Effect of the herbicide MCPA on VA mycorrhizal infection and growth of *Pisum sativum*

Inmaculada Garcia-Romera and Juan A. Ocampo

Departamento de Microbiologia, Estacion Experimental del Zaidin, C.S.I.C., 18008-Granada, Spain

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Summary - Zusammenfassung

The effect of the hormonal herbicide MCPA on VA mycorrhizal infection and on pea plant growth was examined. Plant growth was decreased by MCPA applied at the rate of 120 ppm. The VA infections of pea roots inoculated with *G. mosseae* was decreased by the MCPA when applied at high dosis (120 ppm), but those of indigenous endophytes were decreased at the rate of 12 and 120 ppm. MCPA applied to VA inoculated pots eight weeks before pea planting also decreased the VA infection of these plants. These results suggest that VA mycorrhizas was affected not only through the plant but also directly by the application of the herbicide.

Glomus mosseae may help plants recover from the deleterious effect of MCPA when applied at intermediary (12 ppm) but not at high dosis (120 ppm).

Wirkung des Herbizids MCPA auf die Mykorrhizabildung und das Wachstum von *Pisum sativum*

Die Wirkung des Herbizids MCPA auf die VA-Mykorrhizabildung und auf das Wachstum von Erbsen wurde untersucht. Bei einer MCPA Konzentration von 120 ppm wurde eine Depression des Pflanzenwachstums beobachtet. Die gleiche MCPA Konzentration verminderte die Mykorrhizabildung in Erbsen die mit *G. mosseae* inokuliert waren. Bei einheimischen Endophyten kam es bei Konzentrationen von 12 ppm und 120 ppm zu einer Verminderung der Mykorrhizabildung. Auch bei einer MCPA-Gabe in inokulierte Töpfe, acht Wochen vor dem Pflanzen der Erbsen, wurde eine negative Wirkung auf die Mykorrhizabildung beobachtet. Diese Ergebnisse deuten darauf hin, daß die VA-Mykorrhiza nicht allein über die Pflanzen, sondern auch direkt durch das Herbizid beeinflußt wurde.

Glomus mosseae kann, bei mittlerer MCPA Dosis (12 ppm), den Pflanzen helfen, sich von der nachteiligen Wirkung des Herbizids zu erholen. Bei hoher Dosierung (120 ppm MCPA) tritt dieser Effekt nicht ein.

Introduction

Vesicular-arbuscular (VA) mycorrhizas are components of most plant systems (*Jeffries*, 1987). Thus, herbicides can affect VA fungi either directly or through its effect on plants. However, there are few papers about the effect of herbicides on VA mycorrhizas, and groups of chemicals, such as hormone herbicides, has received very little attention (*Trappe* et al., 1984). The 2-methyl-4O-chlorophenoxyacetic acid (MCPA) is a hormone herbicide which produces cessation of growth, tumor formation and secondary root induction. Root elongation stops and root tips swell. These chemicals are known to modify nucleic acid metabolism in plants and they may interact with numerous enzyme systems (*Audus*, 1976). These alterations through plant metabolism and growth can affect the VA mycorrhizal development. MCPA is usually not used when growing *Pisum sativum*, but this plant also has been used for physiological studies of hormone herbicides (*Fedtke*, 1982).

Although no effect of 2,4-D (an hormonal herbicide) on VA mycorrhizal infection has been observed (*Golubinskaya*, 1973), different results within herbicides belonging to the same groups of chemicals or within the same herbicide have been found (*Burpee* and *Cole*, 1978; *Pellet* and *Sieverding*, 1986). This probably, can be due to the different experimental conditions under which the herbicides have been used (*Trappe* et al., 1984). A combination of pre-inoculation technique and sand:vermiculite as plant growing medium has been proposed for studing the effect of herbicide on VA mycorrhiza (*Garcia-Romera*, 1986), and will be used in this work.

The potential role of VA mycorrhiza in stress resistance against several physico-chemical and biological factors is well known (Hayman, 1983). Recent examinations of VA mycorrhizal symbioses have indicated that VA fungi may improve host resistance to pesticide stress (*Menge*, 1982; *Ocampo* and *Barea*, 1985; *Garcia-Romera*, 1986). Because of the sensitivity of pea to MCPA, either applied directly or by its residues, after successive application in other plants (*Audus*, 1976), this plant was used in order to see the possible improvement of the resistance to MCPA by VA mycorrhizal infection.

Materials and methods

The experiments were carried out in open pots (500 ml) with sand: vermiculite mixture (1:1 v/v) and with soils collected from Granada Province, Spain. The sand:vermiculite mixture was autoclaved at 120° C 20 min. The

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soils were a "red brown" (Soil no. 1) and a "grey brown", (Soil no. 2) types (Table 1). These soils were mixed with 25% (w/w) of sand, and used either unsterile to allow infection by the indigenous VA endophytes or sterilized by steaming.

Table 1: Chemical and physical properties of soil no.1 and no.2.
Tabelle 1: Chemische und physikalische Eigenschaften von Boden Nr.1 und Nr.2.

	Soil no.1	Soil no.2
Sand (%)	13.2	34.8
Loam (%)	56.9	51.9
Clay (%)	29.8	11.3
Organic matter (%)	1.25	1.93
N (ppm)	1440	1720
P (ppm)	41	34
K (ppm)	226	282
$CaCO_3$ (%)	37.3	19.5
Fe (%)	2.4	0.8
pH	8.4	8.1
Na (meq/100g)	0.1	0.1
Ca (meq/100g)	40.4	29.5
Mg (meq/100g)	1.7	2.9

Pea (*Pisum sativum* var. Lincoln) was used as test plant. Seeds were sown in moistened sand, and two-week-old seedlings were transplanted to pots. Sterilized soil and sand:vermiculite pots were inoculated with rhizosphere soil from maize plants stock cultures of a isolate of *Glomus mosseae* which contained spores, mycelium and infected root fragments. To each pot 5 g soil inoculum mixed with 5 g sand was added. Soil filtrate (filter paper Whatman no. 1) from the rhizosphere of mycorrhizal- infected maize plants was added to the uninoculated controls. Soil filtrate contained common soil microorganisms with no propagules of *Endogonaceae*. Plants were grown under greenhouse conditions (Supplementary light (Sylvania incandescent and cool- withe lamps, 400 nmol · m^{-2} s^{-1} 400-700 nm), 16/8 h light/dark cycle, 25/19°C and 50% R.H.). Plants were watered from below using a capillar system and fed with Hewitt's nutrient solution (5 ml wks^{-1}) containing phosphate for uninoculated soil pots, lacking phosphate for inoculated pot and diluted (1/2) plus 50 mg/l of P for sand:vermiculite pots. All plants were inoculated with *Rhizobium meliloti* strain 128C53 isolated in our laboratory.

The MCPA herbicide was used at concentrations of 1.2, 12 and 120 ppm. These concentrations were based on the weight of air-dried soil. The herbicide was applied to pots after two weeks of plant growth (Experiment 1) or eight weeks before planting (Experiment 2). Pots without herbicide application were used as controls.

Plants were harvested after 4 (Experiment 2) or 6 (Experiment 1) weeks of growth and the dry matter was recorded. After harvesting parts of the root system were cleared and stained (*Phillips* and *Hayman*, 1970) and the percent root length infected measured by the gridline intersect method (*Giovannetti* and *Mosse*, 1980).

Results

Experiment 1

The VA infection of pea plants inoculated with *G. mosseae* was decreased by MCPA when applied at the rate of 120 ppm either in sterilized soil no.1 and no.2 (Tables 2 and 3) or in sand:vermiculite (Table 4) pots. The application of 12 ppm of MCPA decreased already the percent of root length infected by the indigenous endophytes in both soils (Table 2 and 3).

Table 2: Effect of MCPA herbicide on VA mycorrhizal infection and on the dry wt. of pea *(Pisum sativum)* plants grown in unsterilized and sterilized soil no. 1.
Tabelle 2: Wirkung von MCPA auf die VA-Mykorrhizabildung und auf das Trockengewicht von Erbsenpflanzen in nicht-sterilisiertem und sterilisiertem Boden (Boden Nr. 1).

Treatments	Amount of herbicide applied (ppm)	Dry weight (mg)		% mycorrhizal root length
		Shoot	Root	
Sterilized soil	0	236 b	400 a	–
	1.2	240 b	390 a	–
	12	128 c	240 b	–
	120	50 e	164 c	–
Sterilized soil plus *G. mosseae*	0	356 a	386 a	65 a
	1.2	316 a	340 a	57 a
	12	268 b	338 a	56 a
	120	110 c	135 c	10 d
Unsterilized soil	0	358 a	308 a	40 b
	1.2	320 a	300 a	32 b
	12	126 c	386 a	18 c
	120	96 d	210 b	5 d

Each figure is the mean for five pots. Within dry wt. and within % root length infected values followed by the same letter are not significantly different according to Duncan's multiple range test P = 0.05.

Table 3: Effect of MCPA herbicide on VA mycorrhizal infection and on the dry wt. of pea *(Pisum sativum)* plants grown in unsterilized and sterilized soil no. 2.
Tabelle 3: Wirkung von MCPA auf die VA-Mykorrhizabildung und auf das Trockengewicht von Erbsenpflanzen in nicht-sterilisiertem und sterilisiertem Boden (Boden Nr. 2).

Treatments	Amount of herbicide applied (ppm)	Dry weight (mg)		% mycorrhizal root length
		Shoot	Root	
Sterilized soil	0	268 b	392 a	
	1.2	258 b	382 a	
	12	108 c	184 c	
	120	66 d	66 d	
Sterilized soil plus *G. mosseae*	0	330 a	398 a	54 a
	1.2	290 a	318 a	54 a
	12	212 b	272 b	46 a
	120	140 c	110 c	9 c
Unsterilized soil	0	306 a	332 a	46 a
	1.2	275 a	282 b	49 a
	12	138 c	263 b	22 b
	120	80 d	150 c	2 c

Legend as in Table 2.

Table 4: Effect of MCPA herbicide on VA mycorrhizal infection and on the dry wt. of pea *(Pisum sativum)* plants grown in sand:vermiculite pots inoculated with *G. mosseae*.
Tabelle 4: Wirkung von MCPA auf die VA-Mykorrhizabildung und auf das Trockengewicht von, mit *G. mosseae* inokulierten, Erbsenpflanzen in Sand:Vermiculit Substrat.

Treatments	Amount of herbicide applied (ppm)	Dry weight (mg)		% mycorrhizal root length
		Shoot	Root	
Mycorrhizal uninoculated	0	238 b	332 a	
	1.2	216 b	360 a	
	12	116 c	100 c	
	120	46 e	70 d	
Mycorrhizal inoculated	0	304 a	310 a	64 a
	1.2	346 a	326 a	59 a
	12	232 b	237 b	57 a
	120	82 d	136 c	12 b

Legend as for Table 2.

The herbicide decreased dry weights of pea plants when applied at the concentration of 12 and 120 ppm (Tables 2, 3 and 4). However, the shoot dry weight of *G. mosseae* infected plants, grown in both soils, was significantly higher than that infected by the indigenous endophytes when the MCPA was applied at concentrations of 12 and 120 ppm (Table 2 and 3).

Experiment 2

Table 5 shows the dry matter and the percentage of root length infection of pea planted in soil no.1 and in sand:vermiculite pots, eight weeks after the herbicide application. Non significant differences in plant dry weight between the different doses of herbicides applied and control were observed. The percent of root length infection was decreased by the application of 12 and 120 ppm of MCPA either in soil no. 1 as well as sand:vermiculite pots.

Table 5: VA mycorrhizal infection and dry wt. of pea *(Pisum sativum)* plants grown in pots treated with MCPA herbicide and inoculated with *G. mosseae* eight weeks before planting.
Tabelle 5: VA-Mykorrhizabildung und Trockengewicht von Erbsenpflanzen *(Pisum sativum)*. Applikation von MCPA in Töpfe und Inokulierung mit *G. mosseae* 8 Wochen vor Setzen der Pflanzen.

Plant growth medium	Amount of herbicide applied (ppm)	Dry weight (mg)		% mycorrhizal root length
		Shoot	Root	
Soil no. 1	0	28 a	12 a	54 a
	1.2	29 a	20 a	56 a
	12	28 a	23 a	38 b
	120	25 a	21 a	17 c
Sand:vermiculite	0	25 a	22 a	52 a
	1.2	29 a	15 a	55 a
	12	20 a	17 a	32 b
	120	24 a	19 a	13 c

Legend as for Table 2.

Discussion

MCPA decreased the growth and the VA mycorrhizal infection of pea plants. These results disagree with those found for other hormonal herbicide (*Golubinskaya*, 1973). Different effect of the same herbicide or group of herbicides on VA mycorrhiza (*Garcia-Romera*, 1986) have been found. Thus, it is almost impossible to establish a general mode of action of herbicides on VA mycorrhizal fungi, perhaps, because of the great variety of crops, soils and ambiental conditions under which herbicides were studied (*Trappe* et al., 1984). The use of sand:vermiculite medium may be suitable to test the effect of herbicides overall in physiological experiments, because less number of variables must be considered.

It is difficult to know the effect of herbicides on VA mycorrhizal fungi, because what influence the fungus can also affect the plant and viceversa (*Trappe* et al., 1984). However, when the MCPA was applied to soil no.1 or to sand:vermiculite pots, eight weeks before pea planting, the root length infection decreases as the concentration of the herbicide increases. On the other hand, the percentage of root lenght infected by the indigenous endophytes was decreased by the application of MCPA at lower concentration than those infected by *G. mosseae*, but, the same trend of the effect of MCPA on the percentage of pea root infected with *G. mosseae* either in soils or in sand:vermiculite pots were observed. These results indicates that VA mycorrhizas was directly affected by the MCPA.

The results of the pre-inoculation experiments cannot be generalized because no close-relationship between the effect of MCPA applied to the fungus, eight weeks before pea planting, in soil or in sand:vermiculite (Table 5) and as mycosymbiont with pea plants (Tables 2 and 4) has been observed. However, the pre-inoculation technique can be usefull to test differences in response between fungal isolates and soils to herbicides.

Variations in effects of pesticides upon mycorrhizal fungi can be attributed to the mycorrhizal species involved (*Spokes* et al. 1981). Indigenous endophytes were as effective as *G. mosseae* on plant growth, but were more sensitive to the action of MCPA, especially those of soil no.2 in spite of the same infection level reached in the control treatments (Table 3). No positive effect of indigenous endophytes on plant growth at any rate of herbicide application was observed. The amount of VA inoculum present in the soil can determine the efficiency of the fungus (*Haas* and *Krikun*, 1985; *Adelman* and *Morton*, 1986). Thus, the possible differences in inoculum potential between *G. mosseae* and indigenous endophytes can be a factor which influences the different sensitivity to MCPA observed and will be investigated.

As has been found with other pesticides (*Menge*, 1982; *Ocampo* and *Barea*, 1985) VA mycorrhizas improved host resistance to MCPA stress. The infection of *G. mosseae* alleviate the negative effect of the herbicide on pea growth

when applied at intermediate (12 ppm) but not at high (120 ppm) levels. These results suggest that under agricultural practices herbicide residues in soil accumulated from repeated applications may eliminate the tolerance of VA mycorrhizal plants to herbicides.

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