ISSN: 0126-6128
Pertanika J. Trop. Agric. Sci. 19(1): 33-41 (1996)

Effects of Formaldehyde Fumigation and Fytolan Drench on VAM Fungi and Nodulation in Some Leguminous Forest Tree Seedlings in India

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Keywords: formaldehyde fumigation, Fytolan drench, VAM colonization, nodulation, field survival

ABSTRAK

Anak benih 12 spesies pokok kekacang (Acacia caesia, A. farnesiana, A. holosericea, A. leucocephala, A. nilotica, Albizia lebbeck, Dichrostachys cinerea, Leucaena latisiliqua, Prosopis cineraria, Dalbergia latifolia *dan* Pterocarpus marsupium) *dibesarkan dalam formaldehid yang dipewasap/batas Fitolan basah ditapak semaian. Anak benih dalam batas formaldehid yang di pewasap terbantut membesar dan klorotik; mempunyai perkolonian akar VAM yang lemah (18-25.3%) dan densiti spora (3.1 – 0.16 g tanah^{-1}), jumlah bintil yang rendah (3 – 8 tanaman^{-1}) dan biojisim berbintil (100 – 870 mg plant^{-1}); jumlah biojisim (15.5 – 72 g plant^{-1}) dan kadar terus hidup lapangan 31.2 – 40.4%) anak benih adalah sangat rendah. Spesis mikoriza yang diasingkan adalah* Acaulospora bireticulata, G. flasciculatum *dan* G. geosporum. *Sebagai perbandingan, anak benih dari batas Fitolan yang basah menunjukkan pertumbuhan biasa, biojisim yang bertambah (18 – 82.2 f g plant^{-1}) dan kadar terus hidup lapangan lebih tinggi (71 – 86%); penglolonian akar VAM yang sangat menggalakkan (53.4 – 100%) dan jumlah bintil densiti spora lebih tinggi (36 – 82.8 g. tanah^{-1}) dan jumlah bintil yang lebih tinggi (7.4 – 17.6 plant^{-1}) dan biojisim berbintil (195 – 950 mg plant^{-1}) dibandingkan dengan anak benih terkawal. Akar untuk tanaman-tanaman ini mempamerkan perkembangan struktur arbuskular dan vesikular yang sangat menggalakkan. Daripada tujuh spesis VAMF yang direkodkan dari tanah-tanah rizosfera terkawal dan batas basah Fitolan,* A. bireculata, G. fasciculatum *dan* G. geosporum *merupakan spesis dominan. Secara statistik, perbezaan antara rawatan adalah ($p < 0.05$).*

ABSTRACT

*Seedlings of 12 legume tree species (*Acacia caesia, A. catechu, A. farnesiana, A. holosericea, A. leucocephala, A. nilotica, Albizia lebbeck, Dichrostachys cinerea, Leucaena latisiliqua, Prosopis cineraria, Dalbergia latifolia *and* Pterocarpus marsupium*) were raised in formaldehyde-fumigated/Fytolan-drenched beds in a nursery. Seedlings in the formaldehyde fumigated beds had stunted growth and were chlorotic; had poor VAM root colonization (18-25.3%) and spore density (3.1 – 10.6 g. soil^{-1}) and lower nodule number (3 – 8 plant^{-1}) and nodular biomass (100 – 870 mg plant^{-1}); the total biomass (15.5 – 72 g plant^{-1}) and field survival rate (31.2 – 40.4%) of the seedlings were very low. The mycorrhizal species isolated were* Acaulospora bireticulata, Glomus fasciculatum *and* G. geosporum. *In contrast, seedlings form Fytolan-drenched beds showed normal growth, enhanced biomass (18 – 83.2f g plant^{-1}) and higher field survival rate (71 – 86%); intense VAM root colonization (53.4-100%) and higher spore density (36 – 82.8 g soil^{-1}) and higher nodule number (7.4 – 17.6 plant^{-1}) and nodular biomass (195 – 950 mg plant^{-1}) compared with the control seedlings. Roots of these plants exhibited extensively developed arbuscular and vesicular structures. Of the seven VAMF species recorded from the rhizosphere soils of control and Fytolan-drenched beds,* A. bireticulata, G. fasciculatum *and* G. geosporum *were the dominant species. The differences between treatments were statistically significant ($P < 0.05$).*

INTRODUCTION

In India, 68% of the population is rural based and depend on forests for fuelwood, timber, cattle fodder and other minor products. Over-exploitation of forest lands has led to the dwindling of forest cover, causing severe ecological imbalance and environmental disaster. As part of the national effort to open new forests, barren substandard soils and low productivity agricultural lands have been used to raise multi-utility trees through plantation programmes. The seedlings needed for these programmes are usually raised in forest tree nurseries.

Legumes often have double symbiotic associations with *Rhizobium* spp. and mycorrhizal fungi (VAM and/or ECM). Such associations often benefit the leguminous plants through improved P and N supplies and also from N-P interactions (Munns and Mosse 1980). Nitrogen fixation by members of Papilionoideae and Mimosoideae needs high phosphate levels, which could probably be satisfied through mycorrhiza symbiosis. Mycorrhizal fungi not only help the plant itself, but also aid the bacterial system to fix N in the nodular tissue. Hence, legumes with dual symbioses are preferred for forestation programmes in marginal environments.

The use of fumigants and fungicides is an important management practice in nurseries to control root pathogenic fungi, soil-inhabiting insects, nematodes and weed seeds. Properly applied fumigation also usually eliminates the beneficial mycorrhizal population (Kormanik *et al.* 1977; Riffle 1980; Maronek *et al.* 1981; Udaiyan *et al.* 1995) as well as nitrogen-fixing and symbiotic bacteria (Trappe and Strand 1969; Abrahamson 1980; Molina and Trappe 1984; Kishinevsky *et al.* 1992). Since most fungicides are not pathogen-specific, they affect a wide range of non-pathogenic fungi (Bollen 1979; Vyas 1988), including those which are beneficial to plant growth, such as VAMF. VAMF are also affected by fungicide application (Manjunath and Bagyaraj 1984; Kough *et al.* 1987; Fitter and Nichols 1988; Plenchette and Perrin 1992; Sukarno *et al.* 1993; Udaiyan *et al.* 1995).

Studies on the effect of different fungicides, both systemic and non-systemic, on the development of VAM infection do not, however, show consistent overall trends (Trappe *et al.* 1984). Non-systemic fungicides have been reported to be ineffective on VAM (Jalali and Domsch 1975; Sutton and Sheppard 1976; Nemec 1980) to reduce VAM development (Nesheim and Linn, 1969; El-Giahmi *et al.* 1976; Plenchette and Perrin 1992) or, surprisingly, to stimulate it (Sugavanam *et al.* 1994).

The purpose of the present study is therefore to assess the effects of formaldehyde fumigation and Fytolan drench on the VA mycorrhizal and nodular endophytes and their subsequent effects on seedling quality and field survival of some leguminous forest tree species.

MATERIALS AND METHODS

The study was carried out in the nursery in the experimental plot of Bharathiar University, Coimbatore, Tamil Nadu, India. The red alfisol - calcareous soil had a pH of 8.1 and electric conductivity of 0.2 $mScm^{-1}$. Soil nitrogen (N), phosphorus (P) and potassium (K) concentrations were 104, 4 and 380 kg ha^{-1} respectively. Total N and available P were respectively determined by the micro Kjeldahl and molybdenum blue method (Jackson 1973). Exchangeable K was measured using a digital flame photo meter (Jackson 1973). The soil was ploughed to a depth of 50 cm, levelled and 1.5 × 1.5 m nursery beds prepared with 2-m intervals between them.

The treatments of the beds were: a) fumigated with 0.4% formaldehyde applied at the rate of 2 l/m^2 covered with polyethylene sheets for 48 h and then exposed to air for 15 days prior to sowing; b) drenched with 0.2% Fytolan, a non-systemic fungicide with protectant properties containing 88% (w/w) copper oxychloride, applied at the rate of $75ml/m^2$ for six hours prior to sowing and; c) untreated control. Each treatment was replicated five times. The plots were arranged in a completely randomised block design.

Fully mature, uniform size, viable seeds of 12 forest tree species (*Acacia caesia* (L.) Willd., *A. catechu* (L.F) Willd., *A. farnesiana* (L.) Willd., *A. holosericea* A. Cunn., *A. leucocephala* (Roxb.) Willd., *A. nilotica* (L.) Willd ex. Del. subsp. *indica* (Benth.) Brenan, *Albizia lebbeck* (L.) Willd., *Dichrostachys cinerea* (L.) Wight & Arn., *Leucaena latisiliqua* (L.) Gills, *Prosopis cineraria* (L.) Douce, *Dalbergia latifolia* Roxb. and Pterocarpus marsupium Roxb.) from the Institute of Forest Genetics and Tree Breeding (IFG &TB), Coimbatore, were sown in nursery beds in June 1992 and watered by irrigation at weekly intervals. Uniform 60-day-old seedlings were transferred to 30 × 12 cm polyethylene bags, each filled with ca. 3 kg soil from the respective seedling beds. Holes were punched in bags for drainage. The bags were arranged closely for sprinkle-irrigation. Samples of feeder root and rhizosphere soil were collected randomly from 10 seedlings for each species and each treatment 60 days after planting.

Root Colonization

Randomly selected root segments were cleaned and stained for assessment of mycorrhizal colonization. The cleared root segments were washed in distilled water, acidified with 5N HCl and stained in trypan blue (0.05% in lactophenol) adopting the technique of Phillips and Hayman (1970). Stained root segments were then examined for the presence of VAM structures, and the percentage of mycorrhizal infection determined by the root slide technique of Read *et al.* (1976).

Spore Population

Total spore count in soil samples was estimated by a modified wet sieving and decanting technique of Gerdemann and Nicolson (1963). Spore population was expressed as the number of individuals per gram of dry soil.

Field Survival Rate

The respective 150-day-old seedlings (100 seedlings for each species from the different treatments) were subsequently transplanted to degraded, barren land at the foot of the Maruthamalai hills, Western Ghats in the Bharathiar University campus in the monsoon month of October 1992. A 4 × 4 spacing was maintained for all seedlings. Data on the field survival of these seedlings were collected in February 1993.

Statistical Analyses

The data were analysed by analysis of variance (ANOVA) and the means were separated by Duncan's new multiple range test ($P < 0.05$). Pearson's coefficient correlations were performed for plant dry weight with nodule number, nodule dry weight, root colonization, spore number and field survival rate.

RESULTS

Soil

The soil at the study area was sandy loam, and low in available nutrients especially phosphorus (4 kg ha^1). However, supplementary fertilizers were not added.

Formaldehyde Fumigation

Seedlings in the fumigated beds were found to be stunted and chlorotic. They had very poor biomass and stunted growth. Maximum reduction was found in *Prosopis cineraria* followed by *Acacia caesia*, *A. catechu* and *Pterocarpus marsupium*. After 60 days in polythene bags, VAM root colonization and spore density, the number, size and biomass of nodules and field survival rate of these seedlings decreased significantly ($P < 0.05$) compared with control seedlings. This effect was maximum in *Acacia nilotica* and *Dichrostachys cinerea*. Spores of *Acaulospora bireticulata*, *Glomus fasciculatum*, *G. geosporum* and *G. macrocarpum* were isolated from the respective rhizosphere soils.

Fytolan Drench

Seedlings from the Fytolan-drenched soils showed significantly ($P < 0.05$) higher biomass and field survival compared to the control beds. *Acacia caesia*, *A. holosericea*, *Leucaena latisiliqua* and *Prosopis cinerea* had increased biomass. Field survival rate increased by 21.7, 14.5 and 12.8% in *Acacia leucocephala*, *Pterocarpus marsupium* and *Dalbergia latifolia* respectively. VAM root colonization, spore density, legume nodule number and biomass were significantly greater ($P < 0.05$) in *Dichrostachys cinerea*, *Acacia catechu*, *A. farnesiana* and *A. holosericea* respectively, than in control seedlings (Table 1). Well-developed VAM structures were observed in treated and control seedlings. Of the seven VAMF species isolated (*Acaulospora bireticulata*, *A. sporocarpia*, *Gigaspora margarita*, *Glomus australe*, *G. fasciculatum*, *G. geosporum* and *G. macrocarpum*), *A. bireticulata*, *G. fasciculatum* and *G. geosporum* were the dominant species, contributing 30, 25 and 20%, respectively, to the total spore count. The field survival rate of the transplanted seedlings from formaldehyde-fumigated, Fytolan-drenched and control soils were 31-40, 71-86 and 64-80%, respectively (Table 1).

A significant positive correlation was established (Table 2) between plant dry weight and nodule dry weight in *Acacia caesia* ($P < 0.001$), *A. catechu* ($P < 0.05$), *A. holosericea* ($P < 0.001$) and *A. nilotica* ($P < 0.05$), but not in the others. The field survival rate of *A. nilotica* and *Pterocarpus marsupium* significantly and positively correlated ($P < 0.05$) with plant dry weight.

DISCUSSION

The results showed that seedlings raised in formaldehyde-fumigated soil were chlorotic and had stunted growth. Their field survival rate was about half that of seedlings from control as well as Fytolan-treated beds. These adverse effects on the quality and performance of seedlings are correlated with a reduction in nodular biomass and poor root colonization by VAMF. Similar results on formaldehyde fumigation in leguminous crops have been reported by Udaiyan *et al.* (1995).

Poor root colonization by VAM was probably due to reduced spore density in the fumigated nursery soil. A similar reduction in VAMF spore density was reported in the rhizosphere of wheat (Hayman 1970) and citrus (Nemec 1980) as a consequence of formaldehyde fumigation. The reduction in nodule number may be due to destruction of the rhizobial population by fumigation (Kishinevsky *et al.* 1992); and the non-availability of sufficient P supply for nodulation (Mosse *et al.* 1976).

It has been suggested that VAMF may play a role in satisfying the high P demand for good nodulation and nitrogen fixation in the control beds (Asimi *et al.* 1980). The synergistic interactions between the bacterium *Rhizobium* and the mycorrhizal endophytes not only enhanced nutrient content in the above-ground plant material, but also seemed to provide well-balanced nutrients to the plants. This subsequently resulted in an improvement in biomass production. Furthermore, mycorrhizal in-

TABLE 1

Effects of formaldehyde fumigation and Fytolan drenching on growth, root colonization, sporulation and field survival of forest tree seedlings

Parameters	Treatments	*Acacia caesia*	*Acacia catechu*	*Acacia farnesiana*	*Acacia holosericea*	*Acacia leucocephala*	*Acacia nilotica*	*Albizia lebbeck*	*Dichrostachys cinerea*	*Leucaena latisiliqua*	*Prosopis cineraria*	*Dalbergia latifolia*	*Pterocarpus marsupium*
Plant d. wt. (g plant)$^{-1}$	Control	58.0 ab	43.0 a	30.0 a	42.5 b	67.0 b	72.8 a	43.2 a	28.0 a	34.1 b	80.0 a	16.2 b	34.0b
	Formaldehyde	55.0 b	40.0 ab	28.0 b	41.0 b	66.5 b	71.0 b	41.3 b	26.3 b	32.4 c	72.0 b	15.5 b	31.0 b
	Fytolan	61.0 a	45.0 a	31.0 a	45.0 a	68.0 a	73.0 a	44.0 a	29.1 a	36.3 a	83.2 a	18.0 a	35.6 a
No. nodules (plant)$^{-1}$	Control	14.6 a	18.0 a	12.0 b	16.1 b	20.3 a	21.4 a	8.2 a	20.3 a	19.4 a	15.4 a	7.5 a	9.4 a
	Formaldehyde	4.8 b	6.0 b	8.0 c	4.4 b	7.5 c	6.0 b	3.0 c	6.0 b	7.1 c	4.1 b	5.2 b	4.2 c
	Fytolan	12.0 a	17.6 a	14.3 a	15.0 a	13.0 a	16.4 a	7.4 b	12.2 a	11.0 b	16.3 a	8.1 a	11.3 a
Nodule d. wt (mg plant)$^{-1}$	Control	400 ab	380 b	475 b	900 b	193 a	250 a	200 a	620 a	700 a	195 a	285 b	270 a
	Formaldehyde	370 b	320 c	460 c	870 c	180 b	100 b	120 b	400 b	520 b	170 b	150 c	250 b
	Fytolan	430 a	427 a	510 a	950 a	195 a	280 a	210 a	630 a	725 a	200 a	300 a	300 a
Root colonization (%)	Control	58.3 a	98.4 a	70.0 a	40.7 b	90.6 a	100.0 a	87.4 b	37.4 b	57.5 a	85.2 a	77.3 a	81.2 a
	Formaldehyde	21.0 b	18.0 c	21.5 b	22.3 c	25.3 b	23.1 b	19.0 c	18.3 c	16.6 b	25.0 b	22.0 b	16.0 b
	Fytolan	53.4 a	89.6 b	77.2 a	56.2 a	95.1 a	100.0 a	94.3 a	56.0 a	56.0 a	91.3 a	85.2 a	83.3 a
No. of spores (g soil)$^{-1}$	Control	70.2 a	41.1 a	50.6 a	82.8 a	48.0 a	27.5 a	42.2 a	94.2 a	55.3 a	40.7 a	38.6 b	37.7 b
	Formaldehyde	5.1 b	4.5 b	8.3 b	3.1 c	6.2 b	8.3 c	8.4 b	10.6 c	5.7 b	9.1 b	7.3 c	6.3 c
	Fytolan	62.6 a	58.2 a	60.6 a	70.4 b	58.5 a	36.0 a	48.1 a	82.8 b	50.6 a	44.5 a	43.4 a	49.4 a
Field Survival rate (%)	Control	68.3 a	76.8 b	80.3 a	69.0 a	64.3 b	80.6 a	69.0 a	73.0 a	80.0 a	78.3 a	65.2 b	68.6 b
	Formaldehyde	38.2 b	32.3 c	40.4 b	34.5 b	37.2 c	31.2 b	38.6 c	39.2 b	38.3 b	37.6 b	32.1 c	37.2 c
	Fytolan	71.0 a	82.0 a	84.2 a	72.4 a	86.0 a	73.5 a	82.8 a	78.5 a	82.2 a	79.2 a	78.0 a	83.1 a

Means within a parameter followed by the same superscript are not significantly different according to Duncan's new multiple range test ($P < 0.05$)

TABLE 2
Pearson's correlation coefficient (r) for plant dry weight (PDW) with nodule number (NN), nodule dry weight (NDW), root colonization (RC), spore number (SN) and field survival rate (FSR)

Sl. Tree Species	PDW × NN	PDW × NDW	PDW × RC	PEW × SN	PDW × FSR
1. *Acacia caesia*	+0.6053	+1.0000**	+0.7992	+0.8076	+0.9007
2. *A.catechu*	+0.9056	+0.9989*	+0.8734	+0.9957	+0.7513
3. *A. farnesiana**	+0.9993f	+0.9142	+0.9771	+0.9869	+0.9682
4. *A. holosericae*	+0.7302	+1.0000**	+0.9814	+0.6880	+0.8332
5. *A. leucocephala*	+0.2497	+0.8305	+0.7925	+0.8664	+0.9679
6. *A. nilotica*	+0.9152	+0.9978*	+0.9958	+0.9773	+0.9985*
7. *Albizia lebbeck*	+0.9064	+0.9818	+0.9718	+0.9880	+0.9849
8. *Dichrostachys cinerea*	+0.5397	+0.9351	+0.9933	+0.8645	+0.9635
9. *Leucaena latisiliqua*	+0.2392	+0.8844	+0.8881	+0.7754	+0.8422
10. *Prosopis cineraria*	+0.9770	+0.9922	+0.9805	+0.9832	+0.9658
11. *Dalbergia latifolia*	+0.8391	+0.7766	+0.7915	+0.7963	+0.8781
12. *Pterocarpus marsupium*	+0.9961	+0.9585	+0.9485	+0.9964	+0.9993*

*, **Correlations are significant at P = 0.05 and 0.001 respectively.

fection has also been reported to increase shoot nitrogen content in nodulated plants (Ross and Harper 1970; Ross 1971). This probably explains the normal growth of the control seedlings.

In general, fungicides are less damaging/deleterious to mycorrhiza population than are fumigants. Nesheim and Linn (1969) suggested that stunting of seedlings can be avoided by using fungicides that eliminate root pathogens but are harmful to mycorrhizal fungi. Sugavanam *et al.* (1994) have reported that Fytolan promoted VAM root colonization, rhizosphere spore population and nodulation in *Arachis hypogaea*. In the present study, drenching of the nursery beds with Fytolan was found to increase endomycorrrhizal colonization and *Rhizobium* nodulation in the legume seedlings. But the extent of increase varied among the host species. The differential response to treatments probably reflects the difference in the genetic constitution as well as the microfloral composition in the rhizosphere of the host species. The higher root colonization and spore density in the Fytolan-treated beds is probably due to the following reasons: i) increased survival of VAM fungal propagules at seedling emergence stage, made possible by the suppression of microbes antagonistic to VAMF (Groth and Martinson 1983; Afek *et al.* 1990; Hetrick and Wilson 1991); ii) The insensitivity of certain *Rhizobium* strains to fungicides (Chiranjeevi 1982; Kataria *et al.* 1985; Radhakrishnan and Chatrath 1989; Singh and Agarwal 1990); iii) The synergistic interaction between *Rhizobium* and the mycorrhizal endophytes, where the mycorrhiza fungi enhance P availability for greater nodule formation (Kucey and Paul 1982).

Results from the present study also showed that seedlings with high biomass with roots extensively colonized by the VA mycorrhiza fungi also have higher field survival rate. Seedlings from Fytolan-drenched beds in particular showed significantly higher field performance than the control. The effective synergistic interactions between the symbionts could probably have provided the necessary prerequisites for the high performance in the field environment. Presence of VAM probably helps to alleviate drought stress during transplanting (Michelsen and Rosendahl 1990) and enhances seedling growth, vigour and survival after transplanting (Brandeau 1970; Biermann and Lindermann 1983). Fytolan drench favours the establishment of the VAM and nodular endophytes of legumes in nutrient-deficient soils and should therefore be employed in nursery management along with other cultural practices for the production of high quality seedlings. The very hazardous chemical, formaldehyde, is not recommended for use in the nursery management practices, if it can be avoided.

ACKNOWLEDGEMENT

One of us (V.S.) thanks the Council of Scientific and Industrial Research, New Delhi, India for the award of Senior Research Fellowship, that supported this research.

REFERENCES

ABRAHAMSON, L.P. 1980. Pesticides in forest tree nurseries, primary and secondary effects. In *Proceedings North American Forest Tree Nursery Soils Workshop*, ed. L.P. Abrahamson and D.H. Bickelhaupt, p. 191-204. Syracuse: State Univ. New York, Coll. Environ. Sci. and Forestry.

AFEK, U., J.A. MENGE and E.L.V. JOHNSON. 1990. Effects of *Pythium ultimum* and metalaxyl treatments on root length and mycorrhizal colonization of cotton, onion and pepper. *Plant Diseases* **74**: 117-120.

ASIMI, S., V. GIANINAZZ-PEARSON and S. GIANINAZZI. 1980. Influence of increasing soil phosphorus levels on interaction between vesicular-arbuscular mycorrhiza and *Rhizobium* in soybean. *Canadian Journal of Botany* **58**: 2200-2205.

BIERMANN, B. and R.G. LINDERMANN. 1983. Use of vesicular-arbuscular mycorrhizal roots, intraradical vesicles and extraradical vesicles as inoculum. *New Phytology* **95**: 97-105.

BOLLEN, G.J. 1979. Side effects of pesticides on microbial interaction. In *Soil-borne Plant Pathogens*, ed. B. Schippers and G. Gams, p. 451-481. New York: Academic Press.

BRANDEAU, J. 1970. El cacao: *Teenicas grricolas* and transfer of nutrients in vesicular-arbuscular mycorrhizas. II. Uptake and translocation of phosphorus, zinc and sulphur. *New Phytology* **81**: 43-52.

CHIRANJEEVI, V. 1982. Studies on persistence and translocation of carbendazim in soil plant ecosystem. Ph.D. thesis, P G School, Indian Agricultural Research Institute, New Delhi.

EL-GIAHMI, A.A., T.H. NICOLSON and M.J. DAFT. 1976. Effects of fungal toxicants on mycorrhizal maize. *Transactions British Mycology Society* **67**: 172-173.

FITTER, A.H. and R. NICHOLS. 1988. The use of Benlate to control infection by vesicular-arbuscular mycorrhizal fungi. *New Phytology* **110**: 201-206.

GERDEMANN, J.W. and T.H. NICOLSON. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Transactions British Mycology Society* **46**: 235-244.

GROTH, D.E. and C.A. MARTINSON. 1983. Increased endomycorrhizal infection of maize and soybean after soil treatment and metalaxyl. *Plant Diseases* **67**: 1377-1378.

HAYMAN, D.S. 1970. *Endogone* spore numbers in soil and vesicular-arbuscular mycorrhiza in wheat as influenced by season and soil treatment. *Transactions British Mycology Society* **54**: 53-63.

HETRICK, B.A.D. and G.W.T. WILSON. 1991. Effects of mycorrhizal fungus species and metalaxyl application on microbial suppression of mycorrhizal symbiosis. *Mycologia* **83**: 97-102.

JACKSON, M.L. 1973. *Soil Chemical Analysis*. New Delhi: Prentice Hall (India).

JALALI, B.L. and K.H. DOMSCH. 1975. Effect of systemic fungitozincants on the development of endotrophic mycorrhiza. In *Endomy-*

corrhizas, ed. F.E. Sanders, B. Mosse and P.B.H. Tinker, p. 619-626. New York: Academic Press.

KATARIA, K.R., J.S. YADAV, F.C. GRAG and R.K. GROVER. 1985. Inactivation of seed treatment fungicides by *Rhizobium*. *Pesticide Science* **16**: 337-340.

KISHENIVSKY, B.D., R. LOBEL, D. GURFEL and NEMAS. 1992. Soil fumigation with methyl bromide as a means of increasing the occurrence of the inoculum strain in peanut nodules. *Soil Biology and Biochemistry* **24**: 845-848.

KORMANIK, P.P., W.C. BRYAN and R.C. SCHULTZ. 1977. Influence of endomycorrhizae on growth of sweetgum seedlings from eight mother trees. *Forest Science* **23**: 500-505.

KOUGH, J.L., V. GIANIZAZZI-PEARSON and S. GIANINAZZI. 1987. Depressed metabolic activity of vesicular-arbuscular mycorrhizal fungi after fungicide application. *New Phytology* **106**: 707-715.

KUCEY, R.M.N. and E.A. PAUL. 1982. Carbon flow photosynthesis and N_2 – fixation in mycorrhizal and nodulated faba beans (*Vicia faba* L.) *Soil Biology and Biochemistry* **14**: 407-412.

MAJUNATH, A. and D.J. BAGYARAJ. 1984. Effect of fungicides on mycorrhizal colonization and growth of onion. *Plant and Soil* **80**: 147-150.

MARONEK, D.M., J.W. HENDRIX and J. KIERNAM. 1981. Mycorrhizal fungi and their importance in horticultural crop production. *Horticultural Review* **3**: 172-213.

MICHELSEN, A. and S. ROSENDAHL. 1990. The effect of VOA mycorrhizal fungi, phosphorus and drought stress on the growth of *Acacia nilotica* and *Leucaena leucocephala* seedlings. *Plant and Soil* **1**: 7-14.

MOLINA, R. and J.M. TRAPPE. 1984. Mycorrhiza management in bareroot nurseries In *Forest Nursery Manual: Production of Bareroot Seedlings*, ed. M.L. Durgea and T.D. Landis, p. 211-233. The Hague: Martinus Nijhoff.

MOSSE, B., C.L. POWELL and D.S. HAYMAN. 1976. Plant growth responses to vesicular-arbuscular mycorrhiza. IX. Interactions between VA-mycorrhizae, rock phosphate and symbiotic nitrogen fixation. *New Phytology* **76**: 331-342.

MUNNS, D.N. and B. MOSSE. 1980. Mineral nutrition of legume crops. In *Advances in Legume Sciences*, ed. R.J. Summerfield and A.H. Bunting, p. 115-125. London: H.M. Stationery Office.

NEMEC, S. 1980. Effect of 11 fungicides on endomycorrhizal development in sour orange. *Canadian Journal of Botany* **58**: 522-526.

NESHEIM, O.N. and M.B. LINN. 1969. Deleterious effects of certain fungitoxicants on the formation of mycorrhizae on corn by *Endogone fasciculatum* and on corn root development. *Phytopathology* **59**: *297-300*.

PHILLIPS, J.M. and D.S. HAYMAN. 1970. Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions British Mycology Society* **55**: 158-161.

PLENCHETTE, C. and R. PERRIN. 1992. Evaluation in the greenhouse of the effects of fungicides on the development of mycorrhiza on leek and wheat. *Mycorrhiza* **1**: 59-62.

RADHAKRISHNAN, P. and M.S. CHATRATH. 1989. Interaction of carbendazim with *Rhizobium* on groundnut seed as measured by symbiotic relationship and disease response to *Macrophomina phaseolina*. *Indian Phytopathology* **44**: 206-213.

READ, P.J., H.K. KOUCHEKI and J. HODGRON. 1976. Vesicular-arbuscular mycorrhizae in natural vegetation system. I. The occurrence of the infection. *New Phytology* **77**: 641-653.

RIFFLE, J.W. 1980. Growth and endomycorrhizal development of broad leaf seedlings in fumigated nursery soil. *Forest Science* **26**: 403-413.

ROSS, J.P. 1971. Effect of phosphate fertilization on yield of mycorrhizal and non-mycorrhizal soybeans. *Phytopathology* **61**: 1400-1403.

ROSS, J.P. and J.A. HARPER. 1970. Effect of *Endogone* mycorrhiza on soybean yields. *Phytopathology* **60**: *1552-1556*.

SINGH, B.P. and V.K. AGARWAL. 1990. Effect of fungicide and *Rhizobium* seed treatment on nodulation in soybean. *Indian Phytopathology* **43**: 467-469.

SUGAVANAM, V., K. UDAIYAN and S. MANIAN. 1994. Effect of fungicides on vesicular-arbuscular mycorrhizal infection and nodulation in groundnut (*Arachis hypogaea* L.). *Agriculture Ecosystem Environment* **48**: 285-293.

SUKARNO, N., S.E. SMITH and E.S. SCOTT. 1993. The effects of fungicides of vesicular-arbuscular mycorrhizal symbiosis. 1. The effect on vesicular-arbuscular mycorrhizal fungi and plant growth. *New Phytology* **25**: 139-147.

SUTTON, J.C. and B.R. SHEPPARD. 1796. Aggregation of sand-dune soil by endomycorrhizal fungi. *Canadian Journal of Botany* **54**: 326-333.

TRAPPE, J.M. and R.F. STRAND. 1969. Mycorrhizal deficiency in a Douglas-fir region nursery. *Forest Science* **15**: 381-389.

TRAPPE, J.M., R. MOLINA and M. CASTELLANO. 1984. Reaction of mycorrhizal formation to pesticides. *Annual Review of Phytopathology* **22**: 331-359.

UDAIYAN, K., S. MANIAN, T. MUTHUKUMAR and S. GREEP. 1995. Biostatic effects of fumigation and pesticide drenches on the endomycorrhizal-*Rhizobium*-legume tripartite association under field conditions. *Biol. Fertil. Soils* **20**: 275-283.

VYAS, S.C. 1988. *Nontarget Effects of Agricultural Fungicides*. Boca Raton: CRC Press.

(Received 29 July 1995)
(Accepted 28 November 1995)