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RESPONSE OF AN ENDOMYCORRHIZAL FUNGUS IN *ALLIUM PORRUM* L. TO DIFFERENT CONCENTRATIONS OF THE SYSTEMIC FUNGICIDES, METALAXYL (RIDOMIL®) AND FOSETYL-AL (ALIETTE®)

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Summary—Two systemic, anti-oomycete fungicides were tested for their effects on colonization of leek roots by *Glomus intraradices* Schenck and Smith, a vesicular–arbuscular mycorrhizal (VAM) fungus. Foliar application of the symplastic fungicide fosetyl-Al (Aliette®), at concentrations of 0.3, 1.0 or 3.0 mg a.i. ml^{-1}, to VAM *Allium porrum* L. (leek) plants, significantly increased colonization by *Glomus intraradices*, the number of intramatrical vesicles, and plant growth compared to inoculated but untreated plants. These effects did not diminish with time. In a second study, mycorrhizal infection and growth of inoculated leek seedlings were significantly reduced by soil drenches of the systemic fungicide metalaxyl (Ridomil®), at concentrations of 0.5, 1.0 or 2.0 mg a.i. per plant. Inoculated but untreated plants had extensive mycorrhiza. We suggest that the probable adverse effects of metalaxyl on *Glomus intraradices* should be taken into consideration when its use is being considered.

INTRODUCTION

There would appear to be a conflict of interest between our need to control pathogenic fungi by chemical means and our desire to encourage the root symbiosis which involves vesicular–arbuscular mycorrhizal (VAM) fungi. Although the majority of pesticides tested adversely affect the symbiosis (Menge, 1982), others do not appear to damage mycorrhizal fungi and under certain conditions may even increase mycorrhizal colonization and development. The fumigants, 1,3-D (1,3-dichloropropene), DBCP (1,2-dibromo-3-chloropropane) (Bird *et al.*, 1974) and ethylene dibromide (Nemec, 1980); non-systemic fungicides, such as difolatan and sodium azide (Nemec, 1980); and systemic fungicides such as terrazole (Menge *et al.*, 1979), chloroneb (Spokes *et al.*, 1981); ridomil (Groth and Martinson, 1983) and fosetyl-Al (Clarke, 1978) have been reported to increase root colonization and spore production by VAM fungi. It is interesting to note that the systemic fungicides mentioned above are all active against oomycetes. It may therefore be possible to selectively control downy mildew, damping off, and other diseases caused by oomycetes, without substantially interfering with VAM colonization.

The mechanisms by which the fungicides stimulate VAM fungi are unknown. Fumigants and non-systemic fungicides are thought to act indirectly to reduce competition in the rhizosphere and create a favourable environment for the colonization of host roots by VAM fungi. Systemic fungicides, which are translocated within the host either in the apoplast or the symplast, may act either, directly on the VAM fungus, or indirectly by altering host root physiology. For instance, the specifically anti-oomycete fungicides, metalaxyl and fosetyl-Al are both systemic. However, the former is translocated mainly in the transpiration stream (Bruin and Edgington, 1984), while the latter is translocated in the symplast and can move downwards in plants (Bertrand *et al.*, 1977).

We have examined the effects of different concentrations of metalaxyl and fosetyl-Al on colonization of leek (*Allium porrum* L.) roots by the VAM fungus *Glomus intraradices* Schenck and Smith. Our choice of these two fungicides was dictated by two further considerations: (i) it has been suggested by Pirozynski and Malloch (1975) that the VAM fungi have oomycetous affinities; (ii) since both fungicides are systemic with different translocation pathways, their effects on the VAM fungus may well be different.

MATERIALS AND METHODS

Origin of VAM inoculum

A culture of *Glomus* sp. (Herb. DAOM 181602), isolated from roots of ash trees (*Fraxinus americana* L.) growing in a nursery in Québec City, Québec, was supplied by Dr V. Furlan (Agriculture Canada, Ste. Foy, Québec). This fungus forms vesicles in host roots, but no external spores (Plenchette *et al.*, 1981) and has been used in other studies (Plenchette *et al.*, 1982; Furlan *et al.*, 1983). This fungus was recently identified by N. C. Schenck as *Glomus intraradices* (Schenck and Smith, 1982).

Inoculum production

Inoculum was produced and multiplied in roots of leek. Seeds of leek cultivar Giant Musselburgh 170 (Stokes Seeds Ltd, St. Catherines, Ontario, Canada), 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, seedlings were transplanted into 10 cm pots filled with "Turface" (a calcined montmorillonite soil substitute containing virtually no phosphorus, Plant Products Co. Ltd, Bramalea, Ontario, Canada). Leek root inoculum (1 g) colonized by *Glomus intraradices* was spread 5 cm below the surface of "Turface" and two seedlings of similar size, with the first two leaves developed, were transplanted to each pot. All pots were maintained in a controlled environment chamber at night and day temperatures of 18 and 24°C respectively, 80% relative humidity and illumination of 390 $\mu E\ s^{-1}\ m^{-2}$ with a photoperiod of 16 h. The seedlings were watered daily with distilled water and each pot was fertilized weekly with 25 ml of Long Ashton solution. Samples of the potting medium were removed periodically from each pot of check for possible contamination by other fungi and soil fauna.

Fungicide application

Aliette®, a wettable powder containing 80% of the active ingredient fosetyl-Al (aluminum tris-*o*-ethyl phosphonate), was supplied by May and Baker (Canada) Inc. Mycorrhizal (VAM) plants that were 55 days old were sprayed with fosetyl-Al until run-off. To prevent any run-off from reaching the potting medium, this was covered with non-absorbent cotton. Fosetyl-Al was applied at concentrations of 0.3 (lowest), 1.0 (intermediate) or 3.0 (highest) mg a.i. (active ingredient) ml^{-1} of water per plant. These rates are equivalent to 0.3, 1.0 and 3.0 kg a.i. ha^{-1}, the highest rate being the recommended field rate. The fungicide used in a second experiment was Ridomil® 240EC, an emulsifiable concentrate containing 240 $g\ l^{-1}$ solution of the active ingredient (DL-methyl-*N*-(2,6-dimethylphenyl)-*N*-(2-methoxyacetyl) alanine methyl ester), supplied by Ciba Geigy Ltd. Each pot containing two 55-day-old VAM plants was drenched in 100 ml of fungicide solution containing one of three concentrations: 0.5, 1.0 or 2.0 mg a.i. per plant. These rates are equivalent to 2.5, 5.0 and 10.0 kg ha^{-1}, the lowest and intermediate rates being the recommended field rates. For each concentration of both fungicides, three replicates, each consisting of one pot containing two plants, were sampled on each of six dates. VAM plants sprayed or drenched only with water served as the controls.

Assessment of roots for mycorrhizas

Total percent colonization. Three replicates representing each fungicide concentration and each control were harvested 5 days after treatment, and every 10 days thereafter until 55 days (see Figs 1–4). The plants from each pot were separated at the root–shoot junction, and dry weights of shoots were determined. The roots were rinsed thoroughly under a gentle stream of tapwater, blotted dry and 300 × 1 cm root segments from each replicate of each treatment were randomly chosen, cleared and stained according to the method of Kormanik *et al.* (1980), but modified as follows. Root samples from each replicate were bleached with 25 ml of 10% (w/v) aqueous KOH at 80°C for 60 min. Then the roots were stained for 3 h with 0.05% (w/v) acid fuchsin dissolved in lactophenol solution (300 g phenol, 250 ml lactic acid, 250 ml glycerine, 300 ml water). The root segments were destained in lactophenol 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 1.25 cm grid squares (Fischer Scientific Ltd). The presence or absence of colonization in 100 root segments was recorded at each point where a root segment intersected a line.

Assessment of vesicle numbers, and presence or absence of arbuscules and extramatrical hyphae. A subsample of 50 1-cm stained root segments from each replicate of each treatment was randomly chosen, and mounted on microscope slides in melted glycerine jelly. Each root segment was scanned along its entire length and the numbers of intramatrical vesicles, and presence or absence of arbuscules and extramatrical hyphae were recorded.

Statistics. The percentages of root segments at each fungicide concentration and in the control (i) containing more than one kind of fungal structure (**FS**), (ii) with intramatrical vesicles only (**IV**), (iii) with arbuscules only (**AR**), and (iv) with extramatrical hyphae only (**EH**), were plotted as probits against the $\ln(\log_e)$ of days after treatment. Slopes and elevations (intercepts) were statistically compared for equality. A Student–Newman–Keul's (SNK) test was then performed to determine the significance of differences among them.

Shoot weight data were transformed using $\ln(\log_e)$ transformation and plotted against days after treatment using linear regression. Slopes and elevations were statistically compared for equality, followed by an SNK test to determine the significance of differences among them.

The number of intramatrical vesicles per colonized root segment was analyzed using weighted two-way analysis of variance (ANOVA) with concentration vs sampling time as the interaction term. For each replicate, the total number of root segments with vesicles was used as the weighting factor. This was necessary in order to overcome the large variation in vesicle numbers encountered within each replicate and among replicates.

RESULTS

Effect of fosetyl-Al on VAM colonization and plant growth

A positive linear relationship ($P < 0.001$) existed between the percentage of VAM colonized root segments in control and fungicide treated plants and the number of days after treatment (Figs 1a–d). This relationship was expressed as $y = ax + b$, where y is the probit of the percentage of root segments with **FS**, **IV**, **AR** and **EH**, and x is the $\ln(\log_e)$ of days after treatment.

In the case of VAM plants treated with fosetyl-Al, the mean percentage of segments with **FS**, **IV**, **AR** and **EH** at any day after treatment increased significantly ($P < 0.001$) with increasing concentration; however the rate of increase over time (as indicated by the slope of the lines) for all concentrations and the control was the same ($P > 0.1$) (Figs 1a–d). The mean percentage of mycorrhizal segments

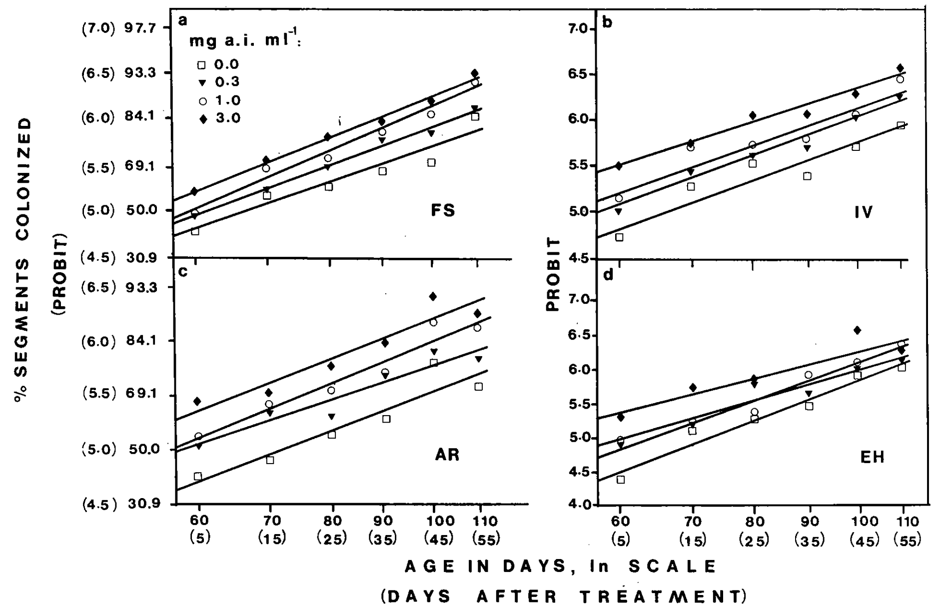

Fig. 1. The effect of different concentrations of fosetyl-Al on *Glomus intraradices* colonization in leek roots. The percentage of root segments colonized with (a) more than one kind of fungal structure (**FS**), (b) intramatrical vesicles (**IV**) only, (c) arbuscules (**AR**) only, and (d) extramatrical hyphae (**EH**) only, is plotted as probits against the ln($\log_e$) of physiological age (days) of plants. Each point represents the average of 3 replicates.

with **FS**, **IV**, **AR** and **EH** of treated VAM plants was significantly higher than that of control VAM plants (Table 1). In addition, fosetyl-Al has a significant stimulatory effect on growth of VAM plants expressed as shoot dry weight. The logarithm ($\log_e$) of shoot dry weight versus days after treatment did not significantly ($P < 0.001$) depart from linearity at all concentrations and in the controls (Fig. 2a) as determined by regression analysis. The slopes of the lines did not differ significantly ($P > 0.1$), indicating that the rate of increase of shoot weight at any day was the same for the fungicide-treated and control VAM plants. However, the elevations (intercepts) of all lines were significantly different ($P < 0.001$), indicating that mean shoot weight of VAM plants increased significantly with increase of fosetyl-Al concentration (Table 1).

The rate of increase in number of intramatrical vesicles per colonized root segments varied with concentration and with the number of days after treatment, as indicated by a highly significant interaction term ($P < 0.001$) (Fig. 3). In general, VAM plants treated with fosetyl-Al had more vesicles per colonized root segment than did control VAM plants 5 days after treatment. The maximum increase in vesicle numbers following fungicide treatment was observed 45 days after treatment, with the highest number attained in VAM plants treated with 3.0 mg a.i. ml^{-1}.

Table 1. Comparison of mean elevations (intercepts) of regression lines of percent segments colonized vs time and dry weight of shoot vs time at different concentrations of fosetyl-Al[1]

Concentration (mg a.i. ml^{-1})	% Segments with[2] FS	IV	AR	EH	Dry weight (mg)
0.0	65.7a[3]	66.5a	59.6a	64.6a	456a
0.3	72.8b	76.3b	70.0b	74.1b	554b
1.0	78.4c	78.8c	75.2c	74.2b	656c
3.0	82.5d	85.9d	81.7d	83.4c	853d

[1]Slopes of all lines were not significantly different ($P > 0.1$).
[2]Mean elevations were detransformed to percentages using Gauss Transformation. **FS**, more than one kind of fungal structure, **IV**, intramatrical vesicles, **AR**, arbuscules and **EH**, extramatrical hyphae.
[3]Values within each column not followed by the same letter are significantly different at the 5% level according to Student–Neuman–Keul's (SNK) test.

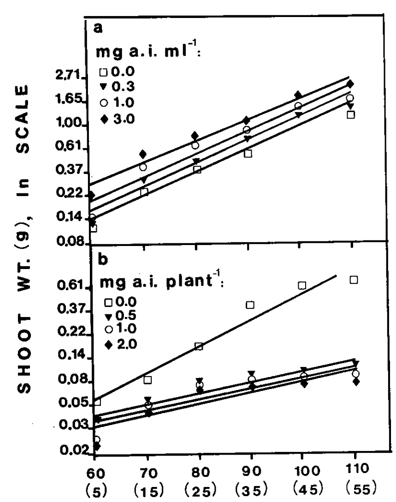

Fig. 2. Dry weight of shoot of plants treated with (a) fosetyl-Al and (b) metalaxyl, is plotted as ln($\log_e$) against the physiological age (days) of plants. Each point represents the average of 3 replicates.

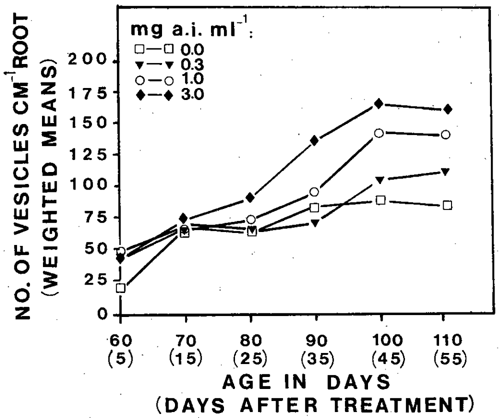

Fig. 3. Mean numbers of intramatrical vesicles per root segment colonized at 4 concentrations of fosetyl-Al, on 6 occasions.

Effect of metalaxyl on VAM colonization and mycorrhizal plant growth

Figures 4 and 2b show that in control VAM plants, there was a steady increase in percentage of segments with **FS** and in plant growth expressed as dry weight of shoot. In the case of metalaxyl-treated VAM plants, although there may have been an initial increase in the proportion of root segments colonized between 5 and 15 days after treatment, there was no apparent increase subsequent to this period (Fig. 4). The slopes of the lines of treated VAM plants were significantly less than that of the control ($P < 0.001$), but were not significantly different from each other (Table 2). Metalaxyl significantly decreased growth of VAM plants with time. Growth rates of treated VAM plants, as indicated by the slopes of the lines, were not significantly different from each other ($P > 0.05$) but were significantly different from the controls.

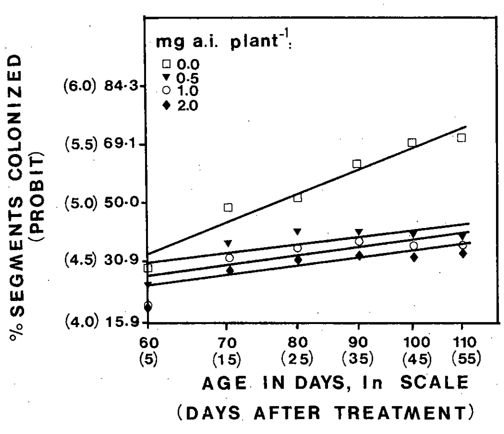

Fig. 4. The effect of different concentrations of metalaxyl on *Glomus intraradices* colonization in leek roots. The percentage of root segments colonized with more than one kind of fungal structures (**FS**), is plotted as probits against the ln($\log_e$) of physiological age (days) of plants. Each point represents the average of 3 replicates.

Table 2. Comparison of slopes of regression lines of percent segments colonized vs time and dry weight of shoot vs time at different concentrations of metalaxyl[1]

Concentration (mg a.i. plant^{-1})	% segments with fungal structures (**FS**)	Shoot dry weight (mg)
0.0	1.71a[2]	55a
0.5	0.53b	23b
1.0	0.59b	22b
2.0	0.57b	24b

[1]Slopes of the lines were significantly different ($P < 0.001$).
[2]Values within each column followed by the same letter were not significantly different at the 5% level according to SNK test.

DISCUSSION

In this study *Glomus intraradices* reacted differently to the fungicides fosetyl-Al and metalaxyl. Differences were reflected in growth of mycorrhizal plants and VAM root colonization expressed as the proportion of root segments colonized. With metalaxyl, colonization of leek roots and its progress, as well as the growth of mycorrhizal plants were reduced. Nemec (1980) observed that in sour orange seedlings, mycelial colonization of *Glomus etuniucatum* Becker & Gerd. was significantly stimulated by metalaxyl at low (2.2 kg ha^{-1}) and high rates (9.0 kg ha^{-1}) but not at the intermediate rate (4.5 kg ha^{-1}), a result which is difficult to interpret. Groth and Martinson (1983) reported that soil incorporation of metalaxyl at 2.9 mg kg^{-1} of soil increased VAM colonization by *Glomus fasciculatum* Gerd. and Trappe in corn roots, but had no effect on the growth of mycorrhizal plants. The differences between our results and those of the above-mentioned studies are difficult to explain, but may be due in part to differences in the species of VAM fungi involved. Different species of VAM fungi are known to react differently to pesticides (Spokes and Macdonald, 1978; Nemec and O'Bannon, 1979). Other factors such as the plant species involved, the rates of application of the fungicides and growth conditions could also contribute to such differences.

The mechanism by which metalaxyl inhibits colonization of leek roots by *Glomus intraradices* is not yet understood. The effect of metalaxyl on mycorrhizal infection may be a direct action or may be mediated through its phytotoxic effect on root function and growth, though, if any toxicity occurred, no symptoms were seen. The benefits of metalaxyl soil treatment are usually assessed in terms of disease control. It appears, however, that the possible adverse effects of this chemical on *Glomus intraradices* and perhaps other VAM fungi should be taken into account when its use is being considered.

With fosetyl-Al, plant growth and the proportion of root segments with **FS**, **IV**, **AR** and **EH**, were significantly increased with increasing concentration and this effect did not diminish. These results confirm the observation of Clarke (1978) who found that lettuce seedlings colonized by a mixture of VAM fungi showed a 10% increase in colonization after being sprayed with fosetyl-Al. It is of interest to note that the increase in growth of mycorrhizal plants (measured as shoot dry weight) strongly paralleled increases in percent colonization by the fungus. Fosetyl-Al not only increased the extent of colonization by *Glomus intraradices* in leek roots, but also increased the production of intramatrical vesicles. Bird *et al.* (1974) and Nemec and O'Bannon (1979) observed similar increases in vesicle formation of *Endogone* sp. in cotton roots and of *Glomus etinucatum* in roots of sour orange, respectively when DBCP was applied to the soil.

It is unlikely that the increase in colonization and vesicle production by *Glomus intraradices* in fungicide-treated mycorrhizal plants can be attributed to the presence of other fungi since: (1) fosetyl-Al was applied only to the foliage; (2) all experiments were conducted in a controlled environment growth chamber using a sterilized potting medium; (3) mycorrhizal cultures were established from an uncontaminated, surface sterile, root inoculum; and (4) periodic sampling of the potting medium did not reveal the presence of disease on roots or of pathogenic and saprophytic fungal propagules. Bacteria were present, and thus, we can not rule out the possibility, however minor, that fosetyl-Al might have suppressed bacterial populations in the potting medium.

The stimulation of *Glomus intraradices* by fosetyl-Al as reported here, may be a direct effect of the chemical on the plant, or the mycorrhizal fungus, or both. The lack of a significant difference among the slopes of the concentration response curves (Figs 1a–d) indicates that fosetyl-Al has its greatest effect early in the colonization process, i.e. before the first sampling time (5 days after treatment). An effect of the fungicide after the first sampling time, might have been expected to produce differences in the rates of colonization measured among the concentrations of the fungicide used. Such an effect was not observed. The mechanism by which fosetyl-Al produces this effect is not certain, but appears to be related to its role in altering root exudation. Fosetyl-Al causes a significant increase in exudation of soluble sugars from roots of VAM plants, the magnitude of which is maximal during the first few days after treatment (Jabaji-Hare and Kendrick, 1985). While these results do not prove that root exudation is responsible for increased *Glomus intraradices* colonization, they do provide support for the hypothesis that root exudates are predisposing factors for the colonization and spread of mycorrhizal fungi (Ratnayake *et al.*, 1978; Graham *et al.*, 1981).

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