NAPROPAMIDE AND PARAQUAT AFFECT DEVELOPMENT OF MYCORRHIZAE IN RED OAK (*QUERCUS RUBRA* L.) SEEDLINGS

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Abstract.--Paraquat and napropamide were applied to fumigated nursery beds as a preemergence treatment in May 1978. Prior to chemical treatment the beds were inoculated with the fungus, *Pisolithus tinctorius* (Pers.) Coker and Couch, at a rate of 200 ml/0.1m^2 and sown with stratified red oak seed. After 4 months, percentage root colonization was significantly greater in the inoculated than in the non-inoculated control plots. Percentage colonization in the inoculated plots was unaffected by napropamide even at the 6 kg rate but decreased with additions of paraquat and was lowest for the combined paraquat-napropamide treatment (2 and 6 kg/ha, respectively). Percentage root colonization was significantly correlated with stem height ($r = 0.574$) and root/shoot ratio ($r = 0.631$). Generally, the application of either herbicide or a rate increase resulted in a decrease in seedling height growth or stem dry weight when compared to inoculated control. Seedlings in non-inoculated plots responded similarly but appeared to be less sensitive to the chemical treatments. For each chemical treatment, the inoculated seedlings were larger. Root dry weight was unaffected by addition of herbicides but, on the average, seedlings in inoculated plots exhibited greater root dry weights.

INTRODUCTION

Certain mycorrhizal fungi improve the nutritional status and increase the growth rate of many plant species (Mosse, 1973; Marx, Bryan and Cordell, 1976). Colonization of hardwood seedling roots by specific fungal symbionts has been achieved in greenhouse (Pope and Chaney, 1979) and tree nursery (Bryan and Kormanik, 1977) tests and expanded use of mycorrhizal inoculum in seedling production appears imminent.

A summary of the effects of pesticides on mycorrhizal fungal development for agronomic crops (Menge, Johnson and Minassian, 1979) indicates that the majority of pesticides inhibit fungal infection and development. However, some pesticides, including captan, maneb and copper sulfate did not significantly affect growth or sporulation of mycorrhizal fungi when applied in certain concentrations. Sutton and Sheppard (1976) reported that

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vesicular-arbuscular mycorrhizae (VAM) infection was stimulated in roots of plants in soil drenched with 0.5 mM captan.

Herbicide use in tree seedling nurseries is increasing. At present, information regarding the effect of herbicides on mycorrhizal development per se is limited (Kelly and South, 1977; Pope and Holt, 1978). Understanding the effects of herbicides on the development and efficacy of mycorrhizal fungal associations is critical if these associations are to be used advantageously in seedling production. This study was conducted to determine the effects of the herbicides paraquat and napropamide on the development and efficacy of the mycorrhizal fungi, Pisolithus tinctorius grown in association with red oak seedlings.

MATERIALS AND METHODS

All nursery beds were fumigated with 312 kg/ha of 98% methyl bromide three weeks prior to the initiation of the study.[2/] Following fumigation, and again at the termination of the study, the surface 30 cm of soil was sampled for analyses of physical and chemical properties (Table 1) and inoculation potential of mycorrhizal fungi.

Table 1.--Physical and chemical properties of a sandy loam nursery soil sampled after fumigation and after one growing season.

	Physical properties		Chemical properties				
	%			kg/ha			
Sample date	Texture	O.M.	pH	Total N	P	K	Ca
May 1978	sℓ	7.25	5.9	93	53	320	1250
September 1978	sℓ	7.25	5.6	103	68	490	1180

Inoculation potential of P. tinctorius was determined by measuring the root infection of Virginia pine (Pinus virginiana L.) seedlings which were grown in a sample of nursery soil. Seeds were germinated in the sampled soil contained in ten, 15 cm pots and the resulting seedlings were evaluated for number of infected root tips after 4 weeks. Similarly, at the termination of the study soil samples, kept separate by herbicide and inoculation treatment, were evaluated for presence of P. tinctorius. Ten Virginia pine seedlings were evaluated for each replicate of each treatment combination.

Paraquat at 0, 1 and 2 kg/ha active ingredient or napropamide at 0, 3 and 6 kg/ha were applied in spray form as pre-emergence weed control treatments in May. Herbicide plots were 5 m long and 1.4 m wide. Within each herbicide plot, prior to seeding or chemical treatment, a subplot 1 m long and 1.4 m wide was inoculated with mycorrhizal forming fungi. Vegetative

[2/] Research for this study was conducted at the Vallonia State Tree Nursery, Vallonia, Indiana.

inoculum of Pisolithus tinctorius[3] was incorporated in the upper 10 cm of soil in the subplot at a rate of 200 ml/0.1 m^2. Control subplots were inoculated with sterilized inoculum at rates equal to the treated subplots. Following soil inoculation, stratified red oak seeds were planted and the herbicide treatments applied. Treatment combinations (5 herbicide treatments and 2 levels of inoculation) were arranged in a completely randomized design with 3 replications of each treatment combination. Treatment effects were examined by analysis of variance (ANOVA) and Tukey's W-procedure.

Developing seedlings received standard nursery treatment in terms of fertilization and supplemental watering. Uniform weed control and seedling density were maintained; study areas were hand weeded to achieve 100 percent weed control and seedling density was kept at approximately 90 seedlings per m^2 (8 seedlings/ft^2).

Seedlings were harvested in September and height, diameter and dry weight by plant component were determined. Seedlings were dug from the beds by hand with care taken to minimize root loss. Five seedlings were selected at random from each subplot and used for analysis. In total, 150 seedlings were sampled. A 10 percent sample (by fresh weight) of the lateral roots of red oak seedlings was collected and percentage of infected root tips determined.

RESULTS AND DISCUSSION

Percentage of lateral roots of red oak colonized by P. tinctorius was significantly greater in inoculated compared to the non-inoculated control treatment (Figure 1). Colonization of lateral roots in the inoculated treatment tended to decline with increasing rates of napropamide application but these reductions were not significantly different from the control, even at the 6 kg/ha rate. Paraquat applied at 1 and 2 kg/ha significantly reduced percentage mycorrhizal colonization of inoculated seedlings but no difference in colonization was evident between the 1 and 2 kg/ha rate of paraquat. For inoculated seedlings the combined herbicide treatment, napropamide (6 kg/ha) and paraquat (2 kg/ha) significantly reduced percentage infection when compared to either chemical used alone. The low levels of infection plus the similarity in infection between inoculated and non-inoculated subplots suggests that the combined treatment eliminated the incorporated inoculum and may have persisted in the soil to delay natural re-infection.

Colonization that occurred in the non-inoculated treatment represents the natural infection that may result following soil fumigation. Natural sources of inoculum include wind disseminated spores, vegetative ingrowth from soil below the effective fumigation zone and fungi within the fumigated zone which escaped death. The relatively constant level of infection for all herbicide treatments suggests that a major re-infestation of the soil within the rooting zone occurred after the application of herbicides.

Soil populations of Pisolithus tinctorius were assessed after fumigation

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[3] Commercial name "Myco Rhiz", product of Abbott Laboratories, Chicago, Illinois.

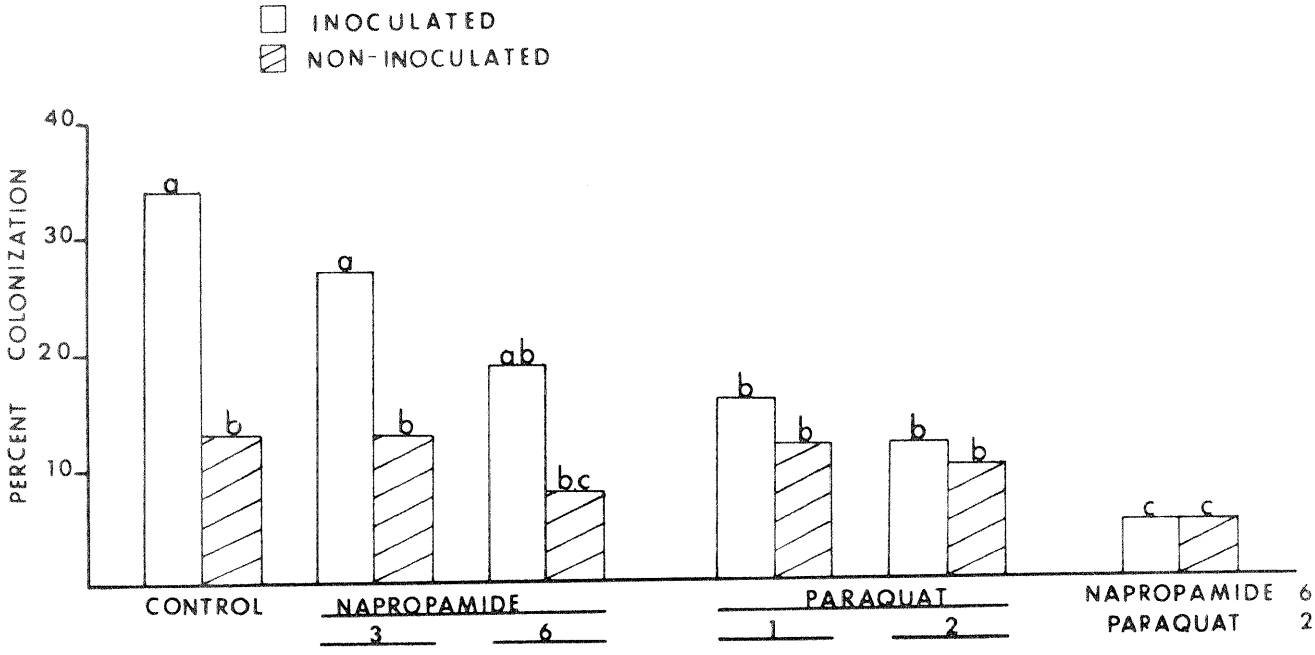

Figure 1.--Effect of napropamide and paraquat on root colonization (%) of red oak seedlings by Pisolithus tinctorius. (Rates of paraquat and napropamide are expressed in kg/ha of active chemical).

but immediately before mycorrhizal inoculation and at the termination of the study. No significant differences in inoculation potential were evident for the various herbicide treatments, consequently, these data were combined for each inoculation treatment (Table 2). The pre-treatment soil samples were taken approximately 3 weeks following the fumigation. Evaluation of 10 Virginia pine seedlings indicated that natural inoculum of P. tinctorius was present in the plant rooting zone. After 4 months there was little difference in the inoculation potential of soil inoculated with P. tinctorius and of the soil receiving no inoculum. No information is available on the duration of these levels of inoculation potential but it is obvious that substantial re-infection of fumigated, non-inoculated soil occurred after one growing season.

Generally, application of either herbicide or an increase in herbicide application rate in inoculated subplots resulted in a decrease in seedling height and stem dry weight when compared to the inoculated control (Figure 2A and B). Seedlings in non-inoculated subplots responded similarly but appeared to be less sensitive to chemical treatments.

Compared to the inoculated control, napropamide applied at 3 and 6 kg/ha reduced height growth of inoculated seedlings by 17 and 38%, respectively; similarly, paraquat applied at 1 and 2 kg/ha reduced seedling height

Table 2.--Mycorrhizal inoculation potential of a nursery soil.

Date sampled	Inoculation treatment	Virginia pine root analysis[a/]		
		Root tips infected	Total no. root tips	Percent infection
4/23/78	Pre-treatment	1.25	31	4
10/15/78	Inoculated	25	35	70
	Non-inoculated	15	27	57

[a/] Pre-germinated Virginia pine seedlings were transplanted into 5 cm plastic pots containing sampled nursery soil and grown for 4 weeks.

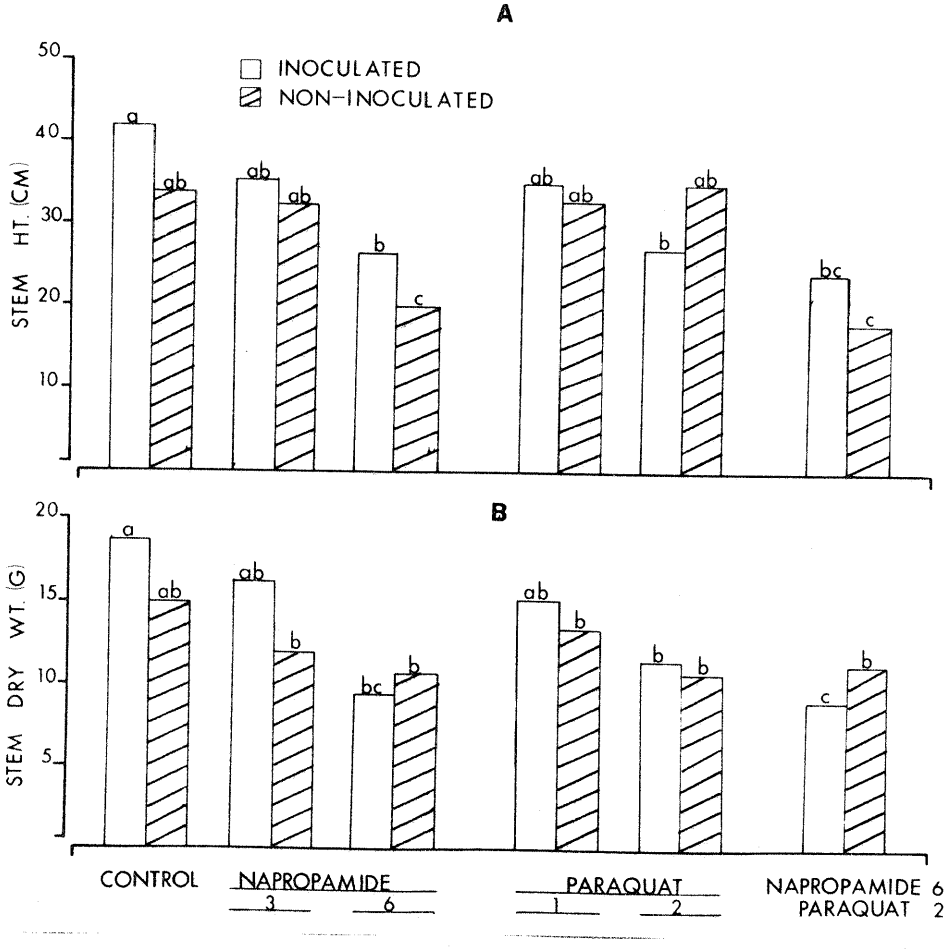

Figure 2.--Napropamide and paraquat influence height growth (A) and stem dry weight (B) of red oak seedlings inoculated or not inoculated with *Pisolithus tinctorius*. (Rates of paraquat and napropamide are expressed in kg/ha of active chemical).

growth by 20 and 36% (Figure 2A). By comparison, stem dry weight of inoculated control seedlings was reduced by 16 and 50% when napropamide was applied at 3 and 6 kg/ha, 21 and 39% when paraquat is applied at 1 and 2 kg/ha and 53% when the combination treatment of 6 kg/ha napropamide and 2 kg/ha of paraquat was applied. For any given chemical treatment, inoculated seedlings tended to be taller and have a greater stem dry weight than non-inoculated seedlings, however, significant differences in height growth occurred only for the 6 kg/ha napropamide treatment and significant differences in stem dry weight for 6 kg/ha napropamide in combination with 2 kg/ha of paraquat.

Napropamide and paraquat, have little effect on red oak seedling height growth or stem dry weight except at high rates of application but paraquat or the combination treatment of paraquat and napropamide do significantly reduce the colonization of red oak roots by P. tinctorius. Percentage root colonization was significantly correlated with stem height, in inoculated subplots (r = .574) and all subplots combined (r = .423), and with root/shoot ratio (r = .631) in inoculated subplots (Table 3). The correlations are not striking but, given the variability of conditions under which the seedlings were grown, they do represent an important biological relationship.

Table 3.--Correlation coefficients between percentage root colonization of red oak seedlings by Pisolithus tinctorius and growth variables.

Dependent variable	Independent variable	Correlation (r value)
Percent colonization a) Inoculated plots b) All plots	Stem height	a) 0.474* b) 0.323*
	Stem diameter	a) 0.115 b) 0.083
	Stem weight	a) 0.178 b) 0.163
	Root weight	a) 0.197 b) 0.236
	R/S ratio	a) 0.531* b) 0.205

*Correlations significant at α = .10 level.

It is logical to conclude that the herbicides used in this study reduce the growth rate of red oak, in part, by reducing the level of mycorrhizal colonization. Additional work is needed to determine the impact of various herbicides on fungal growth, infectivity, and the threshold levels of herbicide required to arrest fungal activity. Further laboratory, greenhouse and field work will be required to answer these and other questions concerning the interactions of herbicides and mycorrhizal fungi.

SUMMARY

1. Application of the herbicide paraquat at rates of 1 or 2 kg/ha or paraquat applied in combination with napropamide at 6 kg/ha significantly reduced the percentage of red oak seedling roots colonized by *P. tinctorius*.

2. For all treatments, inoculated seedlings had a greater percentage of colonized roots than non-inoculated seedlings but high inoculation potential of the non-inoculated soil suggests a significant re-infection of the fumigated soil by native fungi.

3. Stem dry weight and height of inoculated seedlings were significantly reduced by application of napropamide at 6 kg/ha, paraquat at 2 kg/ha or the combination.

4. Percentage root colonization was significantly correlated with stem height and root/shoot ratio.

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