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EFFECTS OF ETHREL ON THE FORMATION AND RESPONSES TO VA MYCORRHIZA IN MEDICAGO AND TRITICUM

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SUMMARY

Ethrel, a compound which readily releases ethylene, depressed VA mycorrhiza formation in *Medicago sativa* and *Triticum vulgare* when it was either applied to the rooting medium or sprayed to the foliage. The axenic germination of *Glomus mosseae* spores was found to be sensitive to ethrel suggesting that at least part of the effect of ethrel on mycorrhization could come from its effect on fungal development. The possible ecological significance of these findings is discussed.

INTRODUCTION

Mutualistic symbiosis involving plants and microorganisms such as vesicular-arbuscular mycorrhiza (VAM) and legume root nodules depend, both for their formation and function, on a series of interactions between the constituent partners. The implication of plant hormones, either synthesized by the host or by the endophytes, on the establishment and development of these biotrophic associations has been demonstrated or suggested[1,2,8,14,15,22]. The synthesis of these substances by microorganisms in the rhizosphere[7] introduces an extra supply which could affect the hormonal balance inherent with the maintenance of the mutualism[18].

Ethylene, a plant hormone, could play a dual role in the establishment of these microbe-plant associations because of its known activity on root formation and promotion[31,32] and its suggested, but discussed[10], implications on soil microbial activity (see[6,24]).

The evolution of ethylene by soils has been described by several authors[19,25,28,30] and, in some instances, the quantities released are far in excess

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of those known to cause root inhibition in cereals[29]. Soil ethylene is of microbial origin being synthesized by most species of soil bacteria and fungi[24].

Although some investigations refute this (see[10]), ethylene has been described to be one of the causes of soil fungistasis[12,17,24,26,27], but as far as we know there is no information about fungistasis towards VAM fungi in soil. The aim of this paper is to study the effects of ethrel (2-cloroethane phosphonic acid), a substance which readily releases ethylene[9] on the formation of and responses to VAM and also its influence on the VAM spore germination. Ethrel is used for some agricultural (horticultural) purposes[4].

MATERIALS AND METHODS

Two types of experiments were designed, both of them using ethrel as ethylene source. Two points are important, 2-chloroethane phosphonic acid (ethrel) is stable at pH values below 4.0 and begins to be hydrolized, releasing ethylene, as the pH rises. It is also known[4] that at pH = 7.1, a 45.4% of ethrel is hydrolized in 18 h at 25 ± 1°C. Consequently, at such temperature and pH conditions, the 91.1% of ethrel is lost in 72 h.

Experiment 1. – Effect of ethrel on VA mycorrhization

This experiment was carried out in open pots of soil as was that previously described[5] (soil no. 8, 18.2 ppm Olsen P, pH = 7.4). This soil was steam-sterilized and then mixed with sterile sand (5:2 mixture).

Two VA mycorrhizal host plants were tested: *Medicago sativa* L. cv. Aragón and *Triticum vulgare* L. cv. Mara. Seeds were germinated on moistened filter paper and two-day-old seedlings were transplanted into pots (seven per pot) containing 300 g of the soil/sand mixture. The VA mycorrhizal endophyte assayed was the yellow-vacuolate spore type (YV)[21], a form of *Glomus mosseae*[11] that was collected from a stock plant culture. The mycorrhizal inoculum was applied to the planting hole and it consisted of spores, hyphae and infected root fragments throughly homogenized and divided into equal aliquots. The alfalfa seedlings were also inoculated with the strain 203 of *Rhizobium meliloti* isolated in this laboratory. Plants were grown in a glasshouse at 19–25°C. They were watered from below and given Long Ashton nutrient solution[16] (5 ml week^{-1}) without phosphate for the wheat and also without nitrogen for the alfalfa.

Three ethrel concentrations were tested (0.01, 0.1 and 1 mg per pot) which were prepared in freshly diluted aqueous solutions and 10 ml per pot were either injected into the rhizosphere by using a syringe ('root application') or sprayed to the foliage with a hand sprayer ('foliar application'). In the later case the soil in the pots was covered with cotton wool during spraying. There is evidence that foliar-applied ethrel will move to the root[9].

Ethrel treatments were applied for the first time after 10 days of plant growth, being repeated twice more at weekly intervals since ethylene is evolved after its release from ethrel[9]. There was also an untreated control. Ten replicate pots for each one of the different experimental situations (ethrel concentrations, ways of application, untreated control and plants) were prepared. Five of these replicate pots were harvested after 5 weeks of plant growth ('first harvest') and the remain 5 pots were harvested after a further five weeks ('second harvest').

Fresh weight of shoots and roots were recorded and the number of sporocarps and nodules was assessed visually at each harvest and after carefully washing the roots. Mycorrhizal infection was also stimated by microscopically examining stained[23] root samples (more than 100 root segments per pot), and is given as 'Total (%) infection'. This was calculated from data of incidence (percent of root

segments with any VA infection) and extent (calculated by multiplying the percentage of the length by the width of the root cortex infected).

Experiment 2. – Effect of ethrel on VA spore germination

Resting spores of *Glomus mosseae* were isolated from sporocarps obtained from a sample of infected roots. After excision from the sporocarps, the spores were surface-sterilized with streptomycin and chloramine[20] which were applied for 25 min before washing the spores in several changes in sterile water[3]. Once sterilized, the spores were transferred with sterile capillary pipettes to replicate Petri dishes. These contained 1% water-agar and the appropriate quantities of ethrel to give the range of concentrations of 0, 0.1, 1.0, 10.0 and 100.0 mg per litre. Ethrel solutions were brought to neutral pH, sterilized by passage through a filter of 0.45 μm pore size and mixed with molten agar at 45°C. Three replicate Petri dishes per concentration were prepared, each one receiving about 50 surface-sterilized spores. The total number of germinated spores was counted after 3, 6, 9 and 16 days of incubation at 24°C in the dark.

RESULTS

Ethrel either applied to the rooting medium or sprayed onto the foliage depressed plant growth and mycorrhizal infection in alfalfa plants at early stages of their development (Table 1). This effect increased in its intensity with the amount of ethrel applied. Lateral roots were short and had swollen tips on ethrel treated plants. However, as data of the second harvest show (Table 2), shoots of plants that received ethrel by foliar spray had a fairly similar weight as that of the controls, indicating a possible recovery of the plants as the ethrel effect disappeared. In spite of that, the degree of mycorrhizal infection of those plants was lower than in the controls.

Nodulation was markedly decreased by ethrel treatments and the observed inhibition was more intense when the ethrel was applied to the rooting medium (Table 2). The formation of sporocarps of *Glomus mosseae* associated with the roots was also negatively affected by ethrel treatments (Table 2). Table 3 records data of the first harvest of wheat plants and shows that ethrel did not significantly affect plant growth although it depressed mycorrhizal infection. These results could be explained on the basis of a direct effect of ethrel on mycorrhiza formation, since there was no effect of the assayed doses on wheat plant development. Nevertheless, the existence of a direct effect on mycorrhization cannot be assumed from data on plant growth since the hormone may induce physiological changes in the plant that might be not reflected in the weight of shoots or roots.

Differences in the response to ethrel treatments between wheat and alfalfa plants might be due to their different pattern of growth. In fact, at the first ethrel application, *i.e.* after ten days of growth, the root and shoot systems in wheat plants were more developed than in alfalfa.

Table 1. Effect of ethrel on plant growth and mycorrhizal infection of *Medicago sativa* inoculated with *Glomus mosseae* (1st harvest)

Ethrel* treatment	Fresh weight (g)**		Total (%)** infection
	Shoot	Root	
Control	0.70 ± 0.03	0.72 ± 0.03	24.9 ± 4.30
Root application			
0.01	0.68 ± 0.03	0.72 ± 0.02	15.0 ± 3.64
0.1	0.59 ± 0.03	0.68 ± 0.04	8.1 ± 4.75
1	0.39 ± 0.03	0.35 ± 0.06	3.9 ± 3.70
Foliar application			
0.01	0.74 ± 0.03	0.73 ± 0.04	19.1 ± 5.80
0.1	0.52 ± 0.03	0.64 ± 0.05	7.9 ± 1.86
1	0.38 ± 0.03	0.39 ± 0.04	7.6 ± 1.97

* mg ethrel/pot given three times at weekly intervals.
** Mean value of five replicate pots ± Confidence limit at 5 % level of significance.

Table 2. Effect of ethrel on plant growth, mycorrhizal infection and nodulation of *Medicago sativa* inoculated with *Glomus mosseae* (2nd harvest)

Ethrel treatment	Fresh weight (g)		Total (%) VA infection	Sporo-carps*	Number of nodules
	Shoot	Root			
Control	2.42 ± 0.10	2.47 ± 0.18	51.86 ± 8.35	4	103 ± 9.1
Root application					
0.01	2.40 ± 0.09	2.31 ± 0.17	36.50 ± 4.61	2	87 ± 8.1
0.1	1.60 ± 0.17	1.10 ± 0.18	14.89 ± 0.97	1	9 ± 2.7
1.0	1.53 ± 0.14	1.10 ± 0.16	18.31 ± 4.80	0	0
Foliar application					
0.01	2.37 ± 0.20	2.49 ± 0.10	30.61 ± 5.15	3	101 ± 8.4
0.1	2.29 ± 0.12	2.21 ± 0.18	29.41 ± 2.77	2	57 ± 9.0
1.0	2.24 ± 0.18	1.65 ± 0.15	32.18 ± 3.29	0	3 ± 0.8

Legend as for Table 1.
* Estimated on a scale from zero (no sporocarps) to 4 (abundant sporocarps).

Table 4 summarizes the response of wheat plants to ethrel at the second harvest. The degree of mycorrhizal infection and shoot growth were fairly similar in foliar treated plants and in the control ones. On the other hand, those plants that had received ethrel in their rhizosphere exhibit a depression both in growth and mycorrhizal infection. This could be a consequence of the effect of ethrel on mycorrhization as shown at the first harvest (Table 3).

Table 3. Effect of ethrel on plant growth and mycorrhizal infection of *Triticum vulgare* inoculated with *Glomus mosseae* (1st harvest)

Ethrel treatment	Fresh weight (g)		Total (%) VA infection
	Shoot	Root	
Control	4.36 ± 0.32	3.91 ± 0.19	15.68 ± 1.94
Root application			
0.01	4.06 ± 0.27	3.81 ± 0.38	4.95 ± 0.44
0.1	3.91 ± 0.22	3.61 ± 0.23	5.02 ± 0.43
1.0	3.92 ± 0.17	3.45 ± 0.30	4.52 ± 0.70
Foliar application			
0.01	4.42 ± 0.28	3.93 ± 0.22	8.61 ± 0.84
0.1	4.03 ± 0.32	3.81 ± 0.19	6.55 ± 1.00
1.0	3.96 ± 0.47	3.85 ± 0.19	6.70 ± 0.70

Legend as for Table 1.

Table 4. Effect of ethrel on plant growth and mycorrhizal infection of *Triticum vulgare* inoculated with *Glomus mosseae* (2nd harvest)

Ethrel treatment	Fresh weight (g)		Total (%) VA infection
	Shoot	Root	
Control	5.43 ± 0.50	5.45 ± 0.17	34.50 ± 3.75
Root application			
0.01	5.18 ± 0.52	4.82 ± 0.28	26.66 ± 3.13
0.1	4.46 ± 0.35	4.52 ± 0.25	19.16 ± 2.93
1.0	4.22 ± 0.35	4.23 ± 0.17	15.00 ± 1.54
Foliar application			
0.01	4.80 ± 0.36	5.15 ± 0.17	31.75 ± 4.75
0.1	4.82 ± 0.35	4.92 ± 0.19	33.15 ± 4.90
1.0	5.22 ± 0.28	4.28 ± 0.21	35.00 ± 6.30

Legend as for Table 1.

Table 5. Effect of ethrel on the axenic germination of *Glomus mosseae* spores

Ethrel concentration (ppm)	Percentage germination (± S.E.M.)* (days)			
	3	6	9	16
0	3.7 ± 1.8	23.0 ± 1.4	46.0 ± 3.9	88.6 ± 4.4
0.1	1.7 ± 0.9	26.2 ± 7.9	60.1 ± 4.8	85.4 ± 1.3
1.0	10.8 ± 4.2	29.0 ± 5.9	64.4 ± 0.8	89.4 ± 4.2
10.0	0.9 ± 0.9	31.0 ± 4.6	67.8 ± 4.6	89.0 ± 4.6
100.0	0	0	0	0

* S.E.M. = Standard error of the mean.

Spore germination was found to be sensitive to ethrel (Table 5), only at the highest concentration assayed 100 μg/litre which inhibited *Glomus mosseae* spore germination completely in contrast to the lower concentrations.

DISCUSSION

The reported data show that ethrel affects the process of VA mycorrhization and this is probably a result of ethylene production from ethrel. At least part of the effect of ethrel on VAM formation came from its inhibition of root growth[9,31,32], but our results support the idea of a possible direct influence on fungal development. These findings agree with previous papers indicating that ethylene could be an inhibitor of the development of fungal propagules in soil (see[24]). The influence of ethrel on legume nodulation found in this paper confirms previous reports showing such effect of ethylene, whatever its source[9,13].

Although our results suggest a possible role of ethylene in the formation of VA mycorrhiza, it is difficult to establish the critical concentration of the gas responsible of this activity because the ethylene produced in, or applied to, the soil is in part being evolved and, in part, degraded by ethylene-oxidizing bacteria[24], thus the concentration of ethylene that is present around roots of ethrel-treated plants is always fluctuating.

Undoubtedly, the possible implications of ethylene in the activity and ecology of members of the Endogonaceae will depend on the conditions existing at several discrete microhabitats in the soil. Ethylene is only accumulated in anaerobic environments, since in aerobic conditions, although it is synthesized at a higher rate, the gas is rapidly oxidized by specialized microorganisms[6,24]. Since, quantities of ethylene as high as 20 ppm have been said to be released by waterlogged soils and soils held at field capacity[30], the hypothesis that ethylene could affect VAM in natural conditions deserves further study.

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