California Avocado Society 1994 Yearbook 78:111-120

Effect of Root Infection by *Phytophthora citricola* on Avocado Root Rot Caused by *Phytophthora cinnamomi*

Z. A. El-Hamalawi and J. A. Menge
Department of Plant Pathology, University of California, Riverside , CA 92521

Abstract

Root systems of *Persea indica* (1, 2, 5, and 8-mo-old) and *Persea americana* (2 and 8-mo-old) were inoculated with macerated mycelia of *Phytophthora citricola* and challenged 48 hr later with zoospores *of Phytophthora cinnamomi.* Partial protection against the root rot disease caused by *P. cinnamomi* was induced by the prior inoculation of the avocado seedlings with *P. citricola.* Simultaneous inoculation of the root systems of the plants with both pathogens resulted in less protection against root rot caused by *P. cinnamomi.* The ability of *P. citricola* to induce resistance to *P. cinnamomi* by prior inoculation with *P. citricola* was greater in the case of 5- and 8-mo-old plants compared to 1- and 2-mo old seedlings *of Persea americana* and *Persea indica.*

Introduction

Avocado root rot caused by *Phytophthora cinnamomi* Rands is the principal disease of avocado in California as well as many areas of the world (13, 17). The disease affects about 60-70% of the acreage in southern California alone and is responsible for almost 30% losses in avocado production (3). Avocado stem canker caused by *Phytophthora citricola* Sawada is the second major disease of avocado throughout southern California (18, 4). Even though *P. citricola* causes its greatest losses by producing cankers on avocado stems and crowns, it is also capable of infecting the roots, but with far less root damage than *P. cinnamomi* (7).

We observed in the field that the severity of symptoms of root rot caused by P. *cinnamomi* varied from one avocado grove to another even though they had similar inoculum densities of P. *cinnamomi* in the soil. It appeared that areas with low symptom expression were infested also with P. *citricola.* Dolan *et al.* (1986) showed that the exposure of *Persea indica* L. and *Persea americana* Miller to *Phytophthora parasitica* infested soil prior to inoculation with *P. cinnamomi* reduced root infection by *P. cinnamomi* compared to controls. The exposure *of Persea indica* to either *Phytophthora capsici, Phytophthora parasitica,* or *Phytophthora parasitica* var *nicotiana* resulted in only slight root infection but reduced the amount of disease caused by *P. citricola* on stems by 65%, 48% and 57%, respectively (5). Partial protection against *Fusarium* and *Verticillium* wilts induced by prior inoculation with pathogenic and non-pathogenic fungi has been demonstrated in tomato and melon (9). The induced resistance of maize to

Helminthosporium carbonum race 1 by prior inoculation with race 2 was consistently associated with the production of compounds which inhibited infection (1).

The objective of this study was to evaluate the effect of avocado root infection by *Phytophthora citricola* on the severity of the disease incited *by Phytophthora cinnamomi.*

Materials and Methods

Plant material. Avocado seedlings of *Persea americana* cv. Topa Topa were grown from seed in the greenhouse at 24°+ 2°C. The seeds were individually planted in 0.5-L plastic sleeves with perforated bases for drainage. The sleeves were filled with UC-mix #4 (10). Plants of *Persea indica* were grown in the greenhouse from seed in flats containing. The seedlings were watered with dilute (14%) Hoagland's solution (16) as needed.

Root inoculation experiments. One-month-old Persea *indica* seedlings were carefully removed from the flats by flooding prior to removal. Forty *Persea indica* seedlings were placed in 250 ml styrofoam cups (5 seedlings per cup) containing 90 ml water and 10 ml of macerated *P. citricola* mycelium. One 7-day old V8C culture plate of *P. citricola* was lightly macerated in 100 ml sterilized water for 25 sec. using a Sorvall Omni mixer. *Persea indica* stems were kept higher than the inoculum level so they would not become girdled by *P. citricola* and die rapidly. The roots of the seedlings were kept in the inoculum suspension at 24°C on a bench top in the greenhouse for 48 hours. The infection of the avocado roots by *P. citricola* after inoculation was confirmed by the recovery of *P. citricola* on *Phytophthora* selective PARPH medium using the revised key of Stamps *et al.* (1990). Twenty of the seedlings were kept in the *P. citricola* inoculum for the duration of the experiment and the other 20 seedlings were removed from the mycelial suspension and rinsed with deionized water and transferred to another set of styrofoam cups containing 100 ml of *P. cinnamomi* inoculum (~100 zoospores /ml) (2). A set of 20 seedlings was inoculated with *P. cinnamomi* alone, and another set of 20 seedlings was kept in a sterile deionized water in absence of any inoculum. For simultaneous inoculation with both *P. citricola* and *P. cinnamomi,* 20 seedlings were placed in cups containing a mixture of both inocula. At 5 day intervals, seedlings were scored for percent wilting or mortality. All experiments were repeated once and the data were combined for both experiments.

Similar experiments were conducted using 5-months-old *P. indica* seedlings. The inoculation of the seedlings was done in 1-L beakers using 25 ml of the macerate *P. citricola* mycelium in 300 ml water, just enough to cover the roots of the plants without contacting the stem. Experiments were repeated once.

For a third experiment, coarsely ground millet seeds (100 g in 500 ml flask) were autoclaved for 1 hour, infested with three V8C culture plugs (10 mm diam.) of either *P. citricola* or *P. cinnamomi,* soaked in 50 ml sterile water, and incubated for 8-10 days at 24°C. The inoculated millet was mixed with UC-mix #4 (4% v/v) and the soil was maintained at about 15% moisture at 24°C. After 5-7 days, the population density of the

fungus in the mix was determined by the dilution-plate technique on *Phytophthora* selective PARPH media (12).

The infested potting mix was diluted with non-infested sterile UC soil mix #4 to give about 30-40 colony-forming units (cfu) of *Phytophthora* per gram dry soil mix.

Two-month-old seedlings of P. *indica* and *P. americana* were potted with their root balls in 4-L pots containing UC-mix infested with *P. citricola* as described above. Plants were kept in the greenhouse at ~2°C for 2 days. Twenty plants were kept in the *P. citricola* infested soil mix throughout the duration of the experiment and twenty plants were removed from the soil and transplanted into 4-L pots containing UC-mix infested with *P. cinnamomi.* Before transplanting, samples of the root segments were plated on PARPH agar medium to verify the presence of P. *citricola.* Another group of 20 plants was potted with their root balls in 4-L pots containing UC-mix infested with *P. cinnamomi* and a group of 20 control plants was grown in UC-mix free of inoculum. Experiments were repeated once and the data were combined.

Another greenhouse experiment similar to the above was carried out using 8-months-old plants of *P. americana* and *P. indica* grown in 2-L pots. For pre-inoculation with *P. citricola* and inoculation with *P. cinnamomi,* 4-L and 8-L pots were used, respectively. Each treatment included 20 plant replicates and the experiments were repeated once. Data were combined for the replicate experiments.

Results and Discussion

After inoculation with *P. citricola,* the roots of *P. indica* and *P. americana* exhibited partial resistance against infection withP *cinnamomi* compared to controls which were not pre-inoculated with *P. citricola* (Tables 1, 2, and 3). This effect was noted for seedlings inoculated and kept in either soil or water (Tables 1, 2, and 3). The protective effect of *P. citricola* against *P. cinnamomi* increased as the plant age increased. Better induction of resistance to *P. cinnamomi* was obtained for 5- and 8-months-old plants compared to 1-and 2-months-old plants of *P. indica* and *P. americana* (*Tables 1, 2, and 3*). Plant protection by *P*. *citricola* resulted in a reduction in the number of plants wilted and the number of plants dead after 40 days of inoculation with *P. cinnamomi.* The reduction off! *cinnamomi* root rot symptom expressions caused by *P. citricola* was 32% and 62% in the case of 2- and 8-months-old *Persea americana* seedlings and 24% and 40% in the case of 2- and 8-months-old *Persea indica* seedlings, respectively. The infection of the avocado roots by *P. citricola* after inoculation was confirmed by the recovery of *P. citricola* on *Phytophthora* selective PARPH medium. Melouk and Horner (1975) showed that peppermint and spearmint were protected against disease caused by a virulent isolate of *Verticillium dahlia* when inoculated first with the weak pathogen, *V. nigrescens.* The protecting effect of *V. nigrescens* was not due to physical competition with *V. dahlia,* but to accumulation of antifungal phytoalexins in the xylem. Relatively small amounts of inoculum of *V. nigrescens* were sufficient to induce protection. It may be that a weak root rot caused by *P. citricola* induces phytoalexins in avocado which inhibit infection by *P. cinnamomi.*

Table 1. Effect of inoculation of roots of *Persea indica* plants with *Phytophthora citricola* on the severity of wilt caused by *Phytophthora cinnamomi* in water.

Treatment	Days	Plants wilted (%)[w]	
		1 -mo-old seedlings	5 -mo-old seedlings
P. citricola /P. cinnamomi[x] (Pre-inoculation with *P. citricola)*	5	0.0 H	0.0 E
	10	6.7 G	0.0 E
	15	33.3 E	20.0 D
P. citricola / P. cinnamomi (Simultaneous inoculation)	5	20.0 F	0.0 E
	10	53.3 D	30.5 C
	15	80.0 B	45.1 B
P. cinnamomi[y] (alone)	5	66.7 C	33.3 C
	10	100.0 A	46.7 B
	15	100.0 A	100.0 A
P. citricola[z] (alone)	5	0.0 H	0.0 E
	10	0.0 H	0.0 E
	15	0.0 H	0.0 H
Control	5	0.0 H	0.0 E
	10	0.0 H	0.0 E
	15	0.0 H	0.0 E

[w] Each value is the mean of 2 experiments with 20 plant replicates each. Values followed by identical letters are not significantly different (P=0.05) according to Waller-Duncan's K-ratio T test.
[x] The root system of the seedlings was kept in the inoculum suspension of *P. citricola* for 48 hr then rinsed with water and exposed to the inoculum suspension of *P. cinnamomi* for 5, 10 and 15 days.
[y] The root system of the seedlings was kept in the inoculum suspension of *P. cinnamomi* alone.
[z] The root system of the seedlings was kept in the inoculum suspension of *P. citricola* alone.

The simultaneous exposure of avocado roots to *P. citricola* and *P. cinnamomi* resulted in more disease than that observed when 48 hours elapsed between inoculation with the two pathogens. These results indicate that an induction period between inoculations with the two organisms may be required for the protective effect to be expressed. Gessler and Kuc (1982) reported that induced resistance in cucumber foliage required a time interval >3 days between induction with the non-pathogenic *Fusarium oxysporum* formae speciales and the challenge by the wilt pathogen *Fusarium oxysporum* f. sp. *cucumerinum* for maximum protection. Sweet potato sprouts inoculated with a mild strain of the foot rot fungus, *Fusarium solani* f. sp. *batatas,* were protected from subsequent infection by virulent strains of the sweet potato wilt fungus, *Fusarium oxysporum* f. sp. *Batatas* (15). This protection occurred when as little as one day or less

elapsed between inoculations with the two pathogens. All of these examples tend to indicate that inoculation with a less virulent pathogen can create resistance to a more virulent pathogen by triggering a chemical resistance response by the host plant.

Table 2. Effect of pre-inoculation of *Persea indica* roots with *Phytophthora citricola* on the symptoms of root rot caused by *Phytophthora cinnamomi* under greenhouse conditions.

Treatment		Symptoms on 2-mo-old seedlings[v]		Symptoms on 8-mo-old seedlings[v]	
	Time[x] (Days)	Wilting %	Dead[w] %	Wilting %	Dead[w] %
P. citricola /P. cinnamomi (Pre-inoculation with *P. citricola)*	10	36	0D	0	0B
	20	46	0D	20	0B
	30	49	0D	30	0B
	40	57	20 B	60	0B
P. cinnamomi [y] (alone)	10	61	0D	56	0B
	20	83	11C	89	0B
	30	84	16 BC	100	0B
	40	61	39 A	89	11 A
P. citricola [z] (alone)	10	0	0D	0	0B
	20	0	0D	0	0B
	30	0	0D	0	0B
	40	0	0D	0	0B
Control	10	0	0D	0	0B
	20	0	0D	0	0B
	30	0	0D	0	0B
	40	0	0D	0	0B

[v] Each value is the mean of 2 experiments with 20 plant replicates each.
[w] Values followed by identical letters are not significantly different (P=0.05) according to Waller-Duncan's K-ratio T test.
[x] Time after inoculation with *P. cinnamomi.*
[y] Plant roots were kept in soil infested with P. citricola for 2 days.
[z] Plants were potted in soil infested with P. *citricola* only.

Results here are not field experiments and the plants were not subjected to natural infection. However, the results are useful because natural inoculum levels are likely to be lower than those used experimentally. Despite the fact that pre-inoculation with *P. citricola* provided avocado plants with partial protection against root rot caused by *P. cinnamomi,* this induced resistance is an energy demanding process. It places demands on the plant that can not be satisfied if the plant is growing under unfavorable nutritional

conditions which impose stress on the avocado roots and pre-disposes the plant to infection (7).

Table 3. Effect of pre-inoculation of *Persea americana* roots with *Phytophthora citricola* on the symptoms of root rot caused by *Phytophthora cinnamomi* under greenhouse conditions.

Treatment		Symptoms on 2-mo-old seedlings[y]		Symptoms on 8-mo-old seedlings[y]	
	Time[x] (Days)	Wilting %	Dead[z] %	Wilting %	Dead[z] %
P. citricola / *P. cinnamomi*[w]	10	31	0 E	0	0 C
	20	38	0 E	6	0 C
	30	51	0 E	16	0 C
	40	62	0 E	38	0 C
P. cinnamomi [y] (alone)	10	74	13 D	94	0 C
	20	75	25 C	100	0 C
	30	51	49 B	87	13B
	40	25	75 A	62	38 A
P. citricola [z] (alone)	10	0	0 E	0	0 C
	20	0	0 E	0	0 C
	30	0	0 E	0	0 C
	40	0	0 E	0	0 C
Control	10	0	0 E	0	0 C
	20	0	0 E	0	0 C
	30	0	0 E	0	0 C
	40	0	0 E	0	0 C

[w] The root systems of the plants were potted in soil infested with *P. citricola* for 48 hrs.
[x] Time after inoculation with *P. cinnamomi.*
[y] Each value is the mean of 2 experiments with 20 plant replicates each.
[z] Values followed by identical letters are not significantly different (P=0.05) according to Waller-Duncan's K-ratio T test.

P. citricola normally causes only a minor root rot of avocado. This root rot can become more serious, under conditions which favor *P. citricola,* and inhibit avocado growth. *P. citricola* can become a destructive pathogen when it infects the upper parts of the stem and crown through wounds occurring occasionally during cultural practices to cause stem canker (8) and can cause death of the tree. For these reasons, it does not appear feasible to utilize *P. citricola* as a biological control for P. *cinnamomi* root rot. The presence *of P. citricola* in the field may reduce the damage caused by *P. cinnamomi.;*

however, rather than permanently reducing the disease, it may simply delay root rot progress.

LITERATURE CITED

1. Cantone, F. A. and Dunkle, L. D. 1990. Involvement of an inhibitory compound in induced resistance of maize to *Helmithosporium carbonum.* Phytopathology 80:1225-1230.
2. Chen, D. W. and Zentmeyer, G. A. 1970. Production of sporangia by *Phytophthora cinnamomi* in axenic culture. Mycologia 65:397-402.
3. Coffey, M. D. 1992. Phytophthora root rot of avocado. Pages 423-444. In: Plant Diseases of International Importance. I. Kumar, H.D. Chauube, V.S. Sing and A.N. Mukhopadhyav. CRC Press 456pp.
4. Coffey, M., Oudemans, P. and Ouimette, D. 1988. *Phytophthora citricola:* Another cause of avocado decline. Calif. Avocado Soc.Yrbk. 72:127-131.
5. Cohen, Y. and Coffey, M. D. 1984. Protecting *Persea indica* seedlings from *Phytophthora citricola* by a prior inoculation with three other *Phytophthora* spp. (Abstr.) Phytopathology 74:807.
6. Dolan, T. E., Cohen, Y. and Coffey, M. D. 1986. Protection of *Persea* species against *Phytophthora cinnamomi* and *P. citricola* by prior inoculation with citrus isolate of *P. parasitica.* Phytopathology 76: 194-198.
7. El-Hamalawi, Z. A. and Menge, J. A. 1994a. Avocado trunk canker disease caused by *Phytophthora citricola.* Investigation of factors affecting infection and disease development. Plant Dis. 78:260-264.
8. El-Hamalawi, Z. A. and Menge, J. A. 1994b. Effect of wound age and fungicide treatment of wounds on susceptibility on avocado stems to infection by *Phytophthora citricola* . Plant Dis. 78:700-704.
9. Gessler, C. and Kuc, J. 1982. Induction of resistance to Fusarium wilt in cucumber by root and foliar pathogens. Phytopathology 72:1439-1441.
10. Matkin,O.A. and Chandler, P. A. 1957. The U.C.-type soil mixes; Pages 68-85 in: The U.C. System for Producing Healthy Container-Grown Plants. K. F. Baker, ed. Calif. Agric. Expt. Station, Ex. Ser. 332 pp.
11. Melouk, H. A. and Horner, C. E. 1975. Cross protection in mints by *Verticillium nigrescns* against *V. dahliae.* Phytopathology 65:767-769.
12. Mitchell, D. J., Kannwischer-Mitchell, M. E., and Zentmyer, G. A. 1986. Isolating, identifying and producing inoculum of Phytophthora spp. Pages 63-66 in: Methods for Evaluating Pesticides for Control of Plant Pathogen. K. D. Hickey, ed. American Phytopathological Society, St. Paul, MN.
13. Pegg, K. G., Forsberg, L. I. and Whiley, A. W. 1982. Avocado root rot. Queensland Agricultural Journal 108: 162-168.
14. Stamps, D. J., Waterhouse, G. M., Newhook, F. J., and Hall, G.S. 1990. Revised tabular key to the species of Phytophthora. Mycological Papers 162. C.M.I., Kew, Surrey. 28pp.
15. Suzuki, H. 1980. Defenses triggered by previous invaders: fungi, in: Plant Disease, An Advanced Treatise, ed. J. G. Horsfall and E. B. Cowling. 5:319-332. New York, Academic Press. 534 pp.

16. Tuite, J., ed. 1969. Page 37 in: Plant Pathological Methods, Fungi and Bacteria. Burgess Publishing Co., Minneapolis,MN. 239 pp.
17. Zentmyer, G. A. 1980. *Phytophthora cinnamomi* and the diseases it causes. Phytopathol. Monogr. 10. American Phytopathological Society, St. Paul, MN. 96 pp.
18. Zentmyer, G. A., Jefferson, L., Hickman, C. J., and Chang-Ho, Y. 1974. Studies on *Phytophthora citricola,* isolated from *Persea americana.* Mycologia 66: 830-845.