Accelerating the Growth of Black Walnut Seedlings

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Large and vigorous seedlings can be produced within 12 weeks from seed in greenhouses using continuous light, carbon dioxide fertilization, gibberellic acid, and long containers.

The traditional method of producing forest tree seedlings in outdoor nurseries allows the grower minimal control over growing conditions, often resulting in a poor quality product. A practical method for overcoming climatic limitations is use of the accelerated-optimal-growth (AOG) concept (*2,3,4*). AOG is based on controlling components of the environment in which the tree is grown. Such components include light, temperature, mineral nutrients, carbon dioxide, growth regulating chemicals, and container dimensions.

Combining the AOG concept with greenhouse production of containerized seedlings facilitates control over growth, development, and physiological status. Thus, high quality seedlings can be produced in a few months rather than years. The concept includes use of a growing cycle that will produce seedlings of desired size and physiological status for outplanting at a predetermined time. Before such a cycle is implemented, it is necessary to understand how the various components of AOG affect growth and development of a species. Many species, of which black walnut (*Juglans nigra* L.) is a prime example, cease height growth after a few weeks under natural environmental conditions and will not resume growth until exposed to prolonged cold (*6*). Such species exhibit strong determinate growth characterized by a single flush of terminal growth followed by formation of a resting bud even though environmental conditions seem to be adequate for continued growth (*6*). If growth of species with strong determinate growth cannot be regulated, then the AOG concept would not be applicable. This study reports on the responsiveness of black walnut seedlings to various variables employed in AOG systems.

Materials and Methods

The growth response of black walnut seedlings to container size, carbon dioxide (CO_2) enrichment, continuous photoperiod, and gibberellin were observed in the following series of experiments.

Experiment 1. The effects of container dimension and continuous photoperiod upon black walnut seedling growth were tested as follows. Seeds bulked from several sources were stratified 120 days at 3° C, germinated under wet burlap, and returned to the cold room for storage in moist peat until enough seeds were accumulated to complete the study. Seedlings were then planted in the greenhouse and the nursery. Those in the greenhouse were planted in Rediearth (a commercial peat-vermiculite soil mix) at 15- by 15-centimeter spacings in rectangular polycoated paperboard plant bands. Band dimensions were 5, 7.6, 10, and 15 centimeters in width by 28 and 56 centimeters in length. Experimental design was a three replicate randomized complete block with four trees per plot in each of eight band dimension treatments. The photoperiod was supplemented by 50 $\mu E\,cm^{-2}s^{-1}$ continuous fluorescent light during the normal dark period. Germinated seeds were also planted in an outdoor nursery at the same spacing and grown for one season . Trees were watered and fertilized as needed. For further comparisons, 1-0 seedlings were also obtained from a commercial nursery.

Experiment 2. This study investigated the growth response of walnut seedlings to CO_2 fertilization. Eleven seedlots from both southern Indiana and southern Michigan were prepared as in experiment 1, except only 7.6- by 28-centimeter plant bands were used. Light conditions were also similar except that 100 $\mu E cm^{-2} s^{-1}$ supplemental light was used. Seedlings were grown 13 weeks in an enriched atmosphere of 700 (ambient greenhouse level), 1,400, and 2,100 parts per million CO_2.

Experiment 3. This study tested the influence of gibberellic acid, defoliation, and time of treatment on the determinate growth pattern of walnut. Seedlings were prepared as in experiment 1 and light was supplemented as in experiment 2. Treatments were GA_3 (800 p/m), ethanol-Tween 80 (0.05%), complete defoliation by hand, and non-defoliation. Two drops of GA_3 (dissolved in ethanol and Tween 80) were applied to the bud weekly for 3 weeks. The influence of duration of budset was investigated by treating trees that had previously set bud for 2, 6, and 12 weeks.

Results and Discussion

Growth of black walnut, a taprooted species, was greatly affected by container (plant band) dimension. Height, dry weight leaf area, and leaf number of seedlings grown in long bands exceeded that of seedlings grown in short bands (table 1). Band width had little effect on these growth characteristics, with the exception that leaf number increased with wide bands. Seedlings produced in outdoor nurseries were much smaller and less vigorous than those produced in the greenhouse. Figure 1 depicts a 12-week-old seedling grown in a long band.

Figure 1.—A *12-week-old black walnut seedling grown under a continuous photoperiod in a 5.2- by 56-centimeter plant container.*

Band dimension had a significant effect on root development; long bands increased taproot length and weight but did not affect lateral root weight (table 1). Increasing band widths caused increased lateral root weight but did not affect taproot weight. It appears that allowing adequate space for taproot growth is an important consideration for improving overall seedling growth. Therefore, container length should be increased to the extent practical in a seedling production system.

Seedlings in the greenhouse under continuous light grew for a longer period than those in the nursery. However, band dimension did not affect duration of growth in the greenhouse. Seedlings set a resting bud in long bands and under continuous light, which suggests that neither growth inhibition of the taproot or phytochrome regulation are the primary factors inducing the determinate growth response.

Results of the second experiment are presented in table 2. Carbon dioxide enrichment greatly increased growth. Doubling the ambient level (700 p/m) increased height growth by half, and tripling it doubled height growth. The increase in dry weight resulting from growing in 1,400 versus 2,100 parts per million CO_2 was less than the increase from growing in 700 versus 1,400 parts per million, indicating less efficient use of CO_2 at the higher concentrations. However, height growth continued unabated at the highest CO_2 level. The height growth response to CO_2, however, did not become apparent until after 6 weeks of exposure.

An increase in CO_2 concentration beyond the saturation level may lead to toxicity effects (5), but no such effects were observed in these experiments.

Carbon dioxide enrichment also increased leaf growth and internode length. Total leaf area per tree and number of leaves doubled upon doubling the ambient level, whereas tripling CO_2 gave no further increase in these traits. This apparent leveling or decrease in leaf area in response to CO_2 enrichment has also been observed to occur in other species (*5*). Although there was no treatment with natural photoperiod plus CO_2 to test the effect of day length, it is evident that CO_2 and continuous light did not act together to overcome budset. Because the trees were grown in a warm en-

Table 1.—*Effect of container dimensions on growth of AOG black walnut seedlings in comparison with nursery-grown controls*

	Container length (cm)[2]		Container width (cm)[3]				Nursery controls	
Parameters[1]	28	56	5	7.6	10	15	University	Commercial
Height (cm)	58a[4]	76b	61a	63a	76a	67a	21	52
Total dry weight (g)	63a	94b	65a	6a	88a	96a	10	16
Taproot dry weight (g)	17a	26b	18a	22a	27a	20a	2	4
Lateral root dry weight (g)	9a	9a	5a	6a	13b	13b	1	1
Tap/lateral	1.9a	2.9b	3.6a	3.7a	2.1a	1.5a	2.1	3.8
Root/shoot	.70a	.59a	.54a	.74a	.83a	.52a	.42	.45
Growth period (days)	69a	76a	70a	75a	72a	72a	41	
Leaf area ($cm^2 \times 10^3$)	4.0a	7.4b	5.3a	5.5a	7.0a	6.8a	0.6	0.9
Leaf number	15a	23b	16a	17a	22b	21b	6	6

[1]Parameters measured at 12 weeks of age.
[2]Container length averaged over all four widths.
[3]Container width averaged over both lengths.
[4]Mean values in any row of treatment comparisons followed by the same letter do not differ at the 5-percent probability level.

Table 2.—*Growth response of black walnut seedlings to carbon dioxide fertilization*

CO_2 Concentration (p/m)	Total dry weight (g)	Root/shoot	Height (cm) 12 weeks	Height (cm) 8 weeks	Height (cm) 4 weeks	Total leaf area (cm^2)	Leaf number	Average leaf area (cm^2)	Average[1] internode length (cm)
700	10.5a[2]	1.76a	25.7a	24.6a	21.3a	812a	7a	116a	1.9a
1,400	15.4b	1.65a	37.1b	36.5b	21.2a	1,740b	13b	145b	1.9a
2,100	18.9c	1.20b	50.6c	49.4c	25.0a	1,457b	13b	121a	3.0b

[1]Based on distance from the first basal leaf to apical tip.
[2]Mean values in any column followed by the same letter do not differ at the 5-percent probability level.

vironment with temperatures in the CO_2-enrichment chambers occasionally near 35° C, continous growth was probably inhibited.

Seedlots from southern Indiana grew taller than trees from southern Michigan. This genetic response in height growth between the two sources has also been observed for trees grown in an outdoor provenance test (1). There was no seedlot X CO_2 interaction.

Carbon dioxide fertilization of walnut should allow a grower to greatly increase seedling size. The 2,100 level seems to be suitable for maximizing production. Any increase above 2,100 parts per million for a prolonged period of time may cause toxicity and decreased growth.

In the third experiment, both GA_3 and defoliation were observed to induce the regrowth of dormant buds when the treatments are applied shortly after budset (table 3). However, both were ineffective if applied 6 weeks or longer after budset. GA_3 also had no effect on bud break after leaf abscission (12 weeks after budset). These results indicate that the determinate growth habit of black walnut seedlings can be controlled by timely GA_3 treatments of buds or by defoliation. Judicious use of these treatments would allow the grower to produce very large seedlings within a relatively short period of time.

Table 3.—*The effect of time since budset on the ability of GA_3 and defoliation treatments to cause bud break of walnut seedlings.*

	Time after budset		
	Week 2	*Week 6*	*Week 12*
Treatments	Percent height growth increase		
GA_3	25	0	0
Ethanol	0	0	0
Defoliation	30	0	0
Nondefoliation	0	0	0

Conclusion

The series of experiments described here indicates that the use of AOG methods, including supplemental lighting, long containers, CO_2 fertilization, and GA_3 or defoliation, can enable a grower to produce larger, more vigorous black walnut seedlings than is possible in conventional outdoor nurseries and in a fraction of the time. Walnut plantings established with accelerated trees could also lead to a reduction in the rotation age. The AOG concept also has potential for use in genetic test plantings to allow earlier evaluation of trees. Although walnut was the only species investigated, other species with taproot and/or determinate, single flush shoot growth may respond similarly to these growth control techniques.

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