Full Length Research Paper

Growth and development of symbiotic *Arbuscular mycorrhizal* fungi, *Glomus mossea* (Nicol. and Gerd.), in alachlor and glyphosate treated soils

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Herbicides are applied to control weeds in agricultural practices and could also be detrimental to the development of some microorganisms living in the soil ecosystem. This study was conducted to determine the growth and development of mycorrhizal fungi, *Glomus mossea* (Nicol. and Gerd.), in soils treated with herbicides. Herbicide treatments were alachlor at 1.8, 3.6, 5.4 and 36 µg active ingredient (a.i.) g^{-1} or glyphosate at 1.1, 2.2, 3.3 and 21.6 µg a.i. g^{-1} dry soil, representing 0.5, 1, 1.5 and 10 × their recommended field application rates. Spore germination percentage and hyphal growth length were determined from spores germinated on cellulose membrane filters, sandwiched between the herbicide treated soil layers in Perti-dish after 30 days incubation in darkness at 22±1°C. External and internal hyphae and their active portions were determined from soil samples of the host growing medium and colonized host plant root systems, respectively. The alkaline phosphatase (ALP) and succinate dehydrogenase (SDH) staining techniques were used to determine the respective active portion of the hyphae. Spores from the prepared inoculum source have high germination percentage (93%) in non-herbicide treated soil. Germination of spores and its hyphal growth were not significantly affected in soil treated with alachlor and glyphosate at the recommended field application rates or less. Alachlor reduced the spore germination and hyphal growth significantly at treatments higher than recommended field application rates, but non-significant effect caused by the glyphosate. The development of external hyphae was insignificantly affected in the herbicides treated soils compared with that of the untreated soil. Colonization and development of internal hyphae on host plant roots were not affected by the herbicide treatments to the soil growing medium at recommended field application rates or less. There was a tendency for the higher treatment rates of alachlor (1.5 and 10×) to affect the development of internal mycorrhizal tissues. Application of alachlor or glyphosate herbicide at their recommended field application rates were not harmful to mycorrhizal development and symbiotic colonization of plant roots. Alachlor, at higher treatments than the recommended field application rates affected the pre-symbiotic stages of the spore germination and the internal mycorrhizal tissues development substantially.

Key words: *Arbuscular mycorrhizal* fungi, *Glomus mossea* (Nicol. and Gerd.), alachlor, glyphosate.

INTRODUCTION

Arbuscular mycorrhiza (AM) fungi are beneficial fungi found in soil existing symbiotically with plant roots. They enhance the growth of many plant species by increasing the efficiency of nutrient uptake, especially phosphorus. The hyphae extend beyond the roots, explore the soil volume intensively and transport the nutrients directly to the plant (Sylvia, 1992). AM fungi undergo four developmental stages in soil and plant roots: spore germination; growth of hyphae through the soil; penetration and successful initiation of colonization in roots; and spread

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of colonization and development of mycorrhizal relationship within the roots (Tomerrup and Briggs, 1981). The hyphae growing along the root surface and those that extend into the soil are known as the external hyphae. These hyphae link with the internal hyphae which spread inter- and intra-cellularly in the cortical region of the roots. Since AM fungi grow and develop in soil ecosystem, they are likely to be exposed directly to toxicants or chemicals, such as pesticides use in agricultural practices, which may be inhibitory to their development. Some pesticides have been reported to affect AM fungi development, inhibiting spore germination and/or hyphal growth (Carr and Hinkley, 1985; Sukarno et al., 1993, 1996; Kling and Jakobsen, 1997; Reddy and Natarajan, 1994; Menendez et al., 1999; Giovannetti et al., 2006). The effect of herbicides on development of AM fungi had also been reported (Busse *et al.*, 2004; Burpee and Cole, 1978; Kelley and South, 1979), but is not well studied. The interaction between AM systems and agrochemicals in soil is an important consideration in the development of appropriate management technologies of AM fungal populations (Nelson and Khan, 1992). This study evaluated the direct effect of herbicides in soil on AM spore germination and external and internal hyphal development.

MATERIALS AND METHODS

Source and preparation of AM inoculum

Whole inoculum of *Glomus mossea* (Nicol. and Gerd.) (Gerd. and Trappe UK 118), consisting of spores, external hyphae and infected root fragments was obtained from international culture collection of VA Mycorrizal Fungi, UK. The inoculum was propagated on Sorghum bicolor for four months in glasshouse pot cultures using the method of Feldmann and Idczak (1991), which gave the most probable number (MPN) of 88.32 infective propagules per 100 g inoculums, and 5.6 spores g^{-1} inoculum.

Spores of *G. mossae* were isolated from the soil inoculum by wet sieving technique of Gerdemann and Nicolson (1963). The spores were examined for size and colour uniformity under the dissecting microscope (25×). Viable spores were selected and isolated singly. The isolated spores were placed on moist filter paper and kept at 4°C for two days. Before use, the spores were sterilized in two changes of freshly prepared 2% (w/v) solution of chloramines T containing 0.2 g l^{-1} streptomycin sulphate (Mosse, 1959), and rinsed thoroughly in three changes of sterile distilled water.

Effect of herbicides on spore germination and hyphae development

Five sterilized spores were placed between each pair of cellulose membrane filters (millipore 0.45 μm pore size, diameter 47 mm) in Petri-dish. Each pair of the filters with the spores was then sandwiched between 2 × 30 g soil layers, earlier treated with the herbicides alachlor at 1.8, 3.6, 5.4 and 36 μg a.i. g^{-1} or glyphosate at 1.1, 2.2, 3.3 and 21.6 μg a.i. g^{-1} dry soil, representing 0.5, 1, 2 and 10 × their recommended field application rates. The controls were treated with sterile distilled water. Each spore containing Petri-dish was wrapped in parafilm and incubated in darkness at 22± 1°C for 30 days. Five replicate plates were used per treatment.

The spores were recovered after the incubation period. The spores and the developed germ tubes were stained with trypan blue in acidic-glycerol, and examined microscopically at 250× magnification for germination and hyphal attachment (Tommercup and Kidby, 1980). The hyphal length (stained hyphae) was estimated by gridline-intersect method of Newman (1966) under a dissecting microscope fitted with 10 × 10 gridline eyepiece micrometer, at 25× magnification. The intersections between the horizontal/vertical gridlines and hyphae at all fields were counted. Hyphal length was calculated using the modified formula of Tennant (1975):

Hyphal length = [C x N x G]

Where, C is the constant 11/14; N is the number of intersection and G is the grid unit

Development and activity of external and internal hyphae

Mycorrhizal development was studied on peanut (*Arachis hypogaea*) plants placed in the glasshouse as completely randomized design (CDR) with four replicates. The experiment was conducted in black plastic pots (6 × 20 cm), filled with 1 kg of sterilized soil: sand mixture (1:3). Inoculum of *G. mossea* was inoculated at ten percent by weight pot^{-1} before sowing of the peanut seeds (one seed pot^{-1}) at 5 cm depth. Prior to planting, the seeds were surface sterilized for 2 min in 30% aqueous hydrogen peroxide, and rinsed in sterile distilled water. Hoagland's solution minus P (Davis et al., 1978) was applied as nutrient source twice a week at 20 ml pot^{-1} until four weeks.

Alachlor and glyphosate herbicides were applied separately as 20 ml soil drench pot^{-1} at four weeks after planting. The herbicide treatments consisted of 1.8, 3.6, 5.4 and 36 μg a.i. g^{-1} or 1.1, 2.2, 3.3 and 21.6 μg a.i. g^{-1} dry soil, respectively for alachlor and glyphosate, representing 0.5, 1, 2 and 10× their recommended field application rates. Distilled water was applied to controls.

External hyphae determination

Five cores (10 mm diameter and to the bottom of the pot) of moist soil were taken randomly with a cork borer from each pot at 28 days after herbicide treatments, and mixed well in a plastic bag. The extraction and measurement of external hyphae were carried out according to the method of Abbott and Robson (1985). A 10 g subsample of soil was extracted in 400 ml distilled water for 1 min, and decanted through 250 and 53 μm sieves. The filtrates were then mixed together and transferred into a 200 ml distilled water in flask as hyphae extract.

The hyphae extract was stirred vigorously for 5 min with a magnetic stirrer, and a 50 ml aliquot was taken and vacuumed on a 1.2 μm millipore filter using vacuum flask. The filtrate was stained in trypan-blue (TB) in acidic-glycerol (500 ml glycerol, 450 ml H$_2$O$_2$, 50 ml 1% HCl and 0.05% trypan blue) (Koske and Gemma, 1989).

Alkaline phosphatase (ALP)-active hyphal length was determined (Dikson and Smith, 1998) by applying the ALP histochemical staining technique of Gianinazzi and Gianinazzi-Pearson (1992) and Tisserant et al. (1993). A 50 ml aliquot of the hyphae extract was transferred into a 100 ml vial, mixed with 15 ml reaction medium containing 0.05 M Tris-citric acid buffer (pH 9.2), 1 mg ml^{-1} Fast Blue RR salt, 1 mg ml^{-1} cx-naphthyl acid phosphate, 0.5 mg ml^{-1} MgCl$_2$ and 0.8 mg ml^{-1} MnCl$_2$ 4H$_2$O. The mixture was agitated and made up to 100 ml with distilled water and incubated overnight at 24°C. The ALP stained hyphae were poured onto the 53 μm sieve, washed and collected in 15 ml distilled water. The mixture was stirred vigorously for 5 min with a magnetic stirrer, and transferred and agitated in 100 ml distilled water, after which the heavier particles were allowed to settle down. A 50 ml aliquot was

filtered through 1.2 μm millipore filter using a vacuum flask. ALP enzyme activity was determined as indicated by a violet-black stain. The filtrate containing hyphae either with TB or ALP stained were observed under a dissecting microscope at 250× magnification. Total and active hyphal lengths were estimated by using a gridline-intersect method of Newman (1966) and Tennant (1975) as described earlier. The eyepiece micrometer which had 10×10 gridlines was used in the counting of the intersection between all horizontal/vertical gridlines and hyphae at all field of filter area. Hyphal length was calculated as follows:

Hyphal length/grid (cm/g soil) = [c x n x g x a/b x 1/s]

Where, c is the constant 11/14; n is the number of intersections; g is the grid unit; a is the area of filter covered by sample, mm^2; b is the area of grid, mm^2 and s is the soil on filter, g.

The proportion of ALP activity of the external hyphae was calculated by using the formula:

$$\% \text{ ALP activity} = \frac{\text{Active external hyphal length (ALP stained)}}{\text{Total external hyphal length (TB stained)}} \times 100$$

Internal hyphae determination

A combined methods of Phillips and Hayman (1970) and Koske and Gemma (1989) were used for the observation of internal hyphae of the AM fungi. Infection intensity of internal hyphae on plant roots was determined by staining with trypan blue. The plants were harvested at 28 days after herbicide application, and the root system was taken, washed thoroughly with tap water, and kept overnight in 50% ethanol in 50 ml McCartney bottles. The roots *ca* 3 g (f. wt.) were fixed in 10% KOH and heated in water bath at 90×C for 1 h. The roots were then rinsed in two changes of 200 mL tap water, and finally acidified by soaking in 1% HCL for 5 min. The acidified roots were stained in 0.05% trypan blue mixed with acidic glycerol solution, and heated in a water bath at 90°C for 60 min, and de-stained in acidic glycerol. The AM root colonization was assessed by cutting the destained roots into 1 cm sections in Petri-dish. Ten cuttings were mounted in glycerol into a glass slide and covered with cover glass. The root segments were examined for the presence or absence of AM arbuscules, vesicles and/or internal hyphae under a compound microscope at 40× magnification.

The metabolically active portion of the mycelium was determined by Succinate Dehydrogenase (SDH) staining (Ocampo and Barea, 1985; Smith and Gianinazzi-Pearson, 1988). The SDH activity was determined histochemically by the deposition of purple formazan following reduction of nitroblue tetrazolium in the presence of succinate. Roots were cut into 0.5 cm lengths and incubated overnight in the reaction medium containing 50 mM Tris-HCl (pH 7.4), 0.5 mM $MgCl_2$, 1 mg ml^{-1} Nitro blue tetrazolium (NBT), 0.25 M Na^2 succinate. The roots were then rinsed with distilled water and cleared by boiling in 20% chloral hydrate for 10 to 15 min, and mounted in glycerol on slides. Enzyme activity was indicated by the presence of a dark purple stain in the root fragments observed under 40× magnification of a compound microscope. The total and active infection intensity of the internal hyphae was calculated using the formula of Trouvelot et al. (1986):

M% = [(95n5 + 70n4 + 30n3 + 5n2 + n1) / N]

Where, M (intensity of infection n5, n4, …. n1, respectively designated number of fragment notes 5, 4, …1 (0 = 0%, 1 = 0-5%, 2 = 6-10%, 3 = 11- 50%, 4 = 51- 90%, 5 = >90 %); N (number of observed fragments).

Proportion of SDH activity of the internal hyphae was calculated using the formula:

$$\% \text{ SDH activity} = \frac{\text{Active infection intensity (SDH stained)}}{\text{Total infection intensity (TB stained)}} \times 100$$

Statistical analysis

Data were analyzed using the analysis of variance (ANOVA), and mean separation by least significant difference (LSD) at 5% probability level.

RESULTS

Spores of *G. mossae* from the prepared inoculum source have high germination percentage. Ninety-three percents of the spores germinated when placed between untreated moist soil layers incubated in darkness for 30 days at 22 ±1°C in the Petri-dish (Table 1). Spores germinated in alachlor or glyphosate herbicide treated soil also produced substantially high germination percentages of60 to 86.7%. Treatments of both herbicides at their recommended field application rates or lower did not reduce spore germination significantly, attaining more than 85% germination (Table 1). Alachlor at 1.5× the recommended field application rate caused 30.3% inhibition of spore germination, but at 10×, it significantly reduced the spore germination by 40%. Spore germi-nation percentage for glyphosate treatments remained high at 80% or more. The subsequent hyphal develop-ment from the germinated spores of *G. mossae* in the alachlor or glyphosate treated soil were not significantly affected at the recommended field application rates or lower compared with those in non-herbicide treated soil of the control, measured at 30 days after spore incubation. The hyphal length in the herbicide treated soil ranged from 15.5 to 24.8 mm compared with 19.8 mm of the control. Significant reduction of the hyphal length was recorded for alachlor treatments at 1.5 and 10× the recommended field application rates, with 9.4 and 7.8 mm, respectively. The effects were not significant for glyphosate treatments at those rates. Thus, alachlor or glyphosate herbicides, at their recommended field application rates or less, did not affect the germination of spores and the subsequent hyphal development of *G. mossae* in the soil. The spore germination and hyphal development were significantly reduced by alachlor at concentrations of higher than 1.5×. Glyphosate caused no significant effect on both the spore germination and hyphal development.

The development of external hyphae of *G. mossae* in alachlor or glyphosate treated soil, determined by the total and active hyphal length, was not significantly affected compared with those in the untreated soil. The range of total external hyphal length measured from soil core samples, from treatments of 0.5 to 10× of the recommended field application rates, were 129.8 to 151.6

Table 1. Percent spore germination and hyphal length of *G. mosseae* in herbicides treated soil.

Herbicide	Rate (µg g^{-1} dry soil)	Spore germination (%)	Hyphal length/spore (mm)
Alachlor	1.8	80.0[ab]	15.5[abc]
	3.6	86.7[ab]	17.7[ab]
	5.4	66.7[ab]	9.4[c]
Glyphosate	1.08	86.7[ab]	24.8[a]
	2.16	80.0[ab]	19.5[ab]
	3.24	86.7[ab]	24.6[a]
	21.6	80.0[ab]	16.8[abc]
Control	-	93.3[a]	19.8[ab]

Means followed by the same letters within each column are not significantly different using DMRT 5% significant level. Data were transformed to arc sin√x.

Table 2. Effect of alachlor and glyphosate on total and active hyphal length of external hyphae of *G. mossae*.

Herbicide	Rate (µg g^{-1} dry soil)	Hyphal length (cm g^{-1} dry soil)	ALP activity (%)
Alachlor	1.8	131.7[a]	26.6[a]
	3.6	129.8[a]	25.6[a]
	5.4	151.6[a]	24.6[a]
Glyphosate	1.08	166.7[a]	24.7[a]
	2.16	166.8[a]	23.7[a]
	3.24	155.4[a]	25.7[a]
Control	-	162.7[a]	27.7[a]

Means followed by the same letters within each column are not significantly different using DMRT 5% significant level. Data were transformed to arc sin√x.

mm and 155.4 to 166.8 mm g^{-1} dry soil, respectively for alachlor and glyphosate, as compared with 162.7 mm g^{-1} dry soil in the untreated soil (Table 2). The active portion of these external hyphae in herbicides treated soils of 0.5 to 10× of the recommended field application rates, as determined through estimation of its ALP enzyme activity, were 19.5 to 26.6% for alachlor and 21.5 to 25.7% for glyphosate. Both ranges of the active hyphae were insignificant compared with that for the untreated soil (27.7%).

Colonization and development of internal hyphae of *G. mossea* on host plant roots were not affected by alachlor or glyphosate treatments to the soil growing medium at their recommended field application rates or less. The total internal hyphae (mychorrizal tissue), determined as proportion of the host roots, of 9.4% for both alachlor and glyphosate were not significant, compared with 10.3% in the untreated soil. Higher treatment rates of alachlor (1.5 and 10×) affected the development of internal mycorrhizal tissues. The total internal hyphae of 6.2% and their active proportion of 3.4 and 3.4% for the alachlor treatments were significantly lower than those in the untreated soil

recorded at 10.3 and 7.6%. The effect of glyphosate on internal hyphae development with the range of 8.4 to 10.3% for total mychorrizal tissue and 6.4 to 9.1% for SDH activity were not significant from the untreated soil (Table 3). Alachlor, at the recommended field application rate in soil, therefore, did not significantly affect the colonization and development of *G. mossea* on the host plant roots, but there was a tendency for the higher treatment rates of 1.5 and 10× the recommended field application rate to affect the *G. mossea* development significantly. Glyphosate treatment of until 10× the field recommended rate did not affect the colonization and development of *G. mossea* on the host plant roots.

DISCUSSION

Correct evaluation of the results of these experiments requires recognition of limitation to studies of this nature, which is the good initial establishment of the mycorrhiza beginning with the good spore germination. It was indicative that *G. mossae* spores from the prepared

Table 3. Effect of alachlor and glyphosate on total and active hyphal length of internal hyphae of *G. mossae*.

Herbicide	Rate (µg g^{-1} dry soil)	Root fresh weight (g)	Total mychorrizal tissue (%)	SDH activity (%)
Alachlor	1.8	12.38	10.4[ab]	6.6[b]
	3.6	13.37	9.4[ab]	5.4[bc]
	5.4	15.17	8.3[b]	3.7[c]
Glyphosate	1.08	12.97	9.4[ab]	6.7[b]
	2.16	10.64	12.3[a]	9.1[a]
	3.24	10.20	8.3[b]	5.8[bc]
Control	-	12.94	10.3[ab]	7.6[ab]

Means followed by the same letters within each column are not significantly different using DMRT 5% significant level. Data were transformed to arc sin√x.

inoculum source have high germination percentage, which was important for the initial establishment and further development of the mycorrhiza used in the study. Spores of mycorrhiza with 80% or more germination percentage are considered to achieve high level of germination (Daniels et al., 1981). While this may be so, the presence of pesticides can determine changes in interactions that occur among the various organisms and microorganisms living in the soil, due to differential toxicity for these organisms (Atilano and van Gundy, 1979; Schuepp and Bodmer, 1991). Treatments of alachlor and glyphosate at their recommended field application rates or less, however, were not detrimental to the mycorrhizal development as shown in this study. Alachlor or glyphosate herbicides in soil in Perti-dish, did not affect significantly, the germination of the spores and/or the subsequent hyphal growth of *G. mossea*, measured as its total length from the germinated spores, compared with those in non-herbicide treated soil of the control. Results of those rates of alachlor or glyphosate herbicide treated to the soil medium also showed that they did not affect the hyphal development of *G. mossea* significantly on, and surrounding the host plant roots. The formation of total external and internal hyphae or their active portions were not significantly affected. The active portion of these external and internal hyphae, as determined respectively through estimations of their ALP and SDH enzyme activities, being proportionately active, also indicated no significant difference between the effect of alachlor or glyphosate treated soil and the untreated control. Reports from separate studies also indicated that mycorrhizal development or ectomycorrhizal formation were not significantly affected by alachlor (Burpee and Cole, Jr., 1978) and glyphosate (Busse et al., 2004) when incorporated into the soil at recommended field rates. However, the herbicides were reported to cause minor short-term effects on soil microbial populations (Ismail and Shamsuddin, 2005; de Andrea et al., 2003), although, no significant effect was detected on soil or root microbial communities (Weaver et al., 2007).

At higher than the recommended field application rates of 1.5 and 10×, alachlor could affect the *G. mossae* development. The spore germination and hyphal development, the internal hyphae and its SDH enzymatic activity of the active portion were significantly reduced, although not completely killed them. Development of the external hyphae, however, was not affected significantly. Some other herbicides such as triclopyr, imazapyr, and sulfometuron methyl at concentrations as high as twice the recommended field rate did not inhibit ectomycorrhizal formation (Busse et al., 2004). Examples of the effect on growth reduction of the external hyphae of *G. mossea* as being less than the control (untreated soil) were reported for some fungicide treatments (Sukarno et al., 1993; Kjoller and Rosendahl, 2000). Glyphosate, at these higher than the recommended field application rate, did not cause detrimental effects on any part or stage of the mycorrhizal development. Haney et al. (2000) and Stratton and Stewart (2006) reported that higher application rates of glyphosate had no adverse effect on soil microbial activities; instead, it showed some stimulatory effect. It is indicative from this study that *G. mossea* could freely extent its external hyphae in the soil with alachlor or glyphosate at 10× the recommended field rates, although, there are reports of effects of higher herbicide concentrations than expected in field can limit the development of mychorrizal fungi (Kelley and South, 1980; Chakravarty and Sidhu, 1987; Chakravarty and Chatarpaul, 1990).

Conclusion

Mycorrhizal fungi showed different sensitivity to the herbicide treatment to the soil growth medium during the various phases of the biological cycle. Alachlor or glyphosate in soil growing medium at recommended field application rates or less are not suppressive to growth, development and colonization of *G. mossea* on host plant roots. Spore germination, external and internal hyphae

development and their active portion of the hyphae were comparable to those in the untreated soil. The AM should be able to freely extent its hyphae in the soil and plant roots, and function normally in assisting absorption of nutrient for the host. Use of alachlor or glyphosate herbicide in agricultural practice as recommended, therefore, is not detrimental to mycorrhizal development and symbiotic colonization of plant roots. While glyphosate produced no significant effect at higher than the recommended field rate, alachlor, however, affected the presymbiotic stages of the mycorrhiza on the spore germination and hyphal growth, and the colonization of roots in the soil, suggesting some detrimental effects to *G. mossea* establishment.

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REFERENCES

Abbott LK, Robson AD (1985). Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi. New Phytologist. 99: 245- 255.

Atilano RA, van Gundy SD (1979). Effect of some systemic, nonfumigants and fumigants nematocide on grape mycorrhizal fungi and citrus nematodes. Plant Dis. Rep. 63: 729-731.

Burpee LL, Cole Jr. H (1978). The influence of alachlor, trifluralin, and diazinon on the development of endogenous mycorrhizae in soybeans. Bull. Environ. Contamin. Toxicol. 19: 191-197.

Busse MD, Fiddler GO, Ratcliff AW (2004). Ectomycorrhizal formation in herbicide-treated soils of differing clay and organic matter content. Water, Air Soil Pollut. 152: 23-34.

Carr GR, Hinkley MA (1985). Germination and hyphal growth of *Glomus caledonium* on water agar containing benomyl. Soil Biol. Biochem. 17: 313-316.

Chakravarty P, Chatarpaul L (1990). Non-target effect of herbicides: I. Effect of glyphosate and hexazinone on soil microbial activity, microbial population, and *in vitro* growth of ectomycorrhizal fungi. Pestic. Sci. 28: 233-241.

Chakravarty P, Sidhu S (1987). Effect of glyphosate, hexazinone and triclopyr on *in vitro* growth of five species of ectomycorrhizal fungi. Eur. J. For. Pathol. 17: 204-210.

Daniels BA, McCool PM, Menge JA (1981). Comparative inoculum potential of spores of six vesicular-arbuscular mycorrhizal fungi. New Phytologist. 89: 385-391.

Davis RM, Menge JA, Zentmyer GA (1978). Influence of vesicular-arbuscular mycorrhizae on *Phytophthora* root rot of three crop plants. Phytopathology, 68: 1614-1617.

De Andrea MM, Perez TB, Luchini LC, Bazarin S, Papini S. Matallo MB, Savoy VLT (2003). Influence of repeated applications of glyphosate on its persistence and bioactivity. Pesq. Agropec. Bas., Brasilia, 38: 1329-1335.

Dickson S, Smith SE (1998). Evaluation of vesicular-arbuscular mycorrhizal colonization by staining. *In* A. Varma (ed.). Mycorrhiza Manual. pp. 77-83. Berlin. Springer.

Feldmann F, Idczak E (1991). Innoculum production of VAM fungi for use in tropical nurseries. In Norris JR, Read DJ and Varma AK (eds.). Methods in Mycrobiology vol. 24. Techniques for the study of mycorrhiza. pp. 339-357. London. Academic Press.

Gerdemann JH, Nicolson TH (1963). Spores of mycorrhizal endogone extracted from soil by wet sieving and decanting. Trans. Br.

Mycological Soc. 46: 235-244.

Gianinazzi S, Gianinazzi-Pearson V, Dexheimer J (1979). Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhiza. III. Ultrastructural localization of acid and alkaline phosphatase activity in onion roots infected by Glomus mosseae (Nicol. & Gred.). New Phytologist. 82: 127-132.

Gianinazzi S, Gianinazzi-Pearson V (1992). Cytology, histochemitry and immunocytochemistry as tools for studying structure and function in endomycorrhiza. In Norris JR, Read DJ, and Varma AK (eds). Methods in Microbiology vol. 24, Techniques for the study of mycorrhiza. pp. 109-139.   Academic Press, London.

Giovannetti M, Turrini A, Strani P, Sbrana C, Avio L, Pietrangeli B (2006). Mycorrhizal fungi in ecotoxicological studies: Soil impact of fungicides, insecticides and herbicides. Prevention Today, 2: 47-62.

Haney RL, Sensemen SA, Hons FM, Zuberer DA (2000). Effect of glyphosate on soil microbial activity and biomass. Weed Sci. 48: 89-93.

Ismail BS, Shamsuddin N (2005). Effects of alachlor and metolachlor on microbial populations in soil. Malaysian Journal of Microbiology 1: 36-41.

Kelley WD, South DB (1980). Effects of herbicides on in vitro growth of mycorrhizae of pine (*Pinus* spp.). Weed Sci. 28: 599-602.

Kjoller R, Rosendahl S (2000). Effects of fungicides on arbuscular mycorrhizal fungiL differential responses in alkaline phosphatase activity of external and internal hyphae. Biol. Fertil. Soils, 31: 361-365.

Kling M, Jakobsen I (1997). Direct application of carbendazim and propiconazole at field rates to the external mycelium of three arbuscular mycorrhizal fungi species: effect on 32P transport and succinate dehydrogenase activity. Mycorrhiza, 7: 33-37.

Koske RE, Gemma JN (1989). A modified procedures for staining roots to detect VA mycorrhizas. Mycological Res. 92: 486-505.

MacDonald RM (1980). Cytochemical demonstration of catabolism in soil microorganisms. Soil Biol. Biochem. 12: 419-423.

Menendez A, Martinez A, Chiocchio V, Venedikian N, Ocampo JA, Godeas A (1999). Influence of insecticide dimethoate on arbuscular mycorrhizal colonization and growth in soybean plants. Int. Microbiol. 2: 43-45.

Mosse B (1959). The regular germination of resting spores and some observations on the growth requirements of an *Endogone* sp. causing vesicular-arbuscular mycorrhiza. Trans. Br. Mycological Soc. 42: 273-286.

Nelson SD, Khan SU (1990). Effect of endomycorrhizae on the bioavailability of bound ^{14}C residues to onion plants from organic soil treated with (^{14}C) fonofos. J. Agric. Food Chem. 38: 894-898.

Newman EI (1966). A method of estimating total length of root in a sample. J. Appl. Ecol. 3: 139-145.

Ocampo JA, Barea JM (1985). Effect of carbamate herbicides on VA mycorrhizal infection and plant growth. Plant Soil, 85: 375-383.

Pearse AGE (1968). Histochemistry, theoretical and applied Vol.I. Edinburgh. Churchill Livingstone.

Phillips JM, Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycological Soc. 55: 158-160.

Reddy MS, Natarajan K (1999). Effect of synthetic pyrethroid on the growth of ectomycorrhizal fungi and mycorrhiza formation in *Pinus patula*. Mycorrhiza, 5: 115-117.

Schuepp H, Bodmer M (1991). Complex response of VA mycorrhizae to xenobiotic substances. Environ. Toxicol. Chem. 30: 193-199.

Schweiger PF, Jakobsen I (1998). Dose-response relationship between four   pesticides and phosphorus uptake by hyphae of arbuscular mycorrhizas. Soil Biol. Biochem. 30: 1415-1422.

Smith SE, Gianinazzi-Pearson V (1988). Physiological interactions between   symbionts in vesicular-arbuscular mycorrhizal plants. Annu. Rev. Plant   Physiol. Plant Mol. Biol. 39: 221-244.

Stratton GW, Stewart KE (2006). Glyphosate effects on microbial biomass in a coniferous forest soil. Environ. Toxicol. Water Qual. 7: 223-236.

Sukarno N, Smith SE, Scott ES (1993). The effect of fungicides on vesicular-arbuscular mycorrhizal symbiosis. I. The effects on vesicular-arbuscular mycorrhizal fungi and plant growth. New

Physiologist. 25: 139-147.

Sukarno N, Smith FA, Smith SE, Scott ES (1996). The effect of fungicides on vesicular-arbuscular mycorrhizal symbiosis. II. The effects on area of interface and efficiency of P uptake and transfer to plant. New Physiologist. 132: 583-592.

Sylvia DM (1992). Quantification of external hyphae of vesicular-arbuscular   mycorrhizal fungi. In: Techniques for the study of mycorrhiza. Norris JR, Read DJ and Varma AK (eds). Academic Press, New York. pp. 53-66.

Tennant D (1975). A test of a modified method of estimating root length. J. Ecol. 63: 995-1001.

Tisserant B, Gianinazzi S, Gianinazzi-Pearson V (1996). Relationships between lateral root order, arbuscular mycorrhiza development, and the physiological state of the symbiotic fungus in *Platanus acerifolia*. Can. J. Bot. 74: 1947-1955.

Tisserant B, Gianinazzi-Pearson V, Gianinazzi S, Gollotte A (1993). In planta   histochemical stainingof fungal alkaline phosphatase activity for analysis of efficient arbuscular mycorrhizal infections. Mycological Res. 97: 245-250.

Tommerup IC, Briggs GG (1981). Influence of agricultural chemicals on germination of Vesicular-Arbuscular endophyte spores. Trans. Br. Mycological Soc. 76: 326-328.

Tommerup IC, Kidby DK (1980). Production of aseptic spores of vesicular-arbuscular endophytes and their viability after chemical and physical stress. Appl. Environ. Microbiol. 39: 1111-1119.

Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986). Measure du taux de mycorhization VA d'un systeme radiculaire. Recherche de methods d'estimation ayant une signification fonctionnelle. In Gianinazzi-Pearson V and Gianinazzi S (eds.). Physiological and Genetic Aspects of Mycorrhizae. Paris. INRA. pp. 217-221.

Weaver M, Krutz L, Zablotowicz R, Reddy K (2007). Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil. Pest Manage. Sci. 63: 388-393.