Fungitoxic Activity of Efosite Aluminum on Growth, Sporulation, and Germination of *Phytophthora parasitica* and *P. citrophthora*

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ABSTRACT

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Efosite Al was not highly inhibitory to mycelial growth but was slightly more active against *Phytophthora citrophthora* than against *P. parasitica*; the ED_{50} values were 1,146 and 285 mg/L at 3 days and 929 and 56 mg/L at 7 days for *P. parasitica* and *P. citrophthora*, respectively. Formation of sporangia, chlamydospores, and oospores was highly sensitive to efosite Al, but zoospore germination, chlamydospore germination, and germ tube growth were insensitive to low concentrations (100 mg/L or less) of the fungicide. Indirect sporangium germination of *P. parasitica* was more sensitive to efosite Al than that of *P. citrophthora*. Because of its activity against sporulation of both *Phytophthora* spp., efosite Al can be considered an antisporulant compound.

Additional key words: Aliette, fosetyl Al, phosethyl Al

The systemic fungicide, efosite aluminum (efosite Al, aluminum tris-0-ethyl phosphonate, phosethyl Al or LS 74-783, Aliette), recently developed by Rhône Poulenc Phytosanitaire in France, is effective in control of diseases caused by some fungi in the class Oomycetes (1,2,5,6,10,15,17–19). However, few studies on its in vitro toxicity toward target oomycetous fungi were made (8,15,19), and the biological and chemical modes of action for its efficacy have not yet been determined.

Efosite Al was effective in control of gummosis and/or root rot of citrus caused by *Phytophthora parasitica* and *P. citrophthora* (3,5,17). The present study was undertaken to investigate the in vitro effects of this fungicide on the different stages in the life cycles of these two *Phytophthora* spp. in an attempt to elucidate the biological mode of action for the control of these fungi.

MATERIALS AND METHODS

Two species of *Phytophthora* were used in the study: *P. parasitica* Dast. sensu Tucker (14) (*P. nicotianae* Breda de Haan sensu Waterhouse [16]), isolate T131, and *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian, isolate T544. Both are pathogenic on citrus.

Efosite Al solutions were prepared by a method described previously (4). The fungicide formulation was a wettable powder containing 80% active ingredient. Final concentrations of efosite Al were expressed as mg a.i./L of water, broth medium, or agar medium. When incorporated in the V-8 juice agar medium (pH 5.9; Campbell V-8 juice, 100 ml; 2% $CaCO_3$, 100 ml; agar, 15 g; deionized water, 800 ml), efosite Al up to 100 mg/L slightly decreased the pH of the medium. The pH ranged from 5.9 to 5.6 in V-8 juice agar containing 0–100 mg/L efosite Al. When dissolved in distilled deionized water (pH 6.2), efosite Al substantially lowered the solution pH; eg, to pH 4.8 and 4.0 in 10 and 100 mg/L solutions, respectively.

Toxicity of efosite Al to linear mycelial growth of *P. parasitica* and *P. citrophthora* was evaluated in vitro by incorporating various concentrations of the fungicide in V-8 juice agar medium, employing an earlier method (4). Linear extension was measured at 3 and 7 days.

The method for studying the effect on sporangium formation was the same as that described by Tsao and Oster (12) and adapted by Farih et al (4). Briefly, 1-day-old colonies on nylon mesh squares were incubated in fungicide solutions or water control for 2–3 days before being mounted on microscope slides for quantification of sporulation. A method similar to that described for sporangium formation (4,12) was used for studying the effect of efosite Al on sporangium germination. Each nylon mesh square supporting a sporangium-bearing mat was incubated in fungicide solutions or water control at 25 C before indirect sporangium germination was induced at 18 C. The method used for zoospore germination was described earlier (4). Germination was read after 7–8 hr of incubation.

Chlamydospores of *P. parasitica* were produced in V-8 juice broth according to the method described by Tsao (11) and adapted by Farih et al (4). The procedure for chlamydospore harvest and germination was a modified method of Holdaway and Tsao (7) as described by Tsao and Oster (12) and later adapted for studying fungicidal effect (4). The effect of efosite Al on zoospore and chlamydospore germination was evaluated on V-8 juice agar medium. All germination data are reported as the percentage of control without fungicide.

A method similar to that described in a study on metalaxyl (4) was used for testing the effect of efosite Al on oospore formation, using a highly oosporogenic culture of *P. parasitica* (13). *P. citrophthora* does not form oospores, could not be induced to form large amounts of chlamydospores with various methods, and therefore was not included in these phases of our study.

Formation of sporangia, chlamydospores, and oospores was quantitated as reported earlier (4).

All experiments were repeated at least once, with or without certain modifications in treatments.

RESULTS

Efosite Al was less inhibitory to linear mycelial growth of *P. parasitica* than to that of *P. citrophthora*. The ED_{50} values were 1,146 and 285 mg/L at 3 days and 929 and 56 mg/L at 7 days for *P. parasitica* and *P. citrophthora*, respectively (Table 1).

Sporangium formation was more sensitive than mycelial growth to efosite Al. Concentration as low as 5 mg/L resulted in 96.6 and

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100% inhibition of sporangium formation of *P. parasitica* and *P. citrophthora*, respectively (Table 2). In a repeat experiment, efosite Al at 10 mg/L resulted in complete inhibition of sporangium formation of both species of *Phytophthora*.

To test the possibility that inhibition of sporangium formation might be due to a pH effect, an experiment with *P. parasitica* sporangia was conducted in which the water control and the efosite Al solutions of 5, 10, and 100 mg/L were prepared each with and without the solutions adjusted to pH 6.5 with KOH or HCl solution. The degrees of inhibition of sporangium formation in the two solution series were similar. Although inhibition was complete in the series of three concentrations with pH not adjusted, in the series of solutions adjusted to pH 6.5, the inhibition by 5, 10, and 100 mg/L efosite Al was 79, 92, and 98%, respectively. In another experiment, inhibition was 85, 87, and 100%, respectively, when adjusted to pH 6.0. The data clearly showed that as the concentration increased, the inhibitory effect of the fungicide solution also increased, even in the pH-adjusted series.

Efosite Al was more inhibitory to indirect sporangium germination (zoospore release) of *P. parasitica* than to that of *P. citrophthora* (Table 2). Concentration as low as 10 mg/L reduced germination of *P. parasitica* sporangia by more than 90%, but with *P. citrophthora*, 100 mg/L resulted in only 21% inhibition (Table 2). A repeat experiment showed similar results.

In the presence of efosite Al at 100 mg/L or lower concentrations, zoospore germination of both *Phytophthora* spp. was not inhibited. At 1,000 mg/L, the fungicide reduced zoospore germination by 98 and 53% of *P. parasitica* and *P. citrophthora*, respectively (Table 3). Germinated zoospores on fungicide-free agar medium produced germ tubes of 330 and 116 μm for *P. parasitica* and *P. citrophthora*, respectively, after 8-hr incubation.

TABLE 1. Effect of efosite aluminum in V8-agar medium on linear mycelial growth of *Phytophthora parasitica* and *P. citrophthora*

Concn (mg/L)	Linear mycelial growth (mm)[z]			
	P. parasitica		*P. citrophthora*	
	At 3 days	At 7 days	At 3 days	At 7 days
0	11 a	35.2 a	24 a	63.9 a
250	10.5 a	30 b	11.9 b	19.6 b
500	9.9 b	25.3 c	10.3 c	15.1 c
750	7 c	19.2 d	8.3 d	13.6 c
1,000	6.1 d	16.8 e	5.5 e	10.3 d
2,000	0 e	1 f	0 f	0 e
4,000	0 e	0 f	0 f	0 e
5,000	0 e	0 f	0 f	0 e

[z]Each number is an average of three replicates. Figures with same letter in each column are not significantly different ($P = 0.05$, Duncan's multiple range test).

TABLE 2. Effect of efosite aluminum on the formation and indirect germination (zoospore release) of sporangia of *Phytophthora parasitica* (Pp) and *P. citrophthora* (Pc)[y]

Concn (mg/L)	Formation (no. sporangia/mm^2)		Germination (%)[z]	
	Pp	Pc	Pp	Pc
0	67.7 a	113.2 a	100 a	100 a
0	60.4 ab	80 b		
1	48.5 b	59.3 b	100.4 a	73.8 a
5	2.3 c	0 c		
10	0 c	0 c	9.5 b	77.8 a
50	0 c	0 c		
100	0 c	0 c	11.8 b	78.7 a
1,000	0 c	0 c	3.7 b	15.5 b

[y]Each number is an average of three replicates. Figures with same letter in each column are not significantly different ($P = 0.01$, Duncan's mutiple range test).

[z]Each number is expressed as percent of appropriate water control (0 mg/L). The actual percent germination of control was 73.7 for Pp and 34.3 for Pc. The percent germination at 0 hr was 6.3 for Pp and 4.3 for Pc.

No effect of efosite Al was found on germ tube growth at 100 mg/L, but 1,000 mg/L reduced that of *P. citrophthora* by 78%. In a repeat experiment, results were similar at concentrations up to 100 mg/L, but 1,000 mg/L of the fungicide resulted in 93 and 44% inhibition of zoospore germination of *P. parasitica* and *P. citrophthora*, respectively.

Chlamydospore formation of *P. parasitica* in liquid culture was not inhibited by efosite Al at concentrations up to 10 mg/L. The inhibition of chlamydospore formation was 98% at 50 mg/L, and complete inhibition occurred at 100 mg/L of efosite Al (Table 4). A repeat experiment showed identical results.

On fungicide-free agar medium, *P. parasitica* chlamydospores germinated and produced germ tubes 1,600 μm or longer after 16–18 hr of incubation. At 100 mg/L or lower concentrations of efosite Al, chlamydospore germination of *P. parasitica* was not greatly inhibited (Table 4). At 1,000 mg/L, chlamydospore germination was reduced by 39% and germ tube length by 83%. Inhibition of chlamydospore germination was also not observed in a repeat experiment with efosite Al concentrations up to 100 mg/L.

Efosite Al at 250 mg/L was inhibitory to oospore formation. The oosporogenic culture of *P. parasitica* grown on fungicide-free agar medium produced oospores that covered 50% of mycelial growth area. In the presence of efosite Al at 250 mg/L, the area containing oospores was reduced to 9.3%, and the density of oospores was decreased to 5.4/mm^2, compared with 19.4/mm^2 in the control (Table 5). In a repeat experiment, complete inhibition of oospore formation was observed with 500 mg/L of efosite Al.

DISCUSSION

Various workers have reported that efosite Al does not inhibit mycelial growth of *Phytophthora* spp. unless used at high concentrations. Efosite Al, 100 mg/L, resulted in 63% inhibition of

TABLE 3. Effect of efosite aluminum on zoospore germination of *Phytophthora parasitica* and *P. citrophthora*

Concn (mg/L)	Zoospore germination (%)[z]	
	P. parasitica	*P. citrophthora*
0	100 b	100 a
1	102.3 ab	102.5 a
10	103.1 a	99 a
100	102.1 ab	97 a
1,000	1.3 c	47 b

[z]Each number is an average of three replicates and is expressed as percent of control (0 mg/L). The actual percent germination of control was 97 for *P. parasitica* and 92 for *P. citrophthora*. Figures with same letter in each column are not significantly different ($P = 0.01$, Duncan's multiple range test).

TABLE 4. Effect of efosite aluminum on the formation and germination of chlamydospores of *Phytophthora parasitica*

Concn (mg/L)	Formation (no. chlamydospores $\times 10^{-3}$/culture)[x]	Germination (%)[y]
0	875 a[z]	100 a
1	810 a	101.3 a
10	956 a	91 ab
50	10 b	...
100	0 b	80.7 b
1,000	0 b	60.5 c
2,000	...	0 d

[x]Each number is an average of readings from three replicate bottles of liquid culture.

[y]Each number is an average of readings from three replicate plates of V-8 juice agar medium. Germination is expressed as percent germination of control and viable spores. Viability of spores used was 93%. The actual percent germination of control was 83.5.

[z]Figures with same letter in each column are not significantly different ($P = 0.01$, Duncan's multiple range test).

TABLE 5. Effect of efosite aluminum on oospore formation of *Phytophthora parasitica*[y]

Concn (mg/L)	Area size (cm^2)		Area of mycelial growth containing oospores (%)	Oospores (no./mm^2)
	Mycelial growth	Oospore formation		
0	23.5	11.9	50.8 a[z]	19.4 a
125	20.3	4.8	24.9 ab	14.3 b
250	16	1.5	9.3 b	5.4 c

[y]Results of growing an oosporogenic culture on V-8 juice agar medium in the dark at 25 C for 7 days.

[z]Figures with same letter in each column are not significantly different (P= 0.05, Duncan's multiple range test).

linear mycelial growth of *P. citrophthora* (8). Linear mycelial growth of *P. cinnamomi* was reduced by 89% in the presence of 1,000 mg/L efosite Al (15), but 250 mg/L did not reduce growth in liquid medium (19). Our data confirmed the findings of other workers that efosite Al was also not inhibitory or was slightly inhibitory to the mycelial growth of two *Phytophthora* spp. pathogenic on citrus except at high concentrations. At 7 days, however, efosite Al was 16.7 times more effective against *P. citrophthora* than against *P. parasitica*.

Our studies with efosite Al were performed in a comparative study with metalaxyl, another systemic antioomycetous compound (4). The dosage-response relations for inhibition of linear mycelial extension in agar can be used to demonstrate that efosite Al was about 7,600 and 23,000 times less active than metalaxyl against *P. parasitica* at 3 and 7 days, respectively; and with *P. citrophthora*, efosite Al was about 1,500 and 100 times less inhibitory than metalaxyl at 3 and 7 days, respectively. Our data also suggest that efosite Al is more inhibitory to linear mycelial growth of *P. citrophthora* than to that of *P. parasitica* although metalaxyl is more inhibitory to that of *P. parasitica* than of *P. citrophthora* (4).

The effect of efosite Al on sporangium formation was similar for both *Phytophthora* spp. but was less than the effect of metalaxyl (4). Vegh et al (15) reported that 10 mg/L efosite Al was required to achieve 93% inhibition of sporangium formation in *P. cinnamomi*. On the other hand, indirect sporangium germination (zoospore release) of *P. parasitica* was more sensitive than that of *P. citrophthora* to efosite Al. Farih et al (4) found that indirect sporangium germination of both *P. parasitica* and *P. citrophthora* was less sensitive to metalaxyl than to efosite Al. As stated earlier, efosite Al lowers solution pH when dissolved in water. However, inhibition of indirect sporangium germination still occurred in pH-adjusted solutions. Furthermore, the degree of inhibition became greater as efosite Al concentration increased. Inhibition of sporangium germination by efosite Al, therefore, was probably a direct fungitoxic effect, although a slight indirect pH effect may also be involved at high fungicide concentrations.

Neither zoospore and chlamydospore germination nor germ tube growth of both *Phytophthora* spp. were inhibited by efosite Al at low concentrations (1–100 mg/L). However, chlamydospore and oospore formation of *P. parasitica* was inhibited by this fungicide at low concentrations, a finding similar to that observed with metalaxyl (4). Because they also inhibit sporangium formation, both efosite Al and metalaxyl can be considered antisporulant compounds. This, however, does not explain how various *Phytophthora* diseases are controlled systemically by efosite Al. Several workers (2,9,18) postulated that efosite Al reduces disease systemically in plants by an indirect action, ie, the fungicide must be metabolized or otherwise changed to another chemical form that is toxic to *Phytophthora*. Our results on in vitro effects of efosite Al on the two citrus *Phytophthora* spp. produced no evidence that could be used to refute this postulation.

In studying the in vitro effects of efosite Al on *Phytophthora*, previous workers have been concerned with only the mycelial growth and sporangium formation of these pathogens. Our study was the first to examine the effect of this systemic fungicide on the formation of chlamydospores and oospores and on the germination of sporangia, zoospores, and chlamydospores.

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