Analysis of Rootstocks and New Fungicides for Control of Phytophthora Root Rot and Gummosis in Arizona Citrus Groves[1]

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Abstract

Experiments were initiated to evaluate potential new citrus rootstocks for their relative tolerance or resistance to root rot and gummosis caused by Phytophthora citrophthora and P. parasitica and to determine the efficacy of potential new fungicides for disease control. In greenhouse trials conducted in 1994 and 1995, the range of root loss due to Phytophthora in the 44 different rootstocks tested ranged from 26-96%. Rootstocks sustaining 80% or less root loss will be evaluated further to identify those with superior tolerance to Phytophthora. In growth chamber experiments, the same rootstocks were inoculated on the stem to evaluate resistance to gummosis. The length of canker that developed on these test plants ranged from 1-25 mm. Rootstocks with canker development in the range of 1-10 mm in length will be tested further to identify the most resistant selections. Laboratory studies were conducted to determine the comparative activity of Aliette, Ridomil, Dimethomorph, Fluazinam, ICIA-5504, and SM-9 at concentrations of 1, 10, 100, and 1,000 mg/l on sporulation and growth of P. citrophthora and P. parasitica. Each of the four new molecules was either comparable or superior to Aliette or Ridomil with respect to activity on at least one component of the life cycle of the Phytophthora species tested. The results presented in this report are preliminary in nature and will be validated in future studies.

Introduction

Phytophthora root rot and gummosis of citrus is a serious disease problem in Arizona. The two species of *Phytophthora* that cause this disease have been isolated from over 85 percent of sampled orchard sites in Arizona (8). A suitable environment for multiplication of the pathogens and disease development usually exists each time a grove is irrigated.

For any plant disease to occur, three conditions must be met. A pathogen capable of causing disease must be present, a plant host susceptible to the pathogen must be present, and an environment conducive to development of disease must exist. When all three conditions are favorable, severe plant disease occurs. On the other hand, if one or more of the three primary components of the so-called "Plant Disease Triangle" are less than favorable, disease

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severity is diminished. Our goal in plant disease management is to identify strategies that will render one or more of the components of the plant disease triangle unfavorable for disease development.

Although the three components of the plant disease triangle can be favorable for development of Phytophthora root rot and gummosis in many citrus groves, progress has been made in reducing disease severity. The availability and use of the fungicides Aliette and Ridomil has restricted the ability of the pathogens to multiply and initiate disease. Recent studies have demonstrated seasonal fluctuations in the degree of colonization of citrus root and stem tissues by *P. citrophthora* and *P. parasitica* (4-5). Also, air and soil temperatures recorded in Arizona citrus groves during some winter and summer months may inhibit sporulation of *Phytophthora* and the resulting development of root rot and gummosis (6). All of this information will be useful for identification of periods of time during the year when disease development is most likely to occur. These periods of high risk for disease development are times when fungicide protection should be in place. With proper timing, the maximum disease control benefits can be achieved from a fungicide application.

An important component of an integrated approach for control of Phytophthora root rot and gummosis is the use of tolerant or resistant rootstocks. Many studies have identified citrus rootstocks "susceptible" or "tolerant" to *Phytophthora* (1-3,9). Unfortunately, these studies are often contradictory. Possible reasons for contradictory results may involve the species of *Phytophthora* used in the study (*P. citrophthora, P. parasitica*, or rarely both), the component of disease studied (root rot, gummosis, or both), and the selections of the various rootstocks that were tested. We need to develop and use consistent and reliable methods to evaluate and compare current as well as new potential rootstocks for their tolerance or resistance to root rot and gummosis caused by each pathogen.

Significant progress has been made in the development of successful management strategies for Phytophthora root rot and gummosis in citrus; however, additional progress is possible. Continuing studies are needed to identify new fungicides for disease control. Aliette and Ridomil are two excellent materials for combating *Phytophthora* in citrus, but there is no guarantee that these materials will always maintain their effectiveness or be available. It is prudent to seek alternative fungicides should one or both of these materials be lost in the future. Some new fungicides, which have shown promising results in the control of Phytophthora root and stem rot of chile pepper, should be tested against the *Phytophthora* species affecting citrus.

The goals of these investigations were to evaluate potential new citrus rootstocks for their relative tolerance or resistance to root rot and gummosis caused by *Phytophthora citrophthora* and *P. parasitica* and to determine the efficacy of potential new fungicides for disease control. The achievement of these objectives should enhance our efforts to improve control of Phytophthora root rot and gummosis in citrus.

Materials and Methods

To test new rootstocks for relative resistance to *Phytophthora*, 6-11 month old seedlings of various test rootstocks were removed from wooden flats in which they were growing, roots were rinsed to remove adhering potting mix, then the roots were immersed in water containing zoospores of *P. citrophthora* or *P. parasitica* and incubated for 48 hr. at 24 C. Inoculated seedlings then were planted in potting mix within 10-cm-diameter X 10-cm-deep plastic pots and maintained in the greenhouse for approximately 4 months. During the experiment, the survival time of each rootstock seedling was recorded. At the termination of the trial, the weight of plant shoots and roots was recorded. Two experiments were conducted in 1994 and two in 1995. For every rootstock tested, each experiment included five replicate noninoculated plants as well as five plants inoculated with each pathogen.

For evaluation of rootstocks for relative resistance to stem lesion development (gummosis), 1-yr-old plants were stem-inoculated with *P. citrophthora* or *P. parasitica* by placing a 5-mm-diameter mycelial disk of one of the pathogens into a similar sized wound on each test rootstock seedling. The wound containing the pathogen then was wrapped with plastic tape and plants were maintained at 21 C for 11-12 days in a growth chamber, after which the length of stem cankers was measured. For each experiment, five replicate plants of each rootstock were inoculated with each pathogen. This experiment was conducted twice in 1994 and twice in 1995.

Before we can test the ability of potential new fungicides to control root rot and gummosis, we had to determine the effective concentrations of each chemical needed to suppress growth and sporulation of *P. citrophthora* and *P. parasitica.* The compounds included in these tests were Dimethomorph, Fluazinam, ICIA-5504, SM-9 and the standard fungicides Aliette and Ridomil. The effect of each material on mycelial growth was investigated by incorporating different concentrations of the material into corn meal agar plates, then measuring the growth of the test pathogens on these plates after incubation at 24 C for 4 days. Inhibition of sporulation was studied by incorporating different concentrations of each compound into 1.5% soil extract, then placing mycelial disks of the different species of *Phytophthora* into small plastic dishes containing this soil extract. After incubation for 4 days at 24 C, the number of sporangia were counted that had formed on the sides of each mycelial disk. The influence of the fungicides on motility of zoospores was tested by placing zoospore suspensions into solutions of dilute V-8 juice broth containing various concentrations of each chemical. The duration of zoospore motility for each experimental treatment then was recorded. The nonmotile encysted zoospores subsequently were incubated in fresh V8-juice broth for 48 hr at 24 C to determine their viability after treatment with various concentrations of each compound.

Results and Discussion

The average degree of root rot in all tested rootstocks ranged from 26-96% in these studies. A preliminary value of 70% or less root loss due to *P. citrophthora* or *P. parasitica* in experiments for two years was selected to identify rootstocks with a promising level of tolerance or resistance to root rot (Table 1). Rootstocks that experienced 70% or less root rot for one year as well as rootstocks showing root losses ranging from 71-80% will be subjected to additional tests. A list of rootstocks that experienced root loss of 70% or less in a least one year of trials is presented in Table 1.

The average length of stem cankers that formed in all tested rootstocks ranged from 1-25 mm in studies conducted in 1994 and 1995. A preliminary value of 1-5 mm for the length of canker development during two years was selected to identify rootstocks with a promising level of resistance to gummosis (Table 2). Rootstocks with canker development of 1-5 mm in length for one year as well rootstocks with canker development in the range of 6-10 mm in length will be subjected to additional tests to identify plant selections with superior resistance to stem canker development. A list of rootstocks where the length of stem canker was in the range of 1-5 mm for at least one year is found in Table 2.

A summary of the effects of Aliette, Ridomil, Dimethomorph, Fluazinam, ICIA-5504, and SM-9 on growth and sporulation of *P. citrophthora* and *P. parasitica* is found in Tables 3 and 4. The lowest concentration of chemical needed to achieve ^{3}90% reduction of the following components for the life cycle of *P. citrophthora* was 1 mg/l of Dimethomorph for sporangium formation, 1 mg/l of Dimethomorph for zoospore motility, 1 mg/l of Fluazinam for zoospore cyst viability, and 10 mg/l of Dimethomorph for mycelial growth. Likewise, the concentration of chemical needed to achieve ^{3}90% reduction in the same factors for *P. parasitica* was 1 mg/l of Aliette or Dimethomorph for sporangium formation, 1 mg/l of Dimethomorph or Ridomil for zoospore motility, 1 mg/l of Fluazinam for zoospore cyst viability, and 10 mg/l of Dimethomorph for mycelial growth. Each of the four new molecules tested was either comparable or superior to Aliette or Ridomil with respect to activity on at least one component of the life cycle of *P. citrophthora* or *P. parasitica.* With this information in hand, efficacy studies on citrus can be initiated.

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Table 1. Rootstocks sustaining root losses of 70% or less when infected with *Phytophthora citrophthora* or *P. parasitica*.[1]

ROOTSTOCK		YEAR	
		1994	1995
ROOTSTOCKS INFECTED WITH *P. CITROPHTHORA*			
S-2.	Sunki x Flying Dragon (62-109-19)	**	+
S-7.	Rangpur lime x Shekwasha (61-169-2)	**	**
S-8.	Kao Pan Shaddock x Swingle Citrumelo (55-21-2)	--[2]	**
S-9.	Sunki x Flying Dragon (RN-94-20)	**	--
S-12.	*Citrus taiwanica*	**	--
S-13.	Rangpur lime x Kao Phung Shaddock (59-121-15)	**	+
S-18.	Rough lemon (YMAC)	**	**
S-19.	*Citrus volkameriana* (YMAC)	**	+
S-20.	*Citrus macrophylla* (YMAC)	**	--
S-23.	Troyer citrange (YMAC)	**	--
S-28.	Shekwasha x English Trifoliate	--	**
S-29.	Soh Jalia	--	**
S-43.	Goutou #1	--	**
ROOTSTOCKS INFECTED WITH *P. PARASITICA*			
S-2.	Sunki x Flying Dragon (62-109-19)	**	+
S-17.	Sacaton Citrumelo (56-70-2)	**	+
S-18.	Rough lemon (YMAC)	+	**
S-19.	*Citrus volkameriana* (YMAC)	**	+
S-20.	*Citrus macrophylla* (YMAC)	**	--
S-23.	Troyer citrange (YMAC)	--	**
S-27.	African Shaddock x Rubidoux Trifoliate	--	**

[1] The average degree of root rot in all tested rootstocks ranged from 26-96% in these studies. At least 10 rootstock plants were tested against each species of *Phytophthora.*

[2] A blank in the table indicates that the rootstock was not tested in that year. A plus (+) in the table indicates that the degree of root loss was higher than 70%. Rootstocks that sustained 70% or less root loss for two years are promising candidates for tolerance to the species of *Phytophthora* tested. Rootstocks that experienced 70% or less root rot for one year will be tested for one additional year. Several rootstocks had root losses ranging from 71-80% in the 1995 trials and these will be tested further as well.

Table 2. Citrus rootstocks on which growth of stem cankers caused by *Phytophthora citrophthora* and *P. parasitica* was limited to 1-5 mm in length in experiments conducted in 1994 and 1995.[1]

	ROOTSTOCK	YEAR 1994	YEAR 1995
INOCULATED WITH *P. CITROPHTHORA*			
S-2.	Sunki x Flying Dragon (62-109-19)	**	**
S-3.	Oklawaha Sour orange (OSO)	**	--[2]
S-4.	Savage Sour orange x Cleopatra mandarin #1 (63-191-22)	**	--
S-5.	C-35 Citrange	**	**
S-6.	Carrizo citrange	**	+
S-7.	Rangpur lime x Shekwasha (61-169-2)	**	+
S-9.	Sunki x Flying Dragon (RN-94-20)	**	--
S-10.	Rough lemon	**	--
S-15.	Rangpur lime x Marks Trifoliate (RN-94-22)	**	--
S-24.	Rangpur lime x Marks Trifoliate (RN-94-23)	**	--
S-25.	*Citrus obovoidea*	**	--
S-26.	Goutou	**	--
S-27.	African Shaddock x Rubidoux Trifoliate	**	**
S-28.	Shekwasha x English Trifoliate	**	**
S-29.	Soh Jalia	**	**
S-30.	Gomiri Rough lemon	**	+
S-31.	Sicilian Sour orange x Cleopatra mandarin #2 (F-163-192-51)	**	+
S-35.	Savage sour x Cleopatra #2 (63-191-69)	--	**
S-37.	Rich 16-6 trifoliate	--	**
S-38.	Citrumelo 4475	--	**
S-39.	Citrumelo 80-8	--	**
S-41.	Benton citrange	--	**
INOCULATED WITH *P. PARASITICA*			
S-2.	Sunki x Flying Dragon (62-109-19)	**	+
S-5.	C-35 Citrange	**	+
S-6.	Carrizo citrange	**	+
S-9.	Sunki x Flying Dragon (RN-94-20)	**	--
S-11.	Citremon 1449	**	--
S-13.	Rangpur lime x Kao Phung Shaddock (59-121-15)	+	**
S-15.	Rangpur lime x Marks Trifoliate (RN-94-22)	**	--
S-23.	Troyer citrange (YMAC)	**	--
S-24.	Rangpur x Marks Trifoliate (RN-94-23)	**	--
S-25.	*Citrus obovoidea*	**	--
S-27.	African Shaddock x Rubidoux Trifoliate	**	+
S-28.	Shekwasha x English Trifoliate	**	+
S-29.	Soh Jalia	**	+
S-31.	Sicilian Sour orange x Cleopatra mandarin #2 (F-163-192-51)	**	+
S-32.	Smooth Flat Seville	**	+
S-35.	Savage sour x Cleopatra #2 (63-191-69)	--	**
S-38.	Citrumelo 4475	--	**

[1] The average length of stem cankers that formed in all tested rootstocks ranged from 1-25 mm in these studies. At least 10 rootstock plants were tested each year against each species of *Phytophthora*.

[2] A blank in the table indicates that the rootstock was not tested in that year. A plus (+) in the table indicates that the canker was greater than 5 mm in that year.

Table 3. Summary of fungicide activity on *Phytophthora citrophthora.*

Fungicide	Minimum rate (mg ai/l) needed to cause ^{3}90% reduction of			
	Sporangium formation	Zoospore motility	Zoospore cyst viability	Mycelial growth
Aliette	1000	100	1000	1000
Ridomil	1000	10	100	100
Dimethomorph	1	1	1000	10
Fluazinam	>1000	10	1	100
ICIA-5504	>1000	10	100	>1000
SM-9	>1000	100	100	100

Table 4. Summary of fungicide activity on *Phytophthora parasitica.*

Fungicide	Minimum rate (mg ai/l) needed to cause ^{3}90% reduction of			
	Sporangium formation	Zoospore motility	Zoospore cyst viability	Mycelial growth
Aliette	1	100	1000	>1000
Ridomil	1000	1	100	1000
Dimethomorph	1	1	100	10
Fluazinam	>1000	10	1	>1000
ICIA-5504	>1000	100	100	>1000
SM-9	>1000	100	100	>1000