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Growth and Ectomycorrhizal Development of Northern Red Oak Seedlings Treated with IBA

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Abstract. **Ectomycorrhizal and nonmycorrhizal, glasshouse-grown northern red oak seedlings *(Quercus rubra* L.) received root treatments of IBA in starch, fired-montmorillonite clay, or starch-encapsulated montmorillonite clay. Clay proved to be superior to starch as a carrier for IBA, inducing significant increases in diameter, root length, leaf area, and shoot dry weight. Positive growth interactions between mycorrhizae and IBA were found with the clay carrier. The typical bare-rooted red oak seedling (grown for 1 year in nurseries and outplanted) performs poorly because of insufficient root size. Container-grown seedlings produced using clay/IBA treatments may perform better under field conditions than stock grown conventionally. Chemical name used: indole-3-butyric acid (IBA).**

Inadequate natural oak *(Quercus* spp.) regeneration and slow initial growth of planted seedlings has led to increased interest in faster-growing planting stock (Dixon et al., 1983; Garrett et al., 1979; Johnson, 1979; Loftis, 1979; Parker et al., 1986). Container-produced seedlings grown with mycorrhizae or in a growth medium containing auxin have more lateral roots, greater comparative absorptive surface area, improved water and nutrient relations (Baser et al., 1987; Dixon et al., 1983; Owston, 1972), and higher rates of growth and survival (Dixon et al., 1983).

Baser et al. (1987) developed a method of auxin application that exposed root systems of containerized seedlings to auxin during early growth. In their study, black oak *(Quercus velutina* Lam.) seedlings were grown in a medium containing IBA incorporated in polyacrylic starch. Although early seedling growth was inhibited, IBA-treated seedlings were 39% taller than controls and had more lateral roots after 16 weeks. However, root weight and mycorrhizal colonization decreased when IBA was added. The intensity of colonization must be maximized while maintaining good root surface area if we are to maximize the "success" of the seedlings following outplanting.

This study was designed to investigate the use of selected IBA carriers on growth of red oak seedlings and ectomycorrhizal development. The effectiveness of fired-montmorillonite clay and starch-encapsulated fired clay as IBA carriers was compared to the starch IBA carrier described by Baser et al. (1987).

Materials and Methods

IBA-carrier preparation

Oven-dried starch-IBA (S-IBA). IBA (Sigma Chemical Co., St. Louis) was incorporated into polyacrylic starch (SGP 104K; Henkel Corp., Minneapolis, Minn.) as described by Baser et al. (1987), but at a concentration of 800 rather than 1000 ppm. Earlier studies (Baser et al., 1987) demonstrated 1000 ppm of IBA in starch to be superior to starch alone in stimulating seedling growth. Fifteen grams of IBA, dissolved in 60 ml ethanol and deionized water, was added to make a total volume of 18.75 liters and a final concentration of 0.055 M ethanol and 3.94 mM IBA. Polyacrylic starch at 220 g was then added to the IBA solution, forming a gel. The gel was oven-dried in shallow trays at 30 to 35C for 50 days. The resulting slab was ground in a Wiley mill to pass through a 1.3-mm screen.

Oven-dried clay-IBA (C-IBA). Indole-3-butyric acid was incorporated into fired-montmorillonite clay in a manner similar to that described above. Fifteen grams of IBA was dissolved in 60 ml ethanol and deionized water was added to a volume of 500 ml for a final concentration of 2.06 M ethanol and 148 mM IBA. Less water was used than with the starch because clay absorbs less water than polyacrylic starch. Clay (500 g) was added to the IBA solution. The clay was allowed to absorb the solution and afterwards was dried at 30 to 35C for 50 days and ground in a Wiley mill to pass through a 1.3-mm screen, as described previously.

Oven-dried, starch-encapsulated, clay-IBA (SEC-IBA). Fifteen grams of IBA was incorporated into 500 g clay and ground as described above for C-IBA. The granules were rolled, until coated, in a polyacrylic starch solution consisting of 5 g starch in 500 ml water. Granules were subsequently dried for 2 days at 30 to 35C and crumbled by hand to a granular consistency.

Seedling culture

Northern red oak half-sib acorns were stratified for 90 days, surface-sterilized (10% sodium hypochlorite), and planted two per cavity in Spencer-LeMaire Super 45 (750 cm^3) root trainers (Spencer-LeMaire, Edmonton, Alberta, Canada). Seedlings were thinned to one following germination and grown in a greenhouse for 18 weeks. The growth medium consisted of a 1 soil: 1 peat: 1 vermiculite: 1 sand mixture (by volume) (pH 5.5) that had been sterilized with methyl-bromide (Dowfume MC-2; Dow Chemical Co., Midland, Mich.). The medium in one-fourth of the cavities contained 2.0 g S-IBA (0.15 g IBA), one-fourth contained 5.10 g C-IBA (0.15 g IBA), one fourth contained 6.00 g SEC-IBA (0.15 g IBA); the medium in the remaining cavities contained no IBA. *Pisolithus tinctorius* (Pers.) Coker and Couch (isolate 270; Don Marx, U.S. Dept. of Agriculture Forest Service, Athens, Ga.) inoculum was prepared as described by Marx and Bryan (1975), and 30 ml was added to the medium in half of the containers of each IBA carrier treatment and the control. Ectomycorrhizal inoculum and IBA earners were uniformly mixed with the growth medium within the appropriate root trainer.

There were eight treatments consisting of inoculated and noninoculated seedlings for each IBA-carrier application and controls (see Table 2). There were four replications of 10 seedlings per treatment replicate for a total of 320 seedlings. The 10 seedlings within each treatment were averaged and treated as a replication. Seedlings were watered every other day, and beginning the fourth week, all seedlings were fertilized twice weekly with 60 ml modified Hoagland's solution (Johnson et al., 1957).

Growth measurements

Emergence, seedling diameter (measured just above the root collar to ±0.1 mm), and height (measured ± 1 mm) were recorded biweekly. Leaf area (model 3000; LI-COR, Lincoln, Neb.), percentage of root tips with mycorrhizae, root length, and root and shoot dry weights were measured and root: shoot ratios calculated. Percentage of ectomycorrhizal colonization of individual seedlings and primary lateral roots was assessed following staining as described by Daughtridge et al. (1986a) on half of each root system. The root system was divided down the center of the taproot, or, if lacking a major taproot, the laterals were separated and divided equally. On the remaining half of the root system, root length was determined photoelectronically by the line intersection method described by Rowse and Phillips (1974). Measured root length was doubled to estimate total root length. Root : shoot dry weight (including both root halves) was obtained by drying roots and shoots for 48 hat 80C and weighing.

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Data analysis

All data were subjected to analysis of variance (ANOVA) using the ANOVA procedure of SAS (SAS Institute, 1985). The percent data were arcsin-transformed before analysis. The least significant difference (LSD) test *($P \leq 0.05$)* was used to separate mean treatment effects for all ANOVA where the $P > F$ was ≤ 0.15. Linear correlations were computed by the CORR procedure of SAS.

Results

Emergence (>93% in all treatments) did not differ significantly *($P \leq 0.05$)* among IBA treatments when the data were pooled between mycorrhizal and nonmycorrhizal treatments. After 18 weeks, seedlings treated with C-IBA and SEC-IBA had significantly greater stem diameters than seedlings not treated with IBA (Table 1). IBA-treated seedlings were significantly taller than control seedlings regardless of the IBA carrier (Table 1). C-IBA-treated seedlings had. significantly longer roots and larger leaves than control seedlings or seedlings treated with S-IBA (Table 1). Similarly, shoot dry weight was significantly higher for seedlings treated with C-IBA than S-IBA or control seedlings, but root dry weights did not differ significantly among treatments (Table 1). Seedlings grown with C-IBA had the lowest root: shoot ratio.

Seedlings inoculated with *P. tinctorius* had significantly greater mycorrhizal colonization than noninoculated seedlings (Table 2). Similarly, there was a higher percentage of colonized primary lateral roots on inoculated than on noninoculated seedlings. Few significant within-treatment growth increases resulted from inoculation. However, the biggest difference in seedling growth due to inoculation was due to the C-IBA treatment; stem diameter was significantly increased, and root length and leaf area were increased 16% and 7%, respectively.

Table 2. Percentage of mycorrhizal seedlings [MYC (%)], percentage of mycorrhizal lateral roots per seedling [MYC LATS (%)], and growth characteristics for inoculated and noninoculated 18-week-old northern red oak seedlings without IBA or treated with 800 ppm IBA in a clay (C-IBA), starch (S-IBA), *or* starch-encapsulated clay (SEC-IBA).

Treatment	MYC[z] (%)	MYC LATS[z] (%)	Stem diam (mm)	Ht (cm)	Root length (cm)	Leaf area (cm^2)	Shoot dry wt (g)	Root dry wt (g)	Root : shoot ratio
Inoculated									
No IBA	77 a	51 a	2.94 cd[y]	16.9 c	966 bc	196 d	0.64 bc	2.2 ab	3.5 ab
C-IBA	94 a	67 a	3.35 a	20.9 a	1395 a	315 a	0.80 b	2.7 ab	3.4 b
S-IBA	88 a	56 a	3.20 ab	22.8 a	721 c	213 cd	0.54 c	1.9 b	3.5 ab
SEC-IBA	90 a	67 a	3.21 ab	20.7 ab	1080 ab	265 a–c	0.80 b	2.3 ab	2.9 b
Noninoculated									
No IBA	6 b	9 b	2.81 d	16.9 bc	1027 b	223 b–d	0.66 bc	2.8 a	4.2 a
C-IBA	7 b	14 b	3.07 bc	21.8 a	1203 ab	294 ab	0.99 ab	2.6 ab	2.6 b
S-IBA	0 b	0 b	2.79 d	19.4 a–c	898 bc	220 cd	0.74 bc	2.3 ab	3.1 b
SEC-IBA	6 b	12 b	3.11 a–c	19.2 a–c	1113 ab	247 a–c	0.69 bc	2.4 ab	3.5 ab

[z] Data arcsin-transformed before analysis.

[y] Mean separation within columns by LSD, $P \leq 0.05$.

Discussion

IBA carrier responses

The presence of IBA in containers had no adverse effect on emergence, early growth, or ectomycorrhizal development of northern red oak. This result contrasts with those of Baser et al. (1987) where IBA treatments inhibited ectomycorrhizal development and slowed emergence. The difference may be attributed to a lower IBA concentration (800 vs. 1000 ppm), the use of a different *Pisolithus* isolate, and to certain advantages of a clay rather than a starch carrier. Regardless of the carrier used, seedling height was increased by adding IBA to the growth medium. Similar findings were reported by Baser et al. (1987) for black oak. The C-IBA treatment yielded seedlings with significantly larger stem diameters, root lengths, leaf areas, and shoot dry weights than seedlings treated with the starch carrier or no IBA. Significant increases in both leaf area and root length would suggest the potential for continued accelerated growth beyond the 18 weeks of this study. Moreover, the larger the size of a northern red oak seedling, the greater the possibility of success (i.e., surviving and attaining acceptable height growth) following outplanting (Johnson, 1984). The enhanced growth response observed with the clay carrier may result from a more rapid early rate of IBA release from the clay than from the starch, which would be more favorable for root initiation. Alternatively, the more stable clay granules may lengthen the duration of release of IBA, prolonging the period promoting root initiation. Observations made at the time of harvest indicated that most of the clay granules were still intact. Because bioassays for IBA activity over time were not conducted in this study, additional research is required to identify the mechanism involved.

Table 1. Growth characteristics of 18-week-old northern red oak seedlings without IBA or treated with 800 ppm IBA in a clay (C-IBA), starch (S-IBA) or starch-encapsulated clay (SEC-IBA).[z]

	Characteristics						
Carrier	Stem diam (mm)	Ht (cm)	Root length (cm)	Leaf area (cm^2)	Shoot[y] dry wt (g)	Root dry wt (g)	Root : shoot ratio
No IBA	2.9 c[x]	17 b	996 bc	209 b	0.7 b	2.5 a	3.6 a
C-IBA	3.2 a	22 a	1247 a	300 a	0.9 a	2.6 a	2.8 b
S-IBA	3.0 bc	21 a	812 c	217 b	0.6 b	2.1 a	3.5 a
SEC-IBA	3.2 ab	20 a	1097 ab	256 ab	0.7 ab	2.3 a	3.3 a

[z] Data pooled between mycorrhizal and nonmycorrhizal treatments.

[y] Shoot dry weight does not include foliage weight.

[x] Mean separation within columns by LSD, $P \leq 0.05$.

The finding of a greater root : shoot dry weight ratio in nontreated seedlings as compared to those treated with IBA in Baser et al. (1987) was not observed in this work. Under the conditions of this study, roots of IBA-treated seedlings were equally long or significantly longer than those of non-IBA-treated seedlings, but no significant differences were found in root weights. This difference suggests an improved root architecture for water and nutrient uptake resulting from adding 800 ppm IBA to the containers, and in particular when fired clay was used as the carrier.

IBA-carrier-mycorrhizal responses

Interactions between mycorrhizae and IBA were absent, except within the C-IBA and S-IBA treatments. Ectomycorrhizal inoculation of seedlings within the C-IBA treatment resulted in a significant increase in seedling stem diameter and in root length and leaf area. A significant diameter growth increase was also associated with mycorrhizal inoculation (i.e., noninoculated vs. inoculated within a treatment) under conditions of S-IBA. The increase in root length resulting from mycorrhizal inoculation in the C-IBA treatment may be of special significance to the practitioner even though it was not found to be significant at the $P \leq 0.05$. Transplanted northern red oak seedlings do not respond well under conditions of outplanting in the Midwest. The increased root length, if consistent, provided through a combination of mycorrhizal inoculation and delivery of IBA to the root system via a clay carrier, could serve to improve survival and early growth after outplanting.

Seedlings inoculated with *P. tinctorius* but without IBA did not differ significantly from noninoculated control seedlings in stem diameter, seedling height, leaf area, root length, or dry weights. Although mycorrhizae often increase seedling growth under conditions of low soil fertility, seedlings in this experiment were fertilized regularly. These higher soil fertility levels may have minimized the effects of the mycorrhizae on growth (Dixon et al., 1985; Maronek and Hendrix, 1979). In a study by Dixon et al. (1981), inoculation of container-grown black oak seedlings with *P. tinctorius* did not affect stem diameter or height growth, but did increase dry weight and leaf area. Daughtridge et al. (1986b) found higher dry weight for inoculated than noninoculated container-grown black oak seedlings but found no difference between similarly treated container-grown northern red oak seedlings. Harley and Smith (1983) have postulated that development of the mycorrhizal fungal mass can act as a drain on the host plant, resulting in decreased early growth in some species.

Summary and Conclusions

To investigate further the feasibility of using IBA carriers to stimulate seedling growth, responses to IBA in fired-montmorilonite clay granules were compared to IBAin a polyacrylic starch as used by Baser et al. (1987). While absolute comparisons with Baser et al. (1987) are not possible due to differences in IBA concentrations and the *Pisolithus* isolate used, general comparisons were made. In general, clay proved superior to starch, resulting in significant increases in seedling stem diameter, root length, leaf area, and shoot dry weight. Furthermore, mycorrhizal colonization was highest within the clay-amended treatment, and ectomycorrhizal development was not inhibited by IBA, as Baser et al. (1987) found with S-IBA. Positive interactions between mycorrhizae and IBA were primarily restricted to the clay-carrier treatment where diameter was significantly increased and substantial improvements in leaf area and root length were observed. If extrapolatable to field conditions, this enhanced growth response could result in a higher probability of success following the outplanting of northern red oak seedlings.

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