Drought-induced leaf abscission and whole-plant drought tolerance of seedlings of seven black walnut families

WILLIAM C. PARKER and STEPHEN G. PALLARDY
School of Forestry, Fisheries and Wildlife, University of Missouri, Columbia, MO, U.S.A. 65211

Received December 31, 1984
Accepted May 6, 1985


Whole-plant drought tolerance and leaf abscission in response to drought of 5-month-old, half-sib black walnut (Juglans nigra L.) seedlings representing seven geographic origins were examined. Seedlings were subjected to six different levels of drought stress and then reirrigated. Mortality, leaf abscission, and refoliation responses were measured. Only one of 404 seedlings actually died. Survival of stem cambial tissues at predawn leaf xylem pressure potential values as low as −4.0 MPa was associated with the capacity for drought-induced leaf abscission. The percentage of seedlings exhibiting at least 80% leaf abscission increased linearly as predawn leaf xylem pressure potential decreased from −1.5 to −3.5 MPa. Although substantial differences in leaf abscission among families were observed, these differences were not statistically significant (P ≤ 0.05). Eighteen percent of all seedlings exhibited some degree of refoliation upon recovery of plant water status. However, the cumulative area of regrowth foliage was insignificant in terms of its adaptive value for resumption of photosynthetic activity following stress relief.


La tolérance à la sécheresse de la plante entière et l’abscission foliaire en réponse à la sécheresse ont été examinées chez des semis de noyers noirs (Juglans nigra L.), descendants uniparentaux âgés de 5 mois et issus de sept provenances. Les semis ont été soumis à sept niveaux différents de stress hydriques puis réhydratés par la suite. La mortalité, l’abscission foliaire et la régénération foliaire ont été mesurées. Des 404 semis impliqués dans l’expérience, un seul n’a pas survécu. La survie du tissu cambial de la tige soumise à une tension hydrique aussi basse que −4.0 MPa, au niveau foliaire peu avant l’aurore, a été associée à l’abscission foliaire induite par la sécheresse. Le pourcentage des semis qui ont présenté 80% d’abscission foliaire augmentait de façon linéaire suivant les mesures de la tension qui diminuaient, peu avant l’aurore, de −1.5 à −3.5 MPa. Même si des différences substantielles ont été observées quant à l’abscission foliaire, ces différences n’étaient pas statistiquement significatives au seuil P ≤ 0.05. La régénération foliaire s’est opérée chez 18% des semis suite à leur récupération sur le plan du statut hydrique. Toutefois, la surface cumulée de la régénération foliaire n’était pas significative quant à la valeur adaptative de la continuation de l’activité photosynthétique suivant la fin du stress.

[Intraduit par le journal]

Introduction

Desiccation avoidance of plants is a function of several physiological, morphological, and anatomical traits which serve to regulate the balance between soil moisture absorption and transpirational water loss (Turner and Begg 1981). Leaf abscission, followed by refoliation upon recovery of adequate hydration, is one drought adaptation by which many Mediterranean and desert plants avoid lethal desiccation during periods of severe drought (Orshan 1954; Kozlowski 1972). Phenological events of many tropical deciduous forest species are also closely synchronized with seasonal precipitation patterns (Addicott and Lyon 1973; Reich and Borchert 1984). Several temperate woody species also shed their leaves in response to drought (Kozlowski 1972; Pereira and Kozlowski 1976; Riev and Goren 1979). Further, slight geographic variation in the capacity for drought-induced leaf shedding has been reported among populations of woody species (Tobiessen 1971; Kellner and Tauer 1980).

Black walnut (Juglans nigra L.) exhibits leaf abscission in response to drought during both the seedling stage (Davies and Kozlowski 1977) and later as a mature forest tree (Ginter-Whitehouse et al. 1983). This study was conducted to investigate leaf abscission as a drought-tolerance adaptation (sensu Kramer 1983) in seedlings of black walnut families representing several geographic origins. The specific objectives were to examine and compare among families the following factors: (i) the relationship between predawn leaf xylem pressure potential (Ψᵢᵩₑ) and seedling mortality, (ii) the relationship between Ψᵢᵩₑ and leaf abscission, and (iii) the capacity for refoliation following drought-induced leaf drop.

Materials and methods

The study was conducted in a greenhouse during the summer of 1983 using half-sib seedlings representing seven families (Table 1). Seed from single-parent trees located across the longitudinal range of black walnut was collected in the fall of 1982, stratified, and planted upon emergence of the radicle in moist vermiculite under greenhouse conditions. Seedlings were grown in 1-L containers filled with a sterilized 2:1 sand ± silt loam soil mixture containing a controlled-release fertilizer (Osmocote, San Milpitas, CA, U.S.A.) and a thin layer (~2 cm) of pea gravel in the bottom. After 120 days, a surface application of this same fertilizer was provided. Supplemental lighting was provided in the greenhouse by 1 kW Lucalox sodium vapor lamps (General Electric, Hendersonville, NC, U.S.A.) which were programmed to give a 14 h light : 10 h dark photoperiod. Photon flux density of photosynthetically active radiation at the plant tops at solar noon was approximately 1000 µmol·m⁻²·s⁻¹. Air temperature in the greenhouse was controlled by exhaust fans set to maintain day and night temperatures at 25°C. During the hottest periods of the summer, however, daytime air temperature was closer to 35°C. Plants were maintained in a well-watered condition prior to the experiment. During this period, leaf abscission did not occur and Ψᵢᵩₑ averaged
Because of the protracted germination period of black walnut, seedlings ranged in age from 135 to 150 days at the beginning of the experiment. To partition out any effects of age, plants were arranged in a randomized complete block design with six blocks, with seedling age decreasing uniformly from block to block. Eleven plants per family were placed in each block for a total of 462 seedlings.

After a 5-month growth period, water was withheld from all seedlings and the soil was allowed to dry so that \( \psi_{10} \) values gradually fell to \(-4.0\) MPa. \( \psi_{10} \) values of \(-2.5\) to \(-4.0\) MPa are similar to those encountered in field-grown black walnut during severe drought (Hinckley et al. 1979; Lucier and Hinckley 1982; Ginter-Whitehouse et al. 1983). At \( 0.5 \pm 0.5 \) MPa intervals between \(-1.5\) and \(-4.0\) MPa, between 9 and 12 seedlings per family were reirrigated and kept in a well-watered condition for a period of 6 weeks. It proved difficult to sample seedlings at selected \( \psi_{10} \) values, as some plants exhibited leaf drop at stress levels between the \( \psi_{10} \) designated for study. Seedlings for which \( \psi_{10} \) exceeded the limits noted above were not included in the statistical analysis. Further, it was not always possible to expose at least nine seedlings of some families to \( \psi_{10} \) at or below \(-3.5\) MPa as total leaf drop had already occurred at a higher \( \psi_{10} \). Therefore, leaf material for \( \psi_{10} \) determination was sometimes unavailable. For these reasons, whole-plant drought response could only be assessed on 404 of the total 462 seedlings.

Leaf abscission typically began with the basal leaves and progressed upward toward the stem apex at a similar rate in all families. While most seedlings exhibited total loss of foliage during drought, some plants retained the top 1 or 2 (of approximately 10) leaves. So that abscission could be more accurately quantified, a loss of greater than 80% of the original foliage, rather than total abscission, was selected as a measurement criterion. Within each particular \( \psi_{10} \) increment, the number of plants that exhibited 80% abscission was noted 2 weeks after rewatering. For all families, seedlings which did not exhibit any leaf abscission in response to mild drought (\( \psi_{10} = -1.5\) MPa) also showed no leaf drop after watering was resumed. This result indicated that increased abscission induced as the soil dried further was a response to drought and not a reflection of normal phenology.

At the end of the 6-week rewatering period, mortality and the number of seedlings exhibiting sprouting from the root collar or flushing of terminal and lateral buds were determined at each level of previously imposed drought stress. Mortality was assessed through visual examination of cambial tissues near the root collar. As black walnut seedlings typically exhibit sprouting from the root collar in response to shoot dieback (Fowells 1965; Schlesinger and Funk 1977), the presence of viable tissues in this region was judged as indicative of seedling survival.

A pressure chamber was used to measure \( \psi_{10} \) on single leaflets (Ritchie and Hinckley 1975). As seedling \( \psi_{10} \) began to decline, it became necessary to make some 75 to 90 \( \psi_{10} \) determinations per sample day. To accommodate this large number of \( \psi_{10} \) measurements, groups of 10–15 leaflets were stored in each of six humidified plastic bags and kept in a refrigerator at 2°C until leaf xylem pressure potential (\( \psi_{x} \)) was determined. Using this technique, it was found that after 3 h of storage, \( \psi_{x} \) did not deviate by more than 0.1 MPa from that at time zero. Similar results have been reported by Kaufmann and Thor (1982), who found that the storage of needles of several conifer species in a cool, humid environment resulted in little change in \( \psi_{x} \) over a 4-h period.

A \( \chi^{2} \) test of a \( 2 \times 7 \) contingency table was performed to assess family differences in abscission and refoliation within individual \( \psi_{10} \) treatments. Lewontin and Felsenstein (1965) have suggested that \( \chi^{2} \) tests for \( 2 \times c \) contingency tables (where \( c \geq 3 \)) remain robust only if expected values of cells are greater than 0.5. As a consequence, \( \chi^{2} \) tests could not be performed on some data where leaf abscission was slight or total.

### Results

During the course of this study, only 1 seedling (of 404) died as a result of drought stress, despite \( \psi_{10} \) values that reached \(-4.0\) MPa for some seedlings. A general drought response of seedlings was leaf abscission, followed in some cases, by flushing of the terminal and a few lateral buds 2–3 weeks after watering was resumed. Only one seedling that exhibited leaf abscission sprouted from the root collar. As a consequence of the absence of mortality, only family differences in abscission and refoliation were analyzed. Age-related effects on drought response of seedlings were not significant (\( p = 0.30 \)), most likely because seedlings were typically within 7–10 days in age of one another and because the study was conducted after the period of active shoot growth. Hence, any differences in abscission or refussing were associated with family differences in drought tolerance.

The percentage of seedlings exhibiting leaf abscission increased almost linearly as \( \psi_{10} \) fell from \(-1.5\) to \(-3.5\) MPa. However, differences in abscission response occurring among families were not statistically significant (Fig. 1). A \( \psi_{10} \) value of \(-1.5\) MPa induced 80% abscission or more in about 8% of all plants, with abscission scores ranging from 0% in NY and ONTX seedlings to 20% in IA7 seedlings. Considerably greater abscission was observed at a \( \psi_{10} \) of \(-2.0\) MPa. While families did not significantly differ in the degree of abscission experienced at this stress level, a trend toward more-
nounced abscission in IA7 seedlings (p = 0.06) was observed. The abscission observed in M15 seedlings was abnormally high at -2.0 MPa, higher than that observed at -2.5 or -3.0 MPa. To avoid any contribution of this apparently anomalous result in the statistical analysis, data for this family were not included in the χ² test performed at -2.0 MPa. Sixty-three percent of all seedlings subjected to a ψ₀₀ treatment of -2.5 MPa exhibited greater than 80% leaf abscission, with the values among families in leaf abscission ranging from 40 to 80% in seedlings of M12 and IA7, respectively. At a ψ₀₀ of -3.0 MPa, greater than 80% leaf abscission was observed in about 83% of all seedlings.

Total abscission in almost all seedlings resulted at a ψ₀₀ of -3.5 MPa or less. Apparent variation in abscission among families at low values of ψ₀₀ and percentage values less than 100% were probably a consequence of two contributing factors. First, measurement of ψ₀₀ with the pressure chamber became quite variable when senescent leaves were sampled. This variability was probably attributable to the effects of biochemical degradation of membranes and other internal processes associated with leaf senescence (Beevers 1976). As a result, measured ψ₀₀ values may not have accurately estimated the true soil moisture availability to which seedlings were responding under extreme drought stress. Secondly, sample size for certain families at these levels of stress was very small (two to five), as abscission was nearly complete before attainment of low ψ₀₀ levels.

The percentage of seedlings which exhibited flushing by terminal and lateral buds following leaf abscission averaged 17.8% with significant differences (p = 0.05) among families (data not shown). Lack of refoliation was not a result of seedling mortality which, as noted earlier, was observed in only one seedling. Newly produced foliage was quite different in form and appearance from original leaves. Leaflets and individual leaves were generally smaller and shorter, with leaflets showing slight surface undulations. However, because the area of this regrowth foliage never exceeded 5% of the original leaf area, it seems doubtful whether family differences in this character could result in differential photosynthetic capacity during postdrought periods; therefore, these data are not presented. Further, the percentage of seedlings which produced new foliage following abscission was not associated in any well-defined manner with the value of ψ₀₀ developed during drought.

Discussion

Black walnut seedlings exhibited leaf abscission in response to decreasing ψ₀₀, a response that was associated with the capacity to survive drought stress as severe as any observed in the field for this species (Hinckley et al. 1979; Lucier and Hinckley 1982; Ginter-Whitehouse et al. 1983). The total percentage of plants which experienced greater than 80% leaf abscission increased from 8.1% at a ψ₀₀ of -1.5 MPa to nearly 100% at a ψ₀₀ of -3.5 MPa or less. Leaf senescence, followed by leaf drop, generally occurred first in the older, more basal leaves and progressed acropetally. The lack of statistically significant variation among families despite large numerical differences in abscission response was probably attributable to the small cell sample size necessitated by physical limitations. It is likely that the variation in abscission shown at -2.0 MPa represents a real difference among families.

In general, leaf abscission appears to be an important means by which the carbon and water economy of black walnut are regulated. Abscission of older, more basal leaves representing a nearly 60% reduction in leaf area resulted in a seasonal increase in root:shoot ratio in 1st-year black walnut seedlings grown under optimal soil moisture conditions (Carpenter and Hanover 1974). Large root:shoot ratios have been associated with increased drought tolerance and survival (Bhan et al. 1973; Venator 1982). Gradual decreases in leaf area with declining soil moisture availability likely represent an adaptation by which a more favorable water balance can be maintained in remaining leaves. Progressive abscission of lower leaves beginning at the onset of drought was associated with a consistently higher ψ₀₀ and stomatal conductance in a mature black walnut tree than in either white oak (Quercus alba L.) or eastern red cedar (Juniperus virginiana L.) located on the same site (Ginter-Whitehouse et al. 1983). These data and those of others suggest that in black walnut, shedding of foliage of lower photosynthetic activity and the presence of an expansive, deeply penetrating and efficiently absorbing root system (Holch 1931; Hinckley et al. 1979; Sprackling and Read 1979; Lucier and Hinckley 1982; Ginter-Whitehouse et al. 1983) result in a reduction in whole-plant water use, while high rates of CO₂ uptake per unit leaf area are maintained.

Black walnut is most commonly found growing in more mesic environments such as coves, lower slopes, and floodplains and only occasionally occurs on drier, less productive, upland sites (Fowells 1965). Its growth rate is relatively sensitive to soil conditions, with soil depths of 3–5 ft (1 ft = 0.3048 m) being reported as critical to successful black walnut cultivation (Fowells 1965; Schlesinger and Funk 1977; Ponder 1982). Dependence on deep rooting and leaf abscission as drought-tolerance adaptations may partly explain the greatly reduced growth (Carmean 1966) and survival of black walnut on suboptimal sites.

On deep soils, the expansive root system of this species may assure avoidance of the ψ₀₀ stimulus for accelerated abscission for most or all of the growing season. In the event of complete leaf abscission during severe drought, maintenance of competitive rates of seasonal CO₂ uptake are assured for long unstressed periods by a photosynthetic rate comparable to that of associated species (Larcher 1969; Carpenter and Hanover 1974).

On sites with shallow soils where tap root penetration is inhibited and the root system is confined to a smaller soil volume, the desiccation-avoidance capacity of black walnut may be greatly reduced. Rapid depletion of moisture in this smaller volume would result in a more rapid development of ψ₀₀ values sufficiently low to induce accelerated leaf abscission during relatively short periods of drought. As black walnut seedlings exhibited no capacity for significant refoliation upon recovery of plant water status, a greater predisposition for leaf abscission would reduce survival and limit growth on these sites.

Acknowledgements

The assistance of the New York State Department of Environmental Conservation, the State of Michigan Department of Natural Resources, the Iowa Conservation Commission, and the Ontario Ministry of Natural Resources in collecting seed is gratefully acknowledged. We also thank James N. Burroughs for his help with the experimental design and statistical analysis.


