Seasonal Changes in Cold Hardiness of *Rhododendron* L. ‘Catawbiense Boursault’ Grown under Continuous and Periodic Water Stress

Tomasz Anisko and Orville M. Lindstrom

Department of Horticulture, University of Georgia, Georgia Experiment Station, Griffin, GA 30223

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Abstract. Water status is known to have an impact on cold hardiness of plants. Cold hardiness of ‘Catawbiense Boursault’ rhododendron was examined under continuous and periodic water stress. Under continuous stress, water content of growing medium was maintained at 0.6 to 0.75, 0.45 to 0.6, or 0.3 to 0.45 m³·m⁻³. Under periodic stress, water content was either maintained between 0.6 to 0.8 m³·m⁻³ or plants were subjected to drought episodes at various times in late summer, autumn, and early winter. During a drought episode, watering was delayed until water content was below 0.4 m³·m⁻³. Watering then resumed and water content was maintained between 0.3 to 0.4 m³·m⁻³. Cold hardiness was evaluated on detached leaves and stem sections. The effect of continuous water stress depended on its severity and duration. Moderate stress did not increase cold hardiness compared to well watered plants during the first winter, but it did so when continued into the second winter. More severe stress increased cold hardiness during the first winter, but it decreased cold hardiness during the subsequent winter. The effect of periodic water stress depended on the timing of application. During initial and final stages of acclimation, cold hardiness increased in response to water stress less than during the intermediate stages. Water-stress-induced cold hardiness gradually decreased after rewatering.

Seasonal changes in cold hardiness are well correlated with the water content of many plants, including Koster azalea (*Rhododendron ×kosterianum* C.K. Schneid.) (Lindstrom et al., 1977), red-osier dogwood (*Cornus sericea* L.) (Li and Weiser, 1971; Chen et al., 1975; McKenzie et al., 1974; Parsons, 1978; Bray and Parsons, 1981), ‘Hetzi’ juniper (*Juniperus chinensis* L. ‘Hetzi’) (Pellett and White, 1969), jack pine (*Pinus banksiana* Lamb.) (Calme et al., 1993), lodge-pole pine (*Pinus contorta* Dougl.) (Jonsson et al., 1981), black spruce [*Picea mariana* (Mill.) B.S.P.] (Calme et al., 1993; Colombo, 1990), white spruce [*Picea glauca* (Moench) Voss] (Calme et al., 1993), and winter cereals (Fowler and Carles, 1979). Also, artificial dehydration of tissues has been demonstrated to increase cold hardiness in apple (*Malus domestica* Borkh.) (Tyler and Stushnoff, 1988), cabbage (*Brassica oleracea* var. *capitata* L.) (Cox and Levitt, 1976), red-osier dogwood (Li and Weiser, 1971), and winter cereals (Cloutier and Andrews, 1984; Cloutier and Siminovitch, 1982; Siminovitch and Cloutier, 1982; Tyler et al. 1981). In contrast, there was no relationship between cold hardiness and tissue water content in spinach (*Spinacia oleracea* L.) (Fennell et al., 1990) and in several species of alder (*Alnus* B. Ehrh.) and birch (*Betula* L.) (Takato et al., 1964).

Despite extensive research, the extent to which cold hardiness can be modified by altering tissue water content culturally is unclear. Attempts to increase cold hardiness of plants by regulating tissue water content through induced water stress have had varying degrees of success (Chen and Gusta, 1978). Water stress increased cold hardiness in several herbaceous plants including alfalfa (*Medicago sativa* L.) (Paquin, 1977), asparagus (*Asparagus officinalis* L.) (Burrows et al., 1989), cauliflower (*Brassica oleracea* var. *botrytis* L.) (Biddington and Dearman, 1988), lettuce (*Lactuca sativa* L.) (Biddington and Dearman, 1988), ryegrass (*Lolium perenne* L.) (Thomas and James, 1993), and winter wheat

(*Triticum aestivum* L.) (Gusta et al., 1982; Macdowall and Lowdon, 1989). Similar increases in cold hardiness induced by reduced watering were reported for woody plants, such as citrus (*Citrus* spp.) (Yelenosky, 1979), evergreen azaleas (*Rhododendron* spp.) (Anisko and Lindstrom, 1995), guava (*Psidium* spp.) (Utsunomiya, 1988), jojoba [*Simmondsia chinensis* (Link) C.K. Schneid.] (Nelson et al., 1993), and red-osier dogwood (Chen et al., 1975, 1977; Chen and Li, 1977, 1978). On the other hand, van den Driessche (1969) demonstrated that reducing water supply had no direct effect on cold hardiness of Douglas-fir seedlings [*Pseudotsuga menziesii* (Mirb.) Franco].

The effect of water stress on cold hardiness may depend on the timing of stress application. Blake et al. (1979) subjected Douglas-fir seedlings to water stress for about 30 days at various times during the summer and observed an increase in cold hardiness only when stress treatment began before photoperiodic induction of dormancy. Calme et al. (1993) found that cold hardiness of black spruce seedlings was not affected by water stress imposed by withholding irrigation for 2 weeks starting 13 Aug. They, similarly, attributed this lack of water-stress-induced hardening to the timing of the stress application after initiation of dormancy.

Little is also known about changes in hardiness following recovery from water stress. Parsons and Li (1979) demonstrated that reducing watering during a 3-week period increased cold hardiness of red-osier dogwood by 8 to 10°C. Upon rewatering, however, previously water-stressed plants lost most of their induced hardiness in the following 3 weeks. Similarly, Amundson et al. (1993) withheld irrigation of red spruce (*Picea rubens* Sarg.) from 18 Aug. to 13 Sept. and found that during and immediately after the water-stress period, the cold hardiness of stressed trees increased. This water-stress-induced hardiness disappeared 3 weeks after resuming irrigation. Burrows et al. (1989) reported that rehydration of water-stressed asparagus nullified immediately the previous increase in cold hardiness. In contrast, Stout (1980) found the increase in cold hardiness of alfalfa remaining for several weeks after the water-stress treatment. Timmis and Tanaka (1976) demonstrated that growing Douglas-fir seedlings for 8 to 9 weeks under reduced irrigation did not affect their cold hardiness imme-

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diately after the stress treatment. Cold hardiness measured 5 and 11 weeks after resuming normal irrigation, however, was increased in seedlings previously under water stress treatment. These studies indicate that the timing of water stress and changes in cold hardiness after recovery from stress should be considered if the effectiveness of water stress in inducing cold hardiness is to be properly evaluated.

The purpose of the present study was to determine whether water stress increases cold hardiness of 'Catawbiense Boursault'. Cold hardiness of plants subjected to continuous or periodic stress was examined. The effectiveness of water stress imposed at various times and changes in cold hardiness following the plants' recovery from stress were investigated.

Materials and Methods

'Catawbiense Boursault' plants were grown for 3 years in an unheated plastic house at the Georgia Station, Griffin, Ga. One-year-old plants were potted in 2.2-liter containers the first year of the study and repotted in 9-liter containers the second year. The growing medium (Metro Mix 300, Grace Sierra Horticultural Co., Milpitas, Calif.) contained Canadian sphagnum peat, vermiculite, processed bark ash, and washed sand. Plants were fertilized (1 g·liter⁻¹) with Sta-green (11N-5P-5K, Progress Growers Supply, Canton, Ga.) three times between March and June of each year.

Continuous stress study. Plants were grown under three watering regimes between 24 Aug. 1992 to 23 Mar. 1994. Water content of the growing medium was intended to remain in a range of 0.6 to 0.75 m³·m⁻³ (wet), 0.45 to 0.6 m³·m⁻³ (medium), or 0.3 to 0.45 m³·m⁻³ (dry). Watering of plants under each regime was discontinued or resumed when the water content reached above or below intended levels, respectively. On several occasions, water content exceeded target ranges. The intended average water content levels, however, were maintained for each watering regime. Between 9 Jan. to 21 Feb. 1993 irrigation of plants under all watering regimes was temporarily withheld to estimate the duration of drought episode sufficient to lower the water content to the minimum level (about 0.30 m³·m⁻³), determination of which was needed for the periodic stress study. There were three replications of 10 plants for each watering regime, and the replications were organized in a randomized complete-block design.

Periodic stress study. Plants were grown under six watering regimes. Water content of the growing medium was either maintained near field capacity, i.e., 0.6 to 0.8 m³·m⁻³, for the duration of the study (no stress) or plants under each of the remaining five watering regimes were subjected to the periodic stress at different times during the year. Based on the preliminary data from the continuous stress study, drought episodes lasting approximately six weeks were used. In 1993-94 and 1994-95, periodic water stress was imposed from 30 Aug. to 9 Oct. (early fall), 9 Oct. to 20 Nov. (late fall), 20 Nov. to 1 Jan. (early winter), and 1 Jan. to 19 Feb. (late winter). In 1994-95, a watering regime with water stress imposed from 15 July to 30 Aug. (late summer) also was used. Watering of the water-stressed plants was delayed until the volumetric water content reached below 0.4 m³·m⁻³. Watering then resumed and water content was maintained in a range of 0.3 to 0.4 m³·m⁻³. Except for the time of the drought episode intended for each watering regime, plants were watered to near field capacity. There were three replications of three plants for each watering regime, and the replications were organized in a randomized complete-block design.

Drought induction methods. The volumetric water content of the growing medium was monitored with a time-domain reflecto-

metry technique (Anisko et al., 1994). The method used a cable tester (Tektronix 1502C Metallic Time Domain Reflectometer; Tektronix, Beaverton, Ore.) to measure the apparent dielectric permittivity of the growing medium, which strongly depended on water content. An empirical relationship between the dielectric constant and water content was developed as a calibration equation (Anisko et al., 1994). A randomly selected pot in each block × watering regime was used for monitoring water content of the growing medium.

Evaluation of cold hardiness. Cold hardiness of plants was evaluated in the laboratory with freeze tolerance tests on detached leaves and stem sections. During the first year of the continuous stress study, the small size of the plants limited the amount of plant material available and prevented the use of stems in freeze tolerance tests. Fully expanded leaves and stem sections about 4 to 5 cm long were sampled from the current season's growth. Samples were collected from all plants and pooled for each block × watering regime. Leaves and stem sections were wrapped in moist cheesecloth, placed in test tubes, and inserted in a freezing bath (Forma Scientific, Marietta, Ohio). Sample temperature was recorded by thermocouples placed next to the stem or leaf. To avoid excessive supercooling of tissue water, when the temperature of the samples stabilized at $-2.0 \pm 0.5^{\circ}\text{C}$, the samples were inoculated with ice crystals. After freezing the samples, the temperature was lowered $2^{\circ}\text{C}/\text{h}$. Samples were removed from the bath at 2°C intervals (three leaves and stem sections for each block × watering regime) and thawed in the refrigerator at $2.0 \pm 0.5^{\circ}\text{C}$. Control samples were kept on ice for the duration of the test. To evaluate injury, thawed samples were placed in polyethylene bags for 7 days and incubated

Fig. 1. Seasonal changes in cold hardiness of leaves (A) and stems (B) of 'Catawbiense Boursault' subjected to wet, medium, and dry watering regimes in 1992-93 (open symbols) and 1993-94 (solid symbols) (mean separation between watering regimes within dates and tissue type by Tukey's studentized range; data points with the same letter do not differ significantly at $P = 0.05$, $n = 3$).

at room temperature. Freezing injury was evaluated visually with oxidative browning as the criterion and rated on a scale: 0 = no browning, 1 = browning light or in small areas, 2 = browning darker or in larger areas, and 3 = all sample dark brown. The Richards function (von Fircks and Verwijst, 1993) fitting to the rating values was performed by modified Gauss-Newton method using the NLIN procedure of SAS (SAS Institute, Cary, N.C.). The inflection point of the Richards function was used as an estimate of the lethal temperature (von Fircks and Verwijst, 1993). Analysis of variance within sampling dates was performed on the estimates of the lethal temperature to test the effect of watering regimes on cold hardiness. Comparisons between means were conducted using Tukey's studentized range test.

Results

Continuous stress study. During Winter 1992–93, watering regimes affected cold hardiness of leaves on 9 Jan. ($F = 20.25$, $P = 0.0021$) and 6 Mar. ($F = 22.21$, $P = 0.0017$), but not on 20 Feb. ($F = 1.74$, $P = 0.2590$), following a drought episode, which lowered water content in all watering regimes to the level of 0.3 to 0.35 $\text{m}^3 \cdot \text{m}^{-3}$ (Fig. 1A). Leaves of plants under the dry regime were more cold hardy than those in the medium or wet regime on 9 Jan. and 6 Mar. 1993. Differences in cold hardiness between the medium and wet watering regimes were insignificant.

During Winter 1993–94, watering regimes affected hardiness of leaves and stems on all dates ($P < 0.05$). Leaves of plants under the dry regime reached a similar level of cold hardiness as in 1992–93 (Fig. 1A). In contrast, plants under medium and wet regimes

acclimated to temperatures about 10 to 12°C lower than during the first winter. As a result, plants under the dry regime were the least cold hardy during late fall (20 Nov.) and winter (1 Jan. and 11 Feb.). Leaves of plants under the medium regime were the most cold hardy, except during the onset of dehardening (27 Mar.), when their cold hardiness was lost quicker than that of the leaves under the dry regime (Fig. 1A). A similar relationship was observed in stems, with the exception of 1 Jan. and 11 Feb., when cold hardiness of stems of plants under medium and wet regimes was not different (Fig. 1B).

Periodic stress study. In 1993–94, the effect of watering regimes on cold hardiness of leaves was significant on 10 Oct. ($F = 9.68$, $P = 0.0358$), 21 Nov. ($F = 20.54$, $P = 0.0002$), and 19 Feb. ($F = 4.53$, $P = 0.0240$), but not on 1 Jan. ($F = 0.73$, $P = 0.5636$) and 27 Mar. ($F = 0.11$, $P = 0.9760$) (Fig. 2A). A similar relationship was observed for stems, except for the 1 Jan. test, when cold hardiness depended on watering regime ($F = 65.42$, $P = 0.0001$) (Fig. 2B). In 1994–95, watering regimes affected hardiness on all dates ($P < 0.05$), except 19 Feb. for leaves ($F = 0.85$, $P = 0.5435$) and 27 Mar. test for both leaves ($F = 2.76$, $P = 0.0693$) and stems ($F = 2.70$, $P = 0.0733$) (Fig. 3).

In 1993–94 and 1994–95, the differences in cold hardiness between well watered control plants (no stress) and plants exposed to drought for 6 weeks before freeze testing were significant during the cold acclimation stage (the late summer, early fall, and late fall regimes). There were no differences when plants reached maximum midwinter hardiness and later deacclimated (the early winter and the late winter watering regimes). This relationship held true for leaves and stems, with the exception of stems under the late

Fig. 2. Seasonal changes in cold hardiness of leaves (A) and stems (B) of 'Catawbiense Boursault' subjected to five watering regimes in 1993–94 (mean separation between watering regimes within dates and tissue type by Tukey's studentized range; data points with the same letter do not differ significantly at $P = 0.05$, $n = 3$).

Fig. 3. Seasonal changes in cold hardiness of leaves (A) and stems (B) of 'Catawbiense Boursault' subjected to five watering regimes in 1994–95 (mean separation between watering regimes within dates and tissue type by Tukey's studentized range; data points with the same letter do not differ significantly at $P = 0.05$, $n = 3$).

winter regime tested on 19 Feb. during Winter 1994–95, which were more cold hardy than those under the no stress regime.

The magnitude of the increase in cold hardiness caused by 6 weeks of drought ranged from about 0.5 to 5C in leaves, and from about 2 to 5C in stems (Fig. 4). Upon returning the watering of stressed plants to control levels, however, this difference gradually decreased. Interestingly, stems of plants water-stressed in the early fall watering regime, later during Winter 1993–94 became about 2C less cold hardy than those of plants under the no stress regime (Fig. 4A). No such relationship was noted during Winter 1994–95 (Fig. 4B). In 1993–94 and 1994–95, the greatest increase about 5C in cold hardiness of leaves and stems was observed in plants under the late fall watering regime tested on 21 Nov. (Fig. 4). Regressing the increase in cold hardiness caused by each drought episode vs. cold hardiness of leaves and stems of plants under the no stress regime revealed a hyperbolic relationship between these variables (Fig. 5). It appears that those plants that begun cold acclimation and those near the completion of the cold acclimation process were less responsive to water stress than plants that had attained intermediate levels of cold hardiness. This dependence of the water-stress-induced increase in cold hardiness on the advance of cold acclimation was more pronounced in leaves (Fig. 5A) than in stems (Fig. 5B).

Discussion

In studies that demonstrated a water-stress-induced increase in cold hardiness, drought conditions were imposed by withholding or reducing watering for a certain time, ranging from days (Biddington and Dearman, 1988; Yelenosky, 1979) to weeks (Anisko and Lindstrom, 1995; Chen et al., 1975; Macdowall and

Lowdon, 1989; Thomas and James, 1993; Utsunomiya, 1988), and months (Utsunomiya, 1988; Nelson et al., 1993). We know of no study that examined changes in cold hardiness of plants growing under continuous reduced watering for more than a year. Data from the continuous stress study indicate that duration of the stress has a profound effect on inducing cold hardiness. Growing plants under the dry watering regime for about 5 to 8 months significantly increased their cold hardiness compared to plants under the wet regime. Continuation of the dry regime through the second year, however, reduced plant cold hardiness. In contrast, growing plants under the medium watering regime, did not increase their cold hardiness compared to wet regime during the first year, but it did so after another year. Parsons and Li (1979) also noticed that severe water stress resulted in less cold hardiness of red-osier dogwood than did moderate stress. Similarly, subjecting guava plants to severe water stress for a long period reduced their cold hardiness, whereas stress applied for a shorter time increased plant freeze tolerance (Utsunomiya, 1988). It may be hypothesized that the relative severity of the water stress (dry vs. medium watering regimes) and its duration together determine a plant's response leading to the increased cold hardiness.

The observed difference in cold hardiness of plants under the wet regime between 1992–93 and 1993–94 suggests that the cold acclimation of one-year-old plants was reduced during Winter 1992–93. This was probably due to their young age and the regeneration processes associated with rooting cuttings and also with transplanting rooted cutting into pots. Even during Fall and Winter 1993–94 plants under the wet watering regime did not

Fig. 4. Lethal temperature differentials between stressful watering regimes and the no stress watering regime in leaves and stems of 'Catawbiense Boursault' in 1993–94 (A) and 1994–95 (B).

Fig. 5. Relationship between lethal temperature changes caused by a drought episode and the lethal temperature of plants under the no stress watering regime in leaves and stems of 'Catawbiense Boursault' (leaves in 1993–94: $y = 11.88 + 2.53x + 0.0987x^2$, $R^2 = 0.85$; leaves in 1994–95: $y = 6.85 + 1.53x + 0.0494x^2$, $R^2 = 0.92$; stems in 1993–94: $y = 0.286 + 0.636x + 0.0208x^2$, $R^2 = 0.99$; stems in 1994–95: $y = 1.20 + 0.641x + 0.0187x^2$, $R^2 = 0.79$).

reach the level of cold hardiness previously reported for this cultivar (Holt and Pellett, 1981; Marcinkowski, 1985) and for other cultivars of the Catawbiense-Hybridum group (Sakai et al., 1986).

The magnitude of the increase in cold hardiness induced by 6 weeks of drought, observed in this study (about 0.5 to 5C), corresponds to the values reported previously for several cultivars of evergreen azaleas (Anisko and Lindstrom, 1995). Larger increases in cold hardiness (8 to 10C) were found in red-osier dogwood subjected to reduced watering (Chen et al., 1975, 1977; Chen and Li, 1978, Parsons and Li, 1979). Red-osier dogwood is capable of acclimating to below -70C (Li and Weiser, 1971), whereas maximum cold hardiness of 'Catawbiense Boursault' rhododendron does not exceed -30C (Holt and Pellett, 1981; Marcinkowski, 1985). This fact suggests that the temperature limit to which a plant can acclimate, in addition to the relative severity of stress (Li and Weiser, 1971; Parsons and Li, 1979; Utsunomiya, 1988) and timing of stress application (Blake et al., 1979; Utsunomiya, 1988), may determine the magnitude of the water-stress-induced increase in cold hardiness.

Utsunomiya (1988) reported that water stress applied beginning on 15 Oct. and 9 Dec. greatly increased cold hardiness of guava. In contrast, water stress applied starting 4 Aug. was ineffective. The author concluded that cessation of growth and exposure to low temperatures are required for water stress to increase cold hardiness. This is consistent with our findings that a drought episode applied between 10 Oct. and 21 Nov. produced a larger increase in cold hardiness than drought stress imposed earlier in the season. Blake et al. (1979) and Calme et al. (1993), on the contrary, reported that water stress applied after induction of dormancy in late summer was ineffective. In the Blake et al. (1979) study, only actively growing Douglas-fir seedlings increased in cold hardiness when exposed to water stress. The present study demonstrated that an increase in cold hardiness caused by exposure to water stress depended on the advancement of the acclimation process as indicated by cold hardiness of well-watered plants. During the initial and final stages of acclimation, plants were less responsive to water stress than during the intermediate stages.

The gradual decrease in water-stress-induced cold hardiness after rewetting observed in this study is consistent with previous findings for red-osier dogwood (Parsons and Li, 1979), winter wheat (Tyler et al., 1981), red spruce (Amundson et al., 1993), and evergreen azaleas (Anisko and Lindstrom, 1995). This gradual decrease disagrees, however, with the observations of Timmis and Tanaka (1976). They found that Douglas-fir seedlings grown under drier conditions were later able to harden to a greater extent than those under wet conditions. In this study, plants exposed to a drought episode during the fall (early and late fall watering regimes), stems (both years) and leaves (1994-95 only) remained about 2C more cold hardy 6 weeks after resuming normal watering. In contrast, plants that were stressed earlier (late summer regime) or later (early and late winter regimes) in the season, lost nearly all of their water-stress-induced increase in cold hardiness in 6 weeks following rewetting. The difference in the residual effects of stress between plants stressed in late summer or late winter and those stressed in the fall (early and late fall regimes) may be in part explained by changes in daylength. Parsons and Li (1979) reported that cold hardiness of water-stressed red-osier dogwood decreased following rewetting more in plants under 16-h photoperiod than in plants under 8-h photoperiod. The changes of the photoperiod, however, cannot account for the smaller residual effect of the early winter drought episode.

Delayed residual effects of water stress imposed in late summer, similar to those caused by the early and late fall watering regimes in 1993-94, were observed by Amundson et al. (1993). In their study, cold hardiness of red spruce plants, which were water-stressed between 18 Aug. and 13 Sept., increased during and immediately after the stress period. However, 2 months later (13 Nov.), plants were less cold hardy than well watered control plants.

This study demonstrated that cold hardiness of 'Catawbiense Boursault' rhododendron can be increased by imposing continuous or periodic water stress. The effectiveness of the continuous water stress depended on its relative severity and duration. The effectiveness of the periodic water stress depended on the timing of application, which reflected the dependence on the stage of cold acclimation process.

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