INOCULATION OF NORTHERN RED OAK SEEDLINGS WITH THE FUNGAL SYMBIONT SUILLUS LUTEUS IN A MICHIGAN NURSERY[1]

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Abstract.--The ectomycorrhizal fungus Suillus luteus (L. ex Fr.) Peck, a symbiont adapted to prevailing environmental conditions in the northern U.S., is a promising candidate for routine nursery inoculation of seedlings. The purpose of this study was to introduce S. luteus into a Michigan nursery and evaluate the effect of inoculation on northern red oak (Q. rubra L.) seedling growth and ectomycorrhizal development. Suillus luteus inoculum was incorporated into fumigated nursery soil using two methods: banding or mixing. Placement of the inoculum influenced the number of seedlings colonized with S. luteus. Moreover, the spread of S. luteus mycelia was limited following banding of inoculum. Subsequent examination of seedling root systems revealed significantly different patterns of ectomycorrhizal colonization in the first year. After two years in the nursery seedling growth was significantly improved by inoculation with S. luteus.

INTRODUCTION

The survival and early growth of container- and nursery-grown oak (Quercus) seedlings following outplanting on reforestation sties in the northern U.S. is generally unacceptable (Anderson, 1982). Early growth is characterized by periodic shoot dieback due to inadequate root-shoot ratios of seedlings (Johnson, 1984; Arend and Scholz, 1969). Frequently, nursery-grown oak planting stock lack mycorrhizal associations common to naturally occurring seedlings (Dixon et al, 1981a; Imshemetshii, 1967). The root-shoot balance and subsequent field growth of seedlings after outplanting can be improved following inoculation of the root system with compatible ectomycorrhizal fungi (Beckjord and McIntosh, 1984; Dixon et al, 1981b; Dixon et al, 1980).

Vegetative inocula of ectomycorrhizal fungi have been successfully introduced into conifer nurseries worldwide (Marx et al, 1984). However, previous reports reveal fungal symbionts and tree hosts often have specific ecological requirements (Perry et al, 1987). Thus, cultural practices in nurseries must be adjusted or developed to promote ectomycorrhizal symbiosis. The identification and evaluation of fungal symbionts compatable with red or white oaks have not been fully considered.

Members of the genus Quercus form ectomycorrhizal relationships with a number of fungal symbionts (Dixon et al, 1984). Ectomycorrhizal fungi in the genus Suillus are compatible with several oak species and stimulate early increases in seedling growth (Palm and Stewart, 1984; Dixon et al, 1984). Some species in the genus Suillus are not able to withstand the mechanical manipulation required during inoculum preparation (Dixon et al, 1984). However, ease of isolation, rapid growth in culture and the host specific tendency of some Suillus species may increase the utility of these fungi in nursery inoculation programs (Trappe, 1977).

The objectives of this study were to: 1) test feasibility of introducing Suillus luteus (L. ex Fr.) Peck into fumigated soil of a northern Michigan nursery, and 2) evaluate the effect of inoculum placement on the spread of fungal mycelia, ectomycorrhizal development and growth of northern red oak (Q. rubra L.) seedlings.

MATERIALS AND METHODS

A single provenance of acorns was collected from the Huron-Manistee National Forest in west central Michigan. Acorns were sorted by flotation, graded to a standard weight and stratified (Dixon et al, 1984). Before planting acorns were surface sterilized with 10% sodium hypochlorite for 10 minutes. Suillus luteus was grown in a vermiculite-peat moss-nutrient mixture

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for three months to obtain vegetative inoculum using modified procedures described by Dixon et al (1984) and Marx and Bryan (1975).

The seedlings were grown at the USDA Forest Service J.W. Toumey Nursery, Watersmeet, Michigan. Nursery beds were tilled and fumigated under polyethylene with 400 kg/ha of methyl bromide (MC-33) before acorn sowing. Soil chemical properties of nursery soils were similar in all beds, pH 5.5 and 272 and 540 ppm of available P_2O_5 and K_2O, respectively. Dolomitic limestone was added to the beds to raise the exchangeable calcium and magnesium levels to approximately 1.0 milliequivalent per 100 g soil. The soil texture was a sandy loam, and organic matter was approximately 2% in all plots (Marx et al, 1984).

The study was implemented in a split-plot experimental design with 4 replications, 2 whole plots and 4 subplots. One whole plot was infested with vegetative inoculum of S. luteus, and the remaining whole plot was maintained as an uninoculated control. In the inoculated sub-plots, the vegetative inoculum was incorporated into the nursery soil using two methods: mixing or banding. Banded inoculum was manually placed approximately 10 centimeters under the acorns rows within the nursery bed. The mixed inoculum was broadcast over the nursery bed and tilled to a depth of approximately 20 centimeters. Inoculum density in both subplots was approximately 3 liters per m^2 of soil surface. The control plots received equal amounts of autoclaved inoculum using the same methods. Acorns were planted to obtain a density of 100 seedlings per m^2 in all plots.

The nursery beds were fertilized with three annual applications of ammonium sulfate ($(NH_4)_2SO_4$) (21-0-0 plus 24% sulphur). The granular fertilizer was applied at a rate of 9.2 kg/ha as a top dressing. The beds were irrigated with tap water as needed. Seedlings in all treatments were horizontally root pruned to a depth of approximately 23 cm in early August of each growing season.

A subsample of 25 randomly selected seedlings from each subplot replication was harvested in late June and September of the first growing season. Seedling root morphology, ectomycorrhizal development and the presence of fungal fruiting bodies in nursery beds were evaluated. In September of year two all nursery plots were undercut with a root pruning bar at a depth of 36 cm and seedlings lifted by hand. Fifty seedlings from each nursery sub-plot replication were randomly chosen for measurement of dry weight of roots and shoots (80°C, 48h), number of primary laterals, and number of S. luteus ectomycorrhizal laterals. Criteria for identifying ectomycorrhizal colonization are described by Dixon et al (1984). Reisolation of test fungi was attempted after seedling harvest using techniques described by Dixon et al (1984). Data was subjected to analysis of variance to determine effects of inoculum placement and inoculation on seedling growth and ectomycorrhizal development.

RESULTS

Suillus luteus was successfully introduced into plots of northern red oak seedlings in the J.W. Toumey nursery (Table 1). Evaluation of seedlings on June 30 in the first growing season revealed the number of colonized plants and the percentage of ectomycorrhizal lateral roots colonized was greater in plots receiving mixed inoculum. Fruiting bodies of ectomycorrhizal

Table 1. Ectomycorrhizal development of northern red oak seedlings inoculated by two methods with Suillus luteus during the first growing season in J. W. Toumey nursery, Watersmeet, Michigan.

Sampling date and seedling variable	inoculation treatment		
	mixed inoculum	banded inoculum	noninoculated
June 30			
Seedlings colonized (%)	47a[1]	20b	13b
Suillus laterals per seedling (%)	36a	19b	0c
Total ectomycorrhizal laterals per seedling (%)	21a	24a	10b
September 30			
Seedlings colonized (%)	93a	80a	73a
Suillus laterals per seedling (%)	89a	92a	66a
Total ectomyccorrhizal laterals per seedling (%)	74a	68a	39b

[1]Means within a row sharing a common letter are not significantly different ($P \leq 0.05$).

fungi were not evident in late June.

Evaluation of northern red oak seedlings at the end of the first growing season (September 30) revealed significant ectomycorrhizal development by S. luteus in the inoculated plots. The percentage of seedlings successfully inoculated and the percentage of ectomycorrhizal lateral roots was greater than eighty percent. Seedlings in the control plots were partially colonized by indigenous fungi at the end of the first growing season. Fruiting bodies of Laccaria, Inocybe, Hebeloma, and Thelephora species were identified in the control plots.

At the end of two growing seasons in the Toumey nursery seedlings inoculated with S. luteus exhibited abundant ectomycorrhizal colonization (Table 2). Suillus luteus was reisolated from seedlings in each inoculated subplot. Seedling dry weight was significantly greater in plots inoculated with S. luteus. Within the control plots, ectomycorrhizal development by C. geophilum, L. laccata and T. terrestris was evident, but seedlings were 25% smaller than inoculated plants.

early development of ectomycorrhizae on northern red oak seedlings. Following soil fumigation, thorough mixing of inoculum into the seedling root zone will result in significant ectomycorrhizal colonization of primary lateral roots. Banding of the inoculum in the nursery bed is a useful operational technique for seedling inoculation. However, ectomycorrhizal colonization of seedlings of year one was less in treatments receiving banded inoculum. Inoculum mixing apparently increases the opportunity for lateral root contact with the vegetative mycelium.

Soil fumigation reduced ectomycorrhizal colonization of northern red oak seedlings in the control plots. Although indigenous ectomycorrhizal fungi eventually invaded the control plots significant quantities of ectomycorrhizae were not observed on seedlings until the end of the first growing season. Reduced ectomycorrhizal colonization resulted in significantly smaller seedling dry weights. Previous reports reveal a delay or lack of ectomycorrhizal colonization of oak will lead to nutritionally deficient and stunted seedlings (Mitchell et al, 1984; Dixon et al, 1981a).

Table 2. Total seedling dry weight and ectomycorrhizal development of 2-0 northern red oak seedlings inoculated by two methods with Suillus luteus after lifting (October) from J.W. Toumey nursery, Watersmeet, Michigan.

Seedling variable	Inoculation treatment		
	mixed inoculum	banded inoculum	noninoculated
Total seedling dry weight (g)	16.9a[1]	16.0a	11.7b
Seedlings colonized (%)	82a	54b	37c
Suillus laterals per seedling (%)	78a	77a	5b
Total ectomycorrhizal laterals per seedling (%)	85a	89a	46b

[1]Means sharing a common letter are not significantly different ($P \leq 0.05$).

DISCUSSION

Ectomycorrhizal inoculation of northern red oak seedlings with Suillus luteus in a northern Michigan nursery is feasible. The 110 day growing season, cool soil temperatures and cultural practices employed in the Toumey nursery did not impede ectomycorrhizal colonization of inoculated oak seedlings (Marx et al, 1984). Previously, inoculation of conifers with vegetative inoculum of Pisolithus tinctorius (Pers.) Coker and Couch in the Toumey nursery was relatively unsuccessful (Marx et al, 1984). These observations suggest the need for further testing of fungal isolates which survive prevailing environmental conditions in the northern U.S.

This study reveals vegetative inoculum of S. luteus can be a suitable form of inoculum for

Early seedling development of ectomycorrhizae by S. luteus resulted in significant increases in seedling total dry weight. Dixon et al (1984) observed a similar growth increase of container-grown white and black oak seedlings following inoculation with this isolate of S. luteus. In contrast, Dixon et al (1984) and Marx (1979) reported that isolates of S. cothurnatus Singer, S. pinorigidus Snell and Dick, S. hirtellus (Pk.) Kuntze and S. granulatus (L.:Fr.) Kuntz failed to form significant ectomycorrhizae with various oak hosts under a range of environmental conditions. These observations suggest oak seedling response to inoculation may be dependent on the Suillus isolate tested, inoculation techniques employed and the host x Suillus genotype combination (Palm and Stewart, 1984).

Growth and survival of oak seedlings following outplanting is the ultimate test of the benefits of ectomycorrhizal inoculation in the nursery (Parker et al, 1986). However, it is obvious from this study that early ectomycorrhizal development was associated with improvements in seedling size in the Toumey nursery. Inferences from previous reports suggest the large ectomycorrhizal oak seedlings with adequate root-shoot ratios will survive and compete with indigenous vegetation on routine and adverse reforestation sites (Dixon et al, 1981b). Oak seedlings with abundant S. luteus ectomycorrhizae from the Toumey nursery have been established in outplanting trials to ascertain benefits in the field.

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