

Effects of fosetyl-Al on root exudation and on composition of extracts of mycorrhizal and nonmycorrhizal leek roots

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Foliar applications of the systemic, symplastic, anti-oomycete fungicide, fosetyl-Al (tris-o-ethyl phosphonate) to mycorrhizal (M) leek plants significantly increased both vesicular-arbuscular mycorrhizal (VAM) colonization by a *Glomus* sp., and plant growth. In a second study, foliar fosetyl-Al caused a significant increase in root exudation of soluble sugars from both mycorrhizal and nonmycorrhizal (NM) plants, depending on fungicide concentration and harvesting time. In general, M plants exuded more sugars than NM plants at 2 and 7 days after treatment. Roots of treated NM plants at 7 days contained more soluble sugars than those of similarly treated M plants. Roots of treated NM plants contained more total free amino acids than roots of treated M plants at 2, 7, and 27 days after treatment. Untreated M roots contained more total lipids than did untreated NM roots. After fosetyl-Al was applied to the leaves, there was a significant concentration-related increase in total lipid of M roots. This increase was not observed in treated NM plants. Fosetyl-Al directly or indirectly influenced the physiology of both host plant and VAM fungus.

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Des applications foliaires du fongicide systémique, symplastique et anti-oomycète fosetyl-Al (tris-o-ethyl phosphonate) sur des plants de poireaux mycorrhisés (M) ont accru de façon significative la colonisation mycorrhizienne (VA) par *Glomus* sp., ainsi que la croissance de la plante. Dans une seconde étude, l'application foliaire de fosetyl-Al a engendré une augmentation de sucres solubles exsudés par les racines de plants mycorrhisés et de plants non mycorrhisés (NM); cette augmentation variait en fonction des concentrations utilisées ainsi que du temps de récolte. En général, les plants M ont exsudé plus de sucres que les plants NM, 2 et 7 jours après le traitement. Au jour 7, les racines de plants NM traités contenaient plus de sucres solubles que celles de plants M traités de façon similaire. Les racines de plants NM contenaient plus d'acides aminés totaux libres que celles de plants M aux jours 2, 7 et 27 après le traitement. Les racines de plants M témoins contenaient plus de lipides totaux que les racines de témoins NM. Après application de fosetyl-Al sur les feuilles, il y eut dans les racines de plant M, une augmentation significative de lipides totaux parallèlement à l'augmentation des concentrations utilisées. Cette augmentation n'a pas été observée sur les plants NM traités. Directement ou indirectement, le fosetyl-Al a influencé la physiologie de la plante hôte ainsi que celle du champignon VAM.

This research was engendered by two apparently contradictory observations concerning vesicular-arbuscular mycorrhizal (VAM) fungi and fungicides. On the one hand, it is to be expected that systemic fungicides will have detrimental effects on endomycorrhizal fungi. Several workers (Jalali & Domsch 1975, Sutton & Sheppard 1976, Bailey & Safir 1978, Nemeč 1980, Rhodes & Larsen 1981, Hale & Sanders 1982) showed that some systemic fungicides such as benomyl, tridemorph, triforine, ethirimol, chloraniformethan, thiabendazole, thiofanate, triadimefon, and carboxin were capable of reducing VAM root colonization and/or development of VAM spores, though other systemic fungicides such as pyroxychlor and prothiocarb appeared to have no effect on the VA-mycorrhiza development (Stewart & Pflieger 1977, Paget et al. 1976). On the other hand, some systemic fungicides actually stimulate root colonization by VAM fungi. Menge et al. (1979) showed that Terrazole when applied as a soil drench, caused a significant increase in root colonization and spore production by *Glomus fasciculatum* (Thaxter) Gerd. & Trappe in *Sorghum vulgare* Pers; while Groth and Martin-

son (1983) reported that incorporation of metalaxyl into soil containing different VAM fungi increased root colonization.

The mechanisms for stimulation of VA mycorrhizal fungi by Terrazole and metalaxyl are unknown. However both are translocated pseudo-apoplastically, mainly with the transpiration stream (Bruin & Edgington 1984), and are effective against the Oomycetes (Bruin 1980, Bruin & Edgington 1984), with no effects on other groups such as Zygomycetes, to which it is currently thought more likely that the VAM fungi may belong.

With the exception of the systemic fungicide pyroxychlor (Stewart & Pflieger 1977), little attempt has been made to study the effects of true symplastic fungicides on VAM fungi. In a very brief report, Clarke (1978) reported that lettuce seedlings colonized by *Glomus microcarpum* Tul. & Tul., and two other unidentified *Glomus* species showed a 10% increase in colonization when sprayed with Aliette (fosetyl-Al), compared with colonized plants which were not sprayed. Our preliminary studies also demonstrated the stimulatory effect of

Aliette on root colonization by a *Glomus* sp. and on plant growth when these were compared with untreated, VAM-colonized plants.

Root exudation is thought to be one of many factors which govern mycorrhizal development (Ratnayke et al. 1978, Graham et al. 1981). It has been conclusively demonstrated that application of certain compounds to leaves affects the kind and amount of root exudates (Rovira 1969). For instance, certain systemic fungicides, when applied as foliar sprays, noticeably increased the exudation of amino acids from wheat roots (Jalali & Domsch 1977). This information led us to study the possible effects of Aliette on certain metabolites present in root exudates and root extracts of VAM-colonized leek plants, in the hope that any changes observed might help us to explain the stimulatory effect of the fungicide on the mycobiont and the plant.

Materials and methods

Origin and production of VAM inoculum. A culture of an unnamed species of *Glomus* (Herb. DAOM 181602) supplied by A. Fortin and V. Furlan, Université Laval, Ste. Foy, Québec, formed vesicles in host roots but produced no external spores (Plenchette et al. 1981).

Inoculum production and multiplication was carried out in roots of leek (*Allium porrum* L.). Leek seeds, cv. Giant Musselburgh 170 (Stokes Seeds Ltd., St. Catharines, Ontario), were surface sterilized with 0.5% sodium hypochlorite solution for 45 min, rinsed in sterile distilled water, then sown in sterile vermiculite saturated with Long Ashton solution (Hewitt 1966). After 21 days, 2 seedlings were transplanted into 10-cm pots filled with Turface (Plant Products Co. Ltd., Bramalea, Ontario, Canada), and inoculated with 2 g of leek root colonized by the *Glomus* sp. spread 5 cm below the surface. Control pots were inoculated with 2 g of clean, uncolonized root segments. All pots were maintained in a controlled environment chamber at night/day temperatures of 18/24°C, 80% relative humidity, and illumination of 390 $\mu\text{E s}^{-1}\text{m}^{-2}$ with a photoperiod of 16 h. The seedlings were watered daily with distilled water, and each pot was fertilized weekly with 25 mL of modified Long Ashton solution and watered with distilled water, following the procedure of Plenchette et al. (1982).

Fosetyl-Al application. Aliette^R, a wettable powder containing 80% fosetyl-Al (aluminum tris-o-ethyl phosphonate) was supplied by May and Baker Canada, Inc. Fosetyl-Al was applied at concentrations of 0.3 (lowest), 1.0 (intermediate), and 3.0 (highest) mg a.i. mL⁻¹ water per plant per pot. Each concentration was applied to 24 mycorrhizal

(M) and 24 non-mycorrhizal (NM) plants (56-day-old) until initial runoff; fungicide accumulation at the Turface surface was prevented by absorbent cotton material. Another 24 M and 24 NM plants, sprayed only with water, served as the controls.

Assessment of roots for mycorrhizae. To estimate the extent of mycorrhizal colonization, six additional 56-day-old plants (2 plants per pot), were sprayed with each fungicide concentration as mentioned above. Four days after fungicide treatment, the plants from each pot were separated at the root/shoot junction, and dry weights of shoots were determined. The roots were rinsed thoroughly with tap water, blotted dry, and 300 root segments each 1 cm long from each replicate were randomly chosen, cleared and stained using the modified method of Kormanik et al. (1980). Root samples from each replicate were cleared with 10% (w/v) aqueous KOH, at approximately 80°C for 60 min., and were acidified with 1% (v/v) HCl for 3 min. The root segments were stained for 3 h with 0.05% (w/v) acid fuchsin dissolved at room temperature in lactophenol and then differentiated in lactophenol solution for 24 h.

All root segments from each replicate of each treatment were spread out evenly in a 90 × 90 mm Phage Typing Grid dish marked with 13 mm grid squares (Fisher Scientific Ltd.). Vertical and horizontal gridlines were scanned and the presence or absence of colonization was recorded at each point where a root segment intersected a line. One hundred intersects were observed and the percentage of infected root segments was recorded.

Root exudation studies. Plants were sampled 2, 7, and 27 days after fungicide application and four replicates of each treatment (2 plants per replicate) were randomly selected. The two plants of each replicate were harvested carefully, rinsed thoroughly under a gentle stream of tap water, placed in Mason jars with the roots completely covered with 150-200 mL aerated distilled water, and left for 24 h at room temperature under continuous low light. The plants were removed at the end of this period, and the exudate solution was immediately passed through a sterile 0.45 μm Millipore filter to remove root debris and microorganisms. The filtered solution was lyophilized, then held frozen at -20°C until analyzed. The plants were severed at the root/shoot junction, freeze-dried, and dry weights of root systems obtained.

Soluble sugars from root exudates were extracted using the modified procedure of Parker (1962). Lyophilized root exudates were dissolved in 2 mL 80% (v/v) ethanol, placed in a 22 × 80 mm cellulose extraction thimble and refluxed for 16 h in 90 mL of 80% (v/v) ethanol. The thimble was

allowed to drain thoroughly, and the extract was roto-evaporated to a volume of 15-20 mL. A 1 mL aliquot was tested for soluble sugars by the colorimetric procedure using phenol-sulphuric acid reagent (Liu et al. 1973). The soluble sugar estimations were done in quadruplicate for each fungicide concentration for both M and NM plants. The concentration of sugars was expressed as μg equivalents of dextrose per gram dry weight of root.

Root extract studies. Freeze-dried tissue (30-100 mg, depending on harvest time) from control and fungicide-treated M and NM plants used in the root exudation studies, was subjected to the modified procedure of Parker (1962) and the colorimetric procedure of Liu et al. (1973). The concentration of sugars was expressed as mg equivalents of dextrose per gram dry weight of root.

Total free amino acids were extracted from roots using a minor modification of the method described by Dumbroff and deSilva (1972). Freeze-dried tissue (20-60 mg, depending on harvest time) from the fungicide-treated M and NM plants was placed in a 40 mL glass tube with 7 mL of 10% ethanol (v/v). The tubes were agitated for 30 min on a Buchler Evapomix. The suspension was filtered through Whatman No. 1 paper. A 1 mL aliquot of 10% (v/v) ethanol filtrate was tested colorimetrically for total amino acids by the acetate-ninhydrin reagent (Clark 1964). The concentration of amino acids was expressed as mg equivalents of glycine per gram dry weight of root.

Total lipids were extracted from freeze-dried root tissue of M and NM plants (27 days after fungicide treatment) by the method of Jabaji-Hare et al. (1984). Lipids from dry root tissue were extracted using chloroform-methanol (1:1, v/v) solvent. The extracted crude lipid was dissolved in 10 mL of chloroform-methanol (2:1, v/v), mixed with 2 mL of 0.7% NaCl and centrifuged. The upper phases were discarded, while the lipid sample was collected and dried down on a rotary evaporator. The lipid extract from each replicate of each treatment was weighed and expressed as mg per gram dry weight of root.

Statistics. Percent root colonization and shoot dry weight data were statistically analyzed using one-way analysis of variance (ANOVA). Three-way analysis of covariance (ANCOVA) and three-way analysis of variance were first performed respectively on soluble sugars data in root exudates and extracts. The results of these tests revealed a significant second-order interaction (fosetyl-Al concentration (C) vs. plant states: M and NM (PS) vs. sampling time (ST), $P < 0.001$). Hence a two-way ANCOVA and ANOVA were performed on each data set collected at each sampling time. Total

free amino acids were analyzed using three-way ANOVA while total lipids data were subjected to a two-way ANCOVA. Student Neuman Keul's (SNK) was performed on all data to determine differences among means within each sampling time. All statistical procedures followed Zar (1974) or Sokal and Rohlf (1981).

Results

Root colonization and shoot dry weight. Fosetyl-Al has a significant stimulatory effect on the percent of root segments colonized by *Glomus* sp. ($P = 0.0005$) and on shoot dry weight of plants ($P = 0.0001$) (Table 1). Both mean percentage of root segments colonized (y_1) and mean shoot dry weight (y_2) were highly correlated ($r_1 = 0.88$, $n = 12$ and $r_2 = 0.82$, $n = 12$) with the concentration of fosetyl-Al. The regression equations were: $y_1 = 5.34x + 50.38$ and $y_2 = 0.121x + 0.438$. The shoot dry weight increased progressively with fungicide concentration.

Soluble sugars in root exudates of M and NM plants. The release of soluble sugars from leek plants varied with the presence of VAM, fungicide concentration, and time of sampling ($P = 0.0001$). The maximum increase in exudation of soluble sugars from roots of treated M and NM plants over the control occurred at 2 days after treatment, and declined later (Fig. 1). At 2 and 7 days, M plants exuded greater amounts of soluble sugars than did NM plants when averaged across all fungicide concentrations. The highest concentration (3.0 mg a.i. mL⁻¹) caused maximum exudation of soluble sugars of both M and NM plants (Table 3). An interaction between the effects of C and PS (Table 2) indicated that the exudation of soluble sugars of M and NM plants varied with concentration at 2 and 7 but not at 27 days after treatment (Fig. 1, Table 3). Control M and control NM plants exuded similar amounts of soluble sugars at all sampling times.

Table 1. Percent colonization of mycorrhizal root segments and shoot dry weight of mycorrhizal plants (60-day-old) treated with fosetyl-Al

Concentration (mg a.i./mL)	Colonization† (%)	Shoot dry weight (g)
0	55.3 ^a	0.39 ^a
0.3	60.7 ^b	0.46 ^b
1.0	75.3 ^c	0.65 ^c
3.0	82.0 ^c	0.77 ^d

Values in the same column followed by the same letter are not significantly different at $P \geq 0.05$, Student Neuman Keul (SNK) test.

†Mean of three replicates followed by the asymmetrical 95% confidence limits. SNK test conducted on the arcsine transformed data.

Table 2. Results of analysis of covariance and analysis of variance for the effect of fungicide concentration (C) and plant states (PS) on soluble sugars in root exudates, and on soluble sugars and lipids in root extracts

Days after treatment:	Probability values*						
	Sugars in root exudates†			Sugars in root extract‡			Lipid in root extract†
	2	7	27	2	7	27	27
PS	0.0001	0.0009	0.9640	0.0001	0.0001	0.0001	0.0001
C	0.0001	0.0001	0.0001	0.6576	0.0001	0.0001	0.0004
PS × C	0.0001	0.0042	0.8283	0.0552	0.4878	0.0100	0.7856

*All second-order interactions from the 3-way ANCOVA and ANOVA were significant at the 5% level (see Materials and methods section).

†Data were analyzed using ANCOVA.

‡Data were analyzed using ANOVA.

Table 3. Effect of foseetyl-Al on soluble sugars in root exudate of mycorrhizal (M) and nonmycorrhizal (NM) plants at three sampling times

Concentration (mg a.i./mL)	Soluble sugars ($\mu\text{g g}^{-1}$ dry root) at days after treatment*					
	2 days		7 days		27 days	
	M	NM	M	NM	M	NM
0	251.7 ± 12.6	249.7 ± 13.2	120.1 ± 9.5	144.1 ± 12.8	53.2 ^a ± 1.2	52.6 ^a ± 6.4
0.3	486.7 ± 58.7	285.2 ± 23.2	175.5 ± 13.8	177.1 ± 12.0	56.2 ^a ± 7.6	59.6 ^{ab} ± 5.0
1.0	862.2 ± 66.4	395.6 ± 37.0	292.5 ± 16.6	210.1 ± 5.5	63.6 ^a ± 4.1	67.4 ^{ab} ± 5.1
3.0	960.1 ± 51.4	479.8 ± 54.6	339.5 ± 6.7	251.7 ± 13.3	87.0 ^b ± 3.5	83.2 ^{ab} ± 5.5

*Means of four replicates ± standard error of mean.

Values within each column not followed by the same letter are significantly different at the 5% level (Student Neuman Keul (SNK) test conducted on the square root transformed data).

SNK was not performed on soluble sugars data at 2 and 7 days due to a significant PS × C interaction term (see Table 2).

Table 4. Effect of foseetyl-Al on total soluble sugars in root extract of mycorrhizal (M) and nonmycorrhizal (NM) plants at each sampling time

Concentration (mg a.i./mL)	Soluble sugars (mg g ⁻¹ dry root) at days after treatment*					
	2 days		7 days		27 days	
	M	NM	M	NM	M	NM
0	11.88 ± 0.38	21.69 ± 0.83	13.46 ^a ± 0.72	22.39 ^a ± 0.67	15.14 ^a ± 0.31	23.04 ^a ± 0.68
0.3	12.19 ± 0.76	23.00 ± 0.44	16.05 ^b ± 0.33	23.86 ^b ± 0.25	19.45 ^b ± 0.35	28.27 ^b ± 0.37
1.0	13.90 ± 1.14	21.46 ± 1.15	18.57 ^c ± 0.53	27.73 ^c ± 0.41	23.24 ^c ± 0.82	29.55 ^b ± 0.25
3.0	11.46 ± 0.50	23.94 ± 1.08	21.48 ^d ± 0.60	30.74 ^d ± 0.32	27.86 ^d ± 0.68	32.68 ^c ± 0.80

*Mean of four replicates ± standard error of the mean.

Column means within each sampling time followed by the same letter are not significantly different according to Student Neuman Keul (SNK) test, P ≥ 0.05.

†SNK was not performed on day 2 because C was not significant (see Table 2).

Root extract studies. The soluble carbohydrate content of root extracts of M and NM plants increased with time (Fig. 2). When the data were averaged over time, NM plants contained significantly higher ($P < 0.05$) soluble carbohydrate than M plants (Table 4). At 2 days after treatment, fungicide concentration had no effect on the carbohydrate status of the roots of M and NM plants (Table 2). Significant increases in soluble sugars

apparently related to fungicide concentrations in M and NM plants were first observed at 7 days, and were still increasing 27 days after treatment (Table 4, Fig. 2). A lack of interaction between PS and C at 7 days (Table 2) clearly indicates that the increase of soluble sugars in plants was not due to the presence of the VAM fungus. However, an interaction at 27 days between C and PS indicated that the increase in soluble sugars varied signifi-

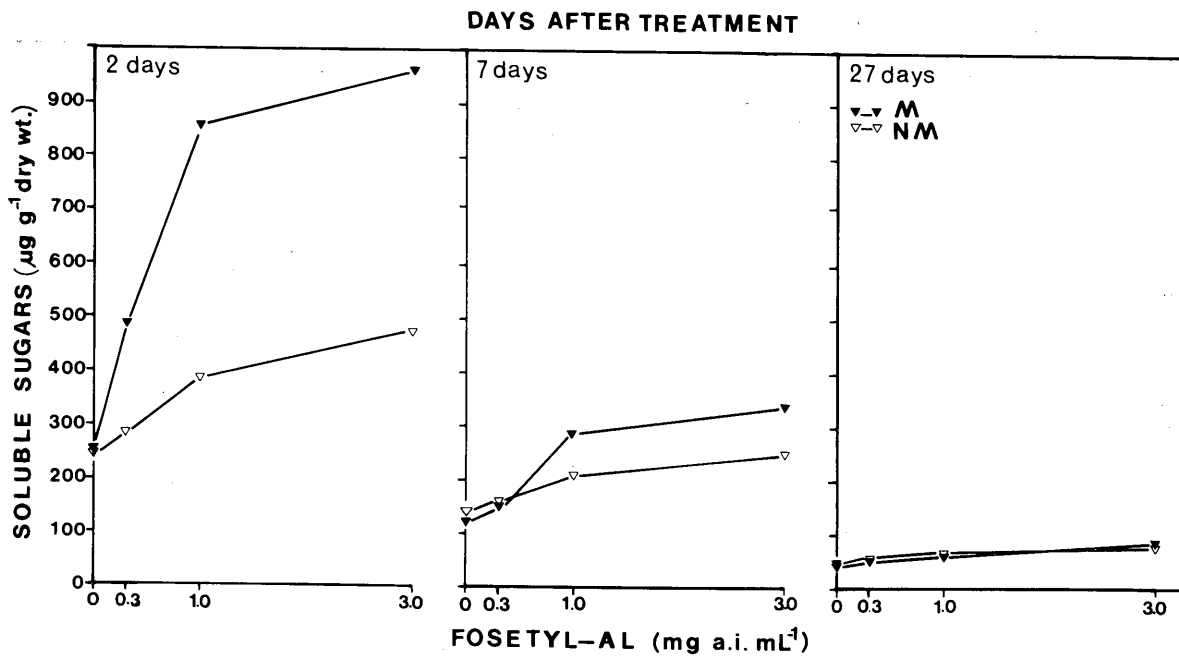


Figure 1. Effect of fosetyl-Al on total soluble sugars in root exudates of M and NM plants at 2, 7, and 27 days after treatment.

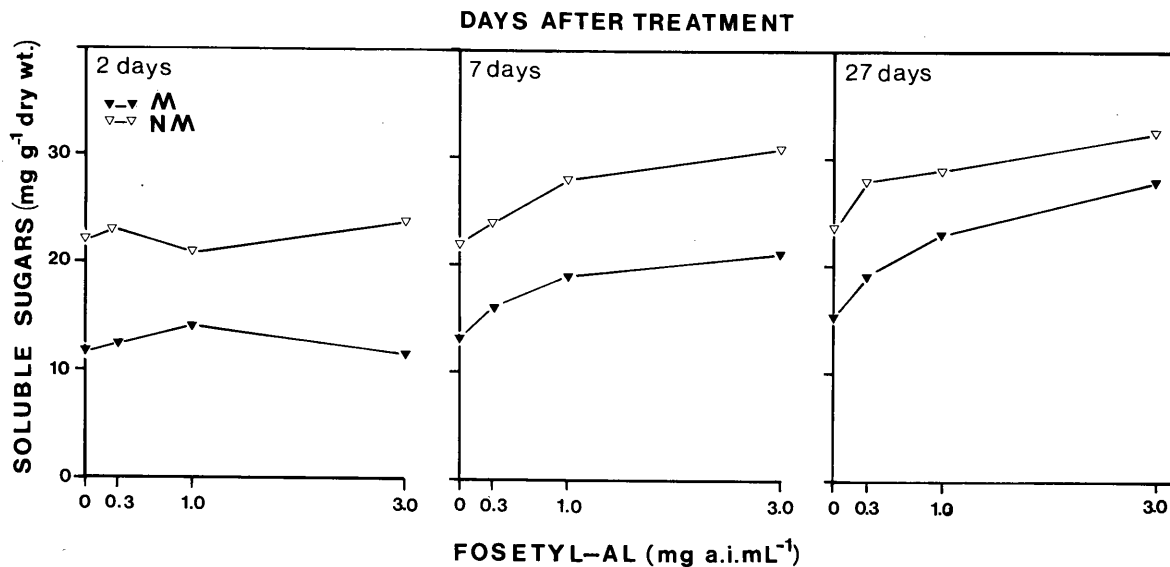


Figure 2. Effect of fosetyl-Al on total soluble sugars in root extracts of M and NM plants at 2, 7, and 27 days.

cantly with concentration of fungicide in both M and NM plants.

Soluble amino acids in root extracts of M and NM plants increased significantly with increasing fungicide concentration ($P = 0.0001$) and with time ($P = 0.0001$). Root extract of NM plants contained significantly ($P = 0.0095$) higher amounts of soluble amino acids than that of M plants (Fig. 3). When the data were averaged over PS, amino acid content was significantly highest ($P < 0.05$) at 7 days;

intermediate at 27 days and significantly different ($P < 0.05$) from that at 2 days. The effects of fungicide concentration on amounts of amino acids in root extract of M and NM plants at all sampling times are presented in Table 5. At 2 days, the highest concentration caused a significant increase in amino acids in root extract of NM and M plants ($P < 0.05$). Amino acid contents of NM plants treated with the highest and intermediate fungicide concentrations were significantly higher than that

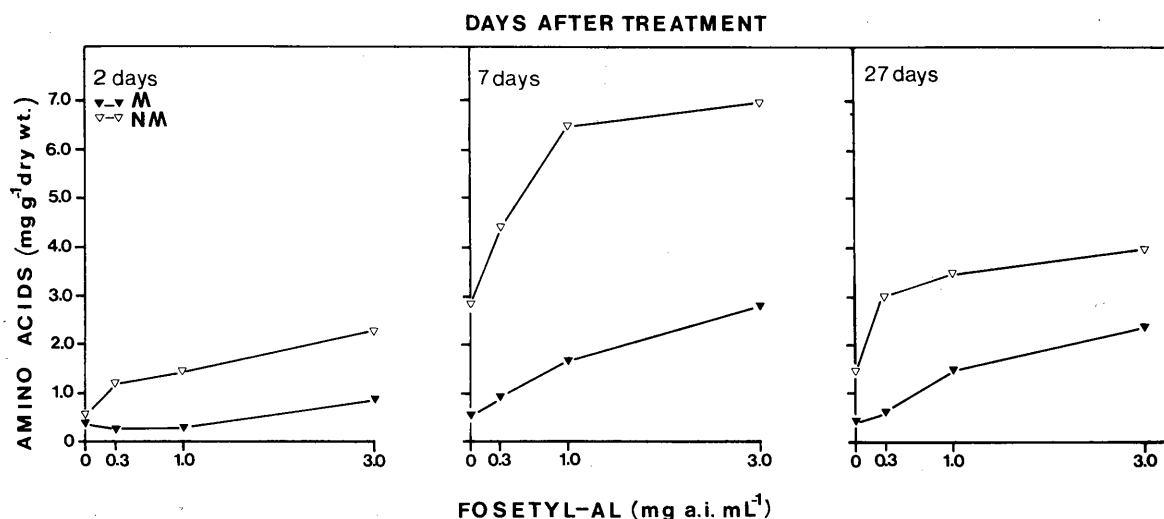


Figure 3. Effect of fosetyl-Al on total free amino acids in root extracts of M and NM plants at 2, 7, and 27 days. Second-order and first-order interactions were not significant ($P \geq 0.1$).

Table 5. Effect of fosetyl-Al on total amino acids in root extract of mycorrhizal (M) and nonmycorrhizal (NM) plants at each sampling time

Concentration (mg a.i./mL)	Amino acids (mg g ⁻¹ dry weight root) at days after treatment*					
	2 days		7 days		27 days	
	M	NM	M	NM	M	NM
0	0.31 ^a ± 0.03	0.45 ^a ± 0.07	0.51 ^a ± 0.10	2.93 ^a ± 0.81	0.43 ^a ± 0.10	1.36 ^a ± 0.32
0.3	0.26 ^a ± 0.01	1.17 ^{ab} ± 0.56	0.94 ^{ab} ± 0.24	4.43 ^{ab} ± 0.87	0.57 ^a ± 0.12	3.03 ^a ± 1.06
1.0	0.29 ^a ± 0.05	1.42 ^b ± 0.10	1.71 ^{ab} ± 0.59	6.50 ^b ± 0.50	1.49 ^a ± 0.86	3.66 ^a ± 0.92
3.0	0.93 ^b ± 0.23	2.29 ^b ± 0.27	2.82 ^b ± 0.76	6.94 ^b ± 0.51	2.51 ^a ± 1.13	4.04 ^a ± 0.93

*Mean of four replicates ± standard error of the mean.

Column means within each sampling times followed by the same letter are not significantly different at the 5% level (Student Neuman Keul test conducted on Ln transformed data).

of the control, but were not significantly different from each other ($P > 0.05$). At 7 days, the amino acid content of the M and NM plants treated with the highest concentration reached a peak (Fig. 3), but at 27 days the amino acid content of M and NM plants at all concentration levels were lower and not significantly different.

A two-way ANCOVA revealed that there was no interaction effect between PS and C on the total amount of lipid root extract (Table 2). Root extract of M plants contained greater amounts of total lipids than NM plants (Fig. 4), and these amounts increased significantly with increasing fungicide concentration. There was no significant increase in lipid content of NM plants with fungicide treatment (Table 6). In M plants the highest concentration caused a significant increase of total lipids ($P < 0.05$), followed by the intermediate concentration, while the total lipid contents of control M plants and those treated with the lowest concentration were not significantly different (Table 6).

Discussion

A single foliar application of fosetyl-Al at rates of 0.3, 1.0, and 3.0 mg a.i. mL⁻¹ effectively increased colonization of leek roots by a *Glomus* sp. and increased the growth of plants. These results confirm the preliminary findings of Clarke (1978) who found that lettuce seedlings colonized by a mixture

Table 6. Total lipids in root extracts of mycorrhizal (M) and nonmycorrhizal (NM) plants 27 days after fungicide treatment

Concentration (mg a.i./mL)	Lipid content (mg g ⁻¹)*	
	M	MN
0	37.03 ^a	28.10 ^a
0.3	39.67 ^{ab}	29.61 ^a
1.0	43.94 ^b	38.43 ^a
3.0	53.91 ^c	41.44 ^a

*Means of four replicates.

Values within each column followed by the same letter are not significantly different at the 5% level (SNK).

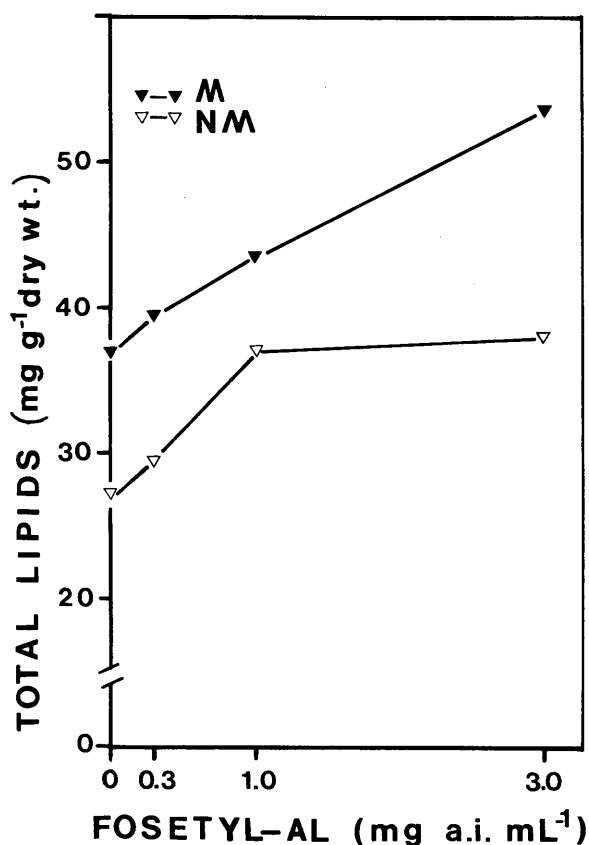


Figure 4. Effect of fosetyl-Al on total lipids in M and NM root extracts at 27 days after fungicide treatment.

of VAM fungi showed a 10% increase in colonization after being sprayed with fosetyl-Al. It is of interest to note that increases in plant growth (measured as leaf dry weight) strongly paralleled increases in percent colonization (measured as percent of root segments colonized). This suggests that increase in dry weight could be partly due to increase in mycorrhizal colonization (Abbot & Robson 1977, Allen et al. 1981). Similar responses of growth due to pesticide application have been reported for sour orange plants inoculated with *Glomus mosseae* and subjected to different concentrations of sodium azide (Nemec 1980), for white ash seedlings inoculated with *G. fasciculatum* and subjected to 0.5 kg ha⁻¹ of paraquat (Pope and Holt 1981), and for soybean plants inoculated with *G. fasciculatum* and subjected to different concentrations of metalaxyl (Groth & Martinson 1983).

This increase in colonization of fungicide-treated leek plants by *Glomus* sp. cannot be attributed to a decrease in organisms pathogenic to leeks, since (1) the experiments were conducted in a controlled environment growth chamber, (2) mycorrhizal cul-

tures were established from surface sterilized root inoculum, (3) there was no evidence of disease on roots, or evidence of pathogenic fungal propagules in the potting medium, and (4) fosetyl-Al was applied only to the foliage.

The root exudation studies indicated that fosetyl-Al caused a significant increase in soluble sugars exuded from roots of M and NM plants depending on the fungicide concentration and harvesting time. We are aware that bacteria may have contributed to the exudates but we consider this source to be insignificant. Schwab et al. (1982) reported a similar response in a non-mycorrhizal species, *Chenopodium quinoa* Willd., when it was sprayed with sublethal doses of simazine. They suggested that the increase of soluble sugars and amino acids in root exudates was responsible for the unusual formation of VAM in this species. In our study, the effects of fosetyl-Al on the increase of *Glomus* colonization in leek plants at 4 days after treatment closely correspond to increased soluble sugar exudates at 2 days after treatment. While these results do not prove that root exudates are responsible for increased *Glomus* colonization, they do provide further corroborative data supporting the hypothesis of Ratnayake et al. (1978) and Graham et al. (1981) that root exudates are predisposing factors for colonization and spread of mycorrhizal fungi.

The fact that fungicide-treated M plants had greater amounts of soluble sugars in their root exudates than similarly treated NM plants suggests a synergistic effect of the VAM fungus and fosetyl-Al on the plant metabolism. The mechanism by which fosetyl-Al stimulates root exudate production is not understood. It has been shown, however, that foliar applications of 0.1% of phosphorus in the form of disodium phosphate increased soluble sugar content in root exudates of crop plants (Balasubramanian & Ragaswami 1969; we could not calculate the actual amount of P these authors applied since the volume of solution sprayed was not reported). It is possible that increased exudation of soluble sugars from NM plants treated with fosetyl-Al may be related to the presence of P in the form of phosphorous acid produced by the breakdown of fosetyl-Al in plant tissue (Bompeix et al. 1980, Trique et al. 1981). However this seems unlikely, when one considers the small quantity of P we added to each plant in the form of fosetyl-Al; 3.0 mg a.i. mL⁻¹ per plant contains only 0.2 mg of P. Sanders (1975) had shown that similar amounts of P (0.17 mg, see Fig. 5) do not affect the colonization of onion plants by *G. mosseae*. Thus further studies are required to understand the actual mechanism(s) by which fosetyl-Al or its degraded form, phosphorous acid, causes increased root exudation.

The root extract studies revealed a quantitative and direct relationship between fosetyl-Al and total soluble sugars and free amino acids. To our knowledge, the significant increase of total soluble sugars at 7 days, and of total amino acids at 2, 7, and 27 days after treatment, is the first evidence presented which reveals the direct effect of fosetyl-Al on host root metabolism. It has been reported that the activity of fosetyl-Al against its target pathogenic fungi results from its stimulation of host defence reactions and the synthesis of phytoalexins (Bompeix et al. 1980), but recently Fenn and Coffey (1984) presented evidence that foliar spray of either fosetyl-Al or its breakdown product, phosphorous acid, which is present in fungicide-treated plant tissue (Vo-Thi-Hi et al. 1979), gave equivalent control of root rot caused in seedlings of *Persea indica* Spreng. by *Phytophthora cinnamomi* Rands, Thorn & Zentmeyer. Their evidence may indicate a direct action of Aliette on the target organism. However, in neither case was any effect of fosetyl-Al on healthy host metabolism demonstrated or sought. Since our root extract studies show that such an effect may exist, further work on the mode of action of fosetyl-Al seems warranted.

Data of Azcon and Ocampo (1981) for mycorrhizal wheat cultivars clearly show that when different cultivars were inoculated with *Glomus mosseae*, their roots contained lower amounts of total and reducing sugars than comparable nonmycorrhizal roots. Our total soluble-sugar data indicate a similar trend. Roots of control M plants had lower soluble sugars than control NM plants at 2, 7, and 27 days after treatment, with the lowest level detected at 2 days. It seems probable that these metabolites are utilized by the VAM fungus or transformed by it into the lipids which are present in large quantities in VAM fungus structures (Nemec 1981, Jabaji-Hare et al. 1984). Significantly more lipid has been extracted from VAM-colonized roots than from uncolonized roots (Cooper & Lösel 1978, Nagy et al. 1980). These findings are in agreement with the total lipid content of root extracts in our study, where control M plants yielded more total lipid than control NM plants. However where fosetyl-Al was applied, a significant increase in total lipid extracted from treated M roots was apparent. This effect, which has not been reported before, is not related directly to effects of fosetyl-Al on the host, as the yield of total lipid extracted from treated NM plants was not significantly affected. Thus the phenomenon may arise from a direct effect of fosetyl-Al on the VAM fungus metabolism.

In our study, total free amino acids in root extracts of control NM plants were considerably

higher than those of control M plants. These results do not agree with those of Young et al. (1972) who found that the content of total free amino acids was three to ten times greater in roots of VAM-colonized than in uncolonized corn plants. Krishna and Bagyaraj (1983) reported that the change in free amino nitrogen and protein fractions of groundnut roots colonized by *Glomus mosseae* depended on plant age. They observed a considerable decrease in these metabolites in mycorrhizal plants as compared with nonmycorrhizal plants after 20 days of growth, with an increase at later stages of growth. The reasons for the differences between our study and the two studies just mentioned are not apparent but may be related to differences in plant species, size, and age, the VAM fungi involved, and other experimental conditions.

In our study, at certain sampling times, the test of significance revealed a highly significant interaction between fosetyl-Al concentration and plant states (See Table 2). One of the factors that can contribute to such results is the physiological age of the plant. The amount and rate of root exudate production as well as the amount of nutrients inside the plant can be affected by plant age (Rovira 1965, Richards & Templeman 1936).

In conclusion, we have presented evidence that foliar application of the systemic, symplastic, anti-oomycete fungicide fosetyl-Al appears to increase VAM colonization and development in roots of leeks and, directly or indirectly, growth of the leek plants themselves. The mechanism by which fosetyl-Al can produce such effects is not known, but we have shown that the fungicide has an effect on both host plant and VAM fungus.

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