THE EFFECT OF CONTAINER TYPE, FERTILIZER TREATMENT, AND MYCORRHIZAL INOCULATION ON THE PRODUCTION OF CONTAINER-GROWN HARDWOODS

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ABSTRACT.--Yellow-poplar and black cherry seedlings were grown in containers for twenty-four and eighteen weeks respectively, under high and low fertilizer treatments with and without an endomycorrhizal fungus. Container seedlings grown in the high fertilizer treatment and inoculated prior to seeding greatly improved outplanting survival and subsequent growth.

Higher survival rates, especially on poorer sites, and faster early growth have made containerization of forest tree seedlings an attractive reforestation technique. In addition, raising seedlings in a controlled environment allows for a more uniform treatment of seedlings prior to outplanting and permits strict control of the early growing environment. Containerization can reduce the time interval from seed collection to outplanting so that seed collected in the fall can be sown and the resultant seedlings outplanted the following spring, thus reducing outplanting time by at least six months. The planting season can also be extended further into the summer months with acceptable survival rates (Ter Bush, 1971; Barnett, 1974; Brooks, 1978). The cost of seed from controlled crosses necessitates a high seed to seedling ratio especially for species that naturally produce a small number of seed per flower, such as chestnut *(Castanea)* and walnut *(Juglans)*. Containerization can be used to maximize the number of seedlings produced from a given quantity of seed and to produce quality seedlings in a shorter time period than is possible with standard nursery techniques. Of particular interest to forest geneticists, containerization can be used as a tool to minimize the time involved in progeny testing and seed orchard establishment.

This study was funded under McIntire-Stennis Project MS-1 through the West Virginia University Agricultural and Forestry Experiment Station, Morgantown, WV. Published as Scientific Paper No. 1562, West Virginia Agriculture Experiment Station.

Most of the experience with container-grown seedlings in the United States has been with conifer seedlings in the Pacific Northwest and the Great Plains regions. Presently, one of every four seedlings produced in the states of Washington and Oregon is container-grown (Stein and Owston, 1977). In contrast, hardwood container programs, production techniques, and subsequent plantation establishment utilizing container-grown seedlings are still in their infancy.

The use of mycorrhizal fungi to stimulate tree seedling growth has been substantiated experimentally (Mosse et al., 1969; Bryan and Ruehle, 1976). Yellow-poplar has been shown to benefit from endomycorrhizal infection (Clark, 1964; Gerdemann, 1965) however, to date there is no description of the mycorrhizal infection as it occurs and affects black cherry. This paper summarizes two years of research involving the production of container-grown black cherry (Prunus *serotina* Ehrh.) and yellow-poplar *(Liriodendron tulipipera* L.) seedlings grown in association with an endomycorrhizal fungus *Glomus fasciculatus.*

Procedure

Seed of black cherry and yellow-poplar was collected from single trees of a local seed source and stratified in moist peat and sand (Schopmeyer, 1976). Black cherry and yellow-poplar seedlings were grown in a glass ridge and furrow greenhouse for fourteen and twenty weeks, respectively. Seedlings were then placed in a cloth shade house (receiving 45 percent of full sunlight) to harden-off. Seedlings were grown in three container types under high and low fertility treatments, with and without an endomycorrhizal fungus.

Containers

The three containers tested were of approximately equal volume; a kraft paper mailing tube 20 cm tall and 5 cm in diameter with a soil volume of 410 cm^3, a paper carton measuring 20 cm X 5 cm X 5 cm with a soil volume of 525 cm^3, and a Spencer LeMaire – Tinus bookplanter having a soil volume of 492 cm3. Groups of four containers were randomly placed in wooden crates lined with a screen material and supported approximately 46 cm from the greenhouse floor to permit air pruning of the roots. Each container was differently shaped to determine the effects of the container on root configuration, specifically the degree of root spiraling.

Fertilizer Treatment

Seedlings were raised in a ground hardwood bark medium, initially fumigated with methyl bromide, under high and low fertilizer treatments. The low fertilizer treatment consisted of unamended bark, fertilized every other week with a water soluble 21-7-7 fertilizer (250 ppm N - 33 ppm P and K). The high fertilizer treatment consisted of bark amended with a shell-drake nutritional mix as modified by Carey (1976) (which includes substituting gypsum [$CaSO_4$] for the lime in the mix) plus the application of a water soluble 21-7-7 fertilizer (500 ppm N - 166 ppm P and K) every other week.

Mycorrhiza

The purpose of the mycorrhizal treatment was to determine the effects of infection on seedling growth and the establishment of the mycorrhizal fungus under differing fertilizer treatments. Half of the seedlings within each fertilizer treatment were inoculated with macerated sorghum (*Sorghum vulgare*) roots infected with an endomycorrhizal fungus, G. *fasciculatus.* The inoculum was placed in the top 3 cm of each container prior to seeding. The remaining seedlings received only root washings that had first been passed through a 35 micrometer mesh sieve (openings smaller than the spore diameter of G. fasciculatus) and then filtered through Whatman No 1 paper. This was to standardize the microflora at the time of seeding (Kormanik et al., In Press). The sorghum pot cultures were established using macerated sorghum roots infected with G. *fasciculatus* obtained from the U.S.D.A. Forest Sciences Laboratory, Athens, Georgia. For microscopic observation, mycorrhizal roots were cleared and stained using a technique described by Philips and Hayman (1970).

Results

Root mass and configuration was significantly affected by container shape and volume. Both species had the smallest root systems (by weight) in the kraft tubes, yet these containers promoted the greatest amount of root spiraling. The largest root systems were from seedlings grown in the paper cartons and the Spencer LeMaire - Tinus bookplanters. The least root spiraling was observed with the Spencer LeMaire bookplanters apparently due to the internal vertical ribs that restricted the growth of roots laterally.

With the exception of root configuration, seedlings of both species grown under the high fertilizer treatment were consistantly larger for all parameters tested (Table 1).

Table 1. Growth Averages of Container Grown Hardwood Seedlings by Mycorrhiza, Fertilizer, and Container Treatment.

Treatment	Container Type	Height (cm)	# Nodes	Green Weight of Roots (g)	Root Collar Diameter (mm)
BC					
MHF	PC	37.1	19	10.05	4.48
	KT	16.8	11	2.97	2.71
	SL-T	33.1	19	8.61	4.24
NMHF	PC	37.2	20	10.06	4.32
	KT	17.5	11	2.10	2.22
	SL-T	33.3	19	7.95	4.08
MLF	PC	7.3	6	1.28	1.71
	KT	6.0	5	0.45	1.35
	SL-T	8.5	7	1.78	1.89
NMLF	PC	8.8	6	1.31	1.72
	KT	7.7	6	0.75	1.61
	SL-T	9.3	7	1.30	1.79
YP					
MHF	PC	23.0	13	8.46	3.96
	KT	12.5	10	2.29	2.37
	SL-T	22.9	13	7.41	3.93
NMHF	PC	24.0	14	6.59	3.92
	KT	16.8	10	2.98	2.79
	SL-T	14.7	11	2.66	3.19
MLF	PC	5.9	9	2.13	2.13
	KT	5.1	7	0.92	1.69
	SL-T	6.1	9	1.27	1.69
NMLF	PC	4.5	7	0.72	1.48
	KT	3.9	7	0.51	1.30
	SL-T	4.9	6	0.55	1.36

MHF - Mycorrhiza - High Fertilizer
NMHF - No Mycorrhiza - High Fertilizer
MLF - Mycorrhiza - Low Fertilizer
NMLF - No Mycorrhiza - Low Fertilizer
YP - Yellow-poplar

PC - Paper Carton
KT - Kraft Tube
SL-T - Spencer LeMaire - Tinus
BC - Black Cherry

Seedlings were largest in the paper cartons and the Spencer LeMaire bookplanters. Seedlings grown in these container types were three times the size of similar seedlings grown under the low fertilizer treatment. Seedlings grown in the kraft tubes were much smaller and occasionally chlorotic. This container is apparently unsuitable for the hardwood container seedlings tested.

Statistically significant differences occurred between G. *fasciculatus* inoculated and uninoculated yellow-poplar seedlings when averaged over all treatments. Inoculated seedlings had a higher number of growth flushes (10 to 9), greater green weight of roots (0.71 g to 0.43 g), and larger root collar diameters (2.7 mm to 2.3 mm) than comparable uninoculated seedlings. In contrast, black cherry seedlings exhibited no statistically significant differences among the growth parameters measured. Root samples that were cleared and stained also revealed differences in infection. Root samples of both species grown under the low fertilizer treatment exhibited typical vesicular-arbuscular infection, by producing intracellular vesicles and arbuscules. However, the location and frequency of these organs was different. Black cherry roots showed numerous vesicles and interspaced arbuscules that were evenly distributed throughout the cortical region. In yellow-poplar samples, infection was concentrated in the layer of four-to-five cortical cells surrounding the stele. This densely stained area contained a few vesicles but was largely composed of cells containing coiled hyphae and arbuscules. The outer region of the cortex was generally free of infection. Only exterior hyphae and limited interior hyphae were present on inoculated roots grown under the high fertilizer treatment. No vesicles or arbuscules were found. Uninoculated treatments showed no infection.

Containerized seedling survival (average of all containers tested), during the first growing season, ranged from 73 percent to 85 percent while bareroot survival never exceeded 39 percent. Inoculated yellow-poplar seedlings had significantly higher (.05 percent level) survival rates on both sites than corresponding uninoculated seedlings (Table 2). Yellow-poplar seedlings raised under the high fertilizer treatment also had higher survival rates on both sites though not statistically higher (Table 2). However, in either case, the 12 percent increase in survival is of sufficient importance to suggest that inoculation of black cherry seedlings would be advisable.

Height growth of containerized black cherry seedlings was greater than that of bareroot seedlings on both sites. Container seedlings grown under the high fertilizer

Table 2. First Growing Season Survival and Growth for Two Container-Grown Hardwood Species.

	Survival Percent		Growth in cm		
Species	Black Cherry	Yellow-poplar	Black Cherry		
Old Field					
Mycorrhizal	80	77*	5.2	Avg. by Container Type	
Non-Mycorrhizal	67	57	6.2		
Container-Grown	73**	67**	5.6	PC	3.9
Bareroot	23	39	1.0		
High Fertilizer	83*	75	8.7	KT	1.9
Low Fertilizer	63	58	1.9	SL-T	8.6
Strip Mine					
Mycorrhizal	90	85*	1.3	Avg. by Container Type	
Non-Mycorrhizal	78	73	1.0		
Container-Grown	84**	78**	1.2	PC	0.8
Bareroot	23	25	0.2		
High Fertilizer	88	82	1.9	KT	0.7
Low Fertilizer	80	73	0.3	SL-T	1.8

* Significant at the .05% level
** Significant at the .01% level

treatment were also larger on both sites. Seedlings planted as root-plugs (grown in the Spencer LeMaire containers) attained the greatest growth following outplanting. No measurement was made for yellow-poplar, as seedlings became dormant soon after planting due to the late planting date.

Conclusions

It has been shown that container-grown black cherry and yellow-poplar seedlings can be successfully grown to field planting size in 18 to 24 weeks respectively, under the high fertilizer treatment in a container of approximately 500 cm^3 of soil volume (Brooks, 1978). These seedlings can be outplanted later in the growing season with acceptable survival and growth rates. Seedlings grown under the high fertilizer treatment in the Spencer LeMaire bookplanters were the tallest and the fastest growing seedlings on both sites. The importance of inoculation with an endomycorrhizal fungus seems to depend on the planting site and the proper choice of a mycorrhizal symbiont for the .species tested. Yellow-poplar showed a higher degree of dependence on infection for improved growth and survival while black cherry seedlings did not exhibit as great a benefit from infection with G. fasciculatus.

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