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

Project No.: **17-PATH15-Adaskaveg/Browne**

Project Leaders: J. E. Adaskaveg
Dept. of Microbiology and Plant Pathology
2317 Webber Hall
200 Office University Building
University of California, Riverside
Riverside, CA 92521
(951) 827-7577
jim.adaskaveg@ucr.edu

Greg T. Browne
USDA-ARS, Crops Pathology & Genetics Research Unit
Department of Plant Pathology, UC Davis
One Shields Ave
Davis, CA 95616
Gregory.Browne@ars.usda.gov

Project Cooperators and Personnel:
D. Thompson, UC Riverside
H. Förster, UC Riverside
W. Hao, UC Riverside
F. Trouillas, UC-KARE
D. Doll, UCCE - Merced County

Objectives

I. Evaluate the in vitro toxicity of new fungicides against *P. cactorum* and *P. niederhauseri* and establish baseline sensitivities.
II. Evaluate oxathiapiprolin, mandipropamid, fluopicolide, and ethaboxam for the management of root of almond in field studies and compare efficacy to the registered mefenoxam and potassium phosphite.
   A. Establish two almond orchards on Hansen and Nemaguard rootstocks and inoculate with *P. cactorum, P. citrophthora,* and/or *P. niederhauseri.*
   B. Apply treatments semi-annually, evaluate for incidence of crown rot and root infection, and enumerate soil populations of the pathogen.
   C. Evaluate trees for phytotoxicity and measure plant growth.

Interpretive Summary:

Phytophthora root rot and crown rot of almond can be caused by several species of *Phytophthora* including *P. cactorum, P. cambivora, P. cryptogea*, *P. megasperma*, and the recently described *P. niederhauseri* that is present at high incidence at some locations. The disease is widely distributed and may cause high levels of tree death and decline in newly planted orchards. The severity and rate of disease development depends on the rootstock and the species of *Phytophthora* involved, but the disease is most severe in waterlogged soils with

poor drainage. Depending on the *Phytophthora* species, survival may be as chlamydospores, oospores, and/or hyphae in plant debris in the soil. Under proper conditions, hyphae start growing or chlamydospores and oospores germinate to produce sporangia that contain zoospores - the main infective propagules. The zoospores are motile in water, they are attracted by root exudates, and they typically infect feeder roots. Management of root rot includes the use of tolerant rootstocks, proper irrigation, and the use of mefenoxam and phosphonate (e.g., potassium phosphite, fosetyl-Al) fungicides. Several new fungicides (mandipropamid - Revus, fluopicolide - Presidio, ethaboxam - Intego, and oxathiapiprolin - Orondis) with high activity against *Phytophthora* species have recently become available for evaluation. All have different modes of action (different FRAC codes) and their respective registrants are supporting registration on almond. The effectiveness of these fungicides for the management of Phytophthora root and crown rots of almond is being evaluated in comparison to mefenoxam and potassium phosphite in field studies. In our evaluations, a total of 18.8% of untreated control trees were dead in a July 2018 evaluation (27.5% of trees on Hansen and 10% of trees on Nemaguard rootstock). Among new fungicides oxathiapiprolin (Orondis) was identified as the most effective with currently no tree death observed, and only one tree showed gumming and a trunk canker. The other three fungicides were highly effective on Hansen rootstock, but on Nemaguard, no significant differences in tree death were found among all treatments and the control. Additional tree evaluations will be done of this plot.

Materials and Methods:

I. Evaluate the in vitro toxicity of new fungicides against selected *Phytophthora* species occurring on almond and other tree crops in California. Eighty isolates of *Phytophthora* were obtained from almond, walnut, or cherry scions, crowns, bark, trunks, roots, or from orchard soil. Species were identified by sequencing of ITS and cox genomic regions and comparison with sequences in GenBank. In vitro sensitivities were determined using the spiral gradient dilution (SGD) method as described previously (Forster et al., Phytopathology 94:163-70. 2004). Mycelial inoculum for the SGD plates was grown on cellophane strips. Fungicide solutions were applied to 15-cm V8C agar plates with a spiral plater using the exponential deposition mode. Mycelium-covered strips were then placed radially along the fungicide concentration gradient. Effective fungicide concentrations or EC_{50}, where 50% growth inhibition is observed, was determined after 3-4 days of incubation using a computer program.

II. Evaluate oxathiapiprolin, mandipropamid, fluopicolide, and ethaboxam in field studies for the management of Phytophthora root of almond and compare to mefenoxam and potassium phosphite. For field studies, an orchard was established at UC Davis in May 2016 with Nonpareil scions on Hansen and Nemaguard rootstocks. A randomized complete-block design with 2 trees of each rootstock (4 trees of each variety for the control) per each of 10 replications per treatment was used. The rhizosphere was inoculated with *P. cactorum*-infested oat seeds in July and Oct. 2016, and with a mixture of *P. cactorum, P. cambivora*, and *P. citrophthora* in March and July 2017. Treatments including mandipropamid (Revus), oxathiapiprolin (Orondis), and mefenoxam (Ridomil Gold) (all Syngenta Crop Protection), fluopicolide (Presidio) and ethaboxam (Intego – both Valent USA), and potassium phosphite (Prophyt - Luxembourg), and selected mixtures at recommended rates were applied to the soil around trees 2 weeks after the first inoculation and in July 2017. The irrigation schedule was adjusted so that the soil in the root zone was exposed to frequent

wetness periods. To evaluate treatment efficacy, soil samples were taken in May 2017, and soil plating and pear baiting were done to determine *Phytophthora* populations. For soil dilutions, 20 g of soil was mixed well with 200 ml of water, 1 ml of the suspension was plated onto PARPH selective medium, and the number of *Phytophthora* colonies was enumerated after 3 days. For pear baiting, 70 g of soil and 200 ml water were placed in a beaker, one green pear (cv. d'Anjou) was added, and beakers were incubated for 7 to 10 days at 20C. The number of firm, dark lesions on the pears was enumerated, and sub-samples re-isolations confirmed the presence of *Phytophthora* spp. In July 2017, trees were evaluated in the field for the presence of crown rot that was visible as profuse gumming at the base of the trunk and poor tree vigor. In July 2018, trees were evaluated as dead or alive and for the presence of gumming and cankers at the trunk base. Data were analyzed using analysis of variance and multiple mean separation methods of SAS ver. 9.4.

A second field trial was established in October 2017 using Nonpareil grafted on Hansen or Nemaguard rootstock. These trees were originally planned for a greenhouse/lathhouse study. Trees were inoculated at planting with *P. cactorum*, in December of 2017 with *P. citrophthora*, and in February with *P. niederhauseri*. The same treatments are being evaluated as in the first trial in six replications of 2 trees for each rootstock and the first application was done in December 2017.

Results and Discussion:

I. Evaluate the in vitro toxicity of new fungicides against selected *Phytophthora* species occurring on almond. Eighty isolates of *Phytophthora* were obtained from diseased tree fruit samples and from orchard soil. Species identification based on DNA sequencing revealed the presence of 11 species, with the majority of isolates belonging to *P. niederhauseri, P. syringae, P. citricola/P. citricola* complex, and *P. cactorum*. Species less common included *P. chlamydospora, P. cinnamomi, P. gonapodyides, P. lacustris, P. megasperma, P. obscura*, and *P. rosacearum.*

All isolates were most sensitive to oxathiapiprolin with EC_{50} values for mycelial growth inhibition of $\leq$0.001 mg/liter (**Table 1**). A rather narrow range of EC_{50} values (0.001 to 0.01 mg/liter) among all isolates was also found for mandipropamid, whereas for ethaboxam and fluopicolide generally higher rates were needed. Higher concentrations of fluopicolide (0.104 - 0.229 mg/liter) were needed to inhibit the 7 isolates of *P. cactorum*, and a wider range of sensitivities (0.021 - 0.318 mg/liter) was determined for the 16 isolates of *P. syringae.* All isolates were also inhibited by mefenoxam, but rates of 0.122 to 0.155 mg/liter were needed for some isolates of *P. citricola*/*P. citricola* complex and *P. rosacearum*. For the 24 isolates of *P. niederhauseri*, a wide range from 0.012 to 0.209 mg/liter was determined. Thus, although a wide range of sensitivities was sometimes present for some of the fungicides and species, no resistance was detected. These isolates were never exposed to any of the new fungicides, and the wide range of sensitivities sometimes encountered for fluopicolide (similarly to our previous evaluations of *Phytophthora* isolates from citrus) may indicate that this fungicide has at higher risk for selection of resistance. Additional isolates will be collected and evaluated in 2018/19.

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. Results for the second evaluation of the first field trial that was established in 2016 are shown in **Table 2**. These results are somewhat different from those reported last year when only gumming and trunk cankers were evaluated. Some trees with gumming and cankers apparently recovered over time and currently appear healthy. Data in **Table 2** are presented for the incidence of dead trees and the incidence of trees with gumming and trunk cankers for both rootstocks combined and for the two rootstocks separately. Tree death in most cases was accompanied with the presence of trunk cankers. *P. cactorum* was recovered from several cankers.

In July 2018, 18.8% of untreated control trees of both rootstocks were dead with 27.5% of Hansen and 10% of Nemaguard trees affected. Among new fungicides, Orondis at three rates tested was identified as the most effective treatment with currently no tree death observed, and only one tree showing gumming and a trunk canker. All treatments, including Ridomil Gold and ProPhyt, significantly reduced death of Hansen trees to low levels and there was no difference among treatments. Due to a high degree of variability, for Nemaguard trees, no significant differences were observed among treatments. Numerically, Intego, Presidio, mixtures of these two fungicides (Intego-Presidio), Ridomil Gold, and Ridomil Gold-ProPhyt had the same number or more dead trees than the control.

For trees still alive with gumming and/or cankers, no significant differences were observed among fungicide-treated and control trees (**Table 2**). Only 5% of control trees still alive showed these symptoms, most trees that had developed cankers at this time were already dead. For live Nemaguard trees, treatments with Orondis, Presidio, Presidio-Intego, or Ridomil Gold-ProPhyt showed a significant reduction in trees with gumming and/or cankers. Additional evaluations will be done later this year, and data will also be obtained from the second trial that was established in 2017.

Soil propagule evaluations using plating on selective media or pear baiting methods currently were very inconsistent. Low or zero levels of Phytophthora propagules were always detected in treated soil, but populations in control soil fluctuated widely between samplings. Root samplings for the presence of Phytophthora so far were also inconclusive. Feeder root samples were difficult to obtain without causing considerable damage to trees. Ultimately, trees will have to be excavated in final evaluations to obtain data on root rot.

Conclusion. New oomycete fungicides that are being evaluated in the laboratory and in field studies are showing promise for the management of Phytophthora diseases of almond. In our evaluations, Orondis was the most effective treatment. Because all four new fungicides represent new modes of action, rotation of these products in semi-annual applications will be an effective fungicide resistance management program. On citrus, we reported resistance in *Phytophthora* species to phosphonate fungicides (FRAC 33). With widespread and indiscriminate usage of phosphite products (fungicides and fertilizer blends of PO_3), resistance likely will also develop in *Phytophthora* populations on almond. Additionally, phosphite residues may remain a problem with some trading partners due to a wide range of residues obtained with different use patterns and differences in regulatory agency reviews of these

fungicides (e.g., potassium phosphate is exempt in the US but not in the EU). The new fungicides under evaluation are effective against phosphonate-resistant populations, will have MRLs established in the EU and elsewhere in the world, will not be systemic, and not have residues in the almond kernels from soil applications.

Table 1. Effective concentrations of four new fungicides and mefenoxam for inhibition of fifty percent mycelial growth (EC_{50} values) of 11 Phytophthora species collected from almond and other tree crops in California[a]

***Phytophthora* spp.**	**No. of isol.**	**EC_{50} value ranges for mycelial growth (µg/ml)**				
		Mefenoxam	**Oxathiapiprolin**	**Mandipropamid**	**Ethaboxam**	**Fluopicolide**
P. cactorum	7	0.009 - 0.023	0.0005 - 0.001	0.007 - 0.009	0.026 - 0.088	0.104 - 0.229
P. chlamydospora	1	0.017	0.0003	0.002	0.053	0.035
P. cinnamomi	5	0.007 - 0.038	0.0002 - 0.0004	0.002 - 0.005	0.006 - 0.017	0.041 - 0.078
P. citricola/citricola complex	14	0.061 - 0.155	0.0003 - 0.0006	0.002 - 0.004	0.083 - 0.258	0.027 - 0.047
P. gonapodyides	4	0.004 - 0.015	0.0002 - 0.0005	0.001 - 0.005	0.007 - 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	0.01 - 0.013	0.0003 - 0.0005	0.002 - 0.005	0.04 - 0.079	0.082 - 0.24
P. niederhauseri	24	0.012 - 0.209	0.0001 - 0.0004	0.003 - 0.01	0.031 - 0.105	0.041 - 0.067
P. obscura	1	0.003	0.0003	0.002	0.033	0.018
P. rosacearum	2	0.105 - 0.122	0.0002 - 0.0004	0.003 - 0.005	0.06 - 0.089	0.06 - 0.08
P. syringae	16	0.002 - 0.041	0.0002 - 0.0004	0.001 - 0.004	0.017 - 0.13	0.021 - 0.318
Total	80					

[a] The majority of *Phytophthora* isolates were recovered from almond, walnut, and cherry plant tissues (scion, crown, bark, trunk, root) or from orchard soil.

Table 2. Evaluation of new fungicides for management of Phytophthora root and crown rot of almond[a]

A. Both rootstocks combined

Treatment[b]	Rate/A	Incidence of dead trees (%)[c]	LSD[d]	Incidence of trees with gumming, canker, or both, but not dead (%)	LSD
Control	---	18.8	a	7.5	ab
Prophyt	64 fl oz	2.5	bc	10.0	ab
Prophyt + Ridomil Gold	64 + 16 fl oz	5.0	bc	2.5	bc
Revus 250SC	8 fl oz	2.5	bc	5.0	bc
Orondis 100 OD A20941A	4.8 fl oz	0.0	c	0.0	c
Orondis 100 OD A20941A	9.6 fl oz	0.0	c	0.0	c
Orondis 100 OD A20941A	19.2 fl oz	0.0	c	5.0	bc
Ridomil Gold 480SL	1 pt	10.0	b	5.0	bc
Intego	10 fl oz	5.0	bc	15.0	a
Presidio	6 fl oz	7.5	bc	5.0	bc
Presidio + Intego	6 + 10 fl oz	7.5	bc	0.0	c

B. Hansen rootstock

Treatment[b]	Rate/A	Incidence of dead trees (%)[c]	LSD[d]	Incidence of trees with gumming, canker, or both, but not dead (%)	LSD
Control	---	27.5	a	5.0	b
Prophyt	64 fl oz	0.0	b	0.0	b
Prophyt + Ridomil Gold	64 + 16 fl oz	0.0	b	5.0	b
Revus 250SC	8 fl oz	0.0	b	5.0	b
Orondis 100 OD A20941A	4.8 fl oz	0.0	b	0.0	b
Orondis 100 OD A20941A	9.6 fl oz	0.0	b	0.0	b
Orondis 100 OD A20941A	19.2 fl oz	0.0	b	10.0	ab
Ridomil Gold 480SL	1 pt	0.0	b	0.0	b
Intego	10 fl oz	0.0	b	20.0	a
Presidio	6 fl oz	5.0	b	10.0	ab
Presidio + Intego	6 + 10 fl oz	5.0	b	0.0	b

Table 2. Evaluation of new fungicides for management of Phytophthora root and crown rot of almond[a] (*continued)*

C. Nemaguard rootstock

Treatment[b]	Rate/A	Incidence of dead trees (%)[c]	LSD[d]	Incidence of trees with gumming, canker, or both, but not dead (%)	LSD
Control	---	10.0	a	10.0	ab
Prophyt	64 fl oz	5.0	a	20.0	a
Prophyt + Ridomil Gold	64 + 16 fl oz	10.0	a	0.0	c
Revus 250SC	8 fl oz	5.0	a	5.0	bc
Orondis 100 OD A20941A	4.8 fl oz	0.0	a	0.0	c
Orondis 100 OD A20941A	9.6 fl oz	0.0	a	0.0	c
Orondis 100 OD A20941A	19.2 fl oz	0.0	a	0.0	c
Ridomil Gold 480SL	1 pt	20.0	a	10.0	abc
Intego	10 fl oz	10.0	a	10.0	abc
Presidio	6 fl oz	10.0	a	0.0	c
Presidio + Intego	6 + 10 fl oz	10.0	a	0.0	c

[a] Trees were planted in May 2016 and Inoculated with *P. cactorum, P. cambivora,* and *P. citrophthora* on 7-5-16, 3-2-17, and 7-6-17.

[b] Treatments were applied to the soil of each tree on 7-19-16 and 7-12-17.

[c] Trees were evaluated as being alive or dead, and for the presence of trunk cankers and gumming in July 2018.

[d] Values followed by the same letter are not significantly different based on analysis of variance and least significant difference mean separation ($P > 0.05$).