotic, many lower leaves were dead, and initiation of flower buds was delayed. The tertiary and quaternary roots were severely rotted, and the remaining root system consisted of brown primary and secondary roots. Plants with mild symptoms were slightly stunted, and flower bud initiation was somewhat delayed. It was difficult to determine whether such plants were infected unless they were placed next to a noninoculated plant. However, their root systems were brown and many of the root tips were rotted. Oospores were observed in infected root tips, and the fungus was isolated from all of the inoculated plants. The noninoculated plant had a large root system consisting of white healthy roots.

When the plants were 4 months old, soil dilution experiments similar to those described above showed that there were substantial amounts of inoculum in soils containing infected plants.

The effect of soil temp on infection was studied. Fertilized 25-day-old plants were inoculated, transplanted to 4-inch clay pots 9 days later, and placed in the greenhouse. Noninoculated 34-day-old plants were also transplanted. When the plants were 8 weeks old, they were transplanted to 7-liter plastic pots. Six inoculated

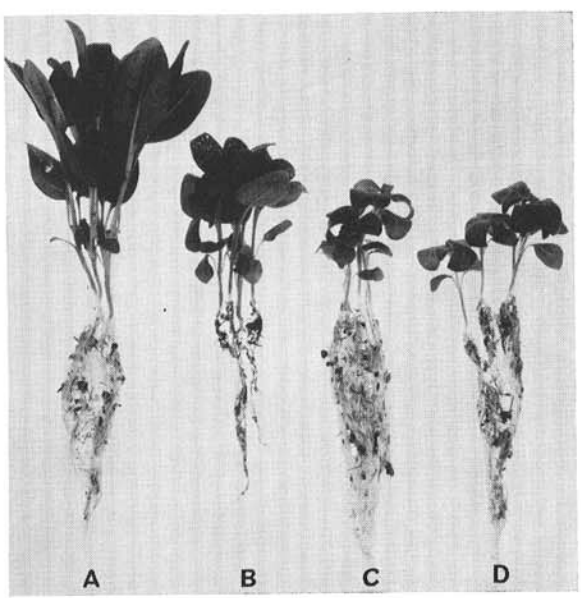

Fig. 2. Effect of fertilization of *Antirrhinum majus* with 0.25 M Hoagland's solution and inoculation with *Pythium ultimum* at age 30 days. Treatments were **A)** fertilized, not inoculated; **B)** fertilized, inoculated; **C)** not fertilized, not inoculated; and **D)** not fertilized, inoculated.

TABLE 2. Effect of age of *Antirrhinum majus* seedling at time of inoculation with *P. ultimum* on incidence of damping-off of peas (*Pisum sativum*) subsequently planted in the soils

% Infested soil in non-sterile U.C. mix	Fertilizer	Emergence count after 9 days: Source of inoculum (days)[a] 10	20	30
16	+	3	1	5
4	+	12	13	6
1	+	40	22	23
0.3	+	40	36	28
16	—	3	4	1
4	—	16	18	11
1	—	24	18	15
0.3	—	53	46	33

[a] Infested soil was obtained by inoculating plants at 10, 20, and 30 days of age. After 12 days, plant remains were removed and the soil diluted. Sixty pea seeds were then planted for each treatment.

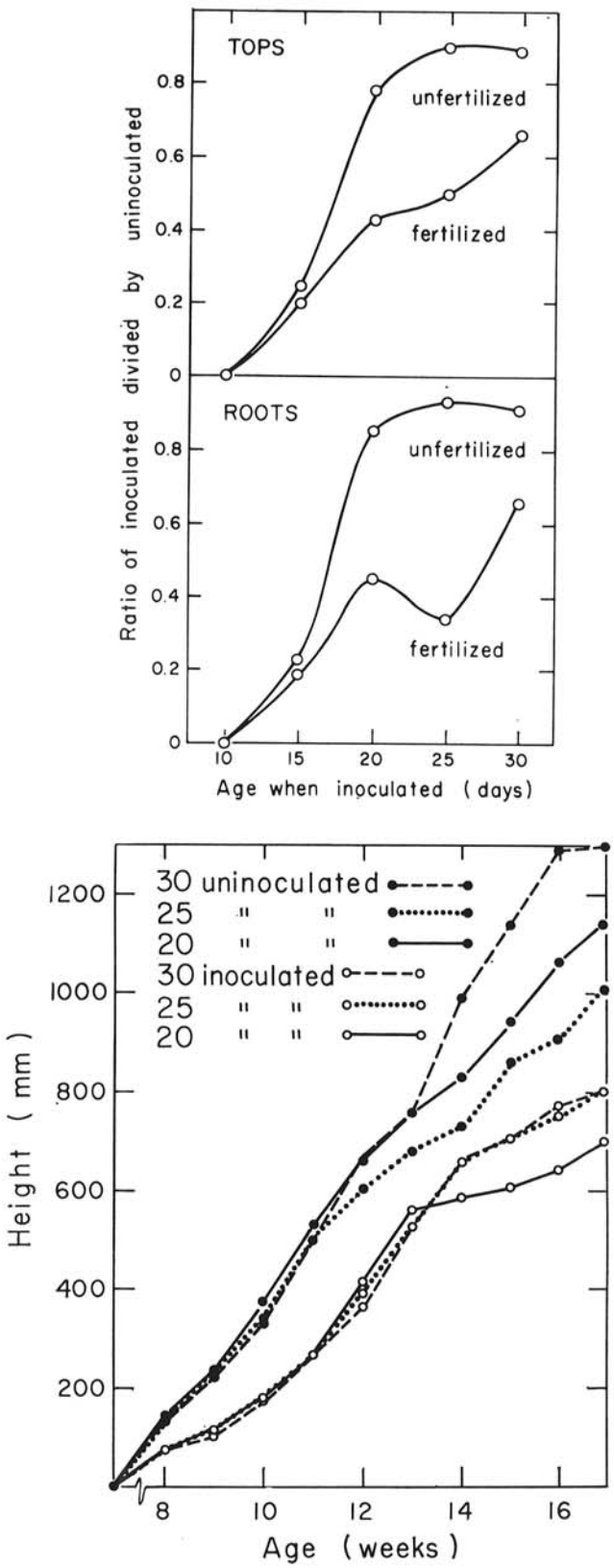

Fig. 3-4. (Above) Effect of age of *Antirrhinum majus* seedlings and fertilization on susceptibility of *Pythium ultimum* 16 days after inoculation. (Below) Effect of infection of *Antirrhinum majus* by *Pythium ultimum* on height of plants grown continuously at soil temp of 20, 25, and 30 C.

and three noninoculated plants were placed in temp tanks held at 20, 25, and 30 C. They were observed daily for 9 weeks and their height was measured weekly. Although the small numbers of plants precluded statistical analysis, certain effects were observed. Inoculated plants were smaller than the noninoculated ones at all three temp (Fig. 4), but none of the inoculated plants wilted. The interrelated effect of temp and *P. ultimum* infection on top growth was not evident until the plants were in the tanks 4 weeks. From this time onward, stunting was most pronounced at 20 C. Roots of inoculated plants were brown and the tips were decayed. After 9 weeks, the fungus was recovered from the roots of all inoculated plants.

In another experiment, seeds were germinated in flats and transplanted weekly to 4-inch clay pots in the greenhouse. When there was a gradation in ages of plants from 3 weeks to well past seed-set, half the plants of each age group were transplanted into gallon cans containing a mixture of *P. ultimum* grown in vermiculite medium and soil, and half were transplanted to noninoculated soil. No difference in susceptibility in relation to age was noted after several weeks. Occasionally during hot weather, infected plants wilted temporarily, but this was not related to the age of the plant. Infected plants gradually developed symptoms described previously.

Thus, snapdragons became tolerant to infection by *P. ultimum* between 15 and 25 days of age; and the tolerance was retained past the seed-set stage of growth. But some infected plants temporarily wilted under heat stress.

Penetration and colonization of susceptible and tolerant seedlings.—Penetration and colonization of the roots of both susceptible and tolerant plants were studied. For in vitro studies, plants were grown in petri plates and inoculated as described. The roots were examined as whole wet mounts after staining for 1 min with neutral red. No zoospores or empty sporangia were observed, and all infection structures observed arose from mycelia. The appressoria formed within 8-10 hr after inoculation, primarily in the regions of elongation and maturation of the root. Occasionally they were formed on the root cap or above the region of maturation, but never on the meristem or the region of differentiation. Colonization of the regions of maturation and elongation was rapid, and several cells were invaded within 4 to 8 hr after the appressoria formed (Fig. 5). Growth typically was intracellular, but intercellular growth occurred, particularly after the tissue was thoroughly colonized. Intracellular mycelium usually formed thick, appressoriumlike structures before penetrating cell walls, and the mycelium passing through the cell was usually constricted. Growth into the regions of differentiation and the meristem was delayed and appeared to depend on death of the tissues before the mycelium penetrated them. The fungus grew rapidly into the stem and leaves; the seedling usually collapsed within 24 hr after initial penetration.

Infected susceptible plants from the above experiments were transferred to deionized water in petri dishes in the laboratory, and observed daily. In two experiments, few oospores were formed in the roots, even after 10 days, but many were formed in the stems and leaves (Fig. 5). In subsequent experiments, oospores were as abundant in the root tissue as in the stems and leaves.

Because we could not grow healthy 25- to 30-day-old plants aseptically for studies of penetration and colonization of tolerant plants, the seedlings were grown in soil in the growth chamber. The roots were washed carefully, and whole plants were placed in deionized water in petri dishes, inoculated 24-48 hr later, and the infection process was observed.

Penetration of the roots of the tolerant plants was essentially the same as that of the susceptible ones. Although appressoria were formed mainly in the regions of elongation and maturation of the root tips of the tertiary and quaternary roots, some also formed on the mature portions of the primary and secondary roots. Colonization of the tertiary and quaternary roots was rapid, and the fungus easily penetrated the cortex of these roots. However, colonization of the cortex of primary and secondary roots was much slower. When appressoria formed on mature portions of the primary and secondary roots, the mycelium colonized only 6 to 8 cells in 24 hr. The tissue below these appressoria became brown about 24-48 hr later. Fungus growth did not stop completely, but the plants were still alive 6 to 7 days after inoculation, indicating extreme resistance of mature root tissues to infection.

Occasionally, lateral roots grew through infected cortical tissue, but they were not invaded until the region of elongation of the root tips had formed. This was additional evidence that the live meristem and region of differentiation in the root tip were resistant to infection. Oospores were formed in infected tissue 4-6 days after inoculation (Fig. 5).

Susceptible and tolerant plants were grown in soil in the growth chamber, inoculated with *P. ultimum,* and examined periodically. The infection of susceptible seedlings was similar to that observed in vitro. It was not determined whether appressoria originated from hyphae or from zoospores. Oospores were difficult to find because infected tissue was difficult to recover intact from soil; they were observed in the occasional tissue that was retrieved.

Penetration of tolerant plants was essentially the same as observed in vitro, but the growth of the fungus stopped completely in the cortex of the mature portions of the primary and secondary roots. When appressoria were formed on mature portions of the primary and secondary roots, the hyphae penetrated the cells to depths of only a few cells. The cells in this infected area rapidly turned brown and formed lesions mentioned earlier. Oospores were observed when infected tissue could be retrieved from soil.

Histochemical determination of lignin in root tissue.—Sections of areas taken from root tips to mature regions of primary, secondary, tertiary, and quaternary roots from each age group were examined to determine whether the presence of lignified tissues was correlated with resistance of tissue to infection by *P. ultimum.*

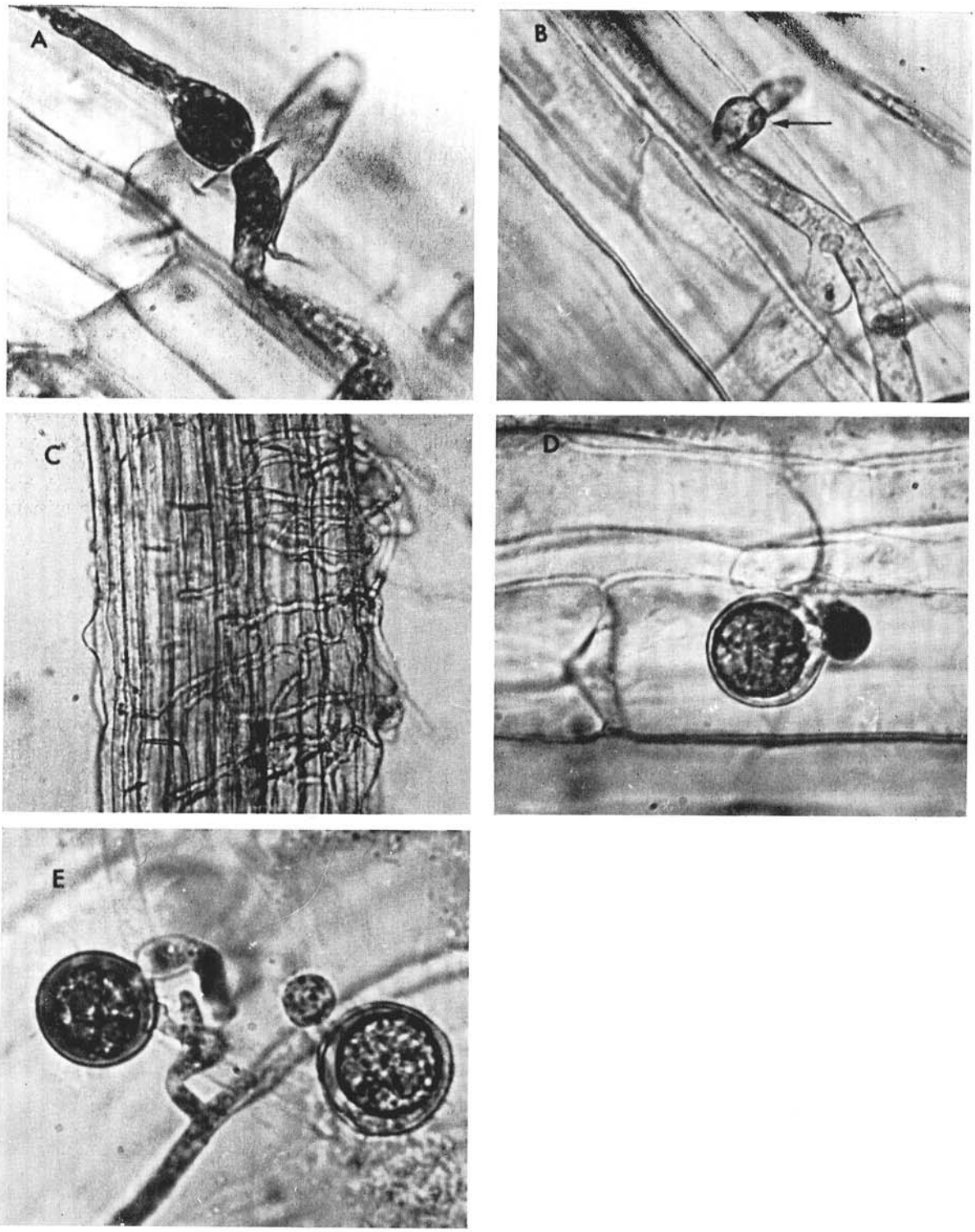

Fig. 5. Penetration and colonization of roots of *Antirrhinum majus* by *Pythium ultimum*. **A)** Appressorium on root hair in region of maturation. **B)** Intracellular mycelium in region of maturation. Note thick, appressoriumlike structure and constriction in mycelium passing through cell wall. **C)** Colonization of region of elongation 6 hr after initial penetration. **D)** Sexual structures in infected stem of susceptible plant. **E)** Sexual structures in roots of tolerant plants.

Lignin was found in the endodermis, vessels, and other tissues centripetal to the endodermis, but not in the cortex of the susceptible or the tolerant plants. In observations previously described, hyphal growth of *P. ultimum* was restricted in cortical tissues of tolerant plants. In contrast, hyphal growth was unrestricted in roots of susceptible plants. Thus, resistance to *P. ultimum* was not correlated with the presence of lignin in the infected tissues.

DISCUSSION.—The greater resistance of older seedlings to attacks by root-inhibiting pathogens such as *P. ultimum* raises an interesting question. How much of the resistance is attributable to anatomical aspects of root growth and how much to physiologic aspects? The system described herein between snapdragon and *Pythium* appears adequate to answer this question, since tolerance to infection and colonization developed sharply between the 15th and 25th day after sowing. Tolerance probably is not directly related to anatomical features of the root, but more probably to other factors.

Our observations indicated that tolerance of roots of older plants to *Pythium* infections was due to a postpenetration host-parasite interaction. In susceptible seedlings, the fungus rapidly colonized the entire root system, where it formed oospores in infected tissue and caused the plants to collapse and die. In tolerant plants, the fungus colonized only root tips of tertiary and quaternary roots but was unable to colonize the mature portions of primary and secondary roots. When the fungus formed appressoria on mature portions of primary and secondary roots and penetrated them directly, it invaded only a few cells that later turned brown. Similar small lesions also were reported by Klisiewicz (12) in safflower. If the fungus grew into the cells of the mature portions of the primary and secondary roots, hyphal growth first slowed and then stopped, and cortical cells of the root adjacent to the mycelium turned brown. Generally, this host reaction would be expected to have an adverse effect on the fungus but the mycelium in this tissue appeared healthy. In fact, oospores formed in infected tissues in a few days. The postpenetration behavior of the pathogen in the tolerant plants was similar to that reported in *Zea* and *Allium* (9, 10).

Thus, tolerance of older plants was due to resistance of mature portions of primary and secondary roots to colonization by *P. ultimum*. Although a browning reaction was observed, the mycelium did not collapse and apparently was not killed. It appears that the fungus lost its ability to colonize these tissues, stopped growing, and formed reproductive structures.

In the past, differences in susceptibility of plants to *Pythium* in relation to age has generally been assumed to be due to tissue maturation associated with thickening and hardening of cell walls (14). This assumption implies that lignification of cell walls is involved in resistance, but we found no evidence in the literature to substantiate this implication. Our work showed that tolerance of snapdragon roots to *P. ultimum* was not related to lignification of cell walls. The presence of lignin was not correlated with age, type of tissue invaded, or resistance. It can, however, be argued that the phloroglucinol test used was not quantitative, and quantitative differences may be involved. This is true, but we found that only the tissue centripetal to the endodermis of the root was lignified. Therefore, this pattern of lignification can account only for restriction of radial invasion by *P. ultimum*. Since longitudinal and tangential movement were also restricted, lignification cannot account for resistance. Griffey & Leach (5) made similar observations and conclusions based upon observations of restricted lesions in bean hypocotyls infected with *Colletotrichum lindemuthianum*. Lignified xylem tissue restricted radial invasion, but could not account for longitudinal and tangential restriction in the cortex.

The fact that hyphae apparently were not killed in the few infected cortical cells of resistant roots was significant because it suggests that if inhibitory factors are produced by the host they are not fungitoxic. Since the fungus produces oospores in infected cortical cells, the conditions necessary for oospore production must exist in the infected cortical cells. Conversely, the conditions favoring continued parasitic activity were apparently absent. So far as we were able to determine, the mycelia in the resistant cortical cells in contact with the host cell walls failed to form either appressorialike swellings or infection pegs.

English & Albersheim (3) showed that changes in host cell walls with age may be involved in determining the amounts of hemicellulose degrading enzymes produced by *C. lindemuthianum,* and Bateman et al. (2) showed a similar phenomenon for hemicellulose and pectin degrading enzymes produced by *Rhizoctonia solani.* Since a similar phenomenon may play a role in snapdragon, this possibility was investigated with respect to pectic enzymes and has been reported (17).

The increase in root rot following fertilization is probably related to the increase in infective inoculum of *Pythium*. Abundant organic matter in the form of peat moss in the U.C. mix may provide substrates for *Pythium* colonization that are enriched nutritionally by fertilization.

More severe root rot at a soil temp of 25 C may be related to a greater growth velocity of *Pythium ultimum* in conjunction with a depressed growth rate of snapdragon.

LITERATURE CITED

1. BAKER, K. F. [ed.]. 1957. The U.C. system for producing healthy container-grown plants. Calif. Agr. Exp. Sta. Ext. Manual 23. 332 p.
2. BATEMAN, D. F., H. D. VAN ETTEN, P. D. ENGLISH, D. J. NIVENS, & P. ALBERSHEIM. 1969. Susceptibility to enzymatic degradation of cell walls from bean plants resistant and susceptible to *Rhizoctonia solani* Kühn. Plant Physiol. 44:641-648.
3. ENGLISH, P. D., & P. ALBERSHEIM. 1969. Host-pathogen interactions: I. A correlation between β-galactosidase production and virulence. Plant Physiol. 44: 217-224.
4. FLENTJE, N. T. 1959. The physiology of penetration and infection. *In* C. S. Holton [ed.] Plant Pathology Problems & Progress. Univ. Wis. Press. p. 76-87.
5. GRIFFEY, R. T., & J. G. LEACH. 1965. The influence of age of tissue on the development of bean anthracnose lesions. Phytopathology 55:915-918.

6. HALPIN, J. E., & E. W. HANSON. 1958. Effect of age of seedling of alfalfa, red clover, ladino white clover and sweet clover on susceptibility to *Pythium*. Phytopathology 48:481-485.
7. HAWKER, L. E., R. W. HARRISON, V. O. NICHOLLS, & A. M. HAM. 1957. Studies on vesicular-arbuscular endophytes. I. A strain of *Pythium ultimum* Trow in roots of *Allium ursinum* L. and other plants. Brit. Mycol. Soc. Trans. 40:375-390.
8. HAWKINS, L. A., & R. B. HARVEY. 1919. Physiological study of the parasitism of *Pythium debaryanum* Hesse on the potato tuber. J. Agr. Res. 18:275-298.
9. HOAGLAND, D. R., & D. I. ARNON. 1959. The water-culture method for growing plants without soil. Calif. Agr. Exp. Sta. Cir. 347. 32 p.
10. HOPPE, P. E. 1949. Differences in *Pythium* injury to corn seedlings at high and low soil temperatures. Phytopathology 39:77-84.
11. JENSEN, W. A. 1962. Botanical histochemistry. W. H. Freeman Co., San Francisco. 408 p.
12. KLISIEWICZ, J. M. 1968. Relation of *Pythium* spp. to root rot and damping-off of safflower. Phytopathology 58:1384-1386.
13. MCKEEN, C. D., & H. J. THORPE. 1968. A *Pythium* root rot of muskmelon. Can. J. Bot. 46:1165-1171.
14. MCNEW, G. L. 1960. The nature, origin and evolution of parasitism, p. 19-69. *In* J. Horsfall & A. E. Dimond [ed.]. Plant Pathology, An advanced treatise 1: Academic Press, N. Y. 715 p.
15. MELLANO, H. M. 1969. Mechanism of tolerance of *Antirrhinum majus* seedlings to infection by *Pythium ultimum* in relation to host sterols and age of tissue. Ph.D. Thesis, Univ. Calif., Riverside. 109 p.
16. MELLANO, H. M., R. M. ENDO, & D. E. MUNNECKE. 1967. Changes in response of *Antirrhinum majus* to infection by *Pythium ultimum* in relation to age and tissue infected. Phytopathology 57:1007 (Abstr.).
17. MELLANO, H. M., D. E. MUNNECKE, & J. J. SIMS. 1970. Seedling resistance. Relationship of pectic enzyme activity and presence of sterols to pathogenicity of *Pythium ultimum* on roots of *Antirrhinum majus*. Phytopathology 60:943-950.
18. RICHARDSON, L. T., & D. E. MUNNECKE. 1964. Effective fungicide dosage in relation to inoculum concentration in soil. Can. J. Bot. 42:301-306.
19. TOMPKINS, C. M., & J. T. MIDDLETON. 1950. Etiology and control of *Poinsettia* root and stem rot caused by *Pythium* spp. and *Rhizoctonia solani*. Hilgardia 20:171-182.
20. VANTERPOOL, T. C. 1952. The phenomenal decline of browning root rot (*Pythium* spp.) on the Canadian prairies. Sci. Agr. 32:443-452.