Plant, Cell and Environment (1985) **8**, 219–222

SHORT COMMUNICATION

Morphological and physiological changes in black alder induced by water stress

J. R. SEILER School of Forestry and Wildlife Resources, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, U.S.A.

Received 31 October 1984; accepted for publication 21 November 1984

Abstract. Black alder seedlings were exposed to 12 weeks of sublethal water stress by watering only when visibly wilted. Control seedlings were watered regularly throughout the treatment period. Stressed seedlings exhibited significant osmotic adjustment of over 0.4 MPa. The water stress treatment also significantly reduced leaf size, increased epicuticular wax content, and increased the root–shoot ratio. The response of leaf conductance to decreasing leaf water potential was influenced by the previous water stress treatment. Stressed seedlings had a much lower initial leaf conductance, but showed a gradual drop in leaf conductance as leaf water potential decreased; whereas, control seedling leaf conductance fell rapidly. These morphological and physiological modifications in response to moisture stress have the potential for significantly improving black alder drought tolerance.

Key-words: *Alnus glutinosa;* Betulaceae; osmotic adjustment; leaf conductance.

Numerous morphological and physiological factors are involved in the overall drought avoidance of woody plants. These factors involve mechanisms that increase absorption or reduce water loss and may include: maintenance of water status in the plant by osmotic adjustment, water storage, and reduction in cell size; reduction of water loss by stomatal closure, wax deposition on leaf surfaces and abscission of leaves; maintenance of water absorption during periods of low soil moisture through the production of deep, highly branched root systems and reduction of the energy load on leaves by production of smaller or fewer leaves, changes in leaf angle and increases in convective cooling (Hinckley *et al.*, 1981; Kozlowski, 1982; Kramer, 1983a). Prior exposure to water stress often modifies these factors responsible for drought tolerance, ultimately resulting in improved drought tolerance or acclimation to water stress (Clemens & Jones, 1978; Matthews & Boyer, 1984; Osonubi & Davies, 1978; Tibbits & Bachelard, 1981).

Black alder [*Alnus glutinosa* (L.) Gaertn.], an actinorhizal, nitrogen-fixing, woody plant capable of reaching commercial timber size has shown strong potential for use in many forestry systems (Gordon & Dawson, 1979). However, this species is very intolerant of water deficits, particularly as a young seedling (McVean, 1956a, b). This study examined the possibility of improving black alder's drought tolerance by exposing seedlings to prolonged, sublethal water stress. Several morphological and physiological parameters known to be important in drought tolerance were evaluated.

Black alder seeds were germinated and grown in a greenhouse in Ray Leach Super Cells (164 cm^3, 4 cm diameter, 21 cm long) for a 5-week period. During this initial growth period seedlings were propagated and inoculated as described previously (Seiler & Johnson, 1984). Seedlings were then transplanted into 1 dm^3 (11 cm diameter) plastic pots containing a nursery soil with a loamy-sand texture. Following 4 more weeks of growth under the conditions described previously (Seiler & Johnson, 1984), one-half of the seedlings were subjected to 12 weeks of sublethal water stress where seedlings were watered only when visibly wilted. The remaining seedlings continued to be watered daily and served as controls.

For the final 2 weeks of the treatment period seedlings were moved into a controlled environment chamber at 23 °C day/17 °C night, a relative humidity of 55% and a 16-h photoperiod with an average of 150 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) at the top of the plant canopy.

Following the treatment period the leaf surface area of the second fully expanded leaf from the terminal apex was measured. To avoid destructive sampling of the leaf, the leaf shape was traced onto paper, cut out, and measured on a model AAM Automatic Area Meter (Far East Mercantile Corp., New York). Leaves from six plants per treatment were measured.

The epicuticular wax content was determined on four seedlings per treatment. The first fully expanded leaf produced during the treatment period was removed from the seedlings and the outline traced on paper for later determination of surface area. Wax was removed from the leaf surface by dipping it for 30 s into 5 cm^3 of chloroform. The chloroform-wax mixture was then filtered through Whatman no. 1 filter paper into a preweighed glass test tube. The chloroform was then evaporated to dryness under nitrogen and the test tube reweighed to determine net wax content (Trimble *et al.*, 1982). Wax was expressed as mg cm^{-2} of leaf surface area.

Leaf water potential components were measured on six seedlings per treatment. All seedlings were watered and leaf water potential measured the following day with a pressure chamber (Scholander *et al.*, 1965). Immediately following the water potential measurement, four leaf strips (approximately 0.3 m × 3.5 cm) cut from interveinal tissue were placed into chambers, frozen in liquid nitrogen and osmotic potential measured using thermocouple psychrometers (model PST55-05, Wescor, Inc., Logan, UT). Sample chambers and procedures were as described previously (Seiler & Johnson, 1984). Turgor potential was estimated as the difference between water and osmotic potential measurements.

To determine the response of leaf conductance to decreasing leaf water potential, three seedlings per treatment were rewatered and drought imposed by withholding water for 2 weeks. Leaf conductance was measured over the 2 weeks as soil water was depleted. This parameter was measured on the same leaf over the course of the experiment using a steady state porometer (Null balance porometer, Interface Instruments, Corvallis, Oregon) followed immediately by a leaf water potential measurement taken on a separate leaf using a pressure chamber. Conductance was measured at ambient humidity (55%) with an average vapour pressure deficit of 1.37 (±0.15) kPa.

Shoot and root dry weight, and root–shoot ratio (g/g) were determined on four seedlings from each treatment after oven drying at 60 °C to a constant weight.

The experiment was arranged as a completely randomized design. Leaf surface area, cuticle wax content, water potential components, and biomass parameters were separated statistically using a *t*-test. Leaf conductance data were analysed using regression techniques.

The water stress treatment reduced leaf osmotic potential over 0.40 MPa, resulting in a significant increase in turgor potential (Table 1). Osmotic adjustment of this magnitude has not been reported previously in black alder; however, Seiler & Johnson (1984) found a decrease in osmotic potential of 0.13 MPa in black alder plants stressed similarly for 5 weeks. It is probable that the longer treatment period used in this study resulted in the further decrease in osmotic potential.

This shift in osmotic potential in water stressed seedlings has important implications for improving black alder drought tolerance. Osmotic adjustment allows the maintenance of both water potential gradients and turgor in plants during prolonged periods of water stress, thus allowing for a continuation of photosynthesis, stomatal opening, and growth (Kramer, 1983b, Turner & Jones, 1980).

Cuticular wax content was enhanced significantly by the water stress treatment, increasing over 60% from 0.086 in control seedlings to 0.138 mg of wax per cm^2 of leaf area in stressed seedlings (Table 2). A similar effect has been found in cotton and soybean where wax deposition was increased through water stress (Clark & Levitt, 1956; Weete *et al.*, 1978).

Levitt (1980) reported that mesic plants, such as black alder, may lose up to half as much water through the cuticle, with stomata closed as they do with stomata open. The importance of leaf waxes in determining water loss through the cuticle has been emphasized by Kozlowski (1982) and Pallardy (1982). Therefore, this response could play a

Table 1. Leaf water potential components of black alder seedlings as affected by 12 weeks of water stress*

Treatment	Water potential	Osmotic potential	Turgor potential
		(MPa)	
Control	−0.63 ± 0.06† A	−1.64 ± 0.08 A	1.03 ± 0.09 A
Water stressed	−0.70 ± 0.16 A	−2.07 ± 0.16 B	1.37 ± 0.15 B

*Water stressed seedlings were watered only when visibly wilted; means followed by the same letter within a column do not differ significantly ($\alpha = 0.05$).

†SE.

Table 2. Black alder seedling morphology as influenced by 12 weeks of water stress*

Treatment	Epicuticular wax content (mg cm^{-2})	Surface area per leaf (cm^2)	Shoot weight (g)	Root weight (g)	Root/shoot ratio (g/g)
Control	0.086 ± 0.01†A	87.2 ± 6.9 A	5.67 ± 0.50 A	5.86 ± 0.28 A	1.05 ± 0.06 A
Water stressed	0.138 ± 0.02 B	28.3 ± 1.3 B	2.50 ± 0.14 B	3.52 ± 0.20 B	1.42 ± 0.09 B

*Water stressed seedlings were watered only when visibly wilted; means followed by the same letter within a column do not differ significantly ($\alpha = 0.05$).

†SE.

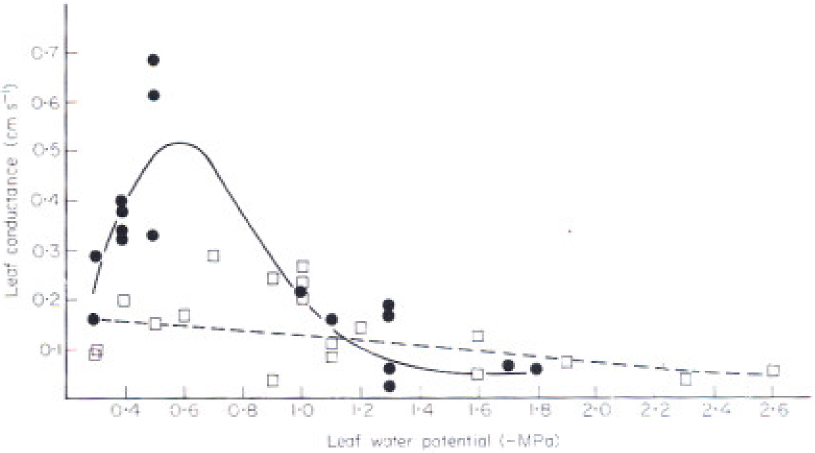

Figure 1. Black alder leaf conductance as affected by leaf water potential and water stress conditioning. Control (●, ——), $\log_e G_l = 3.6 - 8.4\ (WP) + 3.22\ (WP^2) + 3.76\ \log_e\ (WP)$. $R^2 = 0.69$. Stressed (□, - - -), $\log_e G_l = -1.81 - 0.227\ (WP^2)$, $R^2 = 0.35$. WP = water potential, G_l = leaf conductance.

significant role in improving black alder's water conservation, particularly when water stress is developing and stomata are beginning to close.

Water stress in black alder resulted in the production of significantly smaller leaves. Surface area of leaves on stressed seedlings was reduced over 67%, compared to control seedlings (Table 2). In addition, although no actual data were collected, stressed plants were observed to also produce fewer new leaves.

Stressed seedling shoot biomass was reduced more by water stress than was root biomass, resulting in a large increase in root–shoot ratio (Table 2). The relationship between root absorptive capacity and shoot transpirational demand is an important factor in the ability of plants to survive and grow under conditions of soil and atmospheric water stress (Pallardy, 1982). Therefore, reduced leaf size and increased root–shoot ratio are likely to result in an improved water balance, particularly when soil water begins to become limiting.

Smaller leaf size has often been associated with species or seed sources from xeric sites. Smaller leaves not only result in reduced transpirational area, but also improve heat exchange and lessens the distance that water must travel from main xylem to the outermost edge of a leaf (Pallardy, 1982). All of these factors are likely to improve seedling performance during periods of water deficit.

The prior water stress treatment significantly influenced the response of leaf conductance to decreasing leaf water potential (Fig. 1). The lines presented in Fig. 1 are based on regression equations developed by first screening variables using stepwise regression procedures. Final equations were chosen based on residual analysis, C_p statistics, and significance of slope estimates. The lines are presented to illustrate the general trends.

Control seedling leaf conductance was suppressed at the highest water potentials. This initial low leaf conductance is difficult to explain. However, it could be related to the turgor balance between subsidiary cells, epidermal cells and guard cells. Possibly at these highest water potentials, subsidiary cells or surrounding epidermal cells maintain an advantage over guard cells in terms of turgor potential, with the result being decreased leaf conductance. Turgor balance between guard cells, subsidiary cells and epidermal cells has been implicated in the control of stomatal conductance in past studies (Edwards, Meidner & Sheriff, 1976; Johnson & Ferrell, 1983).

Another explanation may be that the initial low leaf conductance was due to soil flooding immediately following rewatering and is simply a lag in the recovery from flooded soil conditions. Whatever the case, conductance quickly recovered, and reached values over 65% higher than stressed seedlings. However, conductance in control seedlings fell very quickly and few measurements could be taken below −1.3 MPa due to severe wilting.

Water-stressed seedlings had much lower conductance values which would greatly reduce transpiration and water use. These seedlings were able to maintain leaf conductance for a longer time and to much lower leaf water potentials. Seedlings did not wilt until water potentials were well below −1.8 MPa and one seedling maintained turgor and conductance to a water potential of −2.6 MPa.

In pre-stressed seedlings, little or no recovery in leaf conductance occurred upon rewatering (Fig. 1). This stable decrease in leaf conductance as a result of the previous water stress treatment has also been found in loblolly pine (*Pinus taeda* L.) (Seiler, 1984). Control of water loss through changes in leaf conductance is known to be an important factor affecting drought tolerance of tree species (Bennett & Rook, 1978; Kozlowski, 1982). The decrease in leaf conductance would enable pre-stressed seedlings to conserve water and function longer during periods of water deficit but would however limit photosynthesis.

Data in Fig. 1 does not appear to support the conclusion that the increased wax content which occurred in the stressed seedlings (Table 2) is actually decreasing cuticular transpiration. Minimum leaf conductance under stress (stomata closed) in both treatments is approximately equal, roughly 0.05 cm s^{-1}. However, leaf conductance under near zero turgor conditions may not accurately reflect cuticular transpiration in a fully rehydrated condition. Possibly, at the higher water potentials, cuticular transpiration is lower in pre-stressed seedlings and this may be contributing to the lower overall leaf conductance found in pre-stressed seedlings (Fig. 1). Data, however, to support this conclusion was not obtained.

In conclusion, exposing black alder seedlings to prolonged, sublethal water stress resulted in the modification of numerous physiological and morphological parameters, all important in drought tolerance. These modifications acting in combination with one another show the potential for greatly improving the water conservation and overall drought tolerance of black alder seedlings.

References

Bennet, K.J. & Rook, D.A. (1978) Stomatal and mesophyll resistances in two clones of *Pinus radiata* D. Don known to differ in transpiration and survival rate. *Australian Journal of Plant Physiology*, **5,** 231–238.

Clark, J.A. & Levitt, J. (1956) The basis of drought resistance in the soybean plant. *Physiologia Plantarum*, **9,** 598–606.

Clemens, J. & Jones, P.G. (1978) Modification of drought resistance by water stress conditioning in *Acacia* and *Eucalyptus*. *Journal of Experimental Botany*, **29,** 895–904.

Edwards, M., Meidner, H. & Sheriff, D.D.W. (1976) Direct measurements of turgor pressure potentials of guard cells. *Journal of Experimental Botany*, **27,** 163–171.

Gordon, J.C. & Dawson, J.O. (1979) Potential uses of nitrogen-fixing trees and shrubs in commercial forestry. *Botanical Gazette*, **140(s),** 588–590.

Hinckley, T.M., Teskey, R.O., Duhme, F. & Richter, H. (1981) Temperate hardwood forests. In *Water Deficits and Plant Growth*, Vol. 6. (ed. T. T. Kozlowski), pp. 153–208. Academic Press, New York.

Johnson, J.D. & Ferrell, W.D. (1983) Stomatal response to vapour pressure deficit and the effect of plant water stress. *Plant, Cell and Environment*, **6,** 451–456.

Kozlowski, T.T. (1982) Water supply and tree growth, part I. water deficits. *Forestry Abstracts*, **43,** 57–95.

Kramer, P.J. (1983a) *Water Relations of Plants*. Academic Press, New York.

Kramer P.J. (1983b) Water stress research: progress and problems. In *Current Topics in Plant Biochemistry and Physiology*, Vol. 2 (eds D. R. Randall, D. G. Blevens, R. L. Larson & B. L. Rapp), pp. 129–144. University of Missouri-Columbia, Columbia.

Levitt, J. (1980) *Responses of Plants to Environmental Stresses*, Vol. 2. Academic Press, New York.

Matthews, M.A. & Boyer, J.S. (1984) Acclimation of photosynthesis to low leaf water potentials. *Plant Physiology*, **74,** 161–166.

McVean, D.N. (1956a) Biological flora of the British Isles: *Alnus glutinosa* (L.) Gaertn. *Journal of Ecology*, **41,** 447–466.

McVean, D.N. (1956b) Ecology of *Alnus glutinosa* (L.) Gaertn. III, seedling establishment. *Journal of Ecology*, **44,** 195–218.

Osonubi, O. & Davies, W.J. (1978) Solute accumulation in the leaves and roots of woody plants subject to water stress. *Oecologia*, **32,** 323–332.

Pallardy, S.G. (1982) Closely related woody plants. In *Water Deficits and Plant Growth*, Vol. 6. (ed. T. T. Kozlowski), pp. 511–548. Academic Press, New York.

Scholander, P.F., Hammel, H.T., Bradstreet, E.D. & Hemmingsen, E.A. (1965) Sap pressure in vascular plants. *Science*, **148,** 339–346.

Seiler, J.R. (1984) Physiological response of loblolly pine seedlings to moisture-stress conditioning and their subsequent performance during water stress. PhD. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Va.

Seiler, J.R. & Johnson, J.D. (1984) Growth and acetylene reduction of black alder seedlings in response to water stress. *Canadian Journal of Forest Research*, **14,** 477–480.

Tibbits, W.N. & Bachelard, E.P. (1981) Effects of fertilizer and frequency of watering on the internal water relations of seedlings of *Angophora costata* and *Banksia serrata*. *Australian Forestry Research*, **11,** 23–34.

Trimble, J.L., Skelly, J.M., Tolin, S.A. & Orcutt, D.M. (1982) Chemical and structural characteristics of the needle epicuticular wax of two clones of *Pinus strobus* differing in sensitivity to ozone. *Phytopathology*, **72,** 652–656.

Turner, N.C. & Jones, M.M. (1980) Turgor maintenance by osmotic adjustment. In *Adaptations of Plants to Water and High Temperature Stress* (eds N. C. Turner & P. J. Kramer), pp. 87–103. Wiley and Sons, New York.

Weete, J.D., Leek, G.L., Peterson, C.M., Currie, H.F. & Branch, W.D. (1978) Lipid surface wax synthesis in water-stressed cotton leaves. *Plant Physiology*, **62,** 675–677.