Stock Quality of Black Walnut (*Juglans Nigra*) Seedlings as Affected by Half-Sib Seed Source and Nursery Sowing Density

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Abstract

Morphological quality of black walnut (*Juglans nigra* L.) seedlings largely dictates their capacity to establish well in plantations and their performance potential for use as rootstocks in grafting. Larger seedlings and rootstocks, attained from propagation at relatively low nursery sowing densities, have comparatively more expansive root systems for exploitation of soil resources and greater nutrient/carbohydrate reserves to support aboveground growth. The relative role of genetic selection in dictating morphological quality of nursery-grown black walnut seedlings, however, has not been thoroughly documented. We collected seed from three half-sib families (A, B, C) of black walnut in Indiana, USA which were sown as sprouted seeds into bareroot nursery beds at three sowing densities (L, 11.2 m^{-2}; M, 24.2 m^{-2}; H, 29.4 m^{-2}). Sowing density significantly affected mean shoot height (H > M > L) and root volume (L > M > H), but not stem diameter. Shoot height was increased by 14.4 cm (43%) in H vs. L and root volume was increased by 32 cm^3 (39%) in L vs. H. Seedling response to family selection was significant for height, root volume, and stem diameter (C > B > A). The magnitude of the difference in response was greater for family selection compared to nursery sowing density, as height and root volume were increased by 15.2 cm (46%) and 44 cm^3 (60%) in C vs. A, respectively. Further, family C had greater mean stem diameter and root volume in H than did family A in L; mean height for these two families were about equal between H and L. Our results suggest that identification of superior genetic sources may be relatively more important than sowing density in nursery propagation of high quality black walnut seedlings.

INTRODUCTION

Black walnut (*Juglans nigra* L.) is an important component of forests in the Central Region of the USA, producing valuable timber and nut resources (Williams, 1990). Black walnut is among the most common species grown in forest tree nurseries in this region (Jacobs et al., 2004), with seedlings being used for afforestation plantings and to a lesser extent as rootstock for grafting of genetically improved germ plasm. Planted hardwood seedlings in this region often grow poorly or fail to survive (Jacobs et al., 2004), which may be partially attributed to variable nursery stock quality. Morphological attributes such as shoot height and stem caliper are easily measured and often used to evaluate nursery stock quality (Thompson, 1985). In addition, large root system morphology appears to provide a useful assessment of potential hardwood seedling performance (Schultz and Thompson, 1996; Jacobs and Seifert, 2004) presumably due to increased access of a more expansive root system to soil resources, as well as supplying greater carbohydrate and nutrient reserves to promote aboveground growth. Benefits to using high quality nursery stock are likely to be realized following seedling planting, as well as when using rootstock for grafting.

Control of black walnut nursery stock quality is largely achieved through cultural practices conducted in the nursery (Jacobs, 2004). Among the most important nursery cultural variables is bed sowing density, with larger seedlings consistently being produced at relatively low sowing densities (Schultz and Thompson, 1996). Low seedbed densities, however, limit overall nursery stock production as only a fixed amount of nursery growing space is generally available for production. Thus, examination of additional factors which might enhance nursery stock quality at a given density could help improve the quality of black walnut nursery stock for seedling planting and grafting.

Though substantial investigation into genetic improvement of black walnut has been conducted on plantation trees (Beineke, 1989), relatively little attention has been given to the role of genetic identity in dictating stock quality of nursery seedlings. Operationally, the majority of black walnut seed attained for nursery stock production is collected from sources of unknown genetic origin (Jacobs, 2003), limiting the positive contribution that genetic identity might have on improving black walnut nursery stock quality. Thus, the objective of this study was to examine the relative role of half-sib seed source and nursery sowing density in the propagation of high quality black walnut nursery stock.

MATERIALS AND METHODS

In October 2002, seeds were collected from three half-sib families (A, B, C) of black walnut in Indiana, USA. The seeds were temporarily stored in mesh bags in an area with adequate air circulation to allow excess moisture in the husks (pericarps) to dry. After drying in November 2002, husks were removed from the seeds, which were then placed in perforated plastic bins filled with lightly moistened and finely milled sphagnum peat moss. The bins were placed into cold storage at 4 °C for 90 days of stratification.

Seeds were pre-sprouted (Davis et al. 2004) to ensure high probability of successful seedling development under nursery density treatments and that all seeds began from a similar physiological state of development following sowing into nursery beds. In February 2003, the seeds were sown in plastic greenhouse flats with Metro-Mix 360® with ScottsCoir® growing medium (O.M Scotts Co., Marysville, OH, USA) and placed into a greenhouse.

Greenhouse environmental conditions were maintained at 24/17 °C (day/night) with natural photoperiod and ambient light intensity. The flats were watered as necessary to maintain moist medium. Twice weekly, the seeds from each flat were removed and carefully rinsed free of planting medium to check for sprouting. Optimum sprouting occurred when the shell had cracked open at the cleavage line but the radicle, while visible, had not yet emerged. This protected the radicle from physical damage. If root formation had begun, or if the epicotyl had emerged above the media, those seeds were discarded. Sprouted seeds were placed in flats and refrigerated at 4 °C until a sufficient number had been collected, at which time the seeds for each family were packed with moistened peat moss into separate plastic bins for transportation to Vallonia Nursery, Indiana, USA (38°48'N, 86°06'W) in April 2003.

Sprouted seeds from each family were sown by hand into bareroot nursery bed plots at three sowing densities (L, 11.2 m^{-2}; M, 24.2 m^{-2}; H, 29.4 m^{-2}). Densities in each plot were kept consistent using planting jigs. The jigs were made of plywood with wooden dowels spaced at regular intervals to mark the desired density. L plots were 2.85 m^2 (32 seeds), M plots were 1.24 m^2 (30 seeds), and H plots were 1.02 m^2 (30 seeds). Plots were arranged in a randomized complete block design consisting of 4 blocks, where every block contained plots for each family x density combination. Seedlings were then grown under operational nursery conditions (Jacobs, 2003) until lifting in March 2004. Seedlings were then stored at 2 °C until measured for morphological characteristics including stem caliper, shoot height, and root volume using the water displacement method of Burdett (1979).

All analyses were performed using SAS 9.1 (SAS Institute Inc., Cary NC, USA). Multivariate analysis of variance was performed using the general linear model where stem diameter, shoot height, and root volume were dependent variables and family, sowing density, family x density interactions, and block were independent variables. For each dependent variable the least-squares means and standard deviations were generated for each combination of family and density by invoking LSMEANS. Means separation was performed using Duncan's multiple range test with $\alpha = 0.05$. Components of variance for estimation of heritability and its standard error were generated using PROC VARCOMP and methods described previously (Woeste, 2002), where block and density were considered fixed effects, and families random. Correlations among the response variables were estimated using PROC CORR.

RESULTS AND DISCUSSION

We found that genetic identity plays a significant role in determining the quality of one-year old black walnut nursery stock based on our relatively small, arbitrary sample of three half-sib families (A, B, C) grown in Indiana, USA. Family effects were very highly significant ($P < 0.0001$) for stem caliper, shoot height, and root volume when averaged over all planting densities, and there was no rank shifting among the families for any of the variables at any density.

Family C consistently had higher stem caliper, height, and root volume than the other two families, irrespective of planting density, and these differences were significant (Figs. 1-3).

Family A was significantly smaller than the other two families for all response variables at every sowing density, with the exception of root volume at the highest density. Though the influence of genetic source in plantation growth of black walnut trees has been well documented (Beineke, 1989), little research has examined the influence of genetic identify in nursery stock quality. However, studies of species within Juglandaceae which have examined this relationship generally report significant family variation among seed sources within a given zone. For instance, Dixon (1988) reported significant variation in black walnut seedling shoot and root growth among three seed sources collected in Minnesota, USA which were inoculated with different strains of mycorrhizal fungi. McGranahan et al. (1988) reported that seed source was a highly significant cause of variation for phenological traits and branching in California black walnut (*Juglans hindsii* Jeps. ex R.E. Smith) seedlings. Furthermore, significant variation was found in seedling growth among nine different full-sib pecan (*Carya illinoiensis* (Wangenh.) C. Koch) sources and it was suggested that parentage plays an important role in pecan nursery operations to increase rootstock vigor (Thompson and Grauke, 2003).

Sowing density also significantly affected mean shoot height (H > M > L) (Fig. 1) and root volume (L > M > H) (Fig. 2), but not stem caliper (Fig. 3). Shoot height was increased by 15.1 cm (45%) in H vs. L and root volume was increased by 35 cm^3 (45%) in L vs. H. Nursery sowing densities have been previously reported to affect black walnut seedling growth, with larger seedlings being produced at lower seedbed densities (Schultz and Thompson, 1996). We found that shoot height increased at higher sowing densities, presumably a carbon allocation response to access limited light resources under high competition. However, Schultz and Thompson (1996) reported that all morphological variables, including shoot height, increased at lower sowing densities. This contrast with our result may have been associated with the much lower range of seedbed densities tested by Schultz and Thompson compared to our study (i.e., 32-96 vs. 11-29 seedlings m^{-2}). Nevertheless, nursery seedling shoot height often provides a relatively inconsistent predictor of field performance (Chavasse, 1977; Thompson and Schultz, 1995), and we contend that root morphology and stem caliper better reflect nursery stock quality.

Interestingly, family effects were found to be more important than nursery sowing density in producing nursery stock plants with larger root volume and stem caliper. For instance, the mean square for family effects of stem caliper was over three fold larger than the mean square for density. Similarly, the mean square for family effects for shoot height was about 15% less than the mean square for density, although both variables were very highly significant ($F = 62.3$ and 72.7 respectively, $P \leq 0.0001$). Further evidence for the importance of genetic identity in black walnut nursery stock quality was reflected in our result that family C had greater mean stem caliper (Fig. 3) and root volume (Fig. 2) in H than did family A in L. The mean shoot height of family A at L was only slightly lower than the mean height of family C at H (373 and 388 mm, respectively) (Fig. 1).

Thus, our results suggest that identification of superior genetic sources may be relatively more important than sowing density in nursery propagation of high quality black walnut seedlings. Family variance was greatest for root volume and stem caliper at low planting densities, and conversely, family variance for height was greatest at high densities. These findings indicate that evaluation and selection of families for root volume and stem caliper should take place at lower sowing densities, and that evaluation and selection of families for shoot height should take place at higher densities.

Heritabilities of stem caliper and shoot height were very high (> 1.0), although the small size of the experiment prompted relatively high standard errors (approximately 0.80). Heritability estimates greater than 1.0 indicate that there were probably systematic biases in the experimental design. We report the high heritabilites for these traits because they correspond with findings that shoot height of 1-0 nursery-grown black walnuts had heritability near 1.0 (Rink, 1984). The heritability of root volume was lower (0.54) than the other two variables, perhaps because the coefficient of variation for this trait was very high (CV = 53.3, overall mean for root volume = 93.9 cm^3). Similarly, phenological traits for seedlings of two California black walnuts had upper estimates of heritabilities ranging from 0.47 to 0.88 (McGranahan et al., 1988).

Pearson correlations among the traits were positive and highly significant with moderate to high R values (Table 1). Correlations among the traits were greater at low planting densities than at high or moderate planting densities (data not shown). This result, taken together with those reported above, may indicate that a single measurement (e.g., stem caliper) evaluated on plants grown at low densities may be adequate to identify families that produce superior quality nursery stock.

CONCLUSIONS

Our study results help to confirm the importance of nursery sowing density in producing high quality black walnut nursery stock for seedling planting and grafting. However, we found that genetic identity of half-sib seed source was relatively more important than sowing density in dictating seedling shoot and root morphological quality. This suggests that the current common operational nursery practice in this region of attaining seed from sources of unknown genetic origin is a limiting factor in the production of high quality nursery stock. Similar to the impact that nursery irrigation, fertilization, weed control, and root culturing practices have on resulting hardwood stock quality (Jacobs, 2003), selection of superior genetic sources could further enhance stock quality, thereby increasing nursery yields and helping to improve plantation success.

Extrapolation of our study results is somewhat limited due to the relatively low number of genetic sources sampled in a single nursery. However, our results suggest that a wider array of genetic sources should be screened over several different nursery locales to help confirm these responses. Additionally, nursery stock produced from different genetic sources should be examined for long-term field response following seedling planting or use as rootstock for grafting. This will help to further validate the effect that genetic identity has on resulting plantation establishment success.

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Tables

Table 1. Pearson correlation coefficients among traits measured in this study.

	Root Volume	Height
Root Collar Diameter	0.78[1]	0.54
Height	0.38	

[1]All correlations highly significant ($P < 0.001$) based on a minimum of 388 observations.

Figures

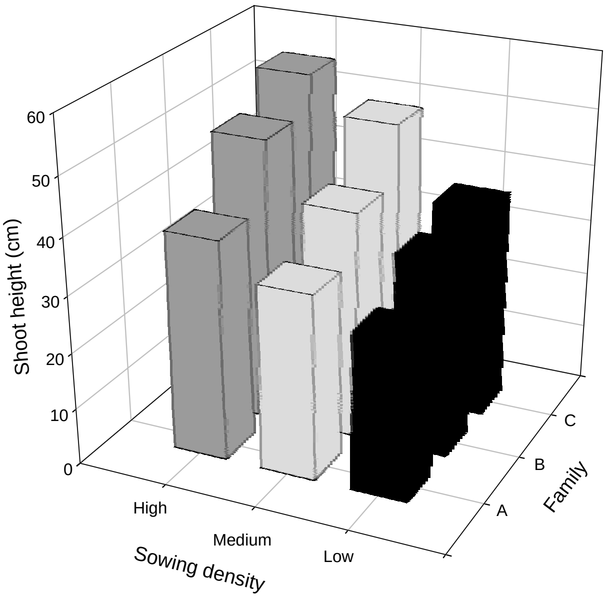

Fig. 1. Mean shoot height for black walnut seedlings from three half sib families (A, B, C) planted at three nursery sowing densities (H, M, L). Seedlings differed significantly ($P < 0.05$) for both family (C > B > A) and sowing density (H > M > L) effects.

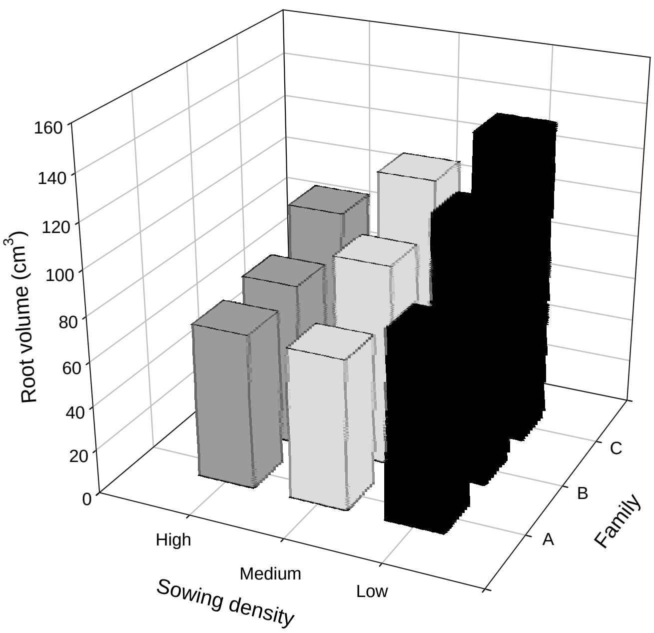

Fig. 2. Mean root volume for black walnut seedlings from three half sib families (A, B, C) planted at three nursery sowing densities (H, M, L). Seedlings differed significantly ($P < 0.05$) for both family (C > B > A) and sowing density (L > M > H) effects.

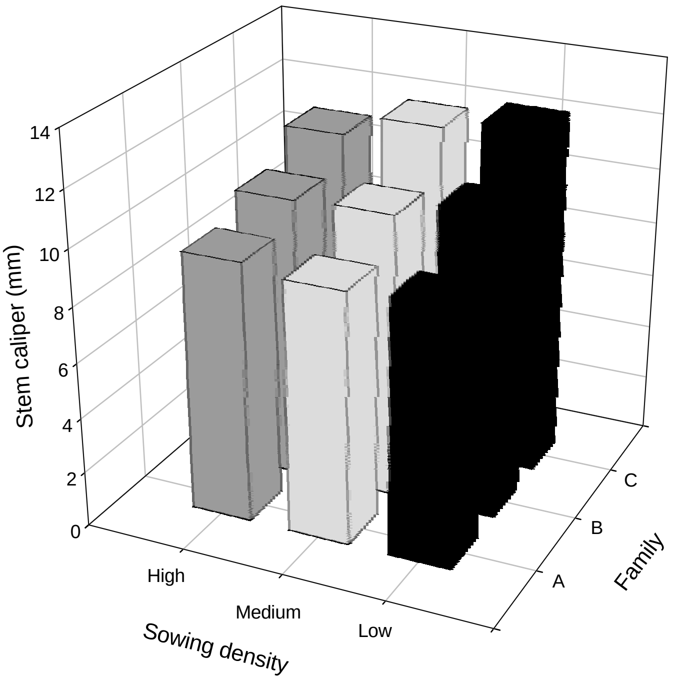

Fig. 3. Mean stem caliper for black walnut seedlings from three half sib families (A, B, C) planted at three nursery sowing densities (H, M, L). Seedlings differed significantly ($P < 0.05$) for family (C > B > A) but not sowing density effects.