Project title:	**Improved flowering of Rhododendron and other woody plant species**
Final report:	**August 2003**
Project number:	**HNS 102**
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Location of Project:	**HRI-East Malling and Efford**
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Key words:	***Camellia*, flowering, hardy ornamental nursery stock, light quality, *Magnolia, Rhododendron*, water deficits, root restriction**

CONTENTS

GROWER SUMMARY

Headline

- In the species tested, no universal stress treatment that improved flowering was identified.
- The flowering potential of *Rhododendron* and *Camellia* is often reduced by imposing mild water deficits but plants appear more compact and uniform.
- Imposing mild water deficits throughout Jul-Oct greatly increased the flowering potential of *Magnolia leobneri* cv. Leonard Messel.
- Irrigation and pruning costs could be reduced by imposing mild water deficits through precision watering of *Rhododendron* and *Camellia* during periods of rapid stem extension.
- Delayed potting-on reduces the number of flower buds per plant in *Rhododendron* but can increase the number of flowers per floral bud. Shoot growth is also reduced so treated plants appear more floriferous.
- Long days (16 h) promote flowering in *R*. cv Hatsugiri. The minimum day length necessary to invoke this response in *R*. cvs. Hoppy and Scintillation is 10 h. The use of inexpensive tungsten bulbs may be an effective way of imposing long day photoperiods over the crop to improve flowering potential.

Background and expected deliverables

Inducing flower buds on finished plants will maximise garden centre sales of many HONS varieties, for example *Rhododendron, Magnolia* and *Camellia*. However, flower bud formation in such species is often limited in the first few years of production. Even in those varieties that form flower buds early in their life, the number of buds can vary significantly from one year to the next. Such inconsistency makes scheduling and marketing of crops difficult.

The aim of this project was to develop practical ways to promote early flower production in container-grown HONS. *Rhododendron* was the main subject but other species were included in some experiments to determine how wide-spread some of the flowering responses were. A major objective was to identify the key environmental stimuli responsible for flower bud initiation and to develop practical techniques to exploit these. The use of controlled stress techniques (mild water stress, root restriction) was assessed to determine if the hormonal balance in the shoot tip could be altered to trigger floral development.

The deliverables from this project are for *Rhododendron* growers and for those nurserymen with a much wider crop range, where a more general understanding of flower initiation is desirable. Anticipated key benefits are:

- Reduced time from propagation to sale, by promoting consistent, precocious flowering.
- Improved quality through more uniform flower bud development and higher numbers of flower buds.
- Improved competition with imports, especially from regions with warmer summers, where higher temperatures can help ensure good bud production.
- Development of procedures for 'stress-induced' flowering across a range of HONS crops.
- Better regulation of flower initiation through environmental control, *e.g.* by altering photoperiod and/or light quality using low cost, tungsten lamps.

Summary of the project and main conclusions

The effects of imposing mild water deficits through Regulated Deficit Irrigation (RDI) or Partial Root Drying (PRD) treatments on shoot growth and flowering potential of *Rhododendron* cvs. Hoppy, Hatsugiri and Jean Marie de Montague, *Camellia japonicum* cv. Kramers Supreme and *Magnolia leobneri* cv. 'Leonard Messel' were investigated at HRI-East Malling and HRI-Efford.

RDI is a method of restricting the daily supply of water to a plant. A percentage of the daily volume of water loss from the plant is applied so that water deficits are imposed gradually. MAFF-funded work suggested that when RDI was applied in late summer, floral initiation in *Rhododendron* was increased. In PRD treatments, one part of the soil dries down with the remainder kept well watered. At set intervals, watering is switched to the other side of the root ball so that all roots experience some water deficits. This differential imposition of water deficits promotes the transport of ABA from the roots to the aerial parts of plants whilst providing sufficient water for growth, photosynthesis and respiration. Increased flux of root-sourced ABA to leaves may affect the processes underlying flower initiation.

The success of RDI and PRD treatments depends on the ability to carefully control the availability of water to the roots, thereby imposing only mild water deficits to the plants. This was achieved successfully under the controlled experimental conditions at HRI. However, we recognise that this will be more difficult to achieve in some larger nurseries, especially on crops such as *Rhododendron* that are susceptible to leaf scorch resulting from severe water deficits.

Our results show that RDI and PRD treatments usually reduced the flowering potential of *Rhododendron* and *Camellia* (for example, see Figure A). In *Rhododendron*, imposing mild water deficits by a PRD treatment throughout Jul-Oct greatly reduced shoot growth (Figure B). However, the effects of PRD on plant growth were not consistent from year to year. Generally, the overall effect of the PRD treatment was to produce compact plants that appeared more uniform (for example, see Figure C).

Figure A. Imposing PRD for different durations did not increase flower and bud production in *Rhododendron* cv. Hoppy.

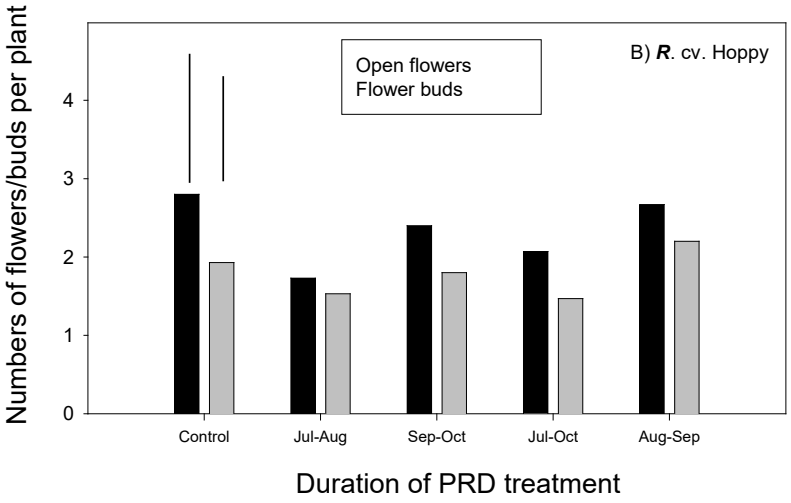


Figure B. PRD effectively reduced plant height and spread in *R.* cv. Hoppy. The photo was taken at the end of October, 2001.

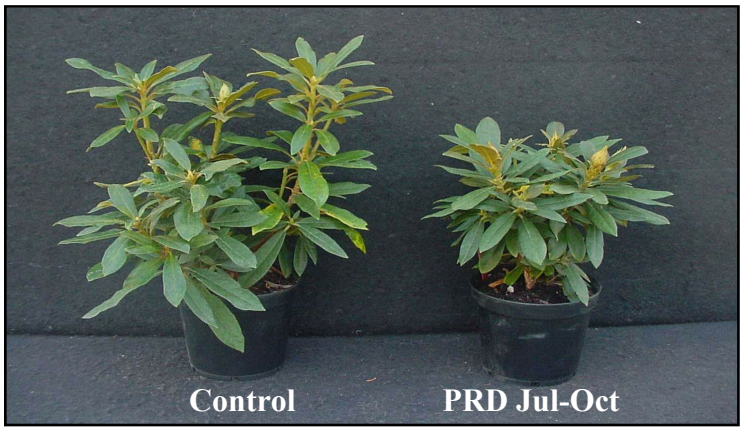
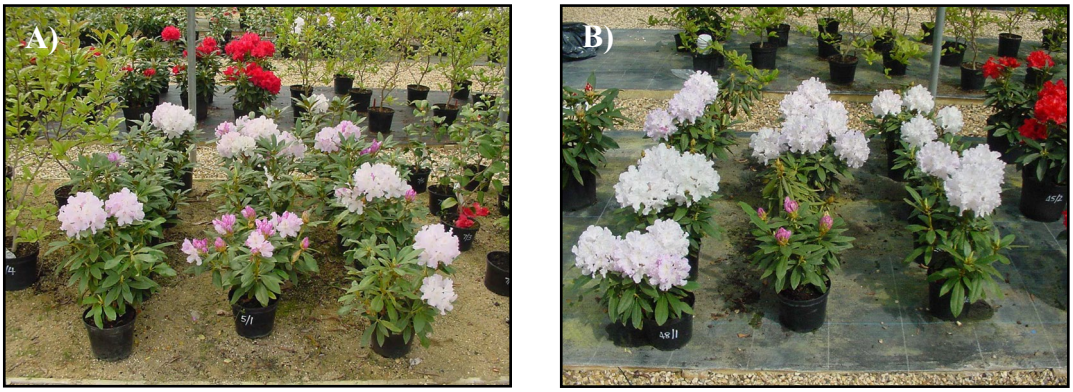


Figure C. PRD increased aesthetic quality in *R.* cv. Hoppy. Plants in A) received overhead irrigation throughout Jul-Oct, 2001. Plants in B) were subjected to PRD from Jul-Oct, 2001. The photographs were taken on May 3rd 2002.



Imposing mild water deficits in *R.* cv Jean Marie de Montague did not always improve flowering potential in our experiments. Indeed, a PRD treatment imposed throughout Sep-Oct adversely affected flower bud production and markedly reduced plant quality (Figure D).

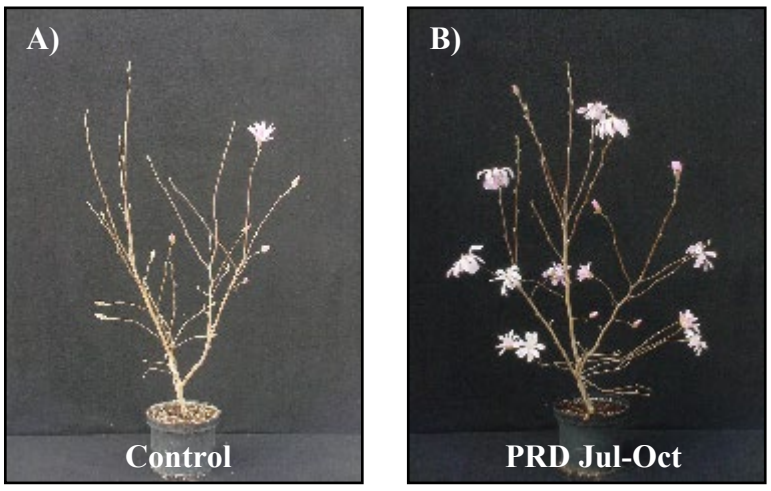
Figure D. Detrimental effects of 'late season' PRD on flowering potential in *R.* cv. Jean Marie de Montague.

Camellia did not respond positively to PRD treatments. Shoot growth was not inhibited so the negative effects of PRD on flowering potential were more apparent (Figure E).

Figure E. Marginal effects of PRD on flowering potential in *Camellia japonicum* cv. Kramers Supreme.

In contrast, *Magnolia leobneri* cv. Leonard Messel responded well to PRD treatments imposed between Jul-Oct. Flowering was greatly increased in trees exposed to PRD treatments in the previous summer (Figure F).

Figure F. PRD greatly increased flowering in *Magnolia leobneri* 'Leonard Messel.'
The photo was taken in March 2002.



Preliminary work under controlled environment conditions indicated that altering the red:far red light ratio may improve flowering potential and reduce shoot growth in *R.* cv Hatsugiri. However, altering the red:far red light ratios in polytunnels using spectral filters did not affect flower bud production and more work is needed to establish the effectiveness of this treatment.

Floral initiation was promoted under long days (between 12 and 16 h). When *Rhododendron* cv. Hoppy was maintained under artificially short days (less than 9 h) for two years, no floral buds were initiated. Very long days (18 h) triggered a second vegetative flush at the expense of floral initiation. Floral initiation in the *Rhododendron* cv. Hatsugiri was improved by placing plants in continuous light for 36 h. The response of other *Rhododendron* cvs. to this treatment is not yet known.

Restricting rooting volume by delaying potting-on reduced plant height and leaf area but also severely reduced the numbers of floral buds per plant. However, the number of flowers per floral bud were increased by delaying potting on of *Rhododendron* cv. Scintillation and, to a lessor extent, cv. Hoppy.

The effects of nutrient stress, imposed by applying triplesuperphosphate or phosphate at low pH, on floral initiation were very variable. Generally, the flowering potential of the *Rhododendron* cvs. Hoppy, Scintillation and Mrs T.H. Lowinsky was reduced or unaffected by 'nutrient stress.'

Floral initiation in *Rhododendron* cvs. Hoppy, Scintillation, Jean Marie, Dopey and Mrs T.H. Lowinsky occurs during late June - August. Thus, injudicious pruning will greatly reduce the numbers of flower buds per plant.

The plant hormone ABA does not seem to regulate flower initiation in the *Rhododendron* cvs. tested. This may explain the variable and negligible effect of controlled water deficit treatments on flowering in *Rhododendron*. In contrast, the naturally-occurring GA_5 and other closely related GAs were found to directly inhibit floral initiation in the *Rhododendron* cv. Hatsugiri.

Suggestions for Further Research

The regulation of flower initiation is complex and involves several separate, but interacting pathways. Without knowing the identity of the hormonal signal(s) that regulate flower initiation in *Rhododendron*, the effectiveness of various cultural and chemical treatments on flowering potential is likely to be limited. At best, results will be variable. In this project, we have identified GA_5 and other closely related GAs as having anti-florigenic activity in *Rhododendron*. Depending on the ubiquity of this effect in other *Rhododendron* cvs., this finding could be of major commercial importance. We can now begin to devise cultural treatments that specifically affect the enzymes involved in the production of the important GAs. It should also be noted that,

in many other species, GA_5 actually promotes flowering. Thus, the potential exists to impose cultural treatments that will greatly improve flowering in a range of HNS species. In particular, the provision of one 'super long day' (continuous light for 36 h) to improve floral initiation is worthy of further investigation.

Financial benefits

- Imposing PRD judiciously throughout Jul-Oct to control the growth of responsive species will reduce irrigation costs.
- Imposing PRD judiciously throughout Jul-Oct to control the growth of responsive species can reduce labour costs associated with pruning to maintain plant shape.
- In the *Rhododendron* cvs. tested, judicious application of PRD will improve plant quality at the point of sale.
- Improved uniformity will facilitate scheduling through bulking of batches.

Action points for growers

- Mild water deficits between Jul-Oct in *Rhododendron* cvs. Hoppy and Jean Marie de Montague sometimes reduce plant growth without adversely affecting flower production. The more compact plants appear more uniform and floriferous.
- PRD can be used to control shoot growth in *Rhododendron* cultivars. The amount of pruning and the associated loss of mature shoots with the potential to form flowers could be reduced if PRD is applied judiciously. However, the effects may not be consistent from year to year.
- If PRD is applied, tilting the pots to an angle of 25^{o} will improve the effectiveness of the treatment.
- RDI and PRD treatments will most effectively control shoot growth if the treatments are applied during periods of rapid stem extension. In *R*. cvs Hoppy and Jean Marie de Montague, the optimum time of application is Jul-Aug. Imposing PRD from Jul-Oct will also be effective.
- Shoot growth and flowering potential of *Camellia japonicum cv.* Kramers Supreme cannot be controlled by PRD treatments consistently.
- Imposing mild water deficits throughout Jul-Oct greatly increases the flowering potential of *Magnolia leobneri* cv. Leonard Messel.
- Nurserymen should ensure that *R*. cv. Jean Marie de Montague receives sufficient water during Sep-Oct. Mild water deficits at this time will greatly reduce flowering potential.

- Nurserymen should be aware that RDI and/or PRD may result in earlier flowering, *i.e.* autumn, in some plants.
- Long days (16 h) promote flowering in *R*. cv Hatsugiri. The minimum number of hours necessary to invoke this response in *R*. cvs. Hoppy and Scintillation is currently being investigated. The use of inexpensive tungsten bulbs may be an effective way of imposing long day photoperiods over the crop.

Additional notes on action points

The success of RDI and PRD treatments depends on the ability to carefully control the availability of water to the roots, thereby imposing only mild water deficits to the plants. This was achieved successfully under the controlled experimental conditions at HRI. However, we recognise that this will be more difficult to achieve in some larger nurseries, especially on crops such as *Rhododendron* that are susceptible to leaf scorch resulting from severe water deficits.

SCIENCE SECTION

1. The control of flowering in *Rhododendron* by imposing water deficits

Introduction

Growers of *Rhododendron* require plants to be in bud or in flower at the point of sale. Retailers will reject entire crops if insufficient numbers of buds have been initiated during production, which requires the grower to retain the crop for a further 12 months. Improving our understanding of floral initiation can provide opportunities to improve plant quality and consistency of cropping.

Moderate plant water deficits can promote flowering in many important horticultural species including *Kalmia* (Carden, 1995), *Litchi* (Stern *et al.,* 1993), *Picea* (Ross, 1986) and *Pyrus* (Mitchell *et al.,* 1986). In *Macroptilium atropurpureum,* greater floral initiation was achieved when water deficits were imposed before a growth flush (Kowithayakorn and Humphreys, 1987). The promotion of floral initiation by water deficits in *Citrus* was nullified by exogenous GA_3 (Nir *et al.,* 1972) (Krajewski and Rabe, 1995). Cameron *et al.* (1999) reported that water deficits enhanced flower bud formation in *Rhododendron* but the extent of the promotion depended on the timing and the degree of stress applied.

The concentration of the phytohormone abscisic acid (ABA) increases rapidly during periods of water deficit. Applications of ABA apparently promoted the synthesis or transport of a floral signal (Nakayama and Hashimoto, 1973; Maeda *et al.,* 2000; Takeno and Maeda, 1996) in several species including *Chenopodium rubrum*, *Lemna pauciostata* and *Pharbitis nil* (El-Antalby and Wareing, 1966).

Conversely, there are many reports that flowering can be inhibited by water deficits. These include three of the most widely studied species in floral initiation, *Lolium temulentum* (King and Evans, 1977), *Pharbitis nil* and *Xanthium strumarium* (Aspinall and Hussain, 1970). Exogenous ABA can delay flowering in *Spinacia oleracea*, *Lolium* sp., carnation and petunia (Vince-Prue, 1985). In *Spinacia oleracea,* ABA levels were higher under long days (LD)

compared to short days (SD), suggesting that ABA is not an inhibitor during SD. In addition, inhibition was not consistent with levels of ABA within the plant. Therefore, the role of ABA in regulating flower initiation remains ambiguous.

Regulated deficit irrigation (RDI) is a method of restricting the daily supply of water to a plant. A percentage of the daily volume of water loss from the plant is applied so that water deficits are imposed gradually. 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 dries down with the remainder kept well watered. At set intervals, watering is switched to the other side of the root ball so that all roots experience some water deficits. This differential imposition of water deficits promotes the transport of ABA from the roots to the aerial parts of plants whilst providing sufficient water for growth, photosynthesis and respiration (Loveys, 1984; Stoll *et al*., 2000). Increased flux of root-sourced ABA to leaves may facilitate the transport of the floral signal.

In contrast to florist azaleas, there is relatively little information about the environmental stimuli of flower initiation in the large-leaved *Rhododendron* hybrids. In these cultivars, juvenility is the major restriction in production. The physiological age of a plant can be a powerful modulator of floral initiation as controlled by environmental signals. Stimuli such as photoperiod can become more pronounced in their effect or in some circumstances be lost completely as the plant ages (Zimmer and Krebs, 1980).

The objectives of the research described in this section were: 1) to determine if stresses imposed on plants by restricting water availability to the roots are translated into physiological responses such as lowered leaf water potentials and reduced stomatal apertures; 2) to investigate if water deficits promoted floral initiation in large-leaved cultivars of *Rhododendron*; 3) to determine whether responses to water deficits differed in the ages of plants commonly used in *Rhododendron* production.

Materials and Methods

Plant material

Thirty plants of *Rhododendron* cvs. Hoppy and Scintillation of two ages were used in each treatment. Two-and-half-year-old plants were potted into 3 L pots and 3.5-year-old plants potted into 7.5 L pots in a medium of peat and Cambark 100 (80:20 v/v), 2g L^{-1} Osmocote® (controlled release fertiliser N:P:K, 15:9:11 + trace elements), 0.9 g L^{-1} $MgCO_3$, 0.15 g L^{-1} Fungaride and 0.6g L^{-1} Suscon® Green (10.34% chlorpyrifos w/w). Experiments were performed in a polythene growth tunnel and plants were watered to container capacity every day prior to treatments.

Experimental design

Blocks of 30 plants consisted of ten plants of each treatment, with plants aligned into two rows of five. Plants numbered one and six were reserved for destructive analysis, non-destructive measurements and spring flower counts were conducted on the remaining eight plants. Treatments commenced in May 2001 and ceased in October 2001 after the cessation of growth and the onset of terminal bud dormancy.

Potential evapo-transpiration calculation and controls

Eight reference plants were located at random throughout the blocking structure and were weighed daily. The reference plants were re-watered to container capacity and the mean water loss calculated. This represented 100% of the potential evapo-transpiration (ETp). Well-watered plants (WW) received 110% ETp at a rate of 4 L per hour *via* drippers. The amount of water supplied was adjusted by altering the open time of a solenoid valve. Water run-off from the containers was collected in saucers for re-absorption.

Establishment of PRD and RDI regimes

In the PRD treatment, water was supplied for the same length of time as the WW treatment but

was not collected; one side of the container medium dried down gradually whilst the other side was kept fully hydrated. Dripper spikes were transferred to the opposing side of the pot every three weeks to ensure that all roots were exposed to a 'wet-dry' cycle. In the RDI treatment, 70% ETp was supplied by adjusting the open time of the solenoid valve in the same manner as the WW treatment. Water run-off was collected in saucers for re-absorption. WW and RDI dripper spikes remained in the same position throughout.

Measurements of water potential (ψ) and stomatal conductance (g_s)

The uppermost leaf of a branch was cut at the base of the petiole and sealed into a Scholander pressure chamber. The chamber was lined with damp filter paper to increase humidity. Compressed air was progressively added to the chamber at a rate of 0.05 MPa min^{-1} until sap first appeared at the cut surface of the petiole when viewed with a x5 hand lens. The magnitude of the applied pressure was equal, but opposite to the leaf water potential.

An upper leaf from the aforementioned branch was placed into the cuvette of a steady state porometer (PP Systems EGM-1, Hertfordshire, UK). The cuvette was positioned onto the leaf with a retort stand. Stomatal conductance (g_s), temperature and PAR levels were recorded after two minutes.

Measurements of relative leaf water content (RLWC)

Following stomatal conductance measurements, the leaf was excised and the petiole re-cut under de-ionised water (DiH_2O). The weight of the leaf was noted and placed into 1.8 ml vials (Chromacol, Welwyn Garden City, UK) filled with DiH_20. Vials were then placed into a misted controlled environment at 20ºC. After 24 h, the leaf was considered to be fully re-hydrated. Leaves were then blotted dry, re-weighed, oven dried for 48 hours at 80ºC and re-weighed. RLWC was calculated as:

RLWC = (fresh weight – dry weight) / (re-hydrated weight –dry weight).

Measurement of diurnal patterns of g_s, ψ and RLWC

The large Hoppy plants numbered one and six were sampled at 20:00, 0:00, 0:700, 10:00, 13:00 and 16:00 h. Gs, ψ and RLWC were measured on leaves originating from the same stem for each time point. Gs, ψ and RLWC measurements were made as described above.

Measurements of organic soil moisture content

Organic soil moisture content was measured with a Theta probe (Delta-T Devices, Cambridge, England). In RDI treatments, measurements were made on both sides of the container and were averaged to account for the position of the dripper on overall water content in the medium. Plant weights were also measured.

Floral parameters

Floral buds per plant, percentage of plants flowering, floral buds per branch and the number of flowers per floral bud were measured in spring, 2002. Owing to different levels of initiation between plants, sampling five inflorescences in the large plants and two in the small plants produced a balanced analysis of flowers per bud. Premature anthesis was measured on all non-destructive plants on 21st September and 29th October, 2001.

Results

Treatment effects on water potentials

Leaf water potentials were significantly reduced by RDI compared to the WW treatment, with the most negative ψ in the small Hoppy plants (Figure 1.1 A). There was no significant effect of PRD on Ψ compared to WW in any cultivar or size of plant.

Treatment effects on plant weight and soil water content

WW treated plants weighed more than those under PRD. The RDI treatment produced the

lightest plants of all the treatments (Figure 1.1 B). Weight was proportional to the water content of soil (Figure 1.1 B). Differences in soil water content between PRD and WW were not significant (Figure 1.2).

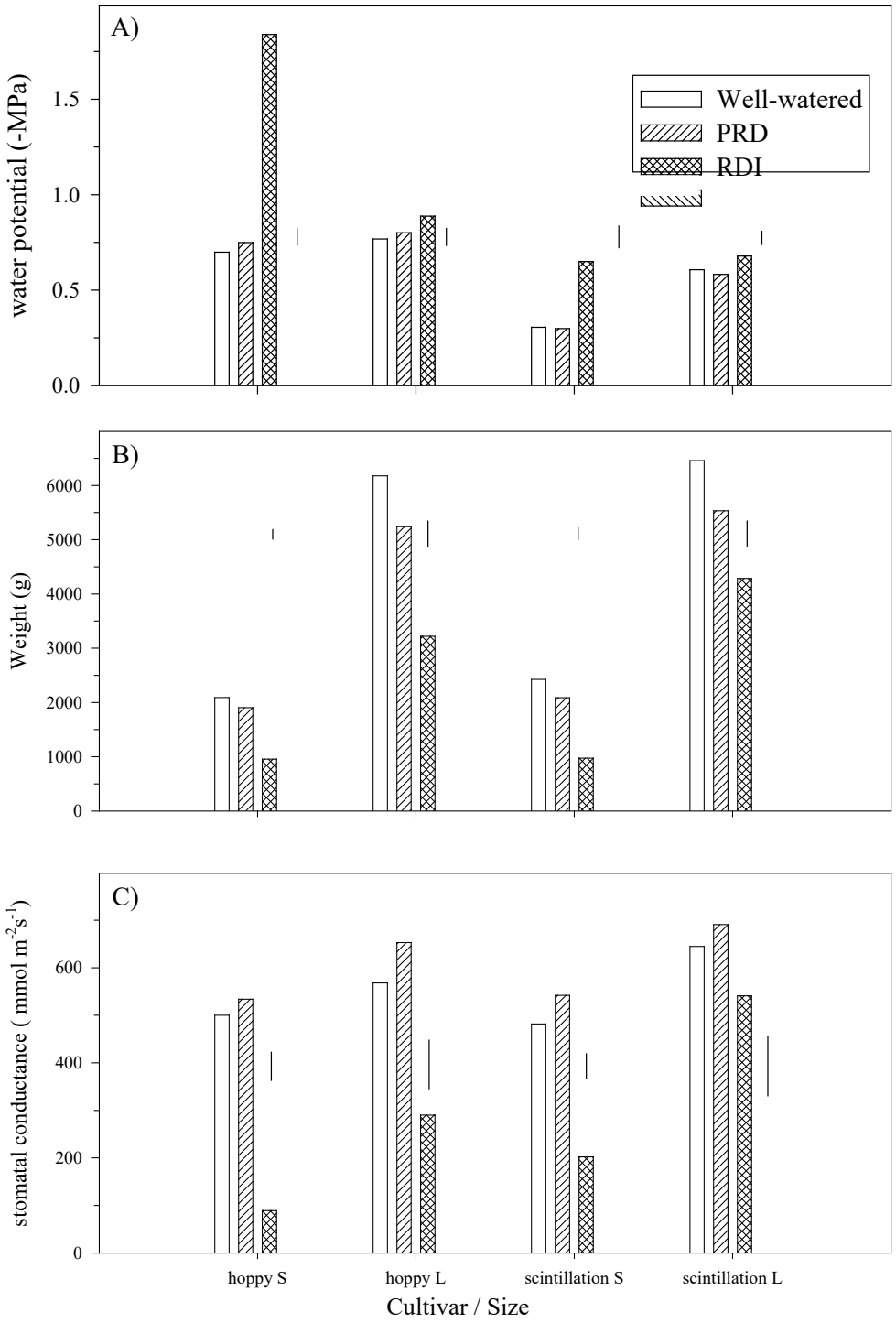
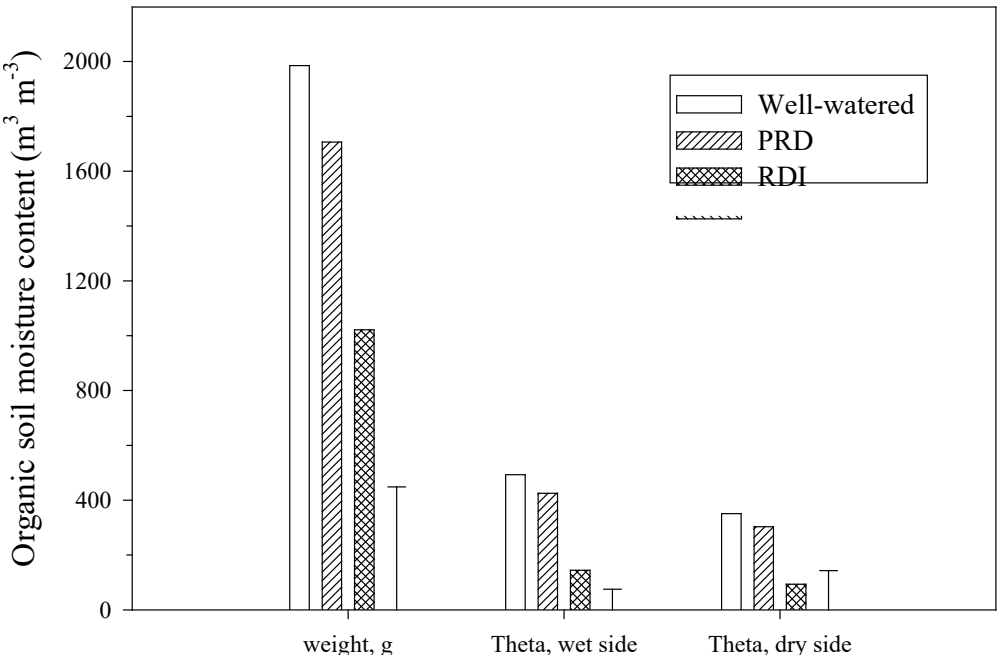


Figure 1.1. Effects of irrigation regimes on A) leaf water potential, B) stomatal conductance and C) plant weight in *Rhododendron* cvs. Hoppy and Scintillation. S = small plants, L = large plants. Measurements were taken at 12:00 h. Vertical lines represent Least Significant Differences (LSD values) at P = 0.05.

Figure 1.2. Plant weight and organic soil moisture content in large Hoppy plants as affected by irrigation regimes. Theta probe measurements were taken on alternate



sides of the plant to account for the swapping of dripper spikes in the PRD treatment on a three week rotation. Vertical lines represent (LSD values at P = 0.05.

Treatment effects on stomatal conductance

Stomatal conductances were significantly reduced in plants subjected to RDI compared to the WW-treated plants in all but the large Scintillation plants (Figure 1.1 C). In the small Scintillation plants g_s was significantly higher in PRD-treated plants compared to those under the WW treatment.

Effects of irrigation regime on diurnal patterns of stomatal conductance

The g_s effect that was observed in Figure 1.1 C was also observed in the diurnal time-course (Figure 1.3 A). The RDI treatment lowered g_s compared to the WW- and PRD-treated plants in the day period. There was no significant difference between the g_s of WW- and PRD-treated plants throughout the 24-hour period. At night, stomatal closed and so treatment differences in g_s were not significant.

Effects of irrigation regime on diurnal water potentials

In both WW and PRD treatments, Ψ were similar and became more negative as the day progressed (Figure 1.3 B). In the RDI treatment, Ψ rose sharply and approached zero from 20:00

– 08:00 h. During the afternoon, Ψ of the RDI-treated plants were similar to those of the other two treatments.

Effects of irrigation regime on RLWC diurnal patterns

There was no diurnal trend in RLWC (Figure 1.3 C). Leaves from PRD-treated plants had higher water content those from the WW and RDI treatments in the day period; RLWC in WW- and RDI-treated plants were not significantly different throughout the 24 hours.

Effects of irrigation regime on floral initiation and flowers per bud

RDI inhibited floral initiation, but this was only significant in small Hoppy and large Scintillation plants (Table 1.1). There was no significant effect of PRD on floral initiation. In the small plants, PRD treatment increased flowers per floral bud compared to the WW plants (Table 1.1). This effect was not significant in the large plants. In the small Hoppy plants, RDI treatment resulted in greater flowers per floral buds compared to WW.

Branch production under different irrigation regimes

The RDI treatment reduced branch production significantly in the large plants (Table 1.1). PRD significantly reduced branch production in large Scintillation plants. The WW plants formed more branches by producing more bypassing branches that arose from the buds subtending a newly formed floral bud.

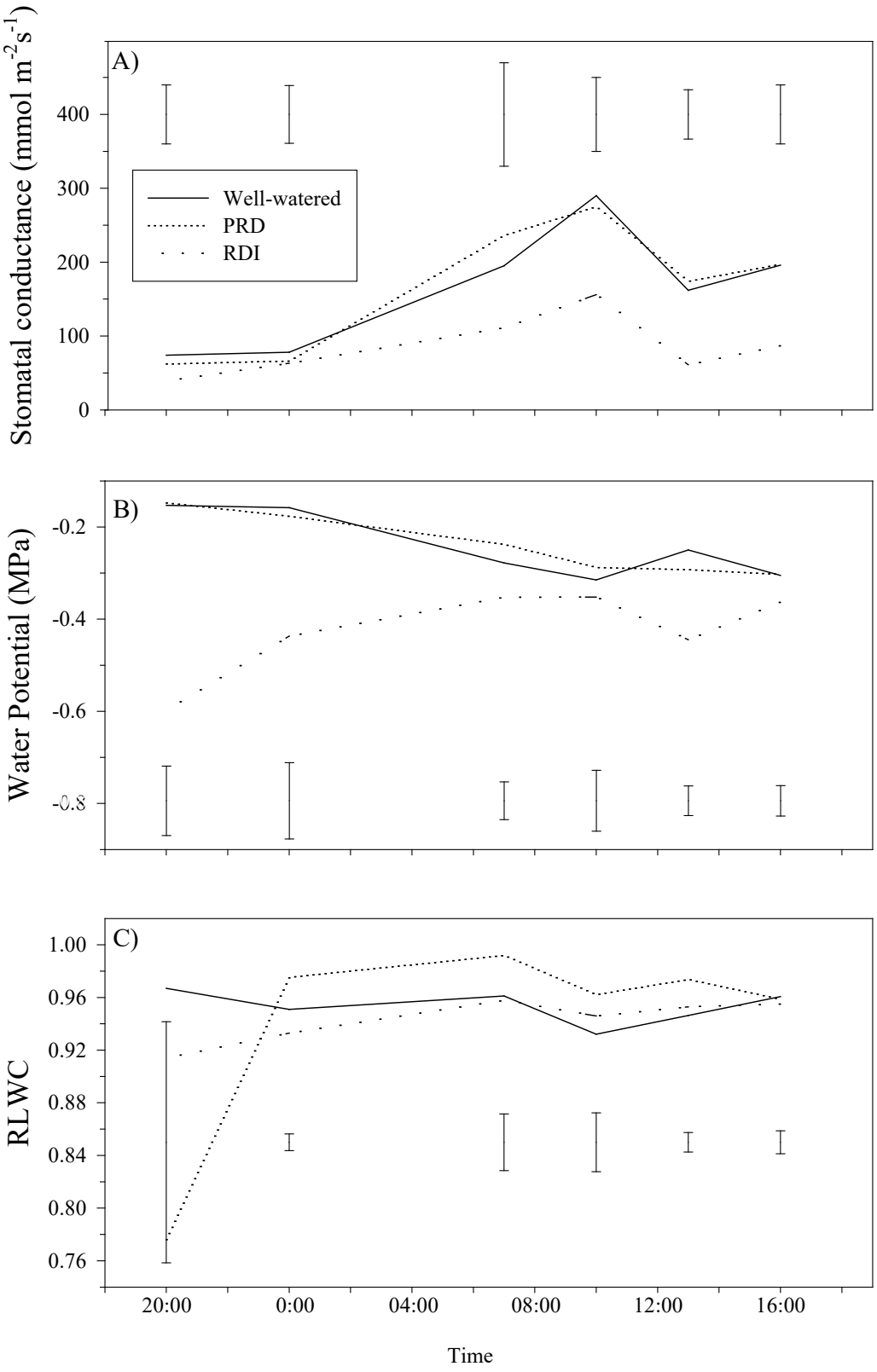


Figure 1.3. Diurnal patterns of A) stomatal conductance, B) leaf water potential and C) relative leaf water content as affected by irrigation regimes. Vertical bars represent LSD values at P = 0.05 calculated for each time point. The first time point measured was 20:00 h.

Table 1.1. Effects of RDI (70 % ETp) and PRD (3 week rotation) on floral parameters and branch production in *Rhododendron* cvs. Hoppy Scintillation. Percentages of potential evapo-transpiration were supplied daily. Data are means of 30 replicate plants, LSD values at P = 0.05, asterisks indicate values that are significantly different from the we-watered control values.

Cultivar size	Treatment	Parameter			
		Floral buds per plant	Flowers per bud	Branches floral (%)	Number of branches
Hoppy small	Well-watered	1.09	12.15	14.25	7.63
	PRD	0.79	14.39*	12.14	7.08
	RDI	0.17*	14.33*	1.71*	7.33
	LSD	0.55	1.16	7.61	0.98
Hoppy large	Well-watered	10.54	16.17	43.52	22.75
	PRD	11.25	16.83	46.95	21.58
	RDI	9.33	15.39	45.68	20.13*
	LSD	3.47	1.01	14.19	2.56
Scintillation small	Well-watered	2.63	17.00	59.17	4.54
	PRD	2.25	17.59*	54.17	4.13
	RDI	2.32	17.31	53.58	4.55
	LSD	0.73	0.58	15.66	0.75
Scintillation large	Well-watered	11.33	15.41	78.75	14.38
	PRD	10.21	15.93	86.32	11.71*
	RDI	9.38*	15.23	77.17	12.13*
	LSD	1.94	0.826	9.04	1.81

Effects of irrigation regime on premature anthesis

All treatments triggered premature anthesis in some plants but the average number of buds that opened prematurely in these plants was increased by RDI (Table 1.2). However, the effects of the treatments on premature flower bud opening were not significantly different when averaged over the population.

Table 1.2. Level of premature flower bud opening in the irrigation regimes on 21st September and 29th October 2001. Floral buds opening in 2001 were not counted in the values for floral initiation in spring 2002. Data are means of 30 replicate plants and LSD values at P = 0.05.

Treatment	21st September 2001		29th October 2001	
	Buds / plant	Buds / population	Buds / plant	Buds / population
Control	1.33	0.33	2.22	0.83
PRD	1.83	0.46	2.30	1.21
RDI	4.17*	1.04	4.02*	1.21
LSD	1.94	0.79	1.15	0.93

Summary

- RDI treatments imposed plant water deficits, PRD treatments did not.
- The RDI and PRD treatments did not promote flower bud production, on the contrary, these treatments reduced numbers of flowers per plant of small Hoppy and large Scintillation plants.
- The effect of RDI on flower bud production varied according to cultivar and plant age but was generally negative.
- There was limited evidence that RDI and PRD treatments increased the numbers of flowers per bud.
- RDI and PRD treatments decreased branch production in large Hoppy and Scintillation plants.
- RDI treatments may promote early flowering (in autumn) in some plants.

2. Effects of partial root drying on flowering in *Rhododendron*, *Camellia* and *Magnolia*

Introduction

Preliminary work funded by MAFF (HH 1608 SHN) demonstrated that flower bud induction was affected by the degree and timing of water stress applied to *Rhododendron* cv. Hoppy (Cameron *et al.*, 1999). Results varied between HRI-East Malling and HRI-Efford, however, indicating that other factors, most notably physiological age of shoot tissue had a strong influence on flower initiation. Nevertheless, results showed that moderate water stress in late summer increased flowering the following spring. Improved flower production resulting from water stress has been documented for other woody plant crops, e.g. *Citrus* (Krajewski and Rabe, 1995), *Kalmia* (Carden, 1995), *Litchi* (Stern *et al.*, 1993), *Picea* (Ross, 1988) and *Pyrus* (Mitchell *et al.*, 1984). More work is now required to confirm these conclusions, identify optimum timing (in relation to a physiological stage rather than a calendar date) and devise practical applications that can be readily used on nurseries.

The induction of water stress in foliage by restricting water availability to the roots increases the biosynthesis of ABA in both the roots and shoots (Gowing *et al*., 1990; Davies *et al.*, 2000). Enhanced foliar concentrations of ABA are thought to restrict shoot growth although the mechanism underlying this response is not known. ABA may have anti-gibberellin type properties (Walton, 1980) and has been implicated in flower formation in some species (Meilan, 1997). If the ratio of ABA to gibberellin is indeed critical in controlling flower development, then there may be a number of practical mechanisms that can be utilised to enhance earlier flowering.

The objectives of the research were 1) to determine whether PRD promoted flower initiation in several HONS varieties, 2) to determine whether the effects of PRD on floral initiation were influenced by the timing of the 'stress' applications.

Materials and Methods

Plant Material

Two cultivars of *Rhododendron*, namely *R*. cvs. 'Hoppy' and 'Jean Marie de Montague,' *Camellia japonicum*. cv. 'Kramers Supreme' and *Magnolia leobneri*. cv. 'Leonard Messel' were evaluated at Efford for their response to PRD treatments in terms of flower bud production, time to flower and plant height. *R*. cv. Hoppy were bought in as well-established plants in 3 L pots, *R*. cv. Jean Marie in 7.5 L pots, *Camellia japonicum* in 3 L pots and *Magnolia leobneri* in 5 L pots. All plants were un-pruned during the trial and were maintained on sand beds at HRI-Efford. Control plants were maintained on uncovered sand beds and PRD-treated plants were maintained on Mypex-covered beds.

In 2003, 30 plants of *Rhododendron* cvs. Dopey and Hoppy were used in each experiment. Half of the plants in 3L pots were potted-on into 7.5 L pots using a potting mix comprised of: Premium bark and Pine bark (75:25 v/v), 3 Kg m^3 Osmocote Plus (12-14 Autumn), 1 Kg m^3 Magnesium Limestone and 1 Kg m^3 Suscon® Green. Plants were maintained on uncovered sand beds and watered to container capacity daily.

Experimental design

In 2001 and 2002, different sand beds were used for each PRD treatment. On each bed, three blocks of five plants of each species were used *i.e.* 15 plants of each species per treatment.

Partial root drying treatments

2001: Drip irrigation was used to provide differential watering within an individual pot, where only half of the root system was watered, leaving the other half in drying soil. The portion of roots exposed to irrigation was altered every two weeks by moving the dripper to the opposite side of the pot. Pots were tilted at an angle of 25° with the dripper positioned in the side of the pot nearest to the ground (Figure 2.1). This helped to ensure that the irrigation water was

confined to the 'wet' side of the pot. The PRD treatments were imposed for different durations, namely July-August, August-September, September-October and July-October, 2001. Control plants received sufficient water *via* overhead irrigation. Prior to and after the PRD treatments, all plants received sufficient water by overhead irrigation. The effect of the PRD treatments on plant height was determined at the end of August, September and October, 2001. Plants were monitored for bud and flower numbers and foliar quality in the following spring (May 2002).

2002: PRD treatments were imposed throughout July-October; control plants received sufficient water *via* overhead irrigation. Prior to and after the PRD treatments, all plants received sufficient water by overhead irrigation. The effect of the PRD treatments on plant height was determined in December 2002 and May 2003. Plants were monitored for bud and flower numbers and foliar quality in the following spring (May 2003).

Figure 2.1. Tilting the pots at an angle of 25° and judicious positioning of the dripper helped to ensure that irrigation water was confined to the 'wet' side of the pot.

Theta probe measurements

A hand-held theta probe (ML2x, Delta-T Devices, Cambridge, UK) was used to confirm that the PRD treatments effectively reduced soil moisture availability in the 'dry' side of the pot. Readings were taken from the 'wet' and 'dry' sides of one pot randomly chosen from each block at fourteen-day intervals just before the dripper positions were switched. Direct outputs (mV)

are presented; high values indicate 'wet' soil and low values indicate 'dry' soil.

Results

PRD effects on soil moisture availability

Theta probe measurements confirmed that the irrigation method successfully limited water availability in the 'dry' side of the pot (Table 2.1). Readings taken for each species/cultivar on this and other dates showed similar differences between the 'wet' and the 'dry' sides of the pot (data not shown). Thus, roots in the 'dry' side of the pot encountered some degree of water deficit and increased concentrations of root-sourced ABA were a likely response to these transient root water deficits.

Table 2.1. The effect of PRD treatments on soil moisture availability in the 'wet' and 'dry' sides of the pot. High theta probe readings indicate 'wet' soils, low readings indicate 'dry' soils. Readings were taken every two weeks throughout Jul-Oct, presented data were taken on 28 September, 2001.

Plant	RDI Treatment	Theta probe reading (mV)	
		'Wet'	'Dry'
Camellia japonicum	Control	871.3	
	Sep-Oct	812.0	274.3
	Jul-Oct	802.0	326.3
	Aug-Sep	793.7	371.7
R. cv. Hoppy	Control	862.0	
	Sep-Oct	680.3	167.0
	Jul-Oct	716.7	278.3
	Aug-Sep	773.0	270.3

Effect of PRD on vegetative growth

The effects of the PRD treatments on plant height varied between species and cultivars. In *Camellia*, stem elongation was reduced by the PRD treatments but this effect was only statistically significant in the Jul-Aug treatment, with plant heights measured at the end of October (Figure 2.2 A). In contrast, all the PRD treatments reduced stem height in *R*. cv. Hoppy with the greatest inhibition caused by the Jul-Oct treatment. Imposing water deficits throughout Jul-Aug also reduced plant height markedly (Figure 2.2 B). Similar inhibitory effects of PRD on

stem extension were apparent in *R*. cv. Jean Marie, with the exception of the Sep-Oct PRD treatment (Figure 2.2 C). Water deficits imposed during Jul-Aug were most effective and presumably coincided with a period of rapid stem extension in well-watered plants. Effects of PRD treatments on stem extension in *Magnolia* were not statistically significant (Figure 2.2 D).

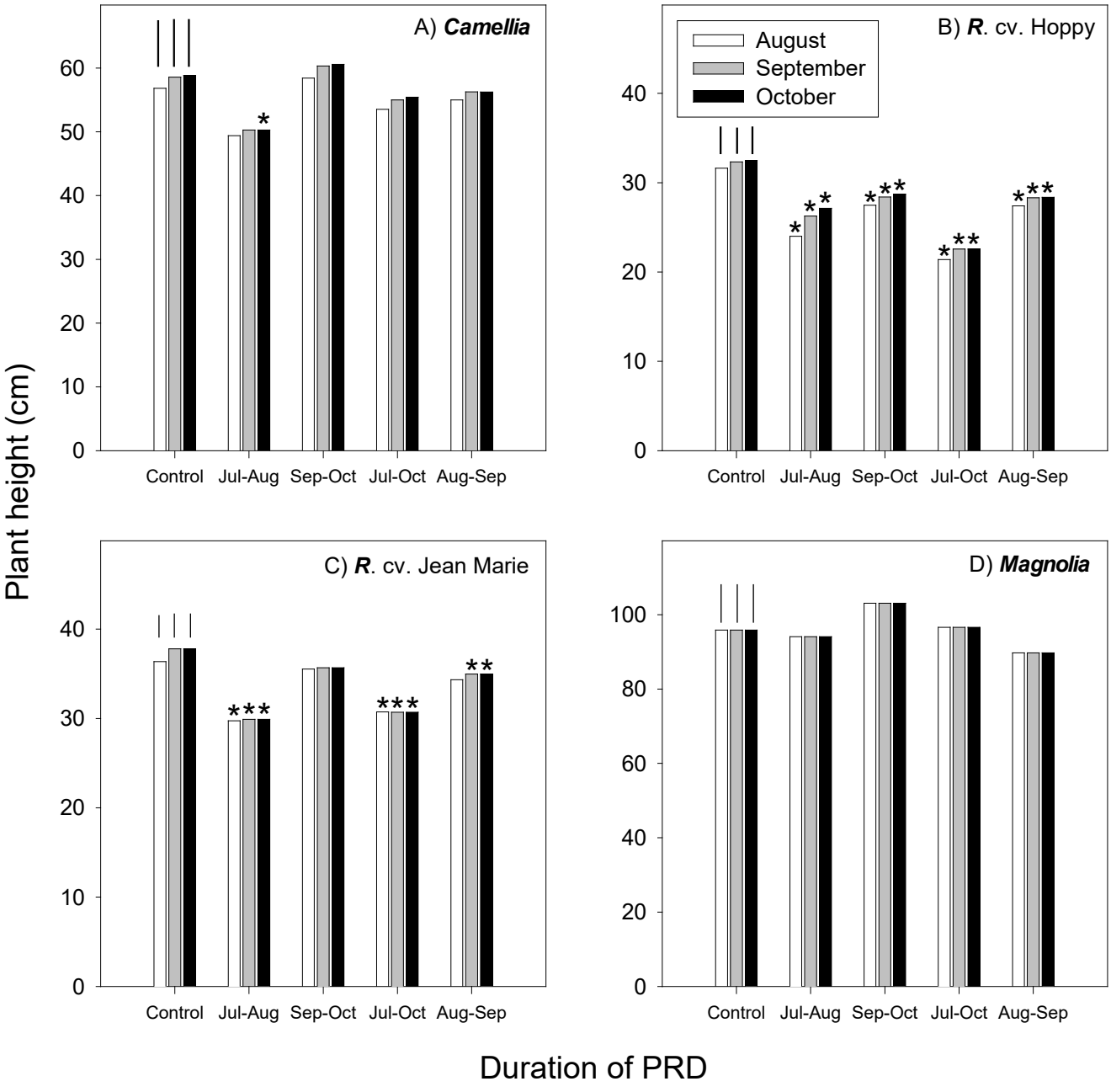


Figure 2.2. Effects of PRD for different durations on plant height in A) *Camellia*, B) *R*. cv. Hoppy, C) *R*. cv. Jean Marie and D) *Magnolia*. Plant heights were measured at the end of August, September and October 2001. Vertical lines represent LSD values at P = 0.05, asterisks indicate values that are significantly different from controls.

The 'spread' of *R*. cvs. Hoppy and Jean Marie was also reduced by the PRD treatments (data not shown). Overall, imposing mild water deficits on the *Rhododendrons via* PRD reduced vegetative growth and produced more compact plants (Figures 2.4 and 2.5).

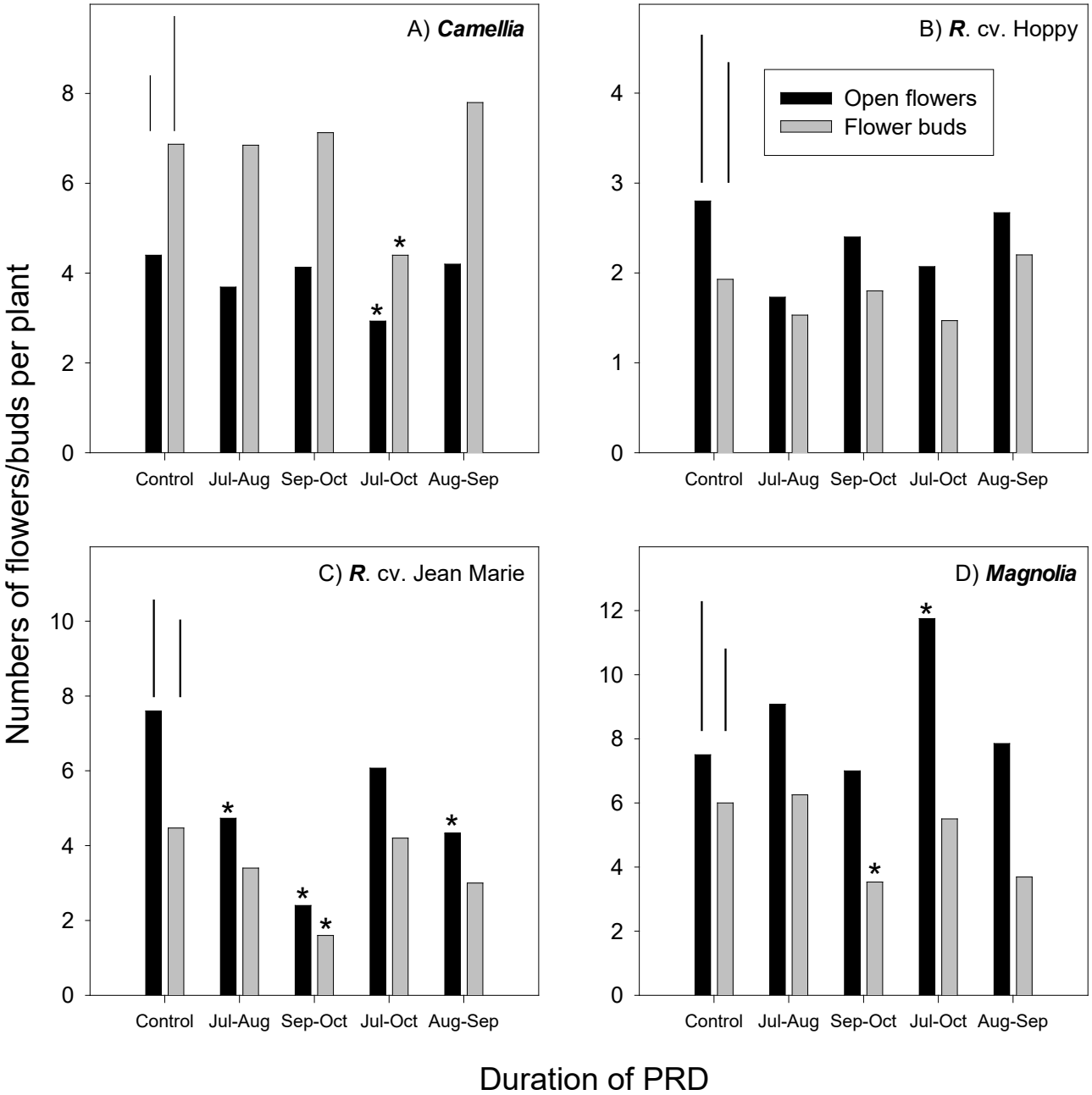


Figure 2.3. Effects of PRD for different durations on numbers of flowers and buds per plant in A) *Camellia*, B) *R*. cv. Hoppy, C) *R*. cv. Jean Marie and D) *Magnolia*. Counts were made at weekly intervals from mid March to the end of May 2002. Values are maximum numbers of flowers and buds per plant. Vertical lines represent LSD values at P = 0.05, asterisks indicate values that are significantly different from controls

PRD effects on flower bud production

In *Camellia* and *Rhododendron*, PRD treatments did not increase flower or bud numbers, any statistically significant effects were in a negative direction *i.e.* numbers were reduced. These

reductions were especially marked in *R*. cv. Jean Marie where PRD applied from Sep-Oct reduced flower numbers and bud numbers by 70% and 65%, respectively (Figure 2.3 C). Imposing PRD throughout Jul-Oct significantly reduced flower and bud numbers in *Camellia*. Combined with the minimal effects of PRD on plant growth, imposing mild water deficits in *Camellia* tended to reduce overall plant quality (see Figure 2.6 A-D).

In *Magnolia*, a PRD treatment imposed throughout Jul-Oct greatly increased the numbers of flowers produced in the following spring, although the number of buds per plant was not affected (Figure 2.3 D). Treatments imposed at other times were less effective and tended to reduce flower and bud numbers.

Some treatments produced plants that were more uniform and of better quality, despite the generally negative effects of PRD treatment on flower and bud numbers per plant. In terms of aesthetic value, *Rhododendron* cvs. Hoppy and Jean Marie appeared to respond particularly well to PRD imposed throughout Jul-Oct (Figure 2.4 C, 2.5 C).

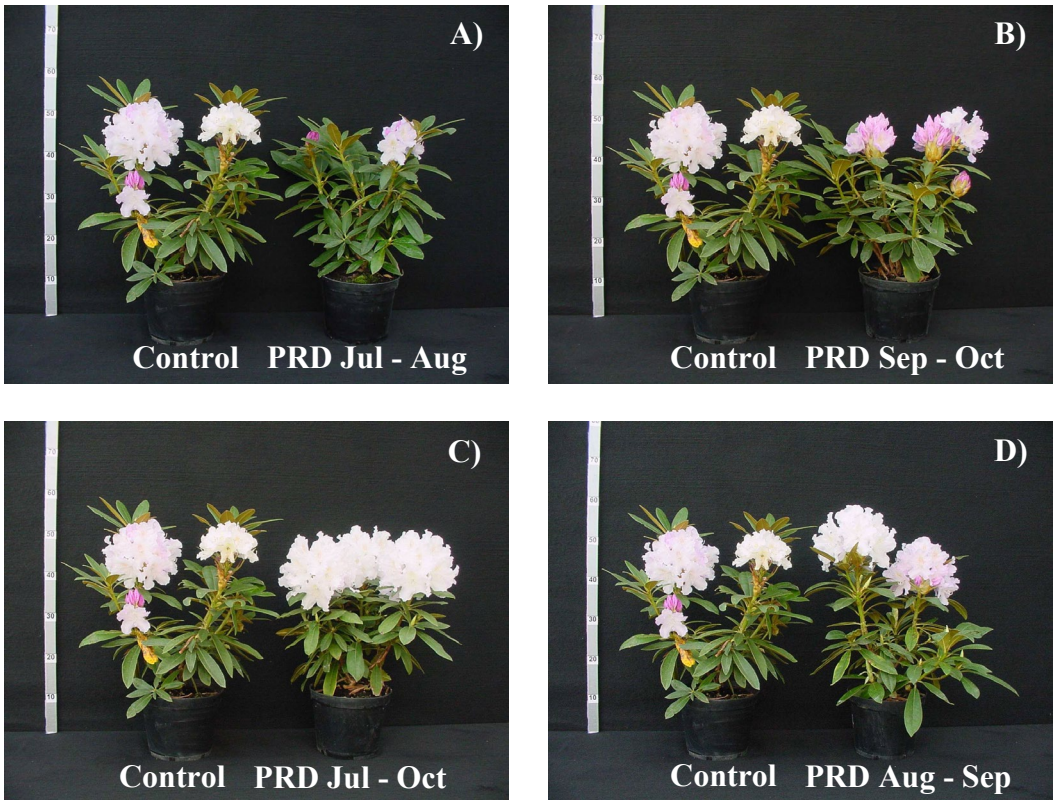


Figure 2.4. Effect of PRD treatments on plant quality in *Rhododendron* cv. Hoppy. The inhibitory effect of PRD on plant height gives the impression of more uniform flowering.

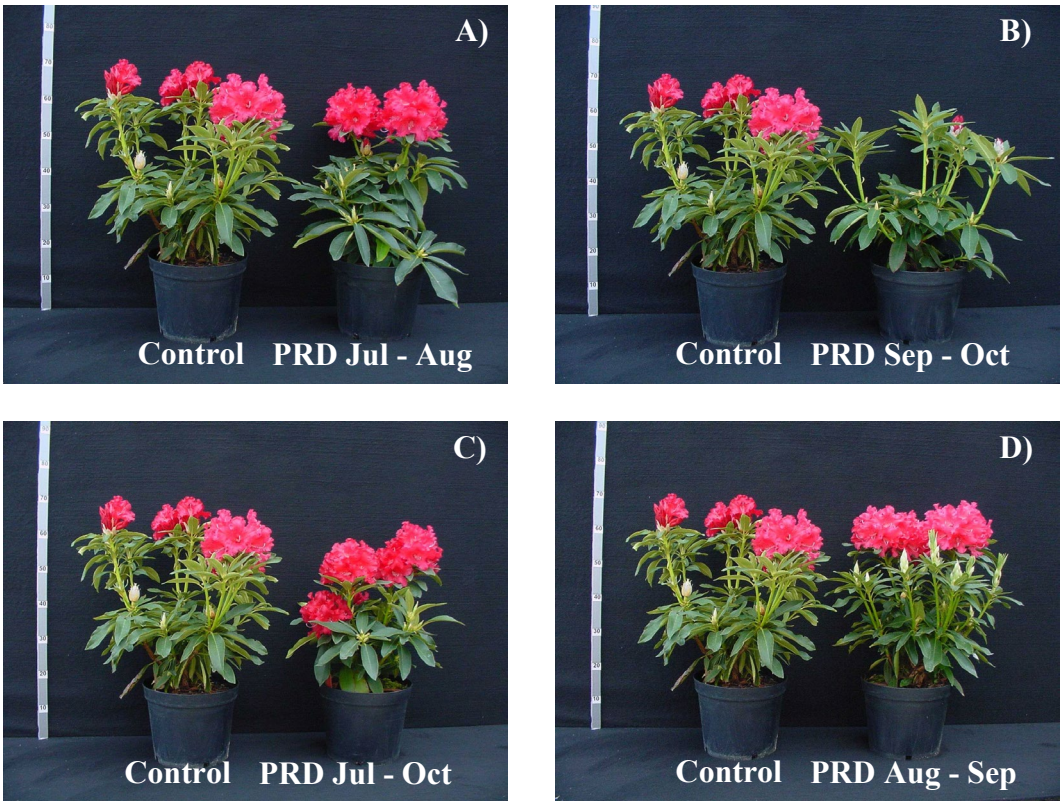


Figure 2.5. Effect of PRD treatments on plant quality in *Rhododendron* cv. Jean Marie. The inhibitory effect of PRD on plant height gives the impression of more uniform flowering.

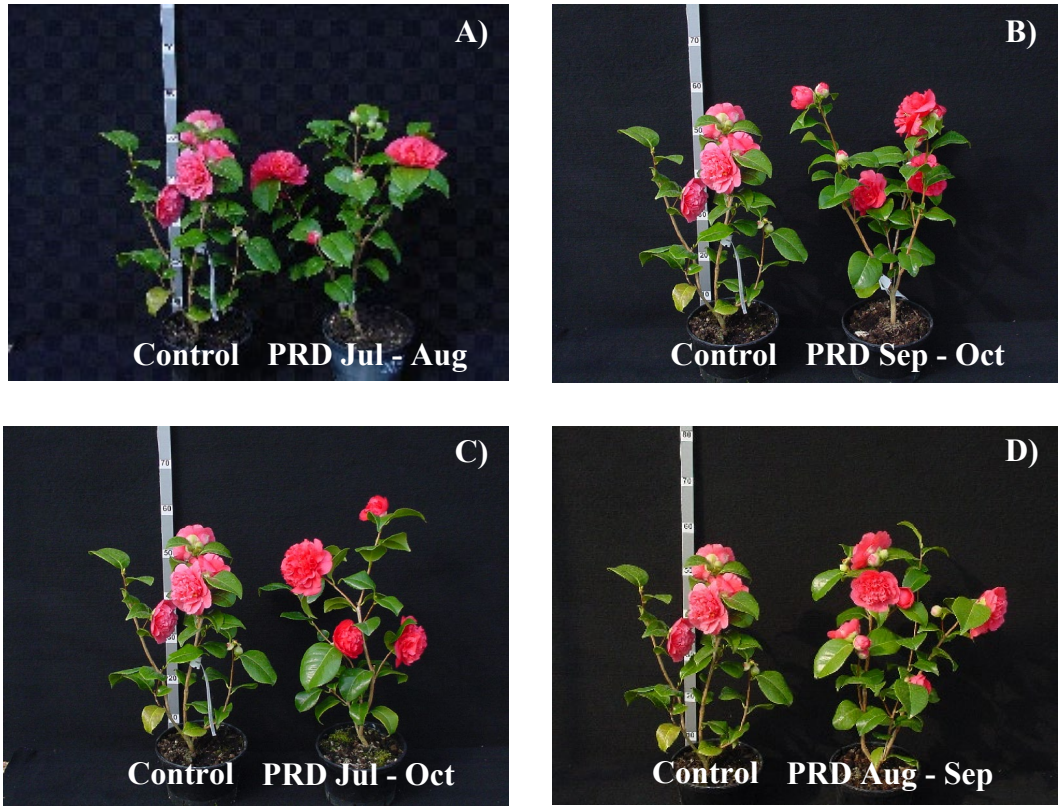


Figure 2.6. Effect of PRD treatments on plant 'quality' in *Camellia japonicum* 'Kramers Supreme.'

These aesthetic differences were due to the inhibitory effects of PRD on plant growth. More compact plants with the same number of flowers appeared more uniform and of a better quality. The effects of PRD on shoot extension in *Camellia* were generally not significant so the quality of the PRD-treated plants did not appear to be improved compared to the well-watered plants (Figure 2.6).

The positive effect of the PRD treatments on flowering in *Magnolia* (Figure 2.7) was not due to reduced plant size. Compared to controls, all of the PRD-treated plants produced a greater number of flowers. However, the disparity between the experimental data (Figure 2.3 D) and the photographs below suggest that the effect of PRD on flowering in *Magnolia* was somewhat variable.

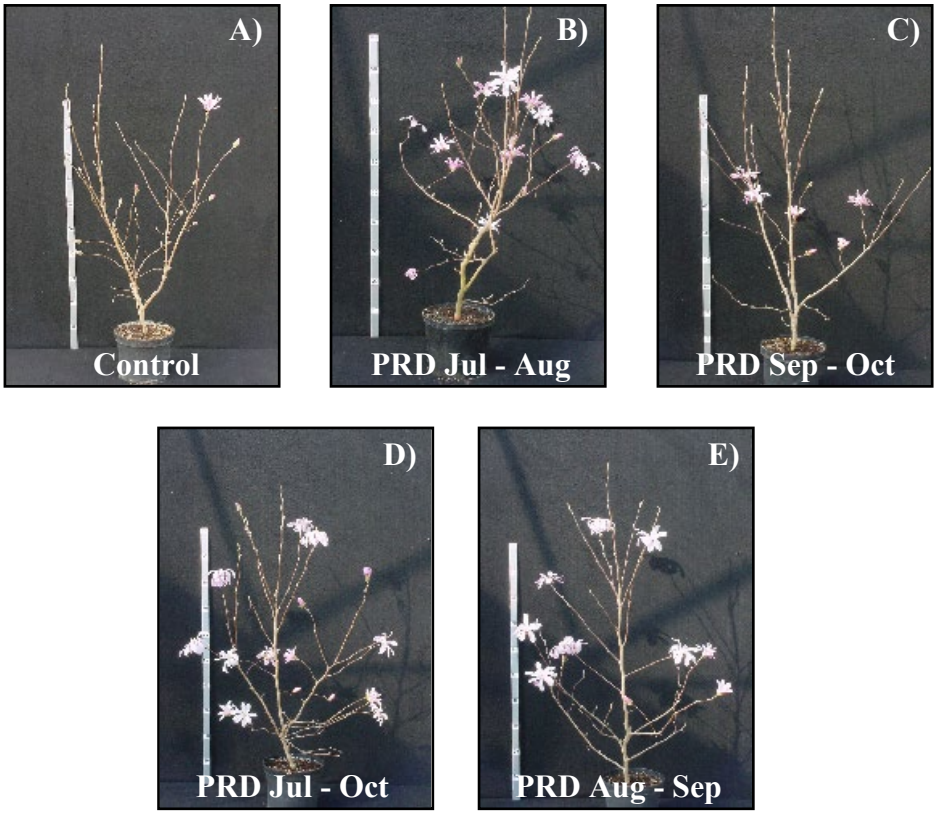


Figure 2.7. Effect of PRD treatments on flowering in *Magnolia leobneri* 'Leonard Messel.'

PRD effects on timing of bud break and flowering

The effects of PRD on the timing of bud break and flower opening varied according to species. Generally, any effect of PRD was to increase the time to bud break and flowering in *Camellia*

and *R*. cvs. Hoppy and Jean Marie. However, in *Magnolia*, all PRD treatments reduced the time to flower opening (Figure 2.8).

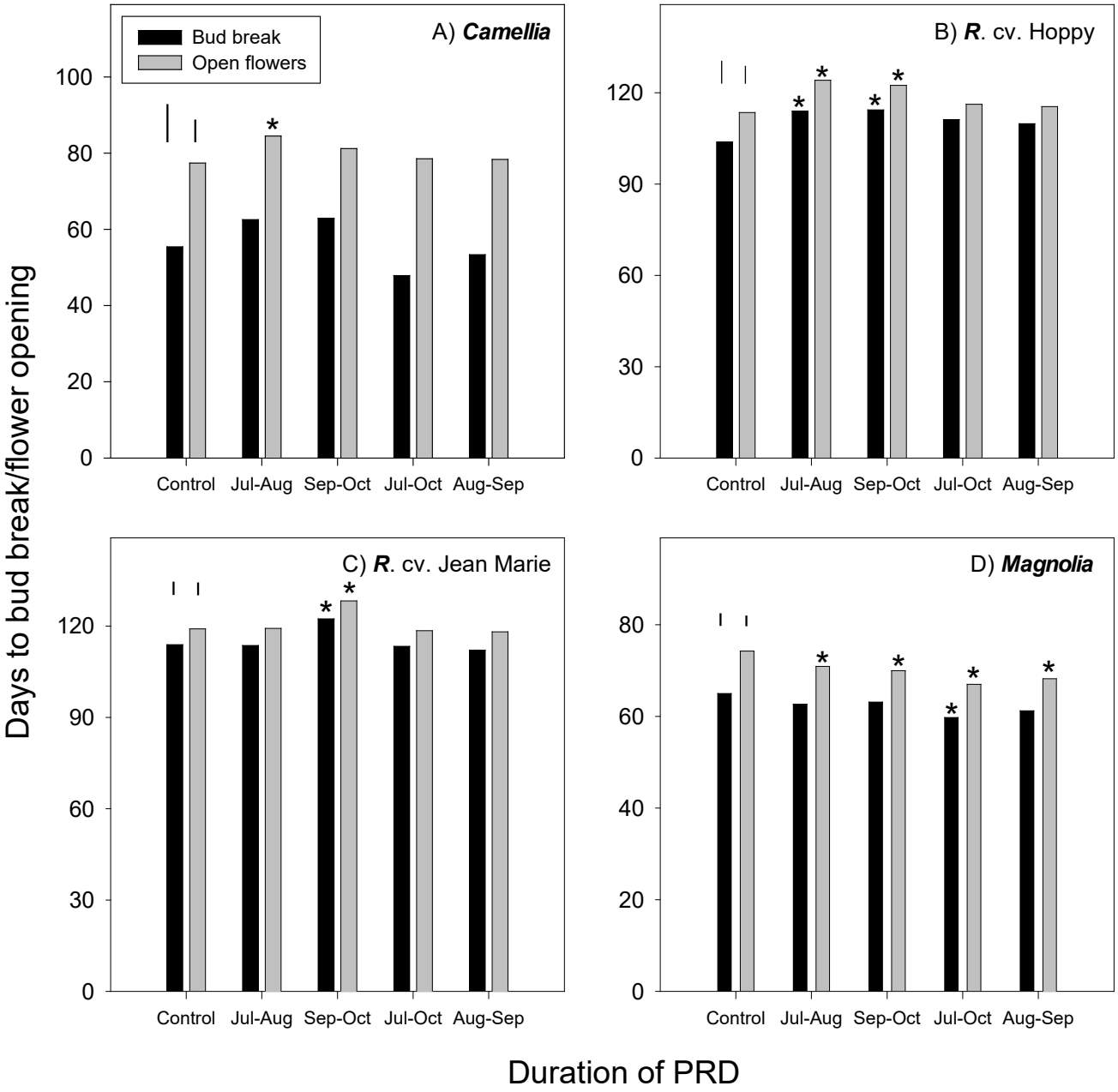


Figure 2.8. Effects of PRD for different durations on the timing of bud break and flower opening in A) *Camellia*, B) *R*. cv. Hoppy, C) *R*. cv. Jean Marie and D) *Magnolia*. Vertical lines represent LSD values at P = 0.05, asterisks indicate values that are significantly different from controls

The Jul-Oct PRD treatment was repeated in 2002 using the *Rhododendron* cvs Hoppy and Dopey. Two different pot sizes were used to assess the combined effects of PRD and restricting rooting volume. In contrast to 2001, the Jul-Oct PRD treatment increased plant height in *Rhododendron* cv. Hoppy plants in 7.5 L pots and had no significant effect on plants maintained

in 3L pots (Figures 2.9A and 2.10). The different watering regimes had no statistically significant effect on the flowering potential of plants in 7.5L pots and reduced the numbers of floral buds in plants in 3L pots (Figures 2.9B and 2.10).

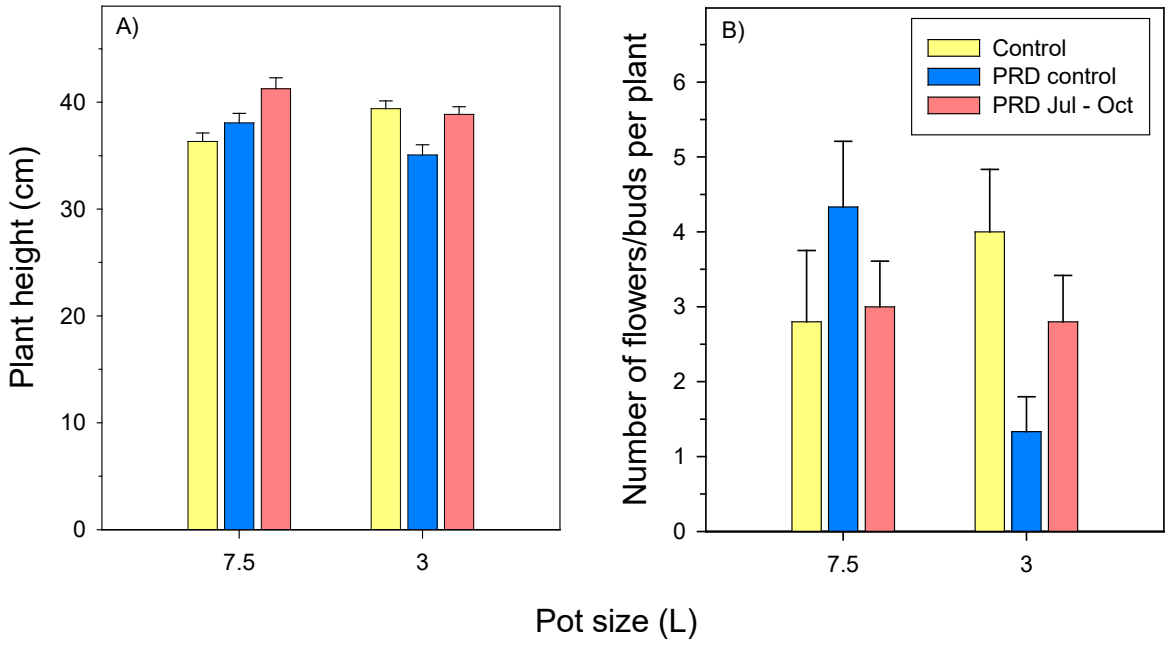


Figure 2.9. Effects of PRD and pot size on flowering and plant height in *Rhododendron* cv, Hoppy**.** Plants in A) were potted into 7.5 L pots at the beginning of July 2002; plants in B) were maintained in 3L pots until November 2002 when they were transferred to 7.5 L pots. Control plants received optimal watering, PRD control plants were watered through drippers and PRD Jul - Oct plants were watered through drippers that were swapped from one side of the pot to the other every 2 weeks. PRD Jul - Oct plants were tilted at 25° as shown in Figure 2.1. Measurements were made in May 2003.

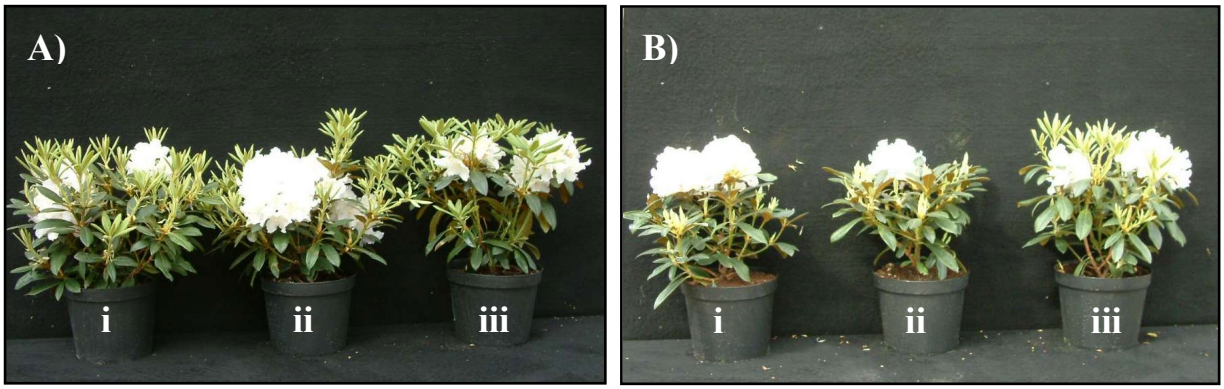


Figure 2.10. Effects of PRD and pot size on flowering and plant height in *Rhododendron* cv, Hoppy**.** Plants in A) were potted into 7.5 L pots at the beginning of July 2002; plants in B) were maintained in 3L pots until November 2002 when they were transferred to 7.5 L pots. Treatment i, ii and iii represent the watering regimes described in Figure legend 2.9 . The photo was taken on 13th May 2003.

In the *Rhododendron* cv. Dopey, PRD treatment again increased the height of plants in 7.5 L

pots and reduced height in plants in 3L pots (Figures 2.11A and 2.12). The different watering regimes had no statistically significant effect on the flowering potential of plants in 7.5 or 3L pots (Figures 2.11B and 2.12).

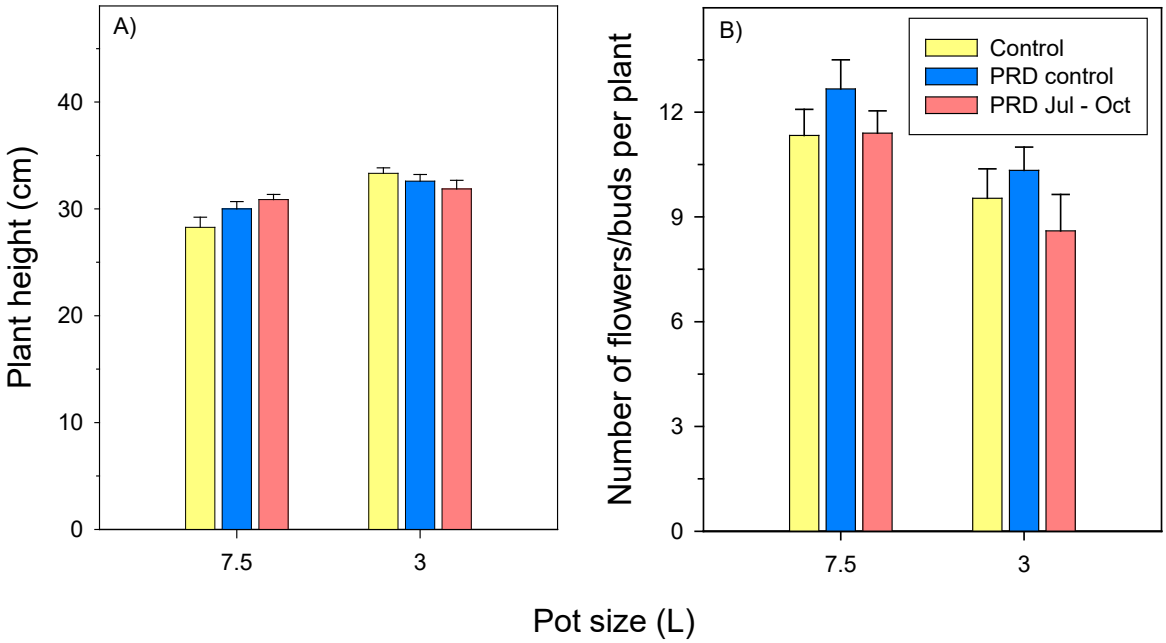


Figure 2.11. Effects of PRD and pot size on flowering and plant height in *Rhododendron* cv. Dopey. Plants in A) were potted into 7.5 L pots at the beginning of July 2002; plants in B) were maintained in 3L pots until November 2002 when they were transferred to 7.5 L pots. Control plants received optimal watering, PRD control plants were watered through drippers and PRD Jul - Oct plants were watered through drippers that were swapped from one side of the pot to the other every 2 weeks. PRD Jul - Oct plants were tilted at 25° as shown in Figure 2.1. Measurements were made in May 2003.

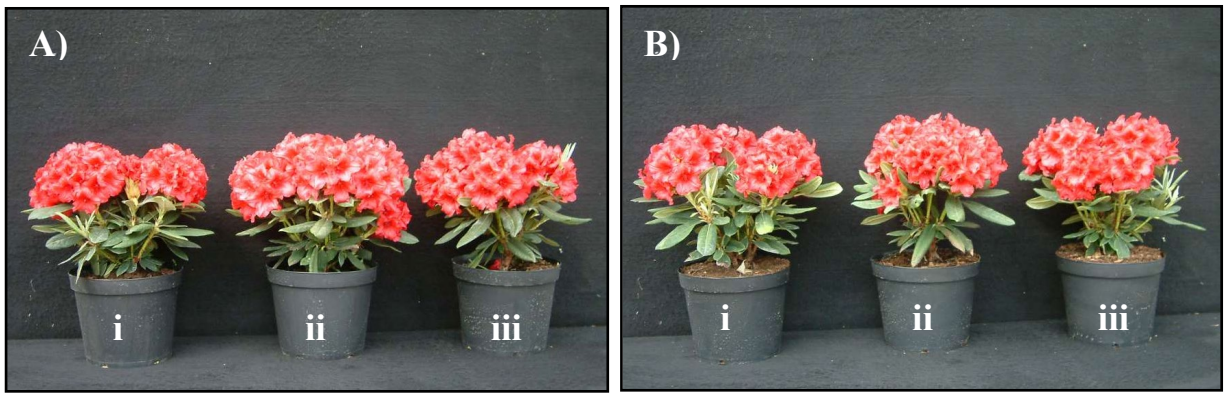


Figure 2.12. Effects of PRD and pot size on flowering and plant height in *Rhododendron* cv. Dopey. Plants in A) were potted into 7.5 L pots at the beginning of July 2002; plants in B) were maintained in 3L pots until November 2002 when they were transferred to 7.5 L pots. Treatment i, ii and iii represent the watering regimes described in Figure legend 2.9 . The photo was taken on 13th May 2003.

Summary

- The PRD treatments reduced the amount of water available to the roots in the 'dry' side of the pot.
- The promotive effect of PRD on plant quality depends on the timing of the application of the stress treatment, the species or cultivar and may not be consistent from year to year.
- PRD reduced vegetative growth in *Rhododendron* cvs. Hoppy and Jean Marie. PRD treatments imposed in Jul-Aug 2002 were particularly effective. PRD did not affect plant height in *Camellia* or *Magnolia*. Again, treatment effects varied from year to year.
- PRD treatments did not increase flower bud production in *Rhododendron* or *Camellia*, any effects tended to be negative *i.e.* numbers of flowers were reduced by PRD.
- In *Rhododendron*, the combined effects of some PRD treatments on plant size and flower bud production sometimes resulted in aesthetic improvements to plant quality and uniformity.
- Flowering in *R*. cv. Jean Marie was very susceptible to mild water deficits imposed during Sep-Oct; flower and bud numbers were reduced by 70% and 65%, respectively.
- Flowering in *Magnolia* was greatly improved by imposing mild water deficits from Jul-Oct. PRD treatments at other times gave variable results.
- Some PRD treatments increased the time to bud burst and flowering in *Camellia* and *R*. cvs. Hoppy and Jean Marie by up to 11 days.
- All PRD treatments decreased time to bud burst and flowering in *Magnolia* by up to seven days.

3. The role of red: far red ratios and day length in the control of floral initiation in *Rhododendron*

Introduction

Some cultivars of *Rhododendron* require several growing seasons before sufficient numbers of floral buds are initiated. This extended production cycle causes many problems for the nurseryman. A better understanding of factors that regulate floral initiation and their potential manipulation by cultural practices may reduce time to flowering and improve plant quality.

Red light (660 nm) and far red light (730 nm) influence many physiological and developmental processes, including flowering, by their action on phytochrome photoreceptors (Smith, 1994). These receptors exist in two isomeric forms, Pr (red absorbing) and Pfr (far red absorbing), at a photoequilibrium that is determined by the ratio of red (R) and far red (FR) light (Figure 3.1) (Furuya, 1989).

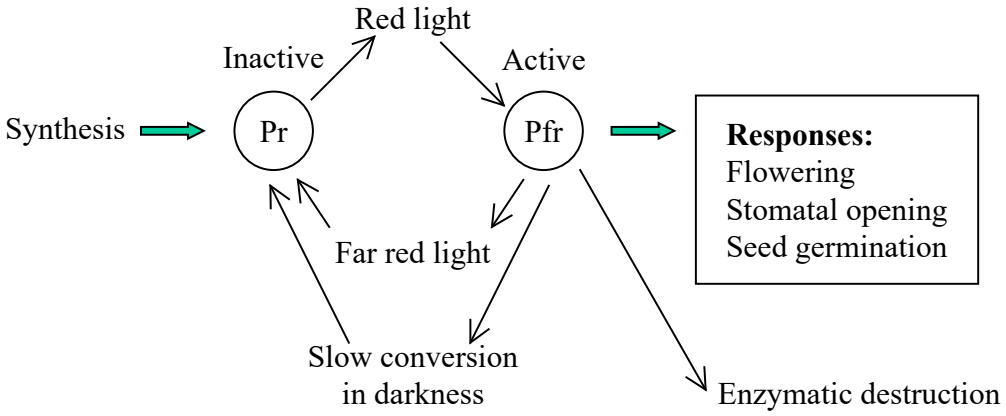


Figure 3.1. The photo-transformation between the Pr and Pfr isomeric forms of phytochrome as modulated by the proportions of R:FR radiation.

The ways in which the R:FR ratio influence flowering are not fully understood. In *Arabidopsis* the most dramatic R:FR ratio response is an acceleration in flowering at very low R:FR ratios (Botto and Smith, 2002). *Rhododendron,* as is the case with many other species, only initiated flowers after a set amount of vegetative growth has occurred (Kohl and Sciaroni, 1956), high 'far red' environments that stimulate shoot elongation (Runkle and Heins, 2001) may reduce the length of time needed before flowers can be initiated. However, R:FR ratio are known to act by

affecting the biosynthesis of gibberellins (GAs) (Martinez-Garcia *et al*., 2000) and GA inhibitors can nullify the effects of far red light on stem elongation (Campbell and Bonner, 1986). In high 'red' environments GA concentrations may be lowered. Inhibitors of GA biosynthesis can promote flowering in *Rhododendron* (Gent, 1995; and see Section 5) suggesting that endogenous GAs inhibit flower initiation (Roberts *et al*., 1999). Therefore, the lowered GA concentrations in high 'red' environments may promote earlier flowering.

Photoperiod can also affect floral initiation in *Rhododendron* (Vainola *et al*., 1999) although the relative importance of the photoperiod signal in relation to other floral stimuli is not yet known. Day length (or more correctly night length) is perceived by the same family of photoreceptors as the red to far red ratio but, in *Arabidopsis*, the phytochromes are different (Garry Whitelam *pers comm*). Furthermore, R:FR ratios may alter the photoperiod response. The R:FR ratio at dusk affects the rate of conversion of active Pfr to inactive Pr during the night period which, in turn, alters the critical night length (Casal and Smith, 1989, Vince Prue, 1985). The response of *Rhododendron* to photoperiod can vary under different environmental conditions and can be influenced by temperature (Petterson, 1972). We reported that *Rhododendron* cv. Hatsugiri was a facultative long day plant (LDP) under controlled environment conditions (Sharp, 2001). The *Rhododendron* cv. Hoppy responded similarly in a controlled environment but was classified as an obligate LDP in outdoor experiments where photoperiod was modified using shading or lighting arrays (Sharp, 2001).

The objectives of this research were: 1) to determine if shoot elongation and floral initiation are influenced by R:FR ratios; 2) to quantify the relative importance of the R:FR response compared with water deficits.

Materials and Methods

Plant material

Rooted cuttings of 18-month-old *Rhododendron* cv. Hatsugiri in 1 L pots containing standard

growth medium were used for the experiments. Plants were watered to container capacity every day. Before the experiment, all plants were pruned to remove apical meristems.

R:FR light ratios in controlled environments

Two growth cabinets (SG C170.PFX.J, Sanyo Gallenkamp, UK) were used to obtain different R:FR ratios. In the first cabinet, a spectral filter (Solatrol, Visqueen UK) was placed under the lighting array to absorb far red wavelengths. In the second cabinet, tungsten bulbs supplemented the incident light with far red wavelengths. Both cabinets were set to a 14 h photoperiod (08:00-22:00), a PAR at plant height of 170 μmol m^{-2} s^{-1} and day/night temperatures of 20 ºC/18 ºC. RH was 60 %.

Plants were subjected to the different R:FR ratios for 70 days and transferred to a cold store (2 ºC) for 28 days to facilitate anthesis. Plants were then maintained under ambient conditions for a further 15 days and numbers of floral buds, flowers per bud and branch lengths were recorded.

Measurement of transmission spectra

Transmission spectra were recorded at 10-nm-intervals between 360 nm and 800 nm using a spectroradiometer (SR 3000B, Macam Photometric Ltd, Livingstone, UK). For greater resolution in the phytochrome active region (640-740 nm), light intensities were recorded at 5 nm intervals. The light sensor was maintained perpendicular to the light source at canopy level using a retort stand. Recording began 15 min into the photoperiod once the light intensity had stabilised. Scans were repeated three times. A light sensor (Type QS, Delta T devices, UK) was used to confirm that PAR was similar in each cabinet.

R:FR light ratios and photoperiod in polythene tunnels

Four polythene tunnels (1.9m x 2.2m x 1.5m) were constructed using conduit piping and

appropriate connectors. FR absorbing filters (Solatrol, Visqueen UK) or FR transmitting (UVA/EVA,Visqueen Agri , UK) filters were draped over the tunnel frames to obtain different R:FR ratios within the tunnels. One 'red' and one 'far red' tunnel were placed under a lighting rig to provide LDs (16 h days); two similar tunnels were covered with silver sheeting to impose short days (SDs) (8 h days).

Treatments were imposed for 70 days. Floral initiation was determined at intervals throughout the treatments. Final floral bud counts, flowers per bud and branch length were recorded during the subsequent spring.

Manipulation of photoperiod on sand beds

Photoperiods of short days (9 h), long days (18 h) and natural day lengths (10-14 h) were supplied for 2 growing seasons. Artificial LD photoperiods were provided by placing a bank of 60 x 25 W tungsten lamps at 1.5 m above part of the crop. This provided irradiance of 2.36 W m^{-2} at the crop canopy; the precise irradiance required to maintain a LD signal is undetermined for *Rhododendron*, however, other species have been quoted as requiring a 0.06-0.30 W m^{-2} (Salisbury and Ross, 1985). Tungsten lamps were illuminated from 6pm-10pm and 6am-8am every day. The SD conditions were implemented by placing a reflective screen (XLS Obscura A/B +B – Svenson) over the other half (from 5pm until 9am). Treatments were in place from 21st June until 15th October 2000. All plants were maintained outside on sand beds for both seasons. Treatments were not supplied during winter.

Water deficit treatments in polythene tunnels

All plants were irrigated automatically to container capacity. RDI treatments were imposed by restricting the volume of water supplied to 50% (moderate stress) and 25% (severe stress) of daily potential evapo-transpiration (ETp). Control plants received 100% ETp. Saucers were placed under the containers to prevent both water run-off and water rising from the sand-bed that

may have rehydrated the droughted plants. Nine reference plants, placed between the blocks, were weighed daily and then re-watered to container capacity. Each RDI treatment consisted of 12 plants per tunnel.

Treatments were imposed for 70 days. Floral initiation was determined at intervals throughout the treatments. Final floral bud counts, flowers per bud and branch length were recorded during the subsequent spring.

Results

Transmission spectra in 'red' and 'far red' controlled environments

The spectra in the 'red' and 'far red' environments were generally similar (Figure 3.2 A & B). In the 'far red' treatment, there was a peak in the green part of the spectrum at 570 nm, however, light at this wavelength is neither photosynthetically or phytochrome active. The light intensity at 660 nm in the 'red' environment was higher than in the 'far red' environment. Since the intensities at 730 nm were the same, the R:FR ratio was higher in the 'red' environment (Figure 3.2 C).

R:FR ratios at different light levels

Spectral filters and tungsten filament bulbs were used to obtain different R:FR ratios in the two growth cabinets. The effect of these treatments was more pronounced at lower light levels but these intensities were too low to sustain adequate growth (Figure 3.3). A light intensity of 170 μmol m^{-2} s^{-1} was considered sufficient to maintain shoot growth, this intensity was achieved at 71% light levels in the far red environment. To obtain equal PAR and yet provide an adequate difference in R:FR ratio between cabinets, the red environment was maintained at 90% light levels.

Effects of R:FR on floral initiation

Floral initiation was significantly greater in the 'far red' environment, where the R:FR ratio

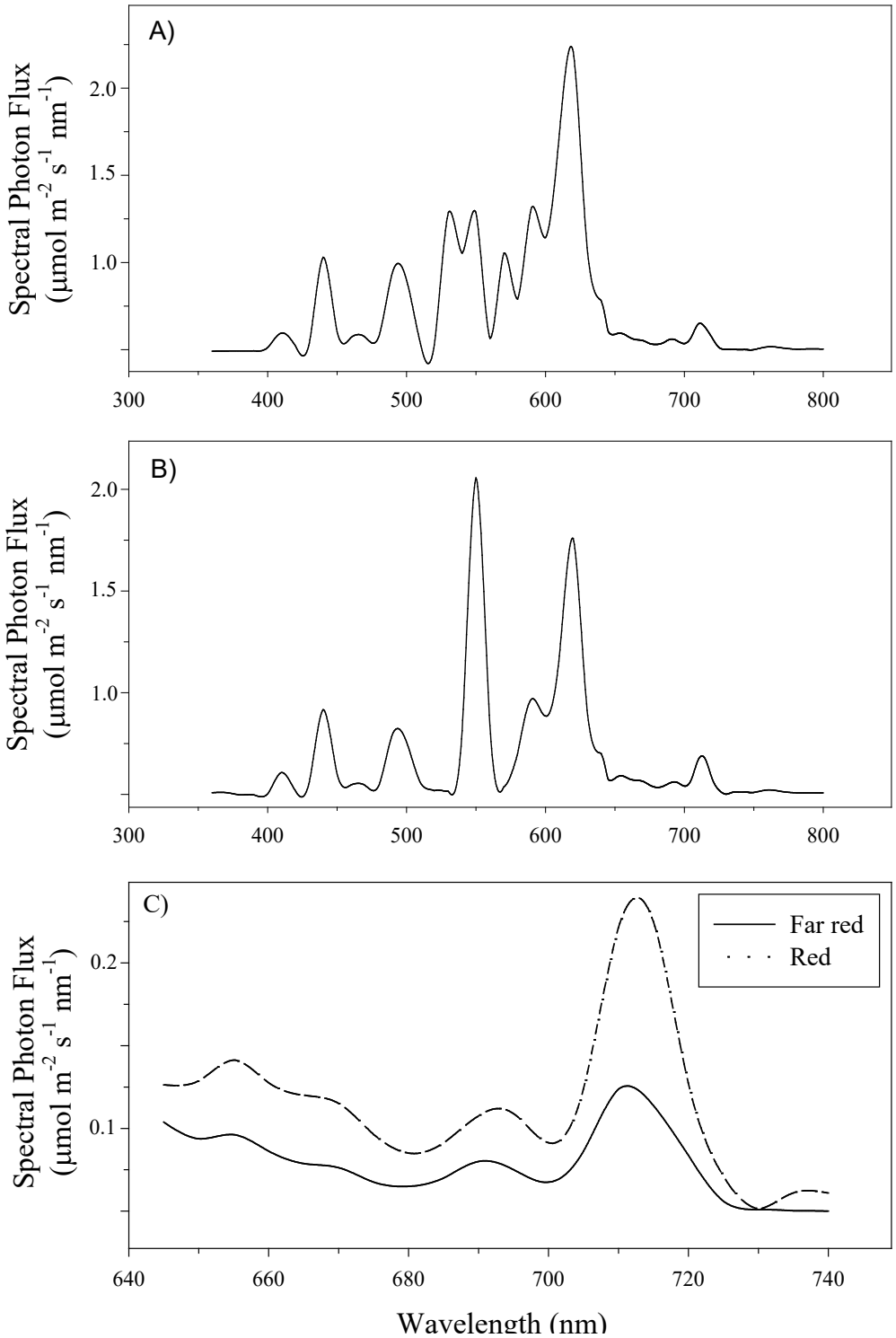


Figure 3.2. Transmission spectra for A) 'red' and B) 'far red' controlled environments. These environments were achieved by the use of a far red absorbing filter (red) and tungsten filaments with a strong far red output (far red).

was lower (Figure 3.4 A). However, 'red' and 'far red' environments did not significantly affect the number of branches per plant or the numbers of flowers per bud (Figure 3.4 B & C). When the number of floral bud was expressed per branch, the effect of R:FR ratio on floral initiation was not significant (Figure 3.4 D).

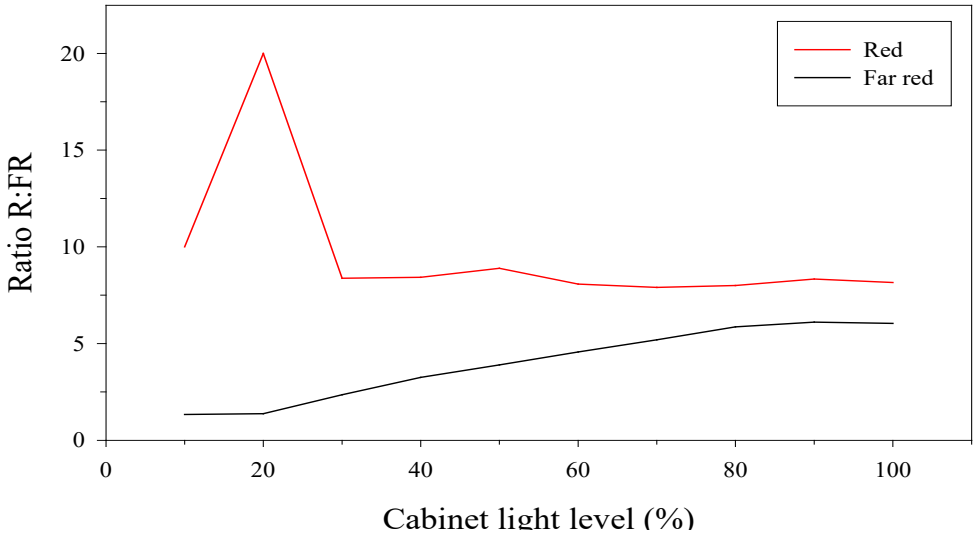


Figure 3.3. Manipulation of the red:far red ratio in the controlled environments by altering the incident light level. In the 'red' environment, the red:far red ratio increased at lower light levels due to the increased absorption of far light by the spectral filter. In the 'far red' environment, the red:far red ratio fell with decreasing light intensity due to greater representation of the tungsten spectra.

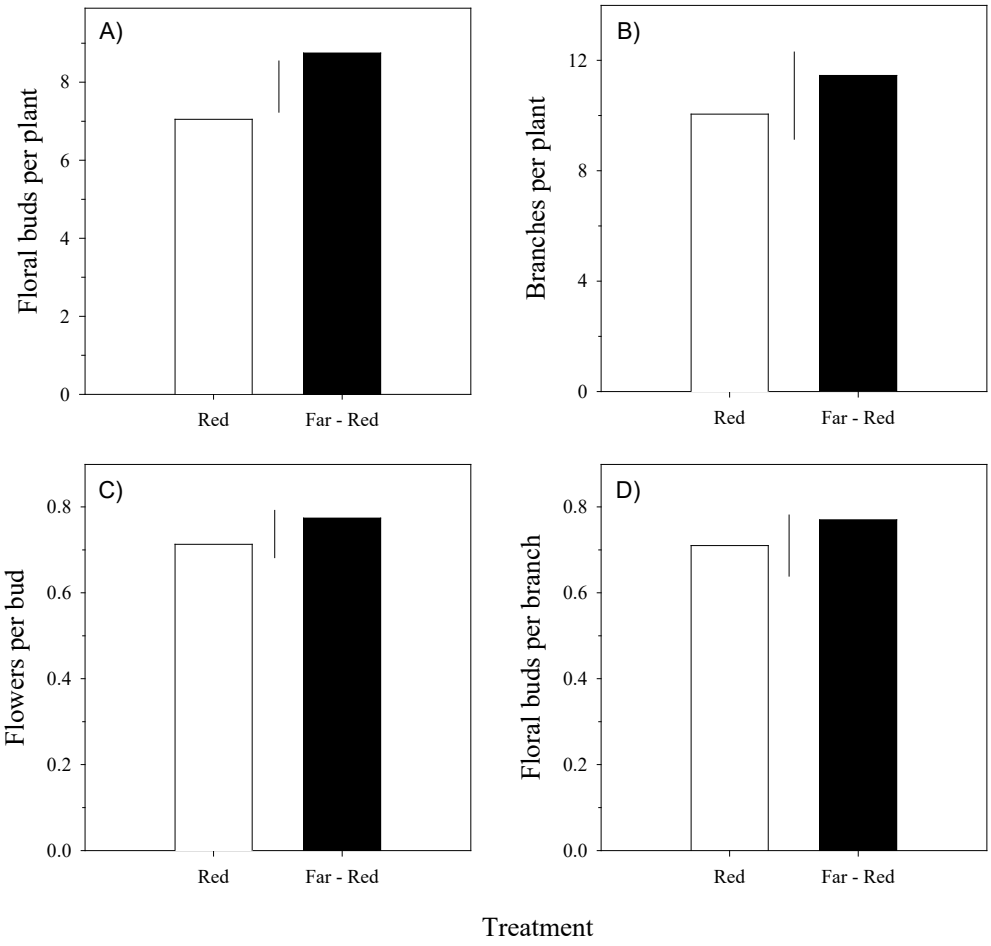


Figure 3.4. Effects of 'red' or 'far red' environments on A) floral buds per plant, B) branches per plant, C) flowers per bud and D) floral buds per plant in Hatsugiri. 'Red' / 'far red' environments were imposed as in Figure 2.1. Data are means of 15 replicate plants, vertical lines are LSD values at P = 0.05.

Effects of spectral filters on transmission spectra in polythene tunnels

Two polythene tunnels of different spectral filters were constructed to obtain differences in R:FR ratios. Since the intensities at 660 nm were higher under the red filter compared to the far red filter (Figure 3.5 A & B), the R:FR ratio was higher in the former.

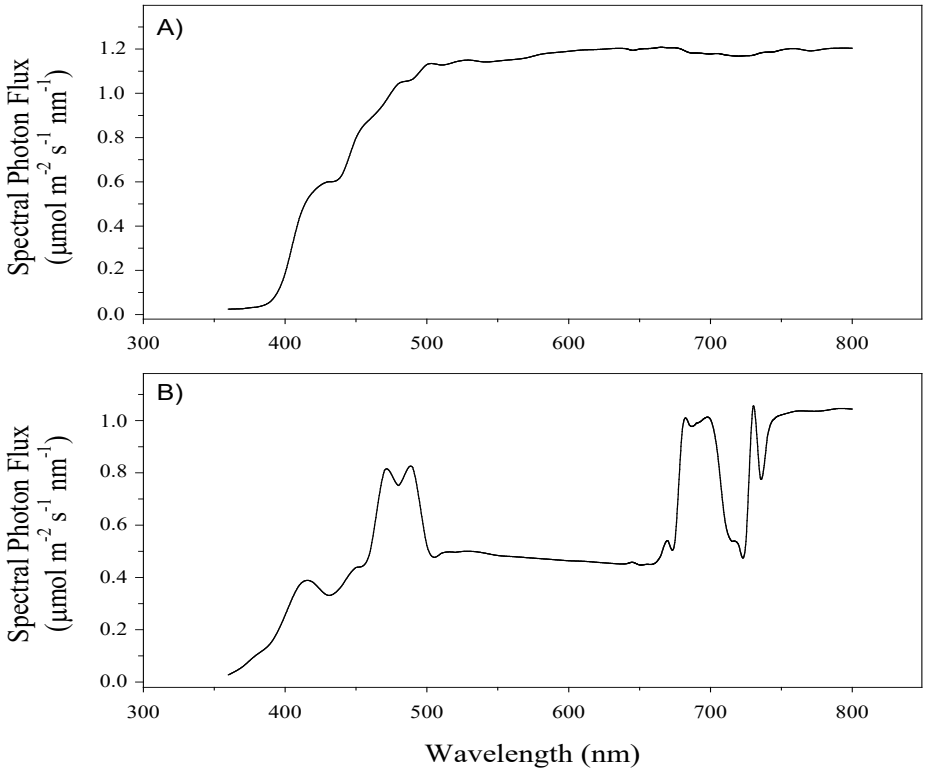


Figure 3.5. Transmission spectra for A) 'red' and B) 'far red' polythene tunnels. These environments were achieved by the use of a far red absorbing filter (red) and a control filter (far red).

Effects of water deficits and photoperiod on floral initiation

In the *Rhododendron* cv. Hatsugiri, moderate and severe water deficits inhibited floral initiation under both SD and LD (Figure 3.6). Floral initiation was significantly greater under LDs than SDs (Figure 3.6). Similarly, in the *Rhododendron* cv. Hoppy, no floral buds were produced under short days (9 h).(Table 3.1 and Figure 3.7). Normal day lengths (10-14 h) were most inducive to floral initiation (Table 3.1). Very long days (18 h) were detrimental to floral initiation and promoted premature flowering (in autumn). The lower number of floral buds per plant under very long days was due, at least in part, to the stimulation of a second vegetative flush in late summer (data not shown).

Table 3.1. The effect of day length on flowering capacity in the *Rhododendron* cv. Hoppy. Plants were either maintained under short days (SD - 9 h), normal days (ND - 10-14 h) or long days (LD - 18 h).

Day length	Floral buds per plant	% plants floral	Flowers per bud
SD	0	0	0
ND	1.781	17.93	14.717
LD	0.625	7.146	14.84
LSD	0.689	7.41	0.907

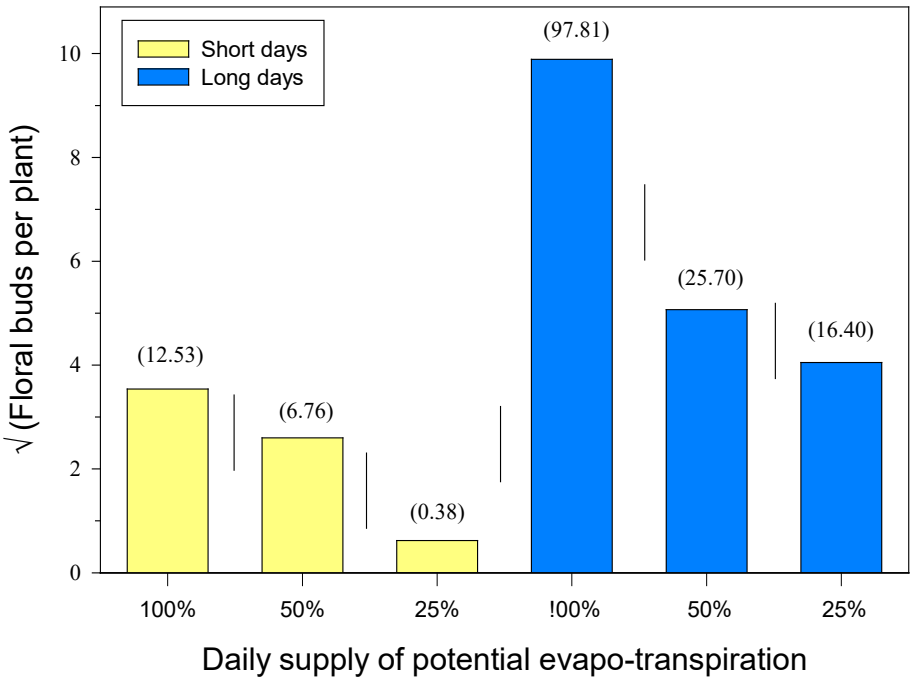


Figure 3.6.. Effects of photoperiod and water deficits on the number of floral buds per plant in *Rhododendron* cv. Hatsugiri. Short days (8 h) and long days (16 h) were imposed by silver screening and daylight extension bulbs, respectively. Percentages of potential evapo-transpiration were supplied daily. Data are means of 12 replicate plants, vertical lines are LSD values at P = 0.05. Values in brackets are back transformed means of flower bud numbers per plant..

Effect of 'super long days' on flora initiation

The imposition of 'one super long day' (36 h) to *Rhododendron* cv. Hatsugiri stimulated floral initiation compared to plants that were maintained under short days (Table 3.2). Floral initiation was detected within 7 days of the super long day treatment. The effects of a 'super long day' on floral initiation in other Rhododendron cvs. has yet to be determined.

Figure 3.7. Seven-year-old *Rhododendron* cv. Hoppy plants maintained under short days (8h) for two years. No floral buds were initiated during the treatment.

Table 3.2. Effect of a 'super long day' (36 h) on floral initiation in *Rhododendron* cv. Hatsugiri. 'Super long days' were imposed in a controlled environment cabinet by supplied by tungsten tubes. Hatsugiri is a facultative long day plant so some floral buds were initiated under short days.

Photoperiod	% buds initiated
Continuous SD	25.7
SD + 1 Super-LD	48.6
LSD	22.61

Summary

- Reducing the R:FR ratio increased the numbers of flower buds per plant under controlled environment conditions.
- The number of branches, flowers per bud and flower buds per branch were increased by altering the R:FR ratio but the differences were not significant.
- The effect of altering the R:FR light ratio on gibberellin concentrations in bud tissues and the implications for floral initiation are currently being investigated.
- Flowering in *Rhododendron* cv. Hoppy was strongly promoted under normal long days (May - September). Short days (9 h) and very long days (18 h) were not conducive to flower initiation in this cv.
- One 'super long day' (36) was sufficient to stimulate floral initiation in *Rhododendron* cv. Hatsugiri.

4. The effect of rooting volume restriction on flowering in *Rhododendron*

Introduction

The restriction of rooting volume often reduces shoot growth, an effect that may, in part, be due to limited supplies of water and nutrients. Impedance to root growth imposed by physical barriers may also contribute to this effect. A reduction in leaf elongation rate is a common response to root restriction stress and is probably mediated by root-sourced chemical signals delivered in the transpiration stream. The nature of the root-derived hormonal signals are unclear but could include increased output of ABA, the ethylene precursor ACC and reduced cytokinin output from the restricted roots.

Anecdotal evidence, *i.e.* from Nurserymen, suggests that *Rhododendron* plants which are not potted-on, sometimes flower more profusely than equivalent plants which have been potted-on into larger containers. Thus, the root-sourced hormonal signals that regulate shoot vegetative growth may also impact on reproductive growth.

The objective of this research was to determine whether root restriction stress, imposed by potting on into different sized pots, increased the flowering potential of *Rhododendron* cvs. Hoppy and Scintillation. The effects of relieving root restriction during periods of rapid shoot elongation (July) or after shoot extension had terminated (September) on flower initiation were also tested.

Materials and Methods

Plant material

At HRI-East Malling, forty five plants of *Rhododendron* cvs. Hoppy and Scintillation were used in each experiment. Treatments were applied at two periods in the annual growth cycle; during July when shoots were still in an active vegetative phase, and during September, when terminal buds have begun to form on the primary laterals. Differing degrees of root volume restriction were imposed by potting-on into different sized pots in July or September, 2002. On each

occasion, two-and-half-year-old plants in 2L pots were either top-dressed with standard growth medium containing Osmocote, potted-on into 5L pots or potted-on into 7.5 L pots. Plants were maintained on an uncovered sand bed and watered to container capacity daily.

At HRI-Efford, 30 plants of *Rhododendron* cvs. Dopey and Hoppy were used in each experiment. Differing degrees of root volume restriction were imposed by potting-on into larger pots in July or November, 2002. On each occasion, plants in 3L pots were potted-on into 7.5 L pots using a potting mix comprised of: Premium bark and Pine bark (75:25 v/v), 3 Kg m^3 Osmocote Plus (12-14 Autumn), 1 Kg m^3 Magnesium Limestone and 1 Kg m^3 Suscon® Green. Plants were maintained on uncovered sand beds and watered to container capacity daily.

Experimental design

At HRI-East Malling, each experiment (July and September) consisted of three pot sizes and two cultivars. Plants were arranged in 10 blocks, each consisting of 3 rows. Every row contained a replicate of each cultivar in 2, 5 and 7.5L pots.

At HRI-Efford, two pot sizes and two cultivars were used. Plants were arranged in blocks, each containing 5 plants. Each treatment consisted of 3 blocks.

Vegetative and Floral parameters

The number of vegetative buds per plant, floral buds per plant, flowers per floral bud, plant height and total leaf area were measured in May 2003. The effects of root volume restriction on total leaf area were determined in cv. Scintillation. Lengths and widths of leaves were measured and total leaf area calculated using a regression equation of leaf length against leaf area.

Results

Root restriction effects on vegetative growth

Restricting root volume (Figure 4.1) by maintaining plants in 2L or 5L pots reduced plant height

in the *Rhododendron* cv. Hoppy in both the July and September experiments, compared to plants that were repotted into 7.5 L pots (Figure 4.2A). Plant height was not affected by root volume when the cv Scintillation was potted-on in July. However, potting-on into 5 and 7.5L pots in September increased plant height compared to plants maintained in 2L pots (Figures 4.2 and 4.3).

Figure 4.1. Effects of restricting potting volume to A) 2L, B) 5L and C) 7.5L in *Rhododendron* cv. Scintillation. B) and C) were potted on in September 2002, the photograph was taken in May 2003.

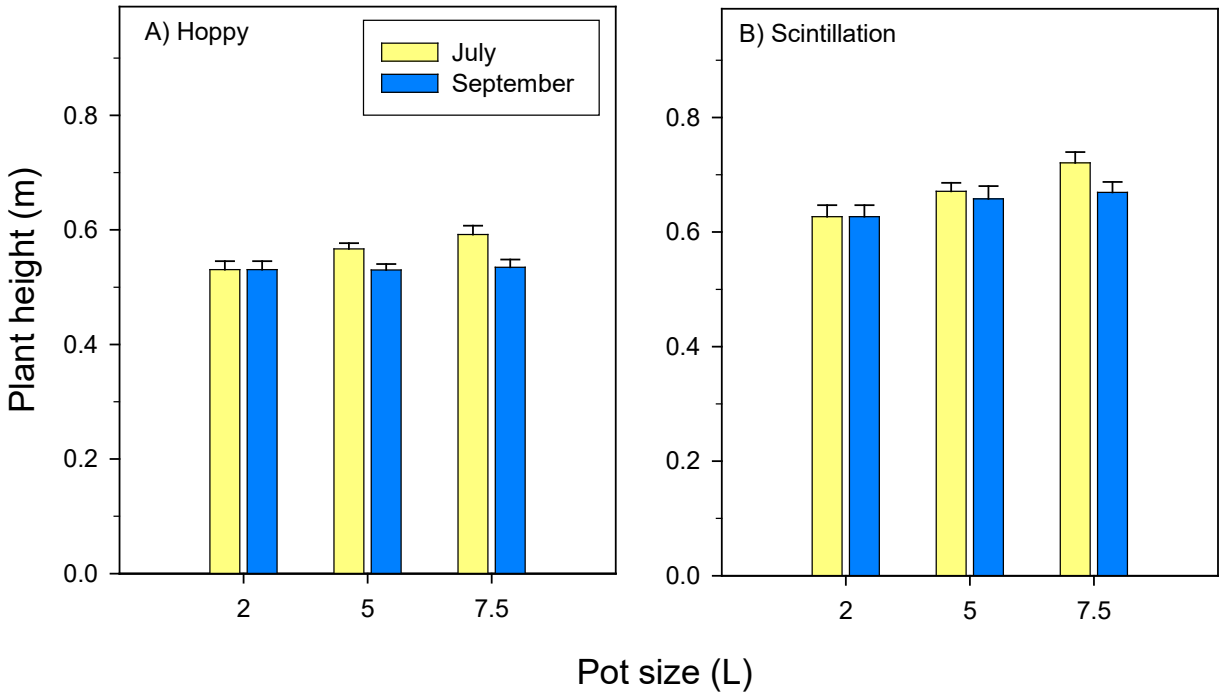


Figure 4.2. Effects of restricted rooting volume on plant height in *Rhododendron* cvs. Hoppy and Scintillation**.** Plants were potted into 7.5 L pots at the beginning of July 2002 or maintained in 2L pots until September 2002 when they were transferred to 7.5 L pots. Plant heights were recorded in May 2003.

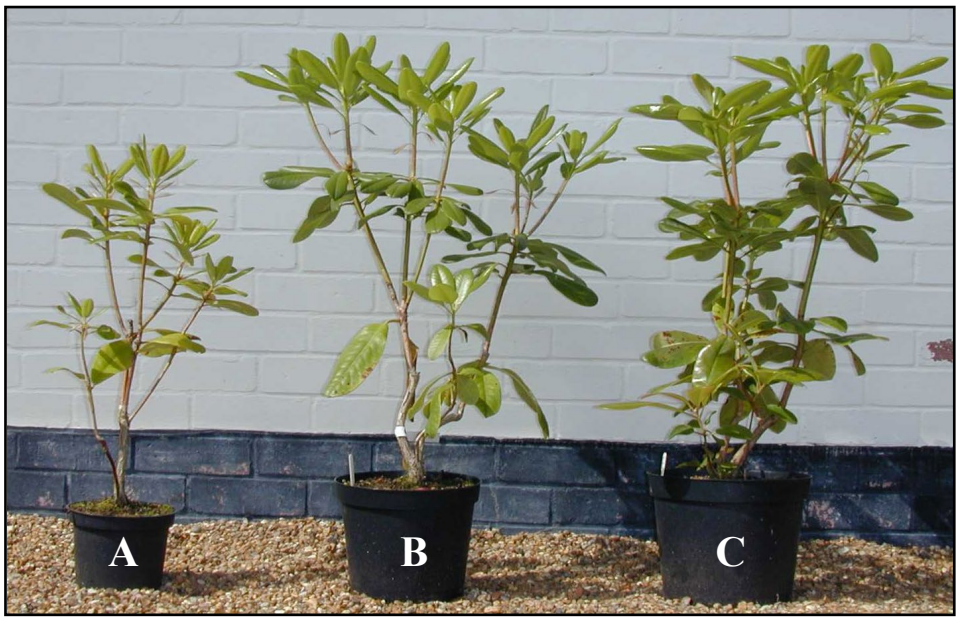


Figure 4.3. Effects on plant height of restricting rooting volume to A) 2L, B) 5L and C) 7.5L in *Rhododendron* cv. Scintilation. B) and C) were potted on in September 2002, the photograph was taken in May 2003.

In experiments at HRI-Efford, restricted rooting volume in *Rhododendron* cvs. Dopey and Hoppy by delaying potting on until November increased plant height (Table 4.1).

Table 4.1. Effects of restricted rooting volume on plant height in *Rhododendron* cvs. Dopey and Hoppy**.** Plants in were either potted into 7.5 L pots at the beginning of July 2002 or maintained in 3L pots until November 2002 when they were transferred to 7.5 L pots. Plant heights were recorded in May 2003. Values are means of 15 plants with associated standard errors.

Cultivar	Potted on	Plant height (cm)
Dopey	July	28.3 ± 0.9
	November	33.3 ± 0.5
Hoppy	July	36.3 ± 0.8
	November	39.4 ± 0.7

The effects of root volume restriction on the number of vegetative buds per plant were similar to those on plant height. Restricting root volume by maintaining plants in 2L pots limited the number of vegetative buds per plant in both cultivars in July and September (Figure 4.4). Numbers of vegetative buds per plant were highest in plants that had been potted on into 7.5 L pots.

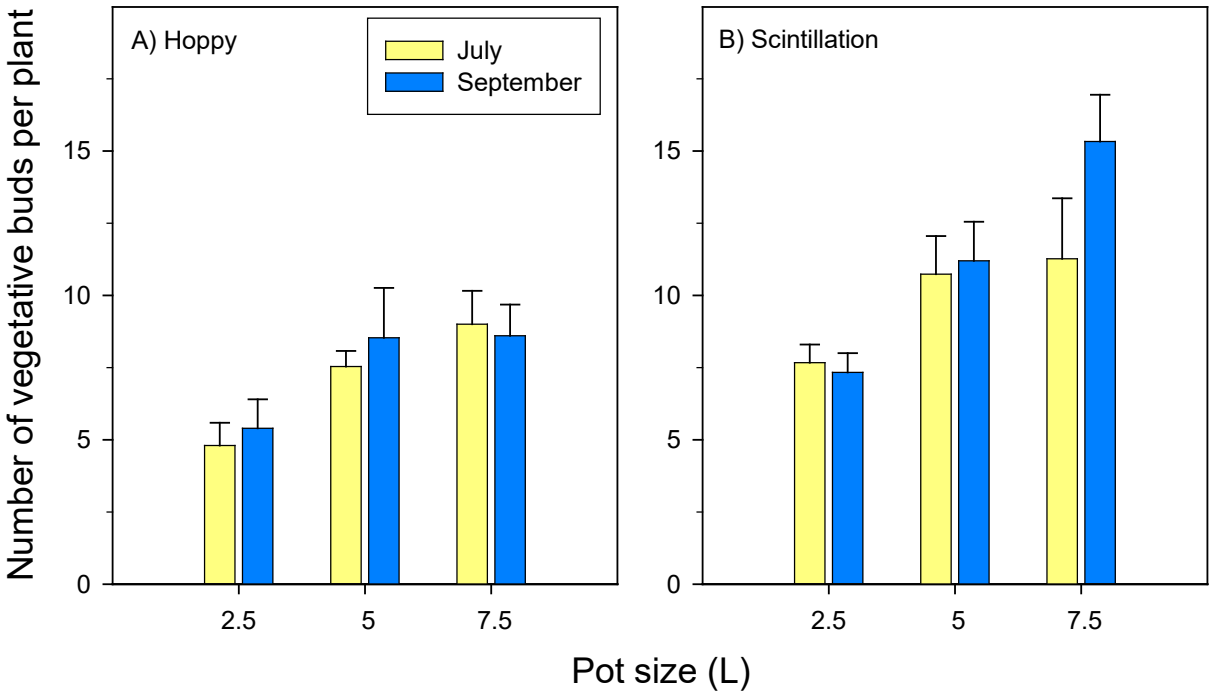


Figure 4.4. Effects of restricted rooting volume on the number of vegetative buds per plant in *Rhododendron* cvs. Hoppy and Scintillation. Plants were potted into 7.5 L pots at the beginning of July 2002 or maintained in 2L pots until September 2002 when they were transferred to 7.5 L pots. Plant heights were recorded in May 2003.

Total leaf area per plant was also significantly reduced by restricting rooting volume (Table 4.2).

Table 4.2. Effect of restricting rooting volume on total plant leaf area in cv Scintillation. Leaf areas were determined non-destructively using a regression analysis of leaf length versus leaf area. Data are means of 15 replicates with associated standard errors.

Pot size (L)	Leaf length (cm)	Leaf area (cm^2)
2.5	44.6 ± 0.9	60 ± 4.5
5	65.3 ± 1.2	126.6 ± 5.1
7.5	72.7 ± 1.4	161 ± 5.2

Root volume restriction effects on floral parameters

Generally, the number of floral buds per plant was reduced by imposing root restriction stress (Figure 4.5). However, the response was variable and differed between cultivars. In cv. Hoppy, rooting volume had little effect when potting-on was carried out in July, in contrast to cv. Scintillation where the number of flower buds per plant was greatly increased the following spring (Figure 4.5).

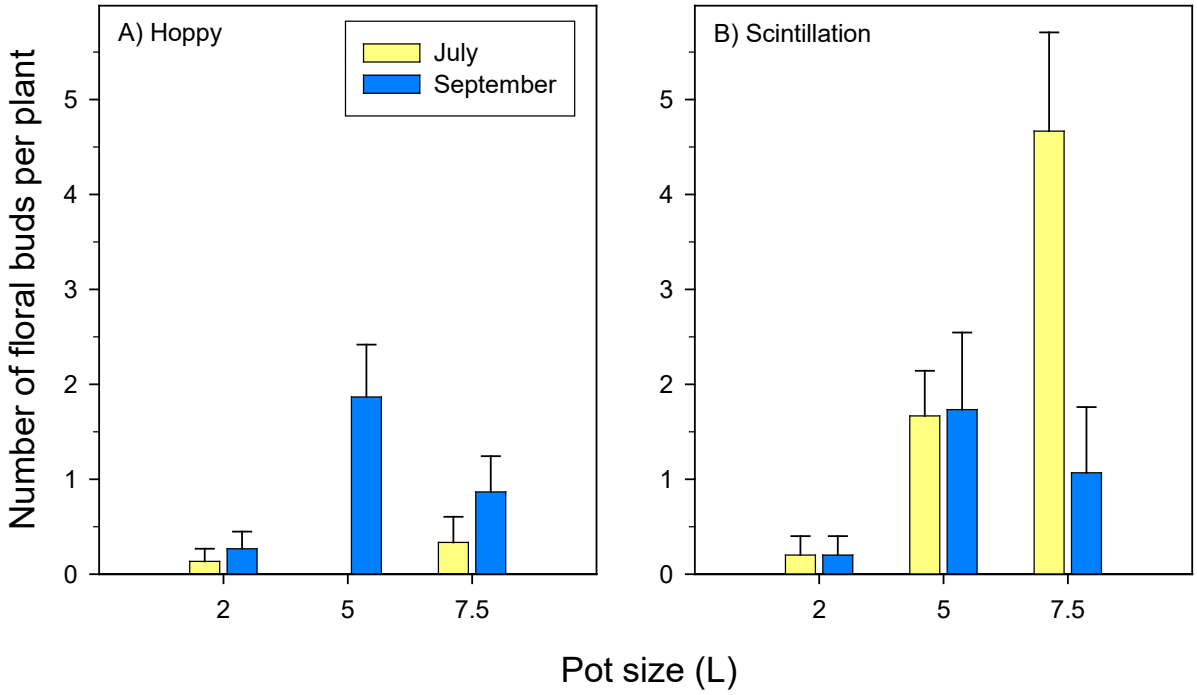


Figure 4.5. Effects of restricted rooting volume on the number of floral buds per plant in *Rhododendron* cvs. Hoppy and Scintillation**.** Plants were potted into 7.5 L pots at the beginning of July 2002 or maintained in 2L pots until September 2002 when they were transferred to 7.5 L pots. Numbers of floral buds were recorded in May 2003.

When potting-on was carried out in September, Hoppy plants in the intermediate rooting volume (pot size 5L) produced the greatest number of floral buds the following spring (Figure 4.5). Delaying potting-on cv. Scintillation to 7.5 L pots for two months caused a marked decrease in the number of floral buds produced per plant (Figure 4.5).

At HRI-Efford, delaying potting-on until November reduced the number of floral buds in *R*. cv. Dopey but increased floral bud numbers in *R*. cv. Hoppy (Table 4.3), although the latter effect was not statistically significant.

Table 4.3. Effects of restricted rooting volume on the number of floral buds per plant in *Rhododendron* cvs. Dopey and Hoppy**.** Plants were either potted into 7.5 L pots at the beginning of July 2002 or maintained in 3L pots until November 2002 when they were transferred to 7.5 L pots. Numbers of floral buds were recorded in May 2003. Values are means of 15 plants with associated standard errors.

Cultivar	Potted on	Number of floral buds per plant
Dopey	July	11.3 ± 0.7
	November	9.5 ± 0.8
Hoppy	July	2.8 ± 1.0
	November	4.0 ± 0.8

Restricting the rooting volume did influence the number of flowers per floral bud, although effects were somewhat variable and not always significant (Figure 4.6). When potting on was delayed until September, imposing root restriction by maintaining plants in 2L pots increased the numbers of flowers per floral bud in both Hoppy and Scintillation (Figure 4.6). However, when the combined effects on floral bud per plant and number of flowers per floral bud were taken into account, rooting volume restriction decreased the flowering potential of both cultivars.

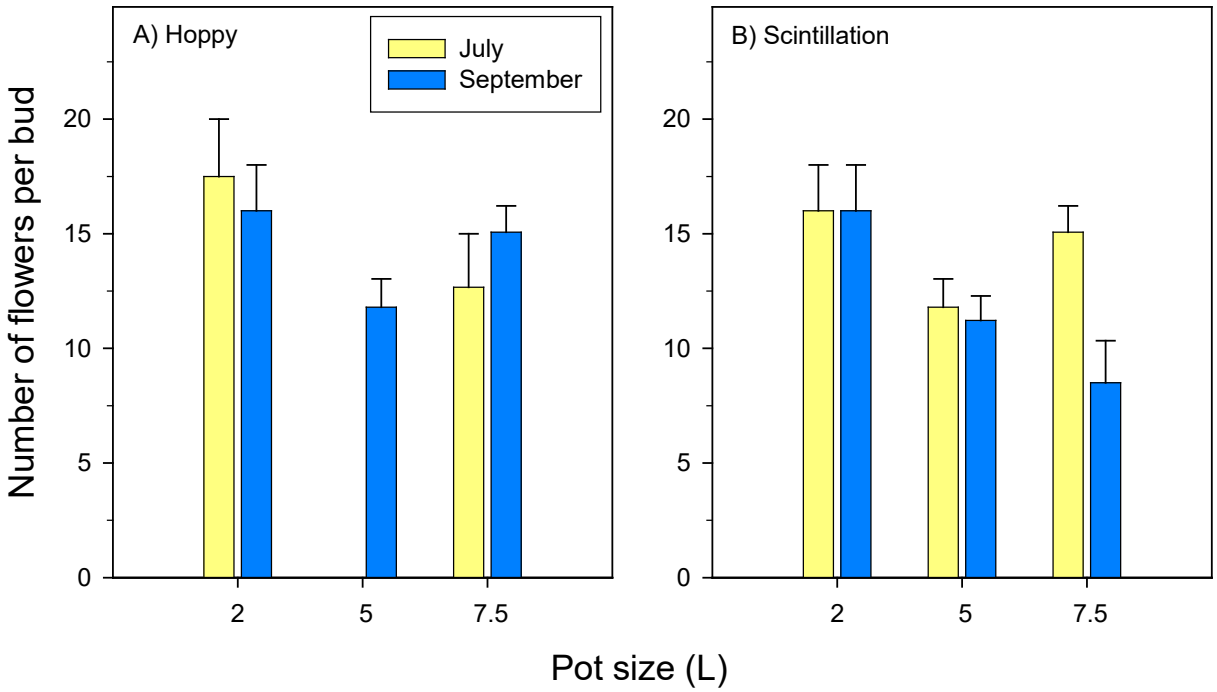


Figure 4.6. Effects of restricted rooting volume on the number of flowers per bud in *Rhododendron* cvs. Hoppy and Scintillation**.** Plants were potted into 7.5 L pots at the beginning of July 2002 or maintained in 2L pots until September 2002 when they were transferred to 7.5 L pots. Numbers of flowers per bud were recorded in May 2003.

Summary

- Restricting rooting volume by delaying potting on into 2, 5 or 7.5L pots reduced plant height and total leaf area.
- Restricting rooting volume greatly reduced the numbers of floral buds per plant.
- The number of flowers per floral bud was increased by root restriction stress.
- The flowering potential of *Rhododendron* cvs. Hoppy and Scintillation was reduced by severe restrictions to rooting volume *i.e.* maintaining plants in 2L pots.
- The flowering potential of *Rhododendron* cv. Hoppy was optimal when plants were transferred to 5L pots, either in July or September.
- The flowering potential of *Rhododendron* cv. Scintillation was optimal when plants were transferred to 7.5L pots in July.

5. The influence of nutrient stress on flower formation in *Rhododendron*

Introduction

The effects of nutrition on flowering in *Rhododendron* have been reported (Ticknor and Chaplin, 1978). In particular, there is some evidence to suggest that high levels of phosphate can improve flowering in *Rhododendron* (Ticknor, 1969) and other woody plants (Taylor and Nichols, 1990). Research carried out at Efford in the 1970's showed good correlation between flower bud initiation and phosphate addition in *Camellia* (Scott, 1977). Additionally, anecdotal evidence indicates that phosphoric acid has been used to improve bud formation of *Rhododendron* in North America. Low pH or alterations in ion levels (EC) associated with phosphoric acid dosing may also be a contributing factor to inducing a 'stress' response in the plants.

Materials and Methods

Three cultivars, namely *R.* cvs Hoppy, Scintillation and Mrs T.H. Lowinsky were evaluated at Efford for their response to abiotic stress treatments in terms of flower production. Plants either received treatments for 8 weeks from 18th July to 11th September 2000 (Summer treatments) or for 9 weeks from 14th September to 15th November 2000 (Autumn treatments), and plants were monitored for bud and flower numbers, and foliar quality in the following spring (May 2001).

There were 15 replicates of each species per treatment:

Control – Plants maintained on an Efford sand bed and hand-watered as required

Phosphate addition (**P**) - Triple superphosphate - $Ca(H_2PO_4)_2$ was added to the growing medium as a liquid feed on a weekly basis. Bulk solution was made up in 100 l batches by dissolving triple superphosphate in warm water to give EC = 2.0 mS cm^{-1}. This solution was watered on to pots by hand once weekly (Wednesday). Plants were irrigated with mains water Monday and Friday to prevent scorching of roots.

Phosphate addition + low pH (Low pH + P) - Phosphoric acid was added to the growing medium as a liquid feed on a weekly basis. Bulk solution was made up in 100 l batches by

dissolving phosphoric acid in water to give pH = 3.5. This solution was watered on to pots by hand three times a week (Monday, Wednesday and Friday).

Results

R. cv. Hoppy proved to be a considerably more floriferous cultivar compared to the other varieties tested, with some individual plants having as many as 12 flower buds. However, application of triplesuperphosphate or phosphates at low pH considerably reduced the number of flower buds that formed (Figure 5.1A).

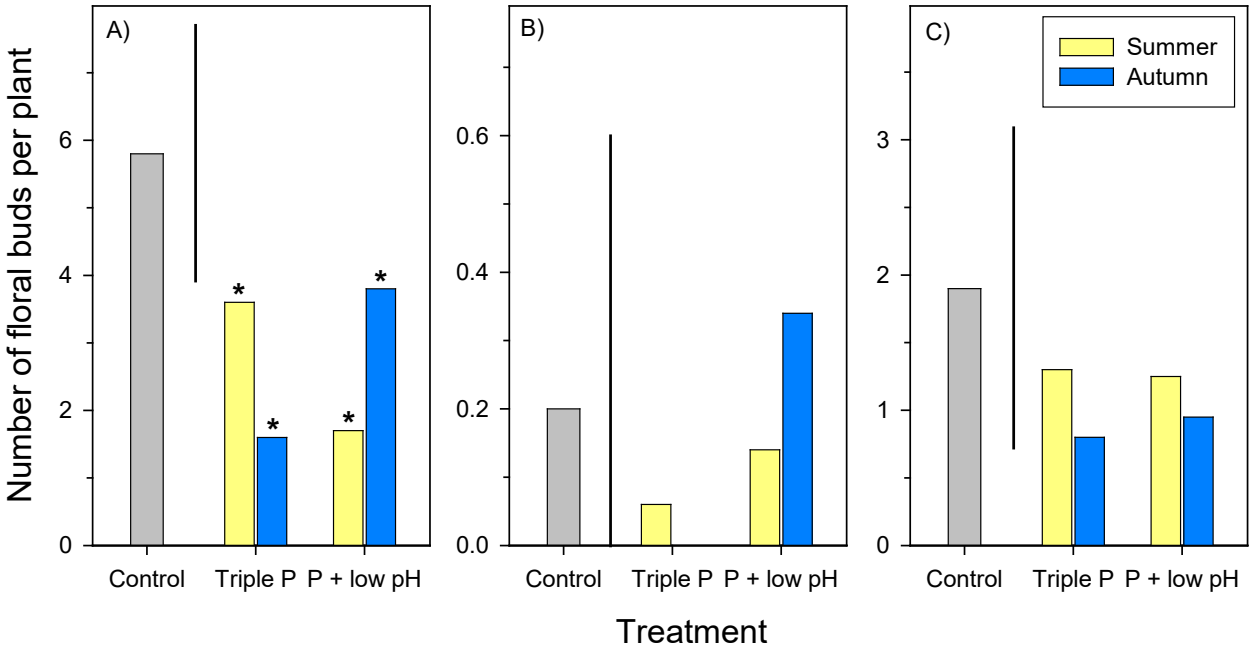


Figure 5.1. Effect of triple phosphate or phosphate applied at low pH on flowering potential of *Rhododendron* cvs A) Hoppy, B) Scintillation and C) Mrs T.H. Lowinsky. Note differences in y-axis scales. Vertical lines represent LSD values at P = 0.05, asterisks indicate values significantly different from control values.

There was a large degree of variance in the flowering results for *R*. cv. Scintillation (reflected by a large LSD value), and no significant treatment differences were apparent (Figure 5.1B). In *R*. cv. Mrs T.H. Lowinsky, a shier flowering cultivar, a similar trend was apparent with the most flowers being induced on the control plants. Again, there was large variation within treatments and no statistically significant treatment effects were apparent (Figure 5.1C)

To assess if any of the treatments had a detrimental effect on plant quality, individual

plants were scored (0-4) for any incidence of leaf necrosis, with 0 = no damage on any leaves and 4 = significant marginal necrosis on more than 1 leaf. Of the three cultivars, *R.* cv. Scintillation was the most susceptible to leaf injury, but there were no significant effects due to treatment (Table 5.1). The large mature leaves of this cultivar suggest that it has been bred from species that have a degree of shade adaptation, and that leaf damage may occur after exposure to high light or high temperatures. Overall, there appeared to be slightly more leaf damage in *R.* cv. Hoppy when treatments were applied in summer compared to autumn. In particular, the application of phosphate (to both *R* cvs Hoppy and Mrs T.H. Lowinsky) resulted in more leaf necrosis.

Table 5.1. Mean scores for leaf necrosis (0 = none, 4 = severe) after the application of triple phosphate and phosphate at low pH.

Treatment and timing of application		*Rhododendron* cultivar		
		Hoppy	Scintillation	Mrs T.H. Lowinsky
Control		0.27	1.47	0.13
Summer	Low pH + P	0.20	0.73	0.00
	P	1.20	1.33	0.43
Autumn	Low pH + P	0.53	1.07	0.20
	P	0.80	1.47	0.20
LSD		0.677	0.646	0.330

Summary

- Results were very variable and no statistically significant treatment effects on flowering were apparent.
- Generally, the application of triple phosphate or of phosphate at low pH reduced flowering potential in *Rhododendron* cvs Hoppy, Scintillation and Mrs T.H. Lowinsky.

6. Identifying the signal that regulates floral initiation in *Rhododendron*

Introduction

Work conducted in Section 3 established that all *Rhododendron* cultivars tested require exposure to long days (short nights) before flowers are initiated. Many *Rhododendron* cultivars will never initiate flowers if maintained under short days (long nights). Day length (or more correctly night length) is detected in the leaves but floral initiation occurs at the apical meristem. Therefore, a signal that ultimately invokes flower initiation must be transported from the leaves to the apical meristem, probably in the phloem. The identity of this signal, the so-called (and elusive) 'florigen', is still not known after decades of research. In the case of inductive long days, the signal may transported from the leaves in sufficient quantities to trigger flower initiation in the apical meristem. Alternatively, the signal that is transported from leaves under non-inductive short days may repress flower initiation; under long days the amount of this repressive signal in the apical meristem may no longer be sufficient to inhibit flower initiation.

The plant hormone ABA has been cited as having anti-gibberellin type properties (Walton, 1980) and having a role in flower formation in some species (Meilan, 1997). ABA can promote flowering in several species including *Rubus fructicosus*, Fragaria spp, and *Pharbitis nil* (El-Antalby and Wareing, 1966). Enhancement of flowering in *Pharbitis nil* occurs only in inductive photoperiods (Lozhnikova *et al.*, 1981). Work on *Pharbitis nil* suggested that ABA acted on the generation and transport of the floral signal (Nakayama and Hashimoto, 1973; Maeda *et al*,. 2000; Takeno and Maeda, 1996; Kulikowska-Gulewska, 1998).

Conversely, ABA delays flowering in *Spinacia oleracea*, *Lolium* sp., carnation and petunia (Vince-Prue, 1985). In *Spinacia oleracea* (LDP) ABA concentrations were higher under LD compared to SD, implying that ABA is not an anti-florigen in this species

Exogenous gibberellins (GAs) can induce or promote flowering in many species and are particularly effective in long day plants. Although several GAs have florigenic activity, including GA_3, GA_4, GA_5, GA_7 and GA_{13}, the floral response to applications of these GAs is

often species specific. These differences in florigenic activity may reflect differences in uptake and transport, and conversion to more, or less, active GAs *in planta*. GA_5 can replace the long day requirement for flowering in *Lolium temulentum*. Furthermore, GA_5 was transported from the leaf to the apical meristem and the floral response was proportional to the amount of GA_5 reaching the apex (King *et al.*, 2001). Under inductive long days, the amount of GA_5 in the shoot apex doubled and was similar to the GA_5 concentration needed to promote flowering in shoot apices from plants maintained under non-inductive short days (King *et al.*, 2003). In addition, GA_6 and 16, 17 Di-hydroGA_5 also induced flowering in *Lolium* without affecting stem elongation (King *et al.*, 2003).

However in many woody perennials, including *Rhododendron*, GAs are antagonistic to the flowering process, *i.e*. they promote vegetative growth at the expense of floral initiation. For example, applications of GA_1, GA_3 and GA_4 to *Rhododendron* cv. Simsii increased vegetative growth and inhibited floral initiation under inductive long days (Bodson and Thomas, 1995). The inhibitory effects of GAs on flower initiation in *Rhododendron* can be overcome by using synthetic GA biosynthetic inhibitors (Scott, 1971). Sprays of paclobutrazol and uniconazol (cyclohexanetriones) in the second year from propagation in April and June increased flowering and reduced stem elongation in field-grown *Rhododendron* and *Kalmia* (Gent, 1995). The timing of application, in relation to the beginning of floral initiation, was critical since spraying in August failed to induce flowering. Another GA biosynthesis inhibitor, Daminozide, increased flowering fivefold in *R.* cv. Hummingbird whereas supra-optimal doses suppressed growth and prevented flower bud initiation (Ryan, 1972).

Photoperiodic induction of flowering in many species, including *Rhododendron,* occurs in the absence of any effects on shoot extension (see Section 3). Thus, in our attempts to try to identify the floral signal, it is important to ensure that the candidate signals affect floral initiation directly, rather than indirectly through effects on stem extension and resource partitioning.

The objective of this research was to try to identify the hormonal signals that regulate

floral initiation in *Rhododendron* cv. Hatsugiri. Applications of candidate GAs to plants under inductive long days were made to try to determine which GAs have antiflorigenic activity in *Rhododendron.* In addition, endogenous concentrations of candidate GAs in the leaves of plants under inductive long days and non-inductive short days were determined. Synthetic inhibitors were also used to block different steps of the GA biosynthesis pathway to help elucidate which GAs control floral initiation and vegetative growth in *Rhododendron*.

Materials and Methods

Plant material and experimental conditions

Two-year-old rooted cuttings of *R*. cv. Hatsugiri were potted into 90 cm^3 pots containing a medium of peat and Cambark 100 (80:20 v/v), 2g L^{-1} Osmocote® (controlled release fertiliser N:P:K, 15:9:11 + trace elements), 0.9 g L^{-1} $MgCO_3$, 0.15 g L^{-1} Fungaride and 0.6g L^{-1} Suscon® Green (10.34% chlorpyrifos w/w). Plants were irrigated daily and fertilised over the course of the experiment with a water soluble acidic fertiliser (N:P:K, 30:10:10 v/v/v + trace elements).

Plants were maintained in a growth cabinet under short days (9 h) at 20ºC for two weeks prior to the experiment. Photosynthetically active radiation at canopy level was 400-500 μmol m^{-2} s^{-1} supplied *via* 400W sodium luminares (Osram Vialox® NAV-T 400W Super). Plants were placed in a randomised blocking structure consisting of either three or four blocks of seven or ten plants per treatment. All apical buds were excised to ensure that all meristems were not induced. Sufficient out growth of axillary buds had occurred 14 d after pruning and stem extension was measured with digital callipers to obtain an initial mean length.

Hormone and inhibitor applications

GAs with florigenic activity in other species or important intermediates of active GA were chosen for applications. ABA, GA_1, GA_3, GA_5, GA_7, GA_{20}, paclobutrazol and prohexadione-Ca were dissolved in 70% ethanol/water (v/v). Various concentrations of hormones or inhibitors

were applied to 30 buds per plant in 2 μL aliquots, using a repetitive pipette. 'Control' buds received 70% ethanol/water (v/v) only. Treatments were re-applied after 7 d.

In a second experiment, the effects of other GAs (GA_6, GA_{32}, GA_{95} and 16, 17 Di-hydro GA_5) on stem extension and floral initiation were tested.

We also attempted to determine whether the effects of GA_5 and GA_7 on floral initiation were direct, or merely a consequence of their effects on stem extension. Two applications of 100 ng of GA_3, GA_5 and GA_7 were applied alone, or in combination with 500 ng of paclobutrazol to apical buds as described above. Applications of 70% ethanol/water (v/v) and 500 ng of paclobutrazol to plants served as 'controls'.

Microscopic determinations of flower induction

At 5 day intervals, 6 buds from each treatment were removed and dissected under a x 50 microscope to determine whether floral initiation had occurred (see Figure 6.1). Once two of these buds had initiated, a full analysis of stem length and apical meristem identity was undertaken. Lengths of stems from the start of the flush to the dorsal section of the buds were measured with digital callipers. The buds of these stems were then dissected and scored either 'Vegetative' or 'Floral'.

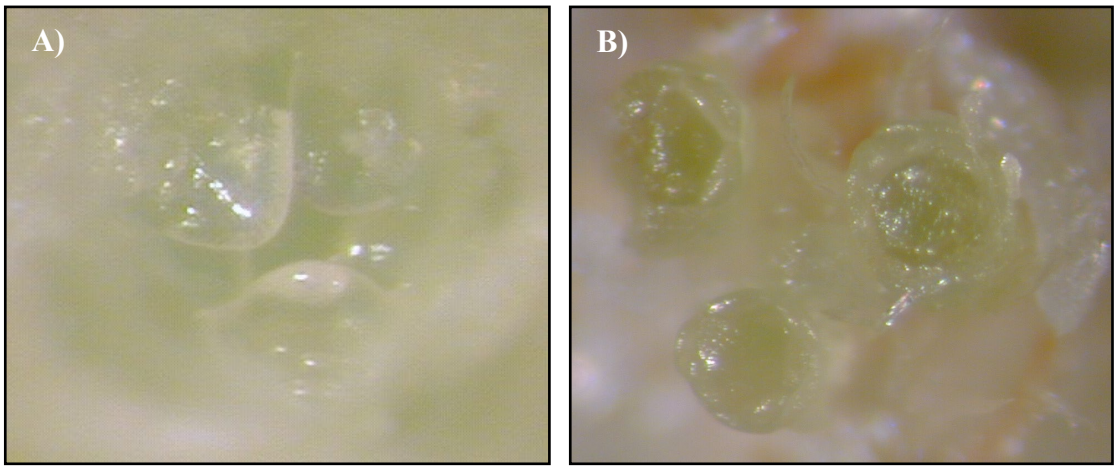


Figure 6.1. A) vegetative meristem and B) florally initiated meristem with bud-scales removed from *R.* cv. Hatsugiri, viewed under x50 magnification.

Extraction and purification of GAs

Apical bud samples were taken from plants under LD and SD at midday, frozen immediately in liquid nitrogen and stored at –80ºC until analysis. Samples were homogenised in cold (4ºC) 80% methanol/water (v/v) containing 20 mg L^{-1} butylated hydroxytoluene (5.0 mL g^{-1} FW) and stirred overnight at 4ºC. To each sample, 50 ng [1,2-^{3}H] GA_1, GA_3, GA_5, GA_8, GA_{19} and GA_{20} were added as internal standards to determine recovery. The samples were centrifuged at 1900 RPM for 20 min and the supernatant collected. Methanol was removed under reduced pressure on a rotary film evaporator (RFE) at 30ºC. An equal volume of pH 8.2 potassium phosphate (0.5 M) buffer was added to the aqueous residue and the pH adjusted to 8.0 with KOH (1 M). The samples were filtered through 10 mm of Celite® (diatomite-cellulose filter media). The samples were added to a column (15 x 50 mm) of insoluble polyvinylpolypyrrolidone (PVP) washed with methanol, thoroughly rinsed with distilled water, then pre-equilibrated with pH 8.2 buffer. After loading, the column was washed with a further 15 mL pH 8.2 buffer. Eluates were combined, adjusted to pH 2.5 with phosphoric acid and partitioned three times against an equal volume of ethyl acetate. The combined organic layer was partitioned three times against 1/5 sample volume 5% w/v sodium bicarbonate, acidified to pH 3.0 with HCl (2 M) and partitioned against ethyl acetate (3 x equal volume). The combined organic layer was washed three times with 10mL of distilled water adjusted to pH 3.0 with HCl. Thirty millilitres of distilled water was added and reduced to dryness using RFE. The extract was adjusted to pH 8.0 with KOH (1 M), added to a column of QAE Sephadex™ A-25 pre-equibrilated with sodium formate (0.5 M) and washed with formic acid (0.2 M) and water (pH 8.0). After loading, the column was washed with pH 8.0 water (30 mL) and GAs were eluted with 0.2 formic acid (40 mL) onto two pre-equilibrated C_{18} Sep-Pak® cartridges in series. After washing with 5 mL pH 3.0 water, the GAs were eluted with 15 mL of 80% methanol and evaporated to dryness.

GAs were purified further by reverse phase High Pressure Liquid Chromatography (HPLC) (Hewlett Packard series 1050). Samples were re-dissolved in 200 µL of 85% methanol

and injected with a blunt end syringe. Fractions 16-20, 22-25 and 29-35 were pooled and reduced to dryness using a centrifugal vacuum concentrator (CVC). The samples were methylated with excess ethereal diazomethane, left for a minimum of 30 minutes and ethereal diazomethane applied again. The samples were reduced to dryness, then 25 μL of N,O-bis(trimethyl)silylacetamide + Pyridine (Trisil BSA) was added under a N_2 stream to prepare the MeTMSi derivatives (methylestertrimethylsilylethers). Five μL of N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) was added to each sample before heating at 90ºC for ten minutes.

GA analysis

Samples were injected into the Gas Chromatograph (GC)(Hewlett Packard 5890 series II). The ion peaks produced on the linked mass spectrometer (MS) (VG TRIO 1) at each relevant retention time were recorded and quantified using single ion monitoring (SIM), (VG TRIO 1).

Results

Effects of ABA on stem extension and floral initiation

Application of ABA to apical meristems of *Rhododendron* cv. Hatsugiri inhibited shoot elongation at all concentrations tested (Figure 6.2A). Exogenous ABA either had no effect or significantly reduced floral initiation (Figure 6.2B).

Effects of GAs on stem extension and floral initiation

Stem extension was promoted by two applications of 10 and 100ng of GA_3, 100ng of GA_5, 100ng of GA_7 and 10ng of GA_{20} (Figure 6.3A). GA_1 did not affect stem extension at any concentration. Floral initiation was inhibited by two applications of 10 and 100 ng of GA_5 and 100ng of GA_7 (Figure 6.3B).

Stem extension was also promoted by GA_6, 16,17 Di-hydroGA_5 and 10 ng applications of GA_{32} and GA_{95} (Figure 6.4A). Floral initiation was reduced by most of the GAs and

concentrations tested, with the exception of 1ng applications of GA_6 and GA_{32} (Figure 6.4B).

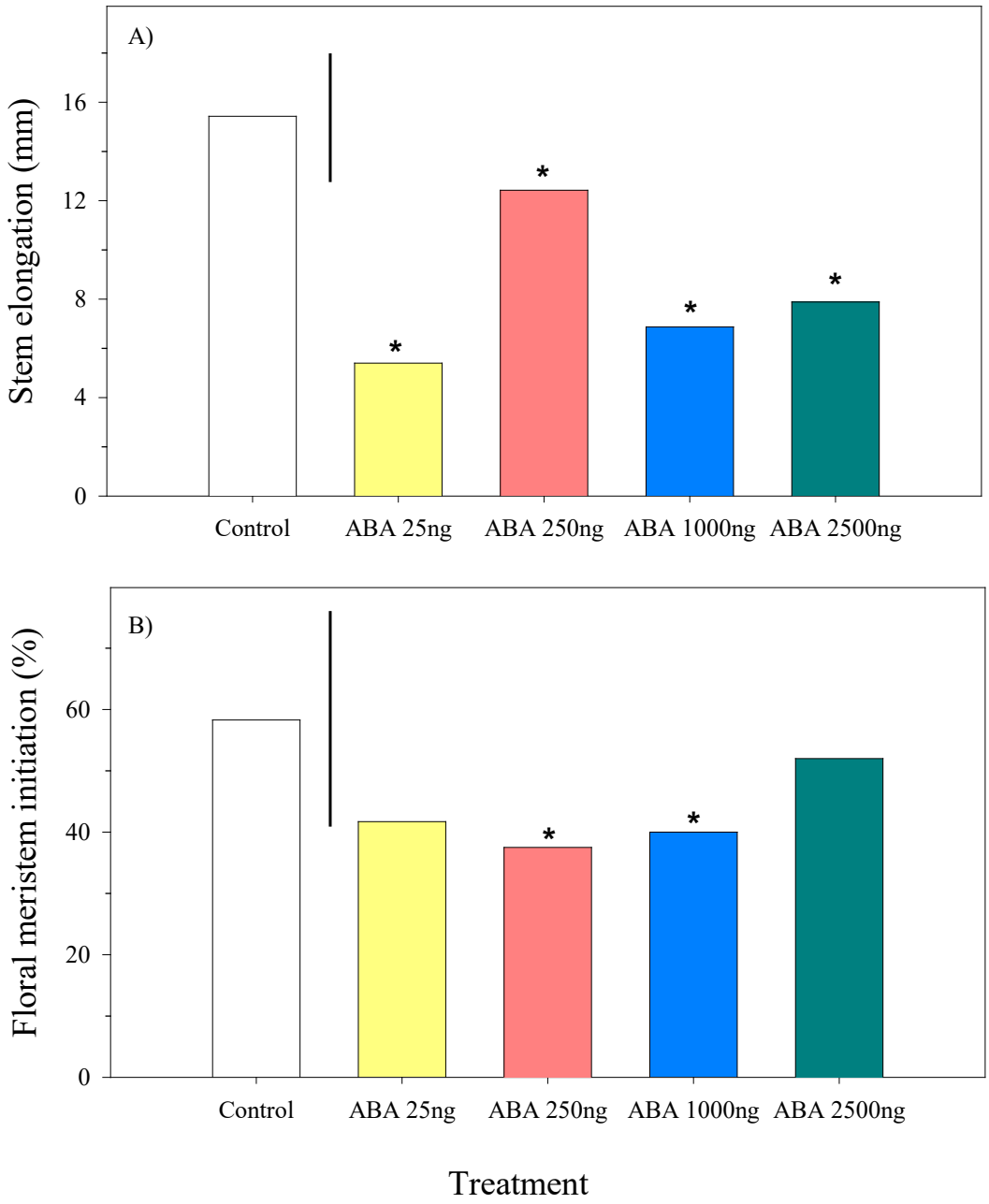


Figure 6.2. Effects of ABA application on A) stem elongation and B) floral initiation in *Rhododendron* cv. Hatsugiri. ABA was applied to apical buds, in a 2 mm^3 droplet of 70 % ethanol, of plants transferred to inductive long days at the beginning of the experiment. Data are means of 60 replicates per treatment, vertical lines represent LSD values at P = 0.05, asterisks indicate values that are significantly different from controls.

Effects of GA biosynthesis inhibitors on stem extension and floral initiation

Prohexadione-Ca, which blocks the latter stages of GA biosynthesis, did not affect stem extension and had only minimal effects on floral initiation (Figure 6.5A). The limited effectiveness of Prohexadione-Ca was confirmed in a repeat experiment (data not shown). In

contrast, paclobutrazol, which blocks steps relatively early in the GA biosynthetic pathway, inhibited stem extension at the higher concentrations and greatly increased floral initiation at all concentrations tested (Figure 6.5B).

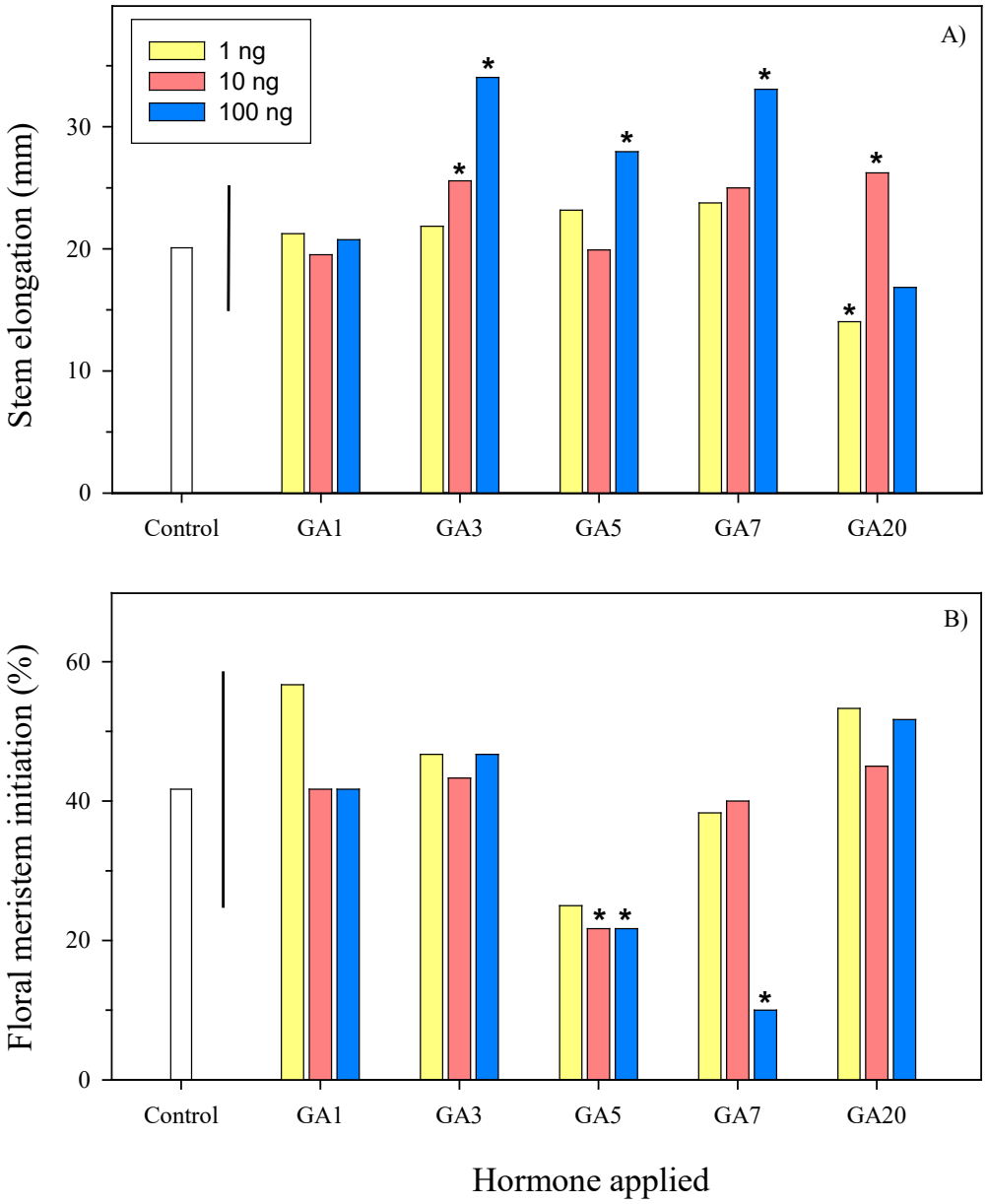


Figure 6.3. Effects of exogenous GA_1, GA_3, GA_5, GA_7 and GA_{20} on A) stem elongation and B) floral initiation in *Rhododendron* cv. Hatsugiri. Data are means of 60 replicates per treatment, vertical lines represent LSD values at P = 0.05, asterisks indicate values that are significantly different from controls.

Separating GA effects on stem extension and floral initiation

It was important to try to determine whether the inhibitory effects of GA_5 and GA_7 on floral initiation were an indirect consequence of their promotive effects on stem extension.

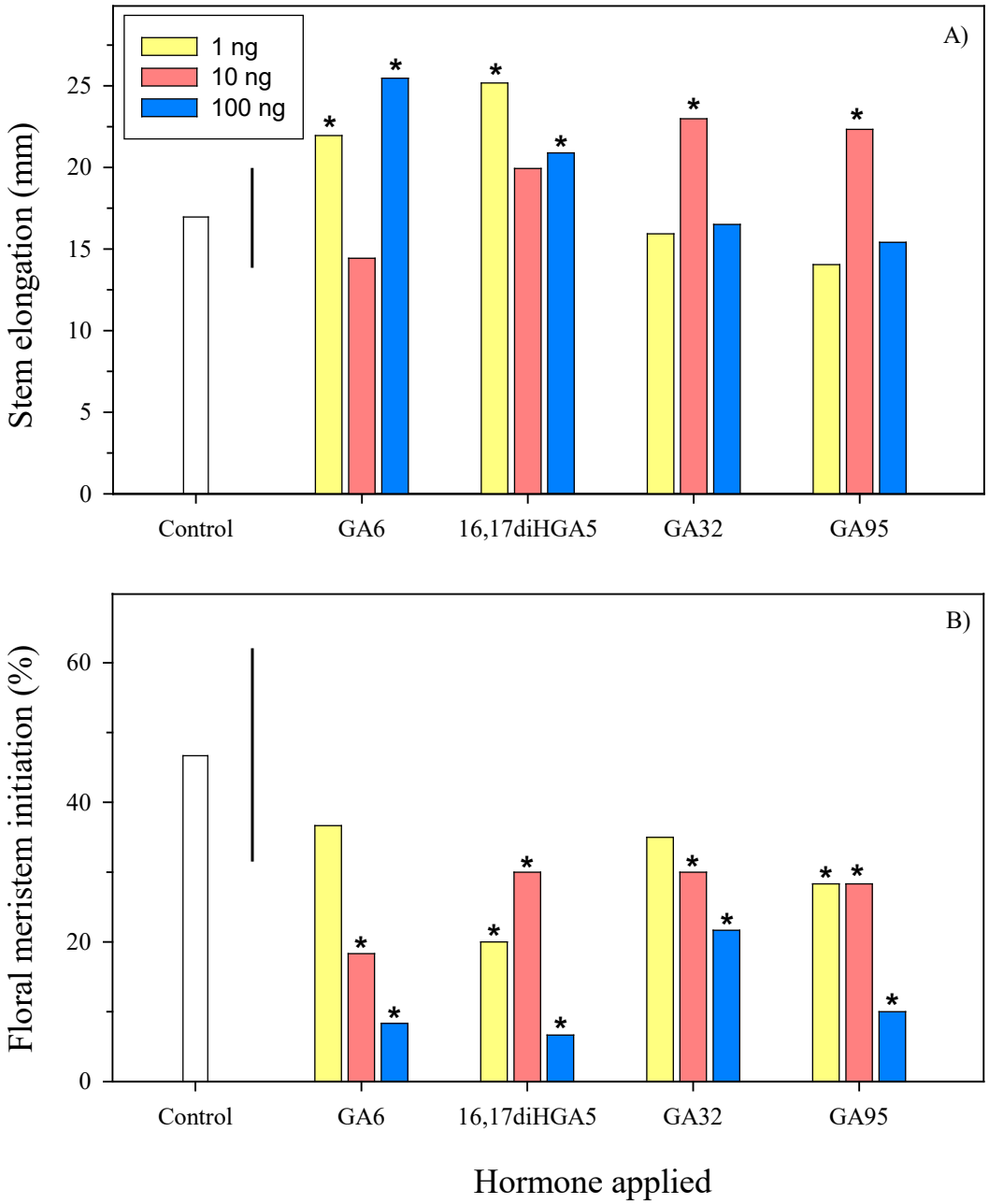


Figure 6.4. Effects of exogenous GA6, Di-hydro GA_5, GA_{32} and GA_{295} on A) stem elongation and B) floral initiation in *Rhododendron* cv. Hatsugiri. Data are means of 80 replicates per treatment, vertical lines represent LSD values at P = 0.05, asterisks indicate values that are significantly different from controls.

Paclobutrazol inhibited stem extension and promoted floral initiation compared to control values (Figure 6.6). GA_3 promoted stem extension slightly and greatly reduced floral initiation. When GA_3 was applied in combination with paclobutrazol, stem extension and floral initiation were similar to control values (Figure 6.6). GA_5 did not affect stem elongation and inhibited floral initiation when applied alone or in combination with paclobutrazol. GA_7 promoted stem

elongation and inhibited floral initiation, but when applied in combination with paclobutrazol, floral initiation remained inhibited despite restoration of stem elongation to control values (Figure 6.6).

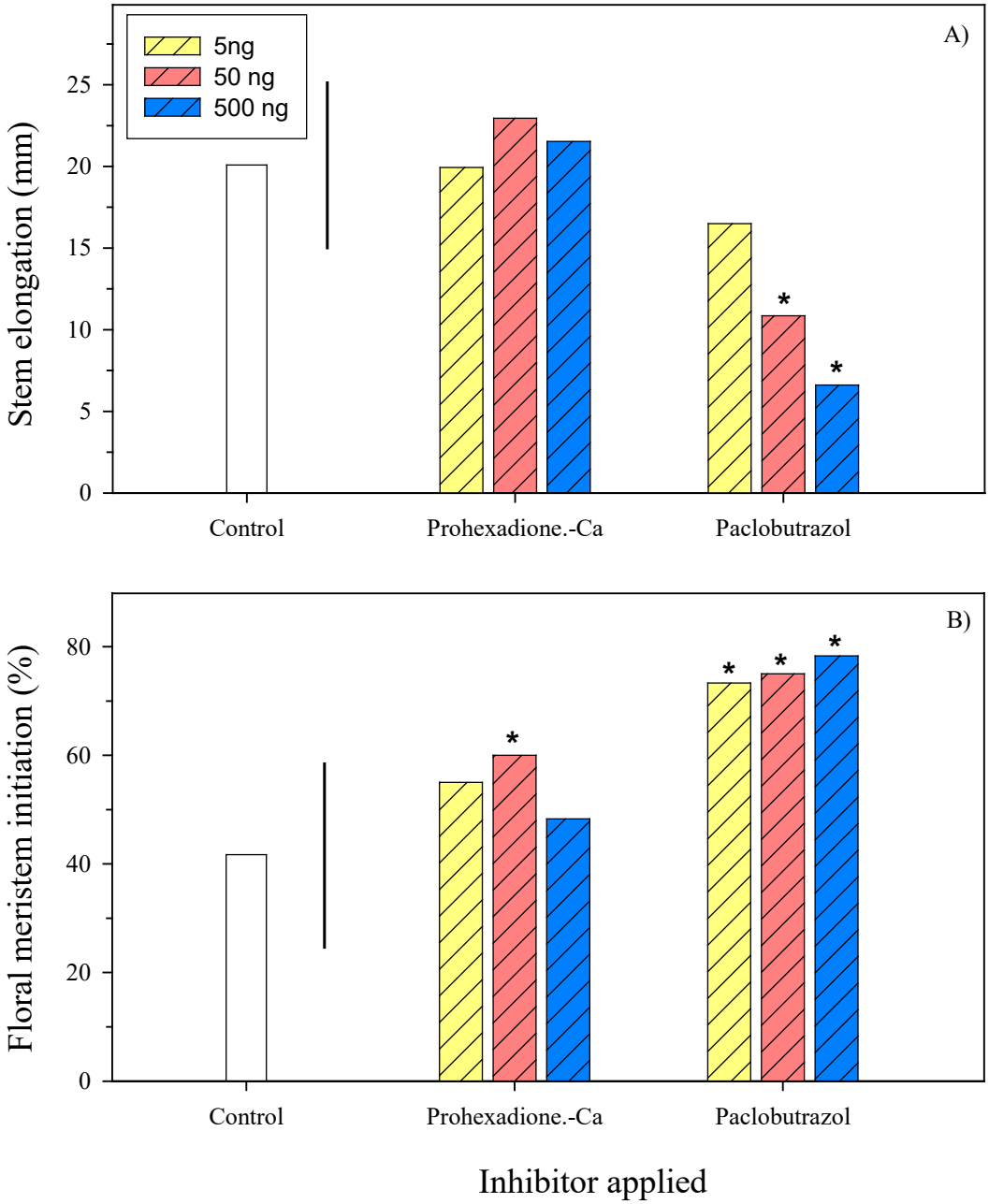


Figure 6.5. Effects of GA biosynthesis inhibitors Prohexadione-Ca and Paclobutrazol on A) stem elongation and B) floral initiation in *Rhododendron* cv. Hatsugiri. Data are means of 60 replicates per treatment, vertical lines represent LSD values at P = 0.05, asterisks indicate values that are significantly different from controls.

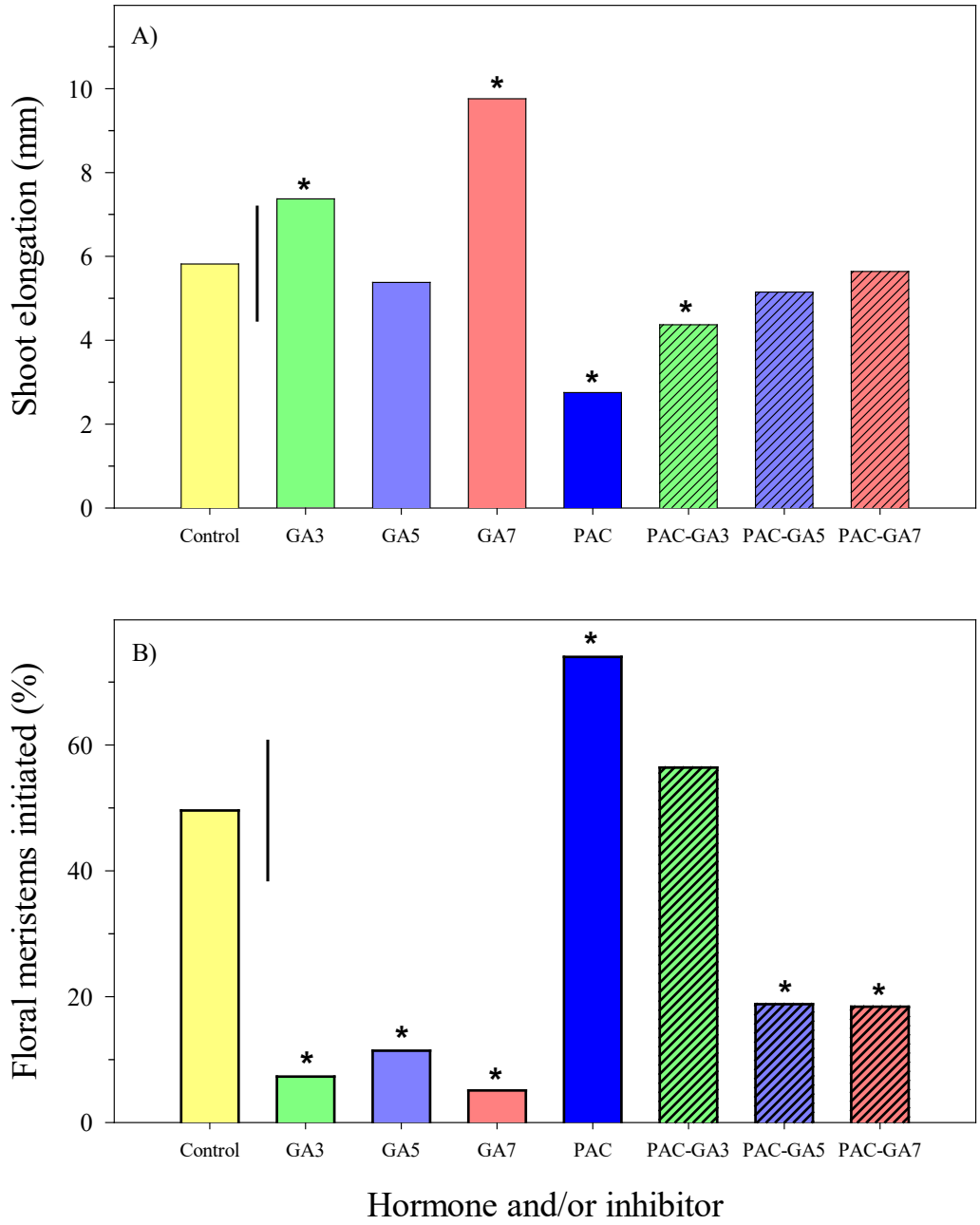


Figure 6.6. Effects of GA_3, GA_5 and GA_7, alone or in combination with Paclobutrazol, on A) stem elongation and B) floral initiation in *Rhododendron* cv. Hatsugiri. Data are means of 60 replicates per treatment, vertical lines represent LSD values at P = 0.05, asterisks indicate values that are significantly different from controls.

Endogenous GA concentrations

Concentrations of the GA precursor GA_{20}, potentially bioactive GA_1, GA_{29} and GA_8 (breakdown products of GA_{20} and GA_1, respectively) were detected in leaf tissue taken from *R.* cv. Hatsugiri maintained under short days, long days and 'super long days' (Figure 6.7 and Figure 6.8).

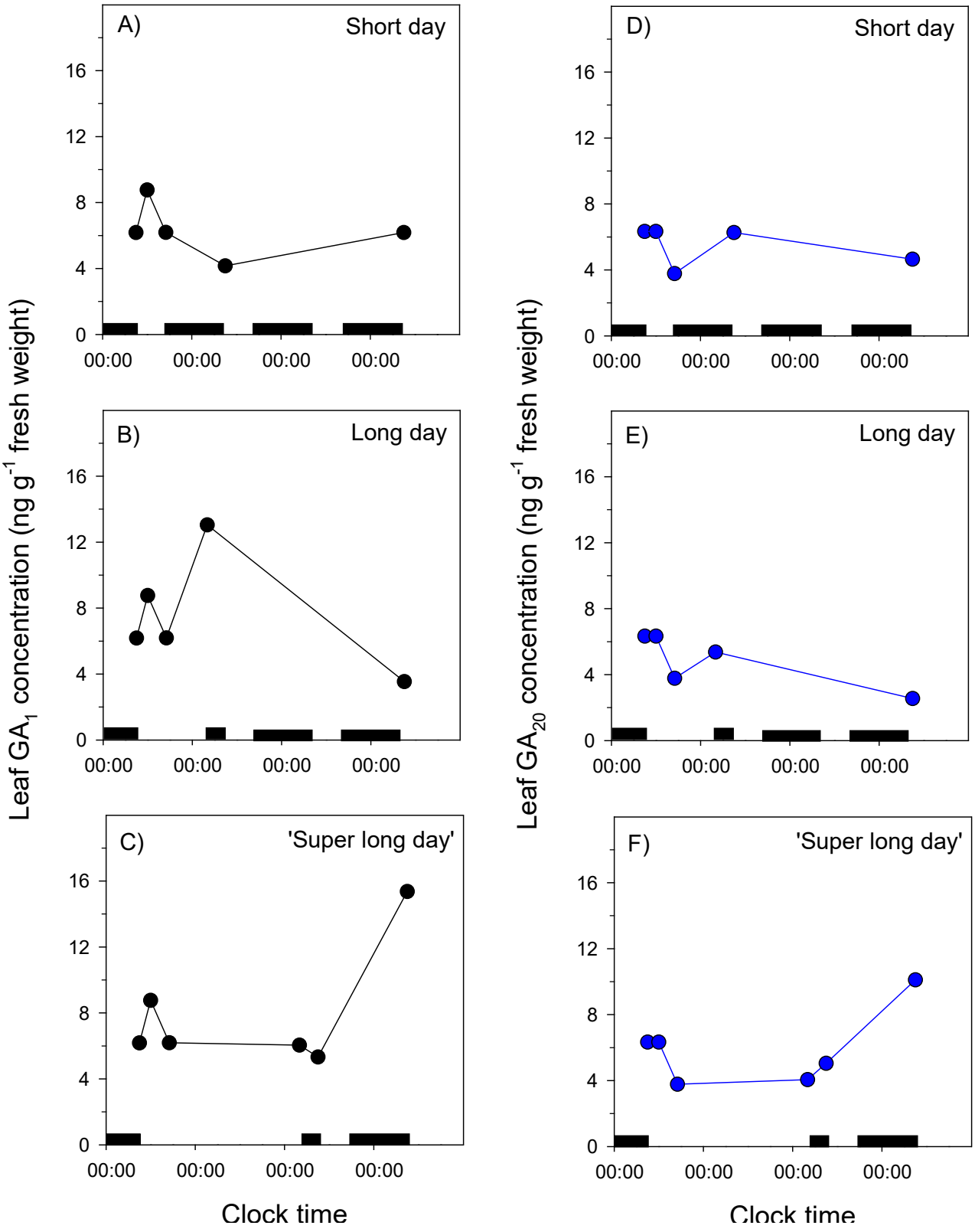


Figure 6.7. Concentration of GA_1 and GA_{20} in leaves of *R.* cv. Hatsugiri. At day 0 all plants were acclimatised under short-days (SD - 9 h) and samples of leaves taken at 09:00, 12:00 and 17:00. At 17:00 one population was left under SD (A & D), one placed in long days (LD - 19h) extensions (B & E) and another under 'super long-days' (SLD - 36 h) (C & F).

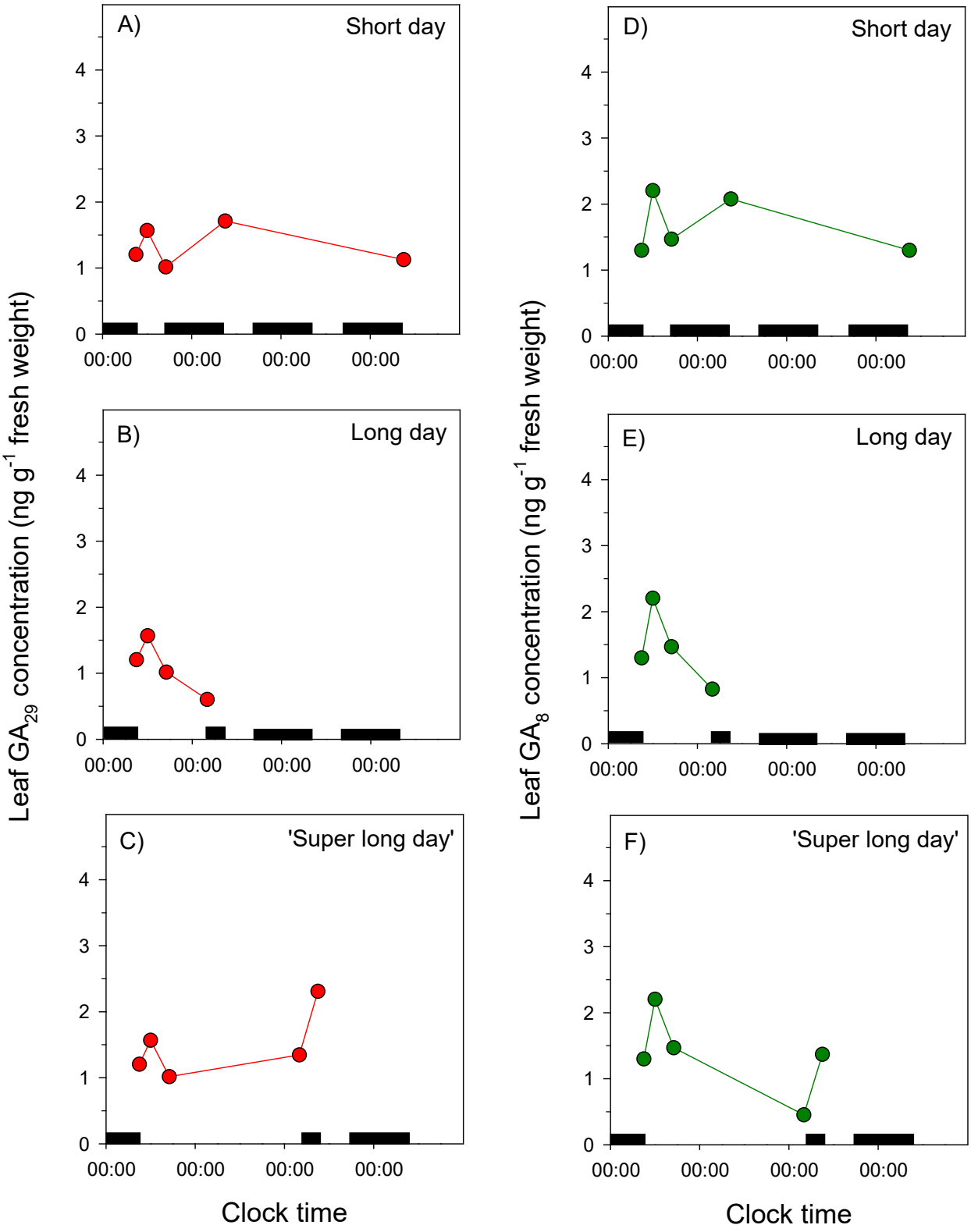


Figure 6.8. Concentration of GA_{29} and GA_8 in leaves of *R.* cv. Hatsugiri. At day 0 all plants were acclimatised under short-days (SD - 9 h) and samples of leaves taken at 09:00, 12:00 and 17:00. At 17:00 one population was left under SD (A & D), one placed in long days (LD - 19h) extensions (B & E) and another under 'super long-days' (SLD - 36 h) (C & F).

Concentrations of GA_1 and GA_{20} in leaves were increased after exposure to one 'super long day', compared to the other treatments (Figure 6.7). However, our earlier results suggested that neither applications of GA_1 nor GA_{20} affected floral initiation (see Figure 6.3B) so the significance of these results in relation to floral initiation is unclear. Concentrations of GA_5 in all samples were below the limits of detection.

Summary

- ABA applications reduced shoot elongation and did not affect floral meristem initiation in *Rhododendron* cv. Hatsugiri.
- Stem elongation was promoted by GA_3 in cv. Hatsugiri; GA_1 had no effect
- Paclobutrazol inhibited stem extension and promoted floral initiation; Prohexadione-Ca did not affect either process.
- GA_5, 16,17 di-hydroGA_5, GA_6, GA_7, GA_{32} and GA_{95} increased stem elongation and inhibited floral initiation.
- The inhibitory effects of GA_3 on floral initiation were indirect and a consequence of its promotive action on stem elongation, perhaps *via* effects on resource partitioning.
- GA_5 and GA_7 directly inhibited floral initiation in *Rhododendron* cv. Hatsugiri
- Endogenous GAs were detected in leaf tissue of *Rhododendron* cv. Hatsugiri maintained under short, long and 'super long' days.
- Concentrations of GA_5 and GA_6 in leaves from plants maintained under short, long and 'super long' days were below the limits of detection.

Conclusions

Regulated Deficit Irrigation (RDI) or partial root drying (PRD) treatments were used to impose moderate water deficits in *Rhododendron*, *Camellia* and *Magnolia*. At HRI-Efford, theta probe measurements confirmed that the PRD method limited water availability to the roots in the 'dry' side of the pot. At HRI-East Malling, the organic soil moisture content was reduced only by the RDI treatment, water availability was apparently not reduced by the PRD treatment. This disparity between sites may have arisen due to minor differences in how the PRD treatments were imposed. At HRI-Efford, tilting the plant pots may have increased the effectiveness of the PRD treatment (see Figure 2.1). The leaf water potentials of plants receiving RDI at HRI-East Malling were lower (more negative) than their well-watered counterparts; these data confirmed that plants subjected to RDI suffered mild foliar water deficits.

In 2001, RDI and PRD treatments reduced vegetative growth in the *Rhododendron* cultivars Hoppy, Scintillation, Jean Marie de Montague and Hatsugiri and, to a limited extent, in *Camellia japonicum*. PRD did not affect plant height in *Magnolia leobneri*. Not surprisingly, the largest reductions in plant height were achieved when the PRD treatment was applied during a period of rapid stem extension (Jul-Aug). Hormone analyses of root, stem and leaf tissues using state-of-the-art equipment is needed to help determine whether the PRD effects on shoot growth are mediated by changes in the production and/or transport of root- and/or shoot-sourced ABA. In 2002, although theta probe measurements indicated adequate soil drying, PRD treatments did not reduce plant height.

Generally, the numbers of floral buds per plant and flowers per bud were not increased in *Rhododendron* and *Camellia* by the RDI or PRD treatments. Indeed, the mild water deficits imposed by these treatments tended to reduce the numbers of open flowers and floral buds per plant compared to their well-watered counterparts. These effects were especially marked in *R.* cv. Jean Marie; imposing water deficits in Sep-Oct greatly reduced flower and bud numbers per plant (see Figure 2.5 B). However, in *Rhododendron*, the reductive effects of RDI and PRD on

plant growth resulted in more compact plants that generally appeared to be more uniform and of a higher quality (see Figures 2.4 & 2.5). The timing of the PRD treatments affected the extent of the inhibition of vegetative growth and, therefore, the overall aesthetic 'value' of the plants. There was limited evidence to suggest that RDI and PRD increased the number of flowers per floral bud in two-and-a-half year-old plants of *R*. cvs. Hoppy and Scintillation; further work is needed to confirm these results.

At the inception of this project, it was thought that ABA may promote flowering in *Rhododendron*, hence the investigation into the effect of transient water deficits on flowering. However, direct application of ABA to the *Rhododendron* cv. Hatsugiri reduced both stem elongation and flower initiation. Accordingly, the lack of success of RDI and PRD treatments in improving the flowering potential of *Rhododendron* is to be expected.

Imposing PRD throughout Jul-Oct significantly reduced flower and bud numbers in *Camellia*. Combined with the minimal effects of PRD on plant growth, imposing mild water deficits in *Camellia* tended to reduce overall plant quality (see Figure 2.6 A-D). The reasons why *Camellia* does not respond positively to mild water deficits are not known. The continued shoot growth under PRD treatments implies that *Camellia* is able to use the available water more effectively than the other species tested. The mechanisms underlying this response are not known. *Camellia* may require more severe water deficits to stimulate the production of ABA in the roots and shoots. Hormonal analysis of root, shoot and flower bud tissues of plants experiencing varying degrees of water stress is needed to help elucidate the responses of *Camellia* to mild water deficits.

In *Magnolia*, a PRD treatment imposed throughout Jul-Oct greatly increased the numbers of flowers produced in the following spring, although the number of buds per plant was not affected. Treatments imposed at other times were less effective and tended to reduce flower and bud numbers. Again, hormonal analysis of root, shoot and bud tissues are needed to help elucidate the responses of *Magnolia* to water deficits.

Reducing the R:FR ratio increased the numbers of flower buds per plant in *R*. cv. Hatsugiri. Greater numbers of flowers per bud, floral buds per branch and branches per plant were also achieved under a 'far red' environment but these differences were not statistically significant. Further work, including hormonal analyses of bud tissues is needed to clarify the effects of R:FR ratio on floral initiation in *Rhododendron*.

Most of the Rhododendron cvs. tested were obligate long days plants. Thus, floral initiation will only take place if plants are exposed to long days *i.e.* a day lengths greater than 9-10 h. If longer days are supplied (*e.g.* 16 h), floral initiation occurs more rapidly so fewer long days are needed. In plants maintained under very long days (18 h), a second flush of vegetative growth was triggered, at the expense of floral bud initiation. In the facultative long day plant *Rhododendron* cv. Hatsugiri, the imposition of a 'super long day' (36 h continuous light) stimulated floral initiation compared to plants maintained under short days. After exposure to a 'super long day', floral initiation occurred within 7 days. Whether other *Rhododendron* cvs respond to 'super long days' in a similar manner remains to be determined.

Restricting rooting volume by delaying potting-on decreased plant height. This effect may have been an indirect effect of resource availability, although attempts were made to ensure that water and nutrient availability to the roots were not limited by the delayed potting-on treatment. The synthesis of the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), is often increased in mechanically impeded roots; an increased delivery to the shoots in the xylem sap may have facilitated extra ethylene production that limited stem extension. Alternatively, the output of GA_3, a promoter of stem extension in *Rhododendron*, in xylem sap from the restricted root system may have been reduced. Interestingly, the numbers of flowers per floral bud was increased by restricting rooting volume. The mechanism underpinning this response is, at this time, unknown. Further work, including hormonal analyses of xylem sap, is needed to clarify the effects of restricting rooting volume on shoot extension floral initiation in *Rhododendron*.

Imposing a root nutrient stress by adding excess phosphate did not increase the flowering potential of *Rhododendron*, irrespective of when the stress was applied.

In contrast to the vast majority of other species, GA_1 had no effect on stem elongation in *Rhododendron* cv. Hatsugiri. GA_3 appeared to regulate stem extension in this cv. The promotive effects of Paclobutrazol on floral initiation may have been mediated through altered resource partitioning arising from its inhibitory effects on stem elongation. Applications of GA_5 and other closely related GAs such as 16, 17 Di-hydro GA_5 and GA_6 inhibited floral initiation without affecting stem elongation. Endogenous GA_5 was not detected in leaf extracts of *Rhododendron* cv. Hatsugiri plants maintained under short days, long days or 'super long days.' Further work is needed to determine whether GA_5 occurs naturally in *Rhododendron* and whether the anti-florigenic activity of GA_5 is direct or is due to conversion to other active GAs *e.g.* GA_6.

Technology Transfer

- Aspects of the work were presented at the nursery stock conference, Contact 2000 - Bromsgrove, January 2001.
- Parts of the project were presented at the HDC HRI-Efford Open Day in October 2001.
- Aspects of the project have been presented to HRI staff and to members of the Biological Sciences Department, Lancaster University, in accordance with PhD requirements.
- An HDC News article is currently being prepared

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