Etiology and control of lethal canker syndrome associated with *Phytophthora* spp. in almonds

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ABSTRACT

Phytophthora cactorum and *P. citricola* were isolated from rapidly expanding and profusely gumming (lethal-type) cankers among six orchards in Kern County. Pure cultures of these fungi were pathogenic in excised almond shoots and in roots and root crowns of peach and almond seedlings. *P. syringae* was isolated only from non-lethal pruning-wound cankers. Results of quantitative surveys in four of the six orchards suggest that lethal Phytophthora cankers can originate from subterranean as well as aerial sites of infection. Therefore, multiple approaches may be required for good control of lethal cankers. Among the aerial lethal cankers, individual sites of canker origin apparently included tree trunks, water pockets, and scaffold branches. By various methods of sampling in orchards affected by the cankers, *P. cactorum* and/or *P. citricola* were detected in soil, water pocket debris, and harvest pick-up machine debris that was blown up into trees during normal harvest operations, which indicates that there are several potentially important reservoirs of primary inoculum of the pathogens that are causing lethal Phytophthora cankers. In one experiment, a concentrated topical post-infection canker spray (non-registered, crop-destruct) with Ridomil Gold EC slowed but did not arrest expansion of lethal Phytophthora cankers. In another experiment, formulations of Kocide, Bordeaux mixture, Ridomil Gold, and Nutri-Phite (a fertilizer) are being evaluated in a commercial orchard for protection and nutritional effects against lethal Phytophthora cankers. As a strategy for control of soilborne infections, work has started on evaluations of genetic resistance to *Phytophthora* spp. in seedling and clonal rootstocks.

INTRODUCTION

In the last several years, many San Joaquin and Sacramento Valley almond orchards have suffered tree loss associated with infection by species of the soilborne "water-mold" fungus *Phytophthora*. The losses in the Sacramento and *upper* San Joaquin Valleys typically have involved crown rot (decay of the root crown). Laboratory isolations from the diseased root crowns detected *P. megasperma*. In the last two years, highest incidence of crown rot has occurred in young non-bearing almond orchards on marginal soils, which are subject to conditions that favor infection by *Phytophthora*, such as prolonged soil saturation due to poor drainage.

On the other hand, in the *lower* San Joaquin Valley, high incidence of tree loss associated with *Phytophthora* has occurred in mature almond orchards in on supposedly well-drained high quality soils. During 1994-97, several of such orchards in Kern County lost up to 5% or more trees due to girdling by cankers that yielded *P. cactorum* and *P. citricola* in laboratory isolations. Our observations indicate that the cankers are perennial and usually expand until affected trees

are girdled and killed. From early field surveys, it appeared that some of the cankers had resulted from aboveground infections, but because of the advanced stage of disease, clear determinations of canker origin were often impossible. We have referred to this disease syndrome as "lethal Phytophthora canker" to describe the symptoms and distinguish the disease from non-lethal "pruning wound" cankers caused by *P. syringae*. In the 1980's, Doster and Bostock documented the "pruning-wound" cankers, which are not usually lethal because of warm temperatures of late spring and summer that limit the causal cool-temperature fungus (1). In comparison to cankers caused by *Ceratocystis*, lethal cankers caused by *Phytophthora* expand more rapidly, especially in vertical direction.

Little is known about conditions and periods that favor the lethal canker almond tree infections by *P. cactorum* and *P. citricola* in the southern San Joaquin Valley, although free water and mild to moderate temperatures are probably required. Phytophthora root and crown rots are favored by mild to moderate temperatures and frequent prolonged periods of water saturation in or on soil; such conditions favor reproduction and spread of motile zoospores that initiate tree infection. Pruning wound cankers caused by *P. syringae* are initiated in cool, wet weather, often during tree dormancy (1).

This project focuses on determining the cause and appropriate control measures for the poorly understood lethal canker syndrome of almonds. Step one of the research is to define the causal organism(s) involved. At the same time, it is important to learn where (i.e., what parts of the tree), when, and how the trees are being infected. Knowledge on where the infections start is critical, because control measures for aboveground infections could differ from control measures for below-ground infections. Depending on knowledge of the pathogens and the disease cycle, appropriate control measures will be tested. Although most of this work occurs in Kern County, many of the findings also will apply to other almond districts.

OBJECTIVES

1. Conduct Koch's postulates to confirm etiology of the rapidly expanding crown, trunk, crotch, and scaffold cankers (lethal canker syndrome) of almond trees.

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

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

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

PROCEDURES, RESULTS AND DISCUSSION

1. Conduct Koch's postulates to confirm etiology of the rapidly expanding crown, trunk, crotch, and scaffold cankers (lethal canker syndrome) of almond trees.

Procedures. Bark samples were collected from cankers on diseased almond trees among six separate orchards in Kern County. The orchards included microsprinkler as well as flood irrigated blocks, with and without water hitting the tree trunks (Table 1). To detect pathogens in cankers, pieces of the diseased bark were cultured on selective medium in petri dishes. *Phytophthora* fungi that grew from the bark were transferred to other culture media to allow species identification according to morphology.

Representative *Phytophthora* species detected in lethal cankers were tested for pathogenicity in excised almond shoots (current-season and 1-yr-old, length about 20 cm). Each shoot was inoculated near its midpoint by removing a 5-mm-diameter disk of bark and replacing the bark piece with a 5-mm-diameter agar disk colonized by either *P. cactorum* or *P. citricola*; sterile agar disks were used for control inoculations. The disks were covered with plastic tape to prevent drying and the shoots were incubated in a humid chamber. Five days after inoculation, pathogenicity was assessed according to canker production in the shoots.

An additional pathogenicity test used potted almond and peach seedlings in a greenhouse. The seedlings were grown for 4 months in 0.2-L pots of noninfested soil and then transplanted into 1-L pots containing either noninfested soil (controls) or soil artificially infested with a *Phytophthora* sp. One week after transplanting, all plants were subjected to flooding for 48 hr once every two weeks. Three months after transplanting, the root systems were assessed for incidence and severity of root and crown rot.

Results and discussion. In 1997, the laboratory culturing methods detected *Phytophthora* from 86 out of 184 total cankers sampled (Table 1). Twenty-one of the isolates were identified as *P. cactorum*, 61 as *P. citricola*, and 4 as *P. syringae*. *P. cactorum* and *P. citricola* were isolated from lethal-type (expansive) cankers, whereas *P. syringae* was isolated from non-lethal pruning-wound cankers.

Within 5 days after inoculation by *P. cactorum* or *P. citricola*, current-season and 1-yr-old Nonpareil almond shoots all developed cankers, but noninoculated control shoots were free from disease (Figs. 1,2). *P. cactorum*, *P. citricola* and *P. megasperma* were pathogenic in potted peach and almond seedlings in the greenhouse, but no significant root or crown rot occurred in noninoculated controls (Table 2). *P. cactorum* and *P. citricola* were reisolated from inoculated shoots and seedlings, thereby completing Koch's postulates.

The results from 1997 indicate that *P. cactorum* and *P. citricola* presently are the *Phytophthora* species primarily responsible for lethal almond cankers in the lower San Joaquin Valley. *P. syringae* was isolated in January and February from pruning wound cankers that ceased development by late spring / early summer without causing serious damage to the trees infected. *P. syringae* was not detected when the cankers were sampled again in June. The pruning wound

cankers are not considered lethal to trees, although they may kill individual branches. Many cankers caused by *P. cactorum* and *P. citricola* were still expanding during warm temperatures of late spring early summer and will likely continue development in 1998. The surveys, pathogen culturing, and pathogenicity tests continue and are needed to confirm 1997 results.

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

Procedures. Four of the six orchard blocks described above (Table 1) were surveyed tree by tree during March-June 1997 to locate cankers in early stages and thereby determine where on individual trees that lethal cankers originate. Using hand tools, each canker was exposed and examined carefully to determine likely sites of tree infection. The cankers were recorded in diagrams and, when possible, categorized according to likely point of origin on the tree. Cankers restricted to aboveground parts of the tree were classified as aerial and those that appeared to expand upwards from below the soil line were called subterranean. When possible, aerial cankers additionally were categorized as infections of a trunk, water pocket (the natural depression that often forms where scaffolds and trunk join), pruning wound, or scaffold. Many cankers were excluded from the classifications because they had killed too much of the tree to allow categorization. Small cankers were the most useful for determining likely points of infection. Samples were collected from each canker for pathogen identification (described above), and records were kept on canker incidence among different varieties.

Results and Discussion. At ranches 1-T and 2-Z, and 4-S, most cankers were aerial (Table 3). However, 76% of surveyed cankers at ranch 3-N and 27% of those at ranch 1-T originated from subterranean crown rot (Table 3). The cankers at ranches 1-T, 2-Z, and 3-N appeared to be the lethal-perennial type that kill trees. Lab isolations detected *P. citricola* predominately from aerial infections and *P. cactorum* predominately from crown infections. A chi-square test on the data from ranch 1-T (where both species were isolated) indicated that the ratio of aerial to subterranean infections was significantly greater for *P. citricola* than for *P. cactorum* ($P=0.001$). Virtually all cankers at ranch 4-S were pruning wound cankers associated with *P. syringae* and had ceased development by summer without serious tree damage (Table 3). Among the four orchards surveyed, lethal cankers were not restricted to a certain method or design of irrigation (Table 1). To date we have found no evidence of differential varietal susceptibility to lethal Phytophthora cankers (Tables 4,5).

Because a substantial proportion of lethal cankers monitored in 1997 apparently started as aerial infections, our research on inoculum sources and control strategies should include an above-ground focus. The results, however, also indicate that Phytophthora crown rot is responsible for a significant proportion lethal canker tree losses in Kern County, so our research should also address prevention of crown rot. Additional orchards will be surveyed in 1998.

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

Procedures. In 1997, attempts began to determine important sources of *Phytophthora* inoculum in orchards affected by lethal canker syndrome. Laboratory and field isolation techniques were applied to samples of soil, water pockets debris, and harvest pick-up machine debris. To detect *Phytophthora* spp. in soil and water pockets, naturally occurring almond seedlings were collected from the samples. Root and stem tissues of the seedlings were observed for symptoms of infection by *Phytophthora* and subjected to isolations on selective medium in petri dishes. Alternatively, soil and water pocket samples were subjected to a pear baiting procedure; firm green pear fruits were placed in the soil or pocket debris, flooded for 48 hr, rinsed off, and then incubated at room temperature while monitoring for development of lesions characteristic of those caused by a *Phytophthora* sp.

Debris blown up into trees during harvest was investigated as a source of *Phytophthora* inoculum. During normal harvest operations, pick-up machine debris was collected in plastic tarps that were stapled into vase-shaped "collection traps" between the bases of scaffold branches. The debris samples were collected separately, weighed, mixed individually with small volumes of potting soil, and placed individually in 0.2-liter pots. Peach and almond seedlings were used as bait for *Phytophthora* spp. in the potted samples. As a control, seedlings were planted in potting soil without debris added.

In orchard 1-T, where some trunk infections had been observed, we investigated the possibility that microsprinklers were depositing *Phytophthora*-infested irrigation water on tree trunks. During a normal irrigation, pear fruits were suspended on tree trunks in plastic bags that caught irrigation water from the microsprinklers. The fruits were suspended in the bags for 48 hr (24 hr of which involved irrigation) and then were incubated at room temperature for 1 wk to observe for lesions caused by a *Phytophthora* sp. Another set of pears was used to bait the on-farm source of the irrigation water, a reservoir receiving surface water from an irrigation district.

Results and discussion. *P. cactorum* and *P. citricola* were detected in soil samples and water pocket debris (via almond seedling baits, Table 6). The presence of *Phytophthora* in the soils of affected orchards is expected; once the pathogens are introduced, the soil is a natural reservoir for these fungi. The isolation of *Phytophthora* from water pockets suggests merit of protection strategies there, but experiments are needed to evaluate this idea. Our survey work and detection of Phytophthora in pick-up machine debris (below) suggests that protection would also be needed for tree scaffolds and trunks, which also served as sites of canker initiation (Tables 3,6).

In 1997, *P. citricola* was detected in one out of 169 pick-up machine debris samples from harvest in 1996 (Table 6). In 1998, a repeat experiment with 1997 samples (still in progress) has again detected *P. citricola* in pick-up machine debris. Isolation of *Phytophthora* from the debris samples is difficult because of the presence of many other (non-pathogenic) fungi, so the results should be viewed qualitatively rather than quantitatively. To date, no *Phytophthora* has been detected in controls for the debris samples.

We do not know with certainty that the inoculum in pick up machine debris contributes to lethal cankers. It is likely that *Phytophthora* is also carried into trees by other agents such as wind and rain and animals. Nevertheless, the debris findings are of interest and raise questions for us about whether soil moisture conditions, irrigation methods, and resulting root distributions could influence surface soil populations of *Phytophthora* and resulting movement from soil into trees.

Phytophthora was not detected in water at any of the reservoir or microsprinkler trapping locations (data not shown). Therefore, to date, there is no evidence that irrigation water is responsible for depositing *Phytophthora* spp. directly on the sites of infection in Kern County. More work is needed to determine mechanisms and times of *Phytophthora* spread up into the trees.

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

Procedures. Three fungicide experiments were initiated. One involved application of copper sulfate powder to water pockets (approx. 1 Tbs. per pocket). After copper application, rainfall was simulated by flooding the pockets for 10 hr by drip emitters connected to water reservoirs. Control water pockets received no copper but were flooded. Efficacy of the copper treatment was to be assessed according to incidence of infection by *Phytophthora* in pear fruits and almond leaf disks that were used as bait for the fungus. In a second experiment, Ridomil Gold EC was applied as a non-registered crop-destruct concentrate spray (1:12, Ridomil Gold EC: water) to test post-infection activity against Phytophthora cankers. In the third experiment, three separate fungicides, including Ridomil Gold EC, Kocide 101, and Nutri-Phite P Foliar (4-30-8 formulation), were applied according to labeled rates and methods to replicated plots at ranch 2-Z on 31 October 1997 (Table 8). On 24 December, Bordeaux (10-10-100) was applied to the plots that had received Kocide earlier (Table 8). Disease incidence was monitored in all plots at the beginning of the experiment and will continue in 1998.

One greenhouse experiment was completed in 1997 to develop screening methods for evaluating resistance to *Phytophthora* spp. in rootstocks and rootstock germplasm for almond. Open pollinated seedlings of almond, peach, peach x almond, and plum were grown in 0.2-liter pots of noninfested soil and then transplanted to 1-liter pots with noninfested soil (controls) or soil artificially infested with isolates of *P. cactorum*, *P. citricola*, or *P. megasperma*. One week after transplanting, all plants were subjected to flooding for 48 hr every two weeks. Three months after transplanting, resistance of the seedling backgrounds to the *Phytophthora* spp. was assessed according to incidence and severity of root and crown rot.

In cooperation with Dave Wilson Nursery, hardwood cuttings of Atlas, Citation, Hansen 536, Lovell, Marianna 2624, Nemaguard, and Viking were rooted during fall and winter 1997/98 and will be used for greenhouse screens of genetic resistance to Phytophthora crown and root rot in 1998. The Lovell, Nemaguard, and Marianna 2624 were included as important standards.

Results and discussion. In the first fungicide experiment, we did not detect *Phytophthora* in the copper sulfate-treated or non-treated control water pockets (data not shown). The experiment will be repeated in 1997/98 with modifications. In the second experiment, the topical spray

treatment of Ridomil on existing cankers slowed but did not arrest canker expansion (Table 9). No results are yet available from the large-scale experiment, but the work will continue and involve repeated applications of the materials according to the registered label schedules. If any of the labeled treatments is effective, it would be immediately available to the growers.

In the screening test with open-pollinated rootstock seedlings, plants from plum backgrounds were relatively resistant to Phytophthora root and crown rot, whereas susceptibility tended to increase successively as backgrounds went from plum to peach, almond x peach, and almond, which was highly susceptible (Figs. 3,4).

Later this year, methods used in the seedling screen will be adapted to begin evaluations of resistance to *Phytophthora* among the clonal rootstocks that were propagated as hardwood cuttings. Repeated evaluations of the clonal rootstocks' resistance to *Phytophthora* under various conditions will be necessary before confidence in assessments of the resistance is justified among growers that are making rootstock choices. Nevertheless, greenhouse screens of the clonal selections should provide valuable information about resistance to *Phytophthora* among peach, peach x almond hybrid, peach x plum hybrid, plum hybrid, and peach-almond-plum-apricot hybrid backgrounds.

Reference

1. 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. Almond orchards surveyed for cankers and results of isolations from "lethal-type"[a] and "pruning-wound-type"[b] cankers in Kern County, 1997.

Ranch no.	Method of irrigation	Irrig. water deposited on trunks directly?	No. of cankers yielding a *Phyt.* sp. / total no. cankers[c]	Species of *Phytophthora* isolated	Respective isolation percentages of *Phytophthora* spp.
1-T	microsprinkler	yes	29/42[a]	*P. cactorum, P. citricola*	17, 83
2-Z	microsprinkler	no	36/72[a]	*P. citricola*	100
3-N	flood	no	11/26[a]	*P. cactorum, P. citricola*	91, 1
4-S	microsprinkler	on some	2/28[b]	*P. syringae*	100
5-K	flood	no	1/3[a]	*P. cactorum*	100
6-R	flood	no	7/13[a,b]	*P. cactorum, P. syringae*	71, 29

[c]To detect *Phytophthora* spp., bark samples were collected from the margins of cankers and cultured in PARP selective medium. Subsequent identifications to species level were based on morphology of isolate the isolates.

Table 2. Pathogenicity and relative virulence of *Phytophthora cactorum* and *P. citricola* isolates from lethal cankers and *P. megasperma* isolates from root crowns[a].

Species of Phytophthora	Isolate no.	Percentage of root crown length rotted		Percentage of roots rotted	
		Almond	Nemaguard	Almond	Nemaguard
Control (non inoculated)	--	0 d	0 c	1 c	3 c
P. cactorum	1040	9 cd	1 c	21 c	3 c
	1098	50 b	6 bc	67 b	11 c
	1100	70 ab	4 bc	82 ab	7 c
P. citricola	819	100 a	41 a	100 a	55 b
	1215	80 ab	31 ab	100 a	24 c
	1217	68 ab	14 abc	77 ab	14 c
P. megasperma	188a	42 bc	39 a	85 ab	72 ab
	1302	60 ab	31 ab	98 a	83 a

[a]At 4 mo of age, potted peach and almond seedlings were transplanted into noninfested soil (controls) or soil artificially infested with the indicated isolates of *Phytophthora*. Starting 1 wk after transplanting, the seedlings were subjected to biweekly 48-hr periods of flooding to stimulate disease development. Three months after transplanting, the roots were washed free from soil and assessed for incidence and severity of disease.

Table 3. Summary of 1997 Kern County almond canker categorizations according to probable site of tree infection.

Category of canker (general part of the tree where infection probably originated)	Ranch designation and percent of cankers in category*			
	Ranch 1-T** (n=33)	Ranch 2-Z (n=70)	Ranch 3-N (n=21)	Ranch 4-S (n=79)
Subterranean (crown rot)	27	11	76	1
Aerial (general above-ground)	73	89	24	99
(trunk canker)	(6)	(24)	(9)	(1)
(water pocket canker)	(18)	(20)	(0)	(2)
(pruning wound canker)***	(0)	(1)	(0)	(89)
(scaffold canker, no p.w.)	(0)	(13)	(9)	(0)

*n= the total number of cankers; *Phytophthora* spp. isolated included: 1-T, *P. cactorum* and *P. citricola* at ranch 1-T, *P. citricola* at ranch 2-Z, *P. cactorum* at ranch 3-N, and *P. syringae* at ranch 4-S.

**ratio of aerial to subterranean infections greater for *P. citricola* than for *P. cactorum* (chi-square, P=0.001).

***pruning wound cankers ceased development by June.

Table 4. Incidence of lethal Phytophthora canker according to almond cultivar, ranch 1-T.

Variety	Total no. of trees surveyed	"Recent"[a] incidence of lethal canker incidence (%)[b]
Butte	496	1.8
Nonpareil	972	2.2
Price	471	1.1

[a] Trees killed by lethal cankers and removed before survey were not included.
[b] Chi square analysis: no evidence for differential susceptibility to lethal cankers (P>0.25).

Table 5. Incidence of lethal Phytophthora canker according to almond cultivar, ranch 2-Z.

Variety	Total no. of trees surveyed	"Recent"[a] incidence of lethal canker incidence (%)[b]
Fritz	355	3.3
Nonpareil	722	3.0
Sonora	416	3.8

[a] Trees killed by lethal cankers and removed before survey were not included.
[b] Chi square analysis: no evidence for differential susceptibility to lethal cankers (P>0.25).

Table 6. Results summary of 1997 efforts to detect possible sources of primary inoculum of *Phytophthora* spp. that are causing lethal cankers in Kern County.

Potential source of inoculum	Detection method	Results
Soil	almond seedlings	(+) *P. cactorum, P. citricola*
Water-pocket debris	pear-fruit baits	(-)
	leaf-disk baits	(-)
	direct plating	(-)
	almond seedling baits	(+) *P. cactorum*
Pick up machine debris	peach seedling baits	(+), 1/169 samples, *P. citricola**

*A repeat (1998) experiment (still in progress) confirms our previous (1997) detection of *P. citricola* in debris that was deposited in trees by a nut pick-up machine during normal harvest operations.

Table 7. Effect of a post-infection topical spray with Ridomil Gold EC on expansion of cankers caused by *Phytophthora.*

Treatment	No. of trees treated	Avg. canker length increase (cm)*
Control (not sprayed)	6	9 a
Ridomil Gold EC spray (wetted thoroughly with 1:12, formulation:water)	8	5 b

*Means without letters in common differ significantly (DMRT, P=0.05), repeated measures model. Treated 5/14/97, measurements averaged from 6/27 and 12/1.

Table 8. Treatments applied 10/30/97 in large-scale replicated trial on control of lethal Phytophthora cankers in almond, Kern County*.

Trt. no.	Material(s) (and type of action)	Trt. dates	Formulation mixture and amount per sprayed acre
1.	Control	--	(no spray)
2.	Kocide 101 (a.i. copper hydroxide; protectant)	10/30/97	8 lb. form. in 175 gal. water applied per sprayed A, air blast, complete coverage.
	Bordeaux (a.i. copper sulfate+hydrated lime; protectant)	12/24/97	10-10-100, 175 gal of mix applied per sprayed A, air blast, complete coverage.
3.	Ridomil Gold EC (a.i. metalaxyl, systemic fungicide)	10/30/97	2 qts. in 250 gal. water applied per sprayed A, banded on ground (6 ft wide) on each side of tree row, partial ground spray
4.	Nutri-Phite P foliar (4-30-8 foliar fertilizer, side benefits?)	10/30/97	4 pts. in 175 gal. water applied per sprayed A, airblast, complete coverage

*Each treatment applied to a total of 210 trees among 5 replicate blocks. Treatments will be repeated as per product labels.

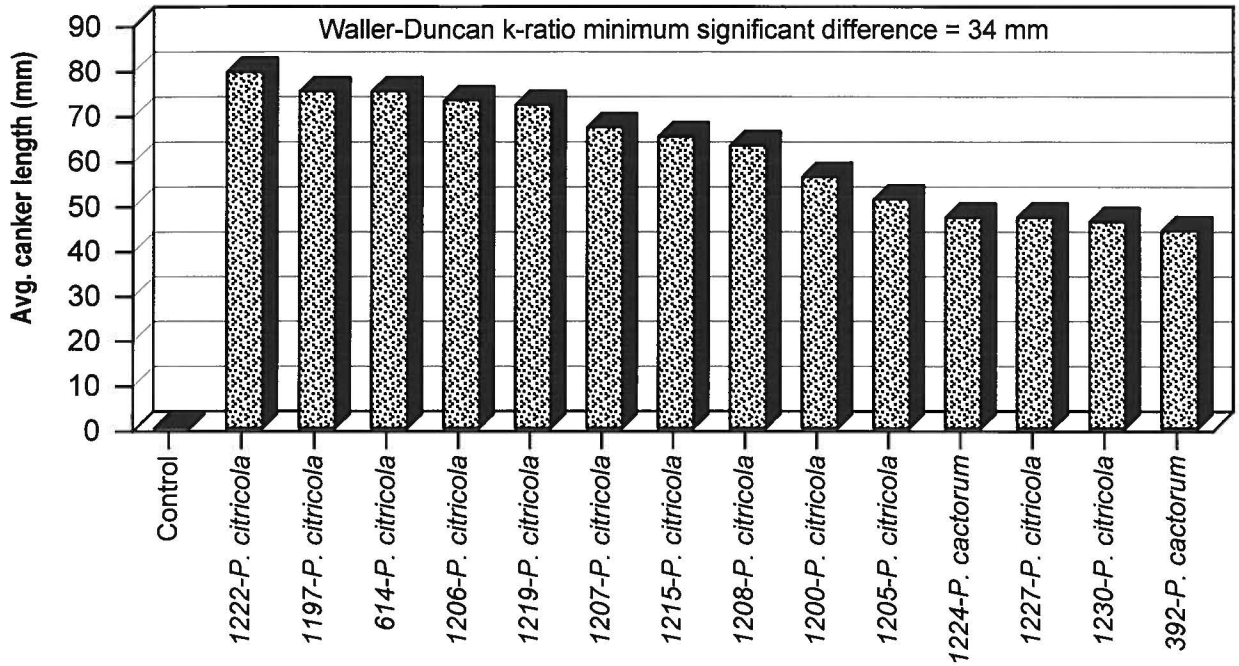

Figure 1. Pathogenicity of lethal canker isolates of *Phytophthora cactorum* and *P. citricola* in "1-yr-old" excized almond shoots. (n=6).

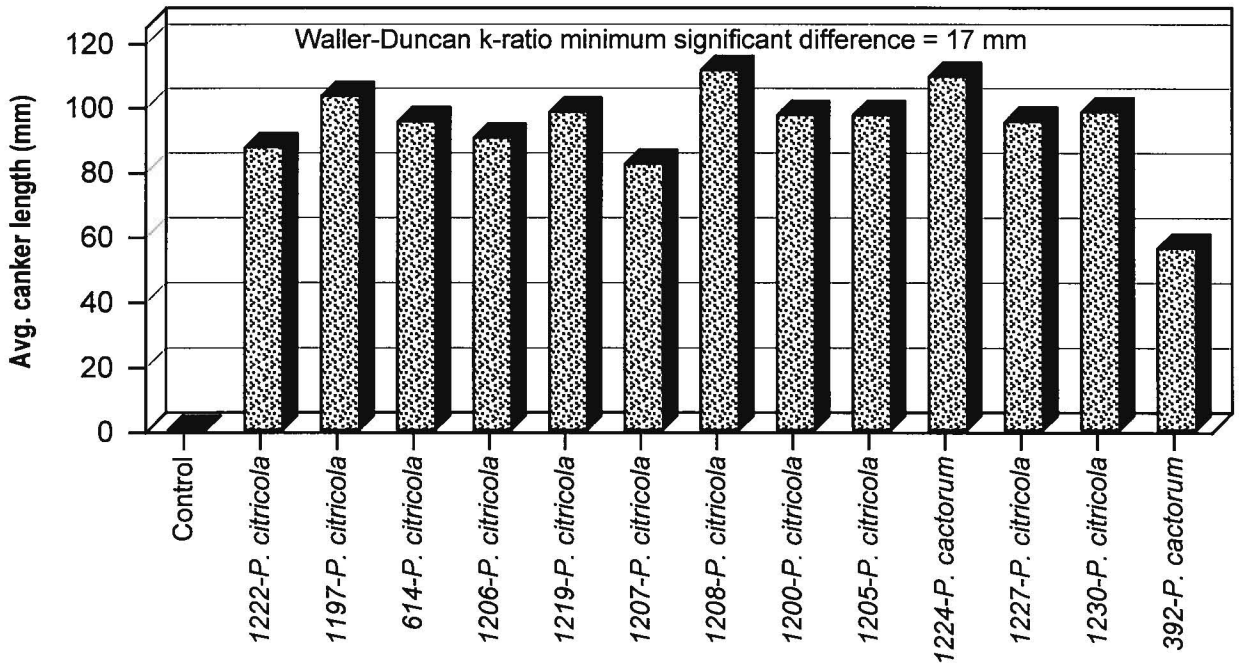

Figure2. Pathogenicity of lethal canker isolates of *Phytophthora cactorum* and *P. citricola* in "current-season" excized almond shoots (n=6).

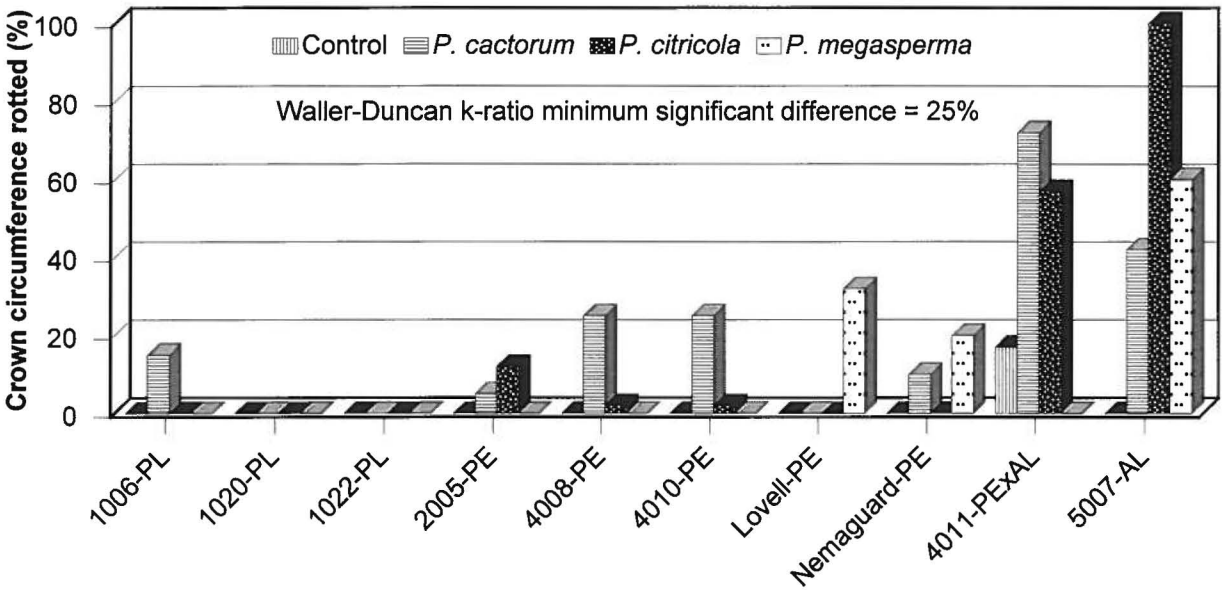

Figure 3. Relative resistance of open pollinated seedlings from maternal sources of plum (PL), peach (PE), peach x almond (PExAL) and almond (AL) to crown rot caused by three species of *Phytophthora* that affect almonds in California.

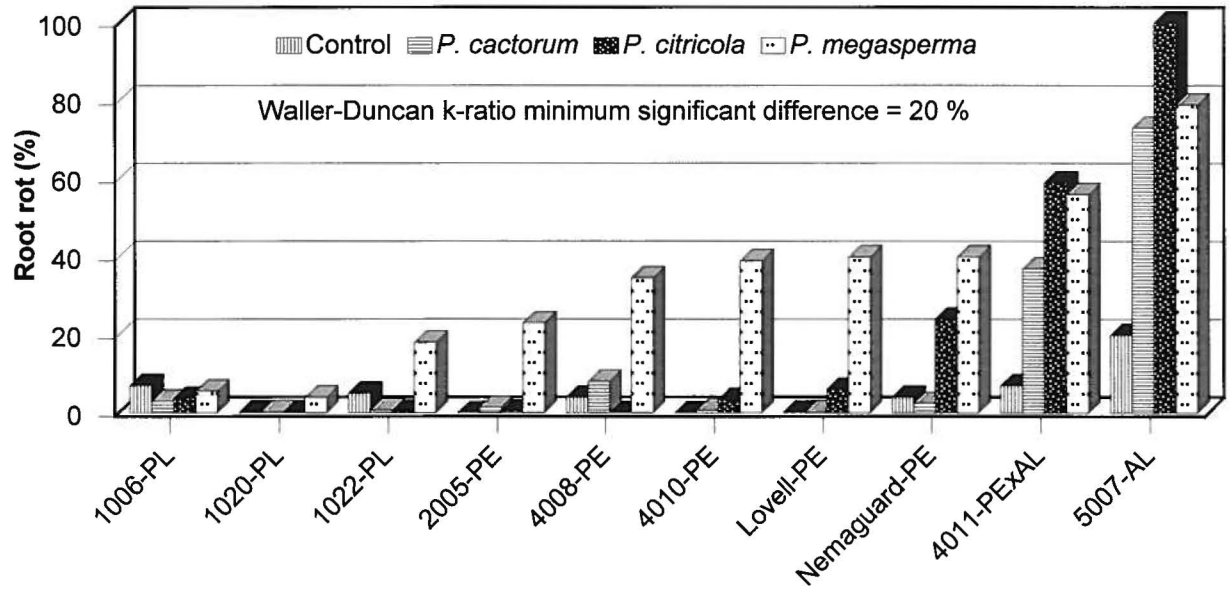

Figure 4. Relative resistance of open pollinated seedlings from maternal sources of plum (PL), peach (PE), peach x almond (PExAL) and almond (AL) to root rot caused by three species of *Phytophthora* that affect almonds in California.