

NODA

notes

NODA • EDNO



NODA Note No. 31

ANTISTRESS ANTIOXIDANT ENHANCES GROWTH AND STRESS TOLERANCE IN CONIFER TRANSPLANTS

F.D. Beall¹, T.J. Blake², S.J. Colombo³, A. Darlington², K. Vishnevetskaia², and G.W. Crook¹

INTRODUCTION

During the past decade, conifer reforestation programs in Canada have expanded rapidly, and, currently, more than a billion seedlings are planted each year. However, according to Farnum et al. (1983), less-than-optimum survival rates and slow seedling growth are reducing the long-term potential benefits of many plantations.

Critical to the reforestation effort is a supply of environmentally tolerant seedlings that can cope with boreal planting sites. One of the principal reasons for seedling mortality and slow growth following outplanting is that fresh, young container stock may be intolerant of stress and slow to adapt to natural conditions (Blake and Sutton 1988). This can reduce their competitiveness and often necessitates later release from competition. Cultural techniques that minimize overwintering damage and planting shock, and yet promote vigorous growth, are needed. Ambiol (2-methyl-4 [dimethylaminomethyl]-5-hydroxybenzimidazole dihydrochloride), an antioxidant, holds promise as a seed treatment that can provide an inexpensive and environmentally sound way to improve seedling growth and survival, and possibly minimize the need for herbicide use.

This research project was designed to consider the hypothesis that seed treatments with Ambiol increase stress tolerance. Seedlings grown from Ambiol-treated seeds were examined to determine changes in seedling morphology,

and to note the response of gas exchange parameters to Ambiol treatment during the early stages of a drought. The possibility that Ambiol treatment increases the resistance of seedlings to frost and heat stress was also considered.

LITERATURE REVIEW

Fitness of Seedlings for Outplanting

A variety of factors play a role in reducing the survival and growth rates of planted seedlings: namely, use of inappropriate phenotypes (e.g., improper cultural practices); use of genotypes, species, ecotypes, or seed sources inappropriate to the planting site; stress to plants caused by transplant shock; insects and diseases; inappropriate site preparation; or environmental stress (e.g., moisture deficiency, high temperatures, freezing, or nutrient deficiencies).

Seedling physiology is more important for survival and growth than is seedling morphology. Blake and Sutton (1987) reported that even relatively small differences in greenhouse procedures influenced the outplanting performance of seedlings grown from the same seed lot but in different greenhouses. Problems with seedling quality may be solved by using genotypes that have superior adaptability to a range of sites, and by improving cultural practices, including seedling conditioning, to increase their stress resistance.

¹ Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre, 1219 Queen Street East, P.O. Box 490, Sault Ste. Marie, Ontario P6A 5M7

² University of Toronto, Faculty of Forestry, Earth Sciences Building, 33 Willcocks St., Toronto, Ontario M5S 3B3

³ Ontario Ministry of Natural Resources, Ontario Forest Research Institute, 1235 Queen Street E., Sault Ste. Marie, Ontario P6A 5N5

Natural Resources
CanadaCanadian Forest
ServiceRessources naturelles
CanadaService canadien
des forêts

Ontario

Ministry of
Natural
ResourcesMinistère des
Richesses
naturelles

Seedling Conditioning

Nursery-produced seedlings are grown under luxurious cultural conditions (nutrients, water, relative humidity); consequently, they often become stressed when planted on boreal forest planting sites (Blake and Sutton 1987). Drought is the most serious environmental stress likely to affect seedlings (Kramer 1986). However, sustained chronic stress may be more damaging, and the importance of multiple stresses has also been highlighted (Mooney et al. 1991).

Plants can be conditioned in several ways, including the use of mild drought, heat, cold, and chemical agents. Cross tolerance has been observed where plants that are hardened to withstand one stress are often more resistant to other types of stress (Levitt 1980). The most limiting environmental and plant factors should be identified before establishing a conditioning regime. In the case of seedlings, early growth is highly correlated with the absolute humidity deficit and stomatal conductance (Blake and Sutton 1988). Thus, conditioning seedlings to improve stomatal control of water loss under stress should improve growth and survival.

Morphological and Physiological Adjustments to Conditioning

Biochemical and structural changes in conditioned plants increase their tolerance to later stress. Adjustments that occur at the cellular, tissue, and organism level increase the ability of a plant to continue to grow in a limiting environment. Adaptations to stress include changes in water relations and carbon allocation between roots and shoots.

Adaptations that allow a plant to tolerate drought increase either its ability to postpone or to tolerate dehydration (Levitt 1980). Dehydration postponement adaptations promote stomatal closure or reduce the transpiring surface area of leaves relative to the root absorption area (i.e., shoot to root ratio). Examples include leaf shedding and rolling, and a reduction in leaf area increment (Blake and Tschaplinski 1992). Root adjustments to drought can be as important as those taking place in the leaves. Increased root growth relative to the shoot helps to maintain plant water status, increases water uptake, and reduces water loss (Kramer 1983, Blake and Tschaplinski 1992).

Dehydration tolerance adaptations allow stomata to remain open, thereby permitting photosynthesis and growth to continue during the early stages of a drought. One such adaptation to drought is called elastic adjustment. This mechanism allows the cell walls to contract more during dehydration, which helps to maintain turgor despite lower relative water contents (Blake and Tschaplinski 1992). In black spruce (*Picea mariana* [Mill.] B.S.P.), drought conditioning increases cell wall elasticity and reduces the effects of water loss on turgor. The tissues of unhardened plants are less elastic. Therefore, their turgor declines more rapidly when compared to hardened plants. These types of adaptations to drought help to sustain growth when water availability decreases (Blake and Tschaplinski 1992).

Methods of Conditioning

Withholding water

Although drought is the simplest and most commonly used method of hardening, it is difficult to apply precisely to a large number of seedlings. Drought may not develop evenly in all seedlings and, consequently, some may be severely affected while others are barely stressed. Using drought to harden seedlings increases the cost of their production because it reduces growth. Also, the beneficial effects of such hardening may only be temporary, because hardening may be lost when the seedlings are returned to normal conditions.

Chemical conditioning

Many chemicals have been used to harden seedlings. These include antitranspirants, which physically or chemically close the stomata; chemicals such as polyethylene glycol or sorbitol, which limit water availability; and plant growth regulators, such as abscisic acid (ABA).

Antitranspirants fall into two categories: (1) film-coating compounds that physically block stomata, and (2) metabolic antitranspirants such as atrazine, which chemically close stomata (Kozłowski 1979, Blake et al. 1990a). Kozłowski (1979) showed that all tested film-coating and metabolic antitranspirants had negative side effects on seedling growth or survival. In contrast, Ambiol reduced the midday transpiration rate and total daily water usage of soybean (*Glycine max* L. Merr.) plants by 25 percent. It also increased some aspects of growth without reducing survival (Darlington et al. 1996).

Although synthetic ABA is an effective antitranspirant, it is expensive and many of its effects wear off quickly (Blake et al. 1990a). Analogues of ABA have also shown some promise (Blake et al. 1990b). However, ABA or analogue treatment results in increased membrane leakage (Fan and Blake 1994) and reduced growth (Tan and Blake 1993).

Antioxidant Defences in Plants—the Role of Ambiol

When plants are stressed by air pollution, drought, disease, insects, or poor nutrition, free radicals, such as singlet oxygen or hydroxyl, increase in concentration (Winston 1990). In turn, this results in biochemical or physiological lesions, cell damage, or even death. Free radicals react with DNA, proteins, and lipids to promote the breakdown of chlorophyll and cause membrane leakage, resulting in growth inhibitions and senescence. Aerobic organisms have evolved antioxidant defences, including vitamins C and E, and carotenoids, to provide protection from oxidative stress by either preventing damage or accelerating repair processes.

Although less is known about synthetic antioxidants, they can also be used to help plants cope with free radicals that are produced under oxidative stress. Ambiol is the only synthetic antioxidant known to increase both stress tolerance and growth in plants. Seed treatment of corn (*Zea mays* L.) using Ambiol increased yields by 13 percent in a

3-year field trial (Kuznetsov et al. 1986). Seed pretreatment of Scots pine (*Pinus sylvestris* L.) resulted in growth promotion that lasted until the end of the second year following treatment. The stimulation of height growth by up to 130 percent differed among half-sib families, indicating a high degree of genetic influence in the response (Vishnevetskaia et al. 1992). Ambiol was also responsible for an increase in the growth of jack pine (*Pinus banksiana* Lamb.) seedlings, due to improved efficiency of water use under mild drought conditions. Ambiol has increased the growth of drought-stressed canola (*Brassica napus* L.) and soybean plants by 25–45 percent relative to the untreated seedlings, yielding plants comparable in size to fully irrigated controls (Darlington et al. 1996).

EFFECT OF AMBIOL ON THE GROWTH AND STRESS TOLERANCE OF CONIFER SEEDLINGS

Laboratory Studies of Drought Responses

Ambiol was applied to seeds of four black spruce half-sib families and to bulk seed collections of black spruce and jack pine. Seeds were soaked for 24 hours in five concentrations of Ambiol (0.0, 0.01, 0.1, 1.0, and 10.0 mg L⁻¹) prior to planting. Seedlings were grown for 6 to 8 weeks, then the roots were washed and the seedlings were placed in root misting chambers. Roots were misted with a nutrient solution containing 20:20:20 (N:P:K).

The plants were drought stressed daily by stopping root misting, starting with a 2-hour period (long enough to reach the wilting point). A computer program extended the stress period by about 2 percent each day over a 21-day period, so that the final drought period was approximately 3 hours. At the end of the experiment all plants were harvested, the dry weights of the roots, stems, and leaves were determined, and the shoot to root and leaf to root ratios were calculated. The effects of Ambiol treatments on stomatal conductance and seedling transpiration rates were also determined. Complete details of the methodology are presented elsewhere⁴.

Response to seed treatment with Ambiol varied greatly depending on the concentrations used and the tree species under consideration. At the second highest level tested, two black spruce seed lots developed a significantly greater root mass (30 percent and 80 percent) than did the controls (Fig. 1). Despite the increase in root mass, the shoot to root ratio also increased significantly in black spruce (Fig. 2). Even the highest level of Ambiol did not cause damage to the seedlings. Interestingly, the morphological changes in shoot to root ratios seem to have occurred only during the drought period, because no differences were apparent prior to the drought treatment. This suggests that Ambiol effects morphology only during times of stress.

Antitranspirant activity

Ambiol seed treatments had no effect on stomatal conductance or transpiration when treated and untreated seedlings were subjected to simulated drought in these experiments.

Isozyme Analysis, Heterozygosity, and Ambiol Response in Black Spruce

Identifying individuals that possess desirable characteristics is essential to the success of forestry breeding programs. However, the breeder must select individuals from a population of seedlings or saplings to capture traits that are expressed only at maturity. Genetically based markers provide an alternative for identifying such desirable traits.

In seedling studies with 2-year-old Scots pine (Vishnevetskaia et al. 1989, Vishnevetskaia et al. 1992), greater levels of heterozygosity (genes composed of different alleles) were correlated with more rapid growth rates. This suggests that the level of heterozygosity should be considered when selecting rapidly growing individuals. Faster growing families of black spruce (Tan et al. 1992) and outcrossed families of jack pine (Blake and Yeatman 1989) were better able to maintain stomatal conductance and net photosynthesis under drought as compared to slower growing and selfed families, respectively. As such, it appears that faster-growing genotypes are more drought tolerant.

To ensure that they will be widely applicable and effective, it is also important to determine genetic variation in the response of seedlings to cultural or chemical treatments. Four open-pollinated, half-sib black spruce families were used in this study. The electrophoretic procedures used are described elsewhere⁵. Two methods were used: namely, starch gel electrophoresis and polyacrylamide gel electrophoresis. Results indicated that the physiological response to Ambiol was positively correlated with the growth rates of the seedlings. A positive association of isozyme heterozygosity with the growth rate and response to Ambiol was detected. The families that had greater diameter growth responses to Ambiol (Fig. 3) also showed a greater mean heterozygosity, more alleles per locus, and a higher percentage of polymorphic loci (genes that produce multiple forms of a protein).

Laboratory Studies of Cold Hardiness and Heat Tolerance

Many environmental factors cause stress to plants, particularly seedling water stress, localized frost, and high air temperatures. All of these result in tissue damage. It is known that an adaptation to one type of stress may increase a seedling's resistance to other types of stress. Compared to unhardened plants, plants that have been exposed to

⁴ Beall, F.; Blake, T.J.; Columbo, S.; Darlington, A.; Vishnevetskaia, K. Low cost, antistress antioxidants for enhanced growth and stress tolerance in conifer transplants. Nat. Resour. Can., Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, ON. NODA/NFP File Rep. No. 29. 83 p. + appendices

⁵ Ibid.

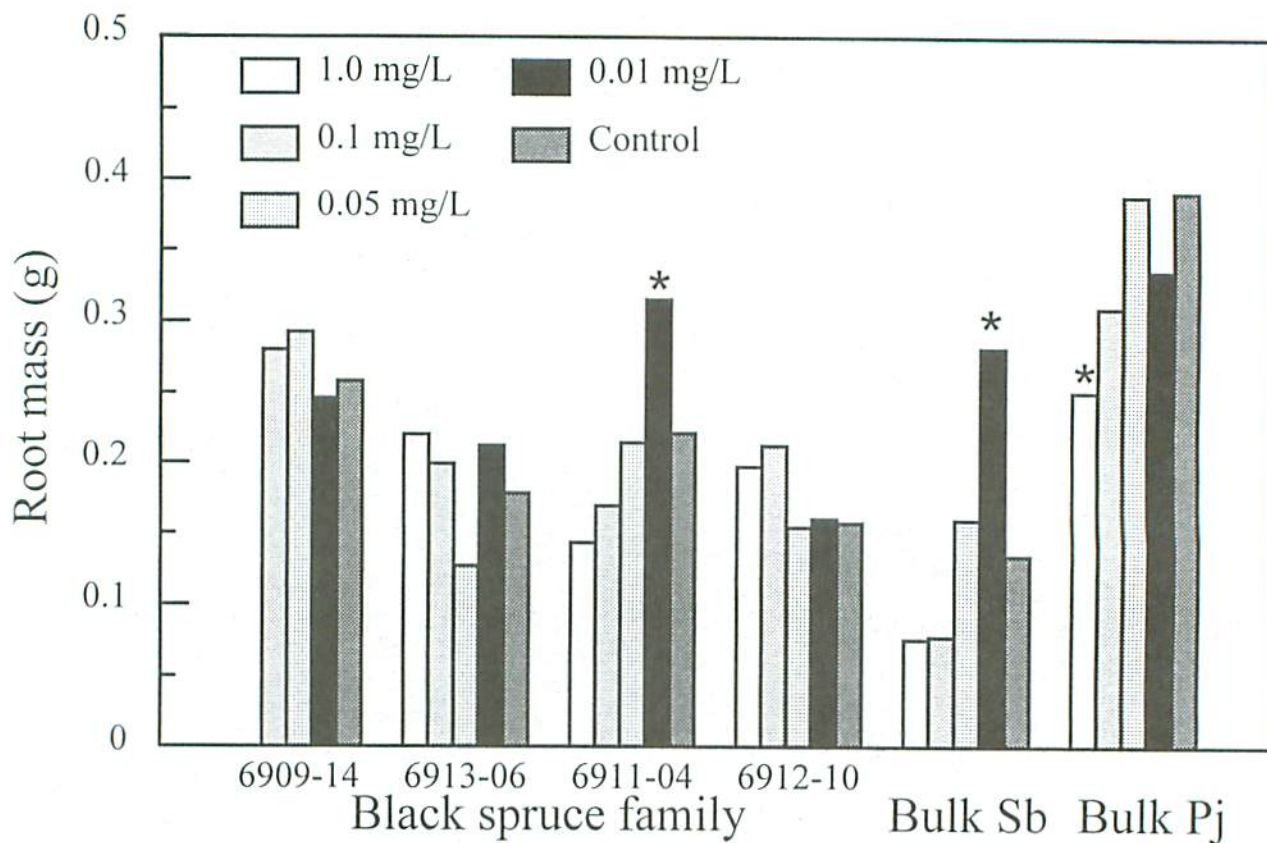


Figure 1. Effect of Ambiol seed treatment on root dry weight in four families of black spruce and bulk seed collections of black spruce (Sb) and jack pine (Pj); "*" indicates a significant difference from the control (0.0 Ambiol) treatment when compared by analysis of variance (ANOVA).

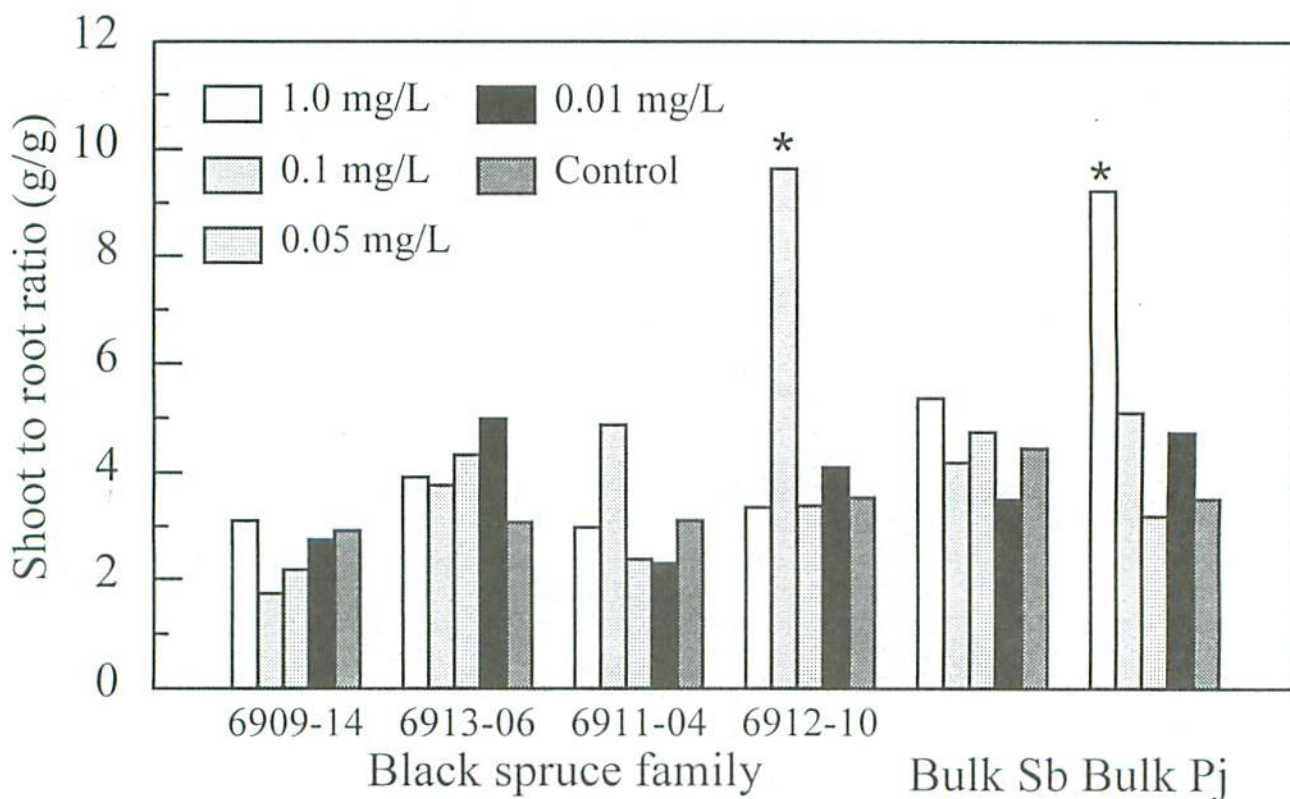


Figure 2. Shoot to root ratios following seed treatment with Ambiol; "*" indicates a significant difference from the control (0.0 Ambiol) treatment when compared by analysis of variance (ANOVA).

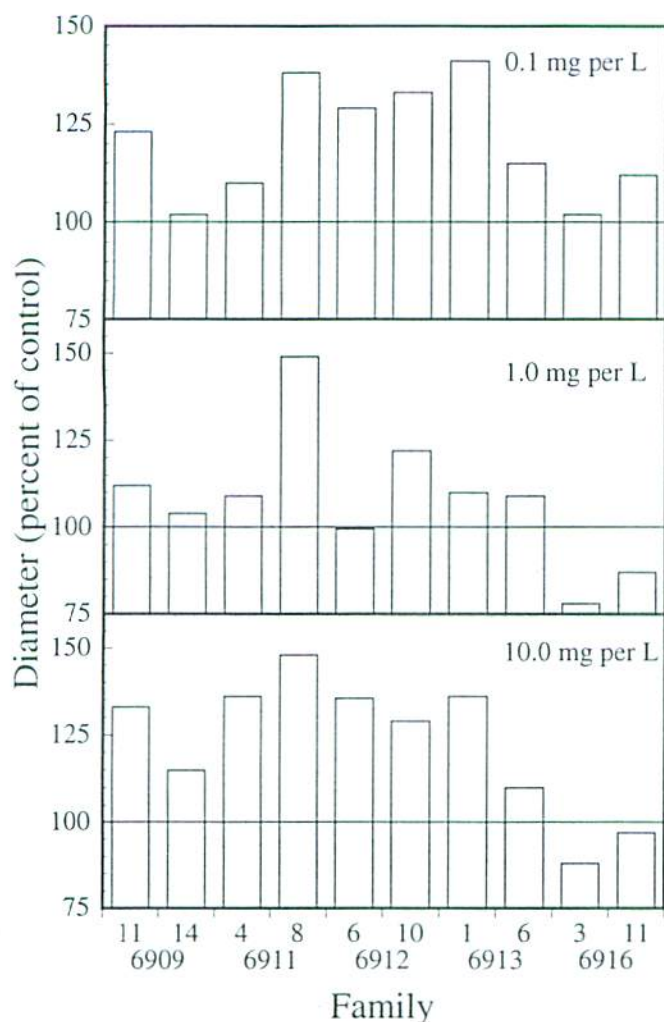


Figure 3. Family variation of black spruce diameter growth in response to Ambiol treatment.

drought stress are usually more tolerant of heat and frost (Kozlowski 1979).

Two slower growing and two faster growing black spruce families, as well as general collection of black spruce and jack pine seeds, were used for these experiments. Seeds were treated with Ambiol by soaking them overnight in five concentrations: 0.0, 0.01, 0.05, 0.1, and 1.0 mg L⁻¹. The effects of Ambiol treatment on heat tolerance were assessed using measurements of tissue damage after exposure to 47°C. Frost tolerance was assessed by electrolyte leakage methods.

When all concentrations were considered, Ambiol seed treatment had no consistent effect on the cold tolerance of black spruce or jack pine — with one exception. When jack pine seeds were treated with Ambiol, injury declined in seedlings that were exposed to a temperature of -10°C.

Initial experiments showed both Ambiol treatment and family to have significant effects on the heat tolerance of black spruce and jack pine seedlings, when analyzed by ANOVA. Seedlings grown from a general collection of black spruce seeds treated with Ambiol exemplify the response of those families that showed a significant increase in heat tolerance

in response to Ambiol treatment (Fig. 4). However, in subsequent experiments no consistent effects were observed. Experiments conducted after completing this research have shown a consistent and significant promotion of heat tolerance in black spruce seedlings (V. Borsos-Matovina and T.J. Blake, pers. comm.).

Field and Greenhouse Studies of Ambiol-treated Seedlings

Black spruce and jack pine seeds (general collection) were soaked in solutions containing 0.0, 0.01, 0.05, 0.1, and 1.0 mg L⁻¹ Ambiol for 24 hours prior to planting. Treated seeds were planted in Spencer-Lemaire containers and grown using standard cultural practices for 15 weeks before being planted at the Ontario Forest Research Institute Arboretum near Sault Ste. Marie, Ontario. Greenhouse experiments were conducted by growing treated seeds in Multi-pots™ using standard cultural practices.

Prior to outplanting the stock produced for the field experiment, seedlings were assessed for two photosynthetic parameters: namely, fluorescence yield and net photosynthesis, and for root growth potential (RGP) using standardized methodologies. This testing did not reveal any significant effects due to Ambiol treatment, and all tests were in the range considered good to excellent for operational crops.

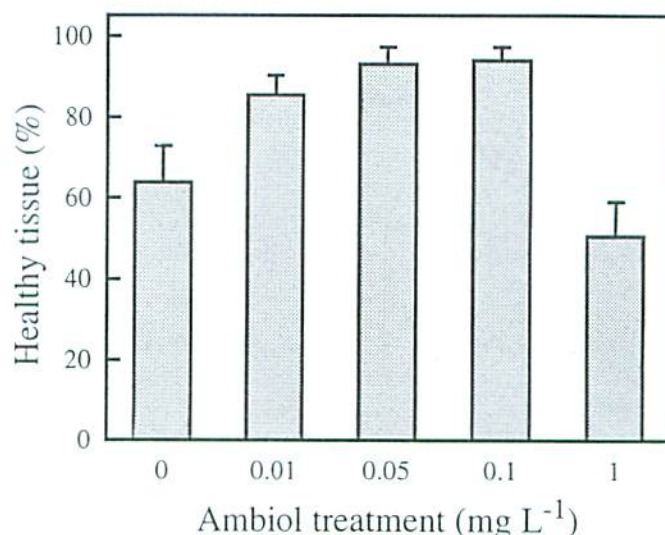


Figure 4. Effect of Ambiol seed treatment on the heat tolerance of black spruce seedlings. Shoots of 13-week-old seedlings were immersed in a 47°C water bath for 30 minutes. After 2 weeks needles were separated into healthy and damaged, and then oven dried. Values are the percent of total dry weight in the healthy fraction with standard errors of the means represented by the capped line attached to each bar. Subsequent to ANOVA, the Bonferroni-Dunn procedure was used to distinguish treatment differences, and bars not headed by the same letter are different at $p = 0.05$.

Ambiol	Black spruce		Jack pine	
	1st year	2nd year	1st year	2nd year
0.0	1.79	2.27	1.27	2.12
0.01	1.43	2.10	1.20	2.26
0.05	1.43	2.27	1.29	1.95
0.1	1.51	2.20	1.13	2.08
1.0	1.46	2.35	1.14	1.66

Table 1. End-of-season shoot to root ratio for black spruce and jack pine seedlings grown from Ambiol-treated seed. Values are derived from measurements taken from six seedlings for each species-treatment combination.

The responses of Ambiol-treated seedlings to cold and heat stress was complicated. Minor variations in light, nutrients, and other environmental factors are known to modify stress responses. Also, the effects of

the response to Ambiol. The observation of differences in species response (Fig. 1), and that black spruce families responded differently to Ambiol, further complicates the picture and suggests that: (1) stress adaptation mechanisms may be species specific, and (2) there is genetic variation in the response to Ambiol. Like plant growth regulators, the effects of Ambiol were dose-dependent. For example, mid-range concentrations of Ambiol may promote a response while higher dosages may inhibit a response (e.g., Fig. 4). This suggests that Ambiol interacts with different physiological systems in a dose-dependent manner. The observation of differences in species response (Fig. 1), and that black spruce families responded differently to Ambiol, further complicates the picture and suggests that: (1) stress adaptation mechanisms may be species specific, and (2) there is genetic variation in the response to Ambiol.

water stress (Figs. 1 and 2). Black spruce and jack pine seedlings grown from Ambiol-treated seeds showed no consistent trend for increased tolerance to cold stress, when all species-treatment combinations were considered. An increase in tolerance to heat stress was observed in some families of black spruce and jack pine, and in seedlings grown from a general collection of black spruce seed. There was some evidence for drought adaptation in jack pine seedlings, since root growth declined and the shoot to root ratio increased following Ambiol treatment without any commensurate increase in water stress (Figs. 1 and 2). Although the diameter growth and the shoot to root ratio were increased in black spruce (but not jack pine) seedlings that had not been subjected to stress (i.e., those grown in a greenhouse), no other consistent and significant differences in morphology were observed. In field-grown seedlings, which may or may not have been stressed, there was a trend for the shoot to root ratio to decline in seedlings produced from Ambiol treated seeds. These results suggest that: (1) the effects of Ambiol treatment may vary depending on the species and whether or not seedlings are stressed; (2) Ambiol has no negative impacts on unstressed seedlings; and (3) the morphological changes observed in Ambiol-treated seedlings suggest that the balance between water absorbing organs (roots) and transpiring organs (shoots) may be improved, since the shoot to root ratio increased in black spruce without any increase in water stress (Figs. 1 and 2).

In laboratory studies of seedlings grown from Ambiol-treated seeds, the authors showed that Ambiol treatment increased the diameter growth, root mass, and shoot to root ratio when seedlings were subjected to mild water

observations had also been made on conifers. In laboratory studies of seedlings grown from Ambiol-treated seeds, the authors showed that Ambiol treatment increased the diameter growth, root mass, and shoot to root ratio when seedlings were subjected to mild water

The authors investigated the use of Ambiol, an antioxidant, applied as a seed treatment to enhance the stress tolerance of conifer seedlings. Ambiol had previously been shown to increase the stress tolerance of a number of agricultural species, thereby resulting in plants indistinguishable from undrugged controls. Some promising observations had also been made on conifers.

SUMMARY AND CONCLUSIONS

The use of luxury levels of hydration, nutrients, and humidity during the production of conifer seedlings for reforestation produces seedlings prone to reduced growth and survival due to environmental stresses after outplanting. Seedling conditioning has the potential to maximize the growth and survival of conifer seedlings planted on environmentally stressful sites. However, most if not all conditioning regimes developed to date have some negative effects on the seedlings.

The effects of Ambiol seed treatment on seedling morphology were small and inconsistent when all species-treatment observations were compared. Results from the greenhouse contrasted with those observed in the field. In experiments conducted in the greenhouse there was a slight trend for Ambiol treatment to increase the shoot to root ratio, as a result of decreased root weight and, in some instances, increased shoot weight. In contrast, in field-grown material the shoot to root ratio was generally decreased as a result of decreased shoot weight (Table 1). This later result is similar to that noted in the root misting chamber studies and suggests that Ambiol effects are most evident when the plant has been subjected to some stress.

Ambiol may not become evident until well after a stress has been relieved, or through further cycles of the stress. Although Ambiol increased growth in some species-treatment combinations, not enough evidence is available at this time to conclude that Ambiol will always increase growth or increase the tolerance of seedlings to heat, frost, and drought. While some evidence points in this direction, it was difficult to obtain consistent results in both the field and greenhouse trials, suggesting that other factor(s) may complicate the response in experiments conducted at different times. For example, Ambiol increased the shoot to root ratio of jack pine seedlings in the greenhouse experiments (Fig. 2), but it had the opposite effect in the field trial (Table 1).

The results of this project and subsequent work suggest that Ambiol treatment does indeed show promise as a conditioning treatment. However, further work is required under controlled-stress conditions to more thoroughly define dose-response relationships. This should be followed by well-designed field experiments to further delineate the potential applications of this technology. Finally, work is needed to determine the costs and benefits of Ambiol use in operational programs, i.e., to find out if the cost of application is justified for all operational crops or just for crops destined for "high risk" planting sites. Until this further research is conducted, Ambiol will remain an experimental treatment.

ACKNOWLEDGMENTS

The authors wish to acknowledge the generous support of the Northern Ontario Development Agreement, Northern Forestry Program, and the support of the Uniroyal Chemical Company. The technical assistance of Ms. Ann Boyonoski, Ms. Cecelia Fernandez, Ms. Nancy Fuselli, Dr. Liu Jian Wei, and Ms. Vera Borsos-Matinova is greatly appreciated. This research was also supported by a Canadian Forest Service grant (to K. Vishnevetskaia), a Natural Sciences and Engineering Research Council (NSERC) operating grant (to T.J. Blake) and an NSERC postdoctoral fellowship (to A. Darlington).

LITERATURE CITED

- Blake, T.J.; Bevilacqua, E.; Hunt, G.A.; Abrams, S.R. 1990a. Effects of abscisic acid and its acetylenic alcohol on dormancy, root development, and transpiration in three conifer species. *Physiol. Plant.* 80:371-378.
- Blake, T.J.; Sutton, R.F. 1987. Variation in water relations of black spruce stock types outplanted in northern Ontario. *Tree Physiol.* 3:331-344.
- Blake, T.J.; Sutton, R.F. 1988. Stomatal responses, the key to adaptation in newly planted jack pine and black spruce. *Plant Physiol. (Life Sci. Adv.)* 7:125-130.
- Blake, T.J.; Tan, W.; Abrams, S.R. 1990b. Antitranspirant action of abscisic acid and ten synthetic analogs in black spruce. *Physiol. Plant.* 80:365-370.
- Blake, T.J.; Tschaplinski, T.J. 1992. Water relations. p. 66-94 in C.P. Mitchell, J.B. Ford Robertson, T. Hinckley and L. Sennerby-Forsse, eds. *Ecophysiology of Short Rotation Forest Crops*. Elsevier Applied Science, Essex, United Kingdom. 308 p.
- Blake, T.J.; Yeatman, C.W. 1989. Water relations, gas exchange and early growth rates of outcrossed and selfed *Pinus banksiana* families. *Can. J. Bot.* 67:1618-1623.
- Darlington, A.; Vishnevetskaia, K.; Blake, T.J. 1996. Growth enhancement and antitranspirant activity following seed treatment with a derivative of 5-hydroxybenzimidazole (Ambiol) in four drought-stressed agricultural species. *Physiol. Plant.* 97:217-222.
- Fan, S.; Blake, T.J. 1994. Abscisic acid induced electrolyte leakage in woody species with contrasting ecological requirements. *Physiol. Plant.* 90:414-419.
- Farnum, R.P.; Timmis, R.; Kulp, J.L. 1983. Biotechnology of forest yield. *Science* 219:694-702.
- Kozlowski, T.T. 1979. *Tree growth and environmental stresses*. Univ. of Washington Press, Seattle, WA. 192 p.
- Kramer, P. 1983. *Water relations of plants*. Academic Press, Orlando, FL. 489 p.
- Kramer, P. 1986. The role of physiology in forestry. *Tree Physiol.* 2:1-16.
- Kuznetsov, Y.V.; Apasheva, L.M.; Poltorak, K.D.; Stoliarova, L.G.; Mustova, L.A.; Grinchenko, A.L.; Smirnov, L.D.; Pikusk, G.R. 1986. 2-methyl-4,6-bismimethylaminomethyl-5-hydroxybenzimidazole hydrochloride possessing growth promoting activity. Author Certificate, SU 1282492 (Cl. C 07 D 235/14).
- Levitt, J. 1980. *Responses of plants to environmental stresses*. Academic Press, New York, NY. 697 p.
- Mooney, H.A.; Winner, W.E.; Pell, E.J. 1991. *Responses of plants to multiple stresses*. Academic Press, San Diego, CA. 422 p.
- Tan, W.; Blake, T.J.; Boyle, T.J.B. 1992. Drought tolerance in faster- and slower-growing black spruce (*Picea mariana*) progenies: I. Stomatal and gas exchange responses to osmotic stress. *Physiol. Plant.* 85:639-644.
- Tan, W.; Blake, T.J. 1993. Drought tolerance, abscisic acid and electrolyte leakage in fast- and slow-growing black spruce (*Picea mariana*) progenies. *Physiol. Plant.* 89:817-823.

Vishnevetskaia, K.D.; Apasheva, L.M.; Dukharev, V.A.; Kuznetsov, Y.V.; Smirnov, L.D. 1989. Method of sensitivity definition of conifers to growth stimulator effect. Author certificate, SU 1470235 (Cl. A 01 N 63/00), 7 April 1989. From Otkrytia, Izobret, 1989 (13). (in Russian).

Vishnevetskaia, K.D.; Apasheva, L.M.; Dukharev, V.A.; Kuznetsov, Y.V. 1992. Genetic polymorphism of proteins and Scots pine growth regulation. Izvestia Academi Nauk. Ser. Biol. 5:807-810. Russian with English summary.

Winston, G.W. 1990. Physicochemical basis for free radical formation in cells: production and defences. p. 57-86 *in* R.G. Alscher and J.R. Cumming, eds. Stress Responses in Plants: Adaptation and Acclimation Mechanisms. Wiley-Liss Inc., New York, NY. 407 p.

This report was produced in fulfillment of the requirements for NODA/NFP Project No. 4011, "Low cost, antistress antioxidants for enhanced growth and stress tolerance in conifer transplants".

Additional copies of this publication are available from:

Natural Resources Canada
Canadian Forest Service
Great Lakes Forestry Centre
P.O. Box 490
Sault Ste. Marie, Ontario
P6A 5M7
(705)949-9461
(705)759-5700(FAX)

©Her Majesty the Queen in Right of Canada 1997
Catalogue No. Fo 29-41/31-1997E
ISBN 0-662-25662-X
ISSN 1198-2233



This report is printed on recycled paper.