Infection of Avocado and Other Species of Persea by Phytophthora cinnamomi

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ABSTRACT

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Zoospores of A^1 (Pc97) and A^2 (Pc40) mating types of *Phytophthora cinnamomi* were attracted to roots of avocado (*Persea americana*), *P. indica*, *P. borbonia*, *P. pachypoda*, camellia, and mandarin orange. Zoospores encysted rapidly on the root surfaces, most commonly just behind the root tip, on cut ends and around wound sites. Cyst germination was stimulated by the presence of roots; germ tubes extended from the side of cysts closest to the root and then grew toward it. Repeated emergence of zoospores was not observed. Germ tubes of Pc40 and Pc97 penetrated the root epidermis and colonized the cortical tissues of both resistant and susceptible plants with no difference in the mode of penetration and post-penetration development of the fungus. Hyphal swellings (vesicles) and chlamydospores were produced by both mating types in the cortex, but oogonia with oospores were found only in Pc40. No anatomical differences were found to account for host resistance. *Persea americana*, *P. indica*, and to some extent, very young seedlings of *P. borbonia* were susceptible to Pc40 and Pc97 whereas *P. pachypoda* was resistant. Pc40 was consistently more virulent than Pc97.

Additional key words: zoospore behavior, scanning electron microscopy, development of root rot.

Phytophthora cinnamomi Rands, first discovered in Sumatra and identified by Rands (21) in 1922 to be the causal agent of bark canker of cinnamon (*Cinnamomum burmanni* Bl.), is now recognized as one of the most devastating and cosmopolitan species of *Phytophthora* that attacks a wide variety of host plants throughout the world (37). In southern California, this fungus causes severe root rot of avocado (*Persea americana* Mill.) (35). Since its description in 1942 (27) the disease has remained the principal problem of avocado industry in California and other areas of the world (33).

Although much is known about the pathogen as well as the disease (33), little is known about the infection of avocado roots by the pathogen. Zentmyer (31) described zoospore chemotaxis to avocado roots and the subsequent chemotropic behavior of germ tubes toward the root surface, but the infection of plant roots at the penetration and post-penetration stages has not been investigated. Furthermore, the discovery of two mating types within *P. cinnamomi* (5, 23), each usually associated with different host plants, has raised the question of differential zoospore behavior of A^1 and A^2 strains on roots of different plant species. Thus, the object of this study was to re-examine the zoospore behavior of *P. cinnamomi* on plant roots and to study pathogenesis.

MATERIALS AND METHODS

Two compatible strains of *P. cinnamomi* were selected for comparative studies: Pc97 (A^1) and Pc40 (A^2) both isolated by G. A. Zentmyer from roots of camellia nursery stock at Fallbrook and a mature avocado tree in Santa Barbara County, California, respectively.

For chemotaxis experiments, zoospores of Pc40 and Pc97 were obtained and suspended in sterile deionized distilled water by the method of Chen and Zentmyer (3) with slight modification (11). Plant roots used were from seedlings of Topa Topa cultivar of avocado (*Persea americana* Mill.) and *P. indica* (L.) Spreng., both susceptible in nature to A^2 isolates; *P. pachypoda* and *P. borbonia* (L.) Spreng., resistant to A^2 isolates by artificial inoculation; Princess Bacciochine variety of camellia (*Camellia japonica* L.), susceptible to A^1 isolates in the nursery; and mandarin orange (*Citrus reticulata* Bl.), a nonhost of A^1 and A^2 isolates (33, 37). The mandarin orange seedlings were 4 mo old, *P. pachypoda* 5 mo old, camellia 16 mo old and the other seedlings were 1 to 3 mo old, and were grown either in sand or UC mix (1). Just prior to inoculation, seedlings were removed from the growth medium and placed in a beaker of distilled water. A piece of root, 1 to 2 cm long, was cut off, rinsed in tap water, then in distilled water, and immediately placed in a zoospore suspension in a petri dish. Zoospore responses could be observed with a dissecting microscope without further disturbance of the root or the zoospores. Only zoospore suspensions with abundant, active zoospores, obtained within 3 to 4 hr after the chilling of sporangia (3) were used. For comparative studies, roots from different plant species were placed side by side, in a 9-cm diameter petri dish, separated only by small glass rods.

To follow the development of the fungus, the roots were left in zoospore suspensions for 24 to 48 hr or longer, up to 11 days. They were examined for lesions and sectioned fresh with a Hooker Plant Microtome, Lab Line Instrument, Inc., Melrose Park, IL 61103 (provided

through the courtesy of R. M. Endo). Sections were stained in 0.01% lactophenol/cotton blue. Infected roots also were transferred to 6-cm diameter petri dishes containing a mixture of concentrated hydrochloric acid and 70% ethyl alcohol (1:3, v/v), and placed in a 50 C oven until the roots became soft. They then were washed thoroughly with distilled water and transferred to 1% ammonium oxalate solution. The treated roots were stained for 5 min in 1% nigrosin, rinsed well in 1% picric acid and mounted in 1% lactophenol. With a little pressure on the cover glass, the root tissues separated readily and the darkly stained fungus could be detected easily. Fresh root sections and macerated tissues were thus prepared at the end of 1, 2, 4, 6, 8, and 11 days.

To test for pathogenicity of Pc40 and Pc97, the isolates were cultured singly in 50 ml of cleared V-8 broth in 250-ml flasks at 25 C. Two-wk-old mycelia from four flasks were blended 2 min in a Waring Blendor and diluted with distilled water to 3 liters. The mycelial suspension then was added to saturate the soil (UC mix) in which the plants were growing, until excess liquid drained from the bottom of the 13.4 cm diameter pots. Six to 10 seedlings of each species were used. Pots were watered regularly to keep the soil moist. After 4 mo in the greenhouse, the general health of the plants was noted; they were removed from the pots, washed with tap water, and examined for root rot. Portions of the rotted roots were treated with macerating agents described previously, stained, squashed on glass slides, and examined for fungal structures of *P. cinnamomi*.

To examine cysts on the root surface with a scanning electron microscope, avocado roots were immersed for 1 hr in a diluted zoospore suspension of Pc40 or Pc97, fixed in 2% aqueous nonbuffered glutaraldehyde solution overnight at 5 C, then washed with distilled water. The water content within the specimens was partially replaced by transferring the specimen daily to an increasing concentration of glycerine–10%, 20%, 30%, 40%, and 50%. Tissues thus infiltrated with 50% glycerine were blotted dry, and a small piece of the root tip was removed with a sharp razor blade and placed onto "double stick" cellophane tape mounted on a scanning electron microscopy (SEM) specimen mount. The specimen was immediately coated uniformly with gold vapor and examined with a Jeolco Model SEM JC3 scanning electron microscope at a beam voltage of 15 kV.

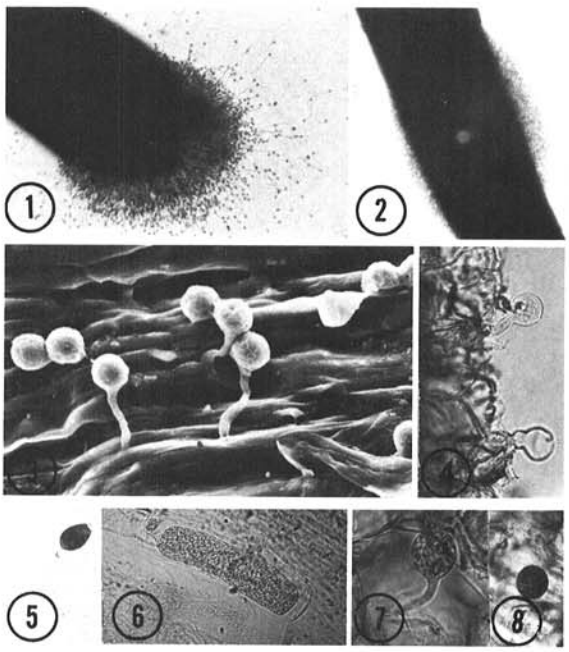

Fig.-(1 to 8). (1-2) Accumulation of zoospores of *Phytophthora cinnamomi* (isolate Pc97) on avocado root. **1)** on cut end and **2)** around wound site (× 120). **3)** Scanning electron micrograph of cysts of *P. cinnamomi* (isolate Pc40) on avocado root (× 800). **4)** Penetration of root surface of *Persea indica* by germ tubes from cysts of *P. cinnamomi* (isolate Pc40) (× 320). **5)** Microsporangium of *P. cinnamomi* (isolate Pc40) on avocado root (×500). **6)** Hyphal vesicle of *P. cinnamomi* (isolate Pc40) in *Persea indica* root tissue (×320). **7)** Hyphal vesicle of *P. cinnamomi* (isolate Pc97) in *Persea borbonia* root tissues (×320). **8)** Chlamydospore of *P. cinnamomi* in avocado root tissue 4 mo after inoculation with isolate Pc40 (×250).

RESULTS

Behavior of zoospores toward plant roots.—Zoospores of both mating types suspended in sterile deionized distilled water were similarly attracted towards cut roots of avocado. Rapid cyst accumulation was greatest in the elongation region immediately behind the root tip. The zoospore responses were similar to those described by Zentmyer (31) with zoospores of an A^2 isolate in nonsterile soil extract. Additionally, zoospores of Pc40 and Pc97 were strongly attracted to cut ends of avocado roots and wound sites inflicted by a small needle (Fig. 1, 2). Although encysted zoospores of *P. cinnamomi* germinate either directly or indirectly in distilled water (11), cysts on or near the root surface germinated by germ tubes oriented towards the root surface. Repeated emergence was not observed. Cyst germination was approximately 100%, in contrast to 40-80% germination in distilled water. There was no apparent difference in the accumulation of zoospores of the two mating types on excised roots of *P. americana*, *P. indica*, *P. borbonia*, *P. pachypoda*, camellia, and mandarin orange, although in mandarin orange, cysts tended to be confined to a small elongation region immediately behind the apex of root tip.

Development of root rot.—Within 24 hr after roots were immersed in zoospore suspensions of Pc40 or Pc97, brown lesions appeared on the roots of avocado and *P. indica*. Lesions formed first in the region of elongation but rapidly extended throughout the entire root segment. Lesions also were observed on roots of *P. borbonia* and *P. pachypoda*, but usually were smaller and more confined to the root tip, whereas in camellia, the lesions were localized as random spots along the length of the root piece. No lesions were observed on roots of mandarin orange.

Observations with SEM revealed that within 1 hr after zoospores accumulated on the root surface, germ tubes of cysts already had penetrated the epidermis (Fig. 3). There was no apparent difference in the mode of penetration by germ tubes of Pc40 and Pc97 cysts.

These findings were substantiated by light microscopy of fresh root sections. Cysts of the two mating types germinated and penetrated roots of avocado, *P. indica*, *P. pachypoda*, and *P. borbonia* (Fig. 4); within 48 hr intercellular hyphae in all cases, and sometimes intracellular hyphae, were found, mainly in the cortex. At the same time, on the surface of the roots, especially those of avocado and *P. indica*, numerous microsporangia of a size only slightly greater than or the same as a cyst (Fig. 5) were evident. Some of these apparently arose from germ tubes of cysts, whereas others were produced by hyphae growing from the root. Within 4 to 6 days, hyphal swellings or vesicles were abundant within cortical tissues of plant roots (Fig. 6, 7). Chlamydospores were observed, but oogonia with oospores were not found until 6 to 11 days and were produced only by Pc40.

Pathogenicity of the two mating types to plant roots.—After 4 mo in the greenhouse, root rot of test plants was classified into 10 groups based on the mean percentage of roots rotted (36). The results are summarized in Table 1.

Control plants were generally healthy, whereas plants infected with *P. cinnamomi* had foliage symptoms in addition to root rot. Avocado plants infected with Pc40 were stunted and had some leaves that were chlorotic and brown-spotted. Stem cankers were common in *P. indica* infected with Pc40 or Pc97; in the former case, some seedlings wilted and died. Leaves of *P. borbonia* infected with Pc40 were pale green, which contrasted strongly with the dark green color of control seedlings. Inoculated plants of *P. pachypoda* were slightly stunted compared with the control. *Phytophthora cinnamomi* was re-isolated from rotted roots of all *Persea* spp. except *P. pachypoda*. The compatibility of the isolates was determined and in all cases was found to match the mating type of the respective inocula.

Examination of macerated, rotted roots confirmed the presence of hyphal swellings or vesicles, chlamydospores (Fig. 8) and in the case of Pc40, a few oogonia with oospores were observed. The only apparent difference between Pc40 and Pc97 at the post-penetration stage was that oospores were never found associated with inoculations with Pc97. Isolate Pc40 was distinctly more virulent to avocado, *P. indica*, and *P. borbonia* than Pc97 (Table 1).

DISCUSSION

Galindo and Zentmyer (5) noted that the H-1 isolate (A^1) from Hawaii was less virulent to avocado than A^2 isolates from California. Recently, Shepherd et al. (24) found that both compatibility types of Australian isolates were equally pathogenic to lupine and *Pinus radiata* seedlings. Weste (28) demonstrated that an A^1 Australian isolate was "more pathogenic" than an A^2 isolate in causing root rot and subsequently death of seedlings of *Nothofagus cunninghamii*.

TABLE 1. Disease Index[a] of avocado and other *Persea* spp. infected with two mating types (A^1 and A^2) of *Phytophthora cinnamomi*

Plant	Pc40 (A^2) (%)	Pc97 (A^1) (%)	Control (%)
Avocado (Topa Topa)	81-90	41-50	0-10
Persea indica	91-100	81-90	0-10
Persea borbonia	41-50	21-30	0-10
Persea pachypoda	11-20	11-20	0-10

[a]Classified into 10 groups based on the mean percentage of roots rotted (0-10%, 11-20%, 21-30%, 31-40%, 41-50%, 51-60%, 61-70%, 71-80%, 81-90%, 91-100%).

Our present study confirmed the earlier findings of Galindo and Zentmyer (5). Isolate Pc40 (A^2) was more virulent to avocado, *Persea indica* and *P. borbonia* than was Pc97 (A^1). Although *P. borbonia* has shown high resistance to root rot (32, 33), the young seedlings (1 mo old) used in this work were moderately susceptible. Presumably, seedling age might be an important factor because seedlings used in other tests (Zentmyer, *unpublished*) were considerably older (3 to 24 mo old). Mellano et al. (15) demonstrated that 15-day-old or younger seedlings of snapdragon were susceptible to *Pythium ultimum* whereas 25-day-old or older plants were resistant. Resistance of *P. pachypoda* seedlings (5 mo old) to both Pc40 and Pc97 was confirmed by low percentage of rotted roots. Since both mating types were pathogenic, it is possible that more serious problems could result, involving a wider host range, if the other mating type were introduced into an area where previously only one type was present. For instance, although the A^2 type of *P. cinnamomi* is commonly found in southern California, A^1 types have been isolated from camellia in nurseries, and on one occasion from avocado roots (34). Should the A^1 type become widespread in the field, the avocado root rot problem could be aggravated.

Although Pc40 and Pc97 displayed differential virulence to avocado and other species of *Persea*, no apparent difference was observed in zoospore behavior towards plant roots. Zoospores were similarly attracted to the roots of resistant or susceptible plants. The significance of zoospore accumulation on plant roots in root diseases has been reviewed by Hickman and Ho (8) and Hickman (7). As with zoospores of other Phycomycetes, zoospores of Pc40 or Pc97 encysted rapidly on the root surface and germinated by germ tubes that grew towards and penetrated the root surface. "Repeated emergence" of zoospores as described for *P. cinnamomi* (11) was apparently suppressed in favor of germ tube formation. This represents another interesting aspect of the morphogenic effect of root exudates on cyst germination (9, 10).

The production of numerous microsporangia on plant roots infected with zoospores of the two mating types is also of special interest. When roots infected with *P. cinnamomi* or other *Phytophthora* spp. are submerged in water or other liquids, abundant sporangia are produced (20, 29), but these sporangia are usually of normal size. In the present study, however, in addition to some normal forms, many sporangia were no larger than the cysts. We do not know what factors contributed to this phenomenon. Possibly, germ tubes of cysts which failed to reach the root surface were terminated by miniature sporangia. Such microsporangia have been found infrequently in *P. cinnamomi* in distilled water.

The direct penetration of the root epidermis by germ tubes of Pc40 and Pc97 was similar to the previous reports on *Phytophthora* (6, 14, 16, 17, 22, 26) and *Pythium* (4,

13, 17, 19, 25). Since no major differences in root structure of *P. pachypoda* and *P. borbonia* were observed before and after penetration of roots by the pathogen, the resistance of these two species probably is due to a post-penetration host-parasite interaction (2, 12). Similarly, Milholland (16) found no anatomical differences between hosts of the highbush and rabbiteye cultivars of blueberry which are susceptible and moderately resistant, respectively, to *P. cinnamomi*. A likely explanation for the resistance of *P. borbonia* and other species of *Persea* to *P. cinnamomi* had been proposed by Zaki et al. who isolated a fungitoxic chemical (borbonol) from the resistant species of *Persea* (30).

Mircetich and Zentmyer (18) reported oospores and chlamydospores in naturally infected avocado root tissues and suggested that these structures play an important role in the survival of the pathogen in soil. However, Milholland (16) found no oospores and only two chlamydospores in the studies of inoculated and naturally infected blueberry roots. In our present investigation, chlamydospores were found in roots inoculated with Pc40 or Pc97 and a few oospores were found in roots inoculated with the A^2 mating type, Pc40. Thus, our findings support the thesis of Mircetich and Zentmyer (18).

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