

## Effect of carbamate herbicides on VA mycorrhizal infection and plant growth

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**Summary** The effect of the carbamate herbicides Chlorpropham, Sulfallate and Phenmedipham, which are believed to inhibit photosynthesis, on VA mycorrhizal infection and on plant growth, were examined. Foliar spraying of Phenmedipham decreased the root concentration of total and reducing sugars and the fungal metabolism (using a staining reaction for succinate dehydrogenase as indicator) 48 h after application. However, all three carbamate herbicides tested, whether applied by foliar spray or directly to soil, did not affect the amount of VA mycorrhizal infection present at the end of the experiment. These herbicides decreased plant growth when they were applied to soil. But when the herbicides were sprayed only Phenmedipham, applied at high concentrations, decreased plant growth. Moreover, our results show that VA mycorrhizas may help plants recover from the deleterious effect of Phenmedipham.

### Introduction

Several authors have reported detrimental effects of pesticides, especially fungicides, on the VA mycorrhizal symbiosis and its well known role in plant growth<sup>15,16</sup>. However, there are few papers on the effects of herbicides on VA mycorrhizas<sup>4,18,20,24</sup>.

Carbamate herbicides are known to repress cell division as a consequence of their disturbing nucleic acid metabolism and protein synthesis (see<sup>2</sup>), but an inhibition of photosynthetic activity by these herbicides has also been reported<sup>7,14</sup>. One such herbicide, Phenmedipham, has been demonstrated to inhibit photosynthesis not only when applied by foliar spray but also when applied to the root<sup>5</sup>.

It is known that the carbon requirements of mycorrhizal fungi must be supplied by the host photosynthate<sup>10,23,25</sup>. Hence, any factor which modifies the photosynthetic products available for distribution might affect VA mycorrhizal development<sup>6,8,11</sup>. Thus, it is to be expected that carbamate herbicides could affect VA mycorrhizal infection through their effect on plant photosynthesis. The effects photosynthesis-inhibition herbicides disappears some time after application<sup>22</sup>. Thus the specific staining reaction for Succinate Dehydrogenase (SDH)<sup>13</sup> was suggested as an indicator for rapid and direct assessment of the part of the mycelium which possesses this metabolic activity<sup>17</sup>.

This would reveal the proportion of fungal development affected by the herbicide.

The interaction between these herbicides and the formation and functioning of mycorrhiza are, however, poorly understood and are examined in this paper.

### Material and methods

The experiments were carried out in open pots of soil collected from Granada Province, Spain. The soil was a 'reddish brown calcareous' type, pH = 7.6. It was steam-sterilized, mixed with 25% (w/w) of sterile sand, and then reinoculated with a soil filtrate containing the common soil micro-organisms except propagules of Endogonaceae. Three mycorrhizal host plants were tested: *Medicago sativa* c. Aragón, *Triticum vulgare* var. Lozano and *Sorghum vulgare*. They were inoculated with rhizosphere soil from stock culture of *Glomus mosseae* which contained spores, mycelium and infected root fragments. Five g of such inoculum were added per pot. The alfalfa plants were also inoculated with strain 203 of *Rhizobium meliloti* isolated in our laboratory.

Seeds were sown in moistened sand, and four-week-old seedlings were transplanted to pots (Two per pot) containing 250 g of the soil: sand mixture. After two weeks growth, plants were treated with the herbicides. Plants were watered from below using a capillarity system and fed with Hewitt's nutrient solution (-P) every fortnight.

#### Experiment 1

Three carbamate herbicides were tested: Clorpropham, Sulfallate and Phenmedipham, which were prepared in freshly diluted aqueous solutions. Ten ml per pot were added to soil at concentrations of 0.006, 0.06, 0.6 and 6 mg/ml for Clorpropham; 0.01, 0.1, 1 and 10 mg/ml of Sulfallate and 0.008, 0.08, 0.8 and 8 mg/ml Phenmedipham or 2 ml/pot were sprayed to the foliage at concentrations: of 0.004, 0.04 and 0.4 mg/ml for Clorpropham, 0.04, 0.4 and 4 mg/ml for Sulfallate and 0.0025, 0.025 and 0.25 mg/ml for Phenmedipham, with a hand sprayer. All treatments were inoculated with VA mycorrhizal fungi. Pots without herbicide application were used as controls.

Plants were harvested after ten weeks and the dry matter production recorded. After harvesting, part of the root system was cleared and stained<sup>19</sup> and the per cent root length infected measured by the gridline intersect method<sup>9</sup>.

#### Experiment 2

Ten ml per pot of Phenmedipham was applied to soil at the rate of 0, 0.08, 0.8 and 8 mg/ml. Half of the pots were inoculated with the mycorrhizal fungus and the remaining pots fed with Hewitt's nutrient solution containing phosphate. In a second part of this experiment Phenmedipham was not applied to soil until plants were four-week-old. Plants were harvested after ten weeks and measurements were as before.

#### Experiment 3

Phenmedipham was sprayed onto the foliage of mycorrhizal sorghum and alfalfa plants as in experiment 1. Half of the pots received the herbicide after two weeks of growth and the other pots after eight weeks. Half of those plants treated when eight weeks-old were harvested 48 h after herbicide application. The rest of the plants were harvested after ten weeks. After each harvest plant dry matter was recorded and the root system from each replicate pot was divided into three portions to record: i) Total mycorrhizal infection. ii) Mycorrhizal fungus with SDH activity and iii) Sugar content of root extracts. Mycorrhizal infection was estimated as above. Succinate dehydrogenase activity (E.C. 3,99.1) in the fungal mycelium was detected by the reduction of tetrazolium salt at the expense of added succinate<sup>12</sup> and the per cent of

Table 1. Effect of soil applied carbamate herbicides on shoot dry weight and on mycorrhizal infection of wheat and alfalfa plants

Herbicides	Amount of herbicide applied (mg/ml)	Wheat		Alfalfa	
		Shoot dry wt (mg)	% root length Mycorrhizal	Shoot dry wt (mg)	% root length Mycorrhizal
Clorpropham	0	406	46	240	68
	0.006	250	56	170	56
	0.06	270	57	180	59
	0.6	250	50	210	71
	6	140	56	50	66
LSD 5%		158	17	124	32
Sulfallate	0.01	280	45	180	60
	0.1	280	56	170	62
	1	360	39	150	66
	10	240	44	100	53
LSD 5%		119	19	134	29
Phenmedipham	0.008	380	46	200	64
	0.08	370	43	200	50
	0.8	240	43	140	56
	8	210	44	100	60
LSD 5%		120	23	81	35

Each figure is the mean for five replicate pots.

VA mycelium with SDH activity was measured under a compound microscope. Total and reducing sugar contents of fresh root were assessed as before<sup>3,21</sup>.

## Results

### *Experiment 1*

Soil application of the carbamate herbicides Clorpropham, Sulfallate and Phenmedipham did not affect VA mycorrhizal infection of wheat and alfalfa plants at the concentrations used (Table 1). However, the three herbicides decreased dry weights of plant shoot<sup>4</sup> when applied at concentrations above than 0.6 mg/ml for Clorpropham and 1 mg/ml for Sulfallate. Phenmedipham also decreased plant dry matter when applied at intermediate concentration (0.8 mg/ml).

When the herbicides were sprayed onto the foliage (Table 2) they did not significantly affect VA mycorrhizal infection and only Phenmedipham decreased plant dry matter at the highest concentration tested.

Table 2. Effect of foliar sprayed carbamate herbicides on shoot dry weight and on mycorrhizal infection of wheat and alfalfa plants

Herbicides	Amount of herbicide applied (mg/ml)	Wheat		Alfalfa	
		Shoot dry wt (mg)	% root length Mycorrhizal	Shoot dry wt (mg)	% root length Mycorrhizal
Clorpropham	0	406	46	240	60
	0.004	480	38	230	54
	0.04	470	41	190	68
	0.4	440	46	200	77
LSD 5%		171	24	59	27
Sulfallate	0.04	460	35	190	63
	0.4	470	54	200	66
	4	440	45	180	83
	LSD 5%	149	26	92	38
Phenmedipham	0.0025	490	44	250	60
	0.025	440	40	290	57
	0.25	240	41	80	66
	LSD 5%	42	8	55	41

Each figure is the mean for five replicate pots.

### Experiment 2

Phenmedipham added to soil when the plants were two weeks old did not affect VA mycorrhizal infection and decreased plant dry matter (shoot and root) of wheat and alfalfa at the end of assay only at high concentrations (Table 3) as in experiment 1. However, the dry weights of uninoculated plants were decreased by the herbicide at all rates of application. The addition of herbicide to four-week-old plants were only affected plant dry matter at concentrations of 8 mg/ml for inoculated and 0.8 and 8 mg/ml for uninoculated plants (Table 4).

### Experiment 3

Tables 5 and 6 show that the Phenmedipham sprayed onto the foliage did not affect VA mycorrhizal infection in any of the situations tested.

Quantities of total and reducing sugars in root extracts and SDH activity in roots were affected, but this was evident only when measured 48 h after Phenmedipham application. There was no dose-response relationship between the amount of Phenmedipham supplied and the sugar content of the root, but the proportion of mycelium of the mycorrhizal fungus with SDH activity depended on the concentration of herbicide applied.

Table 3. Effect of soil-applied Phenmedipham, when plants were 2 weeks-old, on the dry wt. (Shoots and roots) of infected and uninfected wheat and alfalfa plants and on VA mycorrhizal infection after ten weeks of growth

Treatments	Amount of herbicide applied (mg/ml)	Wheat			Alfalfa		
		Dry weight (mg)		% root length mycorrhizal	Dry weight (mg)		% root length mycorrhizal
		Shoot	Root		Shoot	Root	
Mycorrhizal inoculated	0	440	200	45	290	240	81
	0.08	510	190	44	400	220	84
	0.8	320	120	52	170	70	78
	8	250	80	44	170	70	74
LSD 5%		98	60	11	77	50	8
Uninoculated	0	370	150		66	60	
	0.08	250	120		40	40	
	0.8	150	60		20	30	
	8	180	60		8	16	
LSD 5%		10	31		9	28	

Each figure is the mean for five replicate pots.

Table 4. Effects of soil-applied Phenmedipham, when plants were 4-weeks-old on the dry wt (Shoots and roots) of infected and uninfected sorghum and alfalfa plants and on VA mycorrhizal infection after ten weeks of growth

Treatments	Amount of herbicide applied (mg/ml)	Sorghum			Alfalfa		
		Dry weight (mg)		% root length mycorrhizal	Dry weight (mg)		% root length mycorrhizal
		Shoot	Root		Shoot	Root	
Mycorrhizal inoculated	0	1240	810	46	460	340	83
	0.08	1172	850	50	430	300	82
	0.8	1291	910	47	390	341	87
	8	1000	780	49	350	280	88
LSD 5%		116	58	11	85	34	11
Uninoculated	0	1220	960		160	150	
	0.08	1202	960		150	170	
	0.8	820	780		80	130	
	8	790	670		20	30	
LSD 5%		56	62		48	19	

Legend as for Table 3.

Table 5. Effect of Phenmedipham on the *alfalfa* shoot dry weight, sugars content in root extract and VA mycorrhizal infection

Host plant	Treat- ments	Amount of herbicide sprayed (mg/ml)	Shoot dry weight (mg)	Total sugars ( $\mu\text{g/g}$ ) fresh root	Reducing sugars ( $\mu\text{g/g}$ ) fresh root	Total mycorrhizal root length (%)	VA mycelium with SDH activity
	1	0	700	4870	870	80	66
		0.0025	680	3840	840	78	66
		0.025	590	4340	740	80	66
		0.25	380	4340	760	86	75
LSD 5%		164	1050	140	15	20	
	2	0	700	4870	870	80	66
		0.0025	760	4090	890	86	62
		0.025	640	4110	720	88	59
		0.25	560	4820	780	86	63
LSD 5%		165	580	110	26	13	
	3	0	340	3910	700	83	80
		0.0025	300	3150	690	78	88
		0.025	280	3130	610	80	58
		0.25	320	2630	470	84	39
LSD 5%		121	390	110	17	12	

Treatments: 1 = Herbicide sprayed on 2 week-old plants; 2 and 3 = Herbicide sprayed on 8 week-old plants; Plants of treatment 3 were harvested after 48 h of herbicide application; Plants of treatments 1 and 2 were harvested after ten weeks.

Table 6. Effect of Phenmedipham on the *sorghum* shoot dry weight, sugars content in root extract and VA mycorrhizal infection

Host plant	Treat- ment	Amount of herbicide sprayed (mg/ml)	Shoot dry weight (mg)	Total sugars ( $\mu\text{g/g}$ ) fresh root	Reducing sugars ( $\mu\text{g/g}$ ) fresh root	Total mycorrhizal root length (%)	VA mycelium with SDH activity
	1	0	1480	4430	940	65	23
		0.0025	1300	3500	750	57	23
		0.025	1390	3950	840	62	20
		0.25	510	3940	840	61	36
LSD 5%		404	1160	220	21	16	
	2	0	1480	4430	940	65	23
		0.0025	1530	3720	790	52	29
		0.025	1340	3720	790	63	22
		0.25	1210	3470	740	68	19
LSD 5%		300	970	240	23	10	
	3	0	830	3550	750	52	32
		0.0025	920	2860	610	58	28
		0.025	960	2750	680	61	15
		0.25	990	2390	510	57	6
LSD 5%		246	870	190	18	12	

Legend as for Table 5.

## Discussion

Although carbamate herbicides are considered to be photosynthetic inhibitors<sup>7,14</sup>, as corroborated by the staining reaction for SDH 48 h after herbicide application<sup>17</sup>, foliar spraying with these compounds did not reduce the amount of VA mycorrhizal development recorded at the end of the experiment. This agrees with the conclusions of Santakumari and Das<sup>22</sup> that plants recover their normal metabolic activity and growth 72 h after herbicide application. Hence, the deleterious effect upon VA mycorrhizal colonization probably disappears. However, Phenmedipham affected plant growth when applied at high concentrations. Also it is known that mycorrhizal plants occasionally show a growth depression following infection especially when plant growth is limited by external factors<sup>24</sup>. Hence foliar application of 0.25 mg/ml of Phenmedipham at the beginning of the experiment decreased photosynthesis and the flow of carbohydrates to the root at a critical stage when the VA endophyte may have depressing plant growth; such a depression was too strong so that the VA endophyte was not able to achieve the alleviation of plant development.

Carbamate herbicides applied to the soil did not significantly affect VA mycorrhizal development in the roots of plants tested. These results agree with those found for other herbicides<sup>4,18,20,24</sup>. The mechanisms whereby VA mycorrhizal infection was not affected by the application of these herbicides to soil, in spite of their inhibiting effect on plant growth, are unknown.

No clear relationship was observed between the level of VA mycorrhizal infection and its effect on growth of wheat and sorghum plants with similar percentages of root length infected. These results agree with those of other authors<sup>1,26</sup>. Nevertheless, the percent of VA mycelium with SDH activity was 56% for wheat<sup>17</sup> and 23% for sorghum (Table 6) suggesting that SDH activity correlates directly with the VAM growth effects. In contrast to the staining techniques developed by Phillips and Hayman<sup>19</sup>, which does not distinguish between active and inactive part of the fungus, the measurement of SDH activity makes it possible to differentiate metabolically active VA mycorrhizal fungus by staining reactions in intact roots segments<sup>12</sup>.

As expected, Phenmedipham decreased the amount of total and reducing sugars present in the root. The amount of sugars present in the root extracts seems to affect VA mycorrhizal fungus development, and in fact Hayman<sup>11</sup> found that the roots of onion grown under most light contained both the most sugar and the most active infection. In our experiment, although there was no dose-response relationship

between the amount of Phemedipham supplied and the content of root sugars, the proportion of mycorrhizal fungus with SDH activity was dependent on the concentration of herbicide applied. This suggests that either the herbicide decreased a particular sugar not quantitatively detected by measuring the total and reducing sugars or the herbicide decreased another substance besides sugar which was necessary for fungal metabolism and development<sup>5</sup>.

The results obtained with soil applied Phenmedipham (Tables 3 and 4), which is the most effective inhibitor of plant growth (Table 1), indicate that the VA mycorrhizal fungus may alleviate deleterious effect of herbicides on plant growth when applied at low rates. However, our study suggest that under standard agricultural practices herbicide residues in soil accumulated from repeated applications may eliminate the better tolerance of VAM plants to herbicide.

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