

# Comparison of methyl bromide and herbicide effects on endomycorrhizal formation, seedling production, and weed control in sweetgum seedbeds

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## Abstract

Sweetgum seedlings were grown in fumigated and nonfumigated nursery plots where equal portions were treated with a preemergence application of 1.1 kg/ha of trifluralin and a postemergence application of 1.7 kg/ha of napropamide. Methyl bromide at 478 kg/ha significantly decreased average root dry weight. Endomycorrhizal formation on one, two, and three month-old seedlings was not significantly decreased by fumigation or herbicide application. Weeding times for herbicide plots were less than half that for fumigated plots. These results show that herbicides can provide better control of annual weeds and produce better sweetgum seedlings than fumigating with methyl bromide.

## 1 Introduction

Weed populations in hardwood seedbeds often are greater than in pine seedbeds because hardwoods have higher moisture, nutrient, and pH requirements that are more favorable for weed growth. In addition, effective herbicides are not registered for use in hardwood seedbeds. Many nurserymen fumigate hardwood seedbeds with methyl bromide to provide adequate weed control. This practice can be detrimental to endomycorrhizal fungi which are essential to the growth of hardwood species such as sweetgum (*Liquidambar styraciflua* L.) (KORMANIK et al. 1977).

It is suspected that herbicides are less injurious to mycorrhizal fungi than broad spectrum fumigants (MARX and BARNETT 1974); however, information concerning herbicidal effects on endomycorrhiza is scarce. ALTMAN and CAMPBELL (1977) cited an unpublished study on soybeans [*Glycine max* (L.) Merr.] in which it was reported that endomycorrhizal development was not significantly affected by 2 or 4 kg/ha of trifluralin or diazinon; however, alachlor at 4 kg/ha reduced root colonization by these fungi. The authors concluded that because there is a relative lack of knowledge concerning pesticide-mycorrhizal interactions, future research should include an evaluation of potentially beneficial or detrimental interactions whenever herbicides or other pesticides are applied directly to the soil.

Several controlled-environment studies involving effects of methyl bromide fumigation on endomycorrhizal fungi of sweetgum have been conducted in greenhouses and microplots (GRAY and GERDERMANN 1967; MOSSE et al. 1969; MOSSE 1973; BRYAN and RUEHLE 1976; KORMANIK et al. 1977), but very little research has been conducted under nursery field conditions.

Objectives of this study were to compare the effects of methyl bromide fumigation and herbicides on the formation of endomycorrhizae on nursery grown sweetgum and to determine the effects of these practices on seedling production and weed control.

## 2 Materials and methods

### 2.1 Nursery soil management

The experiment was conducted on a Tifton sandy loam at Hauss Nursery, Atmore, Alabama. During early April 1976, 3236 kg/ha of dolomitic limestone was broadcast and disked into the soil. In late April, 151 kh/ha of potash and 547 kg of 4-8-16 fertilizer were broadcast onto the soil. Soybeans were planted in May and disked under in early August. Following a broadcast application of 329 kg/ha  $\text{NH}_4\text{NO}_3$ , the area was seeded to sorghum-sudan (*Sorghum vulgare sudanense*) in late August. In October, the sorghum-sudan was disked under and the area was subsoiled. The soil was bedded for the winter in November.

### 2.2 Plot installation

Four test plots (15.3 m long  $\times$  1.2 m wide) were placed 2 m apart with two replications in each of two nursery beds. Soil chemical and mechanical analyses were made on samples collected from all 16 plots<sup>1</sup>. Two plots per replication were selected at random for fumigation. Each plot was covered with clear plastic and fumigated with 478 kg/ha of methyl bromide and 10 kg/ha of chloropicrin (Pestmaster Fumigant 1, Michigan Chemical Corporation). Soil temperature at 10 cm ranged from 18 to 23°C and soil moisture was adequate for effective fumigation. The plastic was removed after 3 days, and the soil was allowed to aerate for an additional 4 days before sowing sweetgum seed on April 26, 1977. After planting, the plots were mulched with nonfumigated pine straw.

The experimental design was a 2 $\times$ 2 factorial. Each replication contained two fumigated and two nonfumigated plots. Herbicide treatments were randomly assigned to one fumigated and one nonfumigated plot per replication. On April 29, 1977, 1.1 kg/ha of trifluralin were applied to the herbicide plots with a backpack  $\text{CO}_2$  powered sprayer and 1.3 cm of irrigation water were applied immediately. Following the initial root sampling on May 27, 1.7 kg/ha of napropamide were applied to the herbicide plots and immediately irrigated with 1.3 cm of water.

### 2.3 Fertilization and irrigation

All plots were fertilized uniformly with nitrogen (N) as  $\text{NH}_4\text{NO}_3$  and potassium (K) as KCl according to the following schedule: June 30, 33.6 kg of N/ha; July 8, 27.1 kg of N/ha; July 22, 27.1 kg of N/ha; August 8, 27.1 kg of N/ha; September 8, 32.6 kg of K/ha; September 21, 36.6 kg of K/ha. All fertilizers were broadcast, and irrigation was used to wash off fertilizer adhering to seedlings. Approximately 88 cm of irrigation water were applied to seedlings during the growing season to supplement the 60 cm of rainfall. All plots were handweeded when necessary and weeding times were recorded for each plot.

### 2.4 Sampling

Throughout the growing season (when seedlings were one, two and three months old), five seedlings were removed from each plot in order to microscopically examine roots for endomycorrhizae. Points of sampling were located in the transverse middle of the bed and approximately 2.5, 5.0, 7.5, 10.0, and 12.5 m from one end of the plot. Roots were fixed in FAA (13 ml formalin, 5 ml glacial acetic acid, 200 ml of 50 percent ethanol) and transported to the lab.

<sup>1</sup> Chemical analyses of soil samples were performed by the Auburn University Soil Testing Laboratory, Auburn, Alabama 36830.

Root segments were heated at 90°C for one hour in a solution of 10 percent KOH, washed with fresh KOH, and then bleached with H<sub>2</sub>O<sub>2</sub> for approximately 10 minutes. Root segments were then rinsed with H<sub>2</sub>O, acidified in dilute HCl, and stained with acid fuchsin by heating at 90°C for about 10 minutes. Small lateral roots were then mounted on slides with clear lactophenol (PHILLIPS and HAYMAN 1970).

Estimates of endomycorrhizal infection were assigned to each seedling by counting the number of 0.5 cm segments with any evidence of endomycorrhiza and dividing by the total number of segments (HAYMAN and MOSSE 1970).

On August 31, seedlings were lifted from each plot for destructive sampling. Two samples per plot were collected utilizing a frame having side members separated by 30.5 cm and spanning the width of the bed. All seedlings within the frame were lifted by hand. The points of sampling were located 5.0 and 10.0 m from one end of the plot. Heights and root collar diameters were measured, and root and top weights were obtained after drying at 70°C. All data were subjected to an analysis of variance.

### 3 Results and discussion

Soil in the test plots contained 60 kg/ha of available P, 258 kg/ha of exchangeable Ca, 360 kg/ha of exchangeable Mg, and 69 kg/ha of NO<sub>3</sub>-N. This sandy loam soil had a pH of 5.9 and contained 2.3 percent organic matter. These analyses indicate a fertile soil for production of hardwood seedlings.

#### 3.1 Mycorrhizal infection

During the growing season, a lack of seedlings was observed in the general area where the methyl bromide containers were placed (Fig. 1). Since sampling for mycorrhizae was done systematically, some seedlings were sampled from these areas and some were sampled from areas containing many healthy seedlings. This variation became evident in the microscopic evaluations for endomycorrhizal presence. For example, for the first sampling, one plot that had been fumigated contained two non-mycorrhizal seedlings and three mycorrhizal seedlings (0%, 0%, 66%, 74%, and 96%). Because of this variation, seedlings from fumigated plots were not statistically different from controls (Table 2).

By the second sampling date, most of the seedlings that had survived on fumigated plots were mycorrhizal. By the third month, there was little difference in mycorrhizal percentages among various treatments.

Detrimental effects of methyl bromide fumigation on sweetgum seedlings was not as great as had been expected. Over most of the



Fig. 1. The area lacking seedlings indicates the location of the methyl bromide container during fumigation

treated beds, fumigation obviously did not reduce the endomycorrhizal inoculum significantly. However, survival and growth of seedlings was poor in the immediate vicinity of where methyl bromide containers were placed during fumigation. This was probably a result of a higher concentration and deeper penetration of methyl bromide that may have resulted in a more effective elimination of endomycorrhizal fungi. More effective fumigation could possibly have been obtained by applying the methyl bromide by machine instead of by hand. Mechanical application injects the fumigant into the soil to a depth of approximately 15 cm while hand fumigation must rely on downward diffusion of the fumigant.

At two other nurseries, mechanical fumigation with 450 kg/ha of methyl bromide apparently eliminated the majority of endomycorrhizal fungi and resulted in stunted sweetgum seedlings (BARHAM 1978). Seedlings from fumigated controls remained non-mycorrhizal until approximately twelve weeks following sowing. Past cultural practices at these nurseries included frequent fumigation with methyl bromide. In addition, because of a lack of nursery area, one nursery was unable to grow a cover crop between the pine and sweetgum rotations<sup>2</sup>. These practices tend to produce a low level of endomycorrhizal inoculum.

Cultural practices at the Hauss nursery, however, were conducive to producing a large endomycorrhizal inoculum. Methyl bromide fumigation was rarely used at this nursery and had never been applied previously in the experimental area. In addition, the practice of growing soybeans and sorghum-sudan hybrids in alternate years and prior to growing hardwoods would tend to build up the spore population of endomycorrhizal fungi. Fumigating with methyl bromide by hand probably reduced the level of endomycorrhizal inoculum in the soil surface but apparently there was sufficient inoculum surviving for rapid development of mycorrhizae. Past nursery practices that influence endomycorrhizal inoculum levels as well as differences in method of methyl bromide application could possibly account for the difference in response to fumigation reported here and that reported by BARHAM.

One month old seedlings treated only with 1.1 kg/ha of trifluralin were on the average 68 percent mycorrhizal. Examination of these seedlings showed none to be lacking mycorrhiza. This supports the results cited by ALTMAN and CAMPBELL (1977) which reported that trifluralin did not affect mycorrhizal development. The percentage of mycorrhizal roots was not decreased after an additional treatment of 1.7 kg/ha of napropamide was applied. This study indicates that when applied to the soil surface, these herbicides do not significantly affect mycorrhizal development in sweetgum.

### 3.2 Seedling production

In late August, 1079 seedlings were lifted for evaluation. Density of seedlings ( $\bar{x} = 92$  seedlings/m<sup>2</sup>) was not significantly affected by fumigation or by herbicide treatment. Means for all growth parameters were greater for seedlings treated with herbicides alone than for any other treatment (Table 1). Although differences between fumigation and herbicides were not significant for total weight, stem weight, or root collar diameter, fumigation did significantly reduce the average dry weight of roots (Table 2). The average for nonfumigated plots was 5.3 g/root while that for fumigated plots was 4.4 g/root.

There was a significant interaction between fumigation and herbicides for height growth (Table 2). Seedlings from plots treated either with herbicides or fumigation were slightly taller than the controls. However, this increase in height did not occur when both fumigation and herbicides were used (Table 1). The reason for this interaction is not known.

<sup>2</sup> Personal communication with W. C. MILLS, Nursery Supervisor, Mississippi Forestry Commission, Winona, Mississippi.

Table 1

Average growth parameters and percent mycorrhizal infection for sweetgum seedlings treated with herbicides and a fumigant

Treatment	Rate kg/ha	Total dry weight (gm)	Root dry weight (gm)	Stem dry weight (gm)	Root collar diameter (cm)	Height (cm)	% mycorrhizal infection		
							1 month	2 months	3 months
control	0	8.0	4.8	3.2	0.55	39.5	52	58	88
methyl bromide	488	7.7	4.3	3.4	0.54	42.5	51	70	86
trifluralin (pre) + napropamide (post)	1.1 1.7	9.7	5.8	3.9	0.60	43.0	68	71	86
methyl bromide + trifluralin (pre) + napropamide (post)	488 1.1 1.7	8.0	4.5	3.5	0.57	38.7	41	38	81

Table 2

Summary of F-tests from analyses of variance performed on sweetgum seedlings treated with herbicides and a fumigant

Source of variation	Seedling variable tested					% mycorrhizal infection		
	Total weight	Root weight	Stem weight	Root collar diameter	Height	1 month	2 months	3 months
Replication	NS <sup>1</sup>	NS	NS	NS	** <sup>2</sup>	NS	* <sup>3</sup>	NS
Methyl bromide	NS	*	NS	NS	NS	NS	NS	NS
Herbicides	NS	NS	NS	NS	NS	NS	NS	NS
Interaction	NS	NS	NS	NS	**	NS	NS	NS

<sup>1</sup> NS – F not significant. <sup>2</sup> \*\* – F significant at 1 percent level. <sup>3</sup> \* – F significant at 5 percent level

Table 3

Handweeding times in sweetgum seedbeds at the Hauss Nursery in 1977

Treatment	Rate kg/ha	Handweeding times <sup>1</sup>					
		Days after planting					Total
		43	66	83	79	141	
control	0	10.1	9.6	35.0	20.2	7.6	82.5
methyl bromide	488	6.1	8.7	46.4	22.9	8.1	92.2
trifluralin (pre) + napropamide (post)	1.1 1.7	2.0*	2.8*	21.1	9.7	7.5	43.1
methyl bromide + trifluralin (pre) + napropamide (post)	488 1.1 1.7	2.4*	3.2*	17.1	8.7	7.0	38.4

<sup>1</sup> Handweeding time expressed as man-minutes per plot (18.6 m<sup>2</sup>). Weeding season was from planting April 26, 1977 to September 14, 1977 (141 days).  
An asterisk indicates a significant difference from the control at the 5 % level of probability as judged by Dunnett's t-test.

### 3.3 Weed control

Primary weed species occurring on the plots were large crabgrass [*Digitaria sanguinalis* (L.) Scop.], common chickweed [*Stellaria media* (L.) Cyrillo], and prostrate spurge (*Euphorbia supina* Raf.).

The first handweeding indicated that fumigation alone had provided approximately 40 percent weed control while herbicides alone had provided 80 percent control (Table 3). The primary reason for the lack of weed control was caused by reintroduction of crabgrass seed that were brought in with the nonfumigated pine straw mulch. Since methyl bromide has no residual activity, any weed seed blown or carried onto treated areas will grow as well or better than that on nonfumigated soil. An increase in weeding time resulting from using nonfumigated pine straw has been reported previously (SOUTH 1976). Total weeding times for plots treated with herbicides alone were less than half that required for plots receiving fumigation alone. The herbicide treatment was more effective because of the residual activity of the herbicides and because of the followup application of napropamide. Apparently the effectiveness of the herbicides was depleted by the fifth handweeding since weeding times for the herbicide plots were equal to controls.

#### Summary

The following statements are supported by results of this study:

1. Fumigation with methyl bromide can reduce the quality of nursery grown sweetgum by causing a reduction in root weight.
2. Trifluralin and napropamide treatments can provide better control of annual weeds than methyl bromide fumigation.
3. Trifluralin and napropamide can be used in sweetgum seedbeds without causing seedling injury or reducing endomycorrhizal infection.
4. Sweetgum seedlings can be grown at the Hauss Nursery without the use of methyl bromide.

This study has shown that the use of selected herbicides is less harmful to sweetgum seedlings than methyl bromide fumigation. In addition, herbicides can provide better control of annual weeds that are reintroduced after treating with methyl bromide. This has practical applications in nurseries where perennial weeds or hardwood diseases caused by soil borne pathogens are not a problem.

#### Résumé

*Comparaison des effets du méthyl-bromide et des herbicides sur la formation d'endomycorhizes, la production de plants et la lutte contre les mauvaises herbes dans les planches de semis de liquidambar*

Les résultats suivants ont été mis au jour par cette étude:

1. La fumigation au méthyl-bromide peut réduire la qualité des liquidambars poussant en pépinière en provoquant une réduction du poids des racines.
2. Les traitements à la trifluraline et à la napropamide peuvent assurer une meilleure élimination des herbacées annuelles que la fumigation au méthyl-bromide.
3. La trifluraline et la napropamide peuvent être utilisées dans les planches de semis de liquidambar sans causer de blessures aux jeunes plants ni réduire l'infection endomycorrhizienne.
4. Les jeunes plants de liquidambar peuvent être obtenus à la pépinière Hauss sans utiliser le méthyl-bromide.

Cette étude a montré que l'usage d'herbicides choisis est moins dommageable pour les jeunes plants de liquidambar que la fumigation au méthyl-bromide. En outre, les herbicides peuvent aboutir à une meilleure élimination des herbacées annuelles qui recontaminent le sol après traitement au méthyl-bromide. Ceci a des applications pratiques dans les pépinières par les agents pathogènes du sol ne constituent pas un problème.

### Zusammenfassung

Ein Vergleich zwischen Methylbromid und Herbiziden hinsichtlich ihrer Wirkung auf die Endomycorrhizabildung, die Sämlingsentwicklung und die Eindämmung von Unkräutern in Pflanzbeeten mit *Liquidambar styraciflua*

Folgende Feststellungen werden durch die Ergebnisse dieser Untersuchungen belegt:

1. Dämpfung mit Methylbromid kann die Qualität des in Baumschulen angezogenen Liquidambar durch Reduktion des Wurzelgewichtes beeinträchtigen.
2. Trifluralin- und Napropamid-Behandlungen können annuelle Unkräuter besser eindämmen als Dämpfung mit Methylbromid.
3. Trifluralin und Napropamid können in Liquidambar-Saatbeeten eingesetzt werden, ohne daß Schäden an Sämlingen entstehen oder daß endomycorrhizabildende Infektionen herabgesetzt würden.
4. Liquidambar-Sämlinge können in der Hauss-Baumschule ohne Anwendung von Methylbromid angezogen werden.

Diese Untersuchungen ergaben, daß der Einsatz ausgewählter Herbizide die Liquidambar-Sämlinge weniger schädigt als die Dämpfung mit Methylbromid. Außerdem können Herbizide jene Unkräuter besser bekämpfen, die nach Methylbromidbehandlung wieder einwandern. Das wiederum hat praktische Bedeutung für Baumschulen, in denen perennierende Unkräuter oder durch bodenbürtige Pathogene hervorgerufene Laubholzerkrankungen keinerlei Probleme darstellen.

### Literature

- ALTMAN, J.; CAMPBELL, L., 1977: Effect of herbicides on plant diseases. *Ann. Rev. Phytopathol.* **15**, 361–385.
- BARHAM, R. O., 1978: Endomycorrhizal inoculum improves growth of nurserygrown sweetgum seedlings. In: *Proceedings, 1978 Southeastern Area Nurserymen's Conf.* Hot Springs, Arkansas, July 24–27, 1978. p. 93–96.
- BRYAN, W. C.; RUEHLE, J. L., 1976: Growth stimulation of sweetgum seedlings induced by the endomycorrhizal fungus *Glomus mosseae*. *Tree Planter's Notes* **27**, 924.
- GRAY, L. E.; GERDERMANN, J. W., 1967: Influence of vesicular-arbuscular mycorrhizae on the uptake of phosphorous 32 by *Liriodendron tulipifera* and *Liquidambar styraciflua*. *Nature* **213**, 106–107.
- HAYMAN, D. S.; MOSSE, B., 1970: Plant growth responses to vesicular-arbuscular mycorrhiza. *New Phytol.* **70**, 19–27.
- KORMANIK, P. P.; BRYAN, W. C.; SCHULTZ, R. C., 1977: Influence of endomycorrhizae on growth of sweetgum seedlings from eight mother trees. *Forest Sci.* **23**, 501–505.
- MARX, D. H.; BARNETT, J. P., 1974: Mycorrhizae and containerized forest tree seedlings. In: *Proceedings of the North American Containerized Forest Tree Seedling Symposium*, Denver, Colorado, August 26–29, 1974, p. 85–91. Great Plains Agricultural Council Publication No. 68.
- MOSSE, B., 1973: Plant growth responses to vesicular-arbuscular mycorrhizae. IV. In soil given additional phosphate. *New Phytol.* **72**, 127–136.
- MOSSE, B.; HAYMAN, D. S.; IDE, G. J., 1969: Growth responses of plants in unsterilized soil to inoculation with vesicular-arbuscular mycorrhizae. *Nature, London* **224**, 1031–1032.
- PHILLIPS, J. M.; HAYMAN, D. S., 1970: Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.* **55**, 158–161.
- SOUTH, D. B., 1976: Weed prevention in forest nurseries. In: *Proc. 1976 Southeastern Area Nurserymen's Conf.*, Mobile, Alabama, Aug 17–19, 1976, p. 88–93.

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