Comparison of Application Methods of Systemic Fungicides to Suppress Branch Cankers in Clementine Trees Caused by *Phytophthora citrophthora*

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ABSTRACT

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Since 2002, considerable losses of Clementine trees (*Citrus clementina*) have been observed in Spain due to *Phytophthora* branch canker of citrus caused by *Phytophthora citrophthora*. Due to the low efficiency of the available cultural and genetic control measures, application of fungicides is required for economic management of the disease. Fosetyl-Al, metalaxyl, and its enantiomer mefenoxam are the only systemic fungicides registered for control of *Phytophthora* diseases in Spain. However, their efficacy has not been tested against *Phytophthora* branch canker. Greenhouse and field experiments were conducted for 3 years in Spain to evaluate the ability of these fungicides and application methods to reduce lesion expansion. Nevertheless, with the inoculation technique used, it was not possible to evaluate the protective activity of fungicides, which can play an important role in their performance under field conditions. None of the fungicide treatments inhibited lesion expansion when applications were made on a curative basis. The residual effect was better on young than on mature trees. Paint treatments were generally more effective in reducing lesion expansion than drip chemigation or foliar sprays. However, this application method is laborious and becomes uneconomical in Spain. Trunk and branch sprays as well as long-term programs of foliar sprays or drip chemigation for control of spring and autumn infections are proposed as targets for future research.

Phytophthora spp. are soilborne pathogens that cause several serious diseases on citrus worldwide. *Phytophthora* root rot occurs when *Phytophthora* spp. infect the structural and fibrous roots of the rootstock. When the pathogen infects the aboveground part of the rootstock, the disease is known as *Phytophthora* foot rot or crown rot. *Phytophthora* spp. can also infect the scion, usually starting above the bud union near ground level. This disease is then known as *Phytophthora* gummosis, and it causes necrosis of the inner bark and the cambium of the trunk. *Phytophthora* root rot, foot rot, and gummosis may cause tree decline and in severe cases even tree wilt and death. *Phytophthora* brown rot of citrus fruit is caused by inoculum splashed from the soil or infected fruits. Most of the brown rot-affected fruit abscise, but infected asymptomatic fruits develop severe post-harvest rots during storage (17).

All the above-mentioned diseases have been described in Spain associated mainly with *P. citrophthora* (Smith & Smith) Leonian and *P. nicotianae* Breda de Haan (syn. *P. parasitica* Dastur) (4,33,34). They are effectively managed with cultural practices and fungicide applications (34), but since 2002, considerable losses of citrus trees have been observed in Spain due to a new disease caused by *P. citrophthora* (2,35). Infections usually started on the main branches, from which cankers accompanied by gumming expanded upward affecting the secondary branches, and downward affecting the trunk. Rootstocks generally remained healthy and were separated from the affected scion by a definite margin. Cankers progressed, girdling and killing the affected limbs and even the entire tree. Disease incidence was variable, but more than 90% of trees have been affected in some cases. Clementines (*Citrus clementina* Hort. ex Tan.) and their hybrids are the citrus genotypes that are most susceptible to the disease, accounting for more than 81% of the affected orchards in recent surveys conducted in Spain (2).

Adequate soil drainage, proper irrigation schedules, and planting on ridges are cultural practices recommended to avoid overwatering and reduce the incidence of *Phytophthora*-induced diseases in citrus

(17). However, despite the fact that these practices are widely used in Spain, severe epidemics of *Phytophthora* branch canker were observed. The use of tolerant and resistant citrus rootstocks is the most effective control measure for *Phytophthora* root rot and foot rot. *Phytophthora* gummosis is also effectively controlled by grafting the susceptible scion high on a resistant rootstock (17). However, these control measures are ineffective against the new disease detected in Spain because infection takes place on the main branches, well above the bud union. A transgenic line of sweet orange (*C. sinensis* (L.) Osb.) with increased tolerance to *P. citrophthora* infections has been developed by Fagoaga et al. (13). However, due to the regulations established by the European Union on the release of genetically modified organisms, it is uncertain when this new genotype will be available.

Like *Phytophthora* gummosis, the expansion of *Phytophthora* branch canker on the tree can be stopped by scraping the margin of the lesions or just removing the entire affected branch. Hough et al. (20) proposed the use of a heat treatment to eradicate *Phytophthora* lesions on citrus trunks. Nevertheless, no further reports of its efficacy under field conditions are available. In Spain, *Phytophthora* branch canker of citrus is effectively controlled in small orchards by growing the rootstock to form the trunk and the main branches, and then grafting the susceptible scion on them. Although highly effective, all the above-mentioned cultural practices are not economical in Spain due to high labor costs.

As a result of ineffectiveness of the available cultural and genetic control measures, application of fungicides is currently imperative to achieve economic management of the disease. Contact fungicides, such as copper compounds and di-thiocarbamates, can be applied as preventive trunk paints against *Phytophthora* gummosis. Applications should be scheduled to maintain appropriate external deposits of fungicide on susceptible tissues during the critical periods for infection. Contact fungicides may be used also after the scraping of lesions and sanitation pruning. However, topical application of these chemicals is labor intensive, and contact fungicides are currently used in Spain only

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as foliar sprays to prevent *Phytophthora* brown rot of citrus fruit during autumn months.

In contrast to contact compounds, systemic fungicides can penetrate into plant tissues and redistribute to nontreated organs through the vascular system. In some cases, they may eradicate established infections (19). Fosetyl-Al and metalaxyl (currently replaced by its R-enantiomer mefenoxam) are the only systemic fungicides registered in Spain for controlling oomycetes on citrus. Both fungicides are effective for the control of *Phytophthora* root rot, foot rot, gummosis, and brown rot of citrus caused by both *P. citrophthora* and *P. nicotianae* (9–12,14,15,21,23,25, 26,32). Other mobile phosphonates, such as potassium phosphite, also have been effective in controlling *Phytophthora*-induced diseases of citrus (8,18,28). However, these compounds are registered in Spain as foliar fertilizers and cannot be recommended for use as fungicides.

Like contact fungicides, both metalaxyl (mefenoxam) and fosetyl-Al also can be applied as trunk paints for control of *Phytophthora* gummosis, but this application technique is laborious and it has not been widely adopted by citrus growers in Spain. Fosetyl-Al has both downward and upward movement, so it can be applied as a soil treatment or as foliar spray for control of *Phytophthora* root rot, foot rot, and gummosis. Metalaxyl (mefenoxam) exhibits mainly upward movement (19), thus it should be applied as a soil or trunk treatment. Foliar sprays with metalaxyl (mefenoxam) are applied only for control of *Phytophthora* brown rot of citrus fruit, where no translocation is needed because the fungicide is applied directly to the target. To maximize the redistribution into the plant, which is critical to control *Phytophthora* root rot, foot rot, and gummosis, soil applications of metalaxyl (mefenoxam) or fosetyl-Al should coincide with the periods of active root growth, and foliar sprays with fosetyl-Al should be applied during leaf flushes (17).

All available information about the application of systemic fungicides on citrus is in regard to the control of *Phytophthora* root rot, foot rot, gummosis, and brown rot of citrus. However, there are no reports on the performance of these products against *Phytophthora* branch canker of citrus caused by *P. citrophthora*, and few reports of any type on treatment of trunk infections by *Phytophthora* spp. especially under field conditions. Understanding the activity of systemic fungicides and their associated application techniques will assist growers in managing this serious disease in Spain and similar diseases elsewhere. The objectives of this work were to evaluate the activity of systemic fungicides for control of *Phytophthora* branch canker of citrus caused by *P. citrophthora* primarily under field conditions, and to compare their efficacy with the available application methods.

MATERIALS AND METHODS

Inoculum production. Two isolates of *P. citrophthora* recovered from branch cankers on Clementine cv. Hernandina (Phy 033 from Huelva province and Phy 051 from Valencia province) were used in the greenhouse experiment and in field experiments conducted in Castellón and Valencia provinces (eastern Spain). *P. citrophthora* isolates from Huelva HU-03/0313 recovered from branch cankers of 'Fortune' (*C. clementina* × *C. reticulata* Blanco) and HU-03/1981 from Clementine cv. Clemenules were used for experiments conducted in this province (southwestern Spain). Isolates were stored at 6°C in 15-ml flasks containing sterile soil extract. Pieces of mycelia were transferred to petri dishes with potato dextrose agar (PDA) and incubated for 5 days at 24°C in the darkness.

Fungicides. The fungicides used in the experiments were the following: metalaxyl (Ridomil, 25% wt/wt WP; Syngenta Agro, Spain), mefenoxam (Ridomil Gold, 2.5% wt/wt GR; Syngenta Agro), mefenoxam (Ridomil Gold, 48% wt/vol EC; Syngenta

Agro), and fosetyl-Al (Alliette, 80% wt/wt WG; Bayer CropScience, Spain). Fungicides, rates, and application methods used in each experiment are indicated in Tables 1 to 5.

2004 greenhouse experiment. A greenhouse experiment was conducted on 2-year-old 'Hernandina' trees grafted on Carrizo citrange (*C. sinensis* L. Osb. × *Poncirus trifoliata* L. Raf.). Plants were grown in plastic pots containing 4 liters of peat. Fungicides, rates, and application methods used are indicated in Table 1. In foliar applications, plants were sprayed to runoff (~100 ml/tree) with a handgun sprayer (Solo, Germany). Soil drenches were made by pouring 500 ml of aqueous solution of fungicide into each pot in two sequential aliquots. Paints were applied with a paintbrush covering the entire trunk of the scion.

Plants were inoculated with *P. citrophthora* isolates Phy 033 and Phy 051 at 10, 20, 30, and 40 days after fungicide application. For inoculation, a 5-mm-diameter plug of the bark of the scion was removed with a cork borer about 15 to 20 cm above the soil line in each plant. A 5-mm-diameter PDA plug colonized by *P. citrophthora* was placed onto the exposed cambium. The inoculated area was covered by cotton moistened with sterile water, sealed with a strip of Parafilm, and wrapped with aluminum foil to prevent wound desiccation. The experiment was arranged in a completely randomized design. Five plants were inoculated for each fungicide treatment-isolate-inoculation date combination. At all evaluation dates, five nontreated plants inoculated with each isolate were used as controls.

Results were evaluated 10 days after each inoculation date. Bark of the inoculated area was scraped with a scalpel, and margins of the lesions were traced on transparent tape. The area of the lesion was quantified by mean of the Assess software (American Phytopathological Society, St. Paul MN), and the area of the original wound was subtracted to give the actual lesion size. Isolations onto PARBPH selec-

Table 1. Residual activity of fungicide treatments on trunks of 2-year-old 'Fortune' trees inoculated with two isolates of *Phytophthora citrophthora* under greenhouse conditions

Treatments ^y	Dose	Area of lesion (cm ²) ^x							
		Days from fungicide application to inoculation							
		10		20		30		40	
		Phy 033	Phy 051	Phy 033	Phy 051	Phy 033	Phy 051	Phy 033	Phy 051
Metalaxyl – trunk paint	60 g a.i./liter	0.5 a ^z	0.1 a	0.7 a	0.3 a	0.5 a	0.4 a	0.8 ab	0.4 a
Fosetyl-Al – trunk paint	80 g a.i./liter	0.2 a	0.2 a	0.5 a	0.4 a	0.3 a	0.3 a	0.3 a	0.5 a
Fosetyl-Al – foliar spray	24 g a.i./liter	0.2 a	0.1 a	1.5 b	0.4 a	0.7 ab	0.4 a	1.1 b	0.5 a
Fosetyl-Al – soil drench	2.4 g a.i./tree	0.5 a	0.6 a	2.3 b	0.4 a	2.6 c	0.4 a	2.4 c	0.4 a
Metalaxyl – soil drench	0.075 g a.i./tree	1.3 b	0.3 a	1.7 b	0.8 b	1.2 b	1.5 b	2.6 c	0.6 a
Nontreated control		2.0 c	2.4 b	1.8 b	1.8 c	2.4 c	2.5 c	2.4 c	2.7 b

^x Lesion area 10 days after inoculation with *P. citrophthora*. Mean of five trees.

^y Paint treatments were applied to a 20-cm section of trunk. Foliar sprays were applied to runoff (~100 ml/tree). Soil drench treatments received ~500 ml/tree of aqueous solution of fungicide. Metalaxyl (Ridomil, 25% wt/wt WP; Syngenta Agro, Spain), fosetyl-Al (Alliette, 80% wt/wt WG; Bayer CropScience, Spain).

^z Numbers within columns followed by the same letter are not significantly different (LSD at *P* < 0.05).

tive medium (22) from at least one tree arbitrarily selected in each treatment confirmed that lesions were caused by *P. citrophthora*.

2004 field experiments. Experiments were carried out in commercial orchards at the following three locations: (i) a 6-year-old 'Hernandina' orchard grafted on Carrizo citrange rootstock at El Puig in Valencia Province; (ii) a 10-year-old Clementine 'Nour' orchard grafted on Carrizo citrange rootstock at Gibrleón in Huelva Province; and (iii) a 17-year-old 'Hernandina' orchard grafted on Carrizo citrange rootstock at Alcora in Castellón Province. All three orchards were drip irrigated.

Two experiments were performed in each orchard. In one experiment, three lignified branches (~25 to 30 mm diameter) inside the canopy of each tree were inoculated 30 days before application of fungicides to evaluate their curative activity. Inoculation was performed as described above but making an 8-mm-diameter wound. Fungicides were applied on 10 May in the 17-year-old 'Hernandina' orchard, on 17 May in the 6-year-old 'Hernandina' orchard, and on 7 July in the 10-year-old 'Nour' orchard. Fungicides, rates, and application methods used are indicated in Table 2. Lesion length was quantified in one of the three inoculated branches of each tree at 15, 30, and 45 days after fungicide application. In both 'Hernandina' orchards, length was measured only from the inoculation point to the lower margin of the lesion.

In the other experiment, one branch per tree was inoculated with *P. citrophthora* at 15, 30, 45, and 60 days after application of fungicides to evaluate their residual activity. Fungicides were applied on 16 April in the 10-year-old 'Nour' orchard, on 6 May in the 17-year-old 'Hernandina' orchard, and on 7 May in the 6-year-old 'Hernandina' orchard. Fungicides, rates, and application methods used are indicated in Table 3. Lesion length was quantified 30 days after each inoculation date. In all experiments, isolations confirmed that lesions were caused by *P. citrophthora*.

In both 'Hernandina' orchards, drip chemigation treatments were applied individually for each plot by connecting the drip lines to a pump tank. Fungicide concentration in the irrigation water was adjusted to deliver the desired amount of chemical per tree. Chemigation treatments in the 'Nour' orchard were applied individually to each plot with a portable pitot tube injector. In all cases, irrigation periods were similar to the standard in each orchard. Duration of the injection period was at least equal to the pre- and post-injection periods in order to assure a proper distribution of the fungicides into the wetted soil volume. Foliar sprays were applied with a hydraulic sprayer (Mañez y Lozano, Spain) at 3,000 kPa with a hand-held spray gun. Spray volumes varied according to

the size and canopy density of the tree, but all trees were sprayed to runoff (~6 to 10 liters/tree). In paint treatments, aqueous solutions of the fungicides were applied with a paintbrush covering a section 30 cm long of four branches in the residual activity experiments and three branches in the curative activity experiments. Inoculations were made in the center of the treated area.

Two isolates of *P. citrophthora* were used in each experiment (Phy 033 and Phy 051 in 'Hernandina' orchards; HU-03/0313 and HU-03/1981 in 'Nour' orchard). In all experiments, each fungicide treatment-isolate combination was replicated four times on one-tree plots arranged in a randomized block design. Guard trees were located between plots within rows. Four nontreated one-tree plots inoculated with each isolate were included as controls.

2005 field experiments. These experiments were conducted to evaluate the residual activity of fungicides in two commercial orchards: (i) a 11-year-old 'Nour' orchard grafted on Carrizo citrange rootstock at Gibrleón in Huelva Province, and (ii) a 12-year-old 'Hernandina' orchard grafted on Carrizo citrange rootstock at Monserrat in Valencia Province. Both orchards were drip irrigated. In 2005, several treatments utilized two consecutive applications of fungicides. In the 11-year-old 'Nour' orchard, fungicide applications were made on 13 April and 28 April, and in the 12-year-old 'Hernandina' orchard on 14 May and 5 June. Treatments that util-

ized one application were done on the later date. In the 12-year-old 'Hernandina' orchard, chemigation was performed by injecting the fungicides directly into the fertigation system following the schedule described above. Drip lines corresponding to other treatments were turned off during the application. Fungicide rate was increased 13% as the measured difference in flow rate between the closest and farthest emitter from the injector. Foliar sprays and branch paints were applied as described in previous field experiments. Fungicides, rates, application methods, and schedules used are indicated in Table 4.

In the experiment on 'Hernandina', one branch per tree was inoculated at 15, 30, and 45 days after the last fungicide application. Two isolates of *P. citrophthora* were used (Phy 033 and Phy 051). Lesion area was quantified 30 days after each inoculation date by means of the Assess software as described above. In the experiment on 'Nour', one branch per tree was inoculated with the isolate HU-03/0313 at 15 and 30 days after the last fungicide application. Lesion length was quantified 30 days after each inoculation date. Each fungicide treatment-isolate combination was replicated four times on two-tree plots arranged in a randomized block design. Guard trees were located between plots within rows. Four nontreated two-tree plots inoculated with each isolate were included as controls. Isolations confirmed that lesions were caused by *P. citrophthora*.

Table 2. Curative activity of fungicide treatments applied 30 days after the inoculation of branches with two isolates of *Phytophthora citrophthora* in three field experiments conducted in 2004

Treatments ^y	Dose	Length of lesion (cm) ^x		
		Days after fungicide application		
		15 days	30 days	45 days
6-year-old ‘Hernandina’				
Metalaxyl - branch paint	60 g a.i./liter	6.1 a ^z	7.1 a	3.7 a
Fosetyl-Al - branch paint	80 g a.i./liter	7.9 ab	8.1 a	6.4 ab
Fosetyl-Al - foliar spray	3.3 g a.i./liter	7.7 ab	7.2 a	7.0 ab
Metalaxyl – drip chemigation	2 g a.i./tree	9.0 b	9.3 a	5.2 ab
Fosetyl-Al – drip chemigation	20 g a.i./tree	8.1 ab	7.5 a	6.1 ab
Nontreated control		8.1 ab	10.5 a	8.4 b
10-year-old ‘Nour’				
Metalaxyl - branch paint	60 g a.i./liter	5.0 a	3.6 a	4.0 a
Fosetyl-Al - branch paint	80 g a.i./liter	4.0 a	5.4 ab	4.1 a
Fosetyl-Al - foliar spray	2 g a.i./liter	3.6 a	6.7 b	4.6 a
Fosetyl-Al – drip chemigation	20 g a.i./tree	5.5 a	4.6 ab	5.0 a
Nontreated control		4.4 a	5.7 ab	5.6 a
17-year-old ‘Hernandina’				
Metalaxyl – branch paint	60 g a.i./liter	7.4 a	9.2 a	9.0 a
Fosetyl-Al - branch paint	80 g a.i./liter	8.1 a	7.9 a	9.5 a
Fosetyl-Al – foliar spray	2 g a.i./liter	7.2 a	12.4 b	8.6 a
Metalaxyl – drip chemigation	2 g a.i./tree	7.6 a	11.8 b	13.2 ab
Fosetyl-Al – drip chemigation	20 g a.i./tree	7.5 a	12.1 b	12.7 ab
Nontreated control		9.7 a	13.9 b	11.7 ab

^x Lesion length is the mean of eight trees. In 'Hernandina' experiments, data are the length from the inoculation point to the lower margin of the lesion.

^y Paint treatments were applied to the branches covering a 30-cm section. Foliar sprays were applied to runoff (~6 liter/tree for the 6-year-old trees and ~10 liter/tree for the 10- and 17-year-old trees). Metalaxyl (Ridomil, 25% wt/wt WP; Syngenta Agro, Spain), fosetyl-Al (Alliette, 80% wt/wt WG; Bayer CropScience, Spain).

^z Numbers within columns followed by the same letter are not significantly different (LSD at *P* < 0.05).

2006 field experiment. One experiment was conducted to evaluate the residual activity of fungicides in an orchard planted with 3-year-old ‘Hernandina’ trees grafted on Carrizo citrange rootstock at Benifao in Valencia Province. The orchard was furrow irrigated. Fungicides were applied as described in the greenhouse experiment, with the exception of the soil application, which was made with a 20-cm-depth injector connected to a handgun sprayer (Solo, Germany) applying ~1 liter of aqueous solution of fungicide to the root area of each tree. Fungicides, rates, application methods, and schedules used are indicated in Table 5. The first fungicide application was done on 9 June and the second one on 26 June. Treatments utilizing one application were done on the later date. Two trees of each plot were inoculated on the scion part of the trunk as described above at 15, 30, and 45 days after the last fungicide application. Two isolates of *P. citrophthora* (Phy 033 and Phy 051) were used. Lesion area was quantified 15 days after each inoculation by means of the Assess software as described. Each fungicide treatment-isolate combination was replicated four times on six-tree plots arranged in a randomized block design. Four nontreated six-tree plots inoculated with each isolate were included as controls. Isolations confirmed that lesions were caused by *P. citrophthora*.

Analysis of data. Lesion size data were analyzed separately for each experiment and inoculation date. Analysis of variance (ANOVA) was performed to study the effects of the factors “fungicide treat-

ment”, “isolate”, and their interaction on lesion size. Means were separated according to Fisher’s least significant difference test ($P < 0.05$). Mean separation tests for fungicide treatments were conducted separately for each isolate only when the interaction was significant at $P < 0.01$. Statistical analyses were performed with the software Statgraphics Plus 5.1 (Manugistics Inc., Rockville, MD, USA). Additionally, average reduction in lesion size [% reduction = $100 - (\text{Av. lesion size treatment} \times 100) / \text{Av. lesion size control}$] was calculated for the treatments that displayed significant differences from the nontreated control.

RESULTS

2004 greenhouse experiment. Both factors “fungicide treatment” and “isolate” and their interaction were significant ($P < 0.01$). Fosetyl-Al and metalaxyl trunk paints significantly reduced the area of lesions compared with the nontreated control for up to 40 days after application (Table 1). Lesion expansion was also significantly inhibited by fosetyl-Al applied as a foliar spray, with the exception of the isolate Phy 033 inoculated 20 days after application. Lesions recorded in trees treated with fosetyl-Al as soil drench were significantly lower than the nontreated control for up to 40 days after application using the isolate Phy 051 and for up to 10 days with the isolate Phy 033. Metalaxyl applied as soil drench displayed similar results, with the exception of the inoculation with Phy 033 made 30 days after the application, which significantly reduced

lesion size. Average reduction in lesion size for the treatments that displayed significant differences from the nontreated control ranged from 60% with metalaxyl trunk paint with isolate Phy 033 inoculated at 20 days to 95% with fosetyl-Al foliar spray with the same isolate inoculated at 10 days.

2004 field experiments. In the curative activity experiments, none of the studied factors and their interaction were significant ($P < 0.05$). In the experiment on 6-year-old ‘Hernandina’ trees, lesion expansion was inhibited significantly only with metalaxyl branch paint at the last evaluation date (45 days after fungicide application/75 days after inoculation) (Table 2). No significant inhibition of lesion expansion was detected in the 10-year-old ‘Nour’ experiment. In the 17-year-old ‘Hernandina’ experiment, only metalaxyl and fosetyl-Al applied as branch paints at the second evaluation date (30 days after fungicide application/60 days after inoculation) were different from the nontreated control (Table 2). Average reduction in lesion size for the treatments that displayed significant differences from the nontreated control ranged from 34% at 30 days with fosetyl-Al branch paint in 17-year-old ‘Hernandina’ trees to 55% with metalaxyl branch paint at 45 days in 6-year-old ‘Hernandina’ trees.

In the residual activity experiments performed in ‘Hernandina’ orchards, only the factor “fungicide treatment” was significant ($P < 0.05$). In the 10-year-old ‘Nour’ experiment, the factor “fungicide treatment” was also significant ($P < 0.05$), but

Table 3. Residual activity of fungicide treatments on branches of citrus trees inoculated with two isolates of *Phytophthora citrophthora* in three field experiments conducted in 2004

Treatments ^y	Dose	Length of lesion (cm) ^x			
		Days from fungicide application to inoculation			
		15	30	45	60
6-year-old ‘Hernandina’					
Metalaxyl - branch paint	60 g a.i./liter	0.6 a ^z	1.5 a	0.6 a	1.7 a
Fosetyl-Al - branch paint	80 g a.i./liter	3.7 ab	3.4 ab	2.4 b	1.7 a
Fosetyl-Al - foliar spray	3.3 g a.i./liter	5.4 bc	3.4 ab	2.3 b	3.4 a
Metalaxyl - drip chemigation	2 g a.i./tree	6.4 bc	4.7 bc	3.6 b	2.9 a
Fosetyl-Al - drip chemigation	20 g a.i./tree	6.6 bc	6.8 c	3.5 b	3.3 a
Nontreated control		8.3 c	6.1 c	2.3 b	3.3 a
10-year-old ‘Nour’					
Metalaxyl - branch paint	60 g a.i./liter	5.6 a	2.7 ab	2.4 a	2.1 a
Fosetyl-Al - branch paint	80 g a.i./liter	5.2 a	3.0 ab	1.6 a	2.2 a
Fosetyl-Al - foliar spray	2 g a.i./liter	7.4 a	2.5 ab	2.3 a	2.3 a
Fosetyl-Al - drip chemigation	20 g a.i./tree	5.7 a	2.1 a	2.5 a	1.8 a
Nontreated control		5.6 a	3.7 b	3.2 a	2.8 a
17-year-old ‘Hernandina’					
Metalaxyl – branch paint	60 g a.i./liter	1.4 a	2.9 a	1.4 a	0.9 a
Fosetyl-Al - branch paint	80 g a.i./liter	8.4 b	5.8 ab	4.6 a	4.0 bc
Fosetyl-Al – foliar spray	2 g a.i./liter	10.0 b	8.9 bc	4.1 a	4.8 bc
Metalaxyl – drip chemigation	2 g a.i./tree	10.0 b	8.9 bc	5.9 ab	6.5 c
Fosetyl-Al – drip chemigation	20 g a.i./tree	10.2 b	11.2 c	4.3 a	6.3 bc
Nontreated control		10.0 b	11.1 c	9.3 b	5.7 bc

^x Lesion length 30 days after inoculation with *P. citrophthora*. Mean of eight trees.

^y Paint treatments were applied to the branches covering a 30-cm section. Foliar sprays were applied to runoff (~6 liter/tree for the 6-year-old trees and ~10 liter/tree for the 10- and 17-year-old trees). Metalaxyl (Ridomil, 25% wt/wt WP; Syngenta Agro, Spain), fosetyl-Al (Alliette, 80% wt/wt WG; Bayer Crop Science, Spain).

^z Numbers within columns followed by the same letter are not significantly different (LSD at $P < 0.05$).

only in the inoculation made 30 days after fungicide application. In the experiment on 6-year-old 'Hernandina' trees, lesion size obtained in paint treatments was significantly lower than the nontreated control for up to 45 days with metalaxyl, and for up to 30 days with fosetyl-Al (Table 3). The foliar spray with fosetyl-Al reduced lesion expansion only in the inoculation made 30 days after fungicide application. No significant effect was detected with the drip chemigation treatments. In the 10-year-old 'Nour' experiment, fosetyl-Al applied by drip chemigation significantly reduced lesion size compared with the control, but only at the second inoculation date (Table 3). The experiment conducted on 17-year-old 'Hernandina' trees showed that metalaxyl applied as branch paint effectively reduced lesion size in relation to the control for up to 60 days after fungicide application. Fosetyl-Al applied as branch paint significantly inhibited lesion development in the inoculations made 30 and 45 days after fungicide application. Fosetyl-Al applied as either a foliar spray or by drip chemigation significantly reduced lesion development only in the inoculation performed 45 days after fungicide treatment. Metalaxyl applied as drip chemigation was not effective in reducing lesion size for any of the inoculation dates (Table 3). Average reduction in lesion size for the treatments that displayed significant differences from the nontreated control ranged from 50% at 30 days with fosetyl-Al branch paint in 10-year-old 'Nour' trees to 92% with metalaxyl branch paint at 15 days in 6-year-old 'Hernandina' trees.

2005 field experiments. In the 'Hernandina' experiment, the factor "fungicide treatment" was significant ($P < 0.05$), but not "isolate" nor the interaction between fungicide and isolate. In the inoculation made 15 days after fungicide application, lesion expansion was inhibited significantly by all treatments, with the exception of drip chemigation with one application of fosetyl-Al (Table 4). In the inoculation made 30 days after fungicide application, only branch paint with mefenoxam and foliar sprays with fosetyl-Al significantly reduced lesion size. None of the treatments studied were effective in reducing lesion size 45 days after fungicide application. Average reduction in lesion size for the treatments that displayed significant differences from the nontreated control ranged from 35% at 15 days with two consecutive drip chemigations of fosetyl-Al to 83% with mefenoxam branch paint at 30 days. No significant differences from the nontreated control were detected in the experiment on 11-year-old 'Nour'.

2006 field experiment. Data obtained in the inoculation made 30 days after fungicide application with isolate Phy 033 were not included in the analysis due to anomalous results in the nontreated control. The

factor "fungicide treatment" was significant ($P < 0.05$), but not "isolate" nor the interaction between fungicide and isolate. Mefenoxam trunk paint and fosetyl-Al foliar sprays reduced lesion size compared to the nontreated control for up to 45 days after fungicide application (Table 5). A single soil application of fosetyl-Al significantly inhibited lesion expansion in the inoculations made 30 days and 45 days after fungicide application. In contrast, the effectiveness of two consecutive soil applications was only significant at the last inoculation date. Average reduction in lesion size for the treatments that displayed significant differences from the nontreated control ranged from 51% at 15 days with two foliar sprays of fosetyl-Al to 91% with the mefenoxam trunk paint at 30 days.

DISCUSSION

None of the fungicide treatments included in our study reduced lesion expansion when applications were made on a

curative basis 30 days after inoculation with *P. citrophthora*. Residual activity of fungicides in field experiments on mature trees (6 to 17 years old) was generally low, especially in the drip chemigation and foliar spray treatments, and none of the treatments evaluated completely inhibited lesion expansion.

The inoculation technique adopted in our study has extensively been used in previous work with *P. citrophthora* and *P. nicotianae* (11,23,25), and it is recommended by the EPPO guidelines for evaluation of the efficacy of fungicides against *Phytophthora gummosis* (3). Although *Phytophthora* infections on suberized tissues occur through wounds and growth cracks (17), the inoculation technique used involves removing large areas of the bark and placing the inoculum directly in contact with the cambium. Thus, the penetration phase of the pathogen's life cycle is greatly facilitated. With this technique it is possible to evaluate the ability

Table 4. Residual activity of fungicide treatments on branches of citrus trees inoculated with two isolates of *Phytophthora citrophthora* in two field experiments conducted in 2005

Treatments ^y	Dose	Lesion size ^x		
		Days from fungicide application to inoculation		
		15	30	45
11-year-old 'Nour'				
Mefenoxam – branch paint	5 g a.i./liter	2.5 a ^z	3.1 a	
Fosetyl-Al – foliar spray × 2	2 g a.i./liter	3.4 a	3.7 a	
Nontreated control		3.5 a	5.1 a	
12-year-old 'Hernandina'				
Mefenoxam – branch paint	5 g a.i./liter	1.9 a	1.7 a	1.7 a
Fosetyl-Al – foliar spray	2 g a.i./liter	1.8 a	3.7 a	2.1 a
Fosetyl-Al – foliar spray × 2	2 g a.i./liter	4.3 a	3.2 a	2.1 a
Fosetyl-Al – drip chemigation	20 g a.i./tree	8.7 b	10.9 b	4.9 ab
Fosetyl-Al – drip chemigation × 2	20 g a.i./tree	4.6 a	8.9 b	2.7 a
Nontreated control		7.1 b	9.7 b	5.4 ab

^x Lesion size 30 days after inoculation with *P. citrophthora*. Mean of 16 trees. Lesion length (cm) for 'Nour' and lesion area (cm²) for 'Hernandina'.

^y Paint treatments were applied to the branches covering a 30-cm section. Foliar sprays were applied to runoff (~10 liter/tree). Mefenoxam (Ridomil Gold, 2.5% wt/wt GR; Syngenta Agro, Spain), fosetyl-Al (Alliette, 80% wt/wt WG; Bayer CropScience, Spain).

^z Numbers within columns followed by the same letter are not significantly different (LSD at $P < 0.05$).

Table 5. Residual activity of fungicide treatments on trunks of 3-year-old 'Hernandina' trees inoculated with two isolates of *Phytophthora citrophthora* in 2006

Treatments ^y	Dose	Area of lesion (cm ²) ^x		
		Days from fungicide application to inoculation		
		15	30	45
Mefenoxam - trunk paint	5 g a.i./liter	0.4 a ^z	0.1 a	0.3 a
Fosetyl-Al - foliar spray	44 g a.i./liter	0.4 a	0.1 a	0.9 b
Fosetyl-Al - foliar spray × 2	44 g a.i./liter	0.5 a	0.2 a	0.9 b
Fosetyl-Al - soil application	5.4 g a.i./tree	0.8 ab	0.3 ab	1.5 c
Fosetyl-Al - soil application × 2	5.4 g a.i./tree	0.8 ab	0.6 bc	1.2 bc
Nontreated control		0.9 b	0.8 c	2.2 d

^x Lesion area 15 days after inoculation with *P. citrophthora*. Mean of 16 trees.

^y Paint treatments were applied to a 30-cm section of trunk. Foliar sprays were applied to runoff (~140 ml/tree). Soil application of fungicides was made by injecting 1 liter/tree of aqueous solution of fungicide in the root zone. Mefenoxam (Ridomil Gold, 48% wt/vol EC; Syngenta Agro, Spain), fosetyl-Al (Alliette, 80% wt/wt WG; Bayer CropScience, Spain).

^z Numbers within columns followed by the same letter are not significantly different (LSD at $P < 0.05$).

of fungicides to inhibit lesion expansion, but not their preinfection protective activity.

Systemic fungicides generally show good protective activity when applied directly on the target, as in the case of the paint treatments. Nevertheless, soil drench applications of systemic fungicides completely inhibited the germination of *P. nicotianae* zoospores on the trunk of grapefruit trees (31). Besides their postinfection curative activity, the ability of systemic fungicides to prevent infection also should be evaluated to determine their efficacy under field conditions. Unfortunately, our attempts to infect nonwounded branches and bark strips on their external side were unsuccessful (data not shown). The zoospore bark inoculation technique described by Timmer (30) might produce better results and will be used in future experiments to evaluate the protective activity of fungicides.

Although the efficacy of foliar spray and soil drench applications was variable, in general, all the treatments studied on potted plants under greenhouse conditions were effective in reducing lesion expansion. Trunk paint and foliar spray treatments were also highly effective in the field experiment on young, 3-year-old 'Hernandina' trees in 2006. Soil applications of fosetyl-Al were effective as well, but mostly at the last inoculation date. As concluded in previous studies (11,23,25,32), efficacy of systemic fungicides on young trees was usually better than in mature trees. This may be a consequence of greater dilution of the fungicides in the large canopy of mature trees. Time needed for metabolization into the plant might explain this phenomenon in the case of fosetyl-Al (28). However, in our study, young trees were inoculated on the trunk and mature trees were inoculated on the branches. This difference in the inoculation procedure may also have influenced the results.

Soil application of systemic fungicides has proven effective in controlling Phytophthora root rot and foot rot of citrus caused by *P. citrophthora* and *P. nicotianae* (10,11,14,24,26,31,32). However, there are no reports about the efficacy of chemigation through drip irrigation systems on the control of Phytophthora gummosis and branch canker of citrus. Growers in Spain are increasingly implementing surface drip irrigation systems to improve efficiency of water use and to reduce leaching of fertilizers. Compared with foliar sprays and topical paints, drip chemigation can reduce greatly the cost of fungicide application. In addition, soil application of fungicides by drip irrigation has several environmental advantages, such as elimination of drift and waste from spray tanks, reduced worker exposure, and minimization of soil compaction caused by the transit of sprayers.

Although significant differences were detected for some inoculation dates, the efficacy of drip chemigation treatments in reducing lesion expansion was generally low. The available concentration of fungicides in soil can be reduced by sorption, diffusion, volatilization, leaching, and chemical and microbial degradation (29). On the other hand, low performance of soil applications also may be a consequence of poor uptake of the fungicide, reduced translocation, or dilution in the large canopy of mature trees. In our study, fungicide applications were scheduled to coincide with the spring leaf flush to maximize translocation. However, root flushes do not necessarily coincide with shoot growth (5), and the uptake of fungicides from soil might have been affected. In any case, direct measurements to determine the fungicide dynamics from soil to inoculated branches would be needed to validate this hypothesis (28).

Currently, foliar sprays with hand guns or airblast sprayers are the standard methods of pesticide application in Spain. Although foliar fungicide sprays are expensive, application costs can be reduced by applying fungicides in combination with insecticides and acaricides. In our study, the efficacy of fosetyl-Al foliar sprays applied to mature trees was variable among experiments. In 2005, they displayed good residual activity in the 'Hernandina' experiment, but were virtually ineffective in reducing lesion expansion in all three experiments conducted in 2004 on 'Hernandina' and 'Nour'. Besides its fungitoxic activity, the effect of fosetyl-Al against Oomycetes is related also to the induction of plant phytoalexins (1,16). In our experiments, the ability of fungicides to inhibit lesion expansion was evaluated up to 2 months after one or two applications. This experimental approach is generally appropriate to evaluate the fungitoxic activity of fungicides. However, to take into account the effect on host resistance, it would be worthwhile to evaluate the long-term efficacy of fosetyl-Al following a multiyear spray or drip chemigation programs.

As in previous studies (11,14,23,25,31,32), paint treatments were generally effective in reducing lesion expansion. However, despite its efficacy, citrus growers in Spain cannot routinely use this application method because it is laborious and not economically feasible. Trunk and branch sprays are a low-cost alternative to paint treatments (27). This technique may be economical even where labor costs are high as in Spain. However, trees should be skirt-pruned and openings have to be made in the outer canopy to allow spray penetration. In any case, the efficacy of trunk and branch sprays for the control of Phytophthora branch canker of citrus caused by *P. citrophthora* should be evaluated under field conditions.

Differences between metalaxyl (mefenoxam) and fosetyl-Al paint treatments were not consistent among experiments and evaluation dates. As with other phenylamide fungicides, metalaxyl (mefenoxam) has a high inherent risk of resistance problems, particularly when applications are made on a curative or eradication basis (6). Topical applications of metalaxyl (mefenoxam) for control of Phytophthora branch canker of citrus caused by *P. citrophthora* would probably need to follow an anti-resistance strategy, which would imply using the fungicide only as protectant and in mixtures with a multisite compound (6). Although insensitivity to fosetyl-Al has been reported in California for the lettuce downy mildew pathogen, *Bremia lactucae* Regel (7), phosphonates have been used for many years for the control of *Phytophthora* diseases without major field resistance problems (6).

The epidemiology of Phytophthora branch canker of citrus caused by *P. citrophthora* in Spain is still under study. However, due to the rainless Mediterranean summer, critical periods for infection probably occur during spring and autumn months. Moreover, *P. citrophthora* is less active during summer months due to high temperatures (17). In our case, this effect of temperature is illustrated in the residual activity experiments by the smaller lesion size obtained in the latest inoculation dates. Clementine trees are more susceptible to *P. citrophthora* infection in spring (L. A. Alvarez, unpublished), but environmental conditions are more favorable for inoculum spread and infection during the rainy and relatively warm autumns common in Spanish citrus-growing areas. Our experiments were conducted in spring to maximize fungicide translocation and to assure high levels of infection. However, the efficacy of fungicide treatments for the control of autumn infections could not be ascertained. As stated above, future experiments will test the efficacy of long-term application programs against spring and autumn inoculations.

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