Abscisic Acid Analogs Reduce Planting Stress in Newly Planted Seedlings

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Abstract—A research program examined whether specific abscisic acid (ABA) analogs could maintain good water balance of interior spruce seedlings under environmentally stressful conditions, through partial stomatal closure. In addition, the influence of ABA analogs on root growth under optimum conditions was monitored. ABA analogs allowed seedlings to maintain good seedling water balance for extended periods of time, under moderate and severe drought conditions, through greater stomatal closure. ABA analogs caused partial stomatal closure for periods of up to two weeks. Seedlings treated with ABA analogs had the capability to grow an extensive number of new roots, when tested under optimum environment.

INTRODUCTION

Plants require a continuous movement of water from absorbing roots to transpiring leaves to ensure their survival. The capability of a plant to take up water is influenced by available soil water, root system size and distribution, root-soil contact, and root hydraulic conductivity. Transpiration rates are determined by the degree of stomatal opening (i.e., stomatal conductance), needle area and the atmospheric demand for water (i.e., vapor pressure deficit). A plant will be exposed to water stress if transpirational demands exceed their capability to take up water.

Seedlings can be exposed to water stress shortly after being planted on a reforestation site. This occurs because most reforestation sites can have extreme environmental conditions which alter the sites heat exchange processes and soil water relations (Miller 1983). In addition, newly planted seedlings will have root confinement, poor contact of roots with soil, and possibly low root system permeability which can limit water uptake from the soil (Kozlowski and Davies 1975, Burdett 1990). As a result, newly planted seedlings can be exposed to planting stress, where water loss from the shoot, through transpiration, exceeds water uptake by the root system. Planting stress will decline as seedlings start to develop a root system out into the surrounding soil, enabling them to access available soil moisture.

Abscisic acid (ABA) is a plant hormone that naturally accumulates in plants under drought stress conditions (Zeevaart and Creelman 1988). This accumulation of ABA initiates the closure of stomata, thereby reducing plant water use under conditions of restricted water availability (Davies and Zhang 1991). Past studies have found that exogenously applied ABA reduces transpiration in plants by closing stomata

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Mansfield et al. 1978, Davies and Mansfield 1983). As a result, the possibility of using ABA to control water loss in plants has been considered an attractive nursery cultural regime.

Major limitations have existed for using ABA within operational forest regeneration programs. The main problems are that natural ABA is easily degraded in the field (i.e., a very short term effect on stomatal conductance), has poor uptake capability by some plants, and is relatively expensive to synthesize. To address these concerns, scientists at the Plant Biotechnology Institute, National Research Council of Canada, have been developing a series of ABA analogs. These ABA analogs are compounds that are chemically related to natural ABA, are biologically active in plants and produce similar physiological responses as natural ABA. The ABA analogs are chemically simpler to synthesize than natural ABA and slow release forms may be produced ensuring long term effectiveness. This would allow for a large scale production of a cost-effective, ABA analog-based, antitranspirant product to be developed.

A research program has been initiated with the Plant Biotechnology Institute of the National Research Council of Canada, DowElanco Canada Ltd., the University of Victoria and the Forest Biotechnology Centre at BCRI to develop an ABA analog-based antitranspirant product. This product would help alleviate planting stress that occurs during forest regeneration programs. A research program is underway to determine whether specific ABA analogs can: 1) maintain good seedling water balance under environmentally stressful conditions, through partial stomatal closure, and 2) ensure that root growth capability is maintained under optimum conditions and possibly enhanced under environmentally stressful conditions. Preliminary results from this research program are presented in this paper.

MATERIALS

Plant material

Interior spruce (*Picea glauca* (Moench) Voss x *Picea engelmannii* Parry ex. Engelm.) seedlings were grown at Pelton Reforestation Ltd. from March through August, 1993, and placed in frozen storage on November 30, 1993. Interior spruce seedlings were a containerized (410B container) 1+0 stocktype from a single seedlot (seedlot #4061, Kamloops region, Lat. 51° 19' N, Long. 119° 57' W). Testing was conducted from January through May 1994. The seedling population was morphologically screened to ensure a standard height, diameter and root form. Seedlings were removed from frozen storage, allowed to thaw over four days and then ABA analogs were applied.

ABA analog treatments

Compounds that are structurally similar to abscisic acid (ABA analogs) were produced by the Plant Biotechnology Institute, National Research Council of Canada. Two of these ABA analogs (#1 and #5) were applied as a spray-root drench that simulated application through a nursery irrigation system. Seedlings with intact root-soil plugs were placed in a container, and ABA analog treatments were applied (10^{-3} M concentration in an acetone solvent @ 1%) as a top drench. In addition, there were two control treatments; both a water only and acetone only @ 1% (not reported). Treated seedlings were kept in the large container where the treatment flow-through was allowed to be reabsorbed into the plugs over a two day period. This ensured that all of the treatment was taken up by the root plugs and was available to seedlings. During testing, seedlings had their root-soil plugs kept intact. This enabled the ABA treatment to be retained in close contact with the root system.

METHODS

Response under low root temperature conditions

This study determined the needle conductance of seedlings treated with ABA analogs under low root temperature conditions (i.e., spring conditions). This study also determined the long term effectiveness of ABA analogs to maintain a reduction in needle conductance.

Needle conductance (g_{wv}) of seedlings from each analog (n=8) were determined with a LI-COR 6200 gas exchange system. Controlled environment conditions were 22 ^oC air temperature, 50% relative humidity and 16h photoperiod at 400 mmol m^{-2} s^{-1}. Seedlings were potted in a peat growing media, well watered and grown under these controlled environment conditions with root temperatures maintained at 12 ^oC. Needle conductance was assessed six times across the 14 day measurement period.

Response to long term moderate drought stress

This study determined whether ABA analogs enable seedlings to maintain a good water balance, under moderate drought, for extended time periods. Just after ABA analog treatment application, seedlings (n=25) were put through a series of drought cycles. Seedlings were planted in containers containing media of pure sand and placed in the above described controlled environment conditions. These containers were sealed to allow water loss to occur only through needle transpiration. Every four days, each container was watered with 40 ml of water (equivalent to 40%, by weight, of field capacity). This study was conducted over a 12 day period with watering taking place on days 0, 4, and 8 (three four-day cycles). On the first and fourth days of each of the 4-day drought cycles, midday shoot water potential (Y_{min}) readings were taken. These readings corresponded to either periods when seedlings had the maximum or minimum amount of available soil water. For each seedling, these Y_{min} readings were used to determine a mean Y_{min} for each drought cycle.

Response to rapid severe drought stress

This study determined how ABA analogs influence seedling response to severe soil drought. Just after treatment application, seedlings (n=25) were put through a rapid drought. Each seedling root plug was enclosed in a plastic bag and placed in a darkened chamber with the shoot systems exposed to the above described controlled environment conditions. Seedlings were allowed to dry out through shoot transpiration. The g_{wv} of seedlings from each treatment were measured during each daylight period. At each gas exchange measurement time, seedlings were measured with a pressure chamber to determine daylight shoot water potential (Y). Changes in gas exchange parameters and seedling water stress were determined over time until seedlings reached a Y of <-2.0 MPa.

Root growth capacity

Seedlings (n=25) were measured to determine root egress out of the soil plug over 14 days. Root growth capacity was measured on seedlings that were potted in a peat growing media, well watered and grown in the above described controlled environmental conditions.

Root growth was quantified for all roots that developed out of the container plug into the growing media. Total roots were quantified into root length categories of 1) new roots $\geq$0.5 cm, 2) new roots $\geq$2.5 cm, and 3) new roots $\geq$5.0 cm. It should be noted that each subsequently smaller root length category contained all roots measured in the longer root length categories (i.e., root category 1 = categories 1 + 2 + 3, root category 2 = categories 2 + 3).

Experimental organization and analysis of data

Both analogs were tested at the same time within each experimental procedure. Seedlings were set up in a blocked experimental design with all treatments randomized within each block. Where appropriate, an ANOVA followed by a Dunnett's test allowed comparisons between the control treatment and each ABA analog. The rapid drought treatment did not have statistical analysis conducted on results. This was due to differences between treatments in their rate of water loss over time.

RESULTS AND DISCUSSION

Low soil temperatures are one of the main factors that can cause planting stress in newly planted seedlings. Low root temperatures can restrict water uptake resulting in water stress in spruce seedlings (Kaufmann 1977, Grossnickle 1988). This limitation in seedling water movement may result in a hormonal response, analogous to soil drying, (Davies and Zhang 1991) which reduces stomatal opening.

ABA analog #1 had superior long-term drought avoidance capability under low root temperature conditions (Fig. 1). On day 1, this ABA analog reduced g_{wv} by 84% and net photosynthesis was reduced by 50% (unreported data). On days 3 through 9, ABA analog #1 reduced g_{wv} by >37% with only a slight decrease in net photosynthesis (unreported data). Though not significantly different than the control treatment on days 11 and 14, ABA analog #1 reduced g_{wv} by up to 17%.

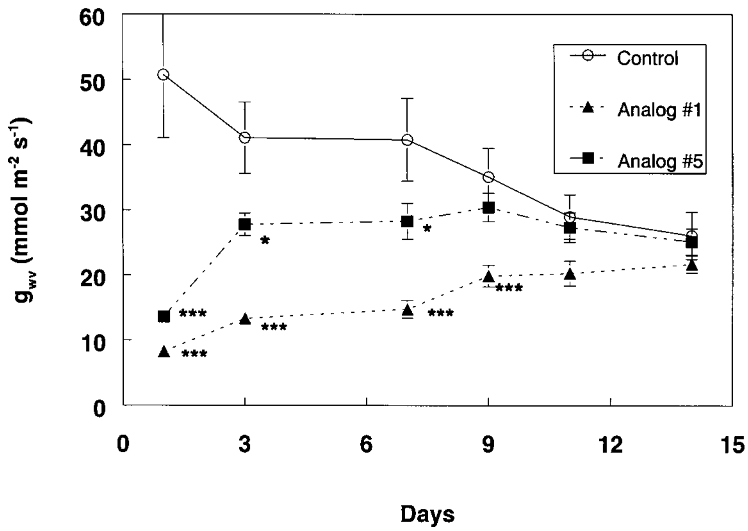

Figure 1. Needle conductance (g_{wv}) of interior spruce seedlings, from a control treatment or treated with an ABA analog, in response to low root temperature conditions. Statistical significance of 0.01 (***), 0.05 (**) or 0.10 (*) were determined by an analysis of variance and a Dunnett's test which compared each ABA analog with the control treatment

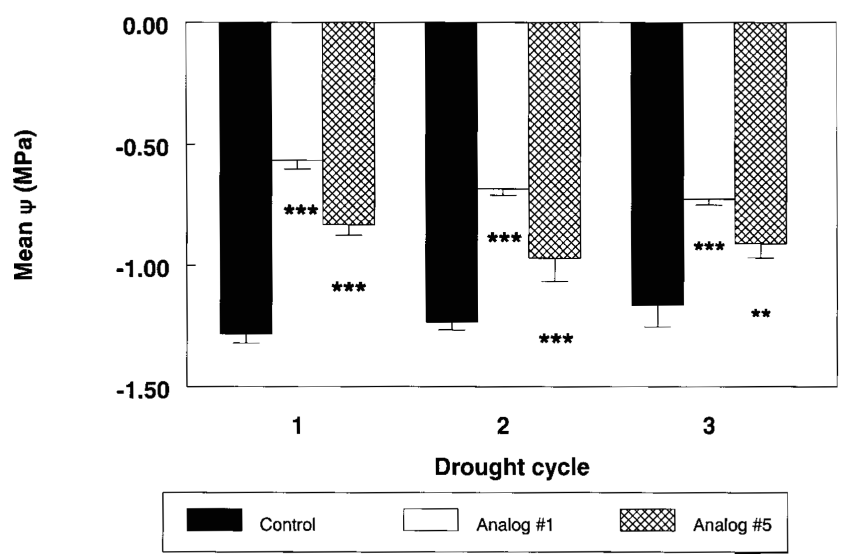

Figure 2. Mean shoot water potential (Y) of interior spruce seedlings, from a control treatment or treated with an ABA analog, over three four-day drought cycles. Statistics as in figure 1.

ABA analog #5 had good long-term drought avoidance capability under low root temperature conditions (Fig. 1). This ABA analog reduced g_{wv} from 30 to 67% for 7 days, with no significant reduction in net photosynthesis (unreported data). By day 9, ABA analog #5 had g_{wv} levels that were not significantly lower than the control treatment, though they were all causing at least a 13% reduction in g_{wv}. ABA analog #5, compared to the control treatment, caused no reduction in g_{wv} after day 10.

Exogenously applied ABA can reduce the transpiration, and/or g_{wv} on a number of woody angiosperm (Davies and Kozlowski 1975a) and coniferous (Blake et al. 1990a, Marshall et al. 1991) species for a limited length of time. Davies and Kozlowski (1975a) found ABA initially reduced transpiration by 60% and that transpiration slowly returned to normal over a period of three weeks. In addition, preliminary testing of synthetic ABA analogs applied on the leaves or via the substrate, having different chemical structures than those tested here, have also been found to reduce g_{wv} (Blake et al. 1990a) or water consumption (Rademacher et al. 1989) in plants.

Drought stress is the other main factor that can cause planting stress in newly planted seedlings. Newly planted seedlings can be exposed to drought through limited soil moisture and/or high evaporative demand conditions of the atmosphere. Drought conditions cause seedling water stress by reducing water uptake from the soil (Kaufmann 1979, Grossnickle and Reid 1984, Sands 1984, Brissette and Chambers 1992) and by increasing transpiration as evaporative demand increases (Grossnickle and Reid 1984, Grossnickle and Blake 1987, Livingston and Black 1987, Grossnickle and Arnott 1992). The result of increased water stress in newly planted seedlings is a reduction in growth (Nambiar and Zed 1980, Margolis and Waring 1986, Livingston and Black 1988, Grossnickle and Heikurinen 1989). As a result, planting stress can be exacerbated by drought conditions that reduce growth and delay a seedling's capability to occupy the site. ABA analogs may provide a means of avoiding moisture stress seedlings can be exposed to after planting on a reforestation site.

ABA analogs #1 and #5 caused superior drought avoidance capabilities in seedlings under moderate drought conditions (Fig. 2). Seedlings treated with these analogs had higher mean Y_{min}, ranging from 20 to 60% higher, in all drought cycles compared to control seedlings. The higher Y_{min} for seedlings treated with the ABA analogs, compared to the control treatment, throughout all of the drought cycles was attributable to lower g_{wv} during the initial stages of each drought cycle (unreported data).

Improvement of drought avoidance under severe drought stress conditions was defined by the length of time interior spruce seedlings maintained nonlethal Y (>-2.0 MPa). ABA analog #1 had superior drought avoidance capability (Fig. 3). This analog reduced g_{wv} and resulted in nonlethal Y being maintained over 400% longer than the control treatment. ABA analog #5 had good drought avoidance capability. This ABA analog reduced g_{wv} which resulted in nonlethal Y being maintained over 150% longer than the control treatment.

Seedlings treated with ABA analogs were capable of avoiding drought stress in either the moderate or severe drought stress experiments. Seedlings treated with ABA analogs, compared to control seedlings, had reduced transpirational water loss through reduced g_{wv}, which resulted in higher shoot Y values over extended time periods. Exogenous application of ABA to conifer seedlings can reduce transpiration and maintain more favorable shoot water potentials under drought conditions (Davies and Kozlowski 1975b, Marshall et al. 1991). In addition, exogenous application of

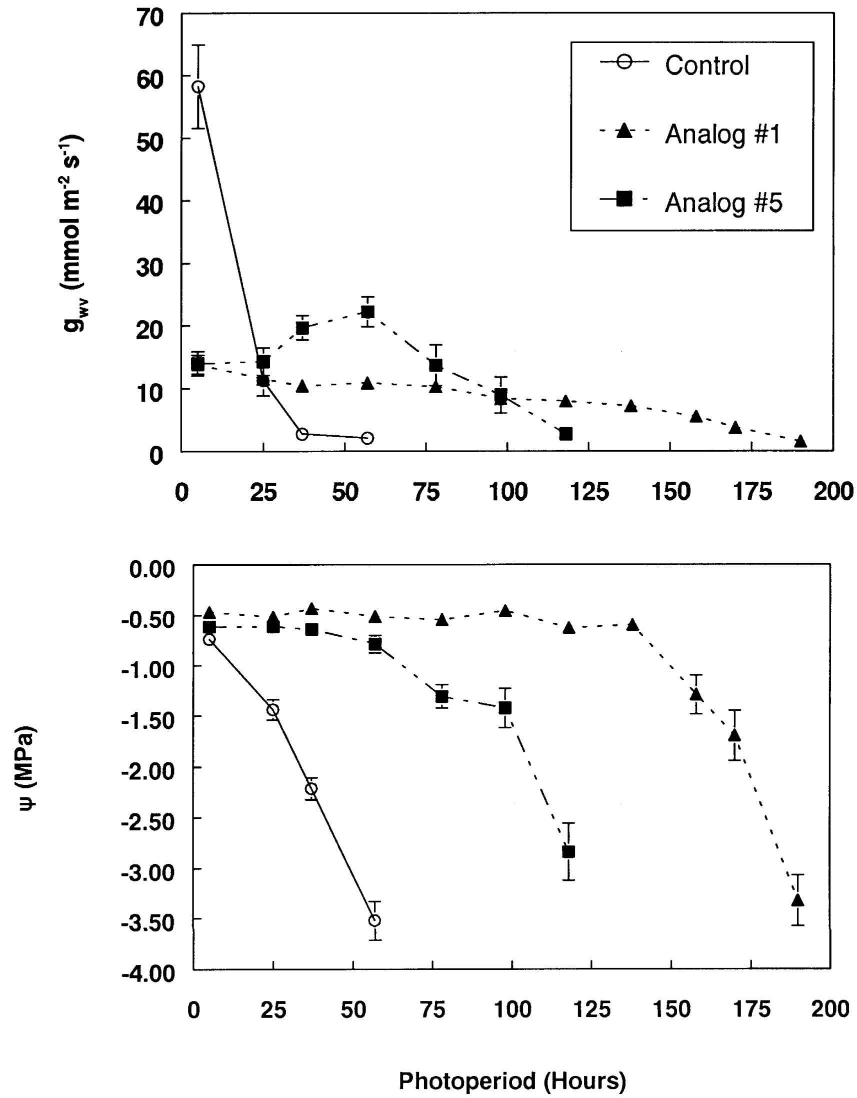

Figure 3. Needle conductance (g_{wv}) and shoot water potential (Y) of interior spruce seedlings, from a control treatment or treated with an ABA analog, over a severe drought cycle.

certain ABA analogs can reduce transpiration and improve water use efficiency in black spruce (*Picea mariana* (Mill.) B.S.P.) seedlings under drought (Blake et al. 1990a).

Improved root growth, in newly planted seedlings, will enhance the ability of seedlings to overcome planting stress and become established. Newly planted spruce seedlings that are able to develop root systems out of the container plug into surrounding soil will have reduced water stress (Grossnickle and Reid 1984). Seedlings in the control treatment, and seedlings treated with ABA analogs #1 and #5 were able to develop over 85 new roots $\geq$0.5 cm in length (Fig.4). Trials comparing laboratory measured root growth capacity with field survival, found greater than 80% survival in interior spruce seedlings that produce >10 new roots (Burdett et al. 1983, Burdett 1987, Simpson 1990, Simpson et al. 1994). This $\geq$0.5 cm measurement length is an indication of the potential number of locations on a root system that can grow new roots. ABA analogs can cause a reduction in g_{wv} and subsequently a reduction in net photosynthesis under optimum environmental conditions (Grossnickle and Folk, unreported data). It has been shown that new root growth in conifers is supported by current photo assimilated carbohydrates (van den Driessche 1987). However, current photosynthates in spruce seedlings have not been strongly related to new root production (Philipson 1988, Thompson and Puttonen 1992). Previous work also found that exogenous application of certain ABA analogs had no effect on the root development of conifer seedlings (Blake et al. 1990b). Seedlings treated with ABA analogs had the capability to grow an extensive number of new roots, when tested in this optimum environment.

Seedlings in the control treatment were able to develop around 70 new roots $\geq$2.5 cm in length, and of these new long roots, 42 were $\geq$5.0 cm in length (Fig. 4). For the longer root length categories, ABA analogs #1 and #5 resulted in approximately a 10% and 35% increase in the ≥2.5 cm and ≥5.0 cm root length categories, respectively. These measurement lengths are an indication of the ability of a root system to develop roots out into the soil and quickly become established.

Root growth results from the reported study indicate that ABA analog #1 increased the number of long roots (>5.0 cm). However, additional testing of interior spruce seedlings did not find a positive influence of ABA analogs on the growth of longer roots when seedlings were tested at a phenological stage having low root growth capacity (unreported data). This indicates the need to further define how ABA analogs influence root growth during different phenological stages that are related to the times of year when seedlings are planted on reforestation sites.

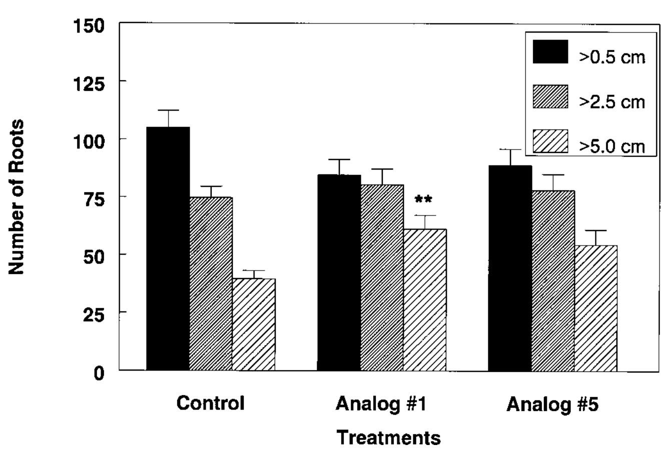

Figure 4. Root growth capacity of interior spruce seedlings, from a control treatment or treated with an ABA analog. Each subsequently smaller root length category contains all roots contained in the longer root length categories. Statistics as in figure 1.

CONCLUSIONS

Results from this research program have found that specific ABA analogs allow seedlings to maintain good water balance under environmentally stressful conditions through partial stomatal closure. These ABA analogs can cause partial stomatal closure for a period of over two weeks. In addition, ABA analogs maintain root growth capability, under optimum conditions, at levels that are conducive to seedling survival and growth when planted on reforestation sites. These results provide evidence that an ABA analog-based antitranspirant product can be developed to help alleviate planting stress in seedlings.

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