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Drought Tolerance and the Physiological Mechanisms of Resistance in Northern Coniferous Seedlings

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ABSTRACT

Abscisic acid and paclobutrazol applied for 2 weeks prior to stress induced a marked increase in the resistance of jack pine (*Pinus banksiana* Lamb.) seedlings to prolonged drought. Pretreatment caused rapid stomatal closure, promoted more favorable water potentials, enhanced water retention and increased survival. Treatment of the actively growing seedlings with paclobutrazol for a full month before exposure to drought conveyed greater stress protection than pretreatment for 2 weeks. In both cases, however, paclobutrazol produced compact, waxy-looking, short-needled seedlings with thickened roots. The treated seedlings also displayed lower water-saturated needle volumes, larger bulk elastic modulus values and higher turgor potentials than the untreated controls during drought. In subsequent tests of three coniferous species, maximum differences in survival were observed late in the drought period with values in 30-day, paclobutrazol-treated, actively growing seedlings versus those in the untreated controls of 100/13%, 100/0%, and 66/0% in white spruce (*Picea glauca* [Moench] Voss), jack pine and black spruce (*Picea mariana* [Mill.] B.S.P.), respectively. Dormant (i.e., in bud set) black spruce seedlings treated with paclubutrazol for 1 month prior to drought exposure were completely protected (100% survival) against drought conditions that were lethal to 87% of all controls.

RÉSUMÉ

Le traitement à l'acide abscissique et au paclobutrazol de semis de pin gris (*Pinus banksiana* Lamb.) pendant 2 semaines avant l'induction d'un stress causé par la sécheresse a provoqué une augmentation marquée de leur résistance à une sécheresse prolongée. Leur traitement préalable a entraîné une fermeture rapide des stomates, favorisé un meilleur potentiel hydrique et amélioré la rétention d'eau et la survie. Le traitement préalable au paclobutrazol des semis en période de croissance active pendant 30 jours avant leur exposition à la sécheresse a assuré une meilleure protection que le traitement préalable pendant 2 semaines.

Le paclobutrazol a toutefois donné dans les deux cas des semis à aiguilles courtes, serrées et d'aspect cireux, à racines épaissies. Pendant la période de sécheresse, les semis traités présentaient également des volumes plus faibles d'aiguilles saturées en eau, des coefficients d'élasticité apparente plus élevés et un potentiel de turgescence plus élevé que les semis témoins non traités. Les différences maximales de survie ont été observées à la fin de la période de sécheresse, les taux de survie des semis traités au paclobutrazol par opposition aux semis non traités étant de 100 et 13 %, de 100 et 0 % et de 66 et 0 % chez l'épinette blanche (*Picea glauca* [Moench] Voss), le pin gris et l'épinette noire (*Picea mariana* [Mill.] B.S.P.), respectivement. Les semis d'épinette noire dormants (c'est-à-dire dont les bourgeons sont en période de dormance, donc recouverts d'écailles) traités au paclobutrazol pendant un mois avant leur exposition à la sécheresse ont été entièrement protégés (taux de survie de 100 %) contre la sécheresse qui a entraîné la mort de 87 % de tous les semis témoins.

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INTRODUCTION

Drought is a major cause of death in seedlings of forest trees that have been grown in a greenhouse and transplanted to a natural setting. Sudden environmental stress is often lethal to plants that have never encountered adverse growing conditions; however, levels of heat and drought that are normally lethal *can* be tolerated by plants that have been conditioned by exposure to sub-lethal levels of stress or treated with growth-regulating compounds. Thus, many plants have an innate potential for substantial stress resistance that may not be apparent in moderate environments. In nature, plants that have developed drought resistance have often undergone morphological changes that occur in response to dry environments (Levitt 1980). Therefore, chemical or cultural treatments that induce stress-adapted morphological types may be powerful tools in activating a plant's natural drought resistance mechanisms.

Resistance to drought is mediated both by drought avoidance mechanisms that regulate the uptake and loss of water from plant tissues and by drought tolerance mechanisms that convey cellular resistance and permit survival of plants in a dry environment. Coniferous seedlings can display both mechanisms and undergo a variety of morphological, biochemical and biophysical changes in response to moisture stress; white spruce (*Picea glauca* [Moench] Voss), for example, can halt the growth process and develop glaucous foliage in response to the gradual onset of drought. Moreover, conifers and other higher plants usually accumulate large amounts of abscisic acid (ABA) in response to moderate and severe moisture deficits, resulting in a reduction in stomatal conductance, thus reducing transpirational water loss; this change may continue for some time after stress is relieved (Roberts and Dumbroff 1986). The accumulation of specific free amino acids in response to drought is common in black spruce (*Picea mariana* [Mill.] B.S.P.), white spruce and jack pine (*Pinus Banksiana* Lamb.) and may constitute a mechanism for increasing drought tolerance at the subcellular level (Cyr et al. 1990).

All three coniferous species develop osmotic potentials that tend to favor the acquisition of water during moisture stress, and their shoots are able to maintain turgor pressure even as their moisture contents decline (Buxton et al. 1985). Moreover, the roots of jack pine can be rapidly desiccated and yet maintain turgor, apparently by a surprisingly large adjustment of their elastic modulus, which seems to be related to the synthesis of a specific set of proteins (Marshall 1990).

Physiological investigations of the drought-stress response in conifers, conducted at the Molecular Plant Physiology and Biochemistry Laboratory of the University of Waterloo, suggest that the fundamental mechanisms of drought adaptation include: (1) the alteration of hormone metabolism, (2) accumulation of metabolites that apparently protect against stress, (3) alteration of gene expression, (4) a reduction in shoot growth, (5) osmotic adjustment, (6) the regulation of gas exchange, (7) a reduction in the volume of water-saturated tissue, and (8) an increase in the elastic modulus of the cell wall. These insights have now been used to develop tentative cultural and chemical techniques for making greenhouse-grown planting stock more resistant to environmental stress. This report describes the effects of four classes of preconditioning treatments on seedlings: adaption to elevated salt levels, manipulation of photoperiods and temperature regimes, the use of antitranspirants, and application of ABA or paclobutrazol (a triazole-type growth regulator) to induce resistance to drought and to a combination of intense heat and prolonged drought.

MATERIALS AND METHODS

Ion-loading, Antitranspirant, Light and Temperature Studies

Ion-loading Tests

Seedlings of white spruce were watered with 120 mM (millimolar) KCl, black spruce were watered with 60 mM KCl and 45 mM $CaCl_2$ and jack pine were watered with 40 mM KCl and 30 mM $CaCl_2$ at 5 days and 1 day before the imposition of a drought caused by withholding water.

Antitranspirant Test

Seedlings of all three species were sprayed with the anti-transpirant Folicote™ (Crystal Soap and Chemical Company, Lansdale, Pennsylvania) until runoff occurred immediately prior to the imposition of drought by withholding water.

Combination of Ion-loading and Antitranspirant Test

Jack pine seedlings were watered with 80 mM $CaCl_2$ and white spruce and black spruce were watered with 80 mM KCl 1 week prior to the start of the test. All seedlings were sprayed with Folicote™ immediately before imposing drought by withholding water.

Light and Temperature Test

Seedlings were placed in a growth chamber with a 4-hour photoperiod, and temperature was gradually lowered to 6°C over a period of 3 days. After 3 weeks, the seedlings were transferred to a second chamber for the drought test.

Paclobutrazol and ABA Studies

Plant Material

Seeds of jack pine (source 32-00-0-00 and lot 75-301), white spruce (source 43-00-0-00 and lot 71-372), and black spruce (source 43-00-0-00 and lot 75-594) were obtained from the Ontario Ministry of Natural Resources. The seeds were disinfected for 15 min in 3% (v/v) hydrogen peroxide, thoroughly rinsed in distilled water and then stratified at 4°C for either 24 hours (jack pine), or for 2 weeks (spruces) before sowing three seeds per tube in RIGI-POT model 67-50 plastic forms (IPL Products Ltd., Brampton, Ontario) lightly filled with a 3:2:2 mixture of peat, perlite and vermiculite. After emergence, the seedlings were thinned to one per tube and grown in a greenhouse or growth chamber.

Growth Chamber Tests

Plastic pots (10-cm diameter) were filled with 30.0 g of dry peat, perlite and vermiculite (3:2:2) and saturated in trays of water for several days. Three 16- to 20-week-old seedlings were planted in each saturated pot and the seedlings allowed to acclimatize in a Conviron growth chamber with day/night (14/10 hours) temperatures of 23°/18°C and 50% relative humidity, under 212 $\mu mol\ m^{-2}\ s^{-1}$ of photosynthetically active radiation (PAR) from a mixture of VHO fluorescent (F96T12-CW-1500, General Electric), incandescent (I-line 130, General Electric) and red light (75R30 Pl, General Electric) for 2 weeks prior to treatment. ABA-treated pots received a total of 50 µmol of the hormone applied in seven root drenches using 143 ml of 50 µM ABA every second day for 2 weeks. Paclobutrazol-treated pots received a total of 200 µmol (60 mg) of paclobutrazol (ICI-Chipmann, Stoney Creek, Ontario) applied in two equal root drenches, each with 100 µmol of paclobutrazol in 50 mL of water on the first and third days of a 2-week or a 1-month pretreatment period. All plants were kept well-watered with 150 mg L^{-1} of commercial 20-20-20 N/P/K fertilizer. Fourteen or 28 days after treatments began, the treated and control seedlings were placed in separate trays of tap water and covered with loose plastic for 12 hours before initiating drought by withholding water for up to 20 days.

Transpiration

Transpiration of seedlings in the growth chamber was recorded with a Li-Cor 1600 steady-state porometer (Li-Cor Instruments, Lincoln, Newbraska), with four pots per treatment measured between 10:00 a.m. and 12:00 noon on each sampling date. The surface area of the needles was estimated from the tissue volume by means of the water displacement method described by Johnson (1984). Pot means for water potentials, water contents and transpiration readings were analyzed statistically by means of one-way analysis of variance followed by Duncan's New Multiple Range Test.

Water Status

Pots were removed from the growth chamber at 12:00 noon and stored in a cool, dark place until all measurements were completed. The components of water potential (Ψ_w) and water contents were measured either on excised lateral branches or on whole shoots. Fresh weights were obtained immediately after cutting. Water potentials and pressure-volume relations were then determined with a PMS model 600 pressure chamber (PMS Instrument Co., Corvallis, Oregon), as described by Marshall (1990). Saturated moisture contents were determined by placing 20 to 30 pre-weighed needles on the bottom of a vial, adding 20 mL of water and reweighing the needles after 24 hours. Tissue was dried in a forced-draft oven at 70°C for 24 hours and then weighed again for calculation of water contents.

Survival

Percent survival at each sampling date was assessed by rewatering pots for a minimum of 3 weeks after cessation of drought. Seedlings that remained wilted, turned brown and became brittle were judged to be dead.

Greenhouse Tests

Seedlings in 10-cm pots were acclimated on a greenhouse bench in natural light during the spring and summer and under natural light supplemented by high-pressure sodium lamps (1400 μmol m^{-2} s^{-1}) for a 16-hour-day/8-hour-night regime during the fall and winter months for up to 14 days before treatment with paclobutrazol, subsequent imposition of drought and measurement of survival as described under growth chamber tests. Diurnal changes in temperature and relative humidity were recorded with a continuous chart recorder (Wilh. Lambrech, K.G., Gottingen, West Germany).

RESULTS

Ion-loading and Antitranspirant Tests

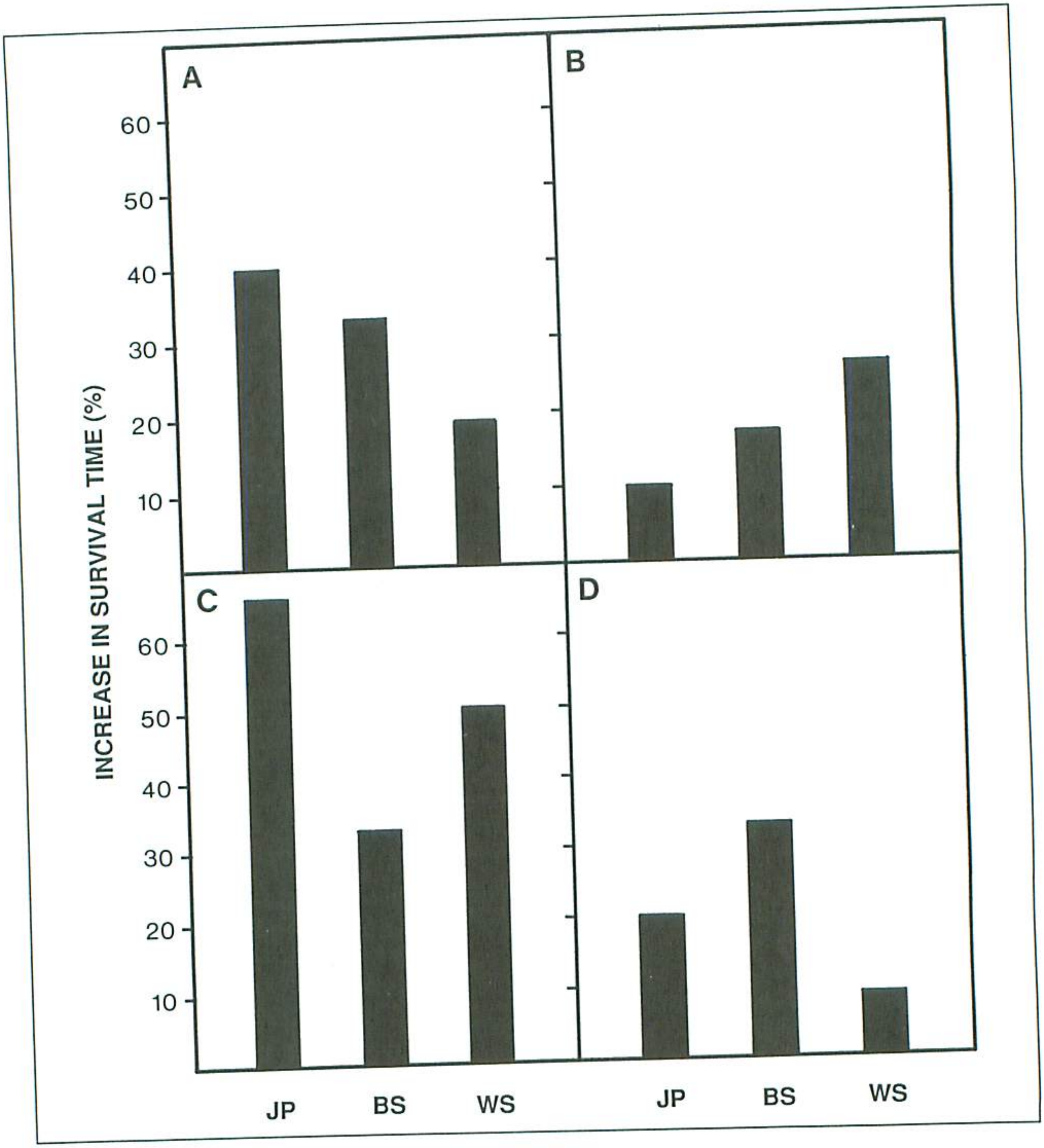
Coating seedlings with Folicote™ delayed drought-induced needle loss by 37% in jack pine, 22% in black spruce and 19% in white spruce (Fig. 1A). Preconditioning seedlings with potassium and calcium salts was found to reduce damage to seedlings during drought (Fig. 1B). Potassium chloride treatments increased the length of drought required to induce needle loss in white spruce by 26%. A combination of potassium and calcium delayed needle loss by 20% in black spruce and 10% in jack pine. The combination of Folicote™ plus ion loading delayed needle loss by 66% in jack pine, 50% in white spruce and 33% in black spruce (Fig. 1C).

Light and Temperature

Growing seedlings under short days and low temperatures delayed needle loss during drought by 20% in jack pine, 33% in black spruce and 9% in white spruce (Fig. 1D).

Morphological Effects of Paclobutrazol

The paclobutrazol treatment generally produced short, waxy-looking needles within 2 weeks. In



jack pine, paclobutrazol inhibited seedling growth and prevented the formation of long, mature fascicled needles for at least 2 weeks (Fig. 2A). In black spruce and white spruce, paclobutrazol often inhibited the growth of the leader and upper lateral branches but stimulated bud break in lower laterals, resulting in reduced height growth and a change in the pattern of shoot extension (Fig. 2B,C). The growth-inhibiting effect of paclobutrazol on white spruce was still apparent for up to 2 months after treatment, but the effect was neither as consistent nor as pronounced as in the case of black spruce.

Paclobutrazol inhibited root extension in all three species. In jack pine, the treated root tips were

Figure 1. (facing page) The effect of Folicote™ antitranspirant [A], ion-loading [B], the combination of ion loading plus the antitranspirant [C] and short-day and low-temperature regime [D], on the 50% survival-time during drought of jack pine (JP), black spruce (BS) and white spruce (WS).

[A] Seedlings of all three species were sprayed to runoff with Folicote™ (Crystal Soap & Chemical Company, Landsdale, Pennsylvania) immediately before imposition of drought.

[B] Jack pine seedlings were root-drenched with 40 mM KCl, black spruce with 60 mM KCl and 45 mM $CaCl_2$, and white spruce with 120 mM KCl at 5 days and 1 day before leaching with water and subsequent imposition of drought by withholding water.

[C] Jack pine were root-drenched with 80 mM $CaCl_2$ and black and white spruce were root drenched with 80 mM KCl 1 week prior to the test. All seedlings were sprayed with Folicote™ immediately before imposing drought by withholding water.

[D] All seedlings were placed in a growth chamber with a 4-hour photoperiod and temperature was gradually lowered to 6°C over a period of 3 days. After 3 weeks, the seedlings were transferred to a second chamber for the drought test.

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thick and often club-like (Fig. 3A,B). Similar effects were not noted for black spruce (Fig. 3C,D), but in white spruce, paclobutrazol induced the formation of nodule-like structures over much of the root system (Fig. 3E,F).

Comparisons of ABA and Paclobutrazol

The physiological effects of a 2-week pretreatment of jack pine with 50 µmol of ABA and 200 µmol paclobutrazol were strikingly similar (Table 1). Preconditioned seedlings displayed significantly less negative water potentials despite water contents similar to or lower than those of the controls. On day 10, the control seedlings, which had an average water content of 68.2%, wilted. In contrast, the ABA- and paclobutrazol-treated seedlings, which had water contents of 65.9 and 65.1%, respectively, remained turgid. Both treatments reduced transpirational water loss and prevented a sharp decline in water content during extended drought. Treatment provided significant protection from drought with survival values of 100% for ABA, 89% for paclobutrazol and 0% for the untreated control seedlings.

Characteristics of Stress Resistance Induced by Paclobutrazol

Dormant (i.e., in bud set) white spruce seedlings that were treated with paclobutrazol for 2 weeks before exposure to drought showed a tenfold increase in the elastic modulus of their shoots and higher turgor values than untreated droughted controls despite similar or reduced water contents (Fig. 4A,B,C). In dormant black spruce, paclobutrazol did not increase elastic modulus values above those in the well-watered control seedlings but it did induce greater turgor at similar moisture contents, and it prevented the precipitous decline in the elastic modulus and moisture content values observed in the untreated stressed seedlings (Fig. 4D,E,F).

The application of paclobutrazol to actively growing seedlings a full month before initiating the drought provided greater stress protection than that induced by the 2-week pretreatment period in dormant seedlings. The paclobutrazol treatment depressed the saturated water volume of the needles in all three species by 4 to 5%. Higher pressure potentials and elastic modulus values were observed in paclobutrazol-treated seedlings despite their lower moisture contents (Table 2).

Paclobutrazol treatment resulted in little or no measurable transpiration during prolonged drought (Fig. 5A,B,C). Maximum differences in survival between paclobutrazol-treated and untreated actively growing seedlings occurred after 17 days for white spruce, 14 days for black spruce and 15 days for jack pine, with survival values (paclobutrazol/untreated) of 100/13%, 66/0% and 100/0%, respectively (Fig. 5D,E,F).

Figure 2. The effect of a 2-week pretreatment with 200 μmol of paclobutrazol on the shoot morphology of jack pine [A], black spruce [B] and white spruce [C].

[A] Typical untreated (left) and paclobutrazol-treated (right) jack pine seedlings.

[B] The different morphologies of untreated (left) and paclobutrazol-treated (right) black spruce seedlings.

[C] The different morphologies of untreated (left) and paclobutrazol-treated (right) white spruce seedlings.

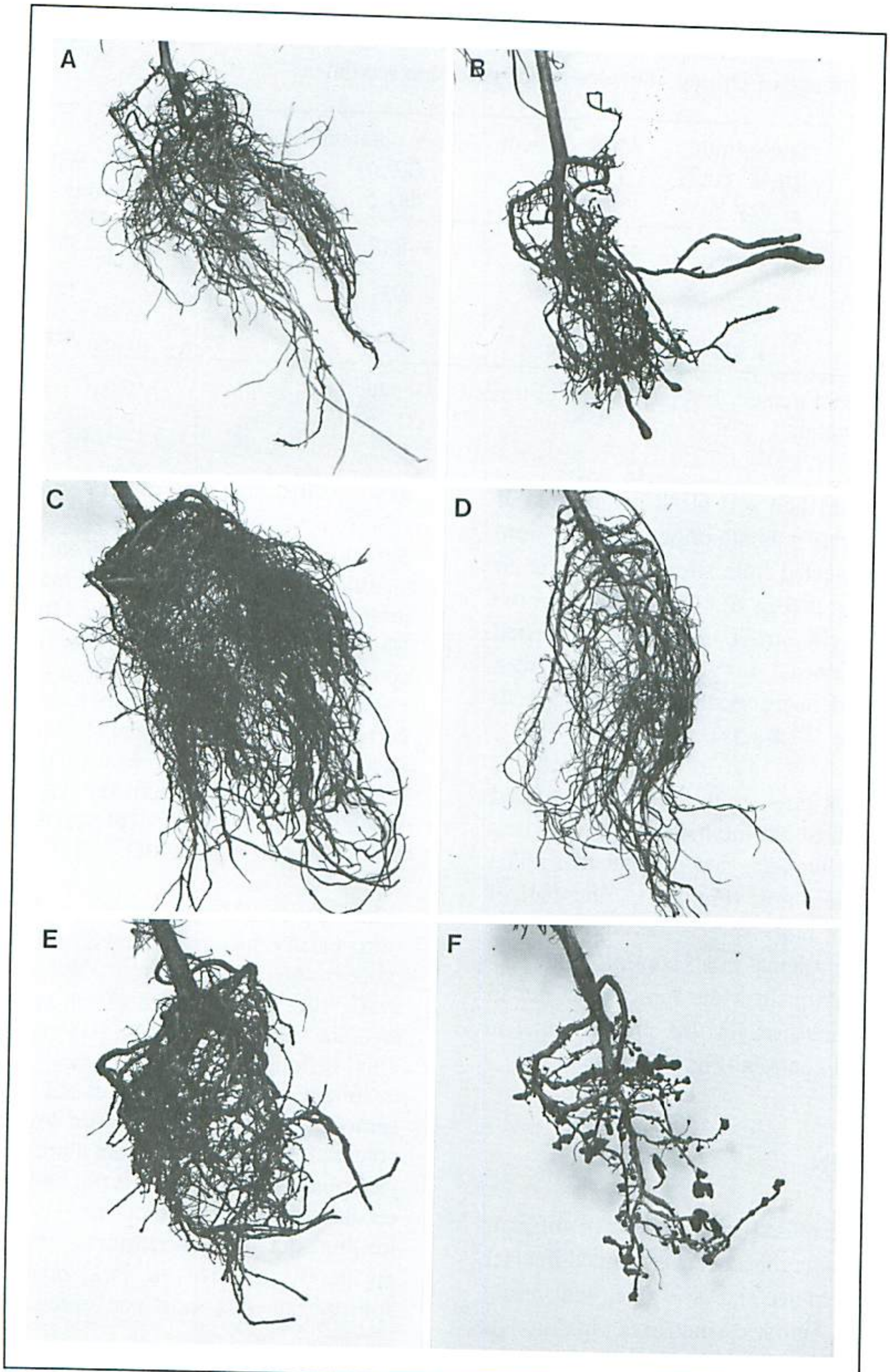

Figure 3. The effect of a 1-month pretreatment with 200 µmol of paclobutrazol on the root morphology of: untreated [A] and paclobutrazol-treated [B] jack pine roots; untreated [C] and paclobutrazol-treated [D] black spruce roots; untreated [E] and paclobutrazol-treated [F] white spruce roots.

Table 1. The effect of drought on transpiration, shoot water content, shoot water potential (Ψ_w), occurrence of wilting and survival of jack pine seedlings.

Treatment[a]	Transpiration ($\mu g\ s^{-1}\ cm^{-2}$) day 8	Water content (% f.wt.)[b] day 5	Water potential (kPa) day 5	Water content (% f.wt.)[b] day 10	Wilt day 10	Survival day 15
A	0.307	70.0	-838	65.9	no	100%
P	0.484	72.4	-931	65.2	no	89%
U	1.243	72.6	-1318	68.2	yes	0%

[a]A – abscisic acid-treated, P – paclobutrazol-treated, U – untreated.
[b]f.wt. = fresh weight

Dormant (i.e., in bud set) black spruce treated with paclobutrazol 1 month prior to drought were completely protected from stress levels lethal to almost all controls (Fig. 6). One month after the relief of drought stress, paclobutrazol-treated seedlings retained a vigorous dark-green appearance and many seedlings resumed shoot elongation (Fig. 7A,B,C).

In dormant jack pine seedlings, 89% of treated seedlings survived a combination of intense heat (≥ 40°C), low humidity and drought that killed all untreated seedlings (Fig. 8A). One-half of treated dormant black spruce survived levels of heat and drought lethal to all controls (Fig. 8B). Fully 86% of dormant white spruce survived 15 days of heat and drought that proved lethal to most untreated controls (Fig. 8C).

DISCUSSION

The protective effects of a variety of different preconditioning regimes were examined in black spruce, white spruce and jack pine, with results ranging from modest increases in drought resistance to very substantial protection. The same pretreatment was often found to have quite different effects among the three species, suggesting inherent differences in the capacity and nature of their drought resistance mechanisms.

Ion-loading and Use of an Antitranspirant

Spraying the needles with Folicote™ produced a significant delay in the relative mortality of all three conifers, but the protective effect conveyed was much higher for jack pine than for the spruces (Fig. 1A). The enhanced effect on jack pine may, in part, be a function of the comparatively large evaporative surface of pine shoots, and it underscores the probable importance of rapid stomatal response during drought as a mechanism of resistance in jack pine (Marshall et al. 1991).

White spruce, and to a lesser extent black spruce, were clearly preconditioned to drought by the process of ion loading. In contrast, the availability of high levels of salt in the growing medium had little effect on jack pine (Fig. 1B). This difference in the efficacy of salt pre-treatment may reflect a general tendency of spruce seedlings to develop lower osmotic potentials (i.e., to accumulate more solutes) than jack pine when soil water is not limiting (Buxton et al. 1985). The protective effects of ion loading and the antitranspirant treatment were apparently additive in jack pine and white spruce, but this was not observed in black spruce.

Short Photoperiod and Low Temperatures

The protective effect of short days and low-temperature regimes on black spruce was particularly striking and demonstrates that these environmental stimuli exert critical control on the expression of the physiological mechanisms of stress resistance in black spruce. The drought-protecting mechanism induced by changes in photoperiod and temperature has yet to be identified nor has its relationship to cold tolerance and dormancy been elucidated. The initiation of an active program of basic research in this area appears to be warranted.

Morphological Effects of Paclobutrazol

Treatment with paclobutrazol markedly reduced the evaporative surface area in the shoots of jack pine and both spruces and strongly depressed water loss via transpiration throughout prolonged drought. Growth of lower lateral branches at the expense of upper laterals and the leader may represent a heretofore unrecognized drought adaptation, since tracheid connections to the leader are continuous and direct whereas the connections from the central stem to the shorter tracheids of the lateral branches are more circuitous (Panshin and de Zeeuw 1980). Thus, a drought-induced gas cavitation in the lower laterals with their large surface area of needles may not readily affect the stem or upper branches. With respect to the roots, triazoles have been shown to induce the accumulation of large starch grains in the epidermal cells of soya bean (*Glycine max*) roots (Upadhyaya et al. 1990), but the reasons for the development and role of the club-like and nodular root thickenings induced by paclobutrazol have not been determined.

ABA and Paclobutrazol

The stomatal response of the paclobutrazol-treated seedlings was remarkably similar to that of ABA-treated plants, which suggests that paclobutrazol may directly or indirectly influence a physiological mechanism normally regulated by ABA. At present, it is unclear whether paclobutrazol mimics ABA through an effect on the ABA titre (Asare-Boamah et al. 1986) possibly via a reduction in the titre of gibberellins, which normally antagonize the effects of endogenous ABA (Coolbaugh and Hamilton 1976; Ho 1983), or through a direct physiological role similar to that of ABA.

Treatment of jack pine seedlings with abscisic acid or paclobutrazol had pronounced effects on seedling water relations. The water relations of plant cells are described by the equation $\Psi_w = \Psi_p + \Psi_\pi$. Cell water potential ($\Psi_w$) is the difference between a positive pressure potential (Ψ_p) and a negative osmotic potential (Ψ_π). An increase in pressure potential results from movement of water along an osmotic gradient into a cell, with measured pressures proportional to the elastic modulus of the cell wall. When water is lost from a cell, pressure potentials and turgor usually fall and can yield highly negative water potentials (Ψ_w), which usually reflect severe drought. In the present work, seedlings treated with ABA and paclobutrazol did not lose turgor at moisture contents that were lower than those in untreated wilted seedlings. The resistance of treated seedlings to wilting when water contents were depressed suggests a mechanism for maintaining turgor that functions in the absence of net water uptake. The regulation of turgor by a decrease in the water-saturated tissue volume, as opposed to an osmotically induced influx of water, has previously been proposed to operate in cabbage and carnation petals and is termed "turgor adjustment" (Eze et al. 1986, Levitt 1986). Observations consistent with this phenomenon were also reported by Buxton et al. (1985), who noted an increase in turgor values despite a decrease in the water content of jack pine.

Mechanisms of Paclobutrazol-induced Tolerance

A reduction in seedling water contents by application of paclobutrazol early in the drought period reflects a phenomenon also commonly observed during the cold adaptation process in crop plants (Krol et al. 1984). Moreover, the result of pretreatment low-temperature trials with conifers (Fig. 1D) suggests a link between frost resistance and drought tolerance. Triazole-type

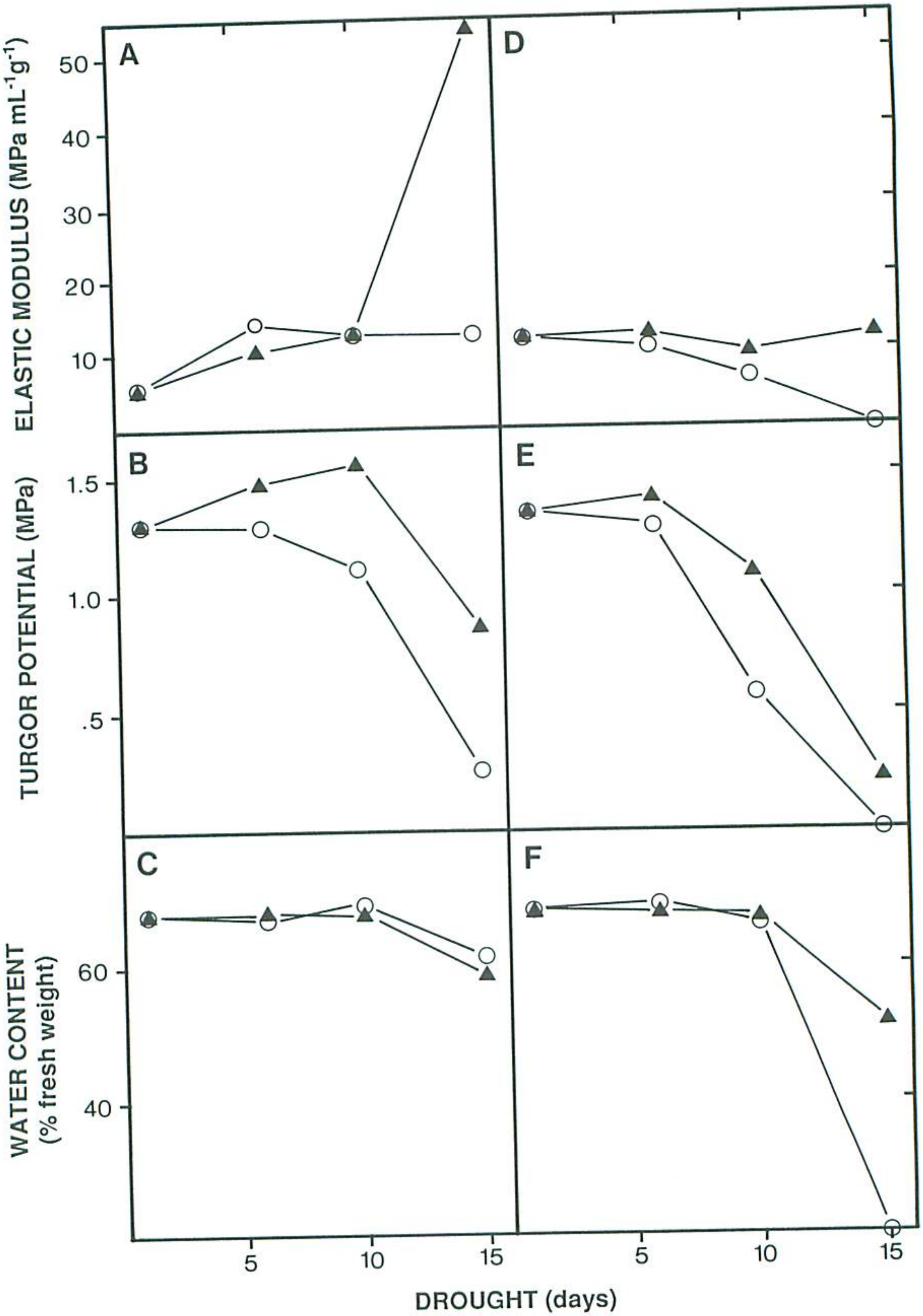
A
D
B
E
C
F
ELASTIC MODULUS (MPa mL-1g-1)
TURGOR POTENTIAL (MPa)
WATER CONTENT (% fresh weight)
50
40
30
20
10
1.5
1.0
.5
60
40
5
10
15
5
10
15
DROUGHT (days)

Figure 4. (facing page) The effect of drought on the elastic modulus, pressure potentials and water contents of dormant (i.e., in bud set) shoots of white spruce [A,B,C] and black spruce [D,E,F] growing in a greenhouse. Untreated seedlings (o) and seedlings treated with 200 μmol of paclobutrazol (▵) 2 weeks before exposure to drought.

[A & D] The effect of drought on elastic modulus values in the shoots of untreated (o) and paclobutrazol-treated (▵) white spruce (A) and black spruce (D).

[B & E] The effect of drought on turgor potential in the shoots of untreated (o) and paclobutrazol-treated (▵) white spruce (B) and black spruce (E).

[C & F] The effect of drought on water contents in the shoots of untreated (o) and paclobutrazol-treated (▵) white spruce (C) and black spruce (F).

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growth regulators have previously been shown to induce frost resistance in higher plants, and the reduction in saturated water contents observed after treatment with paclobutrazol may be one aspect of the frost resistance phenomenon. For example, rye (*Secale* sp.) grown at low temperatures exhibits a reduction in the size of some cell types and an increase in the deposition of hydrophobic compounds on the water-adsorbing carbohydrate matrix of the cell wall (Griffith et al. 1985). In addition, mesophyll cells have been observed to shrink dramatically during freeze-induced dehydration but to regain full size after a return to normal temperatures (Pearse 1988). *In toto*, these results suggest that paclobutrazol may stimulate adaption to drought by a mechanism similar to that expressed during the development of frost resistance.

The apparent significance of the paclobutrazol-induced adjustment of tissue water volume resides in the observation that turgor is still maintained during a significant loss in tissue water content. This paclobutrazol-induced decrease in full-turgor tissue volume is apparently facilitated by a sharp increase in elastic modulus values in response to drought. Turgor adjustment in the absence of net water uptake has been observed only in tissues that are not actively growing, and thus, inhibition of shoot growth by paclobutrazol may facilitate the action of this drought-tolerance mechanism.

Table 2. The effect of drought on the saturated water volume of the needles, water contents as (% fresh weight) of needles, pressure potentials of the shoots (Ψ_p), and elastic modulus values of the shoots of actively growing jack pine, black spruce and white spruce.

Species[a]		[a]Saturated water volume on day 1. (% fresh weight)	Real water content of needles (% fresh weight)	Pressure potential (MPa)	Elastic modulus (MPa $mL^{-1}g$)
White spruce	P	70.6	69.2	1.67	19.8
	U	74.4	70.5	0.51	3.0
Black spruce	P	70.6	65.9	1.87	24.3
	U	74.7	66.2	1.48	12.1
Jack pine	P	75.6	73.8	1.31	4.1
	U	79.9	74.2	0.78	2.4

[a] P = treated with paclobutrazol, U = untreated; white spruce was measured on day 9 of the drought, black spruce on day 6, and jack pine on day 12.

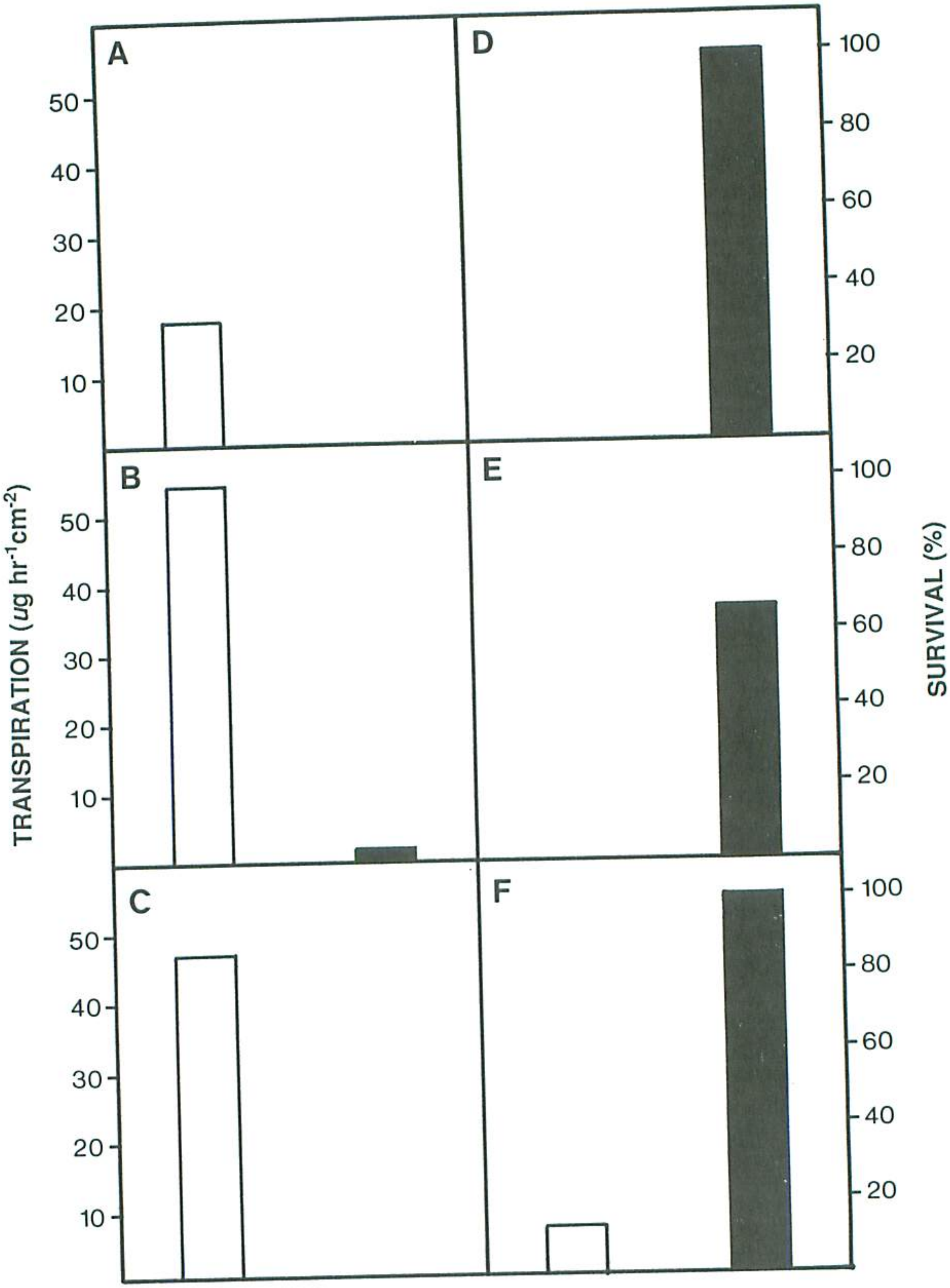

A
B
C
D
E
F
TRANSPIRATION (ug hr-1cm-2)
SURVIVAL (%)
10
20
30
40
50
20
40
60
80
100

Figure 5. (facing page) The effect of drought on the transpiration [A,B,C] and survival [D,E,F] of actively growing jack pine, black spruce and white spruce seedlings in a growth chamber. Untreated seedlings (open box) and seedlings treated with 200 μmol of paclobutrazol (cross-hatched) 1 month prior to the imposition of drought.

[A & D] Transpiration (day 12) and survival (day 17) of untreated and paclobutrazol-treated jack pine seedlings during drought. Seedlings were grown in a greenhouse and allowed to acclimate for 2 weeks between potting and treatment.

[B & E] Transpiration (day 9) and survival (day 14) of untreated and paclobutrazol-treated black spruce seedlings. Seedlings were grown in a greenhouse by Energreen Nurseries (Thunder Bay, Ontario), and bud set was induced prior to shipping. Seedlings were potted, transferred to a growth chamber and allowed 2 weeks to break dormancy before treatment.

[C & F] Transpiration (day 12) and survival (day 15) of untreated and paclobutrazol-treated white spruce seedlings. Seedlings were grown in a greenhouse by the Ontario Ministry of Natural Resources (Swastika, Ontario). Seedlings were potted, transferred to a growth chamber and allowed 2 weeks to acclimate before treatment.

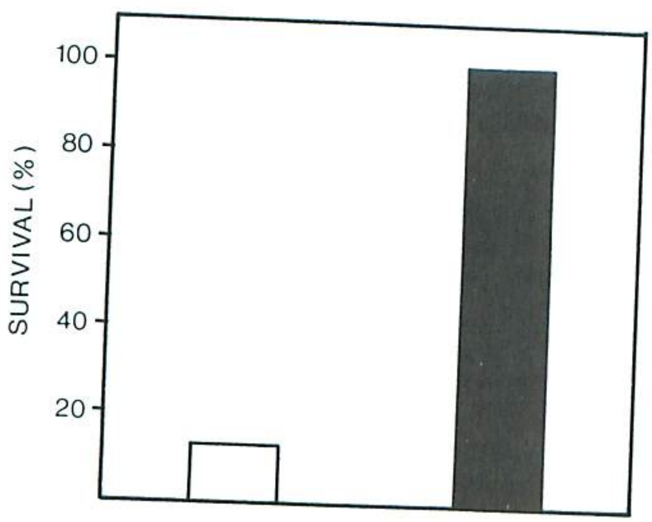


Figure 6. The effect of 12 days of drought on the survival of dormant (i.e., in bud set) seedlings of black spruce. Untreated seedlings (open box) and seedlings treated with 200 μmol of paclobutrazol (solid fill pattern) one month prior to the imposition of drought. Seedlings were grown in a greenhouse by Energreen Nurseries (Thunder Bay, Ontario); bud set was induced prior to shipping. Seedlings were potted, transferred to a growth chamber and allowed 2 weeks to acclimate before treatment.

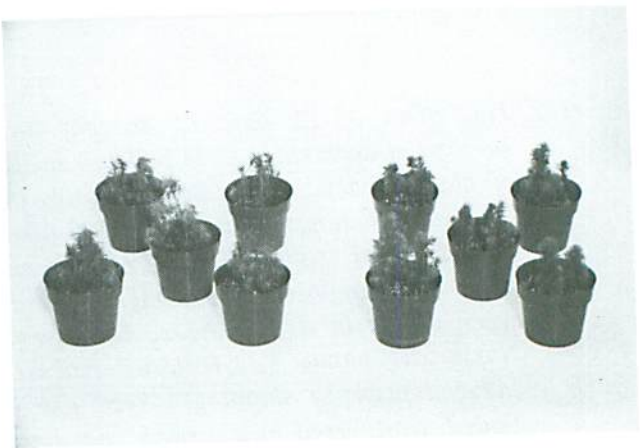
In summary, paclobutrazol stimulates a stress-tolerance mechanism that regulates seedling turgor independently of tissue moisture content; it prevents wilting and thereby increases seedling tolerance to severe drought. In jack pine, treat-ment with paclobutrazol provides a degree of protection similar to that afforded by large doses of ABA. The triazole produces profound morphological changes in both jack pine and white spruce, both of which commonly adapt to dry sites and must therefore readily express their drought-resistance potential to survive. The response induced by paclobutrazol may well represent expression of the inherent drought-resistance capacity of white spruce and jack pine, and field trials of the effects of paclobutrazol would be the next logical step to test this hypothesis.

Paclobutrazol induced significant stress resistance in black spruce during drought, but it did not provide the same high level of protection noted for white spruce and jack pine. The differential effect of paclobutrazol on dormant and active black spruce seedlings (Fig. 6B, 7) and the effects of environmental stimuli on the expression of the drought response indicate significant potential for inducing stress

Figure 7. The appearance of untreated and paclobutrazol-treated seedlings after drought and followed by subsequent rewatering for a minimum of 3 weeks (see Fig. 5A, 6, 5C respectively, for details).

[A] Jack pine seedlings droughted for 17 days

[B] Black spruce seedlings droughted for 12 days



[C] White spruce seedlings droughted for 15 days

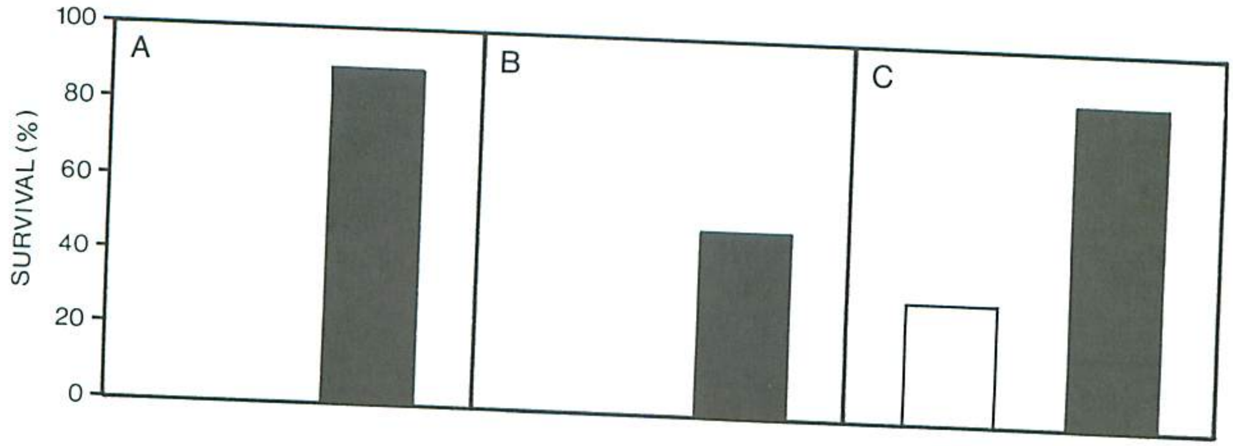


Figure 8. The effect of drought plus intense heat (≥ 40°C) on survival of dormant (i.e., in bud set) seedlings of jack pine [A], black spruce [B] and white spruce [C] in a greenhouse. Untreated seedlings (open box) and seedlings treated with 200 μmol of paclobutrazol (solid fill pattern) 2 weeks prior to the imposition of drought.

[A] Survival of untreated and paclobutrazol-treated jack pine seedlings after 7 days of drought followed by 3 days of drought plus intense heat.
[B] Survival of untreated and paclobutrazol-treated black spruce seedlings after 14 days of drought followed by 1 day of drought plus intense heat.
[C] Survival of untreated and paclobutrazol-treated white spruce seedlings after 12 days of drought followed by 3 days of drought plus intense heat.

resistance. However, at present, it is not clear whether higher doses of paclobutrazol, or other plant growth regulators such as ABA or its analogues, specific growing conditions, or a combination of any of these treatments, will induce the full potential drought resistance in black spruce seedlings.

SUMMARY

Adapting seedlings to high levels of salt (i.e., ion loading) delayed seedling death, as shown by delays in needle loss during drought of 26 and 17%, respectively, in white spruce and black spruce, but it had little effect on jack pine. In contrast, coating seedlings with Folicote™, a commercial antitranspirant, delayed needle loss during drought by 37% in jack pine but by only 23% and 20%, respectively, in black spruce and white spruce. Growing seedlings under a regime of short days and cold nights delayed needle loss by 33% in black spruce and by 20% and 9% respectively in jack pine and white spruce. Treatment with Folicote™ plus ion loading delayed needle loss by 66% in jack pine, 50% in white spruce and 33% in black spruce.

Abscisic acid (ABA) and paclobutrazol, applied for 2 weeks prior to stress, induced a marked increase in the resistance of jack pine seedlings to prolonged drought. Pretreatment caused rapid stomatal closure, promoted more favorable water potentials, enhanced water retention and increased survival. Maximum differences in survival of the pretreated and control seedlings were observed late in the stress period, with survival values of 100, 89, and 0%, respectively, in the ABA, paclobutrazol and control treatments.

Pretreatment of actively growing seedlings with paclobutrazol a full month before exposure to drought conveyed greater stress protection than the 2-week pretreatment. In both cases, however, paclobutrazol produced compact, waxy-looking, short-needled seedlings with thickened roots. Treated seedlings also displayed lower water-saturated needle volumes, lower water contents, larger bulk elastic modulus values and

higher turgor potentials than the untreated controls during drought. Maximum differences in survival were observed late in the drought period, with values in the 30-day paclobutrazol-treated seedlings versus those in the untreated controls of 100/13%, 100/0%, and 66/0% in white spruce, jack pine and black spruce, respectively. Dormant (i.e., in bud set) black spruce seedlings treated with paclobutrazol for one month were completely protected (100% survival) against drought conditions lethal to 87% of all controls.

The 2-week paclobutrazol treatment was also effective in protecting dormant seedlings from a combination of intense heat (≥ 40°C), low humidity and drought. Survival values for the paclobutrazol-treated seedlings were 89% for jack pine (compared with 0% for the control group), 50% for black spruce (0% for the control group) and 87% for white spruce (33% for the control group).

CONCLUSIONS

1. Paclobutrazol provides a degree of drought resistance in conifers that essentially matches the protection conveyed by ABA, the supposed first line of hormonal defense against the effects of environmental stress on plant tissues.

2. Paclobutrazol protects seedlings from drought and the combination of drought plus intense heat. This occurs through alterations in seedling morphology, through the reduction of saturated-tissue water volume, by the reduction of water loss through the needles, and through a large increase in shoot elastic modulus in response to drought. Together, these physiological adaptions delay wilting and enhance both drought avoidance and drought tolerance, thereby enhancing survival during severe drought.

3. Paclobutrazol induces a high level of drought resistance in white spruce and jack pine, but does not appear to completely induce the drought response of black spruce.

4. Short days and low temperatures induce a marked increase in the drought resistance of black spruce, but they are notably less effective with jack pine and white spruce.

RECOMMENDATIONS

1. Field trials of the effects of paclobutrazol on seedling establishment of jack pine and white spruce should be undertaken using the procedures outlined in this report.

2. Paclobutrazol should be applied as follows to current containerized seedlings: (a) Allow the soil around the roots 3 days to become dry; (b) completely saturate the soil with 2 mM (0.6 g L^{-1}) paclobutrazol; (c) wait 3 days while the soil dries and then repeat the application; (d) after 3 days, water and fertilize as usual. One month after the initial treatment date, the seedlings can be exposed to stress.

3. An applied investigation of the induction of drought-resistance in black spruce should be undertaken focusing on the effects of ABA and a variety of triazoles. Dose-response and time-response curves for paclobutrazol should be developed for all three species.

4. Initiate a basic investigation of effects of growing conditions, specifically light and temperature, on the development of drought resistance in conifer seedlings and the roles of endogenous regulator metabolites and specific proteins in the physiological mechanisms that control the stress response.

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