Plant and Soil 88, 3–10 (1985). Ms. 6070

Variation among black walnut seedling families in resistance to competition and allelopathy

GEORGE RINK and J. W. VAN SAMBEEK
Research Geneticist and Research Plant Physiologist, USDA Forest Service, North Central Forest Experiment Station, Forestry Sciences Laboratory, Carbondale, IL 62901, USA

Received 29 August 1984. Revised March 1985

Key words Allelopathy *Festuca arundinacea* Interaction *Juglans nigra*

Summary Of three environmental variables affecting black walnut seedling establishment, moisture stress overshadowed the effects of fescue leachate and fertilizer. Interactions between moisture stress and family and between fescue leachate and moisture stress for both seedling height and dry weight suggested that selection for tolerance to moisture stress is possible, whereas progress from selecting for resistance to fescue leachate would be difficult. Family response to high moisture stress resulted in reductions of 17 to 33 percent in height and 36 to 48 percent in dry weight when compared with seedlings grown under moist conditions. The only effect of fertilization was a 9 percent decrease in seedling survival under high moisture stress conditions.

Introduction

Established guidelines for artificial regeneration of black walnut (*Juglans nigra* L.)[12] require weed control for 3 to 5 years after planting. Such long-term weed control has tended to discourage establishment of black walnut plantations.

One way to reduce the need for intensive weed control is to plant seedlings that can successfully compete with grasses and other vegetation. Such seedlings are presently not available. Namkoong *et al.*[10] state, 'we have not yet used breeding to improve competitive performance.' Bey and Funk[1] considered selecting and breeding walnut for competitive performance, but rejected this approach, assuming that chemical weed control would be more practical for plantation establishment.

As originally proposed by Muller[9], interference refers to the overall influence of one plant on another and thus encompasses competition and allelopathy. Allelopathy is an effect that depends on chemical compounds being added to the environment while competition involves the removal or reduction of some factor such as light, soil moisture, or minerals. Much of the reported effect of fescue (*Festuca arundinaceae* Schreb.) on walnut growth has been labelled 'competition' (*e.g.*[13]) and with few exceptions little evidence is available to indicate whether the effects are actually due to competition, allelopathy or both. Walters and Gilmore[14] showed that fescue leachates applied to *Liquidambar styraciflua* L. seedlings in a greenhouse stairstep apparatus reduced growth by up to 60 percent; an allelopathic component of interference was thereby implicated.

Selection for ability to withstand interference, therefore, involves selecting for increased efficiency in extracting the required growth factors from the environment and/or resisting the effects of phytotoxins. Before establishing a program for increasing competitiveness, we must first determine whether variation in ability to withstand interference (and its components) exists among different genotypes.

The objective of this study was to determine if genetic variation in resistance to interference is present in black walnut. A secondary objective was to identify the most significant environmental variables that contribute to the interference.

Methods

Germinating black walnut seeds of four southern Illinois open-pollinated families were planted in 32 61 × 61 × 61 cm plywood chambers and resulting seedlings grown for a period of 20 weeks (April through August 1983) in a greenhouse. Each chamber contained 30 seeds (8 germinating seeds of each of 3 families plus 6 of a fourth family which had fewer germinating seeds), comparable to the recommended nursery sowing rate for this species[16]. Germinating seeds were refrigerated until planted and were sown in family rows at a depth of 5 cm; families were randomly assigned to rows and family identity was maintained throughout the experiment.

The soil in the chambers was a 1:1 mixture of Hosmer silt loam (Typic Fragiudalf) and coarse river sand. Mixing of sand with the soils was intended to dilute the natural fertility of the silt loam. The silt loam was obtained from the A_1 horizon of an upland oak-hickory forest in Hardin County, Illinois after removal of the litter layer. Each chamber was filled in the field with 257 kg (fresh wt) of soil mix and then moved to the greenhouse. The soil mix averaged 11, 10, and 66 ppm of available NPK, respectively. This is considered low for both N and P and medium for K for woody ornamental plants[7]. The soil pH averaged 5.4.

The 32 experimental chambers were divided into 4 blocks of 8 chambers. Within blocks, each chamber was randomly assigned one of eight possible treatments. The eight treatments were all possible combinations of two moisture stress regimes, two soil fertility levels (fertilized or unfertilized), and two types of watering media (distilled water or fescue (*Festuca arundinacea* Schreb.) leachate). Each factor will be discussed separately.

Watering medium

Fescue leachate was used to water half the treatment chambers and distilled water was used for the other half. Fescue leachate was extracted from fescue sod that had been planted in 16 61 × 61 × 61 cm sand-filled plywood chambers. The sod was watered by periodically spraying the foliage and soil with a hose using unchlorinated water from a local irrigation pond. The leachate was extracted from the bottom of each chamber using the vacuum system described below, and stored in 150 1 containers in coolers at 2–4°C until needed for watering.

Moisture stress regime

Moisture stress was imposed on the soil by a vacuum pump that provided either 0.5 or 0.1 atmosphere (0.05 or 0.01 MPa, respectively) of continuous tension on the soil through a porous ceramic cup. The 0.1 atmosphere tension treatment simulated field capacity for the soil mix. To enable moisture relations to equilibrate at desired levels, tensions were imposed on the soil 4 weeks prior to planting seed; these two negative pressure levels were maintained for the remainder of the experiment.

The vacuum system responsible for providing the soil moisture tension also controlled the watering system. When the soil moisture stress exceeded the preset level, water or fescue leachate was drawn into the soil in response to the moisture gradient; liquid entered the soil through another porous ceramic cup located in the soil profile and connected with a water- or leachate-filled bottle. Thus, the moisture gradient in the soil resulted from opposing tensions between a moisture source and an imposed vacuum and evapotranspiration. Overall, a 1.5 percent difference in available soil moisture between the high and low moisture stress treatments was maintained throughout the 20-week period[6].

Fertility regime

Each chamber intended as a high fertility treatment was fertilized with six grams of 6-24-24 NPK fertilizer uniformly spread over the surface, mixed into the upper 15 cm of soil and watered in with 11 liters of distilled water. The fertilization was equivalent to 168 kg/ha, a commonly recommended rate for agronomic row crops in southern Illinois. The fertilizer was applied one month before planting germinating seed.

Seedlings were measured for total height at the time the experiment was terminated (20 weeks). Each seedling was then labelled with masking tape and all leaves removed. The chambers were turned on their sides, and seedlings washed out intact. Seedlings were severed at the root collar, and the roots and shoots were ovendried and weighed.

Data were analyzed by analysis of variance on an individual seedling basis. The ANOVA model was a randomized complete block design with a split plot arrangement of treatments in which families and associated interactions were assumed to be random and all other treatments (block, nutrient level, moisture stress, and water medium) and their interactions fixed. Where appropriate, Pearson correlation coefficients were calculated on an individual tree basis.

Results

Seedlings averaged 97 percent survival and 42 cm total height after 20 weeks. Although height growth continued throughout the 20-week period, 90 percent of the growth was completed in the first 6 weeks. Aside from a fertilizer × moisture stress interaction, survival was not affected by any source of variation, including family (Table 1). Survival was 90 percent in the fertilized-high stress treatments compared to 99 percent in all other treatments.

Analyses of variance for seedling height and for total dry weight were almost identical (Table 1). In both cases the analyses were dominated by interactions between family and moisture stress and between fescue leachate and moisture stress. The latter interaction resulted from greater suppression of height growth and dry matter accumulation by fescue leachate at low moisture stress conditions than under high stress, a consequence of the method of leachate application. Over the entire five-month experimental period, an average of 37 1 per chamber of leachate was applied under low moisture stress conditions in contrast with only 14 1 under high stress conditions. Presence of leachate under low moisture stress conditions resulted in a 10 percent reduction in seedling height and 14 percent reduction in dry weight (Fig. 1).

Table 1. Probabilities of obtaining greater F-values in analyses of variance of survival, seedling height and dry weight[1]

Variable	Survival	Total height	Total dry weight
Blocks (B)	–	–	0.03
Fertilizer (N)	< 0.01	–	–
Leachate (L)	–	–	–
Moisture stress (M)	< 0.01	< 0.01	< 0.01
N × L	–	–	–
N × M	< 0.01	–	–
L × M	–	0.02	0.02
N × L × M	–	–	–
Family (F)	–	< 0.01	< 0.01
F × N	–	–	–
F × L	–	–	–
F × M	–	< 0.01	0.05
F × N × L	–	–	–
F × N × M	–	–	–
F × L × M	–	–	–
F × N × L × M	–	–	–

[1] Probabilities of statistically significant effects only (*i.e.*, effects where $P < 0.05$).

Family response to increased moisture stress ranged from a 17 to 33 percent reduction in height and a 36 to 48 percent reduction in seedling dry weight. Although the family × moisture stress interaction was significant for both seedling height and dry weight (Table 1), family response to changing moisture stress regime for height was different from that for dry weight (Fig. 2). For example, though family 2 seedlings were tallest under moist conditions, under dry conditions they were only average in height. However, dry weights for seedlings of this family were next to lowest regardless of moisture stress regime. By contrast, seedlings of family 1 were on the average tallest overall, providing good growth and high dry weight under dry conditions as well as in soil at field capacity. Family 4 grew most in dry weight but was next to shortest in height. Although correlations between seedling height and dry weight ranged from 0.52 for family 1 to 0.70 for families 3 and 4, families with the tallest seedlings did not necessarily have the greatest dry weights.

Discussion

Of the variables evaluated in this study, all were involved in first order interactions; there were no second or higher order interactions. In analyses involving interactions, meaningful interpretation of the results entails discussion of the interactions rather than the main effects.

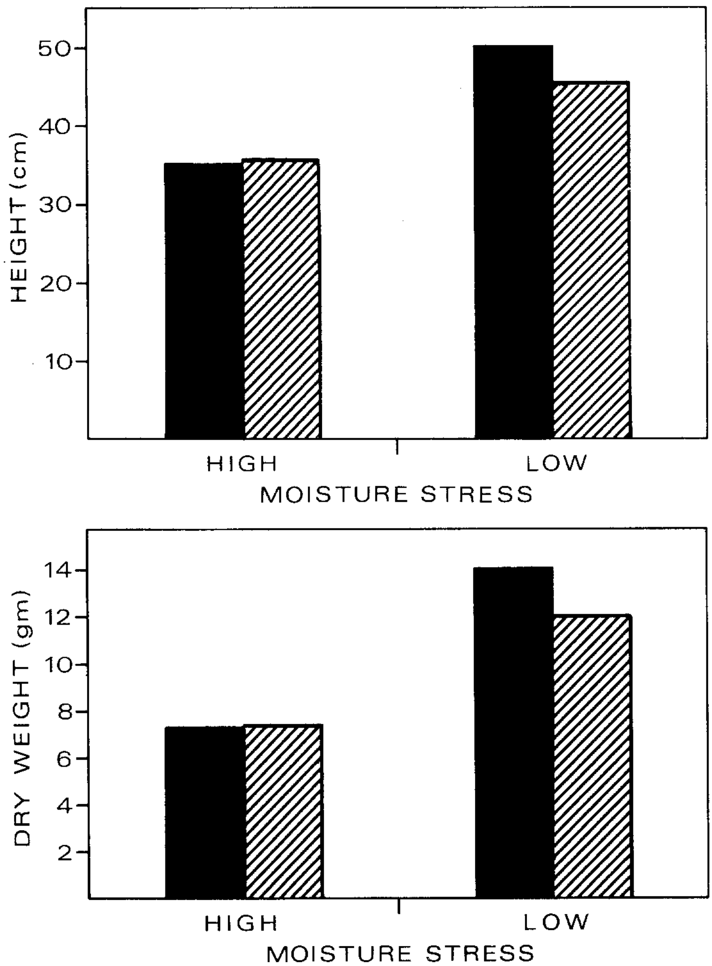

Fig. 1. Height growth and dry weight of seedlings after 20 weeks of growth under two soil moisture regimes and in the absence (*solid bars*) or in the presence of fescue leachate (*cross-hatched bars*).

Although soil moisture stress was the dominant variable in this study, response to moisture stress depended on the level of a corresponding variable. For example, fertilization affected seedling survival under high moisture stress but did not under low stress. This response may have resulted from reduced root growth when exposed to high stress; root dry weights of seedlings growing under high moisture stress averaged 50 percent less than those growing in moist conditions. In an earlier study, Dickson[2] also found that fertilization of walnut seedlings under severe moisture stress resulted in reduced root growth. However, there was no other response to fertilization for any of the seedling characteristics evaluated including root dry weights. Lack of

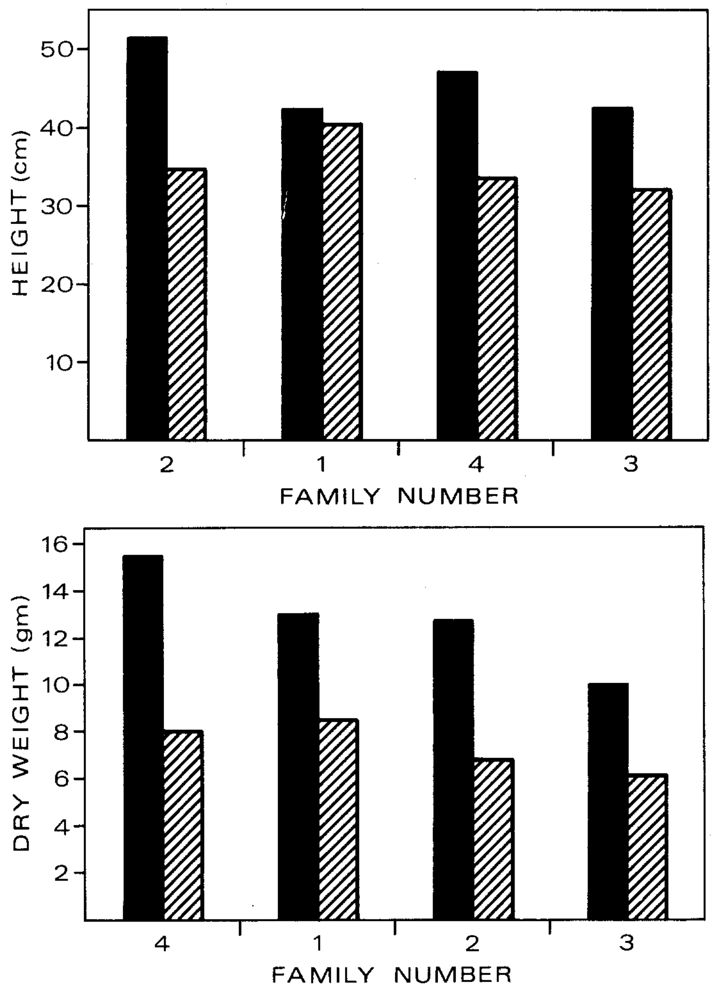

Fig. 2. Height growth and dry weight of black walnut seedlings of four half-sib families to low (*solid bars*) and high soil moisture stress (*cross-hatched bars*).

a fertilizer effect may result from early seedling nutritional requirements being met by cotyledonary reserves; in pecan (*Carya illinoensis* (Waugh) K. Koch), seedling growth was dependent on cotyledons for at least 3 and perhaps as long as 6 weeks after germination[15].

The reduction in seedling height growth and dry weight in the presence of fescue leachate under moist conditions indicates that fescue leachate is phytotoxic to walnut seedlings. Fescue leachate contained low levels of NPK (7, 0.1, 12 mg/l, respectively) and it is unlikely that such low nutrient levels resulted in mineral toxicity. However, because the leachate contained 2 to 3 grams/l of dissolved materials, it is possible that the soil osmotic potential was altered.

The interaction between moisture stress and fescue leachate is believed to result from the method of application; less fescue leachate was applied under high soil moisture stress (14 1) than under low stress (37 1) with a consequent lesser effect.

A rapidly growing body of data suggests that allelopathy affects survival and growth of trees in both plantations and natural stands[3]. Various species of *Aster*, *Solidago*, *Festuca*, *Andropogon*, and *Daucus* have all been implicated as allelopathic to one or more hardwood species. Fescue is especially important because it commonly occurs as the dominant ground cover in old fields in the Central Hardwood area and is often found in walnut plantations. It has also been shown to inhibit growth of *Robinia pseudoacacia* L.[5] and *Lquidambar styraciflua* L.[14]. In most of these cases, leachate-induced growth reductions are interpreted as evidence of allelopathy. However, since such growth reductions can also potentially arise from soil microbial activity[4], definitive statements about allelopathic effects of fescue leachate cannot be made until specific growth inhibitors are identified in the leachate.

The 10 percent reduction in height growth and 14 percent reduction in dry weight shown here is probably a conservative estimate of the effect of fescue leachate, since the fescue leachate used in this study had been stored in coolers for various periods of time and therefore may have been less phytotoxic than fresh leachates. Results of a preliminary exploratory experiment involving sweetgum seed germination demonstrated a trend for fresh fescue leachate to be more inhibitory than stored leachate.

Several studies have examined interactions between black walnut seed source and one or more aspects of interference. Mitchell[8] found significant differences in seedling size among cover types (including fescue) and open-pollinated families, but no interactions after 1 year. Todhunter and Beineke[13], however, demonstrated interactions between families and amount of fescue cover. No effort was made in either of these studies to separate the effects of competition and allelopathy. Because fescue is extremely competitive, it is possible that the interactions observed by Todhunter and Beineke[13] resulted from differential competition for soil moisture or nutrients. The present study showed no evidence of a leachate × family interaction, thus suggesting that selection for resistance to fescue phytoxicity would be difficult. It should be emphasized, however, that only four families were represented in this study and the leachate effect was relatively weak in this short-term study; therefore, these results should be considered preliminary.

The significant interaction between families and soil moisture stress for seedling height and dry weight implied that selection for tolerance to high soil moisture stress is possible. It has been shown that early selection of large seedlings may be reliable when selection is on the basis of high age-age correlations and when genetic variation in size can be separated from environmental variation[11]. In the present experiment statistical significance with only four local, random, open-pollinated families and two levels of stress implied that much more progress from selection for moisture stress is possible from larger populations. Therefore, we recommend initiation of large-scale follow-up progeny tests designed for early selection of stress resistant black walnut.

References

1 Bey C F and Funk D T 1969 Selecting and breeding black walnut for diverse sites and two intensities of culture. Proc. IUFRO/FAO World Consultation on Forest Tree Breeding 2, 12 p.

2 Dickson R E 1971 The effects of soil moisture, texture, and nutrient levels on the growth of black walnut. USDA For. Serv. Res. Pap. NC-66, 6 p.

3 Fisher R F 1980 Allelopathy: A potential cause of regeneration failure. J. For. 78, 346–348.

4 Kaminsky R 1981 The microbial origin of the allelopathic potential of *Adenostoma fasciculatum* H and A. Ecol. Monog. 5, 365–382.

5 Larson M M and Schwarz E L 1980 Allelopathic inhibition of black locust, red clover and black alder by six common herbaceous species.

6 McBride F D and Rink G A. A soil moisture control system for greenhouse plant stress studies. (*In press*)

7 McVey G R 1977 How soil chemistry can work for you. Proc. Internat. Plant Prop. Soc. 27, 277–284.

8 Mitchell R J 1980 The effects of selected cover crops on the growth, development, and mineral nutrition of black walnut (*Juglans nigra* L.). M.S. Thesis, Southern Illinois University, Carbondale, IL, 76 p. (*Unpublished*)

9 Muller C H 1969 Allelopathy as a factor in ecological process. Vegetatio 18, 348–357.

10 Namkoong G, Barnes R D and Burley J 1980 Screening for yield in forest tree breeding. Commonw. For. Rev. 59, 61–68.

11 Rink G 1984 Trends in genetic control of juvenile black walnut height growth. For. Sci. 30, 821–827.

12 Schlesinger R C and Funk D T 1977 Manager's handbook for black walnut. USDA For. Serv. Gen. Tech. Rep. NC-38, 22 p.

13 Todhunter M N and Beineke W F 1979 Effect of fescue on black walnut growth. Tree Planters' Notes 30, 20–23.

14 Walters D T and Gilmore A R 1979 Allelopathic effects of fescue on the growth of sweetgum. J. Chem. Ecol. 2, 469–479.

15 Wetzstein H Y, Sparks D and Lang G A 1983 Cotyledon detachment and growth of pecan seedlings. HortScience 18, 331–333.

16 Williams R D and Hanks S H 1976 Hardwood Nurserymen's Guide. USDA For. Serv. Agric. Handb. No. 473, 78 p.