



## **Influence of the fungicides carbendazim and chlorothalonil on spore germination, arbuscular mycorrhizal colonization and growth of soybean plants**

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**INTRODUCTION.** – Arbuscular mycorrhizal (AM) symbioses are essential components of most plant systems and the beneficial role that AM fungi play in agricultural production is well known (BETHLENFALVAY and LINDERMAN, 1992).

The use of pesticides to control diseases is a fundamental component of soybean crop production. However, despite the frequent application of pesticides, information about their effect on plant growth and on nontarget microorganisms such as AM fungi is limited (OCAMPO, 1993). The fungicide carbendazim have been found to have deleterious effects on AM delaying or preventing the formation of symbioses between fungi and root (DODDS and JEFFRIES, 1989; SUGAVANAM *et al.*, 1994). However, numerous fungicides have been reported to reduce AM fungi under some conditions, but not under others. Environmental factors such as soil, crop system, climate, etc. seem to influence the effects of fungicides on AM symbiosis difficulting the study on their effect on AM fungus formation and function (PERRIN and PLENCHETTE, 1993). As AM fungi cannot be cultured axenically in the absence of the plant host, spore germination test have been used to study the direct effect of pesticides on AM fungi (OCAMPO, 1993). However, very little is known about the effect of the fungicides carbendazim and chlorothalonil on spore germination and hyphal growth of AM fungi.

The present study was designed to determine the influence of carbendazim and chlorothalonil on the formation and functioning of AM in soybean plants.

**MATERIAL AND METHODS.** – The experiments were performed in 500 ml pots of soil collected from the province of Buenos Aires. Half of the soil (Argiudol type), pH 5.4, 2.28% C containing (ppm) 331 N, 9.5 P and 3.2 Ca, was steam-sterilized and mixed 3:2 (V/V) with sterilized quartz sand. The

mixture was brought to field capacity (100 mL) with an aqueous suspension of the fungicides: a) Carbendazim (metyl 1-2-benzimidazole carbamate) from Bayer (Bavistin, 50% active ingredient) at a concentration of 0.0003, 0.0015, and 0.003 ml L<sup>-1</sup> equivalent to 0.6, 3 and 6 l Ha<sup>-1</sup> (agronomical doses). b) Chlorothalonil (Tetrachloroisophthlonitrile) from Fitoquim (Tizonal, 50% active ingredient) at a concentration of 0.00015, 0.00075, and 0.0015 ml L<sup>-1</sup> equivalent to 0.3, 0.15 and 3 l Ha<sup>-1</sup> (agronomical doses). Soybean plants (*Glycine max* cv. Nidera) were grown in a greenhouse with a day/night cycle of 27/19 °C and 50% relative humidity. Plants were watered from below. Half of the pots were inoculated with *G. mosseae*. The AM inoculum consisted of 5 g of rhizosphere soil from alfalfa plant pot cultures of *G. mosseae* isolated from Ciudad Universitaria soil and identified according to GERDEMANN and TRAPPE (1974). Uninoculated plants were given filtered leachings from the inoculum soil. Eight weeks after sowing plants were harvested, the dry matter determined and part of the root system was cleared and stained (PHILLIPS and HAYMAN 1970), and the percentage of root colonization was measured (GIOVANNETTI and MOSSE, 1980). There were 5 replications per treatment.

The effect of the fungicides on germination of *G. mosseae* spores was tested *in vitro* on 1% sterile water agar plus 10 mM 2-(N-morpholin) ethane sulfonic acid (MES), pH 7. The fungicides were added to the agar of each Petri dish at the concentration of 0.01, 0.1, 1, 10, 25, 120 and 250 µl ml<sup>-1</sup> x 10<sup>-3</sup> (agronomical doses) of carbendazim and 0.000001, 0.0001, 0.01, 1, 12, 64 and 120 µl ml<sup>-1</sup> x 10<sup>-3</sup> (agronomical doses) of chlorothalonil. The three higher doses of each fungicide were equivalent to that applied to each pot of the experiment described above. Five to eight surface-sterilized spores per plate were placed in each Petri dish (MOSSE, 1962). Five replications of each fungicide doses and controls were used. The plates were incubated at 25 °C and spore germination was determined after 25 days of incubation under a light microscope. At this time hyphal length was also assessed using the gridline intersect method (HEPPER and JAKOBSEN, 1983). Ungerminated spores exposed to fungicides were washed with sterile water and transferred to freshly-prepared plates of water-agar without fungicides. These were incubated as above and the percentage of germination was determined after 7 days.

Data obtained were subjected to two-way analysis of variance. Data obtained for percentage colonization of root, germination of spores and hyphal length were arcsine-transformed before analysis. The means were compared by Duncan's multiple range test at the 5% level.

**RESULTS.** - No effect of *G. mosseae* on soybean dry matter was observed. The addition of all concentration of carbendazim to plants inoculated with *G. mosseae* decreased the percentage of soybean root length mycorrhization. However, nonsignificant differences in the percentage of AM of plants colonized by *G. mosseae* were observed with all doses of the fungicide chlorothalonil used (Table 1).

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**DISCUSSION.** soybean plants j of root coloniza *seae* strains hav 1997; MENDEZ e of our AM-fung sensitivity to fu 1993; SUGAVANA 1997).

#### Mycorrhizal

TABLE 1. - Effect of length colonization inoculated or u.

Amount of fungicide applied (l Ha <sup>-1</sup> )	Root colonization (%)
0	27
Carbendazim	
0.6	4
0.3	2
6	1
Chlorothalonil	
0.3	1
1.5	1
3	1

Each figure is the m followed by the sam multiple range test ( related with *G. mosseae*

Germination and hyphal length of *G. mosseae* spores was inhibited on media containing at least  $0.1 \times 10^{-3} \mu\text{l ml}^{-1}$  of carbendazim and  $0.01 \times 10^{-3} \mu\text{l ml}^{-1}$  of chlorothalonil (Table 2). The ungerminated spores exposed to  $0.1-120 \times 10^{-3} \mu\text{l ml}^{-1}$  of carbendazim and to  $1-64 \times 10^{-3} \mu\text{l ml}^{-1}$  of chlorothalonil reached 9-37% and 15-16% of germination respectively when were transferred to water-agar plates (Table 2).

DISCUSSION. – Vegetative growth was not improved in AM soybean plants probably because the relatively low percentages of root colonization reached. Similar results with some *G. mosseae* strains have been observed (SCHREINER and BETHLENFALVAY, 1997; MENDEZ *et al.*, unpublished data). However, the responses of our AM-fungal isolates confirm findings by others on AM-sensitivity to fungicides (DODDS and JEFFRIES, 1989; OCAMPO, 1993; SUGAVANAM *et al.*, 1994; SCHREINER and BETHLENFALVAY, 1997).

Mycorrhizal formation by *G. mosseae* was less sensitive to

TABLE 1. – Effect of carbendazim and chlorothalonil on the percentage of root length colonization and on the shoot and root (mg) of soybean (*Glycine max*) inoculated or uninoculated with *G. mosseae*.

Amount of fungicide applied (1 Ha <sup>-1</sup> )	Root length colonization		Shoot dry weight (mg)		Root dry weight (mg)	
	M+	M-	M+	M-	M+	M-
0	23.5c	0	1364a	1449a	1714a	1569a
Carbendazim						
0.6	4.8b	0	1489a	1263a	1679a	1651a
0.3	2.5ab	0	1598a	1679a	1639a	1658a
6	1a	0	1679a	1579a	1678a	1686a
Chlorothalonil						
0.3	19.3c		1432a	1204a	1642a	1527a
1.5	16.6c		1355a	1219a	1566a	1530a
3	19.5c		1455a	1175a	1633a	1641a

Each figure is the mean of five replications. Whithin each fungicide column values followed by the same letter are not significantly different according to Duncan's multiple range test ( $p = 0.05$ ). +M = Inoculated with *G. mosseae*, -M = Noninoculated with *G. mosseae*.

TABLE 2. - Effect of carbendazim and chlorothalonil on the percentage of germination and hyphal length of *Glomus mosseae* spores.

Amount of fungicide applied ( $\mu\text{l ml}^{-1} \times 10^{-3}$ )	Percent spore germination	Hyphal length	Percent germination of spores transferred to water-agar
<b>Carbendazim</b>			
0	78.2a	172.1a	nd
0.01	77.7a	79.7b	nd
0.1	33.5b	7.6c	37
1	28.3c	1.3d	20.1a
10	8.2d	0	20.3a
25	0	0	16.6a
120	0	0	9.2a
250	0	0	0
<b>Chlorothalonil</b>			
0	70.3a	103.2ab	nd
0.000001	95.1a	175.5a	nd
0.0001	85.7a	94.7b	nd
0.01	69.4b	52.5c	70
1	63.2b	3.6d	17.6a
12	25.4c	0	16.6a
64	0	0	15.7a
120	0	0	0

Legend as Table 1.

fungicides than spore germination and hyphal growth on water-agar, suggesting that distinct growth phases of AM fungi may tolerate different concentration of fungicides or that some of the fungicide was absorbed onto the soil components decreasing the amounts to which the spores were exposed to levels insufficient to affect either spore germination or hyphal growth (DODDS and JEFFRIES, 1989; OCAMPO, 1993; SCHREINER and BETHLENFALVAY, 1997). These findings may also explain the differences between carbendazim and chlorothalonil on the inhibition of AM colonization of root.

The ability of *G. mosseae* spores to germinate once removed from media containing  $120 \times 10^{-3} \mu\text{l ml}^{-1}$  of carbendazim and  $64 \times 10^{-3} \mu\text{l ml}^{-1}$  of chlorothalonil indicates it to have had a fungistatic rather than a fungicidal effect. The fungistatic effect of

these fungicides could be being able to inhibit  $250 \times 10^{-3} \mu\text{l ml}^{-1}$  chlorothalonil.

Our findings suggest that these fungicides do not affect AM fungi e

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- BETHLENFALVAY G.J. and LIN  
cial Publication no. 54  
DODD J.C. and JEFFRIES P. E  
ciated with winter whe  
GERDEMANN J.W. and TRAPP  
(1974).  
GIOVANETTI M. and MOSSE  
mycorrhizal infection  
HEPPER C.M. and JACQUESSEN  
*donum*, effect of amir  
MOSSE B.: The establishment  
*Microbiol.* 27, 569 (19  
OCAMPO J.A.: Influence of  
CRC, BOCA RATON, FL  
PERRIN R. and PLENCETTE C  
infectivity of two culti  
*tion* 12, 127 (1993).  
PHILLIPS J.M. and HAYMAN  
vesicular-arbuscular  
55, 156 (1970).  
SCHREINER R.P. and BETHLE  
different fungicides -  
(1997).  
SUGAVANAM V., UDAJAN K.  
infection and nodulati  
(1994).

SUMMARY - T  
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these fungicides could help explain the soil inoculum used still being able to initiate AM colonization of roots in presence of  $250 \times 10^{-3} \mu\text{l ml}^{-1}$  of carbendazim and  $120 \times 10^{-3} \mu\text{l ml}^{-1}$  of chlorothalonil.

Our findings provides further evidences that fungicides can affect AM fungi even at rates below recommended levels.

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#### REFERENCES

- BETHLENFALVAY G.J. and LINDERMAN R.G.: Preface: mycorrhizae in sustainable agriculture. ASA Special Publication no. 54. American Society of Agronomy, Madison (1992).
- DODD J.C. and JEFFRIES P.: Effect of fungicides on three vesicular-arbuscular mycorrhizal fungi associated with winter wheat (*Triticum aestivum* L.). *Biol. Fertil. soils* 7, 120 (1989).
- GERDEMANN J.W. and TRAPPE J.M.: The endogonaceae in the Pacific Northwest. *Mycologia Memoir*, 5 (1974).
- GIOVANETTI M. and MOSSE B.: An evaluation of the techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol.* 84, 489 (1980).
- HEPPER C.M. and JACKOBSEN I.: Hyphal growth from spores of the mycorrhizal fungus *Glomus calodanum*: effect of aminoacids. *Soil Biol. Biochem.* 15, 55 (1983).
- MOSSE B.: The establishment of vesicular arbuscular mycorrhiza under aseptic conditions. *J. Gen. Microbiol.* 27, 509 (1962).
- OCAMPO J.A.: Influence of pesticides on VA mycorrhizae. Pesticide Interactions in Crop Production. CRC, Boca Raton, Florida. pp. 214-226 (1993).
- PERRIN R. and PLENCIETTE C.: Effects of some fungicides applied as soil drenches on the mycorrhizal infectivity of two cultivated soils and their receptiveness to *Glomus intraradices*. *Crop Protection* 12, 127 (1993).
- PHILLIPS J.M. and HAYMAN D.S.: Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55, 158 (1970).
- SCHREINER R.P. and BETHLENFALVAY G.J.: Mycorrhizae, biocides, and biocontrol 3. Effects of three different fungicides on developmental stages of three AM fungi. *Biol. Fertil. Soils* 24, 18 (1997).
- SUGAVANAM V., UDAIYAN K. and MANIAN S.: Effect of fungicides on vesicular-arbuscular mycorrhizal infection and nodulation in groundnut (*Arachis hypogea* L.). *Agric. Ecosystem Environ.* 48, 285 (1994).

SUMMARY. – The application of the fungicides carbendazim and chlorothalonil decreased the percentage of colonization of soybean by the arbuscular mycorrhizal fungus *Glomus mosseae*. However, nonsignificant effect was observed on the colonization of soybean by the application of the fungicide chlorothalonil. Concentrations of  $0.1 \times 10^{-3} \mu\text{l ml}^{-1}$  of carbendazim and  $0.01 \times 10^{-3} \mu\text{l ml}^{-1}$  of chlorothalonil inhibited germination and hyphal length of *G. mosseae* spores. Ungerminated spores, treated with the fungicides, were able to germinate when they were transferred to freshly-prepared water-agar plates indicating a fungistatic rather than a fungicidal effect.