Epidemiology and Management of Phytophthora Diseases of Citrus

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Year 3 of 5 (60% Complete)

Objectives

1) Conduct surveys and studies on the epidemiology of *Phytophthora* spp. causing brown rot in the three citrus-producing districts of California.
2) Evaluate new fungicides treatment for *Phytophthora* for brown rot and root rot disease management.
3) Evaluate the fungicide resistance potential and resistance frequencies to the new mode of action fungicides.
4) Develop guidelines to minimize the risk of harvesting citrus fruit with non-visible (quiescent) brown rot infections.

Problem and Significance

Phytophthora diseases can cause major problems for the citrus industry. Root and crown rots may delay or prevent establishing an orchard, reduce yields, and may cause mature orchards to decline. Brown rot can result in substantial crop losses especially in lower parts of the tree and can cause rejections of grower lots in international markets that may lead to market closure due to quarantine laws. The recent detection of phosphite resistance further emphasizes the need for new management strategies.

Benefit to Industry

In epidemiological studies, models have been developed that help forecast fruit brown rot. Industry notifications have been made available and have helped the industry optimize timing of fungicide applications and have helped in preventing detection of Phytophthora brown rot in international export markets. Three new fungicides are currently available and were nearly simultaneously registered on citrus based on our efforts. Orondis™ and Revus™ were registered for foliar applications, and Orondis™ and Presidio™ were registered for soil application. Still, another fungicide (Elumin™) is pending registration through the IR-4 program, and new products are still being identified as effective against *Phytophthora* spp. With the detection of phosphite resistance in three *Phytophthora* spp. from citrus in CA, integrated rotational programs can be developed to delay further shifts toward resistance in populations. Integrated postharvest hot water and phosphite treatments effectively manage brown rot on symptomless fruit caused by phosphite-resistant isolates.

Progress Summary

Conduct surveys and studies on the epidemiology of *Phytophthora* spp. causing brown rot in the three citrus-producing districts of California. Using the isolates collected in Districts 1 and 2 orchards over the past two years, we continue to characterize populations of *P. citrophthora* and *P. syringae* (the main brown rot pathogens in CA) based on genetic relationships and fungicide sensitivities. For *P. syringae*, we are also incorporating some isolates from other hosts in CA such as almond and cherry. Species identification is verified by qPCR using specific probes. For genetic analyses of populations, we are currently identifying DNA polymorphisms. Isolates from our surveys are also being evaluated for their fungicide sensitivity and will be used for resistance potential studies.

Evaluate new fungicides for management of Phytophthora brown rot and root rot. We continued our evaluation of new preharvest treatments for management of Phytophthora brown rot. Treatments were applied in two orchards to navel orange trees in late January 2021 just before a rainy period as either a concentrate (100 gal/A) or dilute (400 gal/A) treatment. Fruit were harvested periodically, inoculated with *P. citrophthora*, and incubated for decay development. With 41 mm (1.6

inch) of rainfall, one week after application all treatments except ProPhyt™, using both application volumes, resulted in zero or near zero decay incidence as compared to control fruit from the two orchards with 57.3 or 53.1% decay (Table 1). In both orchards, ProPhyt™ applied as a concentrate spray was more effective (8.3% or 1% decay incidence) than as a dilute spray (27.1% or 7.3% decay incidence). With a total of 70 mm rainfall after 8 weeks, Orondis™, Orondis Ultra™, and Revus™ were still highly effective in reducing brown rot of harvested fruit after inoculation. Fruit treated with ProPhyt™ in a dilute application developed 60% brown rot, but for the concentrate application, the incidence was 18.9%. With results we obtained in the winter of 2020 and 2021, we provide information on the most effective treatments and the best application strategy.

Table 1. Efficacy of preharvest treatments using dilute (400 gal/A) and concentrate (150 gal/A) applications for management of brown rot of navel orange at UCR 2020. Applications were done on 1-22-21. Fruit were harvested after 1, 2, 4, and 8 weeks, inoculated with P. citrophthora *and incubated for 7 days at 20C. ProPhyt and copper-lime treatments are included for comparison.*

Application volume 400 gal/A*

	Brown rot incidence (%)			
	1 week**	2 weeks	4 weeks	8 weeks
Untreated control	57.3 a	57.3 a	95.8 a	96.4 a
Orondis 200 SC 2.4 fl oz	0.0 c	0.0 c	6.4 c	2.3 c
Orondis Ultra 5.5 fl oz	0.0 c	0.0 c	1.0 c	2.4 c
Revus 8 fl oz	0.0 c	0.0 c	5.2 c	8.1 c
ProPhyt 64 fl oz	27.1 b	20.2 b	65.6 b	60.0 b
Champ 4 lb + Lime 3 lb	0.0 c	2.1 c	0.0 c	7.0 c

Application volume 150 gal/A

	Brown rot incidence (%)			
	1 week	2 weeks	4 weeks	8 weeks
Untreated control	57.3 a	57.3 a	95.8 a	96.4 a
Orondis 200 SC 2.4 fl oz	1.0 c	0.0 c	6.3 c	3.5 c
Orondis Ultra 5.5 fl oz	0.0 c	0.0 c	1.0 c	3.6 c
Revus 8 fl oz	0.0 c	0.0 c	2.1 c	1.1 c
ProPhyt 64 fl oz	8.3 c	9.4 c	9.4 c	18.9 c
Champ 4 lb + Lime 3 lb	0.0 c	0.0 c	7.3 c	12.8 c

Two biocontrol treatments were evaluated for their efficacy against Phytophthora root rot of sweet orange seedlings in greenhouse studies. The soil around seedlings was inoculated with *P. citrophthora,* treatments of Howler™ (*Pseudomonas chlororaphis*), Theia™ (*Bacillus subtilis*), or Orondis were applied after 1 week, and the incidence of root rot and pathogen soil population sizes were assessed after 2 months.

Table 2. Efficacy of new biocontrol treatments for management of Phytophthora root rot of Madame Vinous sweet orange seedlings in greenhouse studies. The soil around each 11-month-old seedling was inoculated with P. citrophthora*-colonized rice-vermiculite inoculum, and soil treatments were applied after 1 week.* Phytophthora *soil populations and the incidence of root rot were evaluated 2 months after treatment by plating soil or roots, respectively, onto a selective medium.*

		Soil populations**		Root rot incid.	
Treatment	Rate/A	ppg/g soil	LSD***	%	LSD
Control	-	897.8	ab	59.6	a
Orondis 200	9.6 fl oz	0.0	c	1.3	b
Howler	5 lb	1102.8	ab	50.8	a
Howler	2.5 lb	593.9	bc	60.0	a
Howler	1.25 lb	1039.4	ab	45.8	a
Theia	3 lb	925.0	ab	51.7	a
Theia	1.5 lb	1323.9	a	51.3	a

The two biocontrols were not effective in reducing soil populations of *P. citrophthora* or the incidence of root rot as compared to the control, whereas after Orondis™ treatment, the pathogen could not be detected in the soil, and the incidence of root was reduced by over 95% (Table 2). To protect the high efficacy of Orondis™ in the field, rotations and mixtures with other modes of action (Presidio™, potassium phosphite, Ridomil Gold™) are recommended. Another fungicide with yet another mode of action (Elumin™) is pending registration through the IR-4 program. The fungicide was accepted into the program in the fall of 2021.

We finished our studies on the translocation of mandipropamid in citrus seedlings after soil applications.

In our second field study on new fungicides for managing Phytophthora root rot, we had identified effective rates that were lower for mandipropamid and oxathiapiprolin than rates originally suggested by the registrant. In follow-up evaluations of trees, control trees had smaller canopies as compared to treated trees, two control trees had died, and there was no difference among any of the treatments.

Packinghouse studies were conducted to optimize postharvest phosphite treatments when lemon fruit are also treated with soda ash. For this, the phosphite treatment was done before or after the soda ash bath. The soda ash treatment alone significantly reduced the incidence of brown rot from that of the control in both studies, but ProPhyt™ at 2000 ppm was only effective in the second study. When a ProPhyt™ dip at 2000 or 4000 ppm was followed by soda ash and a water rinse, the incidence of brown rot was significantly reduced from the control (Table 3). When a ProPhyt™ flooder treatment was applied after the soda ash dip, however, the incidence of brown rot was reduced from 100 or 75.4% in the controls to the lowest values, i.e., 7.2% and 4.8%, respectively. Thus, phosphite is best applied after soda ash and water rinse treatments and before the fungicide Graduate A+ (GA+)-wax controlled droplet application.

Table 3. Evaluation of the compatibility of soda ash and potassium phosphite treatments for the management of Phytophthora brown rot of lemons in commercial packing house studies. Treatments were done 22-26 h after inoculation with P. citrophthora*. PAA = peroxyacetic acid, GA+ = Graduate A+. Fruit were evaluated for development of brown rot after 8 days.*

	Study 1		Study 2	
Treatments	%	LSD**	%	LSD
Control	100.0	a	75.4	a
4000 ppm PO_3 /PAA dip before soda ash	45.2	b	54.8	b
4000 ppm PO_3 flooder after soda ash, prior to CDA GA+ in storage wax	7.2	c	4.8	c

When after brushing a subsequent storage wax treatment with Graduate A+ was done, the efficacy was slightly reduced in the second study but was not significantly different from the treatment with no fungicide-wax.

Similar results were previously obtained in laboratory studies, and thus, we identified the best application strategy when fruit are to be treated with soda ash and potassium phosphite, and this helps in the integrated management of several decays (e.g., brown rot, green mold, sour rot).

Evaluate the fungicide resistance potential and resistance frequencies to the new mode of action fungicides. Sampling and characterizing large numbers of isolates of *Phytophthora* species and determining their *in vitro* sensitivities provides an indication if natural resistance pre-exists in the pathogen populations that may be further selected for. For example, for fluopicolide we previously identified isolates of *P. syringae* that were 23 times less sensitive than the most sensitive isolates evaluated. Thus, we concluded that fluopicolide may be at higher risk for resistance selection. We are also testing newly collected isolates for sensitivity against phosphite to determine the extent of phosphite (PO_3) resistance in different citrus growing regions. As our previous studies indicate, phosphite-resistant isolates cannot be managed adequately using previously recommended phosphite rates and application methods. The direct toxicity of phosphite to *Phytophthora* species does not support the recent re-assignment of phosphonates from FRAC Code 33 to P07, the latter code indicating that their mode of action is host plant defense induction. This information was submitted to FRAC. Pre-and postharvest strategies for managing phosphite-resistant pathogen populations have been summarized.

Develop guidelines to minimize the risk of harvesting citrus fruit with non-visible (quiescent) brown rot infections. For the last several years, risk forecasts for *Phytophthora* infections were made using a Numerical Risk Model for copper reduction based on rainfall levels (Table 4.1) and wetness periods and temperature was used to predict infection risk by *P. syringae* (Table 4.2). For this latter model, environmental data are being summarized for 30 CIMIS weather stations in 9 counties. Precipitation was relatively high in citrus growing areas in Western Riverside, Ventura, Santa Barbara, San Luis Obispo, and San Diego Co. in the early winter of 2020/21. By Dec. 31, 2020, copper reduction risk levels were at level 2 or 4 for these locations, and the risk for brown rot developing by *P. syringae* was at level 3 (Table 5). Industry advisories by the California Citrus Quality Council suggested that a second application of copper or approved alternative (i.e., PO_3, oxathiapiprolin, or the newly registered mandipropamid) be done to acreage destined for export to China in Western Riverside, Ventura, Santa Barbara, San Luis Obispo, and San Diego Counties by Feb. 12, 2021. With additional rainfall, a second application was also advised for Fresno and Tulare Co. by Feb. 28, 2021.

Table 4. Numerical risk models used for determining copper reduction (top panel) and forecasting brown rot caused by P. syringae (bottom panel). Risk values are assessed when copper reduction is in the red zone (high risk).

	Accumulated precipitation (inch/mm) starting at current Cu reduction level**			
Cu Red. (%)*	<1 (25.4)	<2 (50.8)	<3 (76.2)	<4 (101.6)
<44	0	1	2	3
45-53	1	2	3	4
54-62	2	3	4	4
63-71	3	4	4	4

Temperature		Risk Values			
		Wetness period (h)			
(°F)	(°C)	<10	10-18	19-25	>25
<41	<5	0	0	1	1
>41-50	5-10	2	3	3	3
>50-68	>10-20	1	2	3	3
>68-77	>20-25	0	0	0	0

Based on new insights from the studies we are conducting, guidelines for growers and packers are modified on an annual basis. Thus, forecasting models have been refined, preharvest copper alternatives have become available, and postharvest application methods have been optimized.

Table 5. Summary of risk assessments for six citrus production regions in California as of 12-31-2020.

County (No. of stations)	Date	Avg Temp Infection periods	Pp (mm) Infection Periods	Copper Risk	P. syr. Risk
W. Riverside (2)	12/31/20	8.4	38.6	2	3
Ventura (3)	12/31/20	9.8	28.0	2	3
Santa Barbara (4)	12/31/20	10.5	36.2	4	3
SLO (3)	12/31/20	10.7	35.6	2	3
San Diego (4)	12/31/20	13.2	52.7	2	3

Conclusions

This project will continue next year as part of the Pre- and Postharvest Citrus Disease Management Core Program. We learned that leaf litter and surface soil can harbor *P. syringae;* other species occur deeper in the soil. We identified four new modes of action of fungicides highly effective against *Phytophthora* spp. Three are registered, the fourth is pending. The new fungicides improved the establishment of a new orchard and increased crop production.

The presence of PO_3 resistance in three *Phytophthora* species emphasizes the timely registration of other modes of action. These should be used in rotation with PO_3 to minimize further selection of resistance. PO_3 combined in heated aqueous postharvest treatments was effective against PO_3-resistant isolates.

For trade of California citrus to international markets with quarantines against selected *Phytophthora* species, we helped to minimize the impact to the citrus industry and identified cultural and chemical management practices. Models based on rainfall and temperatures that favor brown rot infections of fruit were developed. Good agricultural practices (GAPs) are prepared, and risk assessments are provided annually as industry advisories.

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Publications and Presentations

1. Gray, M., Nguyen, K., Hao, W., Belisle, R., Förster, H., and Adaskaveg, J. E. 2020. Mobility of oxathiapiprolin and mefenoxam in citrus seedlings after root application and implications

for managing Phytophthora root rot. Plant Disease 104:3159-3165.

2. Hao, A., Förster, H., Adaskaveg, J. E. 2021. Resistance to potassium phosphite in *Phytophthora* species causing citrus brown rot and integrated practices for management of resistant isolates. Plant Disease 105:972-97