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EFFECT OF REGULATED DEFICIT IRRIGATION IMPOSED IN THE FALL ON COLD HARDINESS OF WOODY PLANTS

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Abstract

In temperate zone, early freeze, particularly if preceded by a period of warm and wet weather, can severely injure many woody plants. The young plants are particularly sensitive to frost, especially those grown in containers. The timing and capacity of cold acclimation are modified by environmental cues. We examined whether regulated deficit irrigation (RDI) imposed in the fall affects cold acclimation of containerized Lawson cypress (Chamaecyparis lawsoniana)'Columnaris' and Knaphill-Exbury azalea (Rhododendron) 'Oxydol'. Three-year-old plants were grown in containers placed in the open field and were cultivated according to standard nursery practice. In the end of growing season (from 5th October to 2nd November 2015, half of the plants were expose to moderated water stress by ceasing irrigation and protecting them from the rain (RDI treatment). The volumetric water content of the growing medium gradually decreases in this time from 0.45 to 0.2 m3/m3. Irrigation of the second part of the plants were continued in October and water content was maintained at 0.45 m3/m3 (control treatment). Cold hardiness of stem tissues was assessed two times (2nd November and 7th December) in the laboratory using the ion-leakage test. Stem tissue were exposed to 6 test temperatures, ranging from +4 to – 26˚C. Additionally, plant quality after overwintering was evaluated. Freeze tolerance of plant stems of both woody plants was significantly higher in December than November. Regulated deficit irrigation (RDI) generally increased cold hardiness of Lawson cypress in November as well as in December. At RDI treatment, ion-leakage from plant tissue frozen to – 26˚C was lower by 20% in the first time of assessment, and by 8% in the second time. Regulated deficit irrigation (RDI) increased cold hardiness of azalea stems only in November, but did not affect frost hardiness in December. At water deficit treatment, ion-leakage from plant tissue frozen to – 26˚C was lower by 12% than at control treatment. This study demonstrated that reduced water supply early autumn promoting acclimating to low winter temperatures.

Key words: controlled freezing test, electrolyte leakage, Knaphill-Exbury azalea, Lawson Cypress, regulated deficit irrigation (RDI)

INTRODUCTION

Cold hardiness determines the ability of plants to adapt and withstand freezing temperatures and is a major consideration in the application of woody plants for different landscape settings. The development of cold hardiness (acclimation) is dependent on the genetic potential, physiological status of the plants and is modulated by environmental signals (Wisniewiski et al. 2003, Gusta and Wisniewski 2013, Strimbeck et al. 2015). Container production has been found to be an efficient and cost-effective way to grow ornamental woody plants and is used more than field production in Poland and other countries in temperate zone. A major problem in overwintering container-grown plants is that the roots are significantly less cold-tolerant than the shoots of the plant. Moreover, roots of container-grown plants are subjected to temperature and moisture extremes not normally found in field production (Mathers et al. 2007). Temperatures inside the containers are normally higher than air temperature as a result of heat storage of the substrate and bedrocks which are covered by black plastic. This anomaly results in prolonging shoot growth and interferes with the process of cold acclimation. Early frosts in this situation can severely injure many woody plants (Davidson et al. 2000). Alexander and Havis (1980) found that warm root environment interferes with cold acclimation in roots and lower stems sections of *Rhododendron* and 10 cm mulch around azaleas in the fall kept the soil warm, delay the development of fall color and increases the amount of winter injury, including bark splitting. Numerous studies have implies that changes in cold hardiness and plant water relation are related and tissue water content has been observed to decrease in deciduous woody plants during cold acclimation. Tissue dehydration is beneficial in cold acclimation plants as tissues containing a large amount of free water are highly susceptible to formation of lethal intracellular ice (Guy 2003, Welling and Palva 2006). Increasing of cold hardiness was observed in stems of *Cornus stolonifera* (Parsons and Li 1979) and evergreen azaleas (Anisko and Lindstrom 1995, Anisko and Lindstrom 1996a) after 3-7 weeks of water deficit treatment in the fall. In *Rhododendron* 'Catawbiense Boursault' 6 weeks of water deficit treatment increased cold hardiness by 0.5-5˚C (Holt and Pellett 1981). In contrast, *Fuchsia megellanica* 6 weeks of water deficit treatment followed by four weeks of short day and low temperature did not affect stem hardiness significantly (Pagter and Petersen 2008). Van den Driessche (1969) reported that reducing the water supply had no direct effect on cold hardiness of *Pseudotsuga menziesii* seedlings. The effect of water deficit on cold hardiness may also be determined by the relative severity of the stress, timing and duration of stress application. Water stress applied for few weeks have been shown to increase cold hardiness of several woody plants, but continuation of water deficit treatment for whole vegetative season reduced cold hardiness of *Rhododendron* ‚Catawbiense Boursault' (Anisko and Lindstrom 1996b). Seasonal cold-acclimation patterns in response to deficit irrigation have been established for evergreen azaleas and elepidote rhododendrons characterized by large, leathery leaves and thick bark on the stems, but not for deciduous azaleas with stems covered by a thin bark. Moreover, evergreen azaleas acclimated more slowly than deciduous azaleas (Alexander and Havis 1980) so their reaction on deficit water supply can be different.

Electrolyte leakage resulting from decreased cell membrane integrity of freeze damaged tissues can be easily measured in the laboratory to determine freezing tolerance of diverse species, including rhododendrons and conifers (Flint et al. 1967, Zhang and Willison 1987, Keates 1990, Lim et al. 1998, Morin et al. 2007, Zhang et al. 2009, Baldi et al. 2011, Lim et al. 2014, Strimbeck et al. 2015). This method is simple, repeatable, and rapid, ensures freezing stress on intact tissues, and has been used to predict field performance.

The aim of the study was to determine whether reduced water supply in the fall affects cold acclimation and overwintering of containerized Lawson cypress 'Columnaris' and deciduous Knaphill-Exbury azalea 'Oxydol'. This taxa were chosen because they represent nursery crops of high commercial value with moderately sensitivity to frost of Lawson cypress (6A USDA plant hardiness zone) and winter hardy Knaphill-Exbury azalea (5B USDA plant hardiness zone).

MATERIAL AND METHODS

As plant material, two ornamental woody plants (evergreen Lawson Cypress *Chamaecyparis lawsoniana* 'Columnaris' and deciduous Knaphill-Exbury azalea *Rhododendron* 'Oxydol') were used. The study was located in Research Institute of Horticulture in Skierniewice, Poland (51°57'17" N, 20°09'30" E, 128 m above sea level, hardiness zone 6B) and plants were exposed to the natural day length. The longest and the shortest day during the year occur 21st June (the length 16h 43') and 22nd December (7 h 46').

Three-year-old plants were grown in containers placed in the open field and were cultivated according to standard nursery practice. The plants were potted 20 April 2015 into 3-liter (azalea) or 2.5-liter (Lawson cypress) pots filled in sphagnum peat moss (Klasmann-Deilmann) supplemented with slow release fertilizer Osmocote Standard 5-6M (15N–9P_2O_5–12K_2O 15-9-12 + 2 MgO with micronutrients, Everris Int. B.V., The Netherlands) at the dose 3 g/L. For Lawson Cypress the substrate was amended with chalk at a rate of 5 $g \cdot l^{-1}$ with a target pH of 6.0 pH. The plants were watered using a drip irrigation emitters (PC Junior CNL 2 L h^{-1}, Netafim, Israel).

Plants of were grown under two watering regimes. The volumetric water content of the substrate was maintained at 0.45 m^3/m^3 throughout the whole growing season (well-irrigated control treatment). Plants at regulated deficit irrigation (RDI) treatment were exposed to 4 weeks of moderated water stress in the end of growing season (from 5th October to 2nd November) by ceasing irrigation and protecting them from rain. Water content of the growing substrate gradually decreases in this time from 0.45 to 0.2 m^3/m^3. Water content of the substrate was monitored two times per week with a TetaMeter (DaltaT Devices, England) with the three sensors located at substrate at 5 cm depth. Starting from 2nd November plants at RDI treatment were irrigated as control plants to provide adequate moisture during dormant season and early growth the following year. Plants were organized in three randomized complete blocks with eight plants per each block × species × watering regime. During the winter period, pots were grouped together to provide protection from frost.

At the termination of growing period (28th October), plant height and diameter, relative water content in stems as well as plant quality (visual assessment, scale from 1 to 5, where 1 is the weakest and 5 is the highest quality) were measured. Relative water content in stem tissue was determined by measuring the fresh weight of 5-cm-long sections. The same stem sections were oven-dried to a constant weight and relative water content was calculated. Additionally, overwintering assessment after a regrowth period of 30 days plants were rated using a 1 to 5 qualitative scale, where 1 means dead plant; 2 – alive, but little top growth; 3 to 5 – vigorous regrowth and 5 means the highest quality.

Cold hardiness of stem tissues was assessed two times at 2nd November (before re-irrigating of plants in RDI treatment) and 7th December 2015 in the laboratory using the ion-leakage method. Current season's 5-cm-long stem section for azalea after rinsing in tap water and demineralized water, were wrapped in moist paper towels to ensure ice nucleation and re-closed in polyethylene bags. For Lawson cypress the distal segments of primary shoots (stems with current-year of foliage, approximately 1.5 g per sample), the tissue type most commonly used for cold tolerance assessment of Cypress genera, was used (Schaberg et al. 2005).

Freezing was accomplished in a controlled-climate chamber (Binder, USA) according to a stepwise lowering freezing program starting from the prevailing outdoor temperature and with a test temperature interval of 5˚C. The freezing rate was $5^{\circ}C \cdot h^{-1}$, and the duration of each test temperature 2 h. There were five test temperatures (-10; – 14; – 18; – 22 and – 26°C). Control shoots were maintained at +4˚C for the duration of the freezing. Samples were thawed at 0˚C in closed Styrofoam boxes for 15 to 24 h. Ions were extracted with 35 mL demineralised water for 20-24 h at room temperature and the electrical conductivity measured (L_{1T}) using a Orion 4-Stars Meter (Thermo Scientific, USA) with temperature-corrected display. After determination of the electrical conductivity the samples were autoclaved at 121˚C for 1 h to allow maximum leakage of ions. After autoclaving the samples were allowed to cool to room temperature and the electrical conductivity was measured again (E_{2T}). The electrical conductivity of demineralised water was measured to give the zero level of electrical conductivity. The measurements were converted into indices of injury according to Flint et al. (1967): Index of injury (IT) = 100 *(RT – R0)/(1 – R0); RT = L_{1T}/L_{2T}; R0 = L_{1C}/L_{2C}, where L_{1T} and L_{2T} are the initial and final leakage values for a sample exposed to temperature T, and L_{1C}, andL_{2C} are the corresponding values measured from respective control samples. Three stems/shoots from randomly selected plants representing each block × watering regime × test temperature was used for ion-leakage measurements for each species.

The effects of regulated deficit irrigation (RDI) treatment on a range morphological and plant quality parameters were examined using one-way ANOVA. Data obtained in the ion-leakage test were analyzed using a two-way ANOVA, where watering regime (2 levels i.e. well-irrigated control for whole cultivation period and moderated water stress (RDI) for 4 weeks in the end of growing season) and temperature (6 levels i.e. +4˚C; – 10; – 14; – 18; – 22 and – 26°C) were the fixed factors. The analysis of variance (ANOVA) was conducted using STATISTICA software. When the ANOVA indicated significant effects, means were separated using Tukey's HSD test, with $p < 0.05$ considered to be statistically significant.

RESULTS AND DISCUSSION

The volumetric water content of the growing medium for plants exposed to 4 weeks of regulated deficit irrigation (RDI) in the end of growing season gradually decreased from 0.45 to 0.22 m^3/m^3 for Lawson cypress 'Columnaris' and from 0.45 to 0.19 m^3/m^3 for azalea 'Oxydol' (Figure 1). Re-watering resulted in a rapid increased water content to control level 0.45 m^3/m^3.

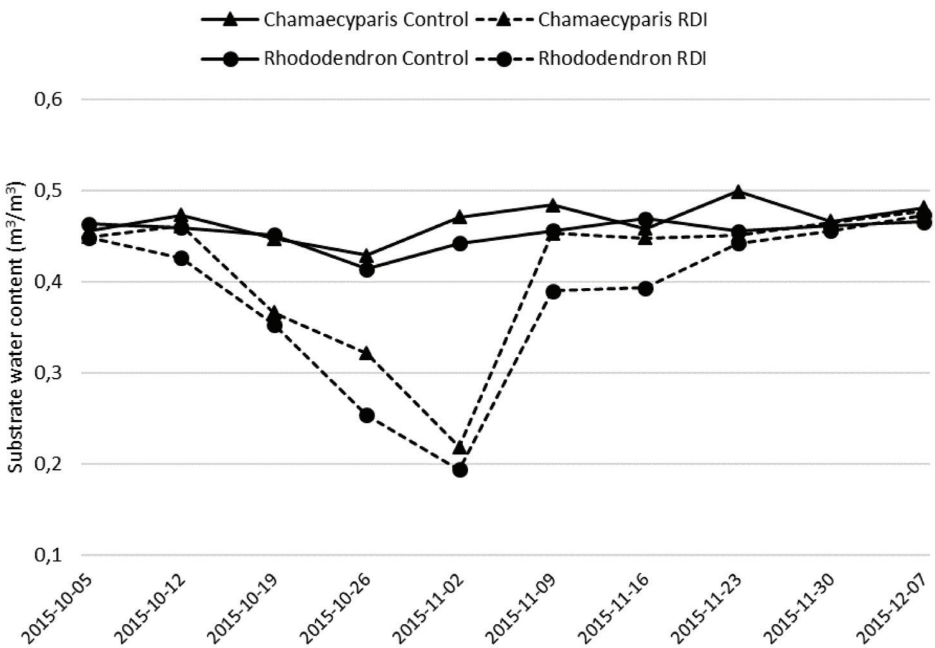

Figure 1. Substrate water content (m^3/m^3) of potted *Chamaecyparis lawsoniana* 'Columnaris' and *Rhododendron* 'Oxydol'. Data are means of 9 plants per treatment

Table 1. Effect of regulated deficit irrigation (RDI) imposed in the fall on plant growth, shoot water content, visual assessment and regrowth quality of *Chamaecyparis lawsoniana* 'Columnaris' and *Rhododendron* 'Oxydol'

Plant species	Irrigation	Plant height (cm)	Plant diameter (cm)	Shoot water content (%)	Visual assessment	Regrowth quality
Chamaecyparis lawsoniana 'Columnaris'	Control	39.5 a	22.4 a	64.1 b	4.4 a	4.2 a
	RDI	39.0 a	18.7 a	57.3 a	4.5 a	4.4 a
Rhododendron 'Oxydol'	Control	38.9 a	20.8 a	50.7 b	4.2 a	4.5 a
	RDI	38.7 a	22.6 a	44.2 a	4.9 a	4.6 a

Plants exposed to 4 weeks of moderated deficit irrigation (RDI) experienced a degree of water stress indicated by the lower water content in stems (10.6% less for Lawson cypress and 12.8% less for azalea) comparing to well-irrigated plants (Table 1). However this moderate water stress did not affect plant size (height and diameter), plant quality assessed in the end of growing season as well as plant quality after overwintering outdoors. All plants regrew vigorously and had similar quality rating. The minimum air temperatures during the winter 2015/2016 was – 17.8˚C (5th January). The Knaphill-Exbury deciduous azaleas are relatively tolerant to water stress (more tolerant than evergreen azaleas). Lawson cypress requires humid environment and moist substrates, however this species is able to effective control change in water potential in drier conditions during growing season (Zobel and Liu 1980).

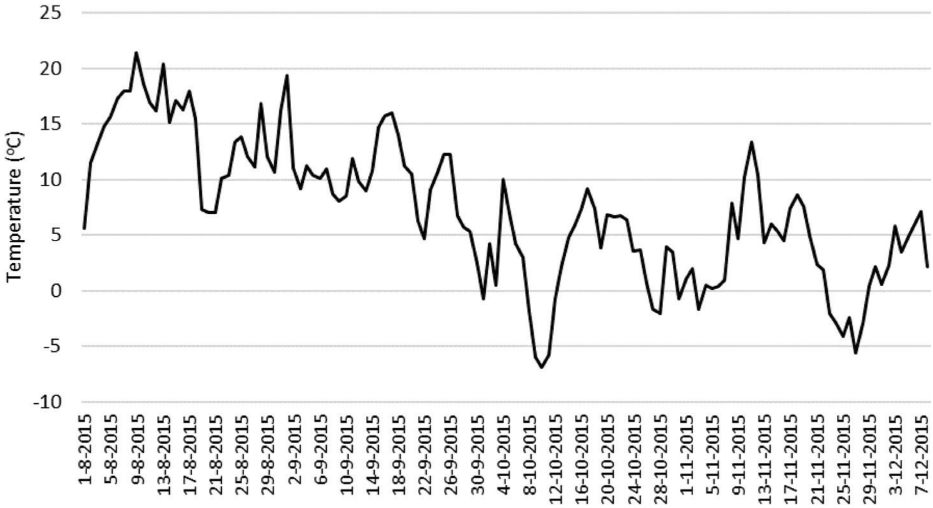

Figure 2. The lowest daily temperatures (°C) in the period from 1st August to 7th December 2015

Freeze tolerance of the two species studied was significantly higher in December than in November. In the period until 7th December when frost resistance tests were finished, the lowest outdoor temperature was – 7.0˚C (Figure 2). Lawson cypress 'Columnaris' and azalea 'Oxydol' in this time cold acclimated, as index injury I_T decreased at successively lower treatment temperature over the fall. Index injury of stem tissue of control plants increased gradually from – 18˚C with decreasing temperature reaching the value 48% at – 26˚C in November and 29% in December for Lawson cypress (Figure 3) and 38% and 24% respectively for azalea (Figure 4). In December, when plants were cold acclimated index of injury of control stems exposed to – 18˚C was relatively low (5% for azalea and 10% for Lawson cypress).

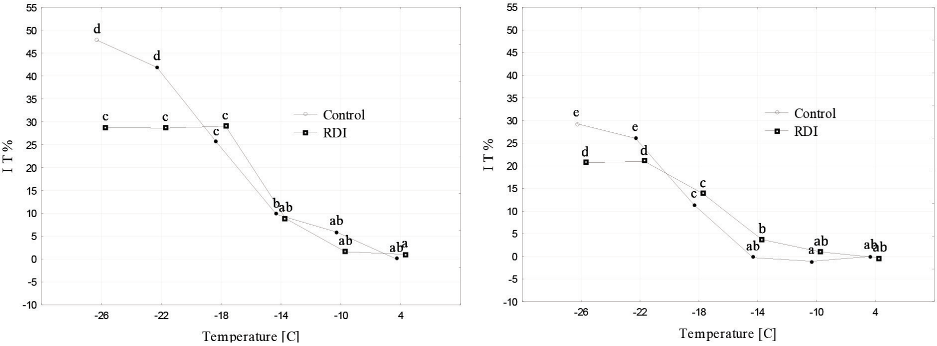

Figure 3. Index of injury (IT) of *Chamaecyparis lawsoniana* ‘Columnaris’ at T temperature assessed 2nd November 2015 (on the left) and 7th December 2015 (on the right) as affected by 4 weeks regulated deficit irrigation (RDI) imposed in the fall (mean separation by Tukey’s HSD test, data points with the same letters do not differ significantly with $p < 0.05$)

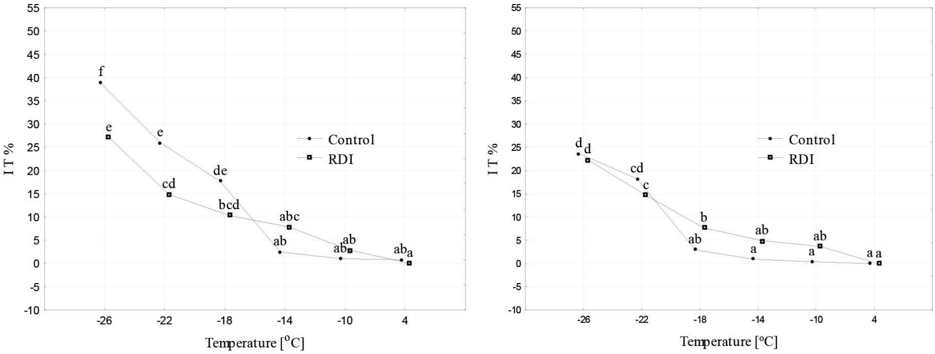

Figure 4. Index of injury (IT) of *Rhododendron* ‘Oxydol’ at T temperature assessed 2nd November 2015 (on the left) and 7th December 2015 (on the right) as affected by 4 weeks regulated deficit irrigation (RDI) imposed in the fall (mean separation by Tukey’s HSD test, data points with the same letters do not differ significantly with $p < 0.05$)

Regrowth measurements and electrolyte leakage tests are useful assays for determining cold hardiness levels after freezing test and the value ≥ 50% are highly correlated with killing temperatures. Tissue viability data are easier to interpret during the early stages of acclimation, but much difficult as plant approach maximum winter hardiness (Iles and Agnew 1995). Alexander and Havis (1980) shown that the stems of five selected clones of deciduous azalea exhibit an initial lag before rapid acclimation. In mid-November cold hardiness of the stems ranged from – 25 to – 34˚C and the maximum hardiness was attained in January, when withstood – 40°C. In our study index if injury of stem tissue for both tested species at – 26˚C was significantly higher in November than December. For deciduous Knaphill-Exbury azalea 'Oxydol' index of injury in the beginning of November did not exceed the value 50% suggesting that lethal temperature in this time is lower than – 26˚C, and for Lawson cypress 'Columnaris' is close to this temperature.

Regulated deficit irrigation (RDI) in the end of growing season decreased ion leakage from stem tissue in November at the lowest temperatures – 22˚C and – 26˚C for Lawson cypress 'Columnaris' (Figure 3) and azalea 'Oxydol' (Figure 4) and did not affect significantly this parameter at temperatures in the range from – 10˚C to – 18˚C. Cold hardiness of Lawson Cypress in December was less dependent on deficit irrigation than in November. At water deficit treatment, index of injury of Lawson cypress stem tissue frozen to – 26˚C was lower by 20% in November and by 8% in December compared to control well irrigated plants. For azalea, water deficit treatment increased cold hardiness of stems only in the first time of assessment (2nd November). At water deficit treatment, index of injury of azalea stem tissue frozen to – 26˚C was lower in November by 12% than at control treatment. These finding are supported by earlier observation (Anisko and Lindstrom 1995, Anisko and Lindstrom 1996) where water stress-induced cold hardiness of evergreen azaleas disappeared 6 weeks after normal watering was resumed. The higher water content treatment in this time either prevented or delayed acclimation. The decrease in cold hardiness after re-watering of previously water stressed plants also agrees with the finding of Parson and Li (1979) for red-osier dogwood and Amundson et al. (1993) for red spruce.

CONCLUSIONS

This study demonstrated that 4-weeks reducing water supply in the fall provides a feasible mean of promoting acclimation and hardiness of the Knaphill-Exbury deciduous azalea and evergreen conifer Lawson cypress produced in containers. Reducing irrigation in nursery practice is easy to apply and moreover contribute to a more sustainable use of non-renewable natural resource that is water.

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