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ETIOLOGY AND CONTROL OF LETHAL CANKER SYNDROME ASSOCIATED WITH *PHYTOPHTHORA* SPP. IN ALMONDS

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Abstract

In 1998/99 as in 1997/98, *Phytophthora cactorum* and *P. citricola* were associated with lethal scion cankers in Kern County almond orchards. The cankers caused by *P. cactorum* usually were initiated at or below the soil line and therefore were technically extensions of "crown rot", but those caused by *P. citricola* usually were initiated above-ground on the trunk, in scaffold-crotch depressions, or on scaffold branches and are referred to as "lethal Phytophthora canker" (LPC) disease. Pruning wounds are not required for LPC caused by *P. citricola*. Care should be used to distinguish LPC from non-lethal "pruning wound canker" caused by *P. syringae* and "mallet wound canker" caused by *Ceratocystis fimbriata*. In LPC-affected orchards, *P. cactorum* and/or *P. citricola* were detected in soil, scaffold crotch pocket debris, and harvest pick-up machine debris that was blown up onto trees during normal harvest operations. Thus, there are several reservoirs of *Phytophthora* inoculum that may play a role in LPC epidemiology. Following treatments with formulations of Kocide, Bordeaux mixture, Ridomil Gold, or Nutri-Phite, only Nutri-Phite (a fertilizer formulation) suppressed canker development when almond shoots collected from the treated orchard were inoculated with *P. citricola*. Among rootstocks tested for resistance to Phytophthora crown rot in the greenhouse, Hansen 536 (a clonal peach x almond hybrid) was particularly susceptible, whereas an experimental rootstock Deep Purple (*Prunus salicina* x *Prunus besseyi*) was as resistant as Marianna 2624. A trial was initiated in Kern County to determine if early selection and careful spacing of scaffold shoots may help prevent formation of deep scaffold crotch pockets, which can be particularly susceptible to LPC infection.

Background

Phytophthora is an important soilborne pathogen on many crops, including almond. Various *Phytophthora* spp. can cause root rot, crown rot, pruning wound cankers, and trunk, crotch, and scaffold branch cankers on almond trees of all ages. The occurrence of these diseases tends to be sporadic, but some of them can cause 5% or more tree loss in severely affected orchards. The root and crown rots are favored by cool to moderate temperatures and prolonged soil water saturation due to poor drainage, marginal soils, excessive rainfall, or over-irrigation. In California, Phytophthora crown rot of almond is usually caused by *P. cactorum* or *P. megasperma* and typically kills the affected trees. On the other hand, pruning wound cankers, caused by *P. syringae*, are favored by cool, wet conditions during pruning operations and usually don't damage bearing trees seriously because the pathogen is sensitive to high temperatures and the cankers cease development in summer (2). This project focuses on biology and control of "lethal Phytophthora canker" (LPC) disease (1). LPC is manifested by extensive cankers and

gumming on almond scions. Recently, it has been most apparent in Kern County in bearing almond orchards. LPC has occurred on coarse-textured soils with good internal water drainage.

Specific objectives of the research include determining which species cause LPC, determining where the infections start (i.e., in the rootstock or in the above-ground scion), determining where primary inoculum resides, and developing appropriate control measures. Last year (1997/98), our work emphasized the first two objectives. This year (1998/99), effort continued on the first two objectives, but we emphasized determining inoculum sources and development of control measures. Although most of the orchard work occurs in Kern County, many of the research findings apply to other almond districts.

Procedures, Results and Discussion by Objective

Objective 1. Confirm etiology of rapidly expanding crown, trunk, crotch, and scaffold cankers (lethal canker syndrome) of almond trees.

Previous work. Previous surveys in six orchards determined that *P. citricola* and *P. cactorum* were the species associated with rapid and extensive above-ground canker development and tree death in several Kern County orchards. Inoculations conducted with representative isolates of both species confirmed their pathogenicity in excised almond shoots and potted almond seedlings.

The orchards affected mainly by *P. citricola* were irrigated by microsprinklers. Those affected mainly by *P. cactorum* were flood irrigated. Field observations suggested that chronic water deposition by microsprinklers onto tree trunks was not required for development of LPC.

Procedures (1998/99). Infected bark samples were collected from almond trees with symptoms of Phytophthora infection among five orchards in Kern County. Pieces of the bark were cultured on selective PARP medium in petri dishes to detect *Phytophthora*. *Phytophthora* isolates were transferred to other culture media for species identification. Representative isolates from 1998, along with standard isolates used in earlier experiments, were tested for pathogenicity by inoculating them into the bark of excised almond branch segments (2-5 cm diameter x 15 cm length).

Results and discussion (1998/99). More than 117 canker samples were collected from the surveyed orchards in Kern County and subjected to the diagnostic isolations. *P. citricola* and *P. cactorum* were isolated from trees with extensive above-ground cankers. In addition, *P. syringae* was isolated from pruning wound cankers, which ceased development by June before serious tree damage. *P. megasperma* and an unknown but distinct *Phytophthora* sp. were isolated from trees dying at high incidence in a 2nd-leaf orchard affected by crown rot. The crown decay did not reach far above the soil line, but it had girdled and killed the affected trees. The crown rot and pruning wound cankers were encountered more commonly in 1998 than in the previous year.

In 1998 pathogenicity tests, all isolates of *P. cactorum* and *P. citricola*, as well as root crown isolates of *P. megasperma*, caused cankers in excised almond branches (Fig. 1). The cankers

caused by *P. cactorum* and *P. citricola* were more extensive than those caused by *P. megasperma* (Fig. 1). Control (non-inoculated) shoots developed no disease.

This year's surveys and pathogenicity test results confirm our previous findings that *P. cactorum* and *P. citricola* are the main causes of LPC in the southern San Joaquin Valley. Special care should be used to distinguish LPC cankers from non-lethal pruning wound canker caused by *P. syringae* and Ceratocystis canker caused by *C. fimbriata*. Ceratocystis canker is initiated at wound sites, expands most rapidly during summer months, and exhibits pronounced staining in the xylem under and near cankers, whereas LPC canker is relatively inactive in mid and late summer and tends not to exhibit staining deep in the xylem. The apparent increase in incidence of pruning wound cankers and Phytophthora crown rot among Kern County almond orchards probably resulted from intensive rains in 1998.

Objective 2. Determine locations (i.e., soilborne or aerial) where the lethal cankers originate on individual trees in Kern County.

Previous work. Previous surveys of four orchards in Kern County determined that *P. citricola* infections usually originated above-ground on the trunk, in scaffold crotch pockets (natural depressions that form where scaffold branches join the tree trunk), or on scaffolds, but those of *P. cactorum* usually originated below-ground. Both types of infection resulted in extensive above-ground cankers and ultimate death of many trees.

Procedures (1998/99). Cankers found in 1998 were examined to determine the likely original site of infection. Attention was given to whether the infection originated above or below the soil surface.

Results and discussion (1998/99). *P. cactorum* was isolated from cankers that apparently originated below-ground and extended up the tree trunks, where they became visible due to gumming and death of the bark. In contrast, *P. citricola* usually was isolated from cankers that were limited to aboveground portions of trees including trunks, scaffold crotch pockets, and scaffold branches.

This year's results confirm the previous findings and indicate that management strategies for lethal almond scion cankers should address control of below-ground as well as above-ground *Phytophthora* infections. Technically, scion cankers caused by *P. cactorum* following below-ground infection are just upward expansions of crown rot. One of the most important steps that growers can take to avoid the crown and trunk-base infections is to plant trees so that the graft union will remain above the soil line; almond tissue is much more susceptible than peach rootstock to soilborne *Phytophthora* spp. and should not be buried. Avoidance of rootstocks with high susceptibility to *Phytophthora* also may help minimize losses from below-ground *Phytophthora* infections (see objective 4, below). Control strategies for above-ground LPC infections may require several approaches; we are investigating use of fungicides, a foliar fertilizer, and new approaches to young-tree training (see objective 4, below) that may help avoid the aerial infections.

Objective 3. Determine sources of primary inoculum that contribute to lethal canker syndrome of almond trees in Kern County.

Previous work. In 1997/98 *P. cactorum* and *P. citricola* were detected in samples of soil and scaffold-crotch pocket debris from orchards affected by LPC. In addition, *P. citricola* was detected in one out of 169 samples of "pick-up machine debris" that was deposited on tree surfaces during almond harvest, suggesting that the debris may play at least a partial role in epidemiology of LPC.

Procedures (1998/99). Work continued to determine important sources of *Phytophthora* inoculum in orchards affected by LPC. Almond seedlings and pear fruits were used as bait to detect *Phytophthora* in samples of soil, scaffold crotch debris, and debris deposited on trees during normal harvest operations. The harvest debris was collected by tarps that were nested among scaffold branch bases as nuts were swept and picked up.

Results and discussion (1998/99). *P. cactorum* and *P. citricola*, as well as other *Phytophthora* spp., were isolated from samples of orchard soil, scaffold crotch debris, and debris deposited on trees during harvest (Tables 1,2). The results suggest that in orchards affected by LPC, trees are exposed to *Phytophthora* inoculum from several sources. The detection of *P. cactorum* and *P. citricola* in harvest debris has suggested a novel mechanism of *Phytophthora* dispersal from soil up into trees. Wind-blown rain or dust, or sprayer-driven mist or debris may also carry the pathogens up into the canopy, but these inoculum sources have not been investigated.

Objective 4. Develop appropriate control measures for lethal canker syndrome of almond trees.

Previous work

Efforts began to test chemical and genetic approaches to controlling LPC and related Phytophthora crown rot. Topical NON-REGISTERED applications of Ridomil Gold on LPC cankers suppressed but did not arrest canker development; the crop from the treated trees was not harvested. An additional trial was established to test effects of Ridomil Gold, a copper spray program, and Nutri-Phite foliar fertilizer on LPC. Hardwood and softwood cuttings of commercially available and experimental almond rootstocks were propagated for screens of resistance to *Phytophthora* spp. associated with LPC and Phytophthora crown rot.

Procedures (1999/98)

Fungicide and fertilizer trial. In an orchard affected by LPC, treatments of Ridomil (Gold formulation), copper (Kocide 101 or Bordeaux 10-10-100, depending on date), and Nutri-Phite foliar fertilizer (0-28-26) have been applied since fall 1997 according to label rates and schedules to 5 replicate 56-tree plots per treatment in a randomized complete block design. Details of the 1998/99 treatments are shown in Table 3.

New incidence of LPC has been too low to allow evaluation of the treatments in the orchard, so shoots have been sampled from the plots and inoculated with *P. citricola* in the lab to determine treatment effects. Following the chemical applications (Table 3), 1-year-old shoot segments (15-20 cm in length) were sampled periodically from all of the plots. For each sample date, three replicate shoots per plot (15 per treatment) were inoculated with *P. citricola*, and one replicate shoot per plot (5 total) was inoculated with an agar disk (the control). The shoots were incubated at 100% relative humidity. Five days after inoculation, canker length was measured on each shoot and used to assess effects on canker development.

Rootstock screening. Judicious rootstock selection may help avoid Phytophthora crown rot as well as above-ground Phytophthora cankers that originate from root crown infections. Experimental and commercial almond rootstocks were propagated for evaluations of resistance to *Phytophthora* spp. Softwood cuttings were rooted under intermittent mist. Hardwood cuttings provided from Dave Wilson Nursery and the Foundation Plant Material Service were rooted in potting soil. The stocks were allowed to develop roots for several months and then transplanted into either noninfested soil (as a control) or soil artificially infested with *P. cactorum*, *P. citricola*, or *P. megasperma*. To favor infection by *Phytophthora*, the soil around plants was flooded for 24 hr every week or 48 hr every 2 weeks. Above-ground disease ratings were made at weekly intervals and, three months after inoculation, the root systems were washed free from soil and rated for severity of root and crown rot.

Tree training. Scaffold crotch pockets were identified as one of the important sites of above-ground LPC infection. A tree training study was initiated to determine if early selection of scaffolds that are spread out along the trunk may help to avoid formation of the water-collecting scaffold crotch pockets. In a commercial orchard that was planted January/February 1998, the following tree training treatments were each imposed on 5 replicate plots:

Treatment 1: Spring 1998 selection of three main shoots around the trunk; shoots originating at the same level on the trunk. Shoots not chosen as scaffolds were bent down.

Treatment 2: Spring 1998 selection of three main shoots around the trunk, shoot origins separated vertically by several inches on the trunk. Shoots not chosen as scaffolds were bent down.

Treatment 3: Fall 1998 selection of three main shoots around the trunk; shoots originating at the same level on the trunk.

Treatment 4: Fall 1998 selection of three main shoots around the trunk; shoot origins separated vertically by several inches on the trunk.

Treatment 5: Conventional fall 1998 selection of five main shoots distributed along the trunk.

Results and discussion (1998-99)

Fungicide and fertilizer trial. In the spring, fall, and winter, orchard applications of Nutri-Phite foliar fertilizer suppressed subsequent canker development when shoots were collected from the treated trees and inoculated with *P. citricola.* The effect was evident for more than one month after the spray application. In contrast, neither the Ridomil nor the copper treatments suppressed canker development compared to that in the non-treated control (Fig. 2 A,B). Activity of the copper was not expected in this test, because the inoculation procedure with *P. citricola* bypasses

the external protection that the copper may offer. It is possible that the Ridomil treatment afforded systemic protection in lower parts of the tree (roots, crown, trunk), but these parts were not tested. The assays are being repeated this year.

Rootstock screening. In the rootstock resistance evaluations that used hardwood cuttings, Hansen 536 (a clonal peach x almond hybrid) exhibited high relative susceptibility to Phytophthora root and crown rot (Fig. 3 A-C). Hansen 536 developed more severe crown rot than most of the other rootstocks in soil infested with *P. cactorum* and *P. citricola.* Hansen 536 also developed severe root rot in soil infested with *P. megasperma* (Fig. 3-C). At the other extreme, the resistant standard, Marianna 2624 (clonal *P. cerasifera* x *munsoniana*), developed no measurable disease in soil infested with *P. cactorum*, *P. citricola*, or *P. megasperma.* Most of the other rootstocks appeared to have intermediate levels of susceptibility to the *Phytophthora* spp., except that *P. megasperma* did cause severe root rot in Atlas and Viking (complex hybrids involving peach, almond, plum, and apricot heritage) and Lovell peach.

With softwood cuttings, emphasis was given to screening with *P. megasperma* due to insufficient numbers of some of the rootstocks for evaluations with *P. cactorum* and *P. citricola* (Table 4). Most of the rootstocks, including Atlas, Hansen 536, Lovell, Nemaguard, UC 1-82 were moderately to highly susceptible to root rot caused by *P. megasperma* (Table 4). In contrast, the pathogen caused only mild root rot in Citation (clonal peach x plum) and no significant disease in Deep Purple (clonal *Prunus salicina* x *Prunus besseyi*) and Marianna 2624. Hansen 536 was the most susceptible of the rootstocks tested with *P. cactorum* (Table 3). Hansen 536 was not tested with P. citricola, but UC 1-82, another peach x almond hybrid clone exhibited some susceptibility to the pathogen, while the other rootstocks tested developed relatively little root and crown rot (Table 3) .

A third greenhouse evaluation of resistance to Phytophthora is underway with rooted hardwood cuttings. To date, results of the third experiment are generally consistent with those of the first screen with hardwood cuttings. Disease severity ratings based on above-ground symptoms of crown and root rot (plant stunting, chlorosis, wilting, and death) suggest again that Hansen 536 is more susceptible than Lovell or Nemaguard peach. Citation and Marianna 2624 appear relatively healthy in the infested as well as the noninfested soil.

Overall, the results of the rootstock screens in the greenhouse suggest that careful rootstock selection and continued development of almond rootstocks may play an important role in controlling Phytophthora crown rot, including cases that lead to lethal above-ground cankers. Development and application of field screening procedures for testing rootstock resistance to Phytophthora would help confirm the greenhouse results.

Tree training. To date, it appears that early scaffold selection during the first growing season may help develop a scaffold system that is more widely spaced than that resulting from latter selection of scaffold shoots during tree dormancy. Quantitative assessment of the early training procedure, however, must wait until trees become larger. It is planned to measure the volume of any pockets that form on an annual basis, starting next year.

Literature Cited

1. Browne, G.T., and Viveros, M.A. 1999. Lethal cankers caused by *Phytophthora* spp. in almond scions: Specific etiology and potential inoculum sources. Plant Dis. 83: (In Press).
2. Doster, M.A., and Bostock, R.M. 1988. Incidence, distribution, and development of pruning wound cankers caused by *Phytophthora syringae* in almond orchards in California. Phytopathology 78:468-472.

Table 1. Detection of *Phytophthora* spp. in orchard soils and scaffold crotch pockets

Potential source of *Phytophthora* inoculum	Method of *Phytophthora* detection	Number of sites/trees sampled	Number of sites/trees with *Phytophthora* (and species identified)
Almond orchard soil	culturing roots from volunteer almond seedlings	4	3 (*P. citricola*) 1 (*P. cactorum*)
	pear baiting	10	2 (*P. citricola*)
Scaffold crotch pocket	culturing roots from volunteer almond seedlings	23	2 (*P. cactorum*) 1 (*P. citricola*) 1 (*P. syringae*) 1 (*Phytoph.* sp.)
	pear baiting	23	0

Table 2. Detection of *Phytophthora* sp. in debris deposited on trees during normal harvest operations[a]

		Percent of samples yielding the *Phytophthora* sp. to pear baits[b]				
Source of debris[a]	No. of samples	*P. cactorum*	*P. citricola*	*P. parasitica*	*P. Syringae*	*Phytoph-thora* sp.[c]
None (control)	58	0	0	0	0	0
Orchard 1	46	0	19	4	6	9
Orchard 2	47	2	27	0	13	17

[a]Debris was collected in clear plastic tarps that nested within the bases of scaffold branches during 8-10 days of normal harvest operations.

[b]Each sample was baited with a pear fruit in a growth chamber on one to three separate occasions.

Table 3. Treatments that were compared in large-scale replicated trial on control of lethal Phytophthora cankers in almond , Kern County (See also Fig. 2 A,B)

Trt. no.*	Material(s) (and type of action)	Trt. dates	Formulation mixture and amount per sprayed acre
1.	Control	--	(no spray)
2.	Copper program:		
	Copper (Kocide 101 applied during October and (a.i. copper hydroxide; protectant)	10/27/98	8 lb. form. in 175 gal. water applied per sprayed A, air blast, complete coverage.
	Bordeaux (a.i. copper sulfate+hydrated lime; protectant)	1/21/99	10-10-100, 175 gal of mix applied per sprayed A, air blast, complete coverage.
3.	Ridomil Gold EC (a.i. metalaxyl, systemic fungicide)	4/7/98 and 10/27/98 and 3/30/99	2 qts. in 250 gal. water applied per sprayed A, banded on ground (6-9 ft wide on each side of tree row, partial ground spray)
4.	Nutri-Phite (0-28-26 foliar fertilizer, side benefits?)	4/6/98 and 10/27/98 and 3/30/99	4 pts. in 175 gal. water applied per sprayed A, airblast, complete coverage

*Each treatment was applied to five separate replicate sets of 56 trees (280 trees total per treatment) in a randomized complete block design in an orchard affected by LPC. Efficacy of the treatments was assessed with excised shoot assays (Fig. 2 A,B), because natural incidence of LPC in the orchard during 1998 was insufficient to evaluate the treatment effects.

Table 4. Relative resistance of softwood cuttings of seven commercial and experimental rootstocks for almond to crown and root rot caused by three species of *Phytophthora* in a 1998 greenhouse evaluation

Inoculum	Rootstock	Percentage of crown circumference rotted	Percentage of roots rotted
None (control)	Atlas	0	4
	Citation	0	3
	Deep Purple	0	2
	Hansen 536	0	23
	Lovell	0	5
	Marianna 2624	0	3
	Nemaguard	0	2
	UC 1-82	0	16
	Viking	0	2
P. cactorum	Atlas	23	12
	Citation	0	5
	Hansen 536	34	26
	Marianna 2624	0	3
	Nemaguard	0	6
	UC 1-82	0	16
P. citricola	Atlas	0	10
	Citation	0	4
	Marianna 2624	0	3
	Nemaguard	2	4
	UC 1-82	22	35
P. megasperma	Atlas	29	97
	Citation	0	19
	Deep Purple	0	3
	Hansen 536	19	93
	Lovell	45	96
	Marianna 2624	0	5
	Nemaguard	46	81
	UC 1-82	20	88
	Viking	6	58
Minimum significant difference: (Waller-Duncan k-ratio test)		27	16

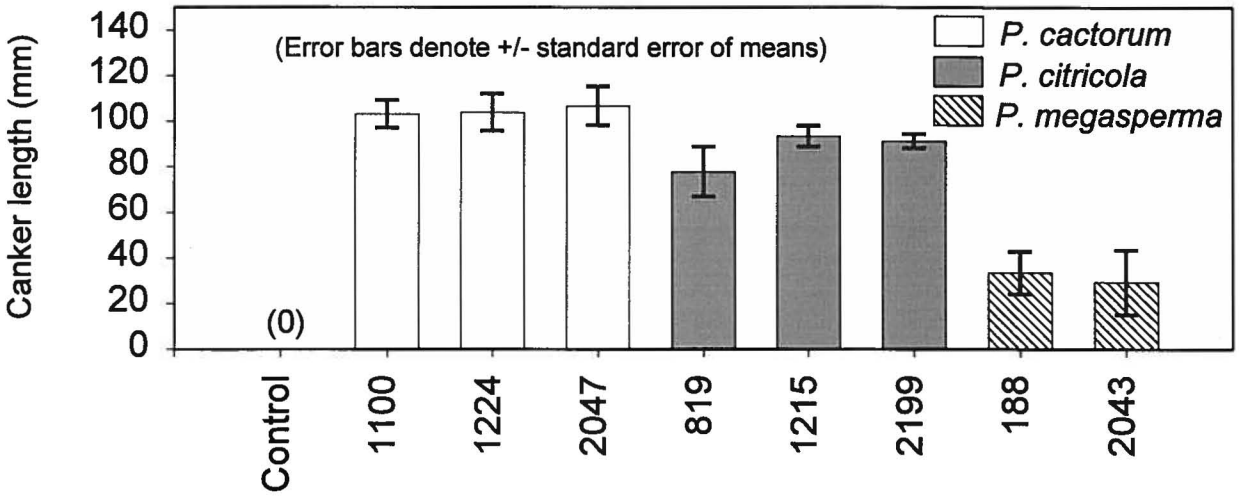

Fig. 1. Pathogenicity and relative virulence of *Phytophthora cactorum*, *P. citricola*, and *P. megasperma* in Nonpareil almond branch segments. Vertical error bars delimit +/- standard error of means.

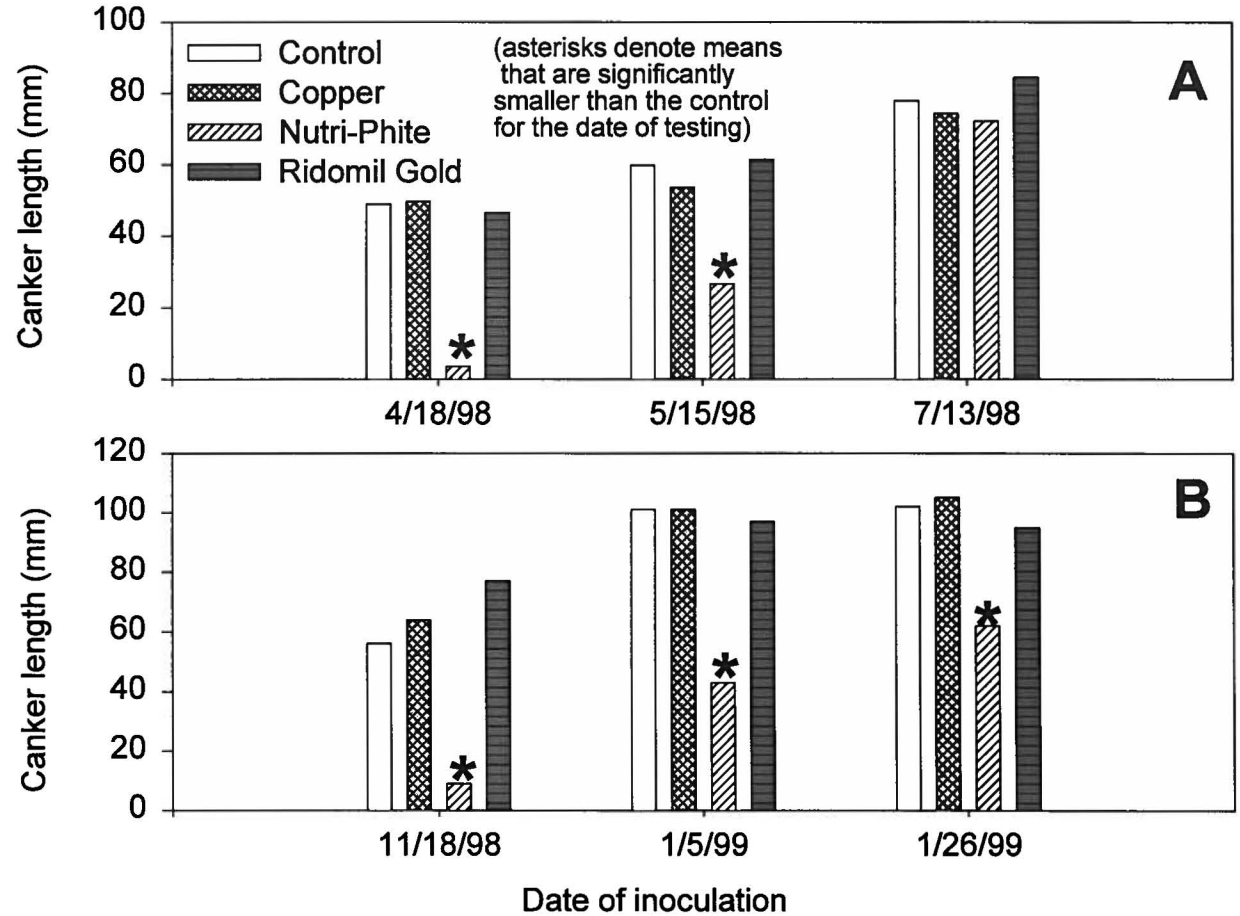

Fig. 2. Effects of some nutritional and chemical treatments (details in Table 3) on subsequent development of shoot cankers caused by *Phytophthora citricola* in **A**, spring/summer 1998 and **B**, fall/winter 1998/99. Following the treatments (Table 3), seperate sets of 1-year old shoots were collected on each date indicated, inoculated with *P. citricola* (or sterile agar, as a control), and incubated for 5 days. Canker lengths were measured 5 days after inoculation. Agar controls developed no cankers.

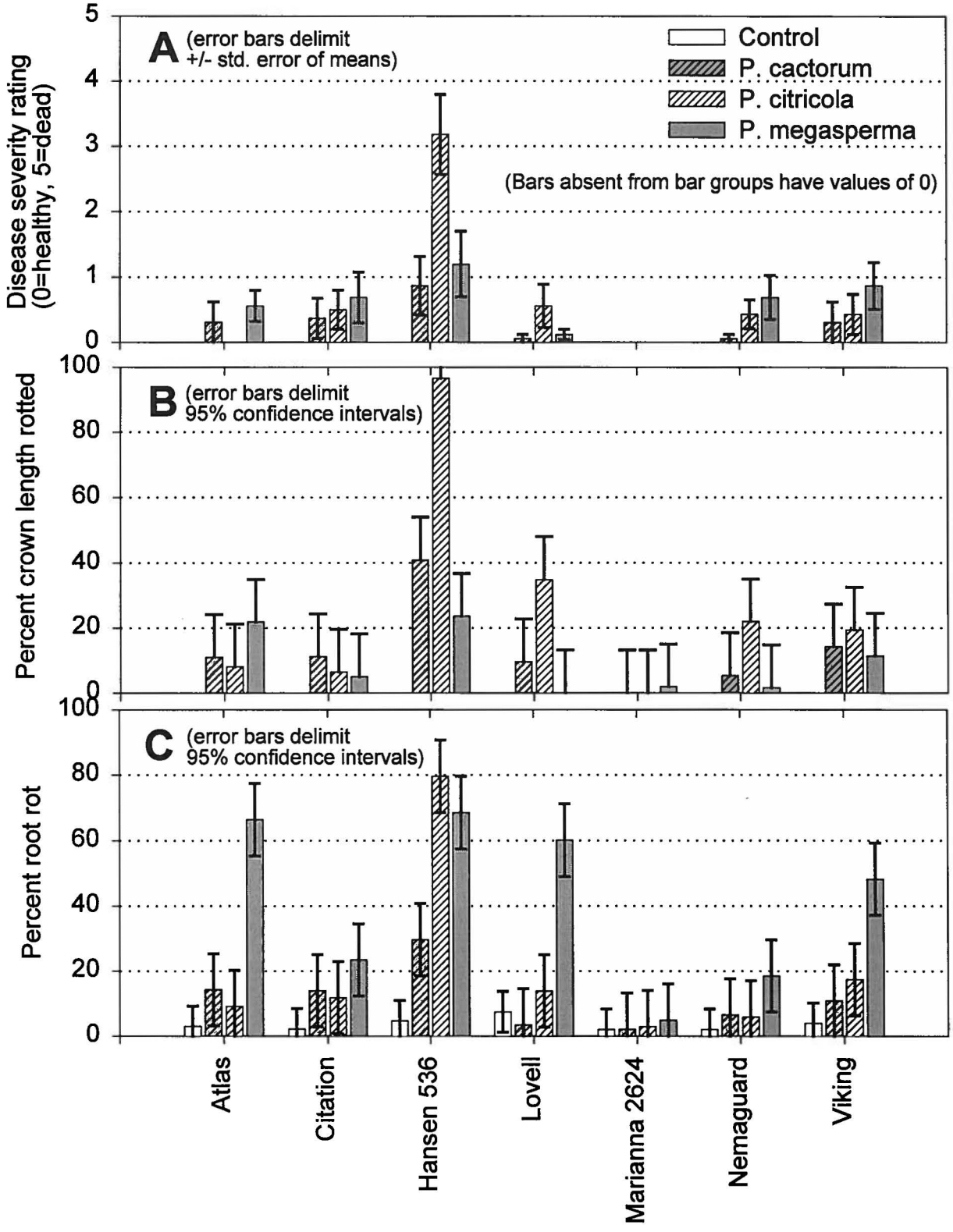

Fig. 3. 1998 greenhouse experiment with hardwood cuttings: relative resistance to three *Phytophthora* spp. in some commercial and experimental rootstocks for almond. The selections were propagated as hardwood cuttings and transplanted into noninfested soil (controls) or soil infested with one of the *Phytophthora* spp. Three months after transplanting, rootstock resistance was assessed according to **A**, above-ground disease severity ratings; **B**, serverity of crown rot; and **C**, severity of root rot.

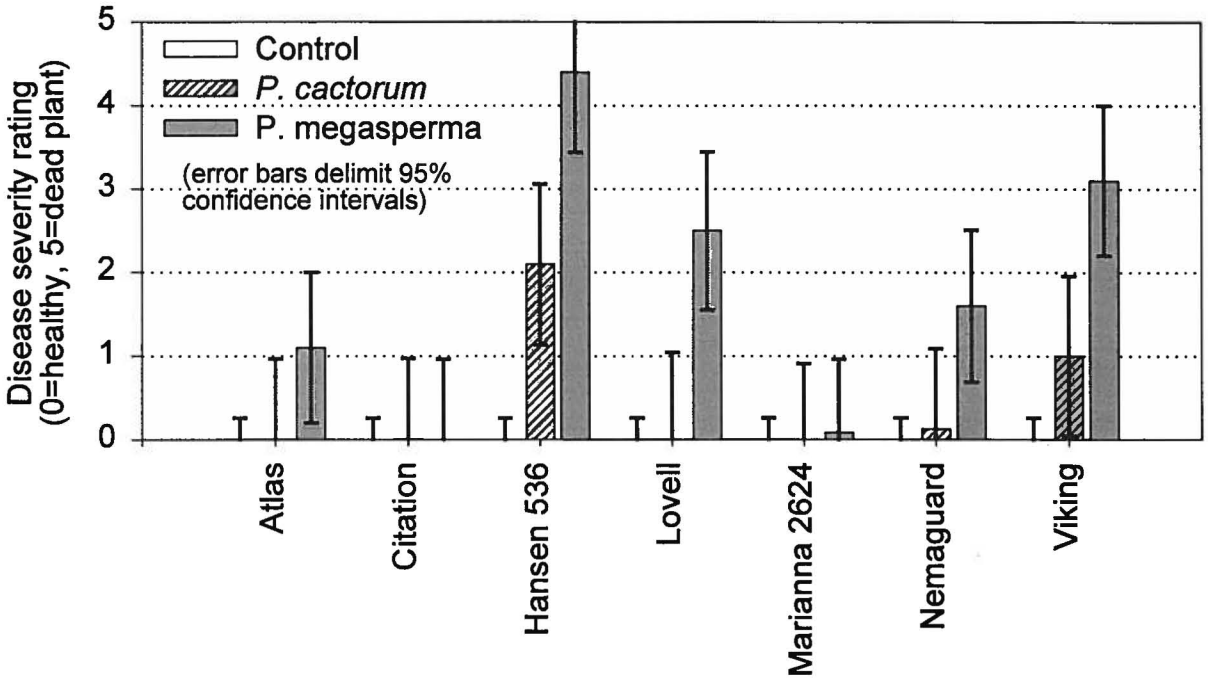

Fig. 4. 1999 greenhouse experiment with hardwood cuttings: relative resistance to two Phytophthora spp. in some commercial and experimental rootstocks for almond. The selections were propagated as hardwood cuttings and transplanted into noninfested soil (controls) or soil infested with one of the Phytophthora spp. The above-ground disease ratings were made 2 months after transplanting. Severity of root and crown rot will be determined on the root systems 3 months after transplanting.