Water deficits promote flowering in Rhododendron via regulation of pre and post initiation development

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ABSTRACT

Flowering is generally considered to be advanced by water deficits in many woody perennial species. A long-standing paradigm being that as a plant senses severe environmental conditions resources are diverted away from vegetative growth and towards reproduction before death. It is demonstrated that in Rhododendron flowering is promoted under water deficit treatments. However, the promotion of flowering is not achieved via an increase in floral initiation, but through separate developmental responses. If regulated deficit irrigation (RDI) is imposed prior to the time of initiation, fewer vegetative nodes are formed before the apical meristems switch to floral initiation, and chronologically, floral initiation occurs earlier. Both RDI and partial rootzone drying (PRD) treatments stimulate the development of more flowers on each inflorescence if the treatments are continued after the plant has undergone floral initiation. However, floral initiation is inhibited by soil water deficits. If the soil water deficit continues beyond the stages of floral development then anthesis can occur prematurely on the fully formed floral buds without a need for a winter chilling treatment. It is hypothesised that inhibition of floral initiation in plants experiencing severe soil water deficits results from the inhibitory action of ABA transportation to the apical meristem from stressed roots. It is demonstrated that ABA applications to well-watered Rhododendron inhibit floral initiation.

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1. Introduction

There are many reports containing circumstantial evidence and preliminary observations of water deficits (drought conditions) promoting floral initiation, while few studies have demonstrated conclusive repeatable induction. Moderate plant water deficits enhance flowering in many important horticultural and forestry species including Kalmia (Carden, 1995), Litchi (Stern et al., 1993), Picea (Ross, 1986) and Pyrus (Mitchell et al., 1986), Macroptilium atropurpureum (Kowithayaakorn and Humphreys, 1987) and Citrus (Nir et al., 1972; Krajewski and Rabe, 1995). Cameron et al. (1999) reported that plant water deficits enhanced flower bud formation in Rhododendron, but the promotion only occurred when treatments were applied after floral initiation. However, there are also many reports that floral initiation can be inhibited by water deficits. Inhibition by water deficits has been demonstrated in Lolio temulentum (King and Evans, 1977), Ipomoea nil and Xanthium strumarium (Aspinall and Hussain, 1970).

In apical buds, the concentration of the phytohormone abscisic acid (ABA) can increase rapidly during periods of plant water deficit (King and Evans, 1977). Exogenous ABA has been demonstrated to promote floral initiation in Chenopodium rubrum, Lemna paucisetata and l. nil (El-Antably and Wareing, 1966; Takeno and Maeda, 1996; Maeda et al., 2000). In C. rubrum and l. nil, enhancement of floral initiation only occurs in inductive photo-periods (Lozhnikova et al., 1981; Nakayama and Hashimoto, 1973). This suggests that ABA does not have a dominant control in the switch to flowering in these species, but probably acts synergistically with other signalling pathways. The findings that floral initiation in l. nil is promoted by ABA application, but inhibited by water deficits in other studies suggests that, in this species, ABA may only act when concentrations increase in tissues not experiencing water deficits. In addition, the effects of ABA remain ambiguous and species specific. Exogenous ABA has been demonstrated to delay flowering in Spinacia oleracea, L. temulentum, Dianthus sp. (carnation) and petunia sp. (Vince-Prue, 1985).

Ethylene is another hormone that regulates plant responses to water deficits by acting to control vegetative growth of plants in drying soils (Sharp et al., 2000; Chaves et al., 2003). However, most studies have reported ethylene applications inhibit floral initiation (Bernier et al., 1981). One explanation for the positive effects of

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exogenous ABA in certain species might be that ABA promotes floral initiation by down-regulating ethylene synthesis, which would otherwise inhibit floral initiation.

The response of plants to water deficits depends upon the methods of application. A complete withholding of water is a severe treatment that does not allow for biochemical or molecular acclimation to the reduced water availability. Most species will eventually require watering to be reinstalled if the stress is to be controlled, maintained, and for the plant to survive. The timing and volume of re-watering is usually related to a physiological parameter such as stomatal conductance, leaf temperature or plant weight, which are indicative of the stress applied. Regulated deficit irrigation (RDI) is a method of restricting plant daily water supply. A RDI application requires a proportion of the daily plant water loss to be reapplied; thus, achieving a soil water deficit that is gradually imposed. Cameron et al. (1999) reported a promotion of floral initiation in Rhododendron when RDI was applied in late summer. In partial root-zone drying (PRD) treatments, one part of the soil is allowed to dry down while the remainder is kept well-watered. At set intervals, watering is switched so that all roots experience some soil drying. This differential imposition of soil drying promotes the synthesis and transport of ABA and other chemical signals from the roots in drying soils to the aerial parts of plants where they act to close stomata (Loveys, 1984). This phenomenon combines with the effect of the water supplied from the well-watered section to maintain cell turgor (Mingo et al., 2003). It was investigated if this increased flux of root-sourced ABA to leaves in PRD and RDI treatments facilitated the transport of the floral signal, and therefore, promotes flowering in Rhododendron.

By imposing soil water deficits only during the time of floral initiation, it was possible to separate the direct effect on flowering from secondary effects resulting from inhibition of growth and photosynthesis during the vegetative phases. ABA was applied to the shoots of well-watered plants to determine if it could elicit the same flowering responses as observed on deficit treated plants and thereby be implicated in a water deficit-flowering signaling pathway. Additionally, water deficits were applied to different ages of plants to determine if water deficits could reduce the long juvenile phase that restricts the commercial production of Rhododendron yakushimanum hybrid cultivars.

2. Materials and methods

2.1. Plant material

All plants were potted in a 90% peat/bark medium (v/v). Plants were irrigated to container capacity before treatment application began by submerging the planted container and medium in tap water for 2 h and allowing them to drain for 1 h before weighing. Apical shoots were pruned at the start of each experiment to ensure that all developing meristems were vegetative.

2.2. Regulated deficit irrigation applied to R. cv. ‘Hatsugiri’ in a controlled environment

2.2.1. Treatments and experimental design

One hundred and twenty, two-year-old ‘Hatsugiri’ plants were potted into 1 L pots. The experiment was conducted in a controlled environment (CE) growth chamber. A mean PAR in 16 h (LD) daylengths at canopy height of 400–500 μmol m⁻² s⁻¹ was supplied by 400 W sodium luminaires (NAV-T Super, Osram, Munich, Germany). The temperature in the canopy was 18 °C day/16 °C night and humidity ranged from 59.2% during the day to 91.7% at night. Plants were maintained in the environment for 10 days before the imposition of treatments.

Water deficit treatments were established by applying daily irrigation in proportions of the estimated potential evapo-transpiration (ETp) of the plants over a 24 h period. Six reference plants, positioned in the crop, were weighed daily (including container and medium). The reference plants were re-watered to container capacity and the mean water loss calculated. This represented the actual evapo-transpiration (1.0 ETa). A drip-irrigation system was used to impose the treatments of well-watered 1.5 ETp, moderate stress 0.5 ETp and severe stress 0.25 ETp. Experimental blocks of 15 plants consisted of five plants of each treatment and were arranged into eight rows.

Stomatal conductance (gₛ), stem elongation, and plant and container weight were measured every 7 days. Three stems per plant were tagged for weekly length measurements using digital calipers. Abaxial stomatal conductance (gₛ) of a leaf on a tagged stem was measured using a steady state porometer (EGM-I, PP systems, Hertfordshire, UK). All measurements were made between 12:00 and 13:00. Diurnal measurements of gₛ, ψstem, RWC, and plant and container weight were taken on a representative plant from each treatment in each block at 00:30, 05:30, 10:00, 13:00, 17:00 and 21:00 at day 20.

The shape and size of the petioles, plus the nature of xylem tissue in ‘Hatsugiri’ was not conducive to measurements of leaf water potential (ψleaf) and relative leaf water content (RLWC). Stems were therefore the unit sampled for these parameters in ‘Hatsugiri’. A selected stem was cut transversely and sealed into a Scholander pressure chamber (Chas W. Cook & Sons, Birmingham, UK) to measure stem water potential (ψstem). The chamber was lined with damp filter paper to increase humidity and compressed air was progressively added to the chamber at a rate of 0.5 MPa min⁻¹ until the vascular tissues darkened or sap first appeared at the cut surface when viewed with a ×15 hand lens. The magnitude of the applied pressure was equal, but opposite, to ψstem.

In order to determine relative water content (RWC) of stems, the upper 40 mm section of a stem was excised and re-cut under distilled water. The weight of the stem was noted and the cut end placed into a 1.8 mL vial (Chromacol, Welwyn Garden City, UK) filled with distilled water. Vials were then placed into a misted controlled environment at 20 °C. After 24 h, the stem was considered to be fully re-hydrated. Stems were then blotted dry, re-weighed, oven dried for 48 h at 80 °C and re-weighed. RWC was calculated as:

\[
\text{RWC} = \frac{\text{fresh weight} - \text{dry weight}}{\text{re-hydrated weight} - \text{dry weight}}
\]

Maximum quantum efficiency of photosystem II photochemistry (Fm/Fm) was measured using a FMS2 pulse modulated chlorophyll fluorimeter (Hansatech Instruments Ltd., Norfolk, UK). Two leaves per plant had leaf clips placed around them to put them into darkness for 20 min. A fibre optic adapter supplied a saturating beam (3000 μmol m⁻² s⁻¹) and the resulting fluorescence intensity recorded. Records were made of the ratio Fm/Fm, which is the difference between the original base fluorescence (F₀) and the fluorescence maximum (Fm) after the beam was supplied.

2.3. Imposition of water deficit treatment in Rhododendron yakushimanum cultivars

2.3.1. Experimental design

Water deficit treatments were imposed on the cultivars R. yakushimanum cv. ‘Hoppy’ (‘Hoppy’) and R. yakushimanum cv. ‘Scintillation’ (‘Scintillation’) to determine if water deficit could induce floral precocity in these cultivars by forcing them to initiate flowers at a younger age. Thirty plants of ‘Hoppy’ and ‘Scintillation’ of two ages were exposed to each treatment. 2.5-year-old plants...
were potted into 3 L containers and 3.5-year-old plants were potted into 7.5 L containers.

Blocks of 30 plants consisted of 10 plants of each treatment with two plants in each treatment reserved for destructive analysis. Non-destructive measurements and spring flower counts were conducted on the remaining eight plants. The experimental plants were surrounded by at least one row of guard plants to minimise boundary effects.

2.3.2. Establishment and monitoring of PRD and RDI treatments

One method for the establishment of PRD treatments physically divides the root system into two equal parts, planting them into separate containers and supply one container with water whilst the other dries down. Irrigation alternates between the pots at regular intervals. However, the PRD treatment in this experiment did not utilise this split pot system, as the physical attributes of a peat-based growing medium made it possible to dry down half of the medium within a pot without separation. Water was supplied for the same length of time as the WW treatment (1.1 ETp), but was not collected. Thus, one side of the medium began to dry whilst the other side was kept fully hydrated. Dripper spikes were transferred to the opposing side of the pot every three weeks. Soil moisture content was determined by inserting a Theta probe (Delta-T Devices, Cambridge, England) into the medium. The signal from the probe in mV was converted into m3 m−3 by a conversion program in the attached Theta meter type HH1 (Delta-T Devices, Cambridge, England), previously established for peat-based soils. The drying side of media was significantly dryer than the side where the dripper spike was located before the location of the dripper was switched over (0.117 m3 m−3, LSD = 0.091). Differences in the means of bulk soil water content between PRD and WW treatments were not significant (0.071 m3 m−3, LSD = 0.091).

In the RDI treatment, 0.7 ETp was supplied by adjusting the open-time of the solenoid in the same manner as the WW treatment. Water run-through was collected in saucers for re-absorption. WW and RDI dripper spikes remained in the same position throughout the experiment. Plant and container weight, Δt, Ψleaf and RWC were measured daily on the six plants per treatment assigned for destructive analysis from 11:30 to 14:00.

2.3.3. Targeting the imposition of RDI and PRD to the time of floral initiation

RDI treatments were imposed on ‘Hatsugiri’, but only during the period of floral initiation, thereby minimising the effect of water deficits on vegetative growth and floral development. ETp was calculated for each individual plant to remove two major sources of error in calculating RDI levels. If ETp is estimated on reference plants, then variability exists between the water use in the reference plants and those in the WW treatment. In addition, as the experiment progresses, the plants in the WW will produce more apical buds and maintained at container capacity for 20 days in a glasshouse where air temperature was maintained at ~18 °C in 16 h (LD) photoperiods. Earlier microscopy studies indicated that floral initiation was not induced until day 28. Treatments were imposed one week before the estimated time of floral initiation to allow the medium to dry down to a sufficiently low water content for mild deficits to be perceived. After 20 days, 1.0 ETp irrigation continued to be supplied to WW and PRD treated plants, while RDI-treated plants received 0.7 ETp. The PRD treatment was established using the methods described above. Two buds from each plant (RDI and WW) were removed daily for analysis under ×50 magnification. Once 20% of the buds had initiated, thirty buds per plant were removed to determine the percentage of apical meristems initiated per plant.

2.4. ABA application experiment

To assess the role of ABA in floral initiation and vegetative growth 25, 250, 1000 and 2500 ng ± ABA were applied to six plants each in 70% ethanol/water (v/v) using a repetitive pipette. Treatments were supplied to ten stems in three aliquots of 2 μL, one aliquot to a single flushing apical bud. From initial studies on endogenous ABA concentrations, these applications were within the natural range of ABA in actively growing and dormant Rhododendron bud and leaf tissue. Floral initiation, stem length and stomatal conductance were measured after 29 days.

2.5. Indices of flowering

In all the experiments outlined, the plants not used for destructive measurements were transferred to a 2 °C cold store for four weeks under SDs (8 h) simulated the chilling requirement. This treatment promoted anthesis once plants were forced in an unheated polythene growth tunnel, where the following measurements were taken:

- the number of floral buds per plant;
- the number of flowers per floral bud;
- the number of flowers per plant;
- the percentage of plants flowering;
- the percentage of plants with one or more floral buds;
- the number of nodes produced before floral initiation;
- date of anthesis.

2.6. Statistical analysis of experiments

Data were processed using Genstat 10.1 (Rothamsted Experimental Station, UK). Results were analysed by ANOVA and significance amongst mean values was determined by least significant difference (LSD) values where P = 0.05. LSD values were calculated from standard error of difference of means (SED) and the relevant degrees of freedom.

3. Results

3.1. The effects of RDI on flowering and vegetative growth in ‘Hatsugiri’

There was no significant effect of either moderate or severe RDI treatment on the percentage of plants with one or more floral buds, the number of floral buds per plant or the number of flowers per floral bud (Table 1). Plants produced fewer nodes before floral initiation in moderate RDI (16.3 nodes) and severe RDI (16.1 nodes) compared to the WW treated plants (23.3 nodes) (LSD = 2.1, P < 0.05, d.f. = 37). The degree to which RDI was imposed did not significantly affect the number of nodes produced.

RDI plants weighed less than WW plants after seven days of treatment. Severe RDI plants weighed less than the moderate RDI

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plants floral (%)</th>
<th>Floral buds per plant</th>
<th>Flowers per bud</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 ETp</td>
<td>72</td>
<td>31.5</td>
<td>2.8</td>
</tr>
<tr>
<td>0.5 ETp</td>
<td>68</td>
<td>32.5</td>
<td>2.9</td>
</tr>
<tr>
<td>0.25 ETp</td>
<td>70</td>
<td>27.4</td>
<td>2.75</td>
</tr>
<tr>
<td>LSD</td>
<td>11.5</td>
<td>6.5</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Table 1

Effects of the irrigation regimes well-watered (1.5 ETp), moderate (0.5 ETp) and severe (0.25 ETp) regulated deficit irrigation on (A) percentage of plants floral, (B) floral buds per plant and (C) flowers per bud in R. cv. ‘Hatsugiri’. Data are means of 40 replicate plants, LSD values at P < 0.05. d.f. = 37.
plants for the majority of the time-points of the experiment (Fig. 1A). For both deficit treatments, the weight loss was initially rapid and then slowed to an almost steady rate towards the end of the treatment. RDI triggered a reduction in stomatal conductance after seven days of the treatment (Fig. 1B). The stomatal conductance of the severe and moderate RDI plants was not significantly different for the majority of data-points recorded. For those times where the difference was significant, \( g_s \) was greater in the moderate RDI. RDI treatment slowed shoot elongation growth rates, with final stem lengths significantly shorter than plants maintained at container capacity. The degree of RDI treatment did not significantly affect stem length. Fig. 1C appears to show a reduction of stem length at the end of the flush of growth, however, this is due to the leaves surrounding the apical bud opening out as the growth flush slowed.

3.2. Diurnal physiological responses to RDI treatment

The same diurnal pattern of \( g_s \) was observed in all treatments, with \( g_s \) max in the WW plants at 13:00 (Fig. 2A). \( g_s \) rapidly increased after dawn (05:30), with the maximum \( g_s \) recorded at 13:00 in all treatments. Severely stressed plants had limited \( g_s \) throughout the 24 h, never rising above 30 mmol m\(^{-2}\) s\(^{-1}\). The diurnal change in \( \Psi_{stem} \) in the WW treatment was gradual and \( \Psi_{stem} \) decreased to a minimum of ~0.5 MPa at 13:00 and did not start to increase until after 17:00 (Fig. 2B). The \( \Psi_{stem} \) of severe RDI plants was lower than WW plants throughout the 24 h period, but the difference between moderate RDI plants and WW plants was insignificant at all measurement times. The \( \Psi_{stem} \) minima were at 13:00 for all treatments. There was no significant difference between treatments in the first RWC diurnal study. A threefold increase in the number of replicates sampled was still insufficient to obtain significant differences or diurnal trends in RWC. \( F_v/F_m \) in the leaves of severe RDI plants (0.787) was significantly lower than in both moderate RDI (0.809) and WW treatments (0.817) (LSD = 0.02, \( P < 0.05 \), d.f. = 13). The difference in PSII efficiency between WW and moderate RDI plants was not significant.

3.3. The effects of RDI and PRD on floral initiation and vegetative growth in R. yakushimanum cultivars

RDI inhibited floral initiation in the 2.5-year-old ‘Hoppy’ and 3.5-year-old ‘Scintillation’ plants (Table 2). There was no significant effect of PRD on the number of floral buds per plant. In the 2.5-year-old plants, PRD treatment increased the number of flowers per floral bud compared to the WW plants (Table 2), while the effect was insignificant in the older 3.5-year-old plants. RDI treatment in the 2.5-year-old ‘Hoppy’ plants increased the number of flowers per buds compared to WW plants. Premature anthesis occurred in plants from all treatments, but the average number of buds that opened prematurely on individual plants was increased by RDI (Table 3). RDI treatment reduced the number of branches produced in the 3.5-year-old plants (Table 2). PRD significantly reduced the number of branch produced in the 3.5-year-old ‘Scintillation’ plants.

3.4. Physiological perception of the irrigation treatments

Leaf water potentials were significantly reduced by RDI compared to the WW treatment, with the most negative \( \Psi_{leaf} \) in...
the 2.5-year-old ‘Hoppy’ plants (Fig. 3A). There was no significant effect of PRD on $\Psi_{\text{st}}$ compared to WW in either cultivar or size of plant. RDI triggered stomatal closure in ‘Hoppy’ and the 2.5-year-old ‘Scintillation’ plants (Fig. 3B). In the 2.5-year-old ‘Scintillation’ plants, $g_s$ was significantly higher in PRD plants compared to those in the WW treatment. WW plants weighed more than those in PRD. The RDI treatment produced the lightest plants of all the treatments (Fig. 3C).

3.5. The effects of targeting RDI and PRD treatments to the time of floral initiation

Floral initiation was observed first on meristems from RDI treated plants. However, after all plants had started to initiate flowers, RDI treated plants had significantly lower percentages of floral initiation than WW treated plants (Table 4). The number of nodes produced before floral initiation were significantly lowered by RDI treatment and was associated with shorter branches.

Floral initiation was observed on PRD treated plant meristems before WW plants. No significant treatment differences in the percentage of meristems initiated were obtained. In WW plants, 16.7% of meristems initiated, compared to 18.9% in PRD plants (LSD = 11.3%).

3.6. The effects of ABA applications on floral initiation and vegetative growth

Applications of 250 and 1000 ng ABA inhibited floral initiation (Fig. 4A). The inhibition of floral initiation was not proportional to the amount of ABA applied. Floral initiation was reduced to 64.3% of control levels when 250 ng ABA was applied and 68.6% of control values when 1000 ng was applied. ABA (25 ng) significantly reduced stem lengths to 35% of stem lengths on control plants (Fig. 4B). Stem lengths were proportional to the mean stomatal conductances in the treatments (Fig. 4C).

4. Discussion

4.1. The flowering response of Rhododendron to water deficits

The effects of deficit irrigation on floral initiation were not consistent with the hypothesis that water deficits promote floral initiation in *Rhododendron*. However, promotion or suppression of flowering depended upon the flowering index chosen to express the data. RDI treatment resulted in a reduction in the number of floral buds per plant in the *R. yakushimanum* cultivars, but no significant difference was observed when RDI was imposed on *R. cv. ‘Hatsugiri’*. The PRD treated plants also consistently maintained the number of floral buds per plant that were formed on plants. PRD treatment increased the number of flowers per bud in shy-flowering cultivars, principally in younger plants. Time to initiation was shorter in RDI treated plants when the treatment was applied only during the time when floral initiation occurs. It is hypothesised that the increase in flower number in the PRD treatment is neither ABA or hydraulically regulated. This is based on the findings that exogenous ABA was found to inhibit floral initiation and that there were no significant changes in $\Psi_{\text{stem}}$ in either PRD experiments.

The number of nodes produced before floral initiation was significantly decreased by RDI treatments. Both the shorter time taken to initiate and the reduced number of nodes produced before floral initiation suggests that both chronological and physiological time to flowering are reduced by water deficits. Other floral indices, such as the number of floral buds per plant and the percentage of plants flowering generally indicated there to be no direct promotion of floral initiation by water deficits. Water deficits did not promote flowering until the photoperiod was raised to 16 h LDs, with this response similar to the case in *I. nil*, where promotion is variable and only occurs in inductive photoperiods (Lozhnikova et al., 1981). The dominance of photoperiodic control over any effect of water deficit, along with
the loss of flowering responses to water deficits as plants age suggests that other regulatory pathways controlling flowering dominate in *Rhododendron*.

When soil water deficits were continued beyond the period of floral initiation and development, precocious flowering was observed in the autumn on the *Rhododendron yakuskimanum* cultivars. The premature anthesis observed may have evolved as a strategy to maintain reproductive capacity in severe environmental conditions. Continued water deficits may substitute for, or reduce, the chilling and SD requirement necessary to induce anthesis (Criley, 1985; Post, 1942). Premature anthesis could feasibly be exploited by *Rhododendron* growers to produce flowering plants available for sale in autumn months.

### 4.2. The sensitivity of flowering responses to water deficits

The water deficits imposed were not too weak to induce a promotion of floral initiation. The Ψ(leaf) generated in the RDI treatments was in the same range (0.7–1.0 MPa) reported to promote floral initiation in *M. atropurpureum* (Kowithayaakorn and Humphreys, 1987). In this study, gs and Ψ(stem) were lower in both moderately and severely stressed plants throughout the 24 h period and showed the same patterns observed in wild stands of *Rhododendron maximum* (Boa and Nilson, 1988), as well as in unrelated plant species (Talbott and Zeiger, 1998; Tyree, 1988). These results demonstrate that a physiological stress was perceived, even if floral initiation was being regulated during the night. Lower chlorophyll fluorescence (Fv/Fm) in the 0.25 ETp treatment compared to the 0.5 ETp treatment indicates long-term damage to photosynthetic apparatus. It is clear that the severe treatment had detrimental physiological effects, but the *Rhododendron* drought-avoidance strategy allowed the maintenance of reproductive capacity at the expense of vegetative growth. The increase in flowers per bud could result from the diversion of photoassimilates away from vegetative growth as a water deficit is perceived by the plant. Work by Chikov et al. (2001) would indicate

### Table 4

<table>
<thead>
<tr>
<th>Variable</th>
<th>WW</th>
<th>RDI</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floral initiation (%)</td>
<td>63.8</td>
<td>33.8</td>
<td>14.7</td>
</tr>
<tr>
<td>Nodes before floral initiation</td>
<td>8.86</td>
<td>7.75</td>
<td>0.91</td>
</tr>
<tr>
<td>Branch length (mm)</td>
<td>38.45</td>
<td>30.56</td>
<td>6.91</td>
</tr>
</tbody>
</table>
that photoassimilates would not be taken up as readily by younger leaves in drought conditions as uptake of photoassimilates is proportional to the transpiration rate of the leaf. Surplus photoassimilates in the transpiration stream might then be utilised instead by actively developing floral buds.

4.3. The effectiveness of PRD treatments

A common feature of PRD treatments is reduced stomatal apertures, promoted through ABA transported from stressed roots (Zhang and Davies, 1991; Stoll et al., 2000). Stomatal closure did not occur in the shy-flowering cultivars when PRD treatment was imposed. However, the treatment was perceived, as it increased the number of flowers per bud. A reduction in stomatal conductance did occur when PRD was calculated from the leaf in the PRD plants. This is a key feature of PRD treatments where plant water balance is maintained, but root-sourced hormonal signals control water loss through reductions in stomatal aperture (Auge and Moore, 2002).

4.4. The role of ABA in the flowering responses of Rhododendron

Drought stress results in large increases in the amount of root-sourced ABA delivered to shoots in the transpiration stream (Jokhan et al., 1996; Shashidhar et al., 1996). It is hypothesised that under severe water deficit, ABA concentrations at the meristems are raised to levels where it inhibit floral initiation. ABA applications significantly reduced delivery of ABA from severely stressed roots inhibits meristem induction. Unfortunately no ABA-deficient mutants of Rhododendron species exist and we were unable to obtain ABA biosynthesis inhibitors that lack strong side-effects, which would help facilitate further testing of the control ABA exerts on the flowering responses to soil water deficits in Rhododendron.

4.5. Implications for horticulturists

It is generally assumed amongst horticulturists that some woody ornamental species (including Rhododendron) initiate more flowers under water deficits as a plant senses the stress to be at a near-critical level. The hypothesis being that all resources are then mobilised for reproductive development. This is not the strategy that has evolved in Rhododendron. Instead, plants respond to soil water deficits by initiating flowers earlier, maintaining reproductive capacity at the expense of vegetative growth, forming more flowers on inflorescences and opening flowers in autumn. All these responses can improve crop quality, and therefore, deficit irrigation treatments have potential for commercial use by Rhododendron growers.

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