Effects of Herbicides on in vitro Growth of Mycorrhizae of Pine (*Pinus* spp.)[1]

W. D. KELLEY and D. B. SOUTH[2]

Abstract. Effects of various herbicides on growth of ectotropic mycorrhizal fungi were determined over a 3-week period on modified Melin-Norcrans agar amended with the herbicides at rates of 0, 1, 3, 5, 10, 40, 80, 100, and 500 μg active ingredients/ml. Considerable variation in effect was observed among the herbicides for certain fungi and among the fungi for certain herbicides. All of the test fungi grew in the presence of each of the test herbicides at a rate of 80 μg/ml, and some growth was observed at the 500 μg/ml rate in a majority of the herbicide-fungus combinations. Significant decreases ($P = 0.05$) in growth were observed for specific herbicide-fungus combinations at herbicide rates of 1 and 3 μg/ml; however, 50% or greater decreases in fungal colony areas were observed in only 12 of 112 of the combinations at such low rates. The herbicides, in decreasing order of activity against the fungi, were: diphenylethers > dinitroanilines > *s*-triazines > substituted amides > perfluidone [1,1,1-trifluoro-*N*-[2-methyl-4-(phenylsulfonyl)phenyl] methanesulfonamide]. With few exceptions, the herbicide concentrations necessary to affect fungal growth significantly were considerably higher than would be expected to occur in soil treated with the test herbicides at recommended application rates.

Additional index words. Pisolithus tinctorius, Thelephora terrestris, Suillus luteus, S. cothurnatus, S. hirtellus, Laccaria laccata, Cenococcum graniforme.

INTRODUCTION

The importance of mycorrhizae to growth and survival of southern pines is well documented. Several researchers have reported detrimental effects of soil fumigants such as methyl bromide, metham (sodium methyldithiocarbamate), and dazomet (tetrahydro-3,5-dimethyl-2-*H*-1,3,5-thiadiazine-2-thione) on ectomycorrhizal fungi in forest nurseries (1, 2, 5, 6, 10, 14, 15); however, few reports are available concerning effects of herbicides on these organisms (3, 4, 9, 13). Herbicides are generally more selective than soil fumigants and are generally believed to be less harmful to mycorrhizal fungi using normal nursery practices (12).

For many years, weed control in forest nurseries was accomplished by hand weeding and the occasional use of organic solvents. Cost and availability of labor relative to the costs of commercial herbicides have prompted increased use of herbicides in such nurseries in recent years. Several herbicides are currently labeled for use in forest nurseries, and others are being tested.

The objective of this study was to determine the effect of selected herbicides on growth of mycorrhizal fungi in vitro and the concentrations inhibiting growth of the fungi by 50% or more.

MATERIALS AND METHODS

Test fungi. Mycorrhizal fungi were obtained from D. H. Marx. Director, USDA Forest Service Mycorrhizal Institute, Athens, GA 30601. Fungi tested were: *Pisolithus tinctorius*, (Pers.) Coker & Couch, *Thelephora terrestris* (Ehrh.) Fr., *Suillus luteus* (L. ex Fr.) S.F. Gray, *S. cothurnatus* Singer, *S. hirtellus* (Pk.) Kuntze, two isolates of *Laccaria laccata*, (Scop. ex Fr.) Berk & Br., and *Cenococcum graniforme* (Sow.) Ferd. & Winge. Cultures were mainted on Modified Melin-Norcrans (MMN) agar medium (11) at 26 C in darkness.

Test herbicides. Herbicides tested and their formulations were perfluidone, 50% active compound as a wettable powder (50 WP); the substituted amides, diphenamid (*N,N*-dimethyl-2,2-diphenylacetamide) (50 WP) and napropamide [2-(α-naphthoxy)-*N,N*-diethylpropionamide] (50 WP); the dinitroanilines, oryzalin (3,5-dinitro-N^4,N^4-dipropylsufanilamide) (75 WP) and trifluralin (α,α,α-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine), 476 g/L as an emulsifiable concentrate (EC); the triazines, atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] (76 WP), dipropetryn [2-(ethylthio)-4,6-bis(isopropylamino)-*s*-triazine] (80 WP), hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione], 90% active compound as a soluble powder (90 SP), prometryn [2,4-bis(isopropylamino)-6-(methylthio)-*s*-triazine] (80 WP); and the diphenylethers, bifenox [methyl-5-(2,4-dichlorophenoxy)-2-nitrobenzoate] (80 WP), nitrofen (2,4-dichlorophenyl-*p*-nitrophenyl ether), 238 g/L EC, and oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene], 238 g/L EC.

Laboratory test. Herbicides formulated as wettable and soluble powder were sterilized dry in a Cryotherm gas sterilizing oven (American Sterilizer Co., Model 62108) in an atmosphere of 12% ethylene oxide: 88% dichlorodifluoro methane (w/w) at 2.1092 kg/cm^2 and 100 C for 4 h. Preliminary tests revealed that such formulations of herbicides contained fungal spores that interfered in the test, and that this method of sterilization killed the spores without affecting the herbicides. Herbicides tested as emulsifiable concentrates were used without prior sterilization.

For each herbicide, a solution, suspension, or emulsion containing 20,000 μg ai/ml was prepared in sterilized water in a 500-ml volumetric flask. This was serially diluted in 100-ml volumetric flasks to provide final concentrations of 0, 1, 3, 5, 10, 40, 80, 100, and 500/μg ai/ml of medium when 10 ml of the appropriate stock was added to 390 ml of MMN agar medium. The herbicide was added to the melted MMN agar (50 C) and thoroughly mixed immediately prior to pouring into petri dishes. Each dish received ca 15 ml of the medium, and for each fungus isolate, five replicates (dishes) were prepared for each herbicide concentration and a non-herbicide control.

Each dish of agar was inoculated in the center with a 6-mm diam disk of the test fungus cut with a flame-sterilized cork borer from the periphery of an actively growing colony. Each disk was positioned mycelium-side down on the agar surface of the test dishes. The cultures were incubated in darkness at

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[2] Asst. Prof. and Res. Assoc., Dep. Bot., Plant Pathol, and Microbiol, and Dep. For., respectively, Agric. Exp. Stn. Auburn Univ., Auburn, AL 36830.

room temperature (26 ± 2 C). The diameter of each colony was measured and recorded after 1, 2, and 3 weeks of incubation.

All tests were repeated at least one time. All data were subjected to analysis of variance and means were compared for significant differences by Dunnetts' test.

RESULTS AND DISCUSSION

Growth of the fungi in medium containing 1 μg/ml of herbicide was not affected in 69% of the possible herbicide-fungus combinations (Table 1). Growth was significantly inhibited in 19% of the combinations. The majority of these occurred with the diphenylethers; oxyfluorfen being the most inhibitory compound. Significant increases in growth were observed in 12% of the combinations, but no single herbicide or group of herbicides was more active than another.

Growth of the fungi in medium containing 3 μg/ml of herbicide was not affected in 56% of the possible herbicide-fungus combinations (Table 2). Growth was inhibited in 35% of the combinations, and significant increases in growth were observed 9% of the combinations. The most active group against the mycorrhizal fungi was the diphenylethers with 58% of the possible combinations exhibiting inhibition. This was followed by the triazines and dinitroanilines each with 31% of the combinations exhibiting inhibition, perfluidone with 25%, and the substituted amides with 19%.

Table 1. Growth of mycorrhizal fungi after 3-weeks incubation on Modified Melin-Norcrans agar containing 1 μg ai herbicide/ml.

Herbicide	Fungus[a] 1	2	3	4	5	6	7	8
	(Growth response)[b]							
Diphenylethers								
Bifenox	0	0	–	–	0	–	0	+
Nitrofen	0	–	–	+	0	–	+	0
Oxyfluorfen	0	0	–	–	–	–	0	–
s-Triazines								
Atrazine	–	+	+	0	0	0	0	0
Dipropetryn	0	0	0	0	0	–	0	+
Hexazinone	0	–	0	–	0	0	–	0
Prometryn	0	0	0	0	0	0	0	–
Propazine	0	0	0	0	0	–	0	+
Simazine	0	–	0	+	0	0	+	0
Dinitroanilines								
Oryzalin	0	0	0	–	0	0	0	0
Trifluralin	0	0	0	0	0	0	+	0
Substituted amides								
Diphenamid	0	0	0	0	0	0	0	0
Napropamide	0	0	+	–	0	0	0	+
Miscellaneous								
Perfluidone	0	0	+	0	0	0	0	+

[a]1 = *Cenococcum graniforme*, 2 = *Laccaria laccata*, 3 = *Laccaria laccata*, 4 = *Pisolithus tinctorius*, 5 = *Suillus cothurnatus*, 6 = *Suillus luteus*, 7 = *Suillus hirtellus*, and 8 = *Thelephora terrestris*.

[b]Growth significantly (P = 0.05) stimulated (+), inhibited (–), or unaffected (0) as compared to control.

Table 2. Growth of mycorrhizal fungi after 3-weeks incubation on modified Melin-Norcrans agar containing 3 μg ai herbicide/ml.

Herbicide	Fungus[a] 1	2	3	4	5	6	7	8
	(Growth response)[b]							
Diphenylethers								
Bifenox	0	0	–	–	–	0	0	0
Nitrofen	0	–	–	–	0	–	0	–
Oxyfluorfen	0	0	–	–	–	–	–	–
s-Triazines								
Atrazine	–	+	+	0	0	0	0	+
Dipropetryn	0	–	0	–	0	0	–	0
Hexazinone	–	–	0	–	0	–	–	0
Prometryn	0	0	–	0	0	–	0	–
Propazine	0	0	–	0	0	–	0	+
Simazine	0	–	0	+	0	0	+	0
Dinitroanilines								
Oryzalin	–	0	–	–	–	0	0	0
Trifluralin	0	–	0	0	0	0	+	0
Substituted amides								
Diphenamid	0	0	0	0	0	0	–	–
Napropamide	0	0	+	–	0	0	0	0
Miscellaneous								
Perfluidone	0	0	+	–	–	0	0	+

[a]1 = *Cenococcum graniforme*, 2 = *Laccaria laccata*, 3 = *Laccaria laccata*, 4 = *Pisolithus tinctorius*, 5 = *Suillus cothurnatus*, 6 = *Suillus luteus*, 7 = *Suillus hirtellus*, and 8 = *Thelephora terrestris*.

[b]Growth significantly (P = 0.05) stimulated (+), inhibited (–), or unaffected (0) as compared to control.

Growth of the fungi in medium containing 500 μg/ml of herbicide was unaffected in only 19% of the possible herbicide-fungus combinations (Table 3). Significant decreases in growth were observed in 79% of the combinations, and significant increases in growth were observed in 3% of the combinations. Differences among the groups of herbicides in affecting growth of the fungi were not of the magnitude observed at 3 μg/ml rate.

Herbicide concentrations which restricted colony areas of fungi by 50% or more are presented in Table 4. In 27% of the herbicide-fungus combinations, colony area was not decreased by 50% even at the highest (500 μg/ml) rate. In 53% of the combinations, colony area was decreased by 50% or more only at herbicide concentrations of 40 μg/ml or higher. Colony area was decreased by 50% or more in 10% of the combinations involving herbicides at 5 to 10 μg/ml, and in 11% of the combinations with herbicide rates of 1 or 3 μg/ml. Activity of herbicide groups in decreasing order for inhibiting growth was: diphenylethers > dinitroanilines > *s*-triazines > substituted amides > perfluidone.

These results indicate that certain herbicides were active against the growth of ectomycorrhizal fungi. However, in most cases where significant decrease in growth of the fungi occurred, the herbicide rates were many times higher than would

Table 3. Growth of mycorrhizal fungi after 3-weeks incubation on modified Melin-Norcrans agar containing 500 μg ai herbicide/ml.

Herbicide	Fungus[a] 1	2	3	4	5	6	7	8
	(Growth response)[b]							
Diphenylethers								
Bifenox	0	0	–	–	0	–	0	0
Nitrofen	–	–	–	–	–	–	–	–
Oxyfluorfen	–	–	–	–	–	–	–	–
s-Triazines								
Atrazine	–	0	–	–	–	–	–	–
Dipropetryn	–	–	–	–	–	0	–	–
Hexazinone	–	–	–	–	0	–	–	–
Prometryn	–	–	–	–	–	–	–	–
Propazine	0	0	–	0	0	–	0	0
Simazine	0	–	–	+	0	–	+	0
Dinitroanilines								
Oryzalin	–	–	–	–	–	–	–	–
Trifluralin	–	–	–	–	–	0	–	–
Substituted amides								
Diphenamid	–	–	–	–	–	–	–	–
Napropamide	–	–	–	–	–	0	–	–
Miscellaneous								
Perfluidone	0	–	0	–	–	–	–	+

[a]1 = *Cenococcum graniforme*, 2 = *Laccaria laccata*, 3 = *Laccaria laccata*, 4 = *Pisolithus tinctorius*, 5 = *Suillus cothurnatus*, 6 = *Suillus luteus*, 7 = *Suillus hirtellus*, and 8 = *Thelephora terrestris*.

[b]Growth significantly (P = 0.05) stimulated (+), inhibited (–), or unaffected (0) as compared to control.

Table 4. Concentrations of herbicides in modified Melin-Norcrans agar necessary to decrease colony area of the test mycorrhizal fungi after 3-weeks incubation by 50% or more.

Herbicide	Fungus[a] 1	2	3	4	5	6	7	8
	(μg/ml)							
Diphenylethers								
Bifenox	(?)	(?)	(?)	1	5	500	(?)	(?)
Nitrofen	(?)	3	10	3	10	5	500	40
Oxyfluorfen	500	40	40	1	1	40	3	10
s-Triazines								
Atrazine	(?)	(?)	(?)	(?)	40	80	100	(?)
Dipropetryn	80	40	100	3	80	(?)	40	80
Hexazinone	500	500	500	1	(?)	5	1	80
Prometryn	500	500	80	40	80	10	80	40
Propazine	(?)	(?)	500	(?)	(?)	1	(?)	(?)
Simazine	(?)	(?)	(?)	(?)	(?)	(?)	(?)	(?)
Dinitroanilines								
Oryzalin	100	500	3	10	3	80	80	40
Trifluralin	100	40	80	10	80	80	80	40
Substituted amides								
Diphenamid	500	500	80	80	100	80	100	40
Napropamide	100	40	100	10	40	40	40	10
Miscellaneous								
Perfluidone	(?)	80	(?)	500	500	80	500	(?)

[a]1 = *Cenococcum graniforme*, 2 = *Laccaria laccata*, 3 = *Laccaria laccata*, 4 = *Pisolithus tinctorius*, 5 = *Suillus cothurnatus*, 6 = *Suillus luteus*, 7 = *Suillus hirtellus*, and 8 = *Thelephora terrestris*. (?) indicates 50% decrease was not attained at the highest rate (500 μg/ml).

be expected to occur in soil treated with these herbicides at recommended application rates. Thus, our results support those reported by other researchers (3, 4, 9, 13) concerning effects of herbicides on ectotrophic mycorrhizal fungi.

It is important to point out that all of the fungi grew in the presence of each of the herbicides at a rate of 80 μg/ml, and for a majority of the herbicide-fungus combinations, some growth was observed at the 500 μg/ml rate. This is in contrast with results obtained in similar studies involving fungicides and ectotrophic mycorrhizal fungi; fungicide concentrations of 1.0 μg/ml or less resulted in no growth by certain ectomycorrhizal fungi (7, 8).

The herbicide rates of 1 and 3 μg/ml approximate field rates. Because no herbicide was equally active at these rates against all of the ectomycorrhizal fungi, the development of mycorrhizal roots would not be affected significantly by field rates of these herbicides, assuming no enhancement of activity by field conditions.

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Ericameria austrotexana and Associated Range Forage Responses to Herbicides[1]

H. S. MAYEUX, JR., C. J. SCIFRES, and R. A. CRANE[2]

Abstract. Canopy cover of false broomweed[3] (*Ericameria austrotexana* M.C. Johnston) on the Rio Grande Plains of Texas was initially reduced by 96% by glyphosate [*N*-(phosphonomethyl)glycine] applied at 2.2 kg/ha in the spring. Acceptable control of this shrub with glyphosate lasted at least 4 yr. Glyphosate severely reduced basal cover of perennial grasses during the year of treatment, but grasses completely recovered within 2 yr after treatment. Standing forage crop increased by an average of 83%, compared to untreated areas, and livestock carrying capacity increased from 1 animal unit (AU)/13 ha to 1 AU/7 ha. Picloram (4-amino-3,5,6-trichloropicolinic acid), paraquat (1,1′-dimethyl-4,4′-bipyridinium ion), and paraquat plus atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] applied in the spring were less effective than glyphosate. Canopy reductions of false broomweed 6 months after applications of 2.2 kg/ha of picloram, paraquat, or paraquat plus atrazine were equivalent to that from 1.1 kg/ha of glyphosate, and control with these herbicides was usually restricted to the season of application. Dicamba (3,6-dichloro-*o*-anisic acid), 2,4-D [(2,4-dichlorophenoxy)acetic acid], atrazine, and 2,4-D plus atrazine were not effective when applied in the spring or fall. Time required for recovery of false broomweed canopy apparently varied with amounts of rainfall received after herbicide application.
Additional index words. Glyphosate, range weed control, forage response, perennial weeds, false broomweed.

INTRODUCTION

False broomweed is a native, evergreen subshrub belonging to the Asteraceae. Its heavily branched, irregularly rounded canopy often reaches a height of 10 to 12 dm at maturity. Stems, other than current year's growth, are woody and may be large as 2 cm in diam. The plants are relatively long-lived; as many as 10 annular rings may be present in stem sections from mature plants.

False broomweed foliage is dark green and somewhat aromatic, but not sticky to the touch. Leaves are less than 3 cm long, linear, and entire. False broomweed blooms throughout the summer and fall, producing floral heads only a few millimeters wide, with both disk and pale yellow ray flowers. False broomweed is similar in appearance to the closely related goldenweeds (*Isocoma* sp.) and perennial broomweeds (*Gutierrezia* sp.), especially broom snakeweed [*Gutierrezia sarothrae* (Pursh) Britt & Rusby]. Its morphological similarity to the perennial broomweeds has caused considerable confusion where the species occur as mixed stands.

False broomweed occurs in isolated, but relatively dense, stands throughout the Rio Grande Plains and southern Coastal Prairie of South Texas, which jointly account for about 12 million ha (7). Jones (9) noted that false broomweed occurs frequently on well-drained loams and clays on the Coastal Prairie, and Correll and Johnston (4) described the species as inhabiting open areas within the mixed brush (*Prosopis-Acacia*) of the Rio Grande Plains. Currently, false broomweed infestations are most severe where brush cover has been removed, whether by mechanical or chemical methods. Within two growing seasons after brush removal, false broomweed populations often develop to such a density as to negate the response of range forages to brush control efforts.[4] The dramatic increase in the abundance of this subshrub during the last 10 yr has caused concern among area cattlemen (11), especially since false broomweed infestations severely reduce forage production, and little information is available relative to control of the shrub. Moreover, there are indications that perennial range weed problems in south Texas, primarily infestations of false broomweed and goldenweeds, are progressively worsening (11, 12).

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[2] Range Sci., U.S. Dep. Agric., Sci. Ed. Admin., Agric. Res., Temple, TX 76501; Prof., Dep. Range Sci., Texas Agric. Exp. Stn., College Station, TX 77843; and Agric. Res. Tech., U.S. Dep. Agric., Sci. Ed. Admin., Agric. Res., Temple, TX 76501.
[3] *Ericameria austrotexana*, formerly *Isocoma palmeri* (Gray) Shinners, has not been formally assigned a common name. False broomweed was adopted by the authors because the plant is often confused with and referred to as broomweed (*Gutierrezia* sp.).
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