Epidemiology and Management of Phytophthora Root and Crown Rot of Almond in California

Project No.: PATH15-Adaskaveg/Browne

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A. Summary

Highly efficacious, non-phytotoxic alternative fungicides with different modes of action that can be applied as soil chemigation treatments are being developed for managing Phytophthora root and crown rot of almond. Alternatives to mefenoxam and phosphites are needed because soil fumigations are being greatly restricted, and resistance has been reported to both fungicides in *Phytophthora* species on other tree crops. The new fungicides oxathiapiprolin (Orondis), mandipropamid (Revus), fluopicolide (Presidio), and ethaboxam (Intego), all with different modes of action (FRAC codes 49, 40, 43, and U5, respectively), were highly toxic in vitro against 11 *Phytophthora* species from almond in California. Oxathiapiprolin had the highest activity with EC_{50} values for mycelial growth inhibition of <0.001 mg/liter. In two field studies at UC Davis with soil-inoculated almond trees on Hansen or Nemaguard rootstocks, Orondis was the most effective fungicide in reducing trunk gumming and canker formation. In the second study where more frequent applications were done, Presidio also was very effective. *P. cactorum* was determined to be the main pathogen in these trials. In studies in commercial orchards, *P.* sp. *ax* was the main pathogen species involved. Orondis was most effective when applied soon after tree planting, but management strategies for this aggressive pathogen still need to be improved. Systemic activity of Orondis with uptake into stem tissues after soil application was demonstrated in greenhouse studies with potted plants. This explains the long-lasting efficacy of the fungicide and its potential to manage Phytophthora crown and trunk cankers using soil applications. Orondis was federally registered on almonds in Dec. 2020 (California registration is pending). California registrations of Revus and Presidio is ongoing. The new species *P.* sp. *ax was* found to be highly virulent in greenhouse inoculation studies, and this supports field observations where disease caused by this species progresses very rapidly and often results in tree death.

B. Objectives and Milestones

I. In vitro toxicity of new fungicides against *Phytophthora* species from almond and characterization of the species.

a) Baseline sensitivities and characterization of *Phytophthora* species**. *Significant findings:*** *Oxathiapiprolin, mandipropamid, fluopicolide, and ethaboxam were highly inhibitory to mycelial growth of 94 isolates of Phytophthora from almond belonging to 11 species.*

Oxathiapiprolin was the most toxic with EC_{50} values of <0.001 mg/liter. The baselines being created can be used as references in future resistance monitoring.

b) Comparative virulence of species. ***Significant findings:*** *In inoculation studies, P. sp. ax and P. niederhauserii were the most virulent on almond. Both have a high temperature optimum for growth of 30C.*

c) Resistance potential. ***Significant findings:*** *Studies with outliers of isolates that tolerate higher concentrations of some new fungicides are ongoing to determine the resistance potential.*

II. Evaluate oxathiapiprolin, mandipropamid, fluopicolide, and ethaboxam for the management of root and crown rot of almond in field studies. ***Significant findings:***

a) *In two studies at UC Davis, Orondis (oxathiapiprolin) was highly effective in reducing the incidence of Phytophthora crown rot after inoculation of trees. In the second trial where more frequent applications were done, Presidio (fluopicolide) also reduced the disease to zero levels, and ProPhyt was also very effective. Disease in these studies was caused mostly by P. cactorum.*

b) *In studies in commercial orchards with P. sp. ax as the main pathogen, Orondis using the high-label rate of 9.6 fl oz/A was the most effective in reducing the incidence of crown rot. There was an indication that treatment programs that are started as soon as possible after planting \were more beneficial than programs that are initiated later, especially when an aggressive species such as P. sp. ax is involved*.

c) *Registration update. Orondis was federally registered on almond in Dec. 2020 with California registration pending. California registrations for Revus and Presidio are ongoing.*

d) *Systemic movement of oxathiapiprolin was demonstrated in potted almond plants. Soil applications significantly reduced the severity of trunk cankers after inoculation. Systemic activity in plants can explain the long-lasting efficacy of Orondis after soil application and its efficacy against Phytophthora trunk cankers.*

C. Results and Discussion

I. Evaluate the in vitro toxicity of new fungicides against *Phytophthora* species occurring on almond and characterize the species. We continued orchard surveys for almond trees potentially infected by *Phytophthora* spp. in collaboration with farm advisors, growers, and PCAs. Currently, we have 94 isolates of *Phytophthora* from almond in our collection, and 26 of these were obtained since fall 2020. Isolates belong to 11 species with currently between 1 and 28 isolates per species (Table 1). The five major species were *P. cactorum, P. citricola* complex*, P. niederhauserii*, *P. syringae*, and *P*. sp. *ax.* The new taxon *P*. sp. *ax* was found at several locations which is a concern because it is highly aggressive, similar to *P. niederhauserii* (see below). All isolates were most sensitive to oxathiapiprolin with EC_{50} values for mycelial growth inhibition of <0.001 mg/liter. A rather narrow range of low EC_{50} values (0.001 to 0.009 mg/liter) among all isolates was also found for mandipropamid. Values for ethaboxam were approximately 10-fold higher than for mandipropamid. For fluopicolide, some isolates of *P. cactorum, P. megasperma*, and *P. syringae* had EC_{50} values $\geq$0.2 ppm. Still, EC_{50} values are contiguous with no obvious outliers, and all isolates can be considered sensitive to all of these new fungicides. All isolates were also sensitive to mefenoxam with EC_{50} values between 0.002 and 0.209 ppm. Phosphite sensitivity evaluations are ongoing. We will continue to collect additional isolates of *Phytophthora* spp. to establish baseline ranges.

Baseline sensitivities of non-exposed organisms are important because they can be used as references in future resistance monitoring.

Table 1. In vitro sensitivity of mycelial growth of *Phytophthora* isolates from almond to five Oomycota fungicides

Species	No of isolates**	EC_{50} value ranges for mycelial growth (µg/ml)*				
		Mefenoxam	Oxathiapiprolin	Mandipropamid	Ethaboxam	Fluopicolide
P. cactorum	8 (5)	0.005 - 0.018	0.0005 - 0.0007	0.004 - 0.009	0.013 - 0.079	0.111 - 0.275
P. chlamydospora	1	0.017	0.0003	0.002	0.053	0.035
P. citricola/citricola complex	(10)	0.087 - 0.164	0.0003 - 0.0005	0.005 - 0.007	0.046 - 0.158	0.046 - 0.069
P. gonapodyides	3	0.004 - 0.015	0.0003 - 0.0004	0.003 - 0.004	0.019 - 0.047	0.026 - 0.072
P. lacustris	2	0.003 - 0.004	0.0002 - 0.0004	0.002 - 0.004	0.054 - 0.137	0.015 - 0.025
P. megasperma	4	0.010 - 0.013	0.0003 - 0.0005	0.002 - 0.005	0.040 - 0.079	0.082 - 0.240
P. niederhauserii	28 (4)	0.012 - 0.209	0.0001 - 0.0004	0.003 - 0.009	0.031 - 0.105	0.041 - 0.070
P. obscura	1	0.003	0.0003	0.002	0.033	0.018
P. rosacearum	1	0.105	0.00027	0.004	0.060	0.060
P. syringae	18 (6)	0.002 - 0.043	0.0002 - 0.0004	0.001 - 0.006	0.017 - 0.190	0.021 - 0.318
P. sp. *ax*	18 (1)	0.007 - 0.038	0.0001 - 0.0004	0.002 - 0.007	0.006 - 0.036	0.034 - 0.078

* - In vitro sensitivity was determined using the spiral gradient dilution method.

** - Total number of isolates of each species. Numbers in parentheses are isolates obtained since fall 2020.

The temperature optimum for growth of *P.* sp. *ax* was evaluated in laboratory studies and was determined to be 30C. Growth at 35C was significantly higher than at 25C. Thus, this species is well adapted to the hot California climate, and this is supported by our field observations where disease caused by *P*. sp. *ax* progresses rapidly in the summer season. A high temperature optimum was determined by others also for *P. niederhauserii*.

In greenhouse and growth chamber studies, the virulence of five *Phytophthora* species was compared in wound-inoculations of rootstock and scion portions of the trunks. Plants were incubated at temperatures based on the optimum for mycelial growth of the species used for inoculation (i.e., 12C for *P. syringae*, 25C for *P. cactorum* and *P. citricola* complex, 30C for *P.* sp. *ax* and *P. niederhauserii*). *P.* sp. *ax* and *P. niederhauserii* were the most virulent, and plants were dead two to three weeks after inoculation (Table 2). Numerically, cankers were generally largest for *P. niederhauserii*, and there was variability among the other species in rootstock and scion measurements. The maximum canker length for *P. niederhauserii* and *P.* sp. *ax* was 360.0 and 366.0 mm, respectively, as compared with 93.8 mm for *P. cactorum* (all on the scion). Cankers progressed more rapidly on the Nonpareil scion than on either

Table 2. Virulence of five *Phytophthora* species on Nonpareil almond grafted to Hansen and Brights rootstocks in greenhouse studies

Species inoculated	Tempe-rature	Nonpareil grafted to Hansen				Nonpareil grafted to Brights			
		Rootstock		Scion		Rootstock		Scion	
		Canker length		Canker length		Canker length		Canker length	
		mm	LSD	mm	LSD	mm	LSD	mm	LSD
P. syringae	12C	55.5	b	93.3	a	78.0	b	93.8	b
P. cactorum	25C	96.0	ab	265.5	a	108.5	a	240.3	a
P. citricola complex	25C	99.5	ab	226.8	a	74.5	b	275.8	a
P. sp. *ax*	30C	75.3	ab	366.0	a	119.0	a	357.8	a
P. niederhauserii	30C	117.8	a	319.0	a	104.0	a	360.0	a

The trunks (8 to 12 mm in diameter) of potted 1- to 1.5 year-old trees were wounded (6 mm diameter, cambium was exposed) on the rootstocks and scions and inoculated with a mycelial agar plug that was then wrapped with Parafilm. Plants were kept at selected temperatures with a ca. 12-h photoperiod. Parafilm was removed after 7 to 9 days, and plants were evaluated after another one or two weeks. The maximum canker length was determined at each inoculation site by removing the upper tissue layers. Statistical comparisons are by column.

rootstock. *P. syringae* was generally the least virulent and developed the smallest cankers. These data support field observations where *P. sp. ax* and *P. niederhauserii* are reported as very virulent, and disease caused by *P.* sp. *ax* often results in tree death.

II. Evaluate oxathiapiprolin, mandipropamid, fluopicolide, and ethaboxam in field studies for the management of Phytophthora root rot of almond and compare to mefenoxam and potassium phosphite. In our final evaluation of the first trial at UC Davis in Sept 2019 (two years after the last of two applications), 30% of control trees showed gumming on the lower trunk that often led to tree death (Fig. 1, Plot 1). Isolations indicated that the disease was mostly caused by *P. cactorum*, one of the four species that were used for inoculation. Orondis at three rates was the most effective treatment with ≤5% of trees diseased. Revus and Presidio/Intego only numerically reduced the incidence of disease, and

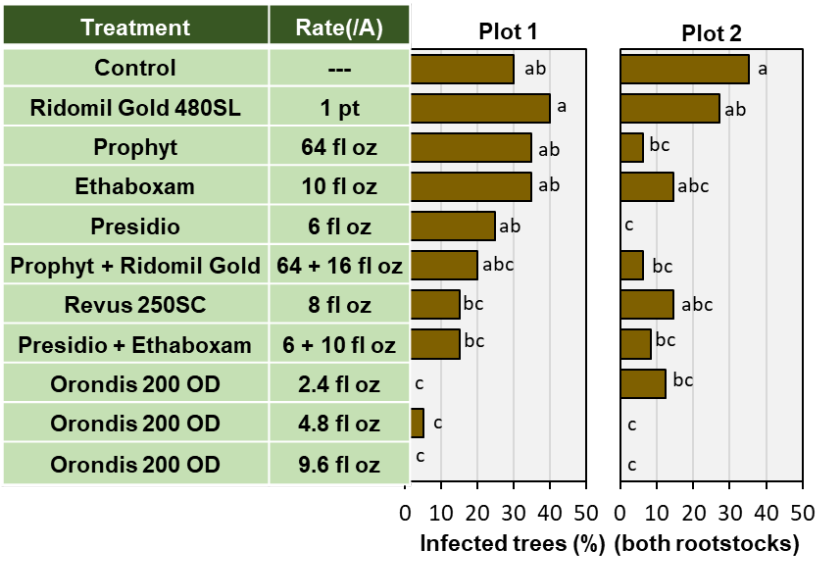

Fig. 1. Efficacy of new and registered fungicides for managing Phytophthora crown rot of almond at UC Davis. Plot 1 was planted in 2016, inoculated, treated July 2016 and 2017, and evaluated in 2019. Plot 2 was planted in Oct. 2017, inoculated, treated Dec. 2017, Oct. 2018, May 2019, and March 2020, and evaluated in Sept. 2020. The observed crown rot was determined to be caused by *P. cactorum*.

Table 3. Evaluation of new fungicides for management of Phytophthora root and crown rot of Nonpareil almond on Krymsk rootstock - Sutter Co. 2020

			Applications		Incidence of diseased trees**	
No.	Treatment*	Rate/A	2-11-20	4-14-20	%	LSD^
1	Control	---	---	---	65.2	a
2	Presidio	6 fl oz	@	@	55.6	ab
3	Orondis 200 + Ridomil Gold	4.8 + 16 fl oz	@	@	43.1	b
4	Orondis Ultra	5.5 fl oz	@	@	38.9	b
5	Orondis 200	9.6 fl oz	@	@	16.7	c

* - The orchard was established in Feb. 2020 with bare root plants at a site where previously planted trees had died from Phytophthora crown rot. Treatments were applied on 2-11 (one week after planting) and 4-14-20 to the soil around the base of the tree and over the trunk. The soil at the base of each tree was inoculated on 5-5-20 with *P. cactorum* and *P*. sp. *ax.*

** -Tree trunks were evaluated on 9-9-20 for the presence of gumming and cankers. Re-isolations indicated that *P.* sp. *ax* was the causal pathogen.

Presidio or Intego by themselves as well as Prophyt and Ridomil Gold were no longer effective. We concluded that Orondis has a long residual life possibly by penetrating the plant tissues, and that the other fungicides will have to be applied more frequently to obtain high efficacy.

In the second plot at UC Davis, isolations from crown-rotted tissues also yielded *P. cactorum*. Orondis at 4.8 and 9.6 fl oz/A completely prevented disease development, but Presidio-treated trees also did not show any crown rot symptoms (Fig. 1, Plot 2). Prophyt, ProPhyt-Ridomil Gold, and Presidio-Ethaboxam showed intermediate efficacy, whereas Revus and Ethaboxam only numerically reduced disease incidence from the control. Four fungicide applications were done in this plot, and this probably contributed to the improved efficacy of Presidio and ProPhyt as compared to the first plot. Ethaboxam (Intego) was not very effective by itself, but in a mixture with Presidio, it may still help in preventing resistance development. Ethaboxam had a similar in vitro toxicity as fluopicolide (Table 1) and therefore, it may interact with soil components or not persist long enough when applied annually or semi-annually for the management of root and crown rots. Based on our studies, Orondis was federally registered on almond in Dec. 2020 (California registration is pending in 2021). Mandipropamid and fluopicolide registrations are ongoing. Thus, in the near future, almond growers will have an increased selection of fungicides available for Phytophthora disease management. Orondis and Presidio, when properly used, will improve orchard establishment, and maintain tree health. The label that we requested for Revus will be for a nursery treatment for potted almond plants similar to its current registration for potted citrus plants. We are also suggesting a premixture of oxathiapiprolin and mefenoxam known as Orondis Gold or co-pack product.

Table 4. Evaluation of new fungicides for management of Phytophthora root and crown rot of Nonpareil almond on Krymsk rootstock - Sutter Co. 2020

			Applications				Incid. of diseased trees**	
No.	Treatment*	Rate/A	Pre-plant	6-12-19	9-18-19	4-14-20	%	LSD^
1	Control	---	---	---	---	---	47.6	a
2	Revus	8 fl oz	@	---	---	@	33.3	ab
	Orondis 200	9.6 fl oz	---	---	@	---		
3	Orondis 200	4.8 fl oz	---	@	---	---	20.8	b
	Orondis 200	9.6 fl oz	---	---	@	@		
4	Revus	8 fl oz	@	---	---	---	16.7	b
	Orondis 200	4.8 fl oz	---	@	---	---		
	Orondis 200	9.6 fl oz	---	---	@	---		
	Presidio	6 fl oz	---	---	---	@		

* - The orchard was established on 4-29-19 using potted plants. Trees were inoculated at planting and on 5-5-20 with a mixture of *P. cactorum* and *P.* sp. *ax.* Pre-plant treatments were applied to the potted trees. Field treatments were applied to the soil around the trunk and over the trunk.

** -Tree trunks were evaluated on 9-9-20 for the presence of gumming and cankers. Re-isolations indicated that *P.* sp. *ax* was the causal pathogen.

Three studies in commercial orchards are currently in progress, two in Sutter Co. where trees were inoculated, and one with natural inoculum in Kern Co. A first evaluation was conducted in the two Sutter Co. locations. In the orchard that was planted in late winter 2020,

65.2% of control trees already showed trunk gumming by early Sept. 2020 (Table 3). The incidence of disease was significantly reduced from the control by Orondis, Orondis-Ultra, and Orondis-Ridomil Gold treatments. Orondis using the high-label rate of 9.6 fl oz/A was the most effective with an incidence of diseased trees of 16.7%. Rates of Orondis in the Orondis-Ridomil Gold mixture (i.e., 4.8 fl oz) and in the premixture of Orondis and Revus (i.e., Orondis Ultra) seem to be not high enough for high disease control.

A combination of a pre-plant treatment with Revus and field treatments with Orondis or a rotation of Orondis with Presidio was evaluated in the second orchard in Sutter Co. Among control trees, 47.6% showed symptoms of crown and trunk gumming and cankers in early Sept. 2020 (disease was not observed earlier that year) (Table 4). Trees that did not receive a field treatment until 19 weeks after planting and inoculation also had a high incidence with 33.3% symptomatic trees, and this was not significantly different from the control. Disease on trees with or without pre-plant treatments that received Orondis treatments 6 and 19 weeks after planting and a treatment with Orondis or Presidio one year after planting developed statistically similar levels of disease with 20.8% or 16.7% incidence, respectively, and these treatment programs had significantly less disease than the control. No disease developed to date in the Kern Co. orchard that was established in 2020.

In summary, a high efficacy of selected treatments was obtained in the UC Davis trials. Phytophthora crown and trunk gumming, and cankers were also significantly reduced at two commercial locations, however, the overall efficacy was lower. *P. cactorum* was the main pathogen in the UC Davis studies, whereas *P.* sp. *ax* was recovered from diseased trees in Sutter Co. As indicated by field observations and confirmed in our greenhouse studies, *P.* sp. *ax* is a highly virulent pathogen, and this may explain the differences in treatment efficacy. Treatments with Orondis or a rotation of Orondis with Presidio that were applied 6 weeks after planting and inoculation were more effective than a Orondis-Revus rotation that started after 19 weeks. This suggests that treatments should be done as soon as possible after planting, especially when an aggressive species such as *P.* sp. *ax* is involved. This should be evaluated in future studies. No phytotoxicity was observed after treatment with any of the new fungicides.

Table 5. Effect of a soil application with Orondis on canker formation on the trunk after inoculation with *P. cactorum* of potted Nonpareil almond grafted to Hansen and Brights rootstocks in greenhouse studies

	Nonpareil grafted to Hansen				Nonpareil grafted to Brights			
	Rootstock		Scion		Rootstock		Scion	
	Canker length		Canker length		Canker length		Canker length	
Treatment	mm	LSD	mm	LSD	mm	LSD	mm	LSD
Control	101.6	a	222.8	a	96.0	a	223.6	a
Orondis 9.6 fl oz	16.0	b	70.0	b	15.8	b	41.2	b

The soil of potted 1- to 1.5 year-old plants was treated with Orondis at the equivalent of a field rate of 9.6 fl oz/A. After 2 weeks, the trunks (8 to 12 mm in diameter) were wounded (6 mm diameter, cambium was exposed) on the rootstocks and scions and inoculated with a mycelial agar plug of *P. cactorum* that was then wrapped with Parafilm. Parafilm was removed after 7 to 9 days, and plants were evaluated after another two weeks. The temperature in the greenhouse ranged from 18 to 27C during the experimental period. The maximum canker length was determined at each inoculation site by removing the upper tissue layers. Statistical comparisons are by column.

The potential systemic activity of Orondis was evaluated in a greenhouse study where potted almond plants received a soil treatment that was followed after two weeks by wound inoculation of the rootstock and scion portions of the trunks with *P. cactorum*. Any reduction of canker length of treated plants as compared with control plants was used as an indication of systemic movement of Orondis. As shown in Table 5, canker length of treated plants was significantly reduced on the Hansen and Brights rootstocks as well on the Nonpareil scion portions of the trunks. Reductions in canker sizes ranged from 68.5% to 84.3%. This systemic activity of Orondis and uptake into tree roots can explain its long-lasting efficacy in field trials. We previously also demonstrated uptake of Orondis into citrus seedlings. Systemic movement in almond plants should also be evaluated on trees in the field by sampling small tissue pieces of the trunk of treated trees. Fungicide residues in tissue extracts are being determined using chemical analyses and bioassays that we developed previously for citrus. These findings have implications for the management of trunk gummosis and canker formation that we observed as major symptoms of *Phytophthora* sp. infections of almond trees in our surveys. Other observations of diseased trees indicate that infections do not seem to originate from the roots or the lower crown. If infections start on the trunk, systemic activity of a fungicide can potentially prevent infections. An additional trunk application may further help reduce the disease. These strategies need to be evaluated and are ongoing.

D. Outreach Activities

Dr. Adaskaveg participated at several grower/PCA meetings at different locations in California over the last two years and gave presentations on almond diseases including Phytophthora root and crown rot and their management. At each meeting, there were approximately 40-50 participants.

a. January 2019, Managing Diseases of Almond in California; Bayer Tree, Nut, and Vine Meeting; Organizer: Bayer CropScience; Universal Studios, Universal City, CA 91608
b. January 2019, Almond Diseases: Key Economic Pests, ID, Biology, and Treatments in Almonds; 2019 Independent PCA Symposium; Organizer: Bayer CropScience; Monterey Plaza Hotel and Spa, Monterey, CA 93940
c. January 2019, Review of Almond and Citrus Diseases of Economic Significance; Independent PCA Meeting; Organizer: Syngenta Crop Protection; Paso Robles, CA.
d. January 2019, Almond Disease Management, Colusa Winter Almond Meeting; Organizer: UCCE; Granzella's Banquet Hall, Williams, CA
e. Feb 5, 2019, Bloom and Foliar Diseases; Annual Almond Production Meeting; Organizer: UCCE; Norton Hall, Woodland, CA
f. Nov 8, 2019, Foliage, Blossom and Nut Diseases; Almond Short Course, Organizer UCCE; Visalia Convention Center, Visalia, CA

E. Materials and Methods:

I. Evaluate the toxicity of new fungicides against *Phytophthora* species occurring on almond and characterize the species.

a. *In vitro toxicity.* Sensitivities were determined using the spiral gradient dilution (SGD) method as described previously (Phytopathology 94:163-70. 2004).

b. *Virulence of selected Phytophthora species on almond.* **T**runks (8 to 12 mm in diameter) of potted 1- to 1.5-year-old trees were wounded (6 mm diameter, cambium exposed) on the rootstock and scion regions and inoculated with a mycelial agar plug that was then wrapped with Parafilm. Plants were kept at temperatures based on the species' optimum (i.e., 12C for *P. syringae*, 25C for *P. cactorum* and *P. citricola* complex, 30C for *P.* sp. *ax* and *P. niederhauserii*) with a 12-h photoperiod. Parafilm was removed after 7 days, and after another one or two weeks, the maximum canker length was determined at each inoculation site by removing the upper tissue layers.

II. Evaluate oxathiapiprolin, mandipropamid, fluopicolide, and ethaboxam in field studies for the management of Phytophthora diseases of almond.

a. *Field studies*. Two orchards with cv. Nonpareil on Hansen or Nemaguard rootstocks were established at UC Davis in 2016 and 2017, respectively. Inoculum was grown on an oat (or rice)-vermiculite-V8 juice substrate. Trees were inoculated at the root zone with *P. cactorum*, *P. citrophthora*, and *P. cambivora* in the first plot and *P. cactorum*, *P. citrophthora*, and *P. niederhauserii* in the second plot. In the first plot, treatments were applied around each tree trunk in July 2016 (after planting) and July 2017. In the second plot, they were applied Dec 2017 (after planting), Oct. 2018, May 2019, and March 2020. A tree was considered infected by *Phytophthora* sp. if profuse, amber-colored gumming was present at the lower parts of the trunk, often associated with canker formation. The presence of the pathogen was confirmed by culturing from infected tissues. Disease incidence was based on the number of diseased trees of the total number of trees.

One commercial trial in Sutter Co. was done in an orchard planted April 29, 2019. Trees were inoculated at planting and May 5, 2020 with a mixture of *P. cactorum* and *P.* sp. *ax*. Pre-plant treatments were applied to some trees. Field treatments were applied to the soil around and over the trunk. The second orchard was established in Feb. 2020 at a site where previously planted trees had died from Phytophthora crown rot. Treatments were applied Feb. 11 (one week after planting) and April 14, 2020, to the soil around the tree base and over the trunk. Soil inoculations were done May 5, 2020 with *P. cactorum* and *P.* sp. *ax*. Data were analyzed using analysis of variance and multiple mean separation methods of SAS ver. 9.4.

b. *Trunk inoculations of treated trees to determine internal movement of fungicides from soil applications.* The soil of potted plants in a greenhouse (18 to 27C temperature range) was treated with Orondis at the equivalent of a field rate of 9.6 fl oz/A. After 2 weeks, plants were wound-inoculated with *P. cactorum* as described above. Evaluation and data analyses were done as indicated above.

F. Publications that emerged from this work

1. This research is ongoing. Information obtained will be summarized in manuscripts for publication once studies are repeated and all objectives have been completed.
2. Our outreach is the form of posters and presentations at the Annual Almond Board Conference and in UCANR and other extension meetings (listed above).