Environmental and cultural factors affecting the production of myoga (*Zingiber mioga* Roscoe) in Australia

by

Kristen Joy Stirling

B.Agr.Sc. (Hons.) University of Tasmania

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Declaration

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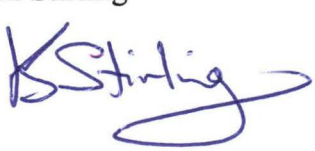


Publications

Refereed journal articles

Section 1:

Stirling, K.J., Clark, R.J., Brown, P.H., Wilson, S.J. (2002) Effect of photoperiod on flower bud initiation and development in myoga (*Zingiber mioga* Roscoe). *Scientia Horticulturae* 95: 261-268

Section 2:

Stirling, K.J., Clark, R.J., (submitted) Effect of low temperature on photoinhibition of photosynthesis in Japanese Ginger (*Zingiber mioga* Roscoe). *Australian Journal of Agricultural Research*

Conference publications

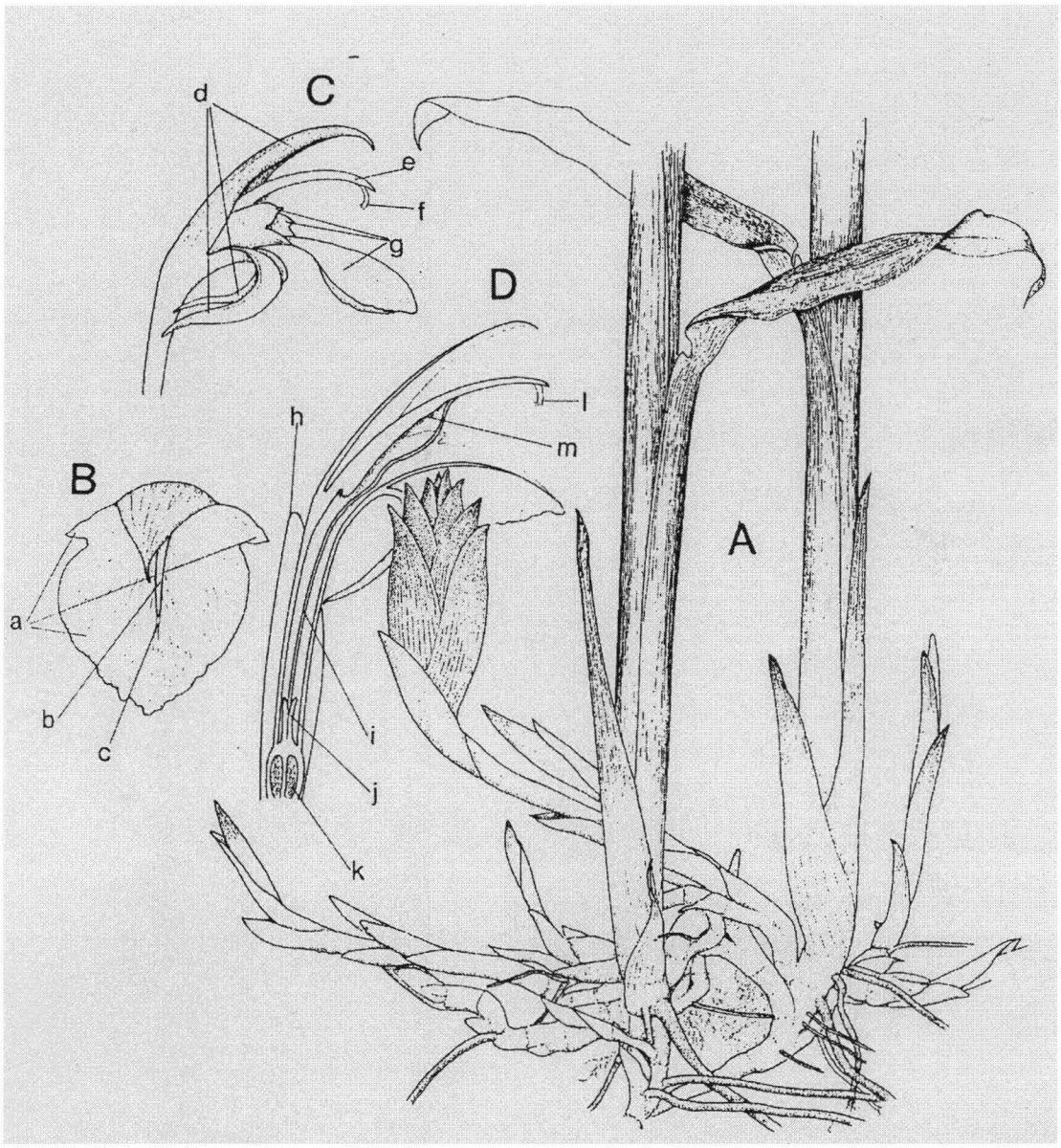
Section 1:

Stirling, K.J., Clark, R.J. (in press) The importance of photoperiod in cultivar and site selection for production of myoga (Zingiber mioga Roscoe). *Acta Horticulturae* – XXVIth International Horticultural Congress, Toronto, August 2002

Section 2:

Stirling, K.J., Clark, R.J. (in press) Investigation of photoinhibition in Japanese Ginger (*Zingiber Mioga* Roscoe). Conference Proceedings - Australian Society of Horticultural Science, Sydney, September 2002

Zingiber mioga Roscoe



Botanical drawing courtesy of Mr. Dennis Morris, Tasmanian Herbarium, Department of Museum and Art Gallery.

A Base of plant showing rhizomes, pseudostems and an inflorescence bud;
B Flower, front view
C Flower, side view
D Section through flower

Abstract

Myoga (*Zingiber mioga* Roscoe) is a new vegetable crop in Australia, cultivated for its edible flower buds. Originating from Japan, the crop was introduced into Australia with the expectation that it could be produced over the summer months, with flower buds then exported to Japan, where they retail at a high out-of-season price. Industry has identified a number of challenges to the successful commercialisation of this crop in Australia. The majority of these relate to cultural aspects of myoga production and manipulation of the growing environment to control crop performance, in particular, extension of harvest season. A lack of knowledge of the effect of environmental factors on the production of myoga flower buds has hampered initial efforts to successfully cultivate this crop in the Australia.

Photoperiod was identified as a potentially important environmental factor affecting the production of myoga flower buds. A series of trials conducted within controlled environment cabinets determined that myoga had dual photoperiod requirements for successful flower bud production, with flower bud initiation having a quantitative short day requirement while flower bud development had a qualitative long day requirement. Differences in the critical daylength required for successful flower bud development in cultivar S and I myoga plants was identified as the reason why cultivar I plants senesced prematurely when field cultivated in Southern Australia. The critical daylength for flower bud development in cultivar S plants was determined to be approximately 13 hours, while cultivar I plants required a daylength closer to 14 hours. Low night temperatures were observed to interact with photoperiod, resulting in successful flower bud development in daylength conditions that at higher night temperatures would have been too short. From the results of these trials, photoperiod was deemed to be a crucial determinant of the location of future production sites and the timing of production seasons.

Environmental factors affecting general plant growth and development were also deemed important areas of research, in particular the tolerance of myoga plants to

strong light conditions. There have been reports of variations in the shade requirement of myoga plants grown in different climatic regions. This led to the hypothesis that these plants were susceptible to cold induced photoinhibition of photosynthesis. This was confirmed during a number of trials which used chlorophyll fluorescence techniques to investigate the function of photosynthetic systems within myoga plants when placed into stressful conditions. Exposure of myoga to light intensities greater than 750 μmol $m^{-2}s^{-1}$ resulted in the engagement of photoprotective mechanisms to prevent photodamage occurring. Sensitivity to low temperatures meant that these plants became photoinhibited at far lower light intensities and the engagement of photoprotective mechanisms was sustained, when plants were exposed to low temperatures in conjunction with light. The acclimation of these plants to low temperature and the successful engagement of photoprotective systems within the photosystem indicate that myoga can cope with strong light intensities over a short-term period. However exposure to strong light intensities at warm temperatures or moderate light intensities at low temperatures, for an extended period of time, is likely to result in permanent photodamage occurring.

Additional cultural factors affecting commercial production of myoga flower buds were identified during the first two years of cultivation. At Albion Park, NSW it was determined that myoga plantings remaining in the ground for longer than one year did not require artificial chilling, and with correct management of vegetation could produce higher flower bud yields than first year plants. Production trials conducted in Rockhampton, QLD investigated the effect of daylength and temperature on flower bud production, and confirmed that myoga was well suited to cultivation in a sub-tropical environment provided due consideration was given to photoperiod and the potential need for artificial chilling.

Recommendations based on the above research findings have already been adopted into commercial production protocols and as such many of the challenges to myoga production in Australia have now been successfully resolved.

Acknowledgements

I would like to thank the many people who made this project possible;

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SECTION 3: INVESTIGATION OF CULTURAL FACTORS AFFECTING COMMERCIAL CULTIVATION OF MYOGA

GENERAL INTRODUCTION

Botanical description and cultivation of myoga

Japanese ginger (*Zingiber mioga* Roscoe) commonly called myoga is the most cold tolerant member of the ginger family. Commonly classified as sterile, myoga is a pentaploid with some reports suggesting that seed is unknown (Palmer, 1984). For this reason, the plant is propagated vegetatively by placing rhizome pieces in the ground at the end of winter. A perennial, rhizomatous plant it displays a distinct periodicity of growth. Under natural conditions, myoga undergoes a period of dormancy or rest over winter months. Growth commences as temperature increases in spring. The rhizome units below the surface bear reduced scale leaves and grow horizontally for a distance before turning to become erect as foliage bearing shoots (Criley, 1985). The foliage bearing shoots grow to approximately 1.5 m in height, and are referred to as pseudostems since they consist of a stem made up of 5 or 6 striate sheaths (Pursglove, 1972). The inflorescence is borne on a separate cladogenous shoot arising from the basal, underground section of pseudostems, in mid summer to early autumn. The bracts enveloping the unopened flowers are thick and fleshy, with a distinctive flavour (Palmer, 1984). The flowers which emerge from the top of the flower bud are zygomorphic and consist of two, large (4 cm long) pale petals with a prominent, yellow style and stigma. Rhizome development continues during the later stages of flower bud development in preparation for the following season of growth. Foliage senescence and abscission is observed in late autumn and rhizomes then enter a dormant or rest period (Gracie *et al*, 2000).

Production in Japan

Myoga grows wild thoughout the Japanese islands of Honshu, Shikoku and Nyshu, however commercial production is also widespread. Unlike the more common species of its taxonomic group, myoga is cultivated for its sterile flower buds and spring

shoots with the thick rhizomatous rootstock, from which it is grown, being considered inedible. The myoga flower buds, sometimes called 'hana myoga' have a distinctive smell, colour and flavour. They are used as a condiment in traditional Japanese soups, salads, fish dishes or prepared individually with a variety of ingredients to enhance the particular myoga flavour.

In Japan the main commercial production areas for flower buds are the Gumma and Kanto prefectures. The annual production of field grown myoga marketed through the wholesale market system in Japan is estimated to be 4,800 tonnes (Clark, 2001). Japanese myoga is produced using traditional and until recently, relatively unsophisticated methods. The availability of field grown myoga is seasonal with the majority of production occurring during June, July and August. Glasshouse production during the winter months has now commenced in the Kochi region. This out-of-season supply of myoga attracts a premium price in the Japanese market. Small semi-commercial production of myoga has been reported in Argentina, Hawaii and New Zealand, however there has been no significant commercial production outside of Japan (Clark, 2001).

Production in the Southern Hemisphere and research impetus

Myoga was initially imported to Tasmania with the expectation that it could be grown in the summer months of the Southern Hemisphere and then exported to Japan to supply out-of-season myoga, which retails at a high price. The climatic conditions of Tasmania were thought to be ideal for the production of myoga since the temperatures over winter are cool enough to initiate flower bud formation while summers are warm enough to promote plant growth (Clark, 2001). Furthermore by cultivating myoga in the field, net returns to producers would be maximised when supplying Japanese markets in the high-demand, low supply season of December to March.

Until recently very little research had been conducted on myoga. This has been due in part, to the ease with which myoga is cultivated in the field in Japan. With the establishment of glasshouse cultivated myoga, a more critical examination of the environmental factors controlling flower bud production became necessary. Similarly the desire to produce myoga in the Southern Hemisphere has meant that traditional methods of cultivating myoga can no longer be relied upon and detailed scientific investigation of the effect of environmental and cultural factors on the production of flower buds is required.

Outline of thesis structure

The general introduction to this project includes information on the botany and cultivation of myoga, its production in the Northern and Southern Hemispheres and the reasons why this research was undertaken. The main areas of research conducted during this project fall into three separate sections of photoperiod, low-temperature induced photoinhibition and cultural factors affecting commercial cultivation of myoga. The first two sections are each comprised of a review of relevant literature, details of materials and methods used in that section and reports detailing experimental work conducted which is pertinent to that topic. The third section does not contain a literature review, however each trial report commences with a comprehensive introduction.

The first section contains all research conducted on the investigation of the photoperiodic nature of myoga. Experimental work undertaken in this area consists of the establishment of the photoperiodic nature of myoga and determination of the critical daylength for flowering. During the identification of the photoperiod sensitive phases of myoga, the differing photoperiodic requirements for flower bud initiation and development were also established. The interaction of photoperiod with temperature resulting in a modification of the flowering responses in myoga was also investigated. In addition, detailed dissections of developing flower primordia enabled the development of myoga flower buds to be recorded and described.

The second section reports on research which investigated the effect of low-temperature induced photoinhibition on the photosynthetic ability of myoga. The review of current literature provides a comprehensive introduction to research conducted in this area while the experiments contained in this section significantly add to that body of work. Preliminary experiments established the light intensity and temperature at which myoga becomes photoinhibited. Subsequent work investigated the effect of preceding night temperatures on the promotion of photoinhibition of photosynthesis in myoga and whether the ensuing photoinhibition could be reversed. In addition, acclimation to low temperatures was investigated as a possible tool in the prevention of photodamage occurring in myoga plants in response to photoinhibiting conditions.

The final section contains research conducted on a number of cultural factors affecting the cultivation of myoga. In Albion Park, NSW four trials were designed to assess: whether rhizome material remaining in the ground between first and second growth seasons required artificial chilling for flower bud initiation, if myoga crops needed to be propagated from whole rhizome pieces, and if vegetation density in two year old crops could be effectively managed so that higher yields could be achieved. In Rockhampton, QLD a production trial investigating the effect of temperature and daylength on flower bud production was established. Treatment factors were applied in a natural, field environment so that results could be quickly integrated into industry practice.

Each section finishes with a conclusion that highlights and discusses the main results of each investigative trial. The implications of these results in developing new industry protocols are also reviewed. The thesis finishes with a general conclusion and recommendations for the future development of this crop in Australia.

GENERAL MATERIALS AND METHODS

LOCATION

Experimental work was conducted in one of three locations within Australia (figure 1). Trials which investigated the photoperiodic nature and shade tolerance of myoga plants (sections 1 & 2) were conducted at the University of Tasmania, Hobart (42^0 South, 147^0 East). Four field trials were conducted at Albion Park, NSW (34^0 South, 150^0 East) to investigate the effect of cultural factors on flower bud production and a field trial was established at Rockhampton, QLD (23^0 South, 150^0 East) to determine the effect of temperature and daylength on flower bud production. The amount of daylight hours throughout the year at each location is given in figure 2 and minimum and maximum temperatures for each location are given in figures 3 and 4.

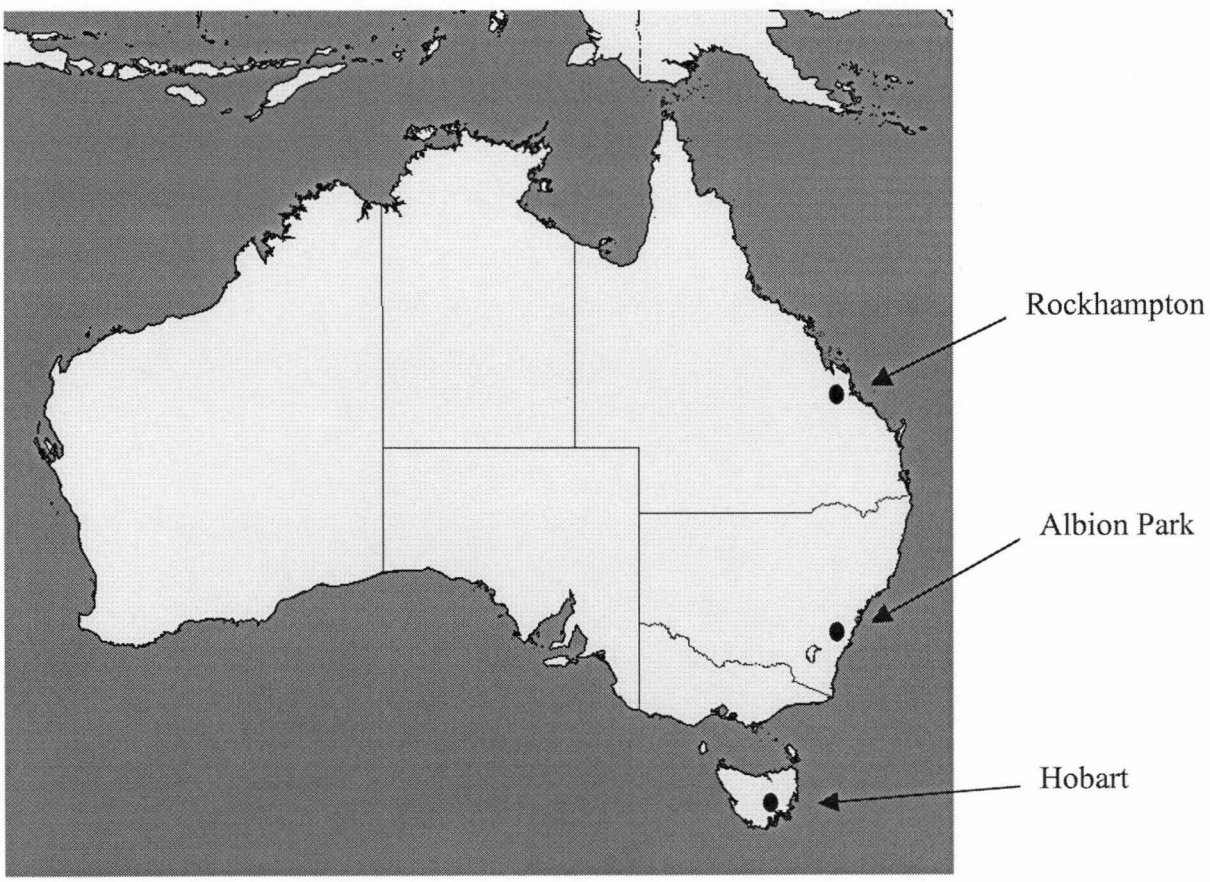


Figure 1: Geographical location of experimental sites within Australia.

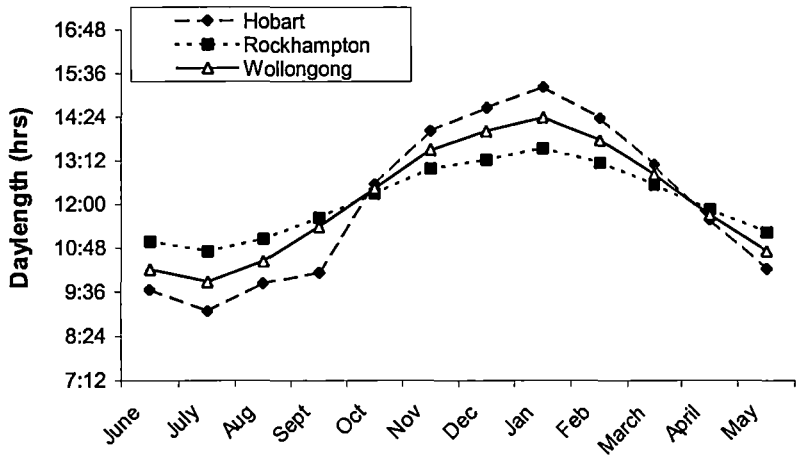


Figure 2: Amount of daylight hours throughout the year at Hobart, Rockhampton and Albion Park (1939-2001).

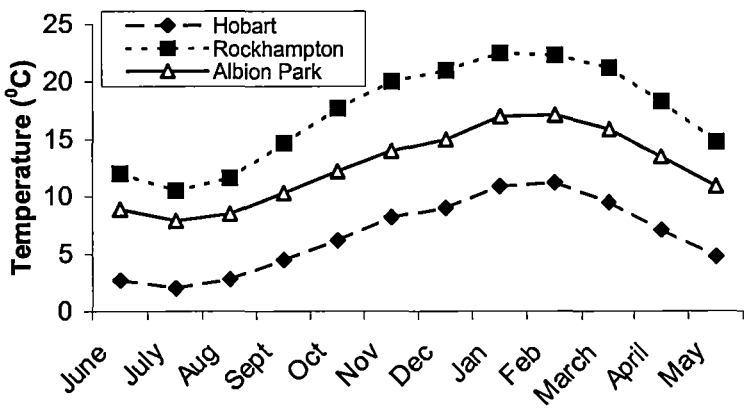


Figure 3: Average minimum temperatures throughout the year at Hobart, Rockhampton and Albion Park (1939-2001).

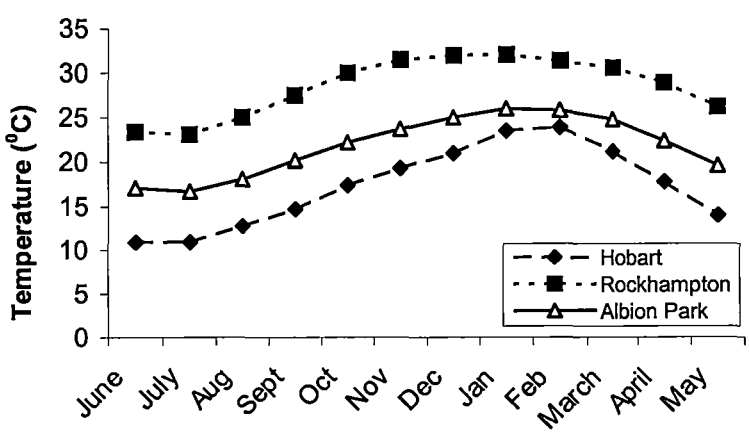


Figure 4: Average maximum temperatures throughout the year at Hobart, Rockhampton and Albion Park (1939-2001).

ENVIRONMENTAL MONITORING

Air temperature was monitored using temperature data loggers (Tinytag *Ultra*, Hastings data loggers) with a temperature range of –30 to 50^0C. Loggers were shielded from direct contact with radiation by the use of plastic covers left open at the base for ventilation. Temperature values were downloaded onto a PC using Gemini Datalogger manager software. Light intensity was measured using a line quantum sensor (LI-191SA, Li-Cor) attached to a 4-channel data logger (LI-1400, Li-Cor) which recorded light intensity every 30 seconds. Data was downloaded onto a PC using LI-COR software.

STATISTICAL ANALYSIS

Experimental layouts for trials conducted in the first two sections of this thesis were of a completely randomised design. Experiments in section 3 utilised both completely randomised and split-plot designs. As with all experiments utilizing controlled environments true replication of some treatments was, at times, not possible (Hurlbert, 1984). Consequently, data in section one was not subjected to analysis of variance and is presented with standard error of sample means. Experiments in sections 2 and 3 contained multiple treatments some of which could be properly replicated and others not. These trials were subjected to analysis of variance, but due caution should be applied to "significant" results for pseudoreplicated treatments. Analysis of variance was carried out using SAS statistical software, version 8 (SAS Institute Inc. 1989). The significance between treatments was assessed at the 5% level using Fisher's LSD (Least Significant Difference) where significant ($P<0.05$) (Steel and Torrie, 1980). Further details of experimental design, treatment of data and statistical analysis pertaining to individual experiments are given in the relevant experimental sections.

SECTION 1: PHOTOPERIODISM - *INTRODUCTION*

Myoga as a new vegetable crop in Australia, is currently undergoing a number of evaluation trials to determine ideal locations and environments for commercial production. Production in Australia coincides with the production of glasshouse-cultivated myoga in Japan, but utilises the favourable climatic conditions of the Southern Hemisphere summer for field cultivation. This allows myoga produced in Australia to compete favourably with the expensive glasshouse-cultivated myoga in Japan. The current unpredictability of flower bud quantity and quality has restricted industry establishment. Greater understanding of the factors regulating flowering in myoga is required to develop crop management strategies for improved yield consistency.

Previous studies in Australia and New Zealand have focused on the effects of light intensity and temperature on flowering (Gracie et al., *in press*). Temperature and shading have been shown to influence the growth and development of myoga, and have been taken into consideration when selecting potential crop locations. A number of potential locations for production of myoga have been identified on the eastern seaboard of Australia. The wide geographical range of these locations indicated that daylength might play an important role in the commercial cultivation of this plant. Photoperiod, a major environmental variable affecting flowering in many plants, has not been investigated in relation to myoga production in Australia or New Zealand.

Myoga (*Zingiber mioga* Roscoe) is a rhizomatous perennial plant endemic to Japan and is cultivated primarily for its edible flower buds (Gracie et al., 2000). The flowers are sterile and the plant is propagated from geophytic units, which are comprised of rhizome segments and tuber-like roots. Field production in Japan is seasonal, with flower buds produced during the summer months. Forced production in glasshouses and heated poly-tunnels occurs over the winter months to provide high quality buds which attract a premium price.

Myoga exhibits a distinct periodicity of growth. Under natural conditions, growth commences after a period of dormancy or rest over winter months. Pseudostems (vegetative shoots) grow from the rhizomes in spring, followed by a flush of flower buds, which develop from the terminal meristem of further branched rhizomes, in mid summer to early autumn. Rhizome and tuberous root development continues during the later stages of flower bud development, followed by pseudostem senescence and abscission in late autumn (Gracie et al., 2000). This distinct periodicity suggests that the growth and development of myoga, like that of many geophytic plants may be affected by temperature and photoperiod (Gregory, 1965: Le Nard and De Hertogh, 1993).

SECTION 1: PHOTOPERIODISM – *LITERATURE REVIEW*

Photoperiodism

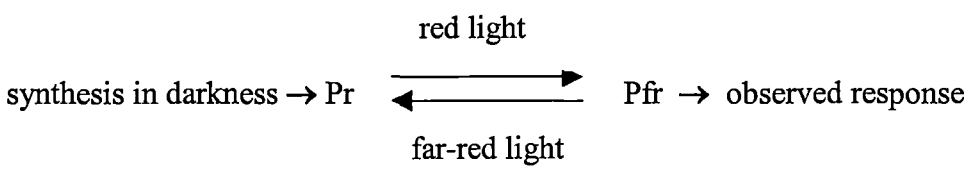
Photoperiodism can be defined as plant responses to the length of the day that enable adaptation to seasonal changes in environment (Thomas & Vince-Prue, 1997). Plants use photoperiodic control of flowering and other vegetative processes to constrain a life cycle to fit a seasonal climate, thus avoiding growth during climatic extremes of winter cold and summer drought. A photoperiodic response can enable a plant to occupy a favourable ecological niche in time and space, while synchronisation of flowering through photoperiodic sensitivity can increase the chances of outbreeding and thus genetic recombination (Rees, 1987).

Photoperiodic responses of plants to environmental factors are both complex and diverse. Interactions occur between plant age and stage of development, with growing temperatures and several aspects of the light climate, on flower initiation and on subsequent development to anthesis. There are qualitative and quantitative responses and several photoperiodic response types, which may be modified by plant morphology (Rees, 1987). For these reasons, it has often been difficult to gain complete information on flowering responses for a given species, or a group of species. Progress in the understanding of plant photoperiodic behaviour, has been made as a result of extensive research conducted on selected crops of commercial importance. However, information on the environmental regulation of photoperiodism still remains limited to less than 2% of all flowering plants.

It is generally accepted that the main site for photoperiodic perception is in the leaves of plants, although the precise location is not known. The leaf measures the length of day or duration of light and/or darkness in each 24 h period. In order to do this, it requires a clock to measure time and a photoreceptor to discriminate between light and darkness. For many years it was assumed that time was measured by a kind of hourglass consisting of a series of catenary steps, which must proceed to completion in order to measure the durations of light and darkness (Thomas & Vince-Prue, 1997). It is now generally believed that photoperiodic time keeping

depends on a circadian oscillator of the kind that underlies many endogenous rhythmic phenomena (Bünning, 1960). The difference between an 'hourglass' clock and a 'circadian' clock is that a circadian clock restarts night length measurement in prolonged darkness. It is evident, however, that 'hourglass' behaviour can also be generated by a circadian clock under some conditions (Vaz Nunes et al., 1991) and there is now a considerable body of experimental evidence for the involvement of a circadian oscillator in photoperiodic timekeeping (Thomas and Vince-Prue, 1997).

The detection of light for photoperiodic responses in Angiosperms is primarily accomplished by members of the phytochrome family of photo-receptors. Phytochrome exists in two forms that are converted from one to the other in the following reaction:



Phytochrome is synthesised in darkness as Pr, the biologically inactive form. When it absorbs red light it is converted to Pfr. Pfr, the active, far red absorbing form results in the observed physiological response. In the reverse reaction Pfr is converted back to Pr when it absorbs light. (Thomas and Vince-Prue, 1997).

Not only do the leaves perceive daylength but they also generate and export a stimulus, which is capable of evoking the observed photoperiodic response at the receptive site. The differentiation of a vegetative shoot apex into floral primordia has long thought to be under the control of a specific floral hormone, termed by Chailakhyan (1936) as florigen. Substances that inhibit flowering termed antiflorigens have also been proposed, with the appropriate daylength leading to the removal of this substance rather than the synthesis of a floral hormone. Extraction and identification of these flower-inducing substances and floral inhibitors has met with limited success.

Critical Daylength

The value of the critical daylength (CDL) which marks the transition between vegetative growth and flowering in obligate photoperiodic plants of both SD and LD types, varies considerably between species and cultivars. No literature is currently available on the CDL for flowering in myoga, largely due to the photoperiodic response of myoga for flowering under Japanese conditions being classified as quantitative. When a particular daylength is essential for flowering, plants are classified as having an obligate or qualitative response. Where a particular daylength accelerates but is not essential for flowering, plants are classified as having a facultative or quantitative response (Thomas and Vince-Prue, 1997). Oga and Ota (1968) and Maeda (1994) both report that optimum production of flower buds occurs under longer daylengths. These long day conditions are reported as being between 15 and 18 hours (Oga and Ota, 1968) and between 12 and 16 hours (Maeda, 1994). At daylengths shorter than these (10 hrs) flower bud production was reported to be greatly reduced but did still occur.

Daylength Response Types

With respect to flowering, the responses to daylength appear to be of three basic types, with some modifications. These are short-day plants (SDP), which only flower, or flower most rapidly, with fewer than a certain number of hours of light in each 24 h period, long-day plants (LDP), which only flower, or flower most rapidly, with more than a certain number of hours of light in each 24 h period and day-neutral plants (DNP), which flower at the same time irrespective of the photoperiodic conditions (Thomas and Vince-Prue, 1997). These classifications usually refer to the daylength required for flower initiation with subsequent floral development viewed as the inescapable consequence of initiation. Most typically, the same daylengths that lead to floral initiation also lead to an increased rate of floral development. But plants are known, that have a photoperiodic requirement for initiation but are day-neutral for floral development or the opposite. Some plants even display contradictory requirements for initiation and development. A strawberry hybrid is reportedly SD for initiation and LD for development and *Callistephus chinensis* is LD for initiation and SD for floral development (Vince-

Prue, 1975). Both Maeda (1994) and Nakamura (1985) report that myoga exhibits a difference in daylength requirement for flower initiation and further development. However while Nakamura (1985) concluded that for an early strain of myoga, initiation is slightly promoted under long photoperiods, Maeda (1994) found that initiation appeared to be delayed under long photoperiods. Both observed that floral development was retarded in the early strain under short photoperiods with a large number of abnormal flowers produced. Oga and Ota (1968) also report a late (autumn) variety where initiation is accelerated, but flower development retarded when grown under short (10 hrs) photoperiods.

Temperature

Photoperiodic requirements for flower initiation and development can be modified by temperature. Examples include a SDP strawberry, which is strictly photoperiodic only at temperatures above about 15^0C (Gutteridge, 1985) and *Clarkia amoena*, which is day-neutral at 20-24^0C but is a LDP at lower temperatures (Halevy & Weiss, 1991). Sub-optimal temperatures may even completely suppress flowering in inductive photoperiods. For example no flowering will occur in *Kalanchoe* (Schwabe, 1969) at temperatures below 8^0C or in *Perilla* (Zeevaart, 1969) at temperatures between 0 and 10^0C. Several photoperiodic plants are unable to flower in inductive daylengths without prior exposure to a period of low temperature. This period of low temperature, termed vernalisation, varies from a few weeks to several months. Although for several plants it is critical, in order to achieve reproductive development, in others a quantitative response can be observed. Vernalisation may reduce the number of inductive photoperiods required to achieve initiation, or may reduce the necessary duration of the photoperiod, implying that the apex has proceeded some ways towards the flowering state and requires less photoperiodic stimulation (Rees, 1987). The effectiveness of vernalisation may be influenced by daylength conditions. In some species, short daylength conditions may partially substitute for the cold requirement, whereas in others it may nullify the effect of vernalisation. Although in a few cases, temperature has been shown to influence the induction process in the leaf (Shinozaki and Takimoto, 1982), the majority of research has been unable to conclude if changes in temperature modify the induction process in the leaf or other components of the overall flowering response.

Interactions between temperature and photoperiod are not always found, however even when plants are responsive to both environmental factors. For example although photoperiod-sensitive genotypes of chickpeas were observed to flower sooner in longer than in shorter days (Summerfield et al., 1980) and flower sooner in warmer temperatures, when exposed to combinations of temperature and photoperiod no interaction was observed (Roberts et al., 1985).
Conjecture in the available literature on myoga also exists on the effect of temperature on flowering in myoga. Nakamura (1985) concluded that temperature did not appear to be critical for floral induction or development, while Maeda (1994) observed that with increasing night temperature and lengthening daylengths, greater numbers of flower buds were produced.

Juvenility

Before a plant can flower in response to its environment (particularly daylength and temperature), the organs that detect the environmental change, usually leaves or meristems, must reach a condition called 'ripeness to respond'. There is a great diversity among species and plant organs in the age at which they achieve this condition. This concept of ripeness to respond is almost identical to that of juvenility, defined as the condition of a plant before it is mature enough to flower (Salisbury & Ross, 1985). Size appears to be important in the transition to maturity and, in general, conditions that promote growth reduce the duration of the juvenile period. The effect of size may be explained by two hypotheses. One is that a plant of sufficient size transmits one or more signals to the apex, which then undergoes a phase change from juvenile to adult. The second is that the apical meristem behaves independently and undergoes the phase transition at a particular time (Vince-Prue, 1984).

The existence of a juvenile phase is obviously one of the factors associated with the lower sensitivity of younger plants to photoperiodic induction, since the daylength cycles given to juvenile plants are not effective (Thomas and Vince-Prue, 1997). It also seems probable that once the flowering stimulus has been irreversibly induced there will be an inevitable delay from the completion of induction until the appearance of the first flower. This implies the existence of a post-inductive phase

which is also insensitive to photoperiod (Roberts and Summerfield, 1987). Between these two photoperiod insensitive phases is the inductive phase in which photoperiod affects initiation and development of inflorescences. The timing of photoperiodic sensitivity can be determined through the use of reciprocal transfer experiments. This can be of great benefit when attempting to separate the often confounding effects of temperature and photoperiod (Major and Kiniry, 1991; Wilkerson et al., 1989). No attempt has been made to establish the duration of these three major phases during the vegetative and reproductive development of myoga.

Industry Application

Basic studies on photoperiodism in plants have been of considerable economic benefit to farmers, horticulturist and plant breeders. Since the time of Garner and Allard, testing plants for their photoperiodic requirements has become standard practice in breeding programs and a major reason for crop failures can now be avoided (Thomas and Vince-Prue, 1997). In particular, photoperiodism has had an enormous impact on horticulture. Daylength manipulation by the use of blackout cloth or supplementary lighting to promote or enhance flowering has been used for a wide range of ornamental species. Crops can be kept in a vegetative state or made to flower as required. Knowledge of the photoperiodic requirements of myoga and use of techniques mentioned above provide the opportunity to substantially increase production of myoga in Australia.

Northern Hemisphere Research

The environmental requirements for flower initiation and development in myoga have not been well researched despite the obvious importance of these events to commercial production of mature flower buds. Previous research points to the importance of daylength in flowering and flower bud yields. However, it is unclear whether the reported responses to varying the length of day were true photoperiodic responses or a result of difference in the length of time available for photosynthesis. In an attempt to grow myoga out of season in Japan, Maeda (1994) imposed various daylength treatments and recorded effects on vegetative growth and flower bud production. Under increasing daylength vegetative growth was more vigorous and

the mean weight and quality of flower buds increased relative to plants grown under the natural daylength of eleven hours. Photoperiod appeared to have a greater effect on flower bud development than flower bud initiation due to the presence of aborted flowers under natural daylengths (Maeda, 1994). Oga & Ota (1968) also observed "summer" myoga strains with seriously retarded development of flower buds under short photoperiods but flower bud initiation only slightly promoted under long photoperiods.

Southern Hemisphere Research

An initial three-year research project conducted at the School of Agricultural Science, University of Tasmania, identified a plant 'condition' which caused one 'type' of myoga to prematurely senesce after two to three years growth, at any site. Originally detected in commercial plantings in New Zealand, plants affected with this 'condition' were observed to senesce prior to flower bud development, resulting in low or non-existent flower bud yields (Clark, 2001). While the cause of the 'condition' remained unknown it did become apparent that not all plants were susceptible. This led to the identification of a second 'type' or cultivar of myoga, labelled 'Superior', which continued to produced high flower bud yields after two to three years growth, often in close proximity to the other 'type' of myoga. PCR "fingerprinting" and gel electrophoresis were unable to detect DNA/protein differences between the 'types'. Elisa testing was unable to detect any existing viruses and a bioassay confirmed that the condition was not transmitted to the Superior 'type' when grown in soil, in which the 'condition' was previously observed (Clark, 2001)

SECTION 1: PHOTOPERIODISM – *SECTION MATERIALS AND METHODS*

Plant Material

Two cultivars of myoga, one high yielding and the other low yielding under Tasmanian conditions (referred to as Superior (S) and Inferior (I) respectively) were used in the majority of experiments investigating the photoperiodic nature of myoga. Plant material was propagated vegetatively using rhizomes from existing myoga plants grown either in the field at New Norfolk or in glasshouses at the University of Tasmania. The rhizome material was soaked in a fungicide treatment consisting of 100ml of Previcur®, 200ml of Baviston®, 100ml of Sumisclex® and 200g of Kocide® per 100L of water. It was then packed into seed trays filled with a moistened mix of 50% perlite, 25% coarse sand and 25% peat and given a standard chilling treatment of three weeks at 4^0C (Gracie *et al*, 2000). Approximately 50g rhizome pieces were then planted into separate 35L pots. The potting soil consisted of peat, sand and pine bark (1:2:7) which was supplemented with slow release fertiliser (nine month Osmocote® 330g/50L), dolomite lime (330g/50L), iron sulphate (25g/50L) and trace elements (Micromax® 20g/50L). The pots were watered daily. Irrigation and fertiliser programs were identical for each of the treatments.

Controlled Environment Cabinets (C.E.C)

A motorised trolley system was used to transfer plants from a common glasshouse space to individual controlled environment cabinets. Supplemental and night break lighting was provided by combined mercury vapour and fluorescent lights with a photosynthetic photon flux density of 30.2 $\mu mol.m^{-2}.s^{-1}$. Glasshouse light levels ranged from 600 to 1200 $\mu mol.m^{-2}.s^{-1}$. Light levels were not significantly different between controlled environment cabinets or trolley positions within the common glasshouse. The average daytime temperature in the glasshouse was 25^0C, and all three controlled environment cabinets were maintained at an average temperature of 17^0C. Due to space and facility constraints true replication was not achievable in the experimental design, however continuous monitoring of the cabinets ensured that the temperature and photoperiod regimes remained constant.

SECTION 1: PHOTOPERIODISM – *EXPERIMENTAL WORK: IDENTIFICATION OF THE PHOTOPERIODIC NATURE OF MYOGA*

Introduction

Selection of suitable production locations in Australia and New Zealand for production of myoga has been limited by lack of information on climatic influences on flowering. This initial investigation focused on photoperiod, as potential production sites within Australia differ considerably in daylength due to the geographical range. This work aimed to demonstrate whether or not myoga is a true photoperiodic plant, and whether the photoperiodic requirement is more important for flower bud initiation or flower bud development. Knowledge of the photoperiodic response of myoga may enable industry to extend the season of production in Australia through either manipulation of growing conditions or through selection of appropriate growing regions.

Initial experience with the crop at high latitudes (43°S) has revealed two apparently distinct types or cultivars with different flowering and vegetative growth characteristics (Clark, 2001). The cultivar currently referred to as Inferior, fails to produce harvestable flower buds and senesces prematurely when grown in Southern Australia. Plants of the Superior cultivar are higher yielding and do not senesce prematurely, however under semi-commercial conditions, flower bud yield fluctuates from season to season. Differences in critical photoperiod between the two cultivars may explain this observation and therefore both cultivars were included in this investigation.

Materials & Methods

Planting material (refer to section materials and methods)

Photoperiod treatments

Three photoperiod treatments were imposed using controlled environment cabinets (for details on cabinets refer to section materials and methods). All plants received 8 hours natural daylight in the glasshouse, this was followed by either 16 hours of darkness (short day (SD) treatment), 16 hours darkness with 60 minutes night break lighting in the middle of the dark period (SD + night break lighting treatment) or 8 hours supplemental lighting followed by 8 hours darkness (long day (LD) treatment). Ten cultivar S plants and ten cultivar I plants were placed into each treatment. There were two harvests, the first at 104 days after planting (DAP) and the second at 201 DAP. At each harvest five cultivar I plants and five cultivar S plants were destructively sampled from each photoperiod treatment. Means and standard errors for the mean for each sample, (n = 5) were calculated.

At each harvest, plants were removed from their pots, potting mix washed off and the above and below ground parts of the plant separated. The above ground material (pseudostems) was weighed and then dried to obtain wet and dry weights. The rhizomes and roots were stored at 2^0C. Rhizomes were later dissected and apical meristems examined to determine if flower bud initiation had occurred and whether initiated flower buds had aborted leaving a senescent primordium. In the second harvest, mature and developing flower buds were removed from the plant and individually weighed and counted.

Results

First Harvest (104 DAP)

Cultivar I plants exhibited similar levels of pseudostem growth across all treatments. Cultivar S plants grown in the LD treatment produced higher pseudostem dry weights than those grown in the SD and SD(+nb) treatments (figure 1).

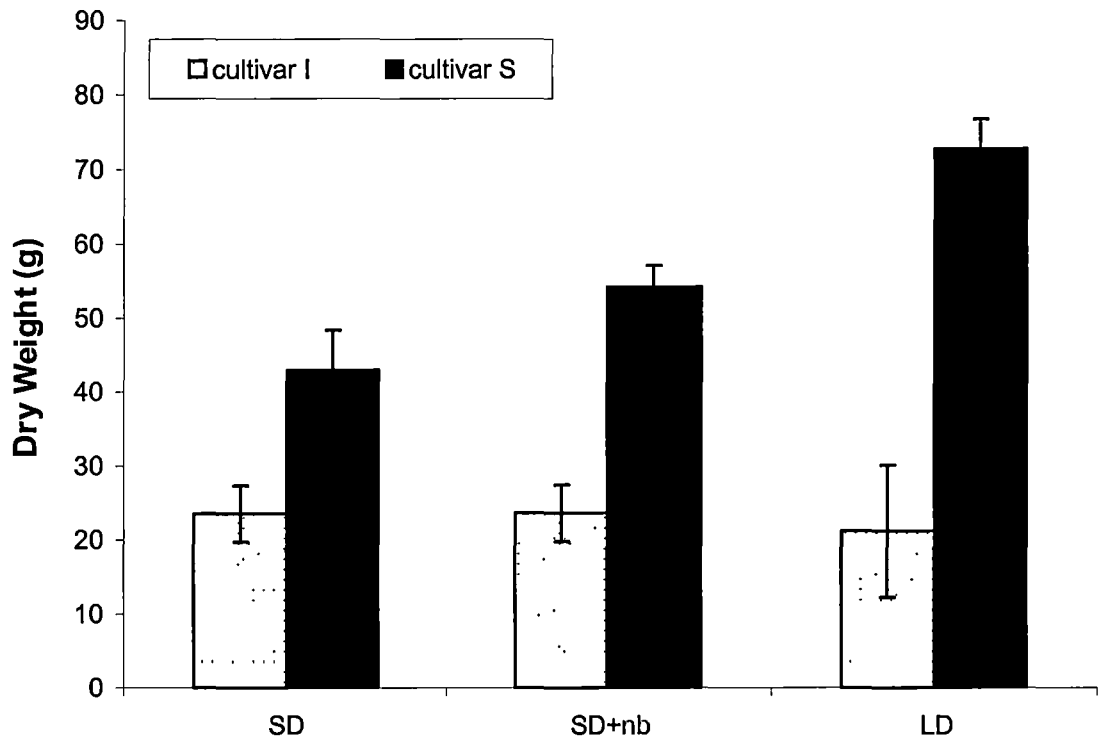


Figure 1: Mean dry weights of pseudostems/plant at 104 DAP (SD = 8 h day/16 h night, SD(+nb) = 8 h day/16 h night + night break, LD = 16 h day/8 h night). Bars show SE (n = 5).

Under short photoperiods (SD), both cultivars failed to produce flower buds (figure 2). Flower bud initiation had occurred in many of the plants, however the initiated flower buds appeared to have aborted. At 104 DAP this was evidenced by meristem tissue that had commenced floral differentiation but failed to continue developing, resulting in desiccated and brown necrotic tissue. Plants under long photoperiods (LD and SD (+nb)) initiated flower buds which developed normally without any evidence of abortion. The total number of flower buds initiated in SD and LD treatments was similar, however all initiated flower buds aborted under SD conditions.

SD (+nb) plants were observed to initiate more flower buds than either SD or LD plants and there was no evidence of any flower bud abortion. Overall the cultivar I plants produced more vegetative rhizomes and fewer flower buds, but like cultivar S plants, only developed flower buds under long photoperiods.

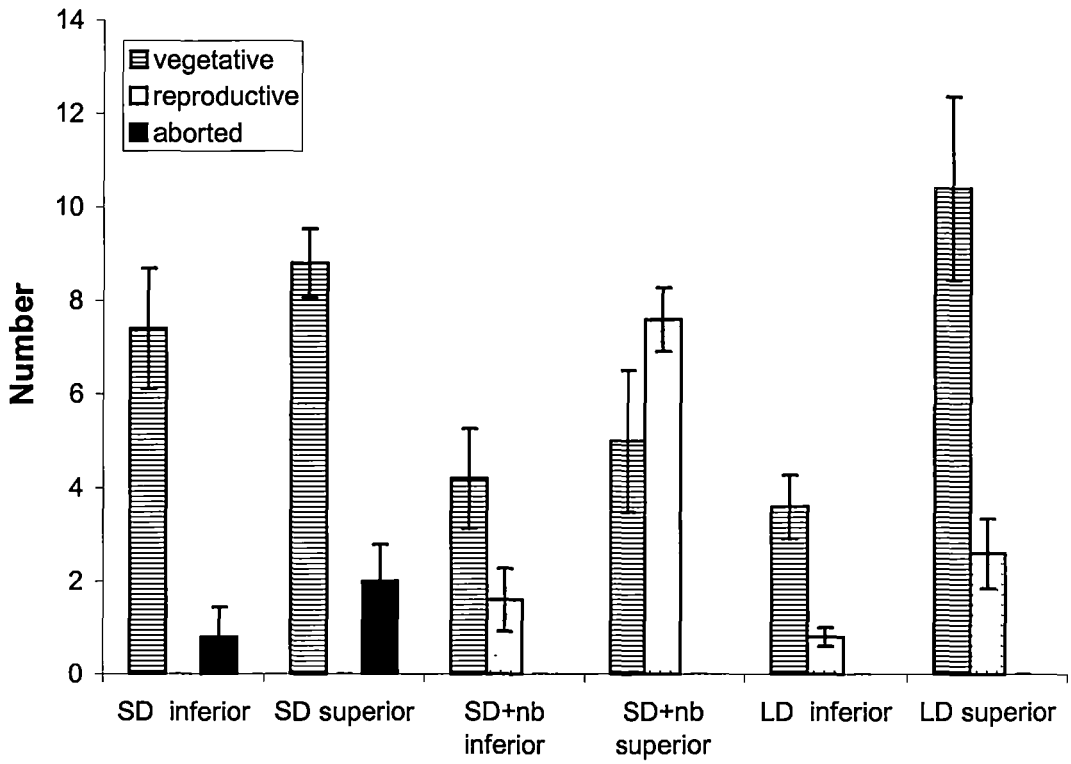


Figure 2: Mean number and type of rhizomes produced per plant in all treatments at 104 DAP. (Vegetative rhizomes are rhizomes that terminate with vegetative shoot primordia whereas reproductive rhizomes are rhizomes that terminate with flower bud primordia. Rhizomes terminating in primordia, which have obviously commenced differentiation but subsequently not developed, resulting in the appearance of degenerated reproductive primordia, are referred to as aborted). Bars show SE (n=5).

Second Harvest (201 DAP)

The data presented in figure 3 confirms the observations made at the initial harvest. No flower buds successfully developed under SD conditions and the addition of a one-hour night break to a short day length resulted in both flower bud initiation and development, to a level exceeding that achieved under LD conditions.

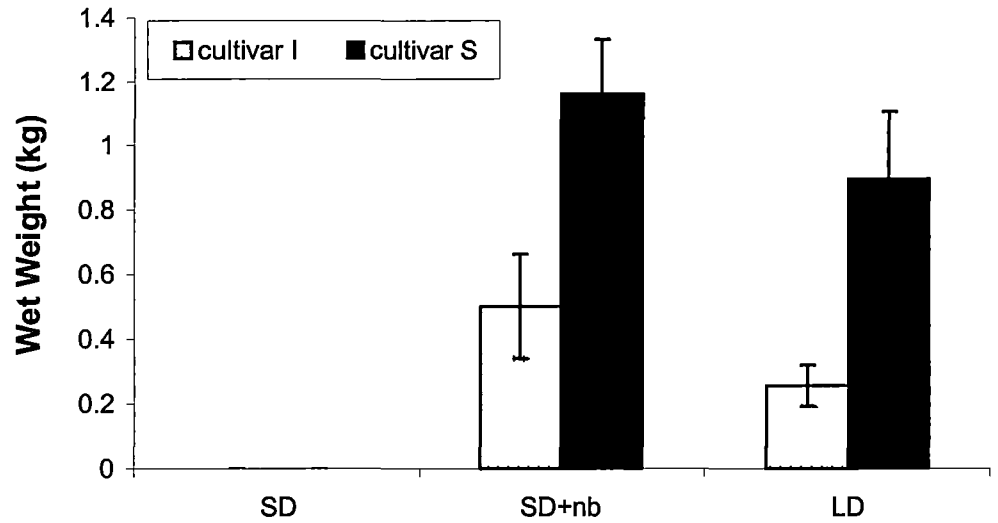


Figure 3: Harvestable yield (kg/plant) of flower buds for all treatments at 201 DAP. (SD = 8 h day/16 h night, SD(+nb) = 8 h day/16 h night + night break, LD = 16 h day/8 h night). Bars show SE (n = 5).

The Superior cultivar developed a greater number of flower buds and exhibited more vigorous vegetative growth than the Inferior cultivar (figures 3 & 4). These results have demonstrated differences in the growth and development of the two cultivars used. The cultivars differed in terms of their sources and this may have confounded the results. The observed differences may be due to cultivar, source or both.

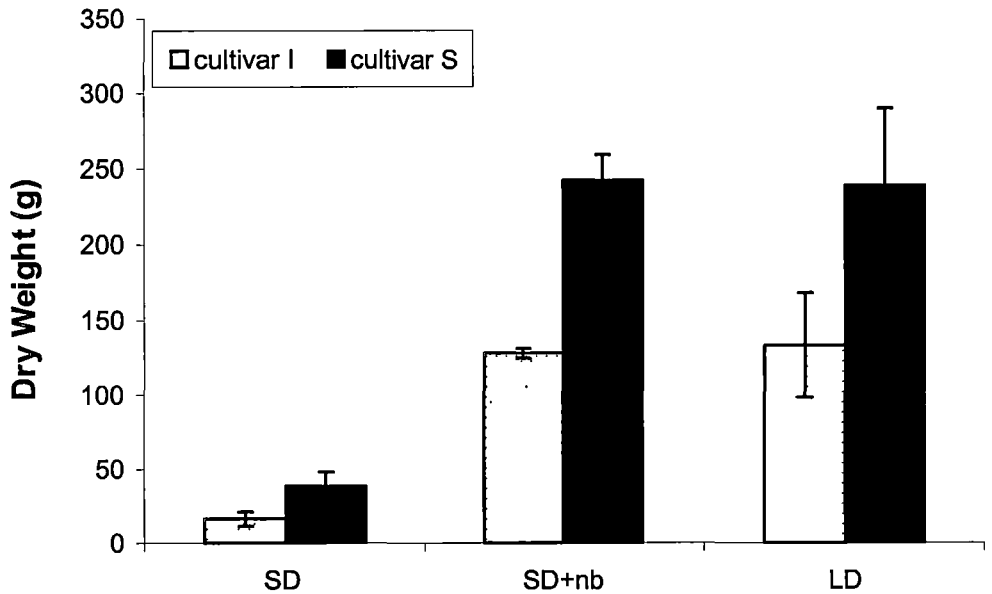


Figure 4: Mean dry weights of pseudostems/plant at 201 DAP. (SD = 8 h day/16 h night, SD(+nb) = 8 h day/16 h night + night break, LD = 16 h day/8 h night). Bars show SE (n = 5).

Plate 1: Effect of short day photoperiod conditions on cultivar S and I plants prior to harvest at 204 DAP.

Pseudostem growth of cultivar I plants growing under short photoperiod conditions (SD) was similar to those receiving long photoperiods ((LD and SD (+nb)) at 104 DAP (figure 1). While cultivar S plants produced higher pseudostem dry weights when grown in the LD treatment than, in the SD or SD(+nb) treatment. However at 201 DAP, plants from both cultivars growing under SD treatments were observed to have substantially lower foliage dry weight than plants growing in long photoperiod treatments and appeared to have entered a state of premature senescence (figure 4; plate 1).

Discussion

The results of this study confirm but significantly add to the work of Maeda (1994) and Oga and Ota (1968) in that it positively identifies the existence of a true photoperiodic flowering response in myoga and that this response is principally via an effect on development rather than initiation. SD plants at 104 DAP were observed to have initiated comparable numbers of flower buds to LD plants but had failed to successfully develop these into flower buds. When a night break

treatment was imposed, all flower buds that initiated proceeded to develop and the extent of initiation and development surpassed the levels recorded in plants grown under the LD treatment, despite the latter plants having received a greater amount of photosynthetically active radiation.

Photoperiod had a greater influence on flower bud development than flower bud initiation. Similar numbers of flower buds were initiated under SD and LD treatments and yet only long photoperiodic conditions resulted in flower bud development (figure 2). In many photoperiodic plants the optimal daylength may change with the stage of development of the reproductive organs and even differ for initiation and further development of the flower. There are four major groups of plants with differing photoperiodic requirements for initiation and flower development. In one of these groups, plants are day-neutral for initiation but have a photoperiodic requirement for flower development. The two woody species, Bougainvillea cv San Diego Red and *Caryopteris* x *clandonensis* are both examples of plants which are day-neutral for initiation but require SD conditions for successful development of inflorescences (Vince-Prue, 1975). Zehni et al. (1970) reported that flower buds of certain varieties of *Phaseolus vulgaris* failed to develop and eventually abscised in LD conditions, although the rate of flower initiation was unaffected. The presence of aborted flower buds on plants under short photoperiodic conditions in the current experiment indicated that flower bud initiation did occur but that flower buds were unable to continue normal development under short photoperiods. Thus myoga may be day – neutral for initiation but LD for development.

The performance of the two cultivars was considerably different under all treatment conditions. Cultivar S plants produced double the weight of pseudostems and flower buds than cultivar I plants. Similar levels of performance have also been observed in field trials conducted in Australia. Although this is likely to be due to genetic differences the different production sources of the parent rhizome material make comparison between the two cultivars difficult. Future work using cultivars where planting material is produced under identical conditions will enable detailed comparisons between the two cultivars to be made.

Maeda (1994) reported that short day conditions accelerated the progress towards senescence and dormancy while long day conditions prolonged growth in myoga. This response to environmental conditions was also observed in this experiment, with plants under short photoperiodic conditions beginning to senesce subsequent to abortion of flower primordia, and at a time when flower buds were observed to emerge from plants grown under long photoperiods. This indicates that the plants under SD treatments tended to enter a type of dormancy far earlier than plants under long photoperiods.

This behaviour may also provide an explanation for the difference in performance observed between cultivars I and S. If conditions are such that poor initiation and/or poor early flower bud development occurs, then plants exhibit early senescence. Inferior plant types, particularly in mature plantings have consistently performed poorly under Tasmanian conditions due to early senescence (Clark, 2001). To date no clear explanation has been found for this behaviour, however it is possible that the inferior type has a longer photoperiod requirement for flower bud development and that this requirement was not being met. Thus initiated flower primordia aborted before flower bud development could commence and the plant was forced into early senescence and dormancy.

This behaviour would become more pronounced in two-year-old stands since these plants have already completed their juvenility phase and are thus able to make the transition to reproduction in response to environmental stimuli. Hence they initiate flower buds earlier and during shorter photoperiods than plants in less mature plantings. To the contrary, later planting would mean later flower bud initiation and the possibility of flower bud development under photoperiodic conditions that were more likely to be conducive. While there is little supporting evidence in the literature, there are reports that early and late flowering varieties are grown in differing areas of Japan (Oga & Ota, 1968) and this theory could be tested in future research.

SECTION 1: PHOTOPERIODISM – *EXPERIMENTAL WORK: DETERMINATION OF CRITICAL DAYLENGTH REQUIRED FOR FLOWER BUD PRODUCTION IN MYOGA*

Introduction

The previous study showed that myoga is a photoperiod sensitive plant (Maeda, 1994; Oga & Ota, 1968; Stirling et al., 2002) and that for flowering to occur a qualitative long-day requirement must be satisfied (Stirling et al., 2002). However the critical daylength at which flowering will or will not occur in myoga has yet to be established. For plants that have a qualitative short day or long day requirement for flowering a 'critical daylength' (CDL) can be identified, which marks the transition between vegetative growth and flowering (Vince-Prue, 1976). Defining the CDL will be of vital importance in determining which locations will be used for establishing further commercial plantings of myoga. Certain environmental requisites such as warm temperatures and frost-free conditions dictate that the best area for commercial plantings of myoga is along the east coast of Australia. The wide geographical range along the eastern seaboard means that daylength will be an important consideration when determining where and at what time of year myoga is to be grown.

Two cultivars of myoga defined as cultivar S and I were included in this trial. Initial research using these cultivars identified that cultivar I senesced prematurely in the second and third year of growth resulting in low or no flower bud production (Clark, 2001). Previously thought to be a pathology issue, it was hypothesised by Stirling et al. (2002) that differing daylength requirements between cultivar S and I plants might be the cause of the observed disparity in plant development. References in Japanese literature (Maeda, 1994; Oga and Ota, 1968), of the existence of early and late flowering varieties indicates that varieties exist that have been bred to flower in different daylength conditions. It is possible that the two types of myoga identified in Australia differ only in their daylength requirement and that the poor production of flower buds from I plants is due to daylength requirement not being satisfied. Both cultivars were included in this trial to determine if cultivar I plants have a longer daylength requirement for flower bud production than cultivar S plants.

Materials and Methods

Planting material (refer to section materials and methods)

Photoperiod Treatments

Three photoperiod treatments were imposed using controlled environment cabinets (for details on cabinets refer to section materials and methods). All plants received one hour of supplemental light from 07:00 to 08:00 hr followed by 8 hours of natural daylight in the glasshouse. This was followed by either one hour of supplemental light in the tunnel from 16:00-17:00 (10 h treatment), three hours of supplemental light in the tunnel from 16:00-19:00 hours (12 h treatment) or five hours of supplemental light in the tunnel from 16:00-21:00 hours (14 h treatment). Nine cultivar S plants and nine cultivar I plants were placed into each treatment. There were three harvests, the first at 99 days after planting (DAP), the second at 132 DAP and the final harvest at 224 DAP. At each harvest three cultivar I plants and three cultivar S plants were destructively sampled from each daylength treatment. Means and standard errors for the mean of each sample, (n = 3) were calculated.

At each harvest, plants were removed from their pots, potting mix washed off and the above and below ground parts of the plant separated. Dry weights were obtained from the above ground material (pseudostems and leaves). The rhizomes and roots were stored at 2^0C. Rhizomes were later dissected and apical meristems examined to determine if flower bud initiation had occurred and whether initiated flower buds had aborted leaving a senescent primordium. In the third harvest, mature and developing flower buds were removed from the plant and individually weighed and counted.

Results

Vegetative Growth

At 99 days after planting (DAP), cultivar S and I plants receiving 10 h daylengths produced similar levels of pseudostem dry weight. At 12 and 14 h daylengths cultivar S plants appeared to produce higher pseudostem dry weights than cultivar I plants. Plants grown under 12 h daylengths were observed to produce higher pseudostem dry weights than plants grown under 10 and 14 h daylegnths. This effect was most noticeable in cultivar S plants (figure 1).

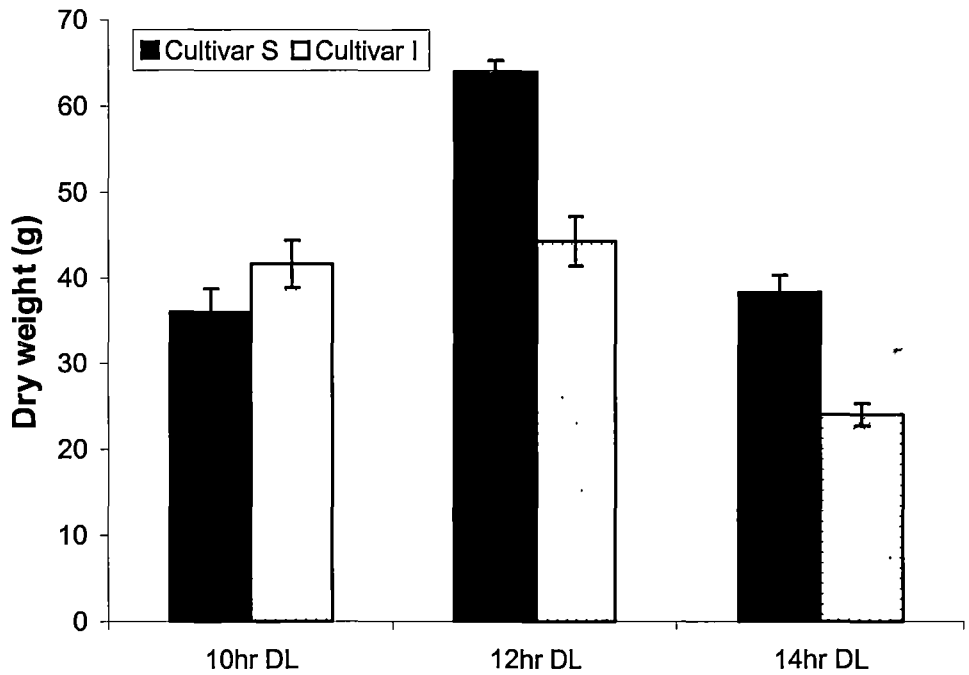


Figure 1: Dry weight of pseudostems at 99 DAP from cultivar S and I plants grown under 10, 12 and 14 hour daylengths. Bars show SE (n=3).

Cultivar I plants produced similar levels of pseudostem dry weight when grown under all treatment conditions. Cultivar S plants also produced similar levels of pseudostem dry weight when grown under all treatment conditions. At 12 and 14 h daylengths, cultivar S plants appear to have produced substantially higher pseudostem dry weights than cultivar I plants. (figure 2). Plants from both cultivars grown under 10 and 12 h daylengths appear to be under some stress as evidenced by their wilted condition (plate 1).

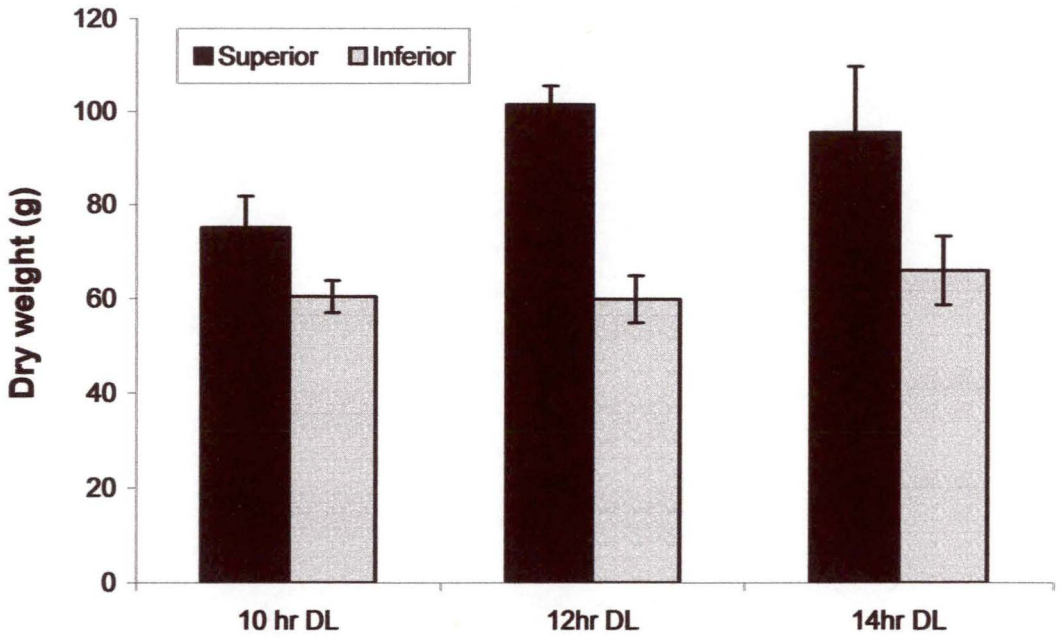


Fig. 2. Dry weight of pseudostems at 132 DAP from cultivar S and I plants grown under 10, 12 and 14 hour daylengths. Bars show SE (n=3).

Plate 1: Effect of daylength on myoga plants prior to harvest at 132 DAP. From left to right, myoga plants were grown under 14, 12 and 10 h daylengths. Cultivar S and I plants are randomly distributed in each controlled environment cabinet.

At 224 DAP plants receiving 14 h daylengths produced substantially higher pseudostem dry weights than plants under 10 and 12 h daylengths. At the 14 h daylength, cultivar S plants appeared to produce higher levels of pseudostem dry weight than cultivar I plants. Both cultivars produced similar levels of pseudostem dry weights under 10 and 12 h daylengths (figure 3).

The effect of daylength can be clearly observed in plate 2 which was taken just prior to harvest at 224 DAP. Plants grown under 10 and 12 hr daylengths clearly displayed symptoms of premature senescence, which resulted in the low levels of pseudostem dry weight in figure 3. Myoga plants grown under a daylength of 14 hrs exhibit normal growth and development and therefore had much higher pseudostem dry weight.

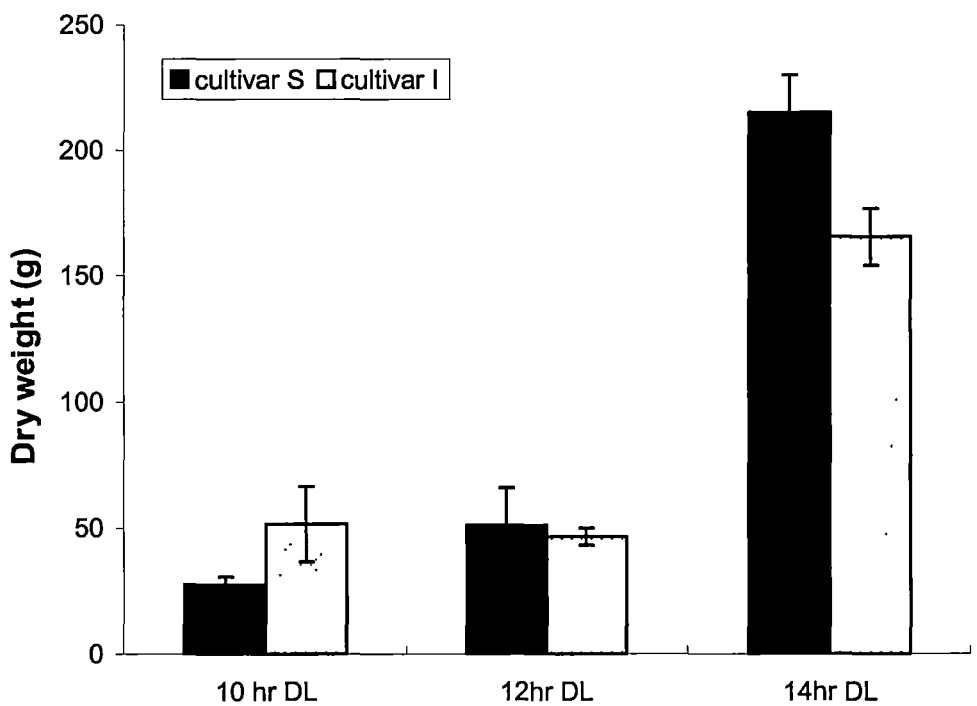


Figure 3. Dry weight of pseudostems at 224 DAP from cultivar S and I plants grown under 10, 12 and 14 hour daylengths. Bars show SE (n=3).

Plate 2: Effect of daylength on myoga plants prior to harvest at 224 DAP. From left to right, myoga plants were grown under 14, 12 and 10 h daylengths. Cultivar S and I plants are randomly distributed in each controlled environment cabinet.

The response of cultivar I and S plants to the 10 and 12 h daylength treatments can be observed in figures 4 & 5. For the two cultivars, in both daylength treatments pseudostem dry weight increased from the first harvest to the second harvest at 132 DAP. After the second harvest a decline in plant growth rate followed by senescence of vegetative material resulted in low pseudostem dry weights at the final harvest. The increase prior to 132 DAP, and subsequent decrease in pseudostem dry weight after the second harvest was more noticeable in cultivar S plants. Cultivar S plants were observed to senesce earlier than cultivar I plants as indicated by the leaf tip necrosis seen in plants grown under 12 h daylengths (plate 1) and by the advanced senescent state of same plants in plate 2. Plants grown under 14 h daylengths also had an increase in levels of pseudostem dry weights from 99 to 132 DAP (figure 6). After the second harvest pseudostem dry weights did not decline and this resulted in higher pseudostem dry weights at the final harvest.

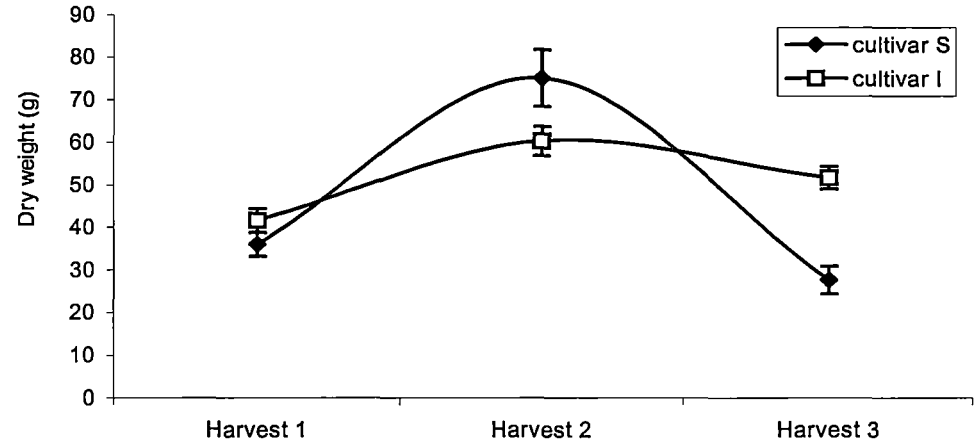


Figure 4. Dry weight of cultivar S and I pseudostems grown under 10 h daylengths at harvest 1, 2 and 3. Bars show SE (n=3).

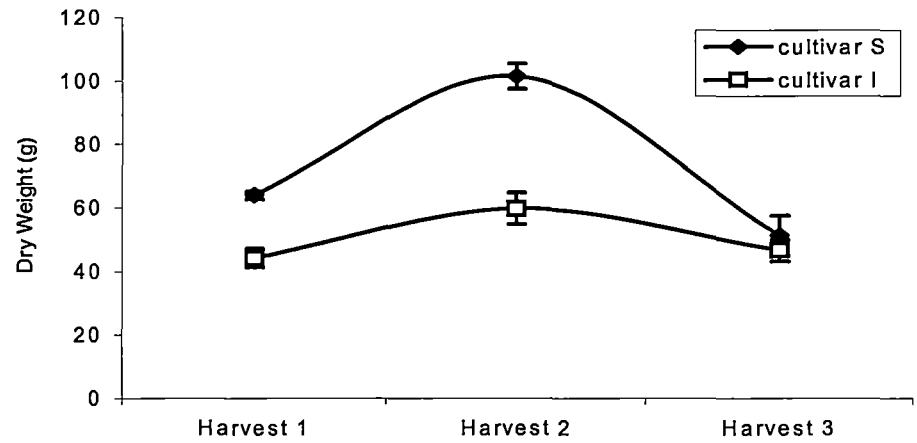


Figure 5. Dry weight of cultivar S and I pseudostems grown under 12 h daylengths at harvest 1, 2 and 3. Bars show SE (n=3).

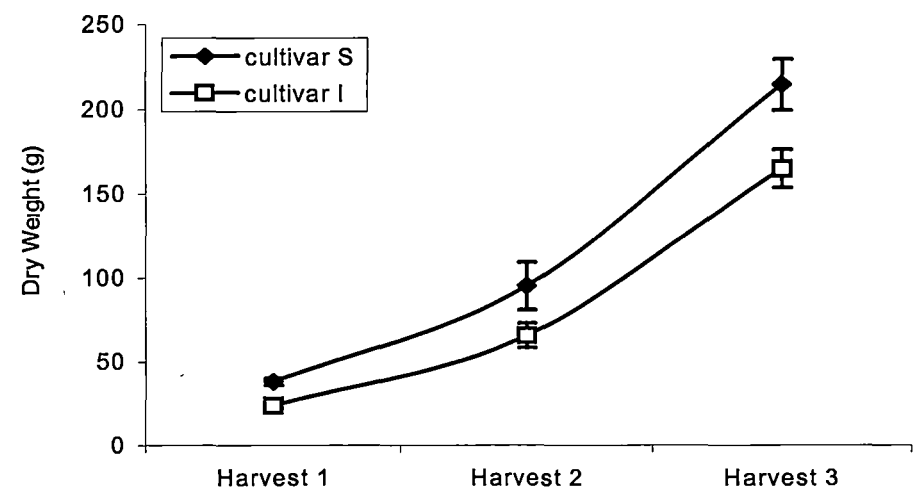


Figure 6. Dry weight of cultivar S and I pseudostems grown under 14 h daylengths at harvest 1, 2 and 3. Bars show SE (n=3).

Reproductive Growth

Initiation of flower buds had occurred in all three daylength treatments by 99 days after planting, with the highest number of initiated flower buds being present in plants receiving 12 h daylengths. Flower bud abortion was evident in plants receiving 12 h daylengths but not in plants receiving other daylength treatments. Cultivar I plants grown under 10 and 14 h daylength conditions had initiated a similar low number of flower buds. Cultivar S plants grown under 14h daylength conditions had not initiated any flower buds, however in the other daylength treatments plants of cultivar S initiated more flower buds than plants of cultivar I (figure 7).

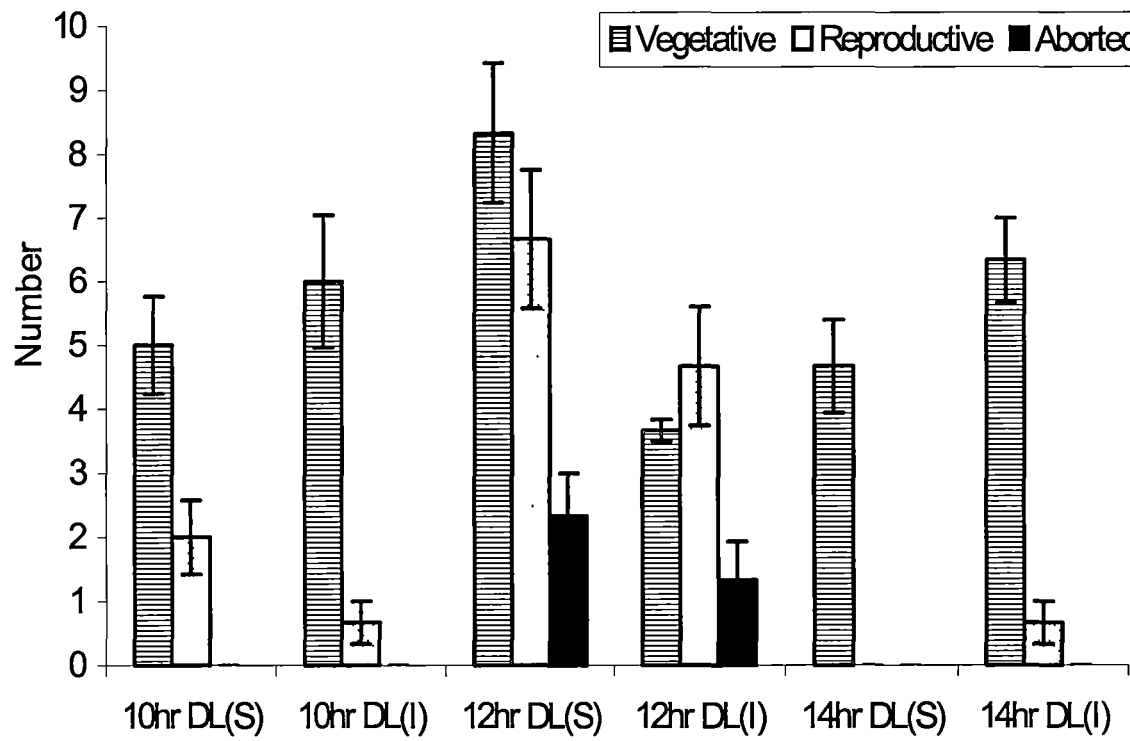


Figure 7. Number and type of rhizome produced at 99 DAP by cultivar S and I plants grown at 10, 12 and 14 h daylengths. Bars show SE (n=3).

At 132 DAP plants receiving 10 and 12 h daylengths had produced high numbers of vegetative rhizomes and low numbers of reproductive rhizomes. There was also a high number of aborted rhizomes present in 10 and 12 h daylength treatments. Plants grown in 14 h daylength conditions produced high numbers of reproductive rhizomes and lower numbers of vegetative rhizomes. There was no abortion evident in these latter plants (figure 8).

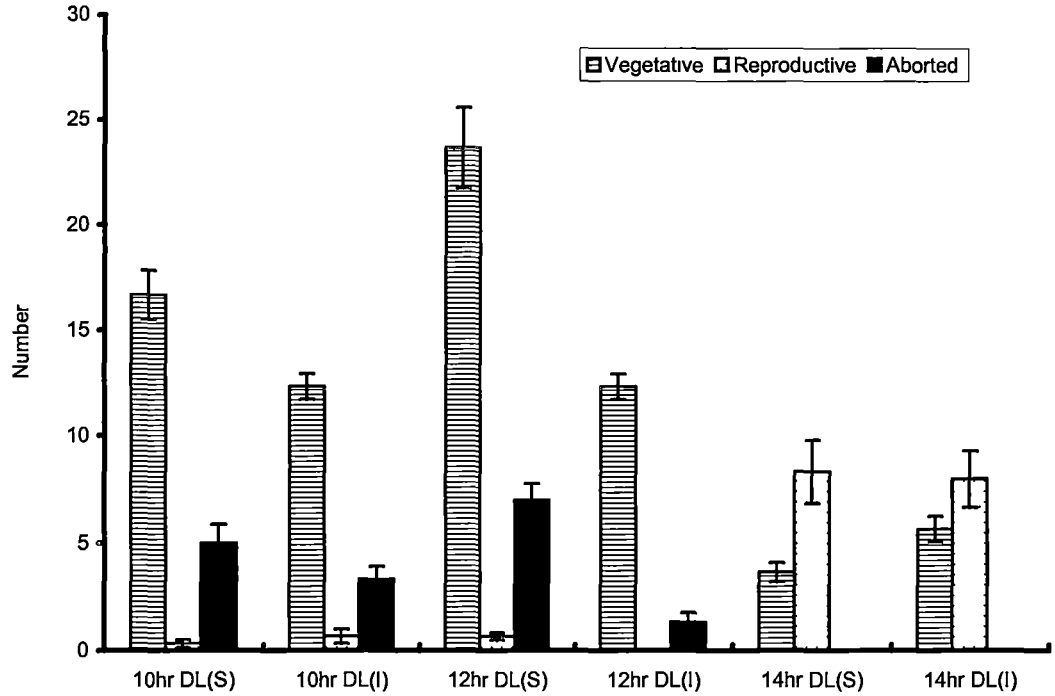


Figure 8. Number and type of rhizome produced at 132 DAP by cultivar S and I plants grown at 10, 12 and 14 h daylengths. Bars show SE (n=3).

At final harvest cultivar S and I plants grown under 14 h daylengths produced similar numbers of flower buds as represented by the harvestable yield of flower buds in figure 9. No yield of flower buds were produced from plants under 10 and 12 h daylengths.

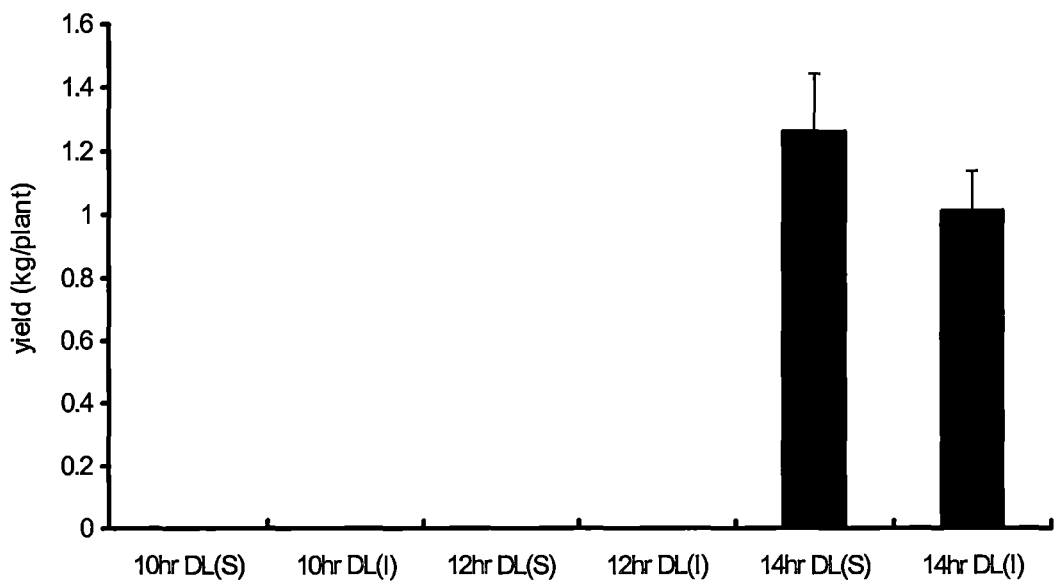


Figure 9. Harvestable yield (kg/plant) of flower buds at 224 DAP from cultivar S and I plants grown under 10, 12 and 14 h daylengths. Bars show SE (n=3).

Discussion

Qualitative photoperiodic plants will only flower when grown under conducive daylengths. Plants which display a quantitative flowering response will eventually flower under any daylength conditions, however flowering is hastened under conducive daylengths (Thomas & Vince-Prue, 1997). Myoga has been shown to only flower in long daylength conditions (Stirling et al., 2002). The fact that flower buds were only produced in this trial at the longest daylength of 14 hours confirms this qualitative response. However this was not the only aspect of myoga reproductive development that was affected. The timing of reproductive development was also affected by the prevailing daylength conditions.

Plants grown under the shorter daylengths of 10 and 12 hours initiated buds earlier and with greater frequency than plants grown in 14 h daylengths. The advanced reproductive state of these plants is confirmed by the presence of aborted flower buds at the first and second harvest and that this abortion was only evident in plants grown under 10 and 12 h daylengths. Advanced initiation of flower buds under short day conditions has also been detected in previous trials conducted on myoga (Stirling et al., 2002). Hastened flower bud initiation in plants grown under short daylengths indicates that myoga has a quantitative short day requirement for initiation. The photoperiodic response for initiation is quantitative since initiation still occurred in plants grown in LD conditions, albeit at a later stage.

The presence of aborted flower buds at these daylengths indicates that although short days may be conducive for flower bud initiation, development still requires long day conditions. Therefore myoga has a quantitative short day requirement for flower initiation and a qualitative long-day requirement for flower development.

Cultivar S and I plants exhibited similar responses in vegetative and reproductive development to the changing daylength conditions in which they were grown. Plants grown under short daylengths displayed rapid reproductive development and high vegetative growth rates until 132 DAP. Continued growth under short day conditions after this point, resulted in flower bud abortion and senescence of above ground vegetative matter for both cultivars. Cultivar S plants appeared to be slightly

more sensitive to the daylength conditions under which they were grown than cultivar I plants. They initiated flower buds earlier and with greater frequency than cultivar I plants. They also had higher rates of vegetative growth from planting to 132 DAP, followed by the greatest degree of senescence. Cultivar S and I plant developmental responses appeared to be much more similar under 14 h daylengths. Levels of above-ground vegetation continuously increased from planting to final harvest and although flower bud initiation occurred at a later stage, similar high numbers of flower buds were achieved at final harvest.

When grown under certain daylength conditions, such as those experienced in Southern Tasmania over summer, cultivar I myoga plants have been observed to show poor flower bud development and early senescence (Clark, 2001). However under the same conditions cultivar S myoga plants will successfully produce flower buds. This difference in plant performance usually becomes apparent in mature plantings which are more likely to start initiating flower buds at an earlier stage and thus in shorter daylengths than first year plants. Stirling et al (2002) hypothesised that these previously observed differences in development and flower bud production in S and I cultivars could be explained by differing daylength requirements. Although this hypothesis could not be confirmed in this trial, it is still possible that cultivar I myoga may have a longer daylength requirement than the S cultivar. The difference in critical daylength (CDL) between the two may be quite small, for example in some short day plants a difference of 15 minutes can determine whether or not flowering occurs (Thomas and Vince-Prue, 1997). Therefore smaller incremental changes in daylength would need to be tested to positively confirm the requirement for different daylengths between cultivar S and I plants.

The critical daylength for myoga which may be defined as that daylength above which flower buds are produced but below which, no flower buds are produced, is likely to be between 12 and 14 hours. This was indicated by the presence of flower buds in myoga plants grown under 14 hr daylengths but not at 12 or 10 h daylengths. This information can be used for more accurate prediction of suitable times of year to produce myoga or alternatively, when to artificially extend daylengths when environmental conditions are unsuitable. Although it can be

reasonably assumed that myoga plants given 14 hours or greater daylength will flower, further trials will have to be conducted in order to determine the exact value of the CDL for myoga.

The requirement for a particular photoperiod may be altered by other environmental conditions, in particular temperature. Temperature plays an important role in the control of flowering time, interacting with daylength and often determining the daylength response. The interaction may control both the initial steps of the flowering process, leading to inflorescence initiation and the speed and direction of the inflorescence's further development (Heide, 1994). Examples of plants which display strong photoperiod and temperature interactions are the short day plant strawberry, which is strictly photoperiodic only at temperatures above about 15^0C (Gutteridge, 1985) and *Clarkia amoena*, which is day-neutral at 20-24^0C but is a long day plant at lower temperatures (Halevy and Weiss, 1991). Myoga may also have a temperature/photoperiod interaction for flower bud production and therefore the results achieved in this experiment may not be the same for myoga grown at temperatures other than those used in this trial. Despite this, the temperatures used in this trial are the most favourable for myoga growth and therefore the critical daylength established in this trial is likely to be very close to that found in the natural environment.

SECTION 1: PHOTOPERIODISM – *EXPERIMENTAL WORK: DETERMINATION OF THE PHOTOPERIOD SENSITIVE PHASES OF MYOGA*

Introduction

In an effort to expand current knowledge of the photoperiodic requirements of myoga it was deemed important to establish the photoperiodic phases of myoga plants. Photoperiodically sensitive plants generally exhibit three phases with respect to developmental responses to photoperiod. During the pre-inductive or juvenile phase immediately following seedling emergence, photoperiod has no effect on subsequent development. This phase is followed by an inductive phase during which photoperiod affects initiation of reproductive development. During the post-inductive phase, reproductive development proceeds independently of photoperiod. Identification of the pre-inductive, inductive and post-inductive phases in photoperiod sensitive plants can be accomplished by transferring plants from inductive photoperiods to non-inductive photoperiods and vice-versa at certain intervals beginning at the time of plant emergence (Patterson, 1995).

Identification of these phases will be of importance not only in expanding current knowledge of the photoperiodic requirements of myoga, but also in the development of myoga as a new vegetable crop in Australia. The establishment of this crop in a multitude of locations across Australia will not only require information on the critical daylength but also information as to when photoperiod becomes a concern during crop development. In particular, information on photoperiodic sensitive phases will be required so that multiple production seasons can be scheduled within one year.

Materials & Methods

Planting material (refer to section materials and methods)

Daylength treatments

Plants were grown in a glasshouse maintained at day/night temperatures of 21/15^{0}C. Plants receiving a 10 h (SD) photoperiod were grown in pots on automated mobile trolleys which were drawn into and out of adjoining night compartments at 17:00 h and at 07:00 h, respectively. Night compartments were maintained at the same temperature as that in the main glasshouse. Plants receiving a 16-h (LD) photoperiod remained in the glasshouse for the entire 24-h period and received supplemental lighting to achieve the LD photoperiod. This lighting consisted of fluorescent tubes and incandescent bulbs from 04:30 h to 08:00 h and again from 16:30 h to 20:30 h EST (Eastern Standard Time); light intensity was approximately 45 μmol $m^{-2}s^{-1}$. Within daylength regimes the plants were arranged into three blocks, each containing one replicate of the 10 treatments ie. nine transfer times and one control. The control treatment remained in the respective daylength treatment until flowering. Transfer treatments were completely randomised within each block. For each transfer time (table 1), three plants were transferred from LD to SD and vice versa. Within each block five additional plants were grown for dissection purposes. These plants remained in their respective daylength treatments and were not transferred. For each harvest time (table 1) three plants from LD conditions and three plants from SD conditions were harvested.

				Days	***After***	***Planting***			
Transfer Times	70	80	90	110	120	130	140	150	170
Harvest Times	-	-	90	110	120	130	140	-	-

Table 1. Days from planting to transfer for plants moved from SD to LD and vice versa.

Harvests

At each harvest, plants were removed from their pots, potting mix washed off and the above and below ground parts of the plant separated. The above ground material (pseudostems) was weighed and then dried to obtain wet and dry weights. The number of pseudostems present was also recorded. The rhizomes and roots were stored at 2^0C. Rhizomes were later dissected and apical meristems examined to determine if flower bud initiation had occurred and whether initiated flower buds had aborted leaving a senescent primordium. Means and standard errors for each sample, (n = 3) were calculated.

At 244 DAP all plants from the transfer experiment were destructively harvested so that fresh and dry weights of pseudostems could be obtained. Mature and developing flower buds were removed from the plant and individually weighed and counted. The presence of buds, whether anthesis had been achieved and the premature senescence of pseudostems were also recorded. Means and standard errors for each sample, (n = 3) were calculated.

Results

Transfer Plants

Plants grown in long day (LD) and transferred to short day (SD) conditions between 0 and 140 DAP (Control SD, 70LD-SD, 80LD-SD, 90LD-SD, 110LD-SD, 120LD-SD, 130LD-SD, 140LD-SD) senesced prematurely and did not develop any flower buds (table 2). Plants grown in LD conditions for greater than 140 days and transferred to SD conditions (150LD-SD, 170LD-SD) did develop flower buds. Plants grown in SD and transferred to LD conditions between 0 and 150 DAP (Control LD, 70SD-LD, 80SD-LD, 90SD-LD, 110SD-LD, 120SD-LD, 130SD-LD, 140SD-LD, 150SD-LD) developed flower buds and many of these reached anthesis by 244 DAP. Plants grown in SD conditions for greater than 150 days and transferred to LD conditions (150SD-LD, 170SD-LD) senesced prematurely.

Treatment	*B.D.*	*A*	*S*
Control SD	-	-	+
170SD-LD	-	-	+
150SD-LD	+ (2)	-	+ (1)
140SD-LD	+	+ (1)	-
130SD-LD	+ (2)	+ (2)	+ (1)
120SD-LD	+	+	-
110SD-LD	+	+	-
90SD-LD	+	+ (2)	-
80SD-LD	+	+ (2)	-
70SD-LD	+	+ (2)	-
Control LD	+	+ (2)	-
170LD-SD	+	-	-
150LD-SD	+	-	-
140LD-SD	-	-	+ (1)
130LD-SD	-	-	+
120LD-SD	-	-	+
110LD-SD	-	-	+
90LD-SD	-	-	+
80LD-SD	-	-	+
70LD-SD	-	-	+

Table 2: Stage of physiological development achieved by LD and SD plants by 244 DAP. Where B.D. is bud development, A is anthesis and S is senescence. (+) sign designates achievement of the above state. (–) sign indicates that the state was not achieved. Sample size (n) = 3, where figures are in brackets the number designates the number of plants in the sample population that achieved that state.

Plants grown under regimes of at least 134 days of LD conditions (after 0-110 days of SD conditions, ie Control LD, 70SD-LD, 80SD-LD, 90SD-LD and 110SD-LD treatments; Figure 1) all produced 100 or more flower buds. As the period of SD treatment increased beyond 110 days, before transfer to LD treatment, the number of flower buds fell, until treatment 170SD-LD (nil flower buds; Figure 1). Conversely, initial LD treatments followed by SD treatments produced flower buds only after the LD treatment lasted for 150 days or more (ie 150SD-LD, 170SD-LD treatments; Table 2, Figure 1).

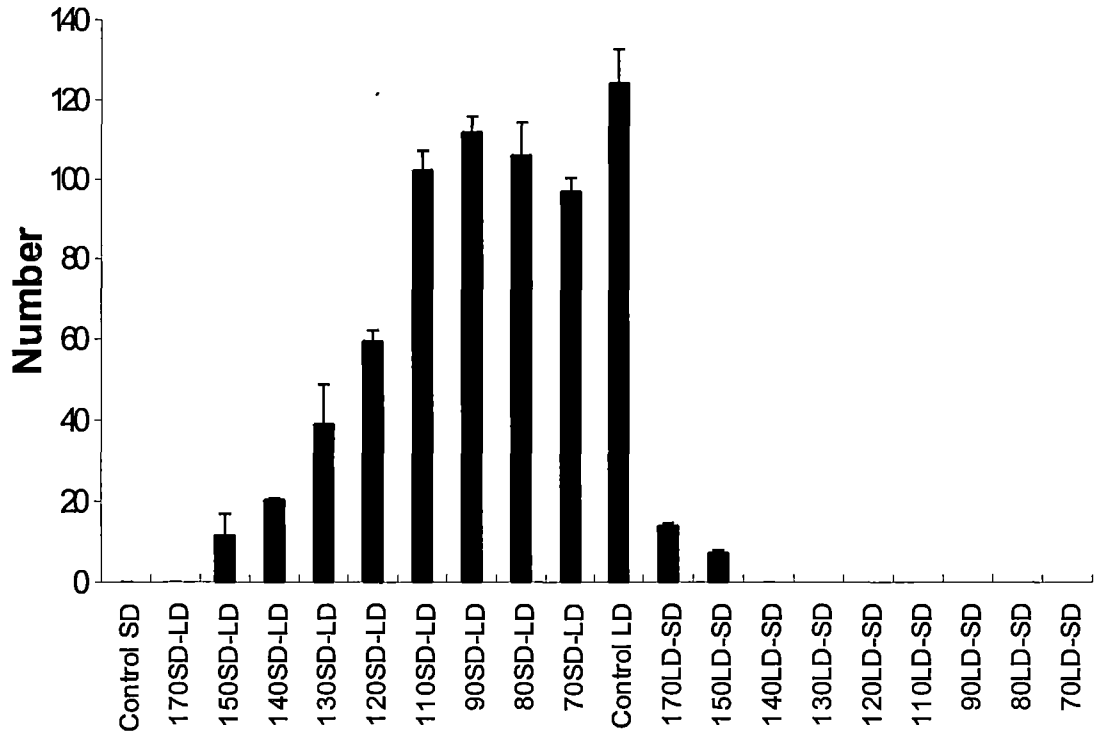


Figure 1: Number of flower buds produced from all treatment plants at 244 DAP. Bars show SE (n=3).

Pseudostem dry weight followed a similar pattern to the development of flower buds (figure 2). Those plants which developed flower buds also produced the highest levels of vegetative growth. Plants grown under SD conditions for greater than 130 days produced similar pseudostem dry weight as those grown initially under LD conditions and then transferred at any time. Pseudostem dry weights were greatest in those plants grown continuously under LD conditions and those grown under short day conditions from 0 up to 130 DAP.

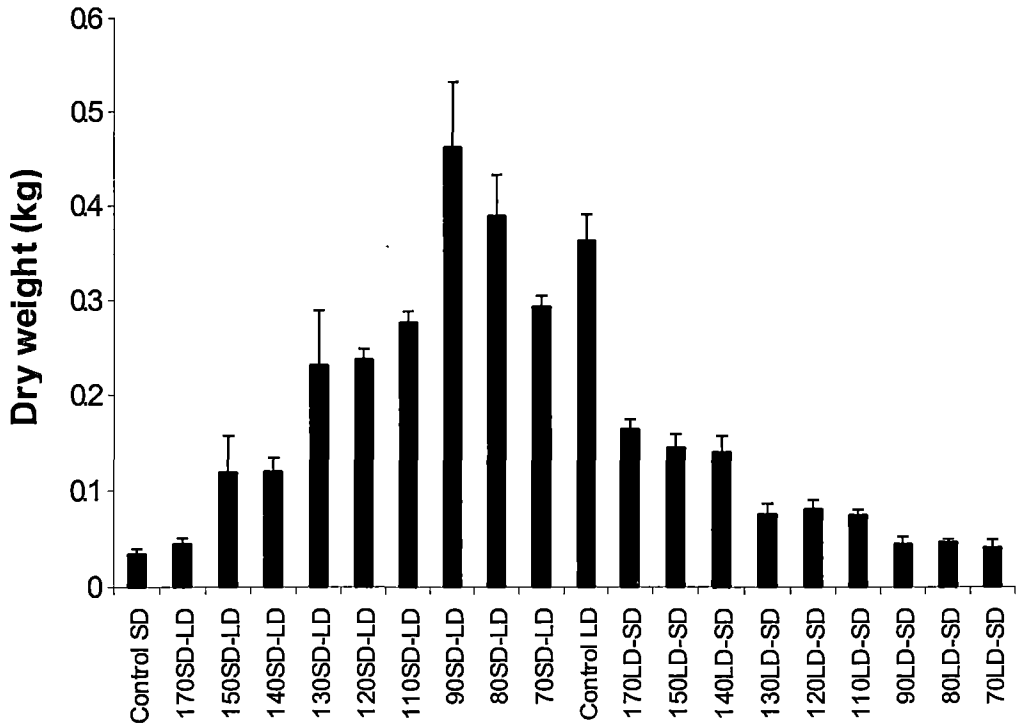


Figure 2: Dry weight of pseudostems/plant at 244 DAP. Bars show SE (n=3).

Dissection plants

Plants grown under LD conditions produced six new vegetative rhizomes between 90 and 110 DAP, after which time new development ceased until 130 DAP, when an additional four rhizomes were produced. New vegetative rhizomes were produced from 90 to 140 DAP in plants grown under SD conditions. The number of vegetative rhizomes produced by plants under SD conditions DAP was less than those produced by plants under LD conditions. Plants under SD conditions began producing reproductive rhizomes after 110 DAP, while plants under LD conditions produced reproductive rhizomes only after 130 DAP. LD plants never aborted initiated flower buds however under SD conditions aborted floral primordia were detected after 110 DAP.

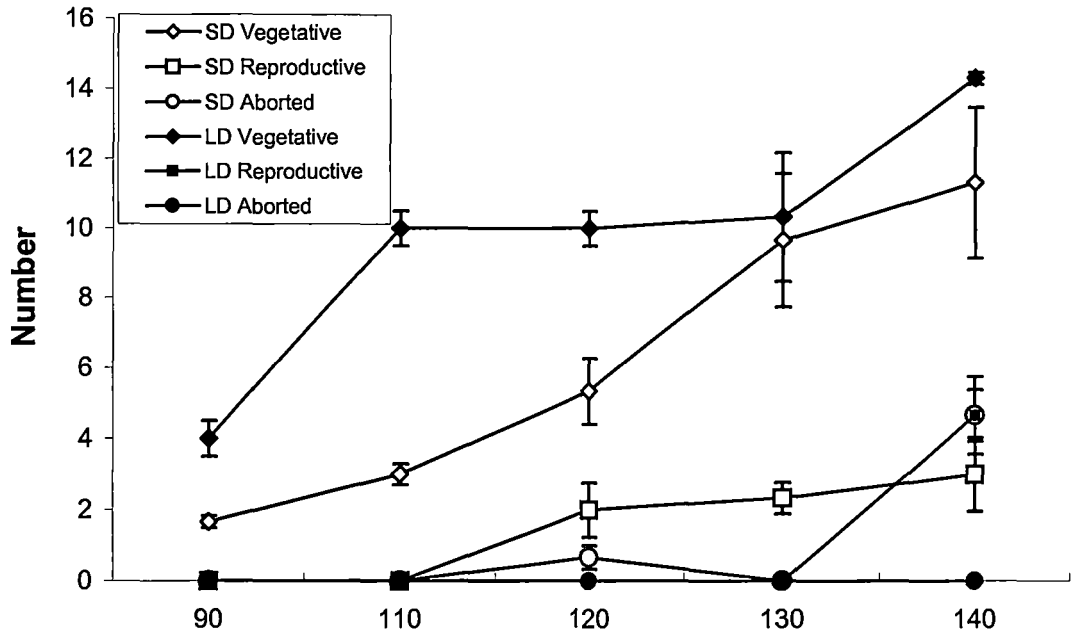


Figure 3: Number and type of rhizomes produced by SD and LD plants between 90 and 140 DAP. Bars show SE (n=3).

Pseudostem dry weight increased in plants in LD conditions between 90 and 110 DAP and between 130 and 140 DAP. Pseudostem dry weight of SD plants increased from 90 to 110 DAP and after 130 DAP (figure 4).

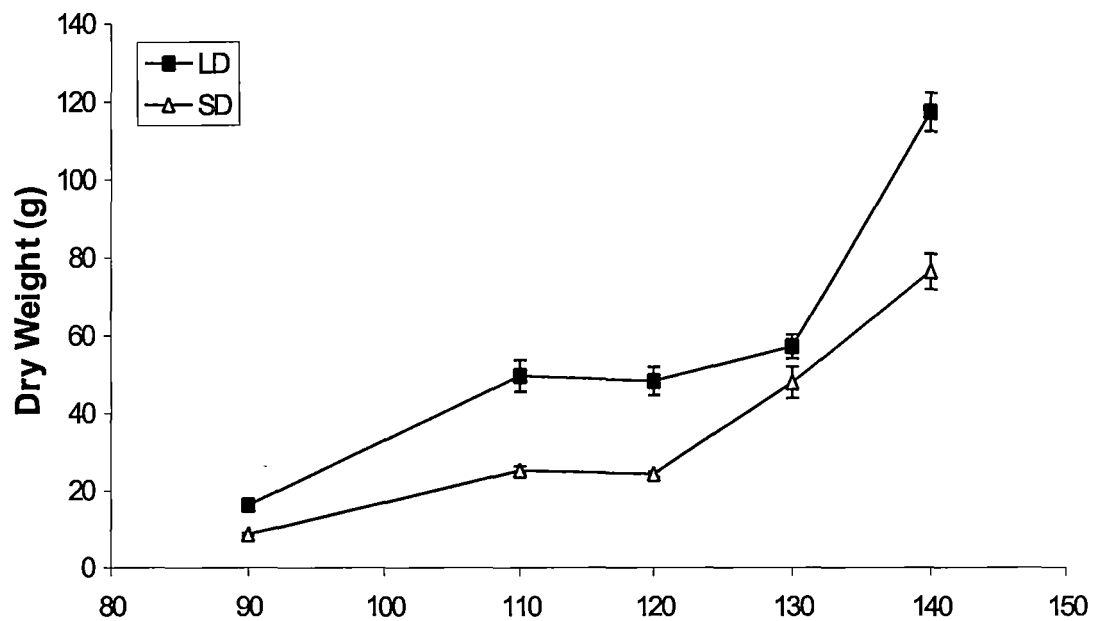


Figure 4: Dry weight of pseudostems produced by plants grown under SD and LD conditions between 90 and 140 DAP. Bars show SE (n=3).

Plants grown under LD conditions produced between 5 and 6 pseudostems by 120 DAP (figure 5). After this point no new pseudostems were produced. Under SD conditions plants had produced between 2 and 3 pseudostems by 120 DAP. Between 120 and 130 DAP, approximately 2 new pseudostems were produced; after this point no new pseudostems were produced.

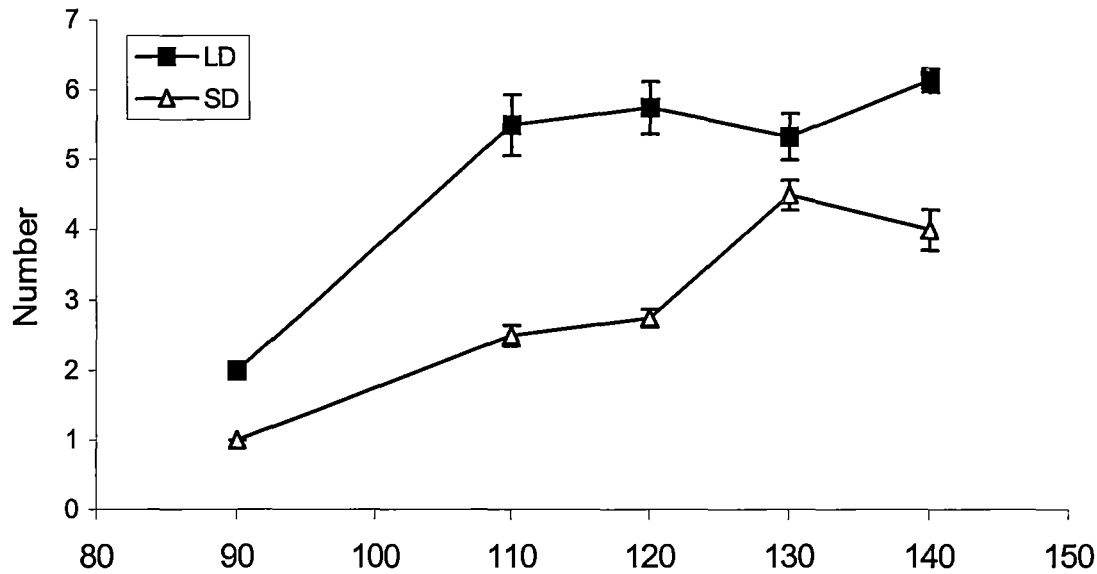


Figure 5: Number of pseudostems produced by plants grown under SD and LD conditions between 90 and 140 DAP. Bars show SE (n=3).

Discussion

Reciprocal transfer experiments can be used to determine the stages of photoperiod sensitivity in day-length sensitive plants. Identification of the stages is achieved by comparing the time taken to reach anthesis by plants grown under varying amounts of inductive and non-inductive daylengths. Although this experiment was designed using this method, identification of the various stages was difficult due to a number of reasons. The main reason was the nature of photoperiod sensitivity in myoga. Typically, plants investigated in reciprocal transfer experiments are photoperiodically sensitive for flower bud initiation rather than flower bud development, with a quantitative rather than a qualitative response. Thus in such experiments all plants will reach anthesis, but this state is delayed or hastened according to the number of inductive days given. A number of plants in the current experiment senesced early and never achieved anthesis, therefore the traditional

methods of data analysis to identify the stages were not possible. However a number of other factors relating to the development of myoga flower buds have been established.

One of the more important findings is that myoga has a dual requirement for flower buds to reach maturity. In figure 3 it was shown that myoga plants grown under SD conditions began to initiate flower buds between 110 and 120 DAP. However, myoga plants grown under LD conditions did not begin initiating flower buds until after 130 DAP. This indicates that myoga has a quantitative SD requirement for flower bud initiation. The requirement is quantitative since initiation occurred in both SD and LD conditions but was promoted by SD conditions. This is in contrast to the photoperiod requirement for development, which is qualitative in nature. This can be seen in Table 2 where flower buds initiated by plants in SD conditions aborted when allowed to remain in SD conditions. Therefore, myoga plants have a quantitative SD requirement for flower bud initiation and a qualitative LD requirement for flower bud development.

Plants grown in LD conditions from 0 up to 130 DAP did not initiate any flower buds during this period. When LD plants were transferred to SD conditions at 70, 80, 90, 110,120 and 130 DAP they exhibited premature senescence (table 2). Based on other data (Stirling et al., 2002) these plants would have initiated flower buds after transfer, however abortion of flower buds due to the SD conditions would have followed and hence no flower buds developed in these plants. Plants which did not develop flower buds eventually senesced in response to the daylength conditions. Flower bud initiation under LD conditions began between 130 and 140 DAP. Plants transferred to SD conditions at 140 DAP had initiated flowers and although they did not senesce, they did not produce any harvestable buds or reach anthesis. Sensitivity of myoga to daylength appears to be greatest at the earliest stages of flower bud development. Therefore, although these plants were able to initiate flower buds in LD conditions, the immediate transfer of plants to SD conditions prevented any development of buds. Plants kept in LD conditions from planting and transferred to SD conditions at 150 and 170 DAP initiated flower buds and were healthy plants which produced low numbers of harvestable buds but did not reach anthesis.

Control plants kept under LD conditions for the entire experiment developed a high number of flower buds, which also reached anthesis.

Plants grown in SD conditions initiated flower buds between 110 and 120 DAP. Aborted flower buds were also observed at 120 DAP, therefore the period between initiation and abortion of floral primordia could be quite short, in the vicinity of 1-10 days. All plants grown in SD conditions and transferred to LD conditions at 70, 80, 90, 110, 120, 130 and 140 DAP produced harvestable buds and reached anthesis. Flowering was most committed in plants transferred from SD to LD conditions from 70 to 120 DAP, while those transferred at 130 and 140 DAP had a 50% chance of achieving anthesis. Plants transferred at the earlier stages would have initiated only a small number of flower buds before transfer and therefore the majority of flower buds would have been initiated and subsequently developed under LD conditions. Plants transferred at the later stages of 130 and 140 DAP would already have initiated and subsequently aborted quite a number of flower buds by this stage resulting in less opportunity for successful development of flower buds through to anthesis. Plants grown under SD conditions and transferred to LD conditions at 150 DAP produced flower buds which did not reach anthesis by 244 DAP. Plants transferred at 170 DAP senesced, as did the control plants grown continuously under SD conditions.

During this trial, plants that developed flower buds but did not reach anthesis by the time of harvesting, were not as mature as those that did flower. Anthesis is the final stage of flower bud development and is used as an indicator of over-maturity for the commercial production of myoga. Plants grown in SD conditions and transferred to LD conditions at 120 and 130 DAP were the first to flower. The rapid achievement of anthesis is expected considering the daylength conditions these plants were grown under. These plants initiated early under promotive SD conditions and when transferred to LD conditions, successfully developed the initiated flower bud to the point of anthesis. SD plants transferred at earlier intervals to LD conditions reached anthesis at a later stage to these plants, however they did produce greater numbers of flower buds.

Plants transferred from SD conditions to LD conditions after 110 DAP would have experienced increasing levels of flower bud abortion according to the amount of time spent over 110 days in SD conditions. However they should have still been able to initiate new flower buds when transferred to LD conditions, especially since plants grown under LD conditions did not begin initiating until the later stage of 130 DAP. It would appear however that when myoga plants abort a certain proportion of initiated flower buds no new flower buds will be initiated, instead the meristematic region of new rhizomes remains vegetative. This conclusion was drawn from successive dissections of developing rhizomes, where all fourth order rhizomes were observed to remain vegetative in plants where flower bud abortion had occurred on second and third order rhizomes. Therefore the reduced number of developed flower buds produced from plants transferred from SD to LD conditions at 120, 130, 140 and 150 DAP may be due to a combination of abortion of initial flower buds initiated and a failure to continue initiating new buds when placed into LD conditions.

Plants that failed to reach anthesis or produce substantial amounts of developed flower buds also had low pseudostem dry weights at final harvest. The low vegetative dry weight of those plants that did not produce flower buds can be explained by the effect of short daylengths on myoga plants. Myoga plants grown under short daylengths were unable to develop flower buds past the stage of initiation. When poor flower bud development occurs the plant begins to senesce at a premature stage in relation to those grown under long day conditions (Stirling et al., 2002). This dormancy, seen here as premature senescence was also observed by Maeda (1994).

Although it was not possible to accurately identify the point at which myoga plants first became sensitive to photoperiod, it was possible to identify the stage at which myoga plants became insensitive to photoperiod following induction and the early stages of development. Reproductive development in myoga plants appears to be insensitive to daylength after 150 DAP. This can be observed in table 2, where plants grown for ≥ 150 days in SD conditions and then transferred to LD conditions did not flower and eventually senesced even though they were in promotive LD

conditions. Furthermore those plants grown in LD conditions for ≥ 150 days still had bud development when placed into SD conditions, whereas if they had been placed into these conditions at an earlier stage they would have senesced.

The patterns of reproductive and vegetative development were quite different in those plants grown under SD conditions compared with those under LD conditions. In LD plants vegetative development ceased both above and below ground between 110 and 130 DAP (figures 3, 4 & 5). After 130 DAP pseudostem dry weight began to increase again and new vegetative rhizomes were produced. Between 130 and 140 DAP floral primordia was observed in LD plants for the first time. This behaviour supports earlier observations of myoga development by Gracie et al., (*in press*), where cessation of pseudostem initiation and the commencement of rhizome development from axillary buds at the base of pseudostems occurred at the same time as the dry weight of the planted rhizome piece ceased to decline. The initiation of flower buds shortly afterwards led to the conclusion that the shift from vegetative to reproductive development was linked to the transition of the planted rhizome piece from source to sink. Although rhizome dry weight was not recorded in this trial, the cessation of all vegetative development twenty days prior to flower bud initiation indicates that assimilates are being utilised in the advancement of reproductive development rather than vegetative development.

The pattern of reproductive and vegetative development displayed by SD plants was quite different to that observed in LD plants in this trial and in myoga plants grown in previous trials (Gracie et al., *in press*). Floral primordia was first detected at 120 DAP in SD plants, twenty days prior to initiation occurring in LD plants. There was no cessation of vegetative development before flower bud initiation. Furthermore vegetative rhizomes were continuously produced from 90 to 140 DAP and new pseudostems were initiated after flower bud initiation has commenced. From the results of this trial, it would appear that the abortion of floral primordia in response to short daylengths affects the normal pattern of reproductive and vegetative development in myoga plants.

It is unfortunate that further dissections of developing plants under long and short daylength conditions was not possible after 140 DAP. However, it would be expected that under long daylengths new vegetative and reproductive rhizomes would continue to be produced while pseudostem dry weight increased. Under short daylengths the number of reproductive rhizomes would eventually decline while the number of aborted and vegetative rhizomes would increase. Pseudostem dry weight would eventually decrease or remain approximately the same due to senescence of plant material as a result of poor flower bud development.

SECTION 1: PHOTOPERIODISM – *EXPERIMENTAL WORK: EFFECT OF TEMPERATURE ON THE PHOTOPERIODIC SENSITIVITY OF MYOGA*

Introduction

Future expansion of the commercial production of myoga will be reliant on locating new sites for further cultivation. A number of sites have previously been identified on the eastern seaboard of Australia, with the wide geographical range of these sites indicating that environmental factors are likely to differ. Daylength has already been identified as an important environmental factor affecting the production of flower buds in myoga and research has been conducted to elucidate the parameters of this sensitivity. The other major environmental factor that will change between production sites is temperature, in particular night temperature. Temperature has been shown in numerous plant species to modify the effect of photoperiod on flowering and may potentially change the parameters of sensitivity. Responses may include an acceleration towards anthesis, inhibition of flower induction, an increase in the number of required inductive cycles; alteration of the required length of the critical dark period and may even change the plant response type to photoperiod, i.e. change a DNP to a SDP (Salisbury, 1963). Therefore the flowering responses observed in myoga plants to photoperiod, in sites such as Hobart, TAS where night temperatures are cool, could be profoundly different to the flowering responses observed in myoga plants grown in areas where night temperatures are warm. For this reason it was deemed important to establish whether the flowering response of myoga to photoperiod could be modified due to an interaction with temperature.

As demonstrated in previous investigations, myoga is sensitive to daylength, requiring long day conditions for successful development of flower buds. Sensitivity to photoperiod is a common aspect of flowering in many species, with a number of species responding to relatively small seasonal differences in daylengths. For example, differences of 10-20 minutes per day have been determined as critical in plants such as rice (*Oryza sativa*) and cowpea (*Vigna unguiculata*) (Roberts, 1991). Although the critical daylength for flower bud development in myoga has

previously been determined to be between 12 and 14 hours, it may be that the range at which myoga will or will not flower is quite narrow, with a difference of 30 minutes being critical. Therefore, in addition to investigating the interactive effects of temperature and photoperiod, the two daylengths in this trial were chosen so that the daylength requirement of myoga could be more accurately defined.

The two cultivars of myoga grown in Australia, (cultivar S and I) were included in this experiment as it was suspected that these two cultivars may have a difference in the critical daylength required for successful flower bud development. This conclusion had been drawn from previous research and observations of both cultivars in the field. Furthermore, by including both cultivars it would be possible to determine if an interaction of temperature with photoperiod resulted in different modifications of flowering response in each cultivar. Although constraints on plant stock meant that cultivar I plants could not be included in all of the destructive harvests it was expected that an indication of different responses to a temperature/photoperiod interaction could be discerned and the difference in critical daylength confirmed.

Materials and Methods

Planting material (refer to section materials and methods)

Photoperiod Treatments

Twelve cultivar S and six cultivar I myoga plants were grown at a night temperature of either 5^0C or 15^0C combined factorially with two photoperiods (12.5 and 13.5 h per day). Controlled lighting and temperature regimes as set out in table 1, were facilitated by the use of controlled environment cabinets (for details refer to general materials and methods). Destructive harvests occurred at 120, 143, 174 and 245 days after planting (DAP). At the first (120 DAP) and third (174 DAP) harvests, three cultivar S and three cultivar I plants were destructively sampled from each factorial treatment. Due to constraints with plant stock at the second (143 DAP) and fourth (245 DAP) harvests only three cultivar S plants were destructively sampled from each factorial treatment. Means and standard errors for each sample, (n = 3) were calculated.

At each harvest, plants were removed from their pots, potting mix washed off and the above and below ground parts of the plant separated. Dry weights were obtained from the above ground material (pseudostems and leaves). The rhizomes and roots were stored at 2^0C. Rhizomes were later dissected and apical meristems examined to determine if flower bud initiation had occurred and whether initiated flower buds had aborted leaving a senescent primordium. Vegetative rhizomes were identified as rhizomes that terminated with a vegetative shoot primordium whereas reproductive rhizomes terminated with flower bud primordia. Rhizomes terminating in primordia, which have obviously commenced differentiation but subsequently not developed, resulting in the appearance of degenerated reproductive primordia, are referred to as aborted.

12 5/LT	time		0600 - 0800	0800 - 1600	1600 - 1830	1830 - 0600	
	position		C.E.C	glasshouse	C E.C	C.E.C	
	light/temp		light/25°C	light/25°C	light/25°C	dark/5°C	
12.5/HT	time	0430 – 0600	0600 – 0800	0800 – 1600	1600 – 1830	1830 – 1930	1930 – 0430
	position	C.E.C	C.E C	glasshouse	C.E C	C.E C	glasshouse
	light/temp	dark/15°C	light/25°C	light/25°C	light/25°C	dark/15°C	dark/15°C
13.5/LT	time		0500 – 0800	0800 – 1600	1600 – 1830	1830 – 0500	
	position		C.E.C	glasshouse	C.E.C	C.E.C	
	light/temp		light/25°C	light/25°C	light/25°C	dark/5°C	
13 5/HT	time		0500 – 0800	0800 – 1600	1600 – 1830	1830 – 0500	
	position		glasshouse	glasshouse	glasshouse	glasshouse	
	light/temp		light/25°C	light/25°C	light/25°C	dark/15°C	

Table 1: Light and temperature regime used to achieve treatment effect on myoga plants.

Results

First Harvest (120 DAP)

Cultivar S and I

Within cultivars all treatment plants had similar pseudostem dry weight at 120 days after planting (table 1). However within each treatment, cultivar S plants appeared to have higher pseudostem dry weights than cultivar I plants. At the longer daylength of 13.5 hours, night temperature had an effect on the number of reproductive rhizomes produced, with cultivar S and I plants grown at the higher night temperature producing more reproductive rhizomes than those at the lower

night temperature (table 2). At the shorter daylength, temperature did not appear to have an effect on number of reproductive rhizomes developed.

For cultivar I plants, initiation of flower buds was only observed in plants grown under longer daylengths with a higher night temperature. The number of reproductive rhizomes present on these plants was similar to the number produced by cultivar S plants from the same treatment. Within cultivars plants from all treatments produced the same number of vegetative rhizomes. The abortion of a flower bud was observed to occur in a cultivar S plants grown at a daylength of 12.5 h with a high night temperature.

Cultivar	*LT/12.5*	*HT/12.5*	*LT/13.5*	*HT/13.5*
Superior	36.78 ± 12.03	51.51 ± 10.51	31.15 ± 10.46	55.36 ± 14.50
Inferior	9.34 ± 3.25	7.24 ± 0.83	4.14 ± 1.88	19.60 ± 3.88

Table 1: Mean dry weights (g) of pseudostems/plant at 120 DAP. Values are shown ± SE (*n* = 3).

Rhizomes	*Cultivar*	*LT/12.5*	*HT/12.5*	*LT/13.5*	*HT/13.5*
Reproductive	*Superior*	4.83 ± 0.86	3.06 ± 1.44	0.89 ± 0.58	7.0 ± 1.44
	Inferior	0	0	0	5.33 ± 0.67
Vegetative	*Superior*	4.17 ± 1.04	6.93 ± 1.48	4.17 ± 0.80	6.17 ± 1.36
	Inferior	3.67 ± 0.33	4.67 ± 2.03	1.67 ± 1.67	4.33 ± 0.33
Aborted	*Superior*	0	0.3 ± 0.17	0	0
	Inferior	0	0	0	0

Table 2: Mean number and type of rhizomes produced by cultivar S and I plants in all treatments at 120 DAP. Values are shown ± SE (*n* = 3).

Second Harvest (143 DAP)

Cultivar S

Cultivar S and I plants grown at 12.5 and 13.5 h daylengths produced higher pseudostem dry weights when grown at a high night temperature (table 3). At both the high and low night temperatures, daylength failed to cause an obvious effect on the weight of pseudostem produced by cultivar S plants.

Cultivar S plants grown at both daylengths with a low night temperature developed similar numbers of reproductive and vegetative rhizomes, with no abortion of

flower buds noted. These plants appeared to be of low vigour with a far smaller, total number of rhizomes developed compared with those grown at the high night temperature. Cultivar S plants grown at the high night temperature displayed different numbers of reproductive, vegetative and aborted flower buds when grown at 12.5 and 13.5 hours daylength. Plants grown at the high night temperature with a longer daylength appeared to be highly reproductive plants with considerably higher numbers of initiated flower buds than those at the same temperature with a shorter daylength. In addition no abortion of flower buds was observed. Plants grown at the high night temperature with a shorter daylength produced low numbers of reproductive rhizomes but large numbers of vegetative rhizomes. Abortion of flower buds was also observed in plants grown under these conditions.

	LT/12.5	***HT/12.5***	***LT/13.5***	***HT/13.5***
Superior	26.87 ± 4.36	63.12 ± 7.30	38.32 ± 17.31	85.94 ± 11.39

Table 3: Mean dry weights (g) of pseudostems/plant at 143 DAP. Values are shown ± SE (*n* = 3).

Rhizomes	***LT/12.5***	***HT/12.5***	***LT/13.5***	***HT/13.5***
Reproductive	3.5 ± 1.54	2.33 ± 1.20	1.5 ± 1.70	19.6 ± 1.76
Vegetative	5.83 ± 1.60	26 ± 4.48	3.38 ± 0.72	2.57 ± 1.0
Aborted	0	4 ± 1.14	0	0

Table 4: Mean number and type of rhizomes produced by cultivar S plants in all treatments at 143 DAP. Values are shown ± SE (*n* = 3).

Third Harvest (174 DAP)

Cultivar S and I

Within cultivars, myoga plants produced the highest pseudostem weights when grown under a daylength of 13.5 hours with a high night temperature (table 5). Under all other light/temperature treatments, similar dry weight of pseudostems was produced within cultivars. Within treatments plants grown under 13.5 hours daylength and a high night temperature displayed the strongest cultivar difference, with cultivar S plants displaying higher pseudostem dry weights than cultivar I plants.

Cultivar S myoga plants grown at both night temperatures with a daylength of 12.5 hours had similar numbers of reproductive rhizomes, however those plants grown at the high night temperature had considerably higher numbers of vegetative rhizomes and aborted flower buds than those at the low night temperature (table 6).

Cultivar S plants grown at the longer daylength, with a high night temperature were more reproductive than those at the lower night temperature as indicated by the higher number of initiated flower buds. Cultivar S plants grown at both night temperatures with a 13.5 h daylength had similar numbers of vegetative rhizomes and no abortion of flower buds was observed.

Cultivar I plants in general, displayed a similar pattern of rhizome development to that observed in cultivar S plants. However a number of differences within treatments exists between cultivars. Although both cultivars grown at long daylengths with a warm night temperature produced similar numbers of reproductive and vegetative rhizomes, abortion of flower buds was observed in cultivar I plants. In addition, no reproductive rhizomes were produced in cultivar I plants grown under long daylengths with a low night temperature. While cultivar S plants grown under high night temperatures with a short daylength produced considerably more vegetative rhizomes than cultivar S plants from any other treatment, cultivar I plants grown under the same treatment conditions produced a similar number of vegetative rhizomes to cultivar I plants from other treatments.

	LT/12.5	***HT/12.5***	***LT/13.5***	***HT/13.5***
Superior	39.81 ± 8.84	50.09 ± 8.76	21.01 ± 3.61	134.71 ± 34.08
Inferior	19.55 ± 2.09	24.59 ± 12.59	4.95 ± 0.35	72.89 ± 6.90

Table 5: Mean dry weights (g) of pseudostems/plant at 174 DAP. Values are shown ± SE (*n* = 3).

		LT/12.5	*HT/12.5*	*LT/13.5*	*HT/13.5*
Reproductive	*Superior*	13.33 ± 8.52	18.6 ± 4.34	1 ± 0.58	28.67 ± 3.28
	Inferior	0.67 ± 0.66	2.33 ± 2.32	0	20.4 ± 3.21
Vegetative	*Superior*	8.97 ± 1.14	44.27 ± 8.29	8.33 ± 1.20	13.67 ± 2.02
	Inferior	6 ± 1.14	12.3 ± 4.58	3 ± 0.88	9.23 ± 1.44
Aborted	*Superior*	0.3 ± 0.33	8.17 ± 5.41	0	0
	Inferior	0	2.67 ± 1.15	0	1.63 ± 0.33

Table 6: Mean number and type of rhizomes produced by cultivar S and I plants in all treatments at 174 DAP. Values are shown ± SE (n = 3).

Fourth Harvest (245 DAP)

Cultivar S

Plants grown at a 13.5 h daylength, with a high night temperature produced substantially higher pseudostem dry weights than those at the same daylength with a low night temperature (table 7). At the shorter daylength plants grown at both night temperatures produced similar amounts of pseudostem dry weight. At high night temperatures, daylength had an effect on growth, with plants grown under longer daylengths producing higher pseudostem dry weights. At the low night temperature, the effect of daylength on the level of vegetation produced was not as obvious.

At this final harvest, plants grown under longer daylengths produced a similar number of reproductive and vegetative rhizomes at both night temperatures (table 8). Plants grown at the shorter daylength with a high night temperature had higher numbers of vegetative rhizomes and aborted flower buds than those grown at the same daylength with a low night temperature.

	LT/12.5	*HT/12.5*	*LT/13.5*	*HT/13.5*
Superior	90.92 ± 10.47	82.5 ± 2.33	111.7 ± 6.05	149 ± 5.35

Table 7: Mean dry weights (g) of pseudostems/plant produced at 245 DAP. Values are shown ± SE (n = 3).

	LT/12.5	*HT/12.5*	*LT/13.5*	*HT/13.5*
Reproductive	11.5 ± 5.94	0	20.3 ± 3.8	26.3 ± 6.8
Vegetative	10.7 ± 5.68	58 ± 1.14	4.63 ± 1.28	12 ± 1.14
Aborted	0	0.67 ± 0.33	0	0

Table 8: Mean number and type of rhizomes produced by cultivar S plants in all treatments at 245 DAP. Values are shown ± SE (n = 3).

Plant Development

The results of this experiment have been re-presented in graph form to aid the interpretation of data with respect to changes in vegetative and reproductive development over time. Only data from cultivar S plants have been included in the graphs since changes were most evident in these plants, which had been monitored more frequently and over a longer time period.

Cultivar S

The pattern of reproductive and vegetative development of myoga plants under all treatment conditions is presented in figures 1 - 4. From 120 to 174 DAP, only plants grown under a high night temperature and a long daylength had a sustained increase in pseudostem dry weight (figure 1). In addition these plants had a large increase in the number of reproductive rhizomes produced during this time (figure 2). Plants grown under these conditions were the most vigorous and by 174 DAP had the highest number of reproductive rhizomes. Plants grown under the same daylength but at the low night temperature produced minimal numbers of reproductive rhizomes from 120 to 174 DAP and did not display an obvious increase in pseudostem dry weight. From 174 to 245 DAP however, these plants showed a dramatic increase in pseudostem dry weight and numbers of reproductive rhizomes. As a result, at 245 DAP, while myoga plants grown at a 13.5 h daylength and a low night temperature had lower pseudostem dry weights than those at a high night temperature, the number of reproductive rhizomes was not appreciably different. The number of vegetative rhizomes produced by plants grown at the longer daylength at both night temperatures remained constant throughout the experiment and no abortion of flower buds was observed for these treatments.

Plants grown at the shorter daylength of 12.5 hours displayed a pattern of development characteristic of myoga plants grown below the critical daylength for flower bud development. This effect was most noticeable in plants grown at the high night temperature. From 120 to 174 DAP, plants grown under the short daylength with a high night temperature produced a high number of reproductive rhizomes but also displayed a substantial amount of flower bud abortion and

produced large numbers of vegetative rhizomes (figures 3 & 4). After 174 DAP, these plants entered a state of premature senescence with the rhizome mass comprised totally of vegetative rhizomes and storage tubers. This behaviour is typical of myoga plants grown under short day conditions.

Plants grown under the shorter daylength at a low temperature did not display these developmental characteristics. Although some abortion of flower buds was noted at 174 DAP, myoga plants grown under these conditions behaved more like those grown at the longer daylength. These plants produced similar numbers of vegetative and reproductive rhizomes at 245 DAP to those grown at 13.5 hours. In addition no further abortion of flower buds was evident in these plants at the last harvest.

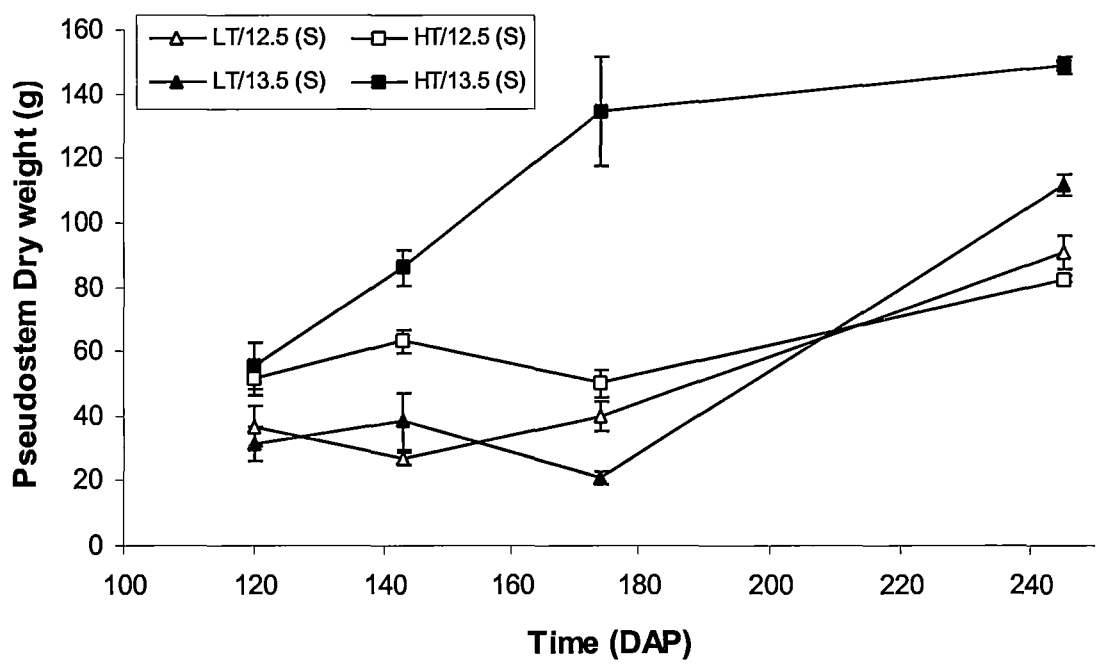


Figure 1: Mean dry weights of pseudostems/plant at 120, 143, 174 and 245 DAP for cultivar S plants grown under all treatment conditions. Bars show SE (n=3).

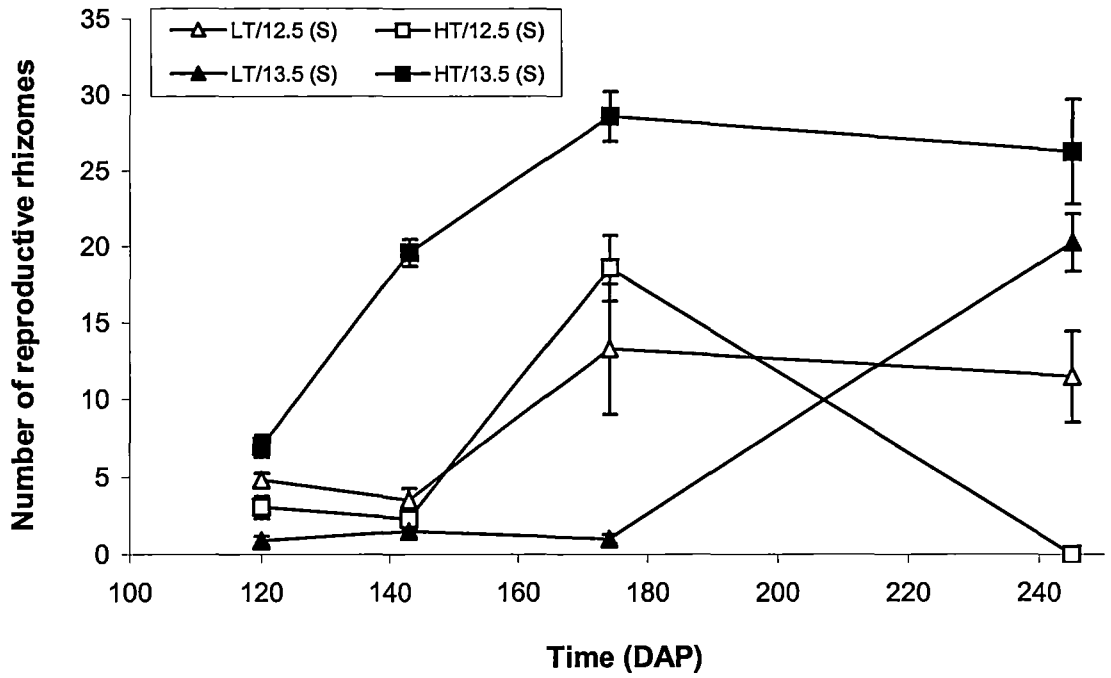


Figure 2: Number of reproductive rhizomes produced by cultivar S and I plants grown under all treatment conditions at 120, 143, 174 and 245 DAP. Bars show SE (n=3).

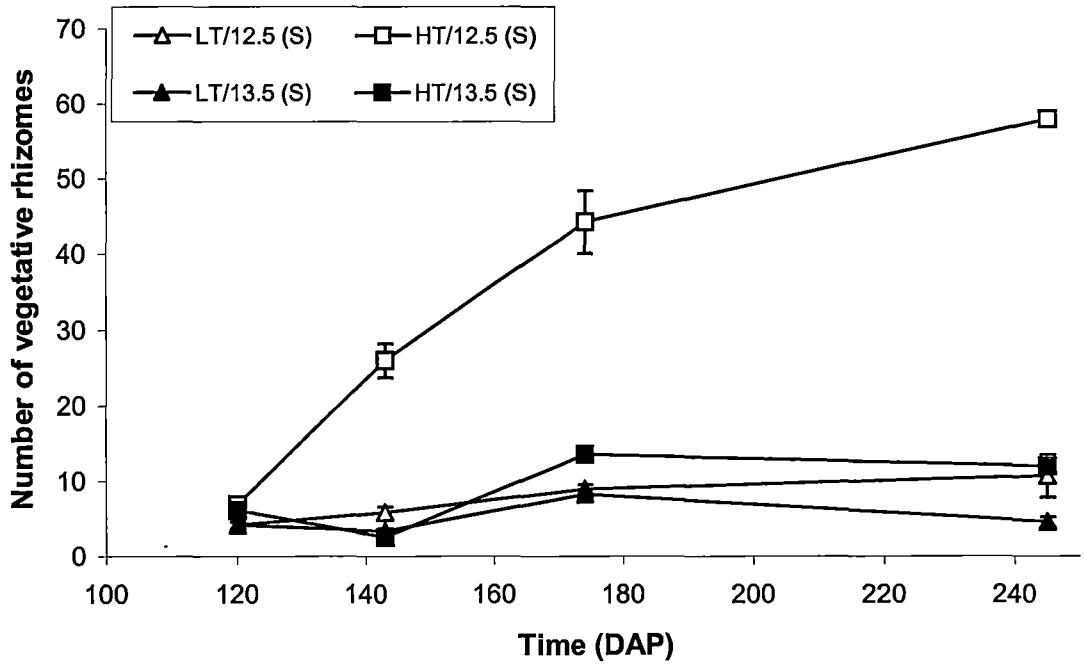


Figure 3: Number of vegetative rhizomes produced by cultivar S and I plants grown under all treatment conditions at 120, 143, 174 and 245 DAP. Bars show SE (n=3).

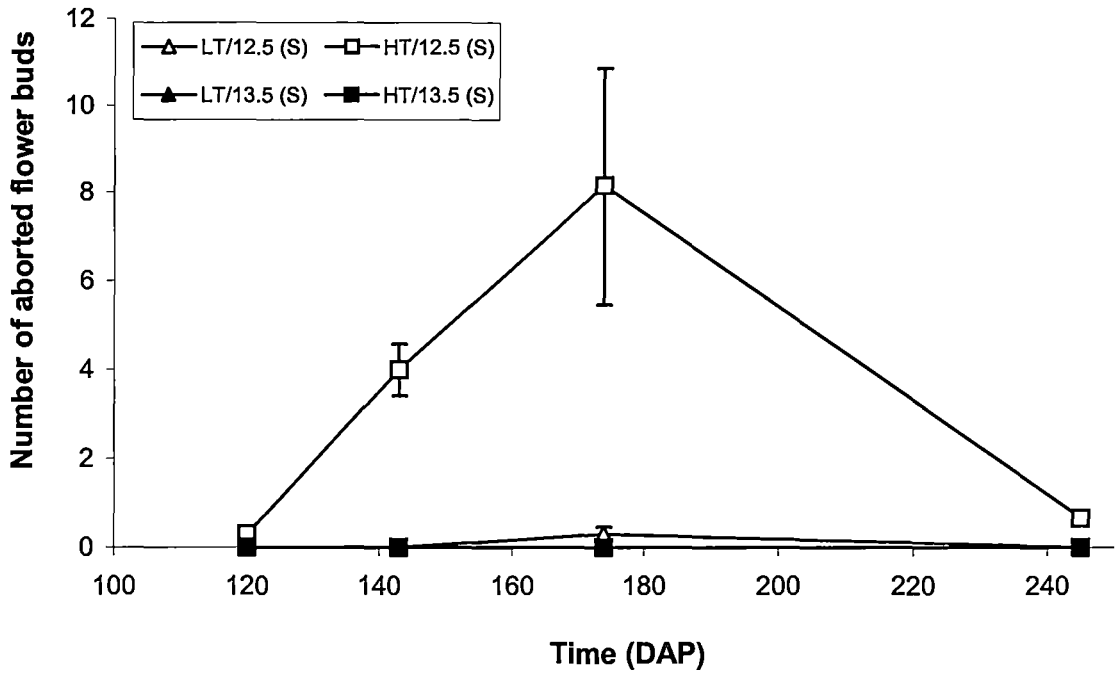


Figure 4: Number of aborted flower buds produced by cultivar S and I plants grown under all treatment conditions at 120, 143, 174 and 245 DAP. Bars show SE (n=3).

Cultivar I

Data on these plants was only available at two harvest dates, however it appeared that cultivar I plants displayed similar responses to cultivar S plants as a result of the treatment conditions (tables 9 & 1; from tables 1, 2, 5 & 6). Although plants grown at the high night temperature/long daylength conditions displayed substantial increases in pseudostem dry weight and number of reproductive rhizomes produced between 120 and 174 DAP, plants grown under the other treatment conditions displayed minimal reproductive and vegetative development. The abortion of flower buds observed in plants grown under the 13.5 h daylength with a high night temperature indicate that for this cultivar the daylength conditions may be marginal for flower bud development.

DAP	*LT/12.5*	*HT/12.5*	*LT/13.5*	*HT/13.5*
120	9.34 ± 3.25	7.24 ± 0.83	4.14 ± 1.88	19.60 ± 3.88
174	19.55 ± 2.09	24.59 ± 12.59	4.95 ± 0.35	72.89 ± 6.90

Table 9: Mean dry weights (g) of pseudostems/plant produced by cultivar I plants at 120 & 174 DAP. Values are shown ± SE (n = 3).

	DAP	*LT/12.5*	*HT/12.5*	*LT/13.5*	*HT/13.5*
Reproductive	*120*	0	0	0	5.33 ± 0.67
	174	0.67 ± 0.66	2.33 ± 2.32	0	20.4 ± 3.21
Vegetative	*120*	3.67 ± 0.33	4.67 ± 2.03	1.67 ± 1.67	4.33 ± 0.33
	174	6 ± 1.14	12.3 ± 4.58	3 ± 0.88	9.23 ± 1.44
Aborted	*120*	0	0.3 ± 0.17	0	0
	174	0	2.67 ± 0.15	0	1.63 ± 0.33

Table 10: Mean number and type of rhizomes produced by cultivar I plants in all treatments at 120 & 174 DAP. Values are shown ± SE (n = 3).

Discussion

The photoperiodic response of myoga should not be considered in isolation from temperature, since interactions between temperature and photoperiod are common and can play a major role in controlling flower initiation and development (Rees, 1987). An example of a plant where the requirement for a particular daylength is modulated by other environmental factors is the "Junebearing" strawberry (*Fragaria* x *anannassa* Duch.). Although this plant has a strict requirement for short day conditions in order to flower, when temperatures are lowered this daylength sensitivity is decreased and flowering will occur in long day conditions (Guttridge, 1985). Many other examples of qualitative SD and LD plants which become increasingly desensitized to daylength at decreasing temperatures have been documented (Bernier et al. 1981). Interactions between temperature and daylength are complex however and although certain environmental conditions will evoke the same response in a number of plants, the complete opposite has also been observed. For example in *Godetia quadrivulnera* (Dougl.) Spach., daylength sensitivity is increased at low temperatures so that at temperatures of 20 – 24^{0}C the plant behaves as a day neutral but at low temperatures is a qualitative LD plant (Halevy and Weiss, 1991).

Cultivar S plants grown at the longer daylength of 13.5 hours during this experiment, displayed a pattern of reproductive and vegetative development characteristic of myoga plants grown under long day conditions. The variation in night temperature did not appear to modify the flowering response at a daylength of

13.5 hours, however the rate of development was altered. It is well known that higher temperatures often accelerate the rate of floral development of plants grown in inductive daylengths (Rees, 1987). Therefore it was not surprising that myoga plants grown at the inductive daylength of 13.5 hours, with high night temperatures were observed to initiate greater numbers of flower buds at the first three harvests compared with those at lower night temperatures. In addition, plants that were exposed to the lower than optimal night temperatures were likely to have delayed reproductive development in response to the low temperatures. The characteristic photoperiodic response was not modified however, as plants at the longer daylength with low night temperatures did produce the same number of reproductive rhizomes as those at the high night temperature at the final harvest. Furthermore, no abortion of flower buds was observed in plants grown at 13.5 hours at either night temperature indicating that the long daylength requirement for flower bud development had been fully satisfied in both temperature treatments.

Temperature did appear to interact with photoperiod to cause a modification in the flowering response of those plants grown at the shorter daylength of 12.5 hours. Plants grown at this daylength with a high night temperature displayed a pattern of reproductive development characteristic of myoga plants grown under short day conditions. These plants were observed to initiate flower buds early but also displayed large numbers of aborted flower buds. In addition these plants produced very high numbers of vegetative rhizomes in relation to the number of reproductive rhizomes that were produced. At the final harvest these plants entered a premature state of senescence; behaviour which had also been observed in myoga plants grown under short day conditions during prior trials. Plants grown at the same daylength but with a low night temperature did not display these developmental characteristics. These plants were observed to behave in a manner attributed to myoga plants grown under long day conditions. Although a minor amount of flower bud abortion was evident at 174 DAP, these plants produced similar numbers of vegetative and reproductive rhizomes to those grown at 13.5 hours at 245 DAP. It seems likely therefore that at the shorter daylength of 12.5 hours, temperature has had a modifying effect on the flowering response. Low night temperatures appear to shorten the critical daylength required for flower bud development in myoga, with a daylength of 12.5 hours becoming permissible. If this conclusion is correct it may

be that in temperate areas such as Hobart, TAS where low night temperatures are commonly experienced, myoga plants could be grown at shorter daylengths in comparison to an area such as Rockhampton, QLD where the high ambient night temperature would require a longer daylength requirement to be satisfied.

Cultivar I plants appeared to follow the same treatment trends as cultivar S plants in terms of general plant growth and development, as observed in the weight of vegetation and number and type of rhizomes produced. However the dry weight of pseudostems and number of flower buds initiated were always lower. Therefore under the conditions tested, cultivar I myoga plants were poorer performers, consistently producing lower yields of flower buds. The only noted exception to this, was the development of cultivar I plants grown under high night temperatures with a daylength of 13.5 hours. Under these conditions both cultivars developed similar levels of vegetation and initiated similar numbers of flower buds. The effect of low temperature on retardation of plant development, was even more pronounced in cultivar I plants grown at the longer daylength, where plants grown under low night temperatures failed to initiate any flower buds by the third harvest at 174 DAP. It is possible therefore that cultivar I plants grown at a daylength of 13.5 hours are more sensitive to temperature than cultivar S plants.

The behaviour of cultivar I plants under the conditions tested suggests that these plants may have a longer critical daylength requirement for flower bud development than cultivar S plants. This conclusion was drawn from a number of results observed in the third harvest of this trial. Although both cultivars initiated similar high numbers of flower buds at the longer daylength of 13.5 hours with a high night temperature, abortion of flower buds was also observed in cultivar I plants grown under these conditions indicating that for this cultivar, the daylength was marginal for flower bud development. Previously observed behaviour of this cultivar during field trials in Southern Australia (Clark, 2001), and results from previous photoperiod trials where both cultivars grown at a daylength of 14 hours had similar reproductive development and no abortion of flower buds support this conclusion.

From the results of this trial it would appear that for cultivar S myoga plants a daylength of 12.5 hours is marginal for successful flower bud development. This

was particularly apparent in plants grown under the high night temperature where abortion of flower buds occurred and the plants entered a premature senescent state. Plants grown at a daylength of 13.5 hours were observed to successfully develop high numbers of flower buds, with no abortion occurring, therefore this daylength was more conducive for successful development of flower buds. It can be concluded that the critical daylength for flower bud production in cultivar S plants is approximately 13 hours. At a daylength range of more than 12 hours and less than 14 hours the response for flower bud development is not qualitative, but quantitative since flower buds in this trial were observed to reach anthesis at a daylength of 12.5 hours. However once daylength is equal to or becomes shorter than 12 hours successful flower bud development will not occur (CDL experiment). For cultivar I myoga plants it is likely that the critical daylength is approximately 14 hours. During this trial it was observed that cultivar I plants when grown at a daylength of 13.5 hours aborted flower buds, indicating that this daylength was marginal for flower bud development. However as with cultivar S plants it is likely that in the range of 13 to 15 hours a quantitative response to the imposed daylength will be observed, since the high numbers of reproductive rhizomes produced by cultivar I plants at this daylength indicated that some development of flower buds was likely to occur.

Results from this trial indicate that the flowering responses of myoga to photoperiodic conditions could be modified by the ambient night temperature. In particular it appeared that at low night temperatures the permissible daylength for flower bud development in cultivar S myoga was shorter compared to that which was acceptable under higher night temperatures. Furthermore, the abortion of flower buds in cultivar I plants, but not cultivar S plants at a daylength of 13.5 hours indicated that a difference in critical daylength did exist between cultivars, with the CDL for cultivar S plants refined to approximately 13 hours and for cultivar I plants approximately 14 hours.

SECTION 1: PHOTOPERIODISM – *EXPERIMENTAL WORK: CHANGES IN APICAL MORPHOLOGY DURING FLORAL INITIATION AND DEVELOPMENT*

Introduction

Floral initiation and development of the apical meristem has not been well documented for members of the Zingiberaceae family. In particular there has been no detailed study of the floral development of Myoga (*Zingiber mioga* Roscoe). During the series of experiments which investigated the photoperiodic nature of myoga, detailed dissections of vegetative and reproductive primordia were conducted. The stages of floral development, including the transition of the vegetative rhizome apex to a reproductive apex were noted and recorded. The development of individual flowers to form the flower bud was also observed. The flower bud represents the commercially harvestable component of myoga. Information on the reproductive phase of this plant is of importance in crop production.

Materials and Methods

Throughout a series of trials investigating the photoperiodic nature of myoga, rhizome material was collected during destructive harvesting of plants. This rhizome material was stored at 2^0C for a maximum period of seven days, until dissections could be conducted. Rhizome material retained structure and quality while stored at this temperature in plastic bags. Rhizomes were dissected under a Nikon stereoscopic microscope with a 6.3X zoom ratio to determine whether the rhizome apex was vegetative or reproductive. Repeated dissections of many rhizomes over a long time period allowed for the identification of stages of floral development. Several fresh specimens were placed into an environmental scanning electron microscope (ESEM) and the stage of development recorded. Meristem tissue which had reached an advanced reproductive stage was larger than was practicable for use in the ESEM and therefore these stages of development were recorded using a Zeiss Tessovar. The branching pattern of developing rhizomes was recorded using the same system as Gracie et al. (*in press*), which permitted the

position of developing flower buds within the plant structure to be identified. The development of the rhizome apex from floral initiation through to anthesis was observed through dissections of rhizome material and while the plant was *in situ*. Once the flower bud had reached anthesis the morphology of the fully developed flower was described.

Results and Discussion

Throughout this thesis the modified floral structure that comprises the commercially yielding part of the myoga plant, has been referred to as a flower bud. Although this terminology is used during commercial cultivation and marketing of myoga, it is not strictly a correct definition, as this structure actually contains many flower buds. The individual flowers of myoga are grouped together in a spike inflorescence. The bracts of each individual flower widen and thicken as the inflorescence develops to form the modified flower bud structure. An 'inflorescence bud' could therefore be a more correct definition of this structure, however to avoid confusion with earlier work, this structure will continue to be referred to as a flower bud. Flower buds were initiated at the apical meristem region of developing rhizomes throughout the current season of growth, once the plant had reached a certain level of maturity. Therefore at each harvest any one plant contained flower buds at differing stages of development. Flower bud initiation was observed to occur predominately on second, third and fourth order rhizomes. Once the rhizome apex had initiated a flower bud the rhizome continued to develop towards the soil surface. Anthocyanin levels within the bracts increased as the flower bud reached maturity giving the flower bud an attractive crimson 'blush'.

The mature flower had petals which were pale yellow, zygomorphic and approximately 5 cm in diameter. The petals and sepals were fused to form a perianth tube which was elongated and exserted from the bracts. Within the perianth tube were modified stamen filaments which distally bore the anther (figure 1). The anther, connected to the stamen filaments by the anther connective, contained two pollen sacs containing pollen grains. The stigma was small with 2 long and narrow glands. The style was slender and the inferior ovary was 3-locular with many-

ovules (Bailey, 1963). According to Palmer, 1984, myoga is a pentaploid with production of seed unknown.

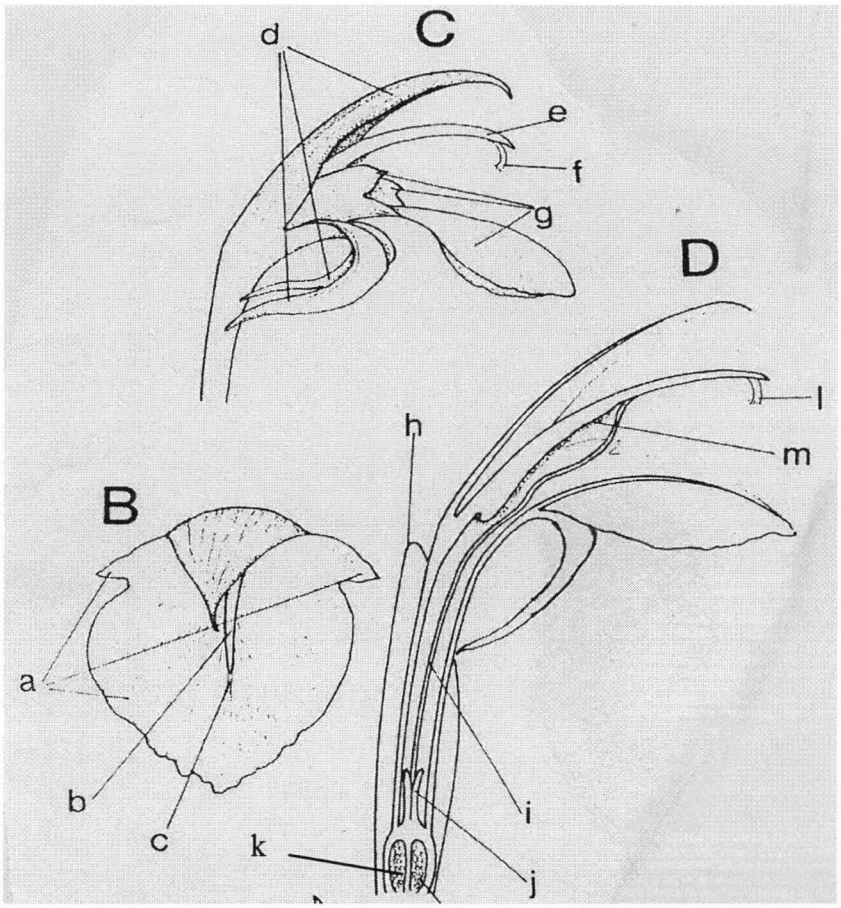


Figure 1: Botanical drawing of a *Zingiber mioga* Roscoe flower (Mr Dennis Morris, Tasmanian Herbarium, Department of Museum and Art Gallery).

Diagram	***Description***
B	Flower, front view: a – modified stamen filaments, b – anther connective, c – stigma;
C	Flower, side view: d – inner perianth lobes, e – anther connective, f – stigma, g – modified stamen filaments;
D	Section through flower: h – outer perianth lobe, i – style, j – nectaries, k – ovary, l – stigma, m – anther.

Table 1: Botanical description of floral diagrams presented in figure 1.

Flower Ontogeny

The morphological changes that were associated with the transition of the rhizome apex from vegetative to reproductive were rapid and obvious when induction occurred. Vegetative meristems were characterized by a small, domed appearance, with the leaf primordium overlapping the meristematic region with an apical diameter of less than 300 um (plate 1). During the first stage of floral development the axis of the rhizome apex began to elongate as the apex widened and became slightly flattened. Initiation of bract primordia also became obvious during this first stage (plate 2). The bracts were distinguishable from the leaf primordia as they were shorter and did not enclose the developing meristematic region. Following bract initiation the development of new meristematic regions was observed. This is shown in plate 3 where three new secondary floral primordia were initiated at the edges of the apical meristem at approximately 90^0 angles to each other. In plate 4, the first secondary flower primordium is evident, while the apical meristem region continues to develop new floral primordia. The secondary floral primordia were distinguishable from the apical meristem by the presence of a new meristematic region completely surrounded by bract primordia. As more secondary floral primordia were formed the meristematic zone in the apex became more domed and reduced in size.

The floral primordia furthest away from the meristematic region of the apex were the most developed, with the most recently formed ones closest to the apex. Development of individual secondary floral primordia was first observed as an elongation of the domed meristematic zone along the vertical axis. This was followed by the formation of a depression in the middle of this zone, with the half of the secondary flower primordia closest to the rhizome apex being raised up so that it was higher than the other half, furthest away from the apex (plates 5 & 6). The upper half of the meristem region divided into two small rounded domes which developed into the anther and upper stamen filament. On the other side of the depression were two smaller oval domes which formed the lower stamen filaments which in the mature flower are split. The depression deepened with the continued development of the anthers, stamen filaments and perianth tube. An inferior ovary developed at the base of this depression, with the style also developing out of this

depression. The perianth tube, consisting of fused sepals and petals would most likely have been initiated prior to the anther and stamen filaments but was not clearly visible until the flowers were quite well developed.

Stage	***Description***
0	Shoot meristem domed, typical of vegetative condition (plate 1).
1	Meristem elongates and flattens and bracts are initiated (plate 2).
2	Meristematic region of secondary floral primordia initiated (plate 3).
3	Separate secondary floral primodia observed. New floral primordial continue to be initiated in apex meristem region (plate 4).
4	The meristematic region of secondary floral primordia elongates along the vertical axis (plate 5).
5	Depression formed in the middle of meristem region (plate 5 and 6).
6	Half of meristem region closest to the apex rises up above half of meristem furthest away from apex (plate 6).
7	Raised half of meristem divides into two round domes (Initiation of anther). Lower half of meristem divides into two oval domes (Initiation of modified stamen filaments). Perianth visible at this stage (plate 7, 8 and 9).
8	Anther and modified stamen filaments are clearly observed. The style and inferior ovary although not visible in the plate have also developed (plate 7 and 8).

Table 2: Stages of development of the apical meristem and developing floral primordia of *Zingiber mioga* Roscoe.

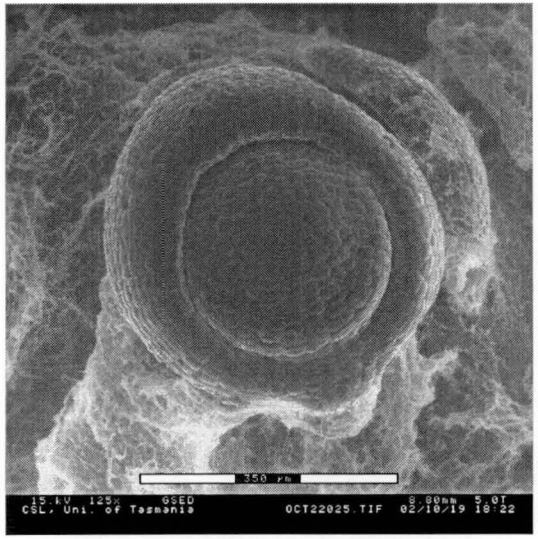


Plate 1: Vegetative bud with leaf primordia overlapping the meristem. Stage 0.

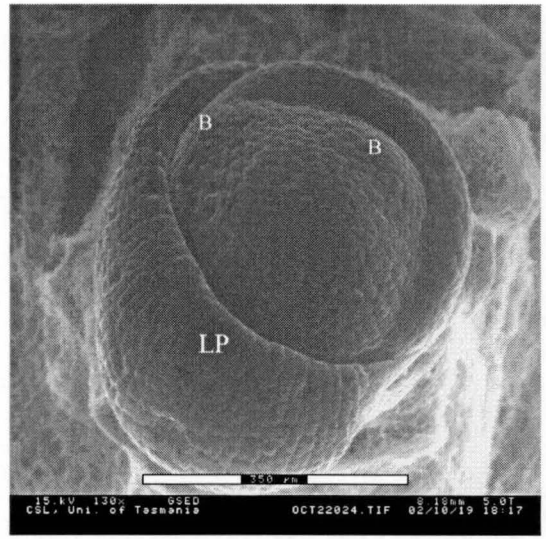


Plate 2: Meristem at stage 1. Two initiated bracts are visible (B). Leaf primordia are also visible (LP).

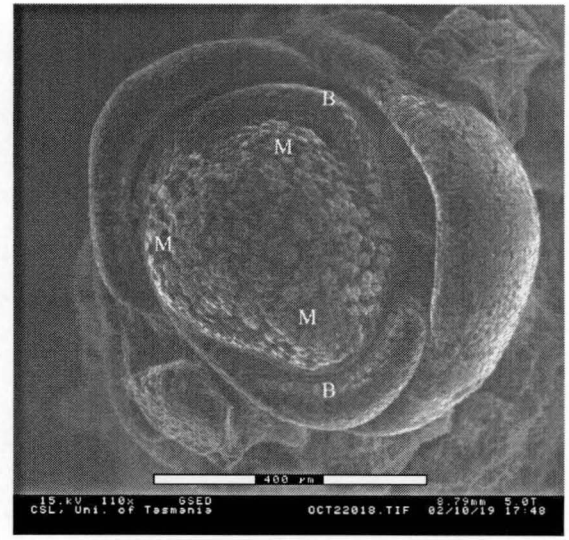


Plate 3: Meristem at stage 2. Two bracts are visible (B). Three new meristem regions are initiated (M).

Plate 4: Meristem at stage 3. Individual secondary floral primordia visible (SF). Apex (A) continues to initiate new secondary floral primordia.

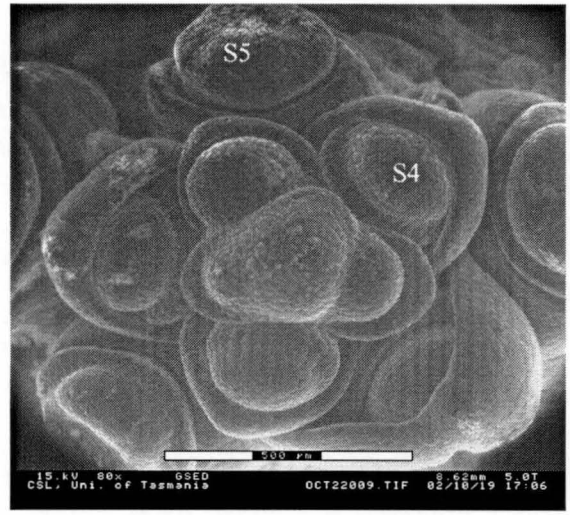


Plate 5: Secondary floral primordia at stage 4 (S4) and 5 (S5). Meristem region of floral primordia at S4 is elongated. Depression observed in middle of floral primordia at S5.

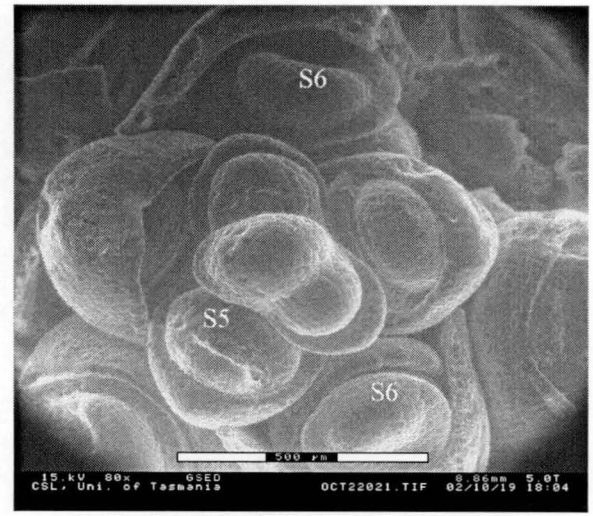


Plate 6: Secondary floral primordia at stage 5 (S5) and 6 (S6). Half of meristem closest to apex is raised above other half furthest away from apex.

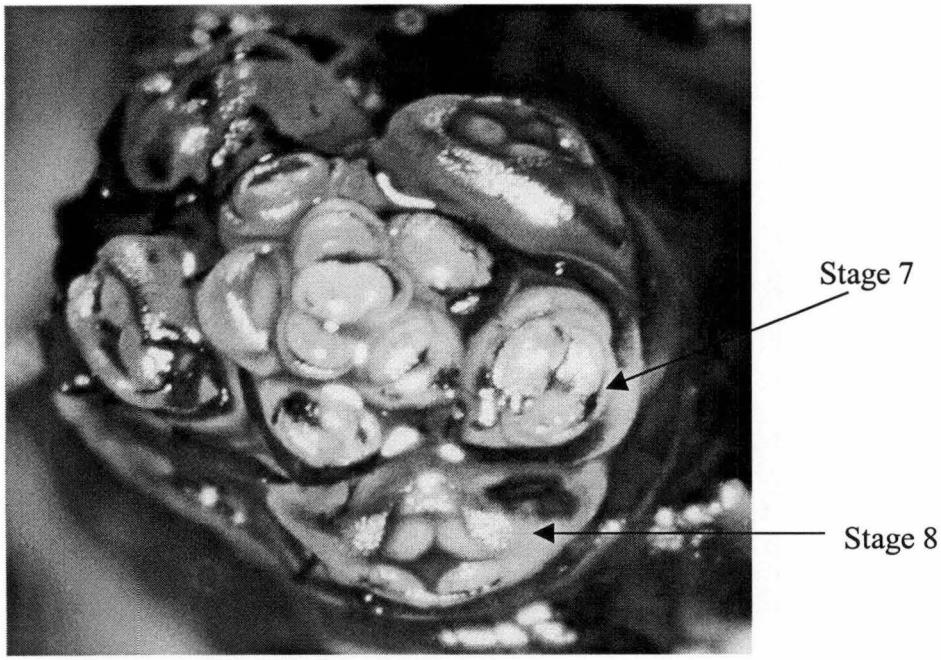


Plate 7: Stages 7, 8

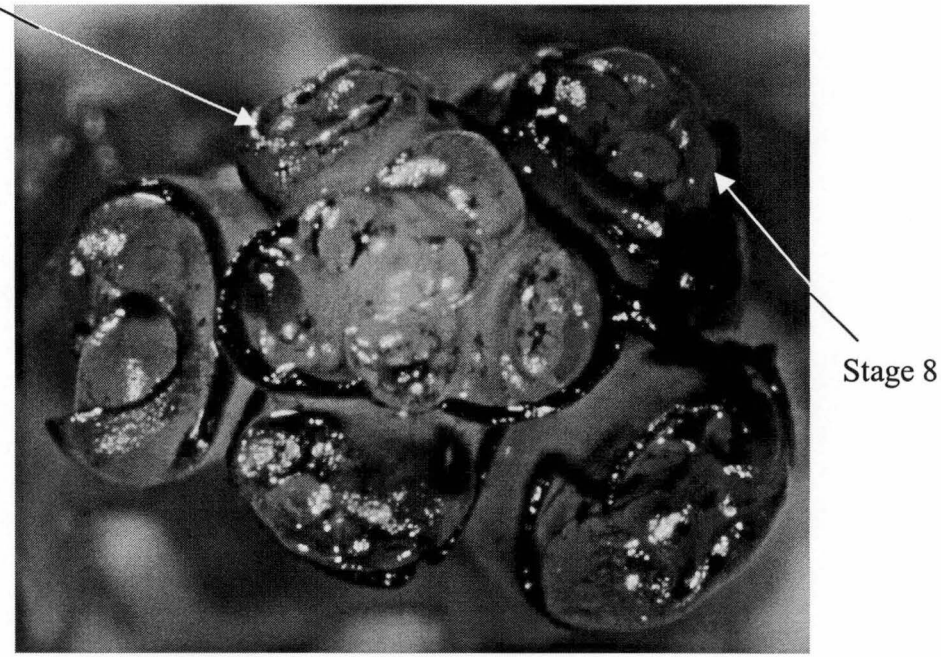


Plate 8: Stages 7, 8

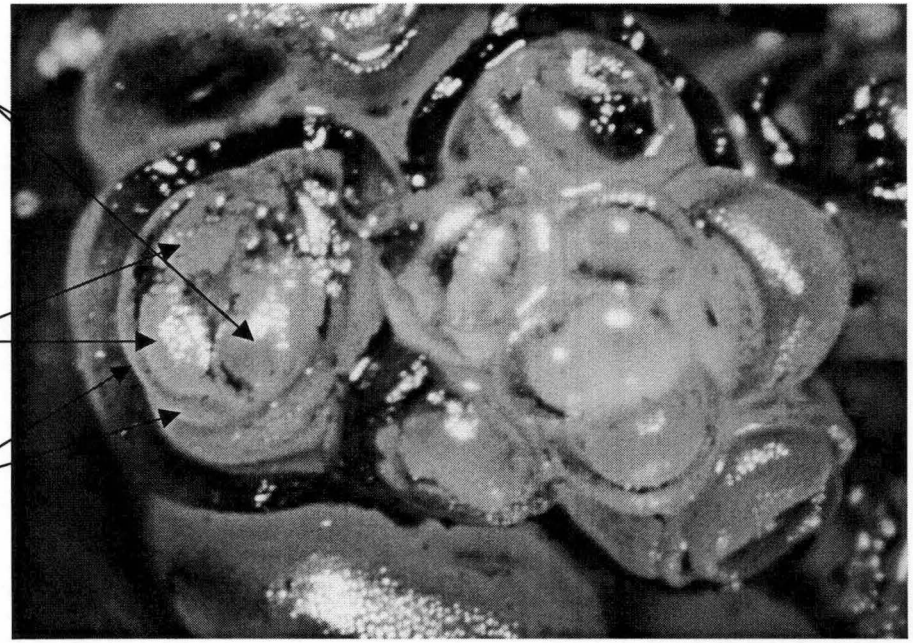
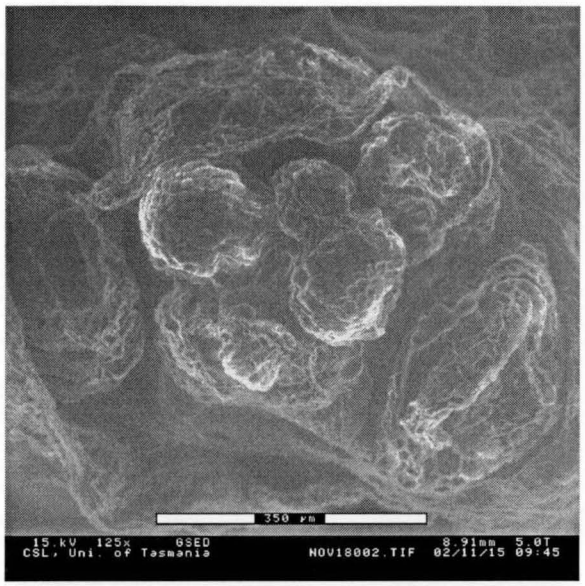
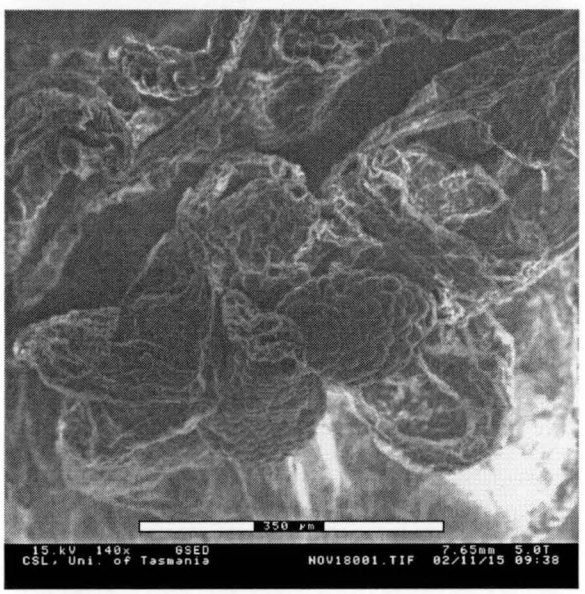


Plate 9: Stage 7

Abortion of initiated floral primordia was also recorded (plate 10). These aborted primordia were characterized by a pink discoloration and loss of primordial structure and turgidity. The stage of reproductive development which had been achieved prior to abortion occurring was difficult to ascertain due to the immediate and complete degradation of flower primordial structure. Abortion of floral primordia was predominately observed in plants that had been exposed to short day conditions. It is unknown whether the rhizome apex reverted back to a vegetative state subsequent to abortion or whether new floral primordia were initiated, since rhizome dissections were not conducted according to a planned time line. From observations of flower bud number during successive destructive harvests of photoperiod trials it seems more likely that the rhizome apex would have reverted back to a vegetative state, instead of re-initiation of new floral primordia occurring.





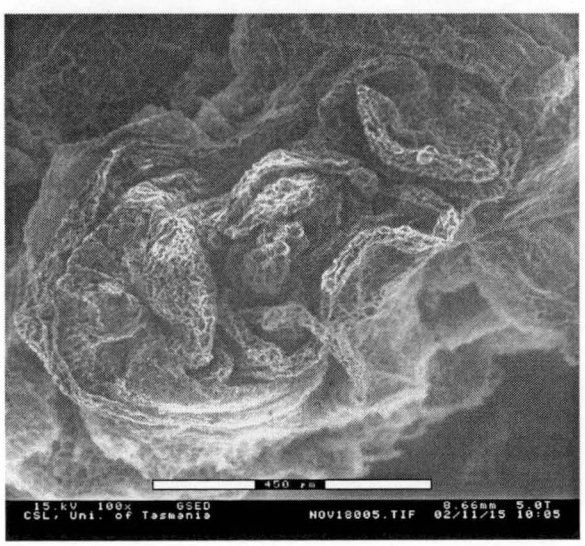


Plate 10: Aborted floral primordia of *Zingiber mioga* Roscoe.

Zingiber mioga Roscoe was shown during this investigation to follow a distinct pattern of floral development. Enlargement and flattening of the meristem was followed by initiation of bract primordia around the rim of the apical meristem. Secondary floral primordia were then initiated and developed centripetally, while the apex meristem continued to initiate new floral primordia. Development of floral organs occurred in a zygomorphic pattern with the anther and stamen filaments closest to the apex developing prior to those organs furthest away from the apex. This work contributes significantly to current knowledge of floral development in the Zingiberaceae family as it appears very little research has been done in this area. The identification of the developmental stages of the floral primordia will also be of assistance in further work which aims to investigate and quantify the effect of environmental factors on flower initiation and development in myoga.

Conclusion

Statistical analysis of data from these experiments was not appropriate considering the lack of true replication. However the conclusions drawn from this work are based on results from a series of trials that often replicated similar environmental conditions. Inferences made in initial experiments on the response of myoga plants to environmental stimuli were supported by following experiments where myoga plants responded comparably to the environmental conditions in which they were grown. It may be concluded therefore, that the developmental responses observed in myoga plants during the preceding experiments were due to the effect of the photoperiod and temperature treatments.

The first of a series of investigations into the photoperiodic nature of myoga established that myoga was a photoperiodically sensitive plant. Plants grown under long day conditions (16h) and short day conditions (8h) with a night break produced flower buds, while those under short day conditions (8h) did not. The failure of plants under short day conditions to produce flower buds was attributed to abortion of developing flower buds rather than a failure to initiate floral primordia. It was concluded that for flower bud development in myoga a qualitative long day requirement must be satisfied. Flower bud initiation did not appear to require a particular daylength, it was therefore thought to be day-neutral.

During the second trial myoga plants grown under 14 hour daylengths produced flower buds, while those grown under 10 and 12 hour daylengths did not. The failure of plants grown under 10 and 12 hr daylengths was once again a result of abortion of developing flower buds in response to short day conditions, rather than a failure to initiate inflorescences. It was further shown that plants grown under the shorter daylengths of 10 and 12 hours initiated flower buds at an earlier stage than those grown under 14 hr daylengths. It was concluded that while flower development in myoga had a qualitative long day requirement, flower initiation was promoted by short daylengths. The critical daylength for flower bud development in cultivar S and I myoga plants was identified as between 12 and 14 hours.

The third photoperiod trial was established so that the photoperiod sensitive and insensitive phases of myoga could be determined. In addition knowledge of the effect of daylength on the pattern of vegetative and reproductive development in myoga was greatly increased. Although it was not possible to identify the point at which myoga first became sensitive to photoperiod, it was established that subsequent to 150 days after planting, photoperiod no longer had an effect on the reproductive development of the plant. It was also shown that abortion of flower buds subsequent to initiation could occur within ten days of exposure to short day conditions. It was also observed that in myoga plants that had experienced flower bud abortion in response to short day conditions, the normal pattern of vegetative and reproductive development in myoga was permanently altered. As a result, plants that had experienced a certain amount of flower bud abortion ceased to initiate new reproductive rhizomes.

Temperature was observed to interact with photoperiod resulting in cultivar S plants grown at low night temperatures developing flower buds at a daylength not permissive for flower bud development at higher night temperatures. At a daylength of 13.5 hours, high night temperatures increased the rate of floral development so that plants grown under these conditions initiated higher numbers of flower buds than those grown at low night temperatures at the first three harvests. The critical daylength for cultivar S myoga plants was further refined to approximately 13 hours but it was noted that between a daylength range of 12 to 14 hours a quantitative response, rather than a qualitative response to daylength was likely to be observed.

Initial experience with myoga plants at high latitudes (43°S) revealed two apparently distinct types or cultivars with different flowering and vegetative growth characteristics (Clark, 2001). The cultivar currently referred to as Inferior (I), failed to produce harvestable flower buds and senesced prematurely after two to three years' growth, when cultivated in Southern Australia. Plants of the Superior cultivar were higher yielding and did not senesce prematurely, however under semi-commercial conditions, flower bud yield fluctuated from season to season.

Myoga plants exposed to short day conditions during this series of photoperiod trials were all observed to senesce subsequent to abortion of flower primordia, and

at a time when flower buds were observed to emerge from plants grown under long photoperiods. This indicated that the plants under SD treatments tended to enter a senescent state far earlier than plants under long photoperiods. This behaviour may provide an explanation for the difference in performance observed between cultivars I and S myoga plants in the field. If conditions are such that poor initiation and/or poor early flower bud development occurs, then plants exhibit early senescence. Inferior plant types, particularly in mature plantings have consistently performed poorly under Tasmanian conditions (Clark, 2001). To date no clear explanation has been found for this behaviour, however it is possible that the inferior type has a longer photoperiod requirement for flower bud development and that this requirement was not being met. Thus flowers aborted before flower bud development could commence and the plant was forced into early senescence and dormancy.

During these trials it was also noted that cultivar S plants performed better when grown at the more marginal daylengths of 10 and 12 hours compared with cultivar I plants, while at the longer daylength of 14 hours the reproductive development of both cultivars was quite similar. In addition the abortion of flower buds in cultivar I plants, but not cultivar S plants when grown at a daylength of 13.5 hours with a warm night temperature indicated that this was a short daylength for cultivar I plants but not for cultivar S plants. The data from these trials support the conclusions drawn above, that cultivar I plants have a longer critical daylength requirement for flower bud development of approximately 14 hours, and that for this reason, differences in growth patterns are observed when cultivar S and I plants are field cultivated in Southern Australia.

Industry Implications

The identification of the photoperiodic nature of myoga has great significance for the future development of this crop in Australia. Areas identified as suitable for future productions sites are predominately situated on the eastern seaboard of Australia. The geographical range of these locations is quite wide and therefore photoperiod will be an important consideration in determining what locations are suitable. Flower buds will only develop in areas where the daylength is equal to or greater than 13 hours. In tropical areas such as Northern Queensland, where it is hoped to cultivate myoga year round, daylength will become a limiting factor during the winter period. Night break lighting can then be used in commercially cultivated crops to interrupt the dark period, artificially creating two short dark periods which will satisfy the long day requirement of myoga plants. The identification of the developmental phases of myoga, which are sensitive to photoperiod will also enable the correct use of artificial lighting and prevent the use of lights when they are not required. This knowledge will also aid the scheduling of crops so that as many production cycles as possible can occur within a year. The geographical range of potential production sites also means that temperature will have a determining effect on the development of this crop. As temperature was found to interact with photoperiod to modify flowering responses, the ambient night temperature will have to be taken into consideration when determining whether the ambient photoperiod is suitable for production of flower buds.

SECTION 2: LOW-TEMPERATURE INDUCED PHOTOINHIBTION – *INTRODUCTION*

Production trials conducted at Rockhampton, Qld (Section 3) demonstrated that myoga plants could be grown in a cultivated field situation without the protection of shade cloth. Although plants experienced some scorching of vegetation and were shorter in height than those cultivated in more protected conditions, flower bud yields were not significantly different. Under New Zealand conditions, myoga pseudostems were reported to burn and become chlorotic without shade, resulting in a reduction of flower bud yield (Douglas and Follett, 1992). For this reason myoga has been cultivated in New Zealand and Southern Australia under 50% shadecloth. The observed differences in plant performance when grown in Southern and Northern Australia led to the hypothesis that myoga plants cultivated in Southern Australia may become photodamaged in response to the combined stresses of strong radiation and low temperature.

Photoinhibition, both of a protective and damaging nature occurs when plants absorb excessive amounts of sunlight. Photoprotective mechanisms exist within plants, which act to reduce the amount of light reaching the reaction centres of photosystem II, however when these mechanisms are exhausted photodamge occurs. In some plants exposure to low temperatures either prior to or during light exposure can increase their susceptibility to photodamage.

The sensitivity of myoga to light intensity, and the effect of low temperature on this sensitivity was determined through a number of experiments. Initial trials conducted within controlled environment cabinets, aimed to establish at what light intensities during low temperature exposure did myoga plants become photoinhibited, and at what rate did they recover. In addition plants were exposed to a moderate light intensity at varying temperatures to determine if susceptibility to photoinhibition was decreased at warmer temperatures and if the recovery rate was increased. Subsequent trials examined the effect of preceding cool nights on the susceptibility of myoga plants to photoinhibition the following day and if myoga plants could become acclimated to low temperatures, therefore increasing their ability to regulate photoinhibitory conditions.

SECTION 2: LOW-TEMPERATURE INDUCED PHOTOINHIBTION – *LITERATURE REVIEW*

Photoinhibition

Absorption of excess sunlight often leads to photoinhibition, which may be defined as a light-dependant down regulation of the quantum yield of photosynthesis (Atwell et al. 1999). Photoinhibition is one of the most important regulatory mechanisms in photosynthesis and results from a series of internal photoprotective mechanisms, which act to reduce the amount of light reaching the reaction centres of Photosystem II (PS II). Photoprotection is normally sufficient to cope with light absorbed by leaves, however when this capacity for photoprotection is exhausted photodamage occurs. Photodamage also known as chronic photoinhibition, is manifest as a decline in both quantum yield and light-saturated rate of photosynthesis (P_{max}) and recovers only very slowly (hours to days), whereas dynamic photoinhibition recovers much faster (minutes to hours). Severe photodamage results in bleaching of pigments and damage to membranes (photo-oxidation) and may lead to tissue death (Atwell et al. 1999).

The primary site of attack within PS II is the reaction centre, where certain protein subunits (e.g. D1, the 32-k Da protein) are rapidly broken down. Photosynthetic electron transport is thus interrupted and the efficiency of PS II is decreased (PS II photoinactivation) (Hamlyn *et al.* 1989). As an immediate protective measure, the excess radiation energy is diverted directly from the photosystems via fluorescence and, more importantly as heat. This non-photochemical conversion of light energy involves a special group of carotenoids known as xanthophylls, which undergo interconversion in response to excess light. Energy is dissipated by the xanthophyll cycle, in which, with the participation of ascorbate and $NADPH_2$, the di-epoxide violaxanthin is reduced to zeaxanthin via the mono-epoxide antheraxanthin. The conversion of violaxanthin to zeaxanthin takes place within a few minutes in the presence of strong irradiance. Reconversion to violaxanthin, which again consumes reductants, also occurs rapidly in weak light or darkness (Hamlyn *et al.* 1989).

As photoinhibition involves photochemical inactivation of PS II and photosystem I (PS I), all photosynthetic organisms should be potentially susceptible at some incident photon flux density (PFD). However the degree of susceptibility is under the influence of many factors, both genotypic and environmental. The imposition of additional stress factors prior to and during exposure to a high PFD exacerbates the adverse effect of light. Low temperature is one stress factor that can increase the sensitivity of plants to photoinhibition (Strand and Lundmark 1987; Ball et al. 1991; Groom et al. 1991). This occurs primarily through an inhibition of the dark reactions of photosynthesis that increases the probability for excessive excitation of PS II (Huner et al. 1993; Oquist et al. 1993a). Low temperature also promotes photoinhibition by suppressing the PS II repair cycle (Greer 1990; Greer et al. 1986, 1991; Krause 1994; Fryer et al. 1995) and by slowing the rate of xanthophyll de-epoxidation (Bilger and Bjorkman 1991; Krause 1992; Adams and Demmig-Adams 1995).

Chilling in the Light

Chilling in the light enhances photoinhibition in both chilling-sensitive and chilling-tolerant plants and the basic mechanism of photoinhibition, insofar as PS II is involved appears to be the same (Krause, 1994). However they differ in the rate of recovery from a photoinhibited state (Baker et al., 1994; Greer, 1986). At low temperatures, chilling-sensitive species are unable to induce an adequate repair mechanism and therefore photo-chemical inactivation results. Chilling-tolerant species can probably tolerate these conditions better than sensitive species because the repair mechanism remains functional at low temperatures (Greer, 1990).

Acclimation to Low Temperatures

Acclimation of plants to low temperatures can significantly reduce their susceptibility to low temperature-induced photoinhibition. Experiments with kiwifruit grown at lower temperatures than normal showed that on subsequent exposure to high PFD's susceptibility to photoinhibition was reduced. Growth temperature therefore had an important role in affecting susceptibility of kiwifruit leaves to photoinhibition relative to the effect of PFD or the exposure temperature

(Greer, 1990). These results agree with those of Krause and Somersalo (1989), Somersalo and Krause (1989) and Mawson and Cummings (1989) that cold acclimation reduces the sensitivity of leaves to photoinhibition. Plants grown at lower than normal growth temperatures at moderate PFD's have however, in response developed chronic photoinhibition. Greer (1990) observed a significant and semi-permanent decline in fluorescence emissions from kiwifruit leaves grown at lower than normal temperatures and a correlated though smaller decrease in photon yield (Greer and Laing, 1989). Somersalo and Krause (1989) also observed pronounced changes in both variable fluorescence (Fv) and the fluorescence ratio (variable fluorescence/maximal fluorescence (Fv/Fm)) and in photon yield in spinach acclimated to low temperatures. Thus, cold acclimation in leaves is a compromise between increased resistance to low temperature-induced photoinhibition and a reduced efficiency for photosynthesis.

Chilling-Induced Stomatal Closure

Many studies have investigated the combined effect of chilling temperatures and high light intensities on both chilling-sensitive and chilling-tolerant species (Terashima et al, 1998; Hetherington et al, 1989; Warren, et al., 1998). However the investigation of chilling in the dark and its subsequent effect on the susceptibility of the plant to photoinhibition has been less widely studied. It has been shown that for some chilling-sensitive herbaceous species, although the development of chilling-dependant injury in the dark is slower, low temperatures alone (i.e. at night) can cause severe reduction in both CO_2 fixation and transpiration on the day following the chill (Crookston et al., 1974; Izhar et al. 1967; Pasternak and Wilson, 1972). The low-temperature effect on transpiration is a reflection of the slow and incomplete stomatal response of chilling sensitive plants. The good correlation often reported between chilling-induced decreases in rates of photosynthesis and transpiration (Pasternak and Wilson, 1972; Crookston et al., 1974) has frequently been seen as indicative of a causal relationship between chilling-induced stomatal closure and a reduced rate of photosynthesis.

The most direct explanation of the persistent post-chilling reduction of stomatal aperture is the frequent coincidence of a decrease of leaf water potential. The leaf

water status deteriorates in many cases during chilling exposure, due to comparatively high transpiration rates resulting from the paralysed stomata closing only slowly and incompletely (Eamus et al., 1983), combined with a decreased hydraulic water conductivity through root plasma membranes (McWilliam et al., 1982) at low temperature.

Stomata remain partly closed subsequent to exposure to chilling temperatures, also due to certain events not related to water stress. The response of stomata to temperature is complex because, in addition to a direct effect of temperature upon the metabolism of the guard cells, temperature will have an indirect effect through vapour pressure differences (VPD) and the intercellular CO_2 concentration (C_i) (Öquist & Martin, 1986). A decrease in temperature generally decreases the stomatal aperture, resulting in a decreased concentration of CO_2 in the intercellular spaces. There are however, also examples of how a low-temperature induced decrease of the stomatal conductance is paralleled by an increase in C_i (Drake and Raschke, 1974; Raschke, 1975; Pearcy, 1977; Mooney et al., 1978; Hällgren et al., 1982). Therefore it is possible that the stomata close as a result of an increased C_i, when the demand for CO_2 decreases because of low-temperature inhibition of the biochemical processes of photosynthesis. Except when low temperatures decrease the water uptake by plants through chilling as discussed above, there are no clear examples of temperature-induced stomatal closure being the primary cause of low-temperature inhibition of photosynthesis. Rather it appears that stomatal conductance adapts or acclimates in concert with the low temperature effects on the biochemical processes of photosynthesis (Öquist & Martin, 1986).

Chlorophyll fluorescence techniques

Chlorophyll fluorescence has been used widely in recent years to understand both the mechanism of photosynthesis itself and the mechanism by which a range of environmental factors alter photosynthetic capacity (Bolhar-Nordenkampf et al., 1989). Fluorescence measurements made over a diurnal course can yield information pertaining to non-photochemical quenching (NPQ), electron transport rates, quantum efficiency and the extent of photoinhibition in response to light, temperature and other single or combined environmental stresses (Bilger et al.,

1995). Early measurements used a sustained decrease in dark-adapted Fv/Fm and an increase in minimal fluorescence (Fo) to indicate the occurrence of photoinhibitory damage in response to high temperature (Gamon and Pearcy, 1989), low temperature (Groom and Baker, 1992) and excess PFD (Ögren and Sjöström, 1990). Despite improvements in technology and the evolution of modulated systems, these observations remain valid and changes in Fv/Fm and Fo are still widely accepted and used as reliable diagnostic indicators of photoinhibition (He et al., 1996; Valladares and Pearcy, 1997).

Light energy absorbed by chlorophyll molecules may be used either in photochemical reactions, dissipated as heat or re-emitted as light emissions called fluorescence. These three processes occur in competition, such that any increase in the efficiency of one will result in a decrease in the yield of the other two. Hence, by measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be gained (Maxwell & Johnson, 2000).

An increase in the yield of chlorophyll fluorescence will occur when photosynthetic material is transferred from the dark into the light. This rise over a period of approximately one second has been explained as a consequence of reduction of electron acceptors in the photosynthetic pathway, downstream of PS II, notably plastoquinone and in particular, Q_A. Once PS II absorbs light and Q_A has accepted an electron, it is not able to accept another until it has passed the first onto a subsequent electron carrier (Q_B). During this period, the reaction centre is said to be 'closed'. At any point in time, the presence of a proportion of closed reaction centres leads to an overall reduction in the efficiency of photochemistry and so to a corresponding increase in the yield of chlorophyll fluorescence. Following on from this, the chlorophyll fluorescence level typically starts to fall again, over a time-scale of a few minutes. This phenomenon, termed fluorescence quenching, is explained in two ways. Firstly, there is an increase in the rate at which electrons are transported away from PS II; this is due mainly to the light-induced activation of enzymes involved in carbon metabolism and the opening of stomata. Such quenching is referred to as 'photochemical quenching'. At the same time, there is an

increase in the efficiency with which energy is converted to heat. This latter process is termed 'non-photochemical quenching' (NPQ). In a typical plant, changes in these two processes will be complete within about 15–20 min after which an approximate steady-state is attained, although the time taken to reach this state can vary significantly between plant species (Johnson et al., 1990).

In order to gain useful information about the photosynthetic performance of a plant from measurements of chlorophyll fluorescence yield, it is necessary to be able to distinguish between the photochemical and non-photochemical contributions to quenching. The usual approach is to 'switch off' one of the two contributors, specifically photochemistry, so that the chlorophyll fluorescence yield in the presence of the other alone can be estimated.

The 'light doubling' technique, allows the contribution of photochemical quenching to be transiently reduced to zero (Bradbury and Baker, 1981; Quick and Horton, 1984). In this approach, a high intensity, short duration flash of light is given. The effect is transiently to close all PS II reaction centres. Provided the flash is short enough, no (or a negligible) increase in non-photochemical quenching occurs and no long-term change in the efficiency of photosynthesis is induced. During the flash, the fluorescence yield reaches a value equivalent to that which would be attained in the absence of any photochemical quenching, the maximum fluorescence, Fm. Comparison of this value with the steady-state yield of chlorophyll fluorescence in the light (F_t) and the yield of chlorophyll fluorescence in the absence of an actinic (photosynthetic) light (Fo) give information about the efficiency of photochemical quenching and by extension, the performance of PS II (Maxwell & Johnson, 2000).

As well as changes occurring in the efficiency of photochemistry, the efficiency of heat dissipation (i.e. non-photochemical quenching) can change depending on various internal and external factors. Such changes are reflected as changes in the level of Fm. Unlike photochemistry, it is not possible to inhibit heat dissipation totally, so it is not possible to measure the yield of chlorophyll fluorescence in the absence of non-photochemical quenching. Hence, all estimations of non-photochemical quenching are strictly relative to some dark-adapted point. For this

reason, it is necessary to design experiments in such a way that a dark-adapted, non-stressed reference point can be estimated. This requirement can be a major limitation in field conditions, where it is usual to estimate the pre-dawn value of Fm (Maxwell & Johnson, 2000).

The Fv/Fm ratio (calculated as the difference between Fo and Fm, divided by Fm) has been widely used in studies as an indicator of changes in quantum efficiency of photosynthesis occurring during photoinhibition (Somersalo & Krause, 1990; Greer 1990; Nir 1997). Dark-adapted values of Fv/Fm reflect the potential quantum efficiency of PS II and are used as a sensitive indicator of plant photosynthetic performance, with optimal values of around 0.83 measured for most plant species (Bjorkman and Demmig, 1987; Johnson et al., 1993). Values lower than this will be seen when the plant has been exposed to stress, indicating in particular the phenomenon of photoinhibition. Dark-adapted Fv/Fm values not only indicate whether plant leaves are photoinhibited but can also provide information as to the extent or degree of photoinhibition. An increase in Fo is characteristic of destruction of PS II centres or impedement of the transfer of excitation energy from the antenna to the reaction centres, whereas a decline in Fm may indicate an increase in non-photochemical quenching (Björkman, 1986; Baker & Horton, 1988). The measurement of chlorophyll fluorescence from leaves both in the dark and in the light can therefore provide information both on the photosynthetic performance of the plant and insight into the mechanisms by which an environmental stress has altered the photosynthetic ability of a plant.

Light tolerance of myoga plants

Myoga grows naturally throughout Japan in the understorey region of trees (Pursglove, 1972; Tomlison, 1969). As a likely shade plant, myoga could be inherently more susceptible to photoinhibition than a sun plant. The higher sensitivity of shade plants to photoinhibition is generally ascribed to factors such as large light-absorbing chlorophyll antenna for PS II and lower rates of light-saturated photosynthesis (Oquist et al., 1992) The photosynthetic apparatus in shade plants, organised to take advantage of low light is poorly endowed with processes to forestall or cope with absorption of excess radiation. In the short term, shade plants

accommodate bright light in sunflecks without photoinhibitory damage, and even exploit it for additional post-illumination CO_2 exchange (Pearcy 1994; Chazdon and Pearcy, 1986). When exposed to sustained bright sunlight, in excess of that encountered during growth plants such as *Alocasia macrorrhiza* suffer photoinhibitory damage. Photoacclimation to bright light, the successful long-term accommodation of photoinhibitory processes, is genetically limited in many species, being dependent on adequate nutrition and favourable temperatures (Anderson and Osmond, 1987).

Cultivation of myoga within Australia

Increased demand for tropical and sub-tropical plants has led to the encouragement of production in cooler areas despite the fact that moderately cool temperatures are known to limit the photosynthetic productivity of many of these species (Schaffer and Anderson, 1994). Previous reports state that exposure to low temperatures and moderate to high irradiances causes a reduction in both the maximum rate and quantum yield of CO_2 assimilation in a number of plants, which can potentially reduce the carbon yield. For instance, lychee (Menzel and Paxton, 1985) and rambutan (Diczbalis and Menzel, 1998) trees held under high light conditions at 14^0C had reduced stomatal conductance of CO_2 and tree growth. Decreased vegetative growth following photoinhibition has also been reported in beans (Farage and Long, 1991; Laing et al., 1995) and snow gum (Blennow et al., 1998).

Although the simultaneous occurrence of low temperatures and moderate to high irradiance levels is likely to cause stress to the photosynthetic systems within myoga, the exposure of plants to cool nights followed by warm, sunny days may also result in photodamage occurring. The reduction of photosynthesis on the day following an overnight chill has been well established in herbaceous crops of tropical and subtropical origin, such as tomato, cucumber (*Cucumis sativus*) and maize (*Zea mays*) (Allen et al., 2000). However the mechanisms of low-temperature induced photoinhibition in chilling-sensitive species is not always agreed on. The ginger family originates from the tropical Indo-Malaya region with additional centres in tropical Africa and Central and South America (Criley, 1985). Myoga, although the most cold-tolerant of its family still requires warm temperatures in the

region of 20 to 30^{0}C for active growth and prefers frost-free, humid conditions (Clark, 2001). These climatic requirements indicate that myoga is also likely to be sensitive to chilling temperatures and may therefore be inherently more susceptible to low-temperature induced photoinhibition.

SECTION 2: LOW-TEMPERATURE INDUCED PHOTOINHIBITION – *SECTION MATERIALS AND METHODS*

Plant Material

Plant material was propagated vegetatively from myoga plants grown in a glasshouse at the University of Tasmania. The rhizome material was soaked in a fungicide treatment consisting of 100ml of Previcur®, 200ml of Bavinston®, 100ml of Sumisclex® and 200g of Kocide® per 100L of water. It was then packed into seed trays filled with a moistened mix of 50% perlite, 25% coarse sand and 25% peat and given a standard chilling treatment of three weeks at 4^0C (Gracie *et al*, 2000). Approximately 100g rhizome pieces were then planted into separate 35L pots. The potting soil consisted of peat, sand and pine bark (1:2:7) which was supplemented with slow release fertiliser (nine month Osmocote® 330g/50L), dolomite lime (330g/50L), iron sulphate (25g/50L) and trace elements (Micromax® 20g/50L). The pots were watered daily. Irrigation and fertiliser programs were identical for each of the treatments. Plants were grown inside the main glasshouse at the Horticultural Research Centre. The glasshouse was maintained at average day/night temperatures of $25/15^0$C. Plants were grown under natural daylight with light levels ranging from 600 to 1200 $\mu mol.m^{-2}.s^{-1}$.

Chlorophyll Fluorescence Measurements

Chlorophyll fluorescence measurements were made using a PAM-2000 fluorometer and 2030-B leaf clip holder equipped with a portable PC and DA-2000 software (Heinz Walz GmbH, Effeltrich, Germany). Single measurements were made on the middle of each leaf of the most recently expanded leaf pair and on a single older leaf one node back from the leaf pair for both dark-adapted and light stable measurements. For repeated measure experiments, where the effect of the preceding night temperature on the photosynthetic performance of myoga was investigated, placement of the leaf clip holder on the leaf was outlined so that each measurement was repeated at exactly the same position. Each measurement was replicated on three plants for both dark-adapted and light-stable measurements.

Following dark acclimation, initial fluorescence intensity (Fo) was measured at the leaf surface under a low irradiance (0.1 μmol $m^{-2}s^{-1}$) modulated measuring beam. A 0.8s pulse of saturating irradiance (12000 μmol $m^{-2}s^{-1}$), sufficient to close all open (oxidized) photosystem II (PS II) reaction centres was delivered from a saturation pulse lamp. Maximal fluorescence (Fm) was recorded after the first pulse and dark-adapted variable fluorescence (Fv = Fm-Fo) over maximal fluorescence (Fv/Fm) was calculated. Dark adapted Fv/Fm quantifies the efficiency of photon capture by open PS II reaction centres (Butler and Kitajima 1975).

Steady-state fluorescence (Ft) and maximal fluorescence in the light-adapted state (Fm') were measured in situ at various times during the day. Actual fluorescence level, Ft, was monitored until it was stable. Fm' was then measured at the indicated time of day in conjunction with Fm measured pre-dawn the same day used for the calculation of NPQ. The quantum yield of PS II was calculated as (Fm'-Ft)/Fm' = ΔF/Fm' (Genty *et al.*, 1989) and NPQ as NPQ = (Fm – Fm')/Fm' as described by Schreiber et al. (1986). For the calculation of qN, Fo' was measured while the sample was transiently darkened and illuminated with far-red light to account for any quenching of Fo. qN was then calculated as qN = (Fm-Fm')/(Fm-Fo').

Gas Exchange Measurements

Gas exchange measurements were obtained using an open-flow infrared gas analyser (Lci Portable Photosynthesis System, ADC BioScientific Ltd. Hoddesdon, Herts) in conjunction with a PLC-broad leaf chamber (area 6.25cm^2). The rate of CO_2 exchange in the leaf chamber (photosynthetic rate) was measured using the following formula:

$A = u_s \Delta C$

Where:

u_s = mass flow of air per m^2 of leaf area, mol $m^{-2}s^{-1}$

ΔC = difference in CO_2 concentration through chamber, dilution corrected, μmol mol^{-1}.

SECTION 2: LOW-TEMPERATURE INDUCED PHOTOINHIBTION – *EXPERIMENTAL WORK: EFFECT OF LIGHT AND TEMPERATURE ON THE PHOTOSYNTHETIC PERFORMANCE OF MYOGA*

Introduction

The light-dependant down-regulation of the quantum yield of photosynthesis, termed photoinhibition, is a common phenomenon in the plant world (Atwell et al., 1999). It is an important regulatory function, which exists to reduce the amount of light reaching the reaction centres of PS II. Moderate light intensities that a plant may normally be able to regulate using dynamic photoinhibition, can become damaging when electron transfer to the Calvin cycle is prevented or delayed for some reason. Situations of this kind develop if the plant has been stressed previously or when an additional stress is imposed in the presence of strong radiation (Hamlyn et al., 1989). An additional stress such as low temperature increases the sensitivity of plants to chronic photoinhibition primarily through an inhibition of the dark reactions of photosynthesis that increases the probability for excessive excitation of PS II (Huner et al., 1993; Oquist et al., 1993a).

The following controlled environment cabinet experiments were conducted to examine how the combined factors of light and temperature affected the photosynthetic performance of myoga plants. The first set of experiments aimed to establish what level of light intensity during low temperature conditions was photoinhibiting and therefore potentially photodamaging for myoga plants. Myoga plants were then subjected to a range of temperatures at a light intensity of 750 $\mu mol\ m^{-2}\ s^{-1}$ to determine at what temperature range normal functioning of the Calvin cycle was disrupted, thus predisposing plants to photoinhibition. The level or extent of photoinhibition experienced by myoga plants during the experiments was assessed by measuring the proportion of light energy being utilised in photochemistry (Yield) and that being dissipated as heat through the xanthophyll cycle (qN).

Materials and Methods

Myoga plants previously grown in a temperate glasshouse at the Horticultural Research Centre (HRC), University of Tasmania for approximately three months were used for the following experiments. Propagation and general plant growth conditions are described in the section materials and methods. Prior to all fluorescence measurements, test plants were dark-adapted (1 h at room temperature) and then randomly allocated to a darkened controlled environment chamber for a further 1 h at the chosen test temperature. Light inside the controlled environment cabinets was provided by a mix of 4 400W GE Kolorarc MBID 400/T/H Tubular Metal Halide Lamps,(GE Hungary), 2 Osram Vialox Planta-T 400W sodium lamps, and 5 incandescent globes (100 W Pearl; Osram, Germany). Desired light intensity conditions were achieved by changing the height of plants and by utilising a combination of the available lights.

The degree of photoinhibition and recovery was determined at light intensities of 250, 500, 750 and 1000 μmol m^{-2} s^{-1} on three leaves of three plants at 5^0C and at temperatures of 25, 15 and 5^0C at a light intensity of 750 μmol m^{-2} s^{-1}. Plants were exposed to light for a period of 70 to 90 minutes followed by a recovery period in darkness of 30 to 70 minutes, during which time the efficiency of photochemical energy conversion and level of non-photochemical quenching were recorded. Chlorophyll fluorescence measurements are outlined in the section materials and method.

Results

During the time course of the experiment, four critical points of time were identified. Measurements taken at these points consisted of those taken before light exposure (quantum yield values) or at the start of light exposure (qN values), the light-adapted yield and qN measurement at the end of the light period and the quantum yield and qN measurements taken during the dark recovery period. These four points most clearly illustrated the performance of the plant in response to the temperature and light treatments. Significant differences between treatment effects were calculated using one-way analysis of variance at each of the four critical

points ($P<0.05$). Least significant differences (LSD) were also calculated; treatment values with the same letter at each point in time are not significantly different (tables 1 - 4).

Temperature Treatments – Quantum Yield

At the beginning of the light period plants maintained at 15^0C had a significantly higher quantum yield value than those at 25 and 5^0C, however at the end of the light period quantum yield values were very similar for plants from all temperature treatments (table 1). Plants in the 25^0C treatment had significantly higher ($P<0.05$) quantum yield values than those kept at 5^0C during the recovery period.

Treatment	pre-light period	end of light period	start of dark period	end of dark period
25^0C	0.763^B	0.296^A	0.709^A	0.749^A
15^0C	0.786^A	0.382^A	0.694^B	0.726^A
5^0C	0.758^B	0.327^A	0.547^C	0.658^B

Table 1: Quantum yield of PSII values of myoga plants maintained at 5, 15 and 25^0C. Light intensity during the light period was 750 μmol m^{-2} s^{-1} ($n = 3$). Treatment values at each point in time with the same letter are not significantly different ($P<0.05$).

In the first five minutes of exposure, myoga plants at all three temperatures exhibited a rapid decrease in the quantum yield of PS II (figure 1). After this time, PS II quantum yield gradually increased from ~ 0.15 to ~ 0.3 at 89 minutes. Plants from each of the temperature treatments displayed similar quantum yield values during this period of gradual increase. After 91 minutes the lights were turned off and the myoga plants displayed an immediate recovery in quantum yield values. Plants at 25 and 15^0C increased to approximately 0.7, which was close to their original relaxed values before exposure. Plants maintained at 5^0C had a smaller rise in yield values to 0.54.

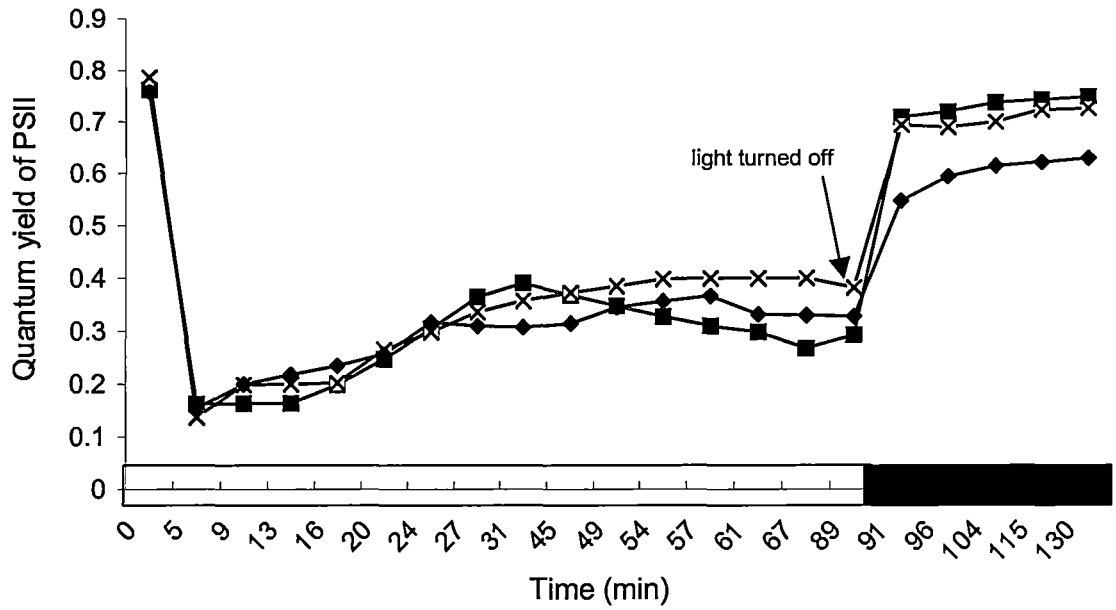


Figure 1: Values depicted during the light period represent the *achieved* quantum yield of PSII of myoga plants maintained at 5 (- -), 15 (-x-) and 25^{0}C (- -). Plants were maintained at a light intensity of 750 µmol m^{-2} s^{-1} for 91 minutes. Values from myoga plants during the subsequent dark period of 39 minutes reflect the *maximum* quantum yield of PSII.

Temperature Treatments – Non-photochemical Quenching

At the start of the light period plants from the three different temperature treatments displayed significantly different qN values, however at the end of the light period there were no significant differences between treatments (table 2). In the period of recovery, plants at 5^{0}C had significantly higher levels of non-photochemical quenching than plants at 25^{0}C.

Treatment	start of light period	end of light period	start of dark period	end of dark period
25^{0}C	1.044^A	0.684^{AB}	0.359^C	0.159^B
15^{0}C	0.964^B	0.591^B	0.396^B	0.298^{AB}
5^{0}C	0.872^C	0.761^A	0.588^A	0.388^A

Table 2: Non-photochemical quenching values of myoga plants maintained at 5,15 and 25^{0}C at a light intensity of 750 µmol m^{-2} s^{-1} (n = 3). Treatment values at each point in time with the same letter are not significantly different (P<0.05).

Levels of qN decreased over a period of 89 minutes from values around 0.9 to values between 0.6 and 0.8 (figure 2). After 91 minutes, the lights were turned off and qN levels further decreased to values between 0.1 and 0.4. Plants at 25^{0}C exhibited the greatest relaxation with values at 130 minutes of 0.16, followed by plants at 15^{0}C with a qN value of 0.29 and 5^{0}C at 0.39.

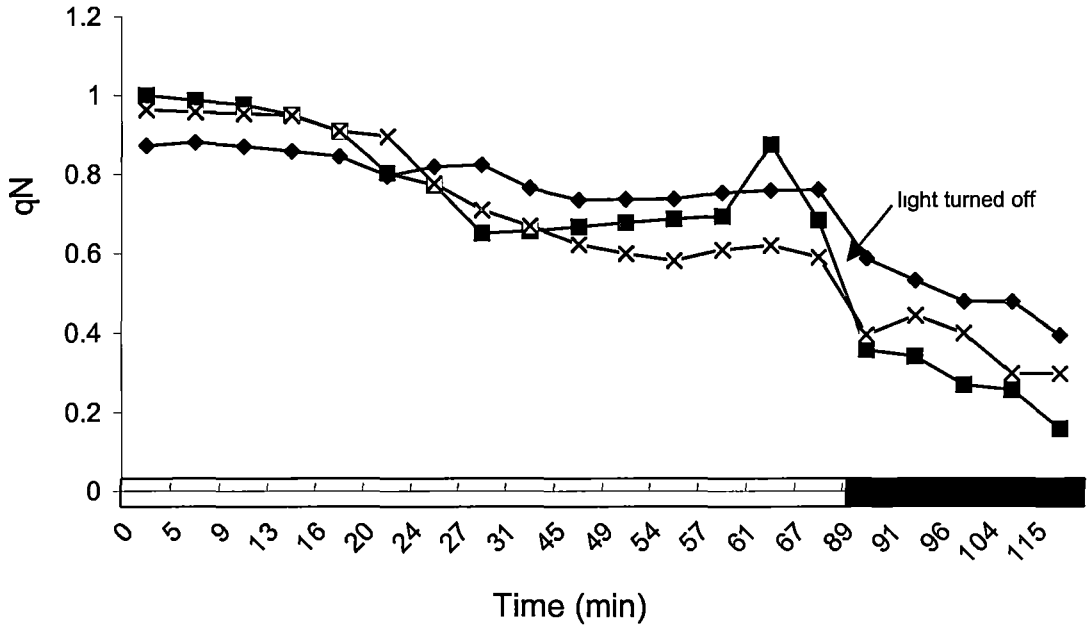


Figure 2: Non-photochemical quenching values of myoga plants maintained at 5 (- -), 15 (- x-) and 25^{0}C (- -) for a period of 91 minutes at a light intensity of 750 µmol m^{-2} s^{-1} followed by a 39 minute recovery period in darkness.

Light Intensity Treatments –Quantum Yield

Subsequent to the pre-light period there were no significant differences in yield values between plants in all four light intensity treatments (table 3).

Treatment	pre-light period	end of light period	start of dark period	end of dark period
1000µmol m^{-2} s^{-1}	0.719^{C}	0.237^{A}	0.618^{A}	0.659^{A}
750µmol m^{-2} s^{-1}	0.758^{AB}	0.327^{A}	0.547^{A}	0.658^{A}
500µmol m^{-2} s^{-1}	0.747^{B}	0.328^{A}	0.608^{A}	0.691^{A}
250µmol m^{-2} s^{-1}	0.780^{A}	0.286^{A}	0.557^{A}	0.680^{A}

Table 3: Quantum yield of PSII values of myoga plants maintained at light intensities of 250, 500, 750 and 1000 µmol m^{-2} s^{-1}, while at 5^{0}C (n = 3). Treatment values at each point in time with the same letter are not significantly different (P<0.05).

Immediate decreases in the quantum yield of PSII occurred when myoga plants were placed into four different light intensities (figure 3). Plants placed into light intensities of 500 and 750 μmol $m^{-2}s^{-1}$ had a slight increase in yield after 6 minutes, plants in 1000 μmol $m^{-2}s^{-1}$ had a slight increase after 11 minutes; yield values then remained at that approximate level until the lights were turned off. Plants placed into 250 μmol $m^{-2}s^{-1}$ continued to decline in yield values after 6 minutes and then remained approximately around the yield value of 0.2. After 67 minutes, plants in 750 and 500 μmol $m^{-2}s^{-1}$ light intensities had yield values of 0.32 and plants in 250 and 1000 μmol $m^{-2}s^{-1}$ had yield values of 0.22. After the lights were turned off plants from all four light intensities had a recovery in yield values to a level between 0.6 and 0.7.

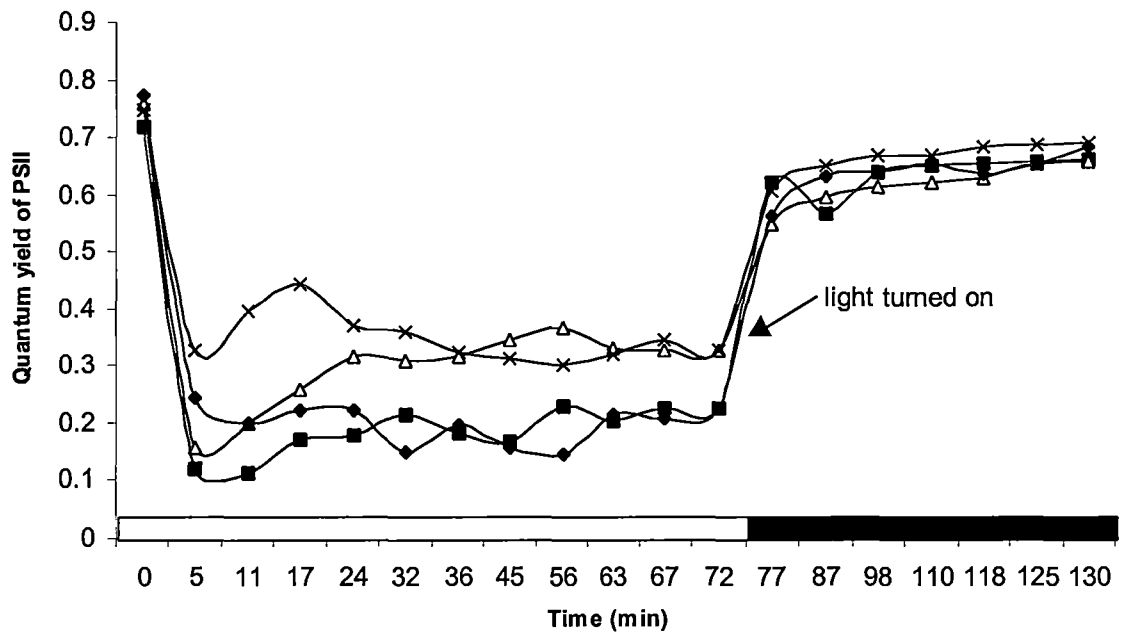


Figure 3: Values depicted during the light period represent the *achieved* quantum yield of PSII of myoga plants maintained at light intensities of 250 (- -),500 (-x-), 750 (-Δ-) and 1000 (- -) μmol m^{-2} s^{-1} while at 5°C for 72 minutes. Values from myoga plants during the subsequent dark period of 68 minutes reflect the *maximum* quantum yield of PSII.

Light Intensity Treatments – Non-photochemical quenching

Non-photochemical quenching levels were similar in plants from all light intensity treatments for each point in time during the experiment (table 4). At the end of the dark period plants that had been exposed to a light intensity of 500 and 1000 μmol

$m^{-2}s^{-1}$ had similar qN values of 0.19 while those exposed to 750 and 250 μmol $m^{-2}s^{-1}$ had similar values of approximately 3.5.

Treatment	start of light period	end of light period	start of dark period	end of dark period
1000μmol m^{-2} s^{-1}	0.883^{B}	0.490^{B}	0.242^{B}	0.193^{B}
750μmol m^{-2} s^{-1}	0.872^{B}	0.761^{A}	0.588^{A}	0.388^{A}
500μmol m^{-2} s^{-1}	1.220^{A}	0.661^{AB}	0.399^{AB}	0.194^{B}
250μmol m^{-2} s^{-1}	0.729^{B}	0.667^{A}	0.534^{A}	0.331^{A}

Table 4: Non-photochemical values of myoga plants maintained at light intensities of 250, 500, 750 and 1000μmol m^{-2} s^{-1}, while at 5°C (n = 3). Treatment values at each point in time with the same letter are not significantly different (P<0.05).

During the 130 minute experiment qN levels of plants at 1000, 750 and 500 μmol $m^{-2}s^{-1}$ declined (figure 4). Plants placed into a light intensity of 250 μmol $m^{-2}s^{-1}$ maintained qN values at approximately 0.8 throughout the period of exposure. After lights were turned off there was an immediate decrease in qN values from all treatment plants followed by a more gradual decline over the remaining recovery period. At the end of the recovery period plants at light intensities of 250 and 750 μmol $m^{-2}s^{-1}$ had qN values of approximately 0.35, which was significantly higher than the qN levels of plants at 500 and 1000 μmol $m^{-2}s^{-1}$ which had a qN value of 0.19.

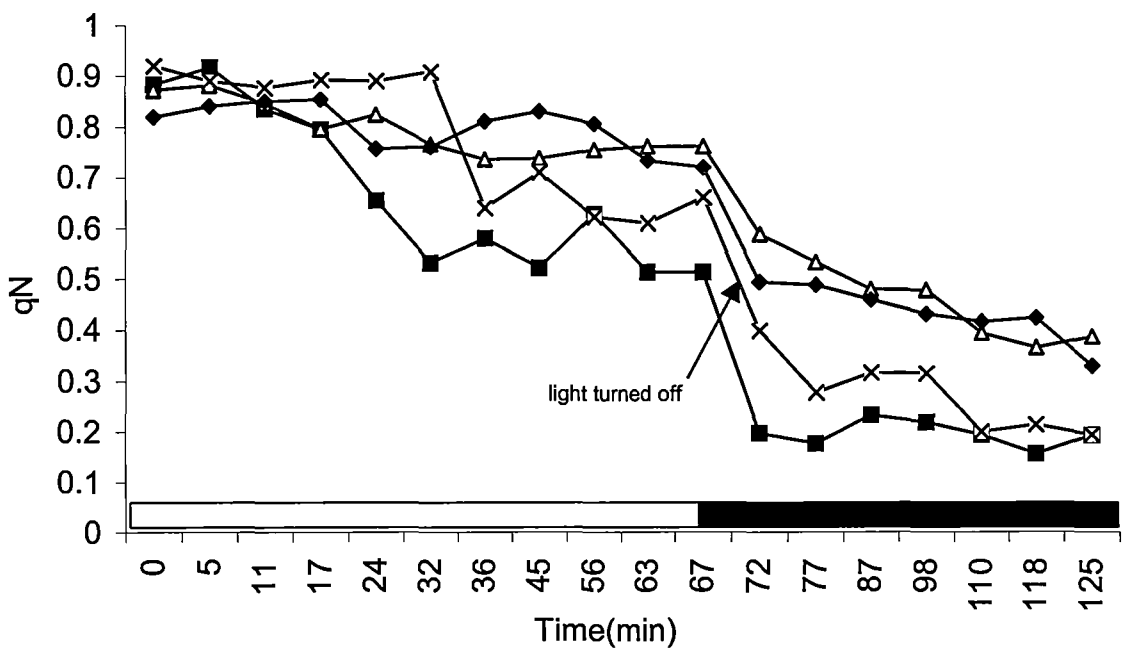


Figure 4: Non-photochemical quenching values of myoga plants maintained at light intensities of 250 (- -), 500 (-x-), 750 (-Δ-) and 1000 (-■ -) μmol m^{-2} s^{-1} for a period of 72 minutes followed by a 68 minute recovery period in darkness while at a temperature of 5°C.

Discussion

Myoga plants placed into light conditions of an intensity of 250 μmol m^{-2} s^{-1} and greater at 5^0C displayed an immediate, substantial decline in quantum yield values. These yield values obtained using Genty's equation ((Fm'-Ft)/Fm'= ΔF/Fm') signified a change in the efficiency of the photochemical energy conversion process resulting in a large decrease in the quantum yield of PS II (Genty et al., 1989). The similar response to all four light intensities implied that even at the lowest light intensity of 250 μmol m^{-2} s^{-1}, myoga plants were severely stressed. The immediate decrease in yield when exposed to light indicates that only low amounts of light energy are being utilised photochemically. This is confirmed by the rapid rise in qN levels to approximately 0.9, indicating that most of the light energy entering the plant is being dissipated as heat through non-photochemical quenching.

Measurements taken at the beginning and end of both the light period and the dark recovery period showed little difference in the level of photoinhibition experienced by plants at different light intensities. Significant differences in yield values were not observed between light intensity treatments due to the extreme sensitivity of myoga plants to light at this temperature; i.e. even plants placed into the lowest light intensity of 250 μmol m^{-2} s^{-1}umol became severely photoinhibited. Plants from all light intensity treatments had significantly lower yield values at the end of the recovery period compared with those before light exposure. The failure of plants to return to pre-light quantum yield values at the end of the recovery period is indicative that photodamage may have occurred within the PS II complex as over an hour had passed since the lights were switched off. However measurements would need to be taken after a longer relaxation period to conclusively establish that photodamage had occurred.

Myoga plants achieve optimum development at daytime temperatures between 20 and 30^0C. Temperatures lower than this are tolerated but plant performance is poor. A daytime temperature of 5^0C is well outside the optimal growth temperature for myoga and it is therefore not surprising to find that at this temperature even low levels of light such as 250 μmol m^{-2} s^{-1} were inhibiting. The second part of this

experiment examined the performance of myoga plants at different temperatures while at a light intensity of 750 μmol m^{-2} s^{-1}. Although it was anticipated that plants at the warmer temperatures would not become photoinhibited, this and subsequent experiments have shown that even at warm temperatures an ambient light intensity of 750 μmol m^{-2} s^{-1}umol is likely to be saturating for myoga. Therefore in this experiment plants in all temperature treatments became photoinhibited to a certain degree.

At the end of the light period plants in all temperature treatments were photoinhibited to a similar level. However during the recovery period there were significant differences in the level of photoinhibition between plants at 25^{0}C and those at 5^{0}C. Plants at 25^{0}C had higher quantum yield values and lower levels of non-photochemical quenching than those kept at 5^{0}C, indicating that myoga plants at the warmer temperature were less stressed than those at 5^{0}C. This result was confirmed by plants at 25^{0}C returning to pre-light quantum yield values while plants at lower temperatures had significantly lower quantum yield values at the end of the treatment compared to the start. The complete recovery of quantum yield values in plants at 25^{0}C indicated that the photoinhibition experienced by these plants was dynamic, that is, of a protective nature and no damage was sustained by PS II. Photoprotective mechanisms such as heat dissipation through xanthophyll de-epoxidation and repair of D1 proteins in PS II reaction centres could have been delayed in plants at lower temperatures, making these plants far more susceptible to photoinhibition which became photodamaging.

These experiments established that myoga plants will become photoinhibited when exposed to a light intensity of 750 μmol m^{-2} s^{-1} at temperatures ranging from 5 to 25^{0}C. Furthermore at a chilling temperature of 5^{0}C all light intensities greater than 250 μmol m^{-2} s^{-1}umol resulted in photoinhibition occurring. In the majority of light intensity and temperature treatments tested the photoprotective mechanisms of the plant appeared to be exhausted resulting in photodamage occurring, although further experiments would need to be conducted to conclusively establish this photodamage.

SECTION2: LOW TEMPERATURE INDUCED PHOTOINHIBTION – *EFFECT OF PRECEDING NIGHT TEMPERATURE ON THE PHOTOSYNTHETIC PERFORMANCE OF MYOGA*

Introduction

In the previous experiment, the susceptibility of myoga leaves to photoinhibition was established for a range of coincident temperature and light conditions. At low temperature myoga leaves were shown to be severely photoinhibited even at moderate light intensities. The failure of quantum yield values to recover to pre-illumination values indicated permanent damage had occurred in PS II, although plants would need to be given longer recovery periods for this to be confirmed. Although it is possible that during field cultivation in southern Australia myoga would be exposed to high light conditions at low temperatures, a more likely situation would be the exposure of plants to low temperatures at night followed by high light conditions the next day.

This experiment was conducted to investigate the response of photosynthesis in myoga leaves to a night chill and a subsequent warm photoperiod. Light energy dissipation through heat and the proportion of light energy being used in photochemistry were measured to determine the extent of photoinhibition in response to these conditions. To distinguish between the effects of direct impairment of biochemical function and low-temperature induced stomatal closure on photosynthetic performance, measures of net assimilation rate of CO_2 in the leaf, stomatal conductance and the concentration of intercellular CO_2 (C_i) were also made. The initial stage of this experiment was conducted over a single 24 hour period to determine if high light conditions preceded by a low temperature night resulted in a photoinhibitory state. The second stage of this experiment was conducted over a period of three days to determine if myoga leaves could re-establish initial levels of photosynthetic capacity given a sufficient period of recovery.

Materials and Methods (first stage)

Planting Material (refer to section materials and methods)

Plant Growth Conditions

Twenty plants were grown in a shadehouse at the Horticultural Research Centre, University of Tasmania. Average day/night ambient temperatures in the shadehouse were 17/8 ^{0}C. In addition to providing shade (50%), the shadecloth extended down the sides of the shadehouse, thus providing some protection from wind.

Photoinhibition Treatment

At 16:00 h eight plants were removed from the shadehouse, four plants were placed into two temperature-controlled rooms at 4^{0}C and 20^{0}C. The plants were left in these rooms overnight and pre-dawn chlorophyll fluorescence measurements were made on the middle part of the two most recently expanded leaves at 05:00 h the next day. Two plants from each night temperature treatment were then moved to a position outside where they received full sunlight and were not shaded by any other structures. The other two plants from each night temperature treatment were placed nearby and covered with 50% shadecloth. Chlorophyll fluorescence measurements were conducted at 06:00, 07:00, 08:00, 10:00, 11:00, 12:00, 14:00, 15:00 and 16:00 h. Net CO_2 exchange rate measurements were conducted at 09:00 h and 13:00 h using an open-flow gas analysis system (Lci, ADC Bioscientific Ltd, Hoddesdon, Herts, UK). Temperature was measured and logged on a tiny-tag recorder; light intensity was measured and logged using a LI-COR quantum sensor.

Chlorophyll Fluorescence and Gas Analysis Measurements (refer to section materials and methods)

Statistical Analysis

Data for the development of photoinhibition over the diurnal course were analysed using a two-factor analysis of variance (ANOVA) with each factor having two levels. Comparisons of different means at each point in time were undertaken using the least significance difference (LSD) at $P<0.05$.

Results (first stage)

In figure 1 the effects of intense light and prevailing night temperatures on the quantum yield of PS II can be clearly observed. Plants in all treatments show a decrease in quantum yield values until at least 10:00 h. Plants placed in the full sun had the largest decreases in quantum yield values. Furthermore, the yield values of plants in the sun remain consistently lower during the trial than the yield values of shaded plants. Apart from the early morning readings at 05:00 and 06:00 h there is a highly significant difference ($P<0.0001$) in quantum yield values between those plants in the sun and those in shade. The prevailing night temperature had a profound effect on those plants under shade. Plants kept at 4 ^{0}C overnight had significantly lower yield values ($P<0.05$) from 05:00 h through to 16:00 h (excepting 15:00 h) than those kept overnight at 20 ^{0}C. Plants in full sun also displayed a significant temperature effect ($P<0.05$) for the majority of readings throughout the day, with low night temperatures resulting in lower quantum yields.

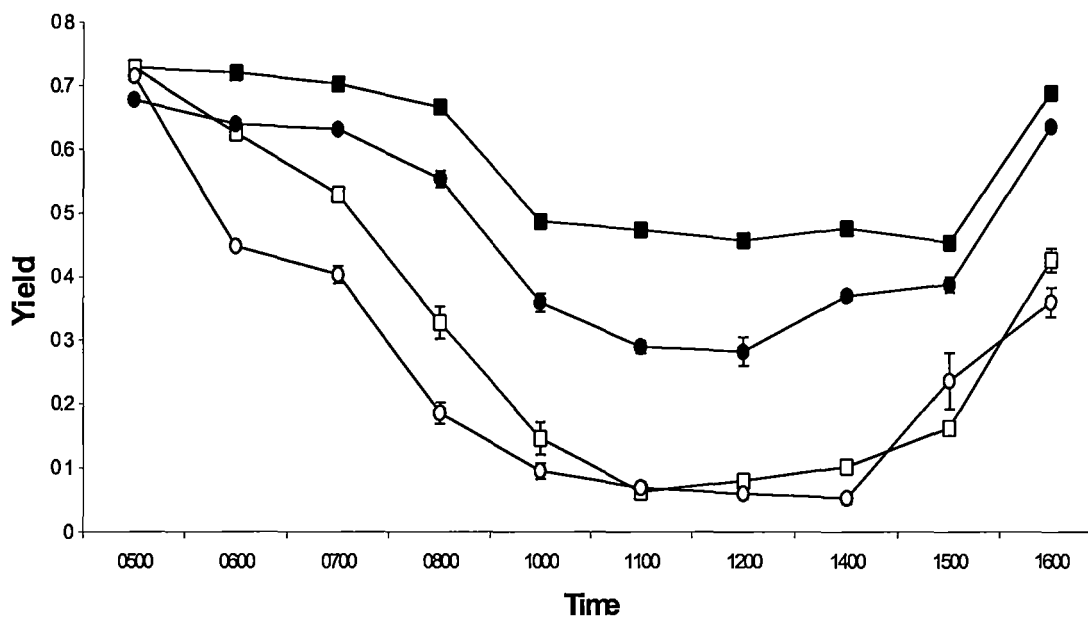


Figure 1: Quantum yield of PSII values from plants which received a preceding night temperature of either 4^{0}C (circles) or 20^{0}C (squares) and were then placed into a non-shaded position (open symbols) or under 50% shade-cloth (fill-in symbols). Hourly measurements were taken from 05:00 to 16:00 hrs. Bars show SE ($n = 4$).

Plants placed into full sun had significantly ($P<0.0001$) higher non-photochemical quenching (NPQ) values from 07:00 to 16:00 h, than plants placed into shade (figure 2). NPQ values are highest in those plants kept at 4^{0}C overnight and then

placed into full sun. Plants kept at 20°C and also placed into full sun show similar levels of NPQ, although the initial values at 06:00 and 07:00 h are much lower. Plants kept at 20°C and 4°C overnight show different levels of NPQ when placed under shadecloth, with plants kept at 20°C the previous night having much lower values. Plants receiving full sun sustained high levels of NPQ from approximately 10:00 h to 15:00 h after which time NPQ levels dropped. Plants in shade conditions had peak levels of NPQ occurring between 10:00 and 12:00 h after which time the levels gradually return to almost zero.

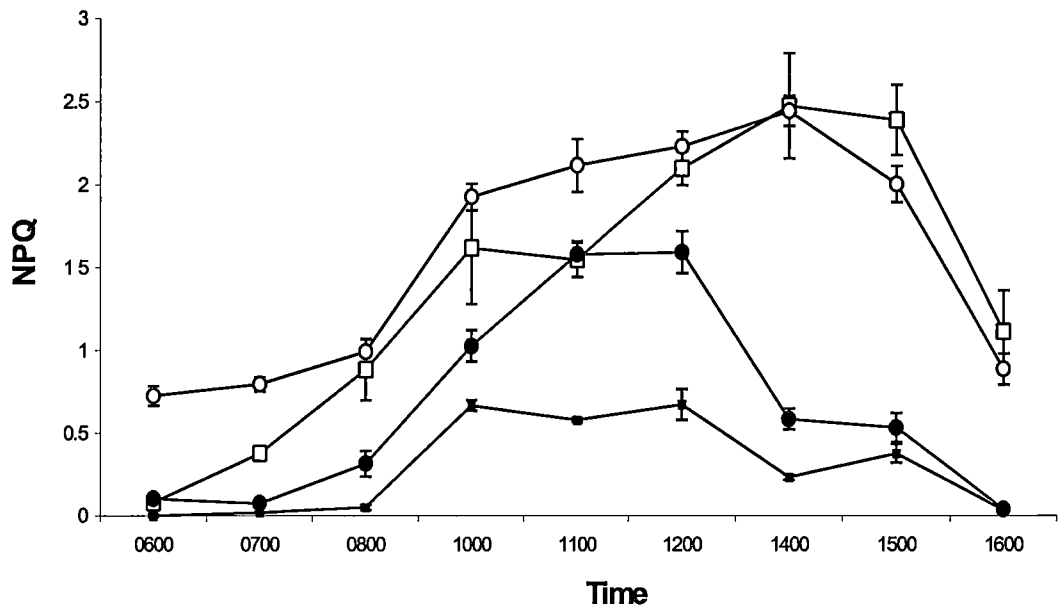


Figure 2: Non-photochemical values from plants which received a preceding night temperature of either 4°C (circles) or 20°C (squares) and were then placed into a non-shaded position (open symbols) or under 50% shade-cloth (fill-in symbols). Hourly measurements were taken from 05:00 to 16:00 hrs. Bars show SE ($n = 4$).

Plants in sun and shade maintained at 4°C overnight had approximately the same rates of CO_2 assimilation occurring at 09:00 h (table 1). At this time 20°C sun and shade plants also had similar rates of CO_2 assimilation but this rate was approximately double that of plants kept at 4°C overnight. At 13:00 h plants from all four treatments displayed different levels of CO_2 assimilation. This is due to an increase in assimilation rate by those plants under shade conditions while those plants in the sun have slightly decreased their rate from 09:00 h. As a result 20°C plants in the shade had the highest assimilation rate followed by 20°C sun, 4°C shade and 4°C sun plants.

Treatment	*09:00 h*	*13:00 h*
4°C - shade	2.728 ± 0.158	4.355 ± 0.065
20°C - shade	5.923 ± 0.483	8.827 ± 0.378
4°C - sun	3.085 ± 0.181	2.247 ± 0.240
20°C - sun	5.9 ± 0.524	5.825 ± 0.238

Table 1: Net assimilation of CO_2 rates (μmol $m^{-2}s^{-1}$) from plants which received a preceding night temperature of either 4°C or 20°C and were then placed into a non-shaded position or under 50% shade-cloth. Measurements were made at 09:00 and 13:00 h ($n = 4$).

Plants kept at 4°C overnight had lower levels of stomatal conductance than plants kept at 20°C at 09:00 h and 13:00 h. Stomatal conductance decreased in all plants from 09:00 h to 13:00 h, except for those plants kept overnight at 20°C and placed into a sun position, which retained the same rate of stomatal conductance. The decrease was most pronounced in plants placed under shade, with the result that at 13:00 h plants kept overnight at 20°C had almost identical rates of stomatal conductance, as did plants kept at 4°C (table 2).

Treatment	*09:00 h*	*13:00 h*
4°C - shade	0.065 ± 0.004	0.02 ± 0.003
20°C - shade	0.172 ± 0.007	0.115 ±0.003
4°C - sun	0.04 ± 0.003	0.013 ±0.008
20°C - sun	0.115 ± 0.011	0.108 ±0.002

Table 2: Stomatal conductance of water in leaves of plants which received a preceding night temperature of either 4°C or 20°C and were then placed into a non-shaded position or under 50% shade-cloth. Measurements were made at 09:00 and 13:00 hrs ($n = 4$).

Ambient light levels rose steadily during the morning reaching a peak of approximately 1500 μmol $m^{-2}s^{-1}$ at midday (figure 5). Light levels fluctuated during the afternoon decreasing to approximately 250 μmol $m^{-2}s^{-1}$ at 16:00 h. Ambient temperatures increased steadily during the morning reaching a peak of approximately 35°C at 14:00 h (figure 6). Temperatures remained high throughout the afternoon.

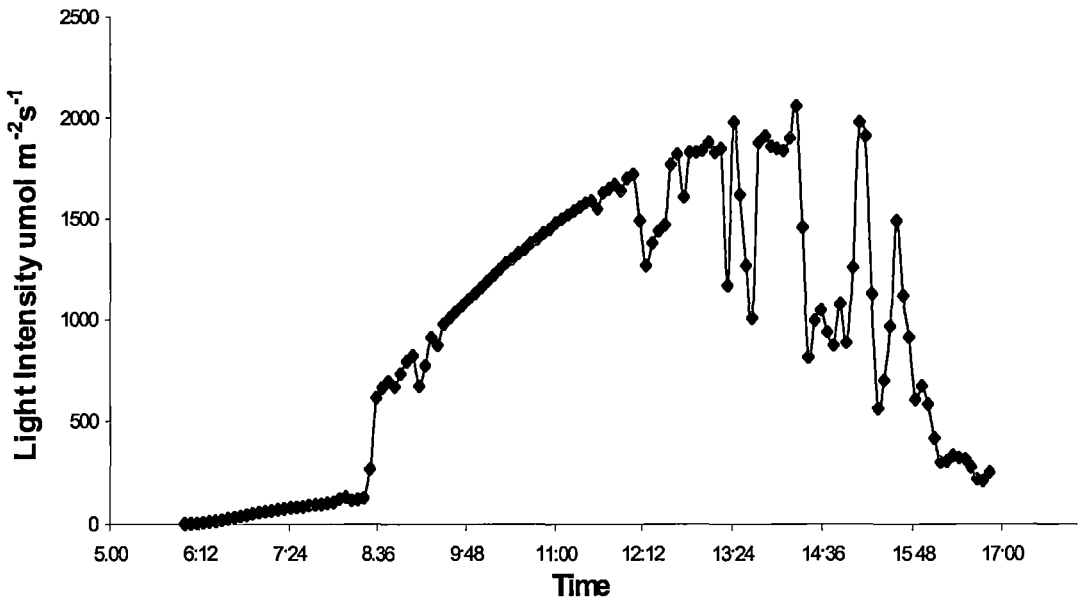


Figure 5: Ambient light intensity values experienced by plants in full sun positions during the light period of the experiment. Plants in the shaded position experienced light intensity values 50% lower than those shown above.

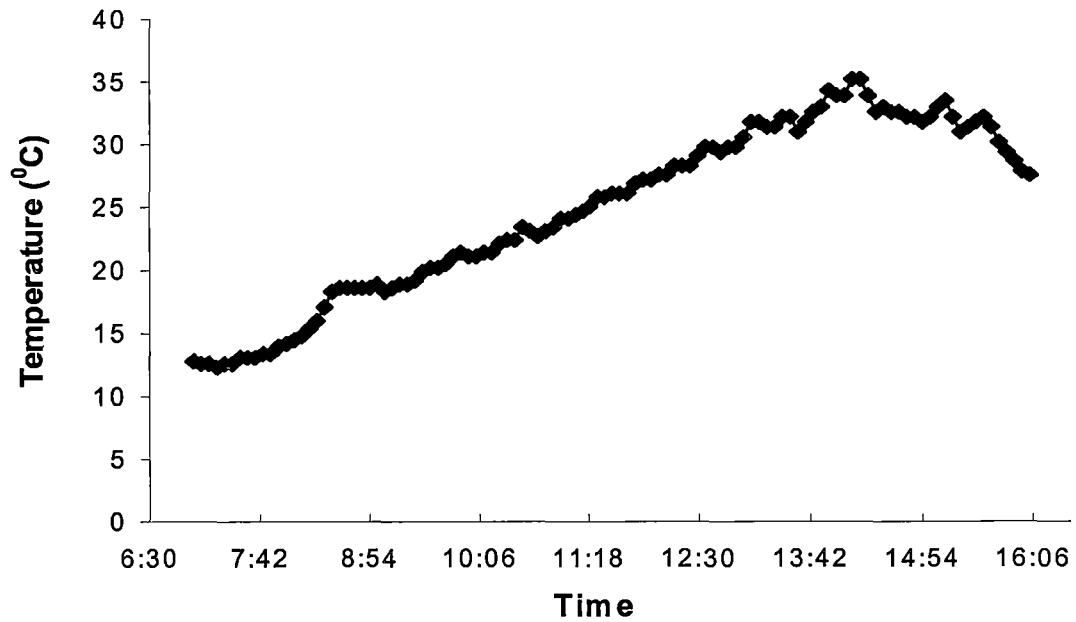


Figure 6: Ambient temperature values experienced by plants during the light period of the experiment.

Materials and Methods (second stage)

Planting Material (refer to section materials and methods)

Plant Growth Conditions

Eight plants were grown in temperature-controlled glasshouse conditions at the Horticultural Research Centre of the School of Agricultural Science at University of Tasmania. Average day/night ambient temperatures in the glasshouse were 25/15^{0}C. Glasshouse light levels ranged from 600 to 1200 μmol $m^{-2}s^{-1}$.

Photoinhibition Treatment

At 16:00 h eight plants were removed from the glasshouse, four plants were placed into a room maintained at 4^{0}C and the other four were placed into a room maintained at 20^{0}C. Plants were left in these dark rooms overnight and pre-dawn chlorophyll fluorescence measurements were made on the middle part of the two most recently expanded leaves at 06:00 h the next day. Two plants from each night temperature treatment were then moved to a position outside where they received full sunlight. The other two plants from each night temperature were placed nearby and covered with 50% shadecloth. Chlorophyll fluorescence measurements were conducted each hour from 07:00 to 16:00 h. Measurements of net CO_2 exchange rate, transpiration and concentration of intercellular CO_2 were made at 09:00, 11:00, 1300 and 15:00 h. Following the last measurement plants were placed back into the same temperature controlled room they had been kept in the previous night. This procedure was repeated for a further two days culminating with a pre-dawn Fv/Fm measurement on the fourth day. Temperature was measured using a data logger (Tinytag Ultra) and light intensity was measured and logged using a line quantum sensor attached to a data logger (LiCor), both instruments were positioned in full sunlight.

Chlorophyll Fluorescence and Gas Analysis Measurements (refer to section materials and methods)

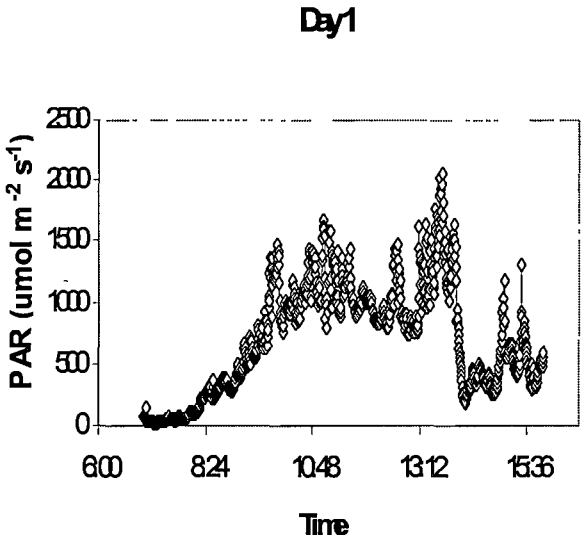
Statistical Analysis

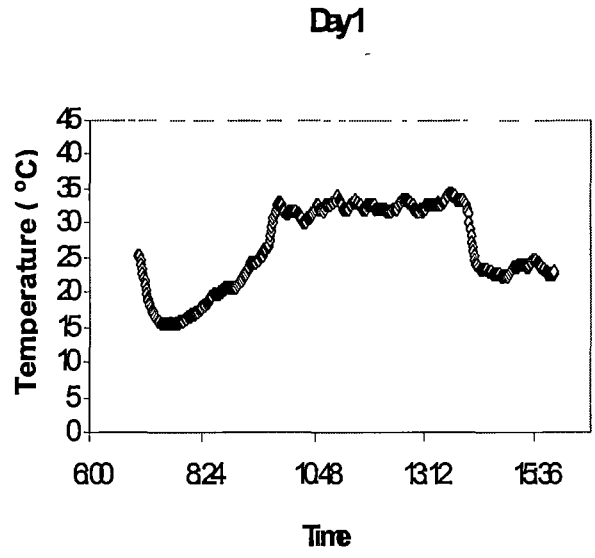
Data for the development of photoinhibition over the diurnal course were analysed using a two-factor analysis of variance (ANOVA) with each factor having two levels. Comparisons of different means at each point in time were undertaken using the least significance difference (LSD) at $P<0.05$. Comparison of pre-dawn Fv/Fm values over time within treatments and of mean diurnal decreases in fluorescence characteristics over time within treatments was made using a t-test of means.

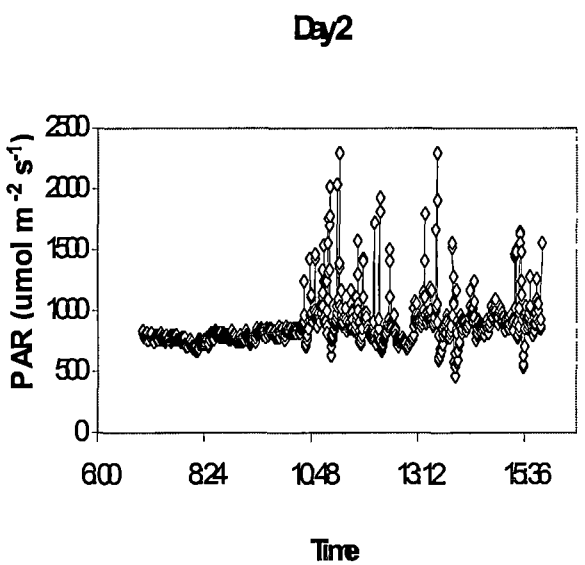
Results (stage 2)

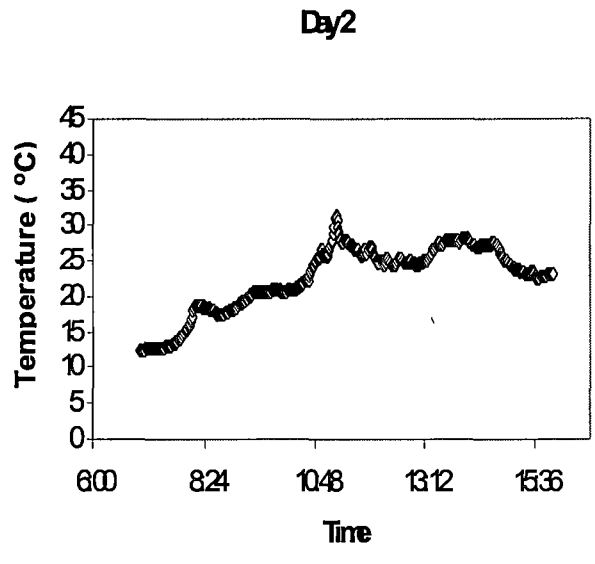
Climatic Data

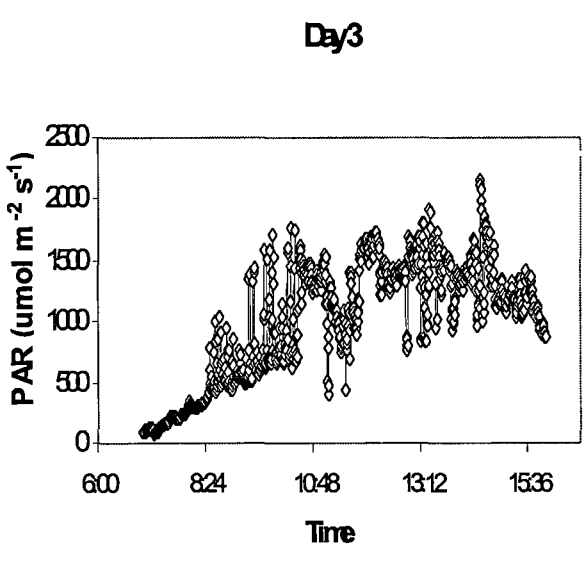
Photosynthetically active radiation (PAR) and ambient temperature values at the University of Tasmania from the 14^{th}-16^{th} of January 2003 are presented in figure 1. The days were mostly cloud-free and sunny, with mean diurnal temperatures ranging from 23 to 30^0C and mean diurnal PAR values of approximately 700 to 1000 μmol $m^{-2}s^{-1}$ in full sunlight.

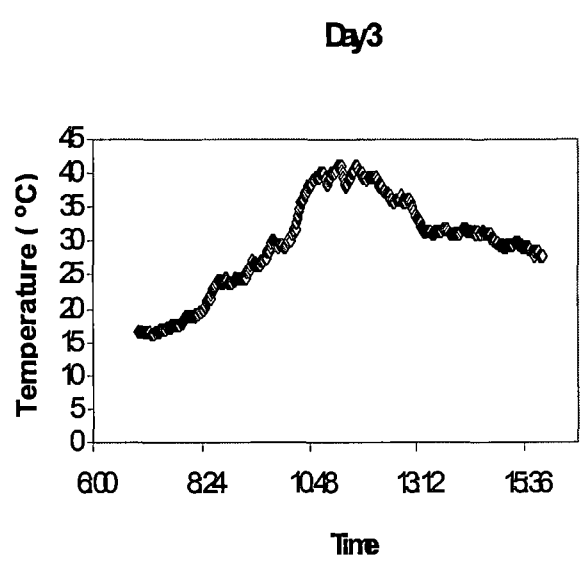

Day1
PAR (umol m -2 s-1)
2500
2000
1500
1000
500
0
600
824
1048
1312
1536
Time


Day1
Temperature (°C)
45
40
35
30
25
20
15
10
5
0
600
824
1048
1312
1536
Time


Day2
PAR (umol m -2 s-1)
2500
2000
1500
1000
500
0
600
824
1048
1312
1536
Time


Day2
Temperature (°C)
45
40
35
30
25
20
15
10
5
0
600
824
1048
1312
1536
Time


Day3
PAR (umol m -2 s-1)
2500
2000
1500
1000
500
0
600
824
1048
1312
1536
Time


Day3
Temperature (°C)
45
40
35
30
25
20
15
10
5
0
600
824
1048
1312
1536
Time

Diurnal chlorophyll fluorescence measurements in shaded conditions

Over the three days of the experiment a similar pattern was observed in quantum yield values from shaded plants in both temperature treatments during the course of the day (figure 7). Generally quantum yield values were observed to decrease during the morning, reaching a prolonged depression from approximately 10:00 h through to 13:00 h. On day 3 quantum yield values begin to rise again during the afternoon period between 13:00 and 16:00 h. The majority of quantum yield values from plants which were kept overnight at 20^0C were significantly higher ($P<0.05$) than those from plants receiving a preceding night temperature of 4^0C. Under both temperature treatments the mean quantum yield values during the course of the day decreased significantly from day 1 to day 2. The mean quantum yield value on day 3 was significantly lower ($P<0.05$) than that on day 1 for both treatments but not significantly different to that on day 2. The quantum yield of PSII in shaded plants recovered overnight from day 1 to day 2 with the initial reading on day 2 at the same level as on day 1. However on the third day the initial quantum yield value had decreased from 0.7 to approximately 0.65.

For these same shaded plants, non-photochemical quenching levels typically increased in the morning, peaked at approximately 12:00 h and then gradually declined during the afternoon, in plants from both night temperatures (figure 8). On day 2 this pattern was altered slightly, with plants maintaining similar levels of NPQ throughout the day. On the first day, only those measurements taken between 10:00 h and 13:00 h showed significant differences in NPQ levels between plants given different night temperatures. However on day 2 and 3 the majority of NPQ values from plants receiving a 20^0C night temperature are significantly lower ($P<0.05$) than those from plants receiving a night temperature of 4^0C. Shaded plants given a night temperature of 4^0C had sustained increased levels of NPQ from the end of day 1 onwards signifying that no relaxation has occurred overnight. This is indicated by the continuance of NPQ values at a level of 1.5 or above from 14:00 h onwards on day 1. NPQ values of shaded plants given a night temperature of 20^0C recovered overnight returning to an initial 07:00 h reading of approximately 0.1 each morning.

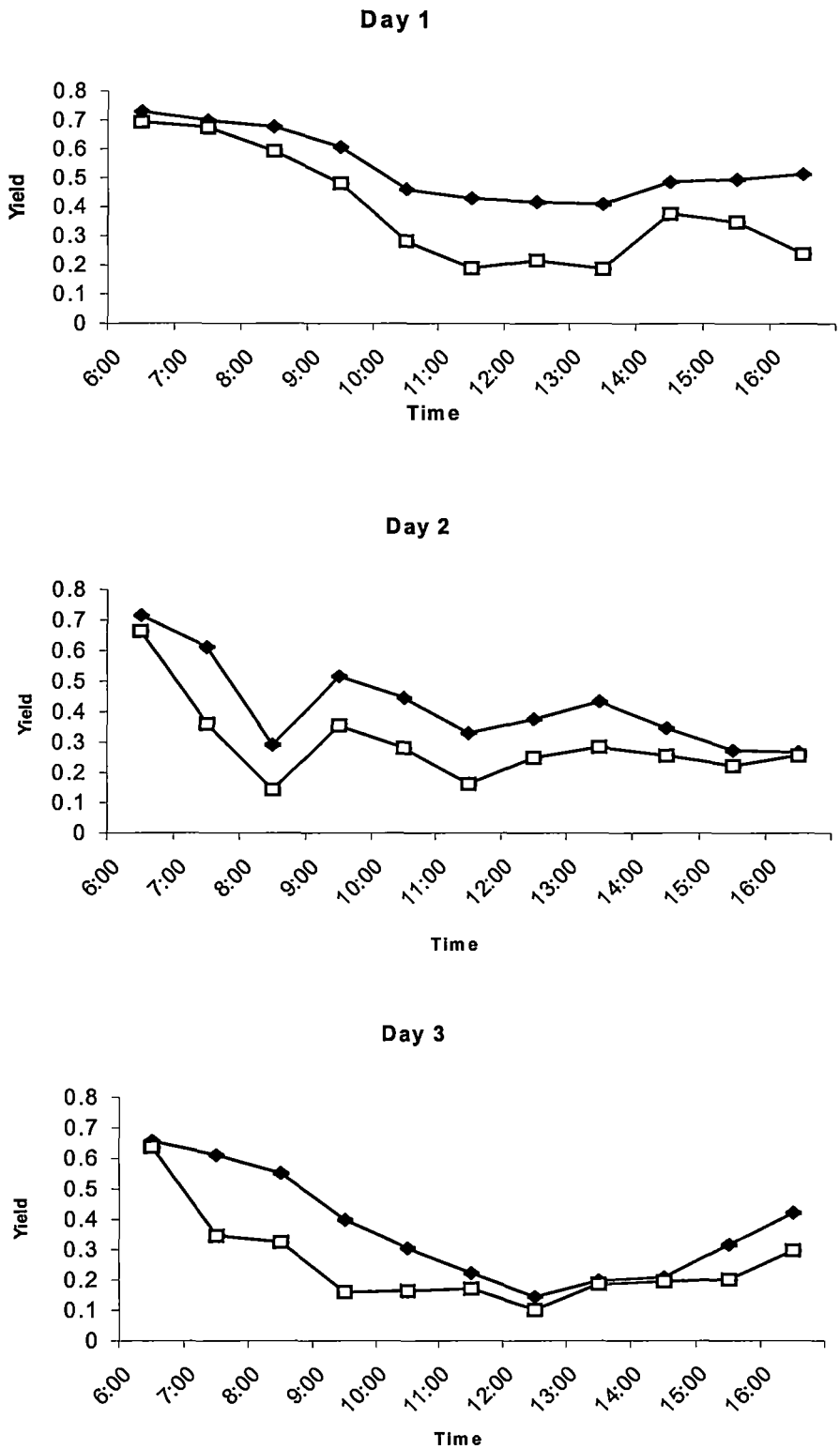


Figure 7: Effect of 20°C (closed symbols) and 4°C (open symbols) night temperature on quantum yield values of myoga plants positioned under 50% shade-cloth for a period of three days. Bars show SE (n=4). Where bars are not visible, SE was very small.

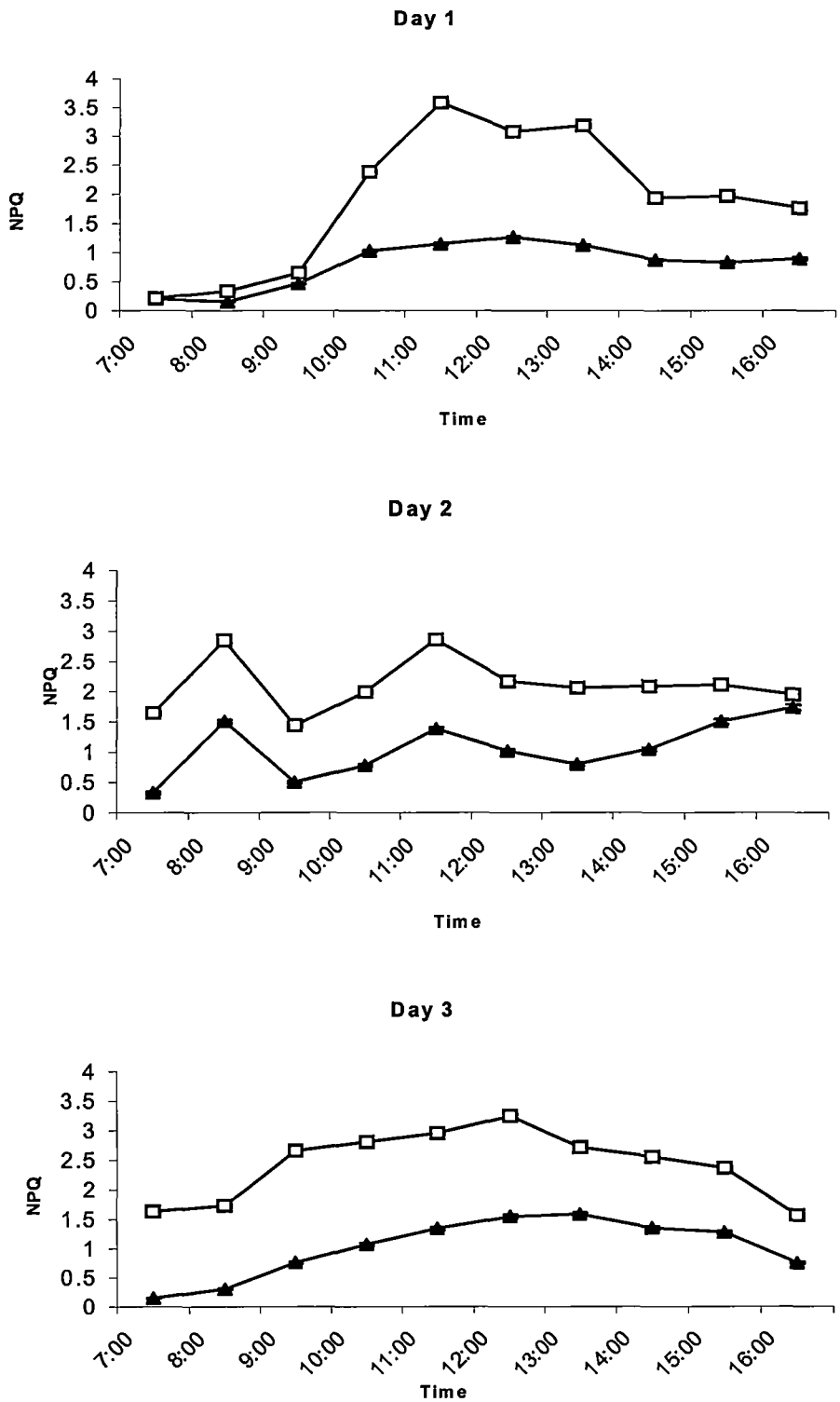


Figure 8: Effect of 20°C (closed symbols) and 4°C (open symbols) night temperature on non-photochemical quenching values of myoga plants positioned under 50% shade-cloth for a period of three days. Bars show SE (n=4). Where bars are not visible, SE was very small.

Diurnal chlorophyll fluorescence measurements in unshaded conditions

Yield values were observed to decrease far more severely for plants exposed to full sun compared with those under shadecloth (figure 9). On all three days a steep decline in quantum yield values was observed from 06:00 to 11:00 h, after which time plants remained at low quantum yield values of approximately 0.1. Quantum yield values for plants exposed to full sun kept at a night temperature of either 4 or 20^0C were not significantly different, except for the readings taken at 07:00 and 08:00 h on day 3. The level of photoinhibition observed for plants from both temperature treatments exposed to full sun did not change over the three days. From day 1, the level of photoinhibition was extreme for both temperature treatments as indicated by the very low yield values. Furthermore the initial quantum yield measurement taken at the start of each day showed that these plants were not making a full recovery overnight with decreases of approximately 0.1 on each consecutive day.

On all three days, levels of non-photochemical quenching in plants from both temperature treatments were observed to increase from 07:00 to approximately 11:00 h, after which time they maintained the same level of quenching (figure 10). Minor decreases were observed towards the end of the day. On day 1 and 3 plants with a night temperature of 4^0C had significantly higher ($P<0.05$) NPQ values than plants with a 20^0C night temperature. On day 2 no significant differences were observed between temperature treatments. NPQ values at the start of day 2 and 3 are higher in plants from both temperature treatments than those at the start of day 1. Although relaxation of non-photochemical quenching occurred during the dark period the higher NPQ values at 07:00 h on day 2 and 3 compared with day 1 indicated that sustained increased levels of non-photochemical quenching was occurring throughout the experiment.

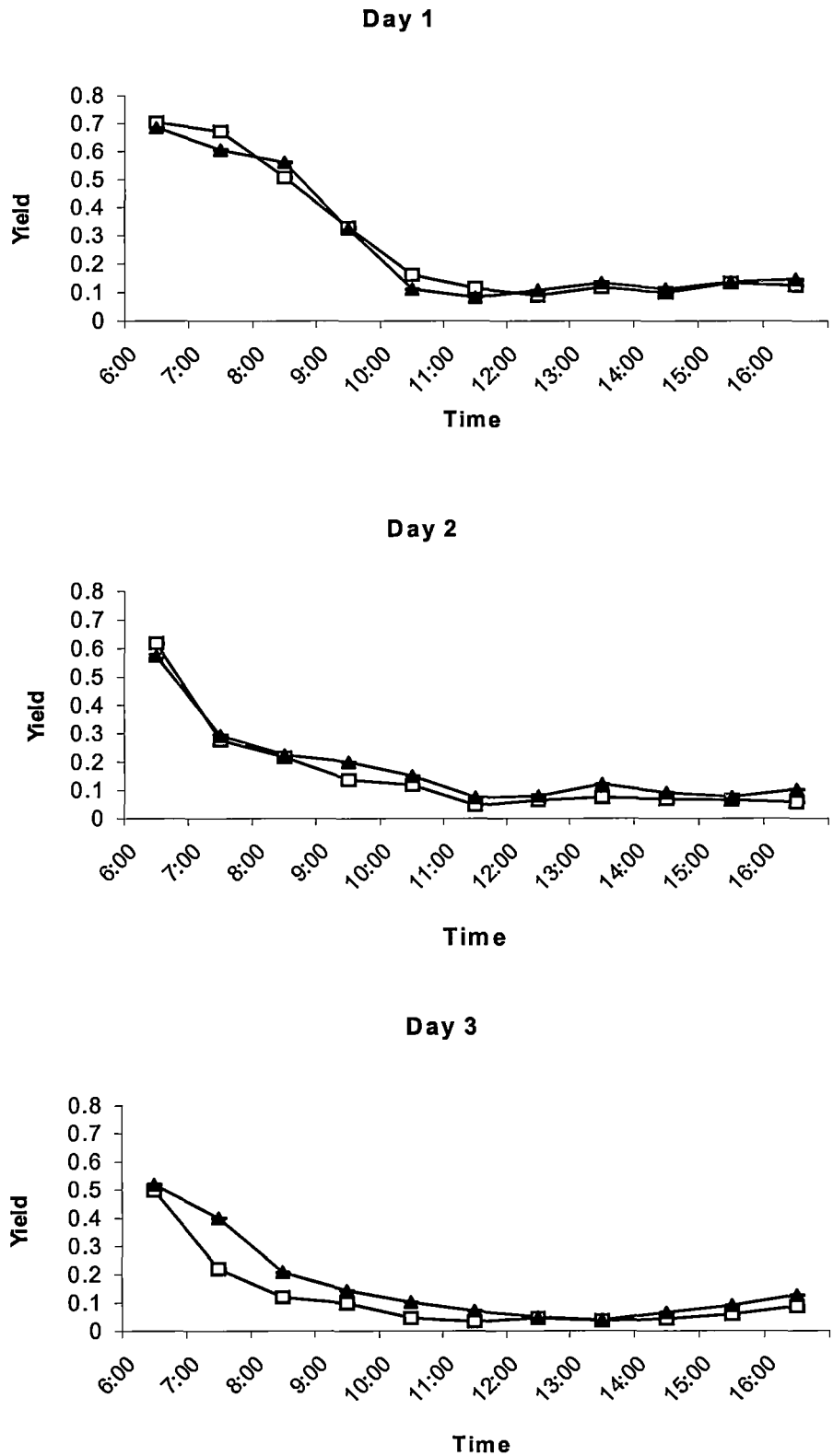


Figure 9: Effect of 20^{0}C (closed symbols) and 4^{0}C (open symbols) night temperature on quantum yield values of myoga plants positioned in full sun for a period of three days. Bars show SE (n=4). Where bars are not visible SE was very small.

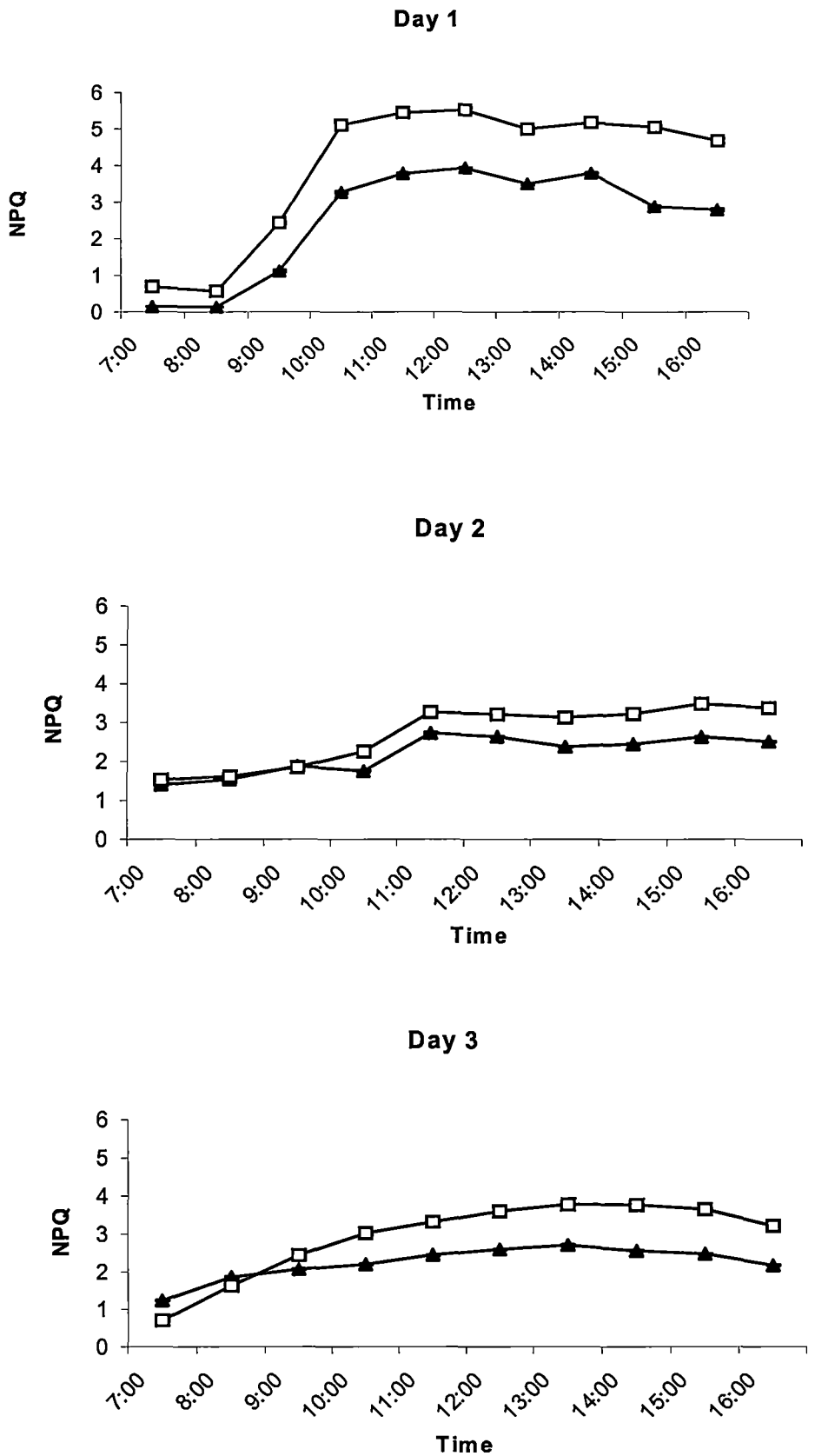


Figure 10: Effect of 20^{0}C (closed symbols) and 4^{0}C (open symbols) night temperature on non-photochemical quenching values of myoga plants positioned in full sun for a period of three days. Bars show SE (n=4). Where bars are not visible, SE was very small.

Gas exchange parameters

On the first day of the experiment, the intercellular CO_2 (C_i), stomatal conductance (g_s) and net assimilation of CO_2 (A) values were not significantly different between treatment plants for the majority of readings (figures 11, 12 & 13). Plants in full sun positions, experienced a gradual decrease in CO_2 assimilation rate and stomatal conductance (figures 11 & 12). Plants under 50% shadecloth at a night temperature of 20^0C were observed to have a gradual increase in CO_2 assimilation and stomatal conductance rates from 09:00 to 13:00 h which was followed by a strong decline through to 15:00 h. Plants under 50% shadecloth with a 4^0C night maintained a similar rate of CO_2 assimilation and stomatal conductance throughout day 1.

On day 2 and 3, the influence of low temperature on gas exchange parameters was clearly observed. Net assimilation rate of CO_2 and level of stomatal conductance was significantly lower ($P<0.05$) and intercellular CO_2 significantly higher ($P<0.05$) in plants under both sun and shade conditions with a 4^0C night temperature than those given a dark period at 20^0C ($P<0.05$) (figures 11, 12 & 13). Plants kept at 4^0C overnight and then placed into both full sun and shaded positions did not have significantly different CO_2 assimilation rates, levels of stomatal conductance or C_i on days 2 and 3. However plants kept at 20^0C overnight generally had significantly higher ($P<0.05$) rates of CO_2 assimilation and levels of stomatal conductance when placed into shaded positions compared with those in full sun. On the third day, plants in full sun positions which were given a 20^0C night temperature exhibited a large decrease in stomatal conductance and assimilation of CO_2 during the course of the day. This is paralleled by a similar increase in intercellular CO_2 over the same time period.

Plants which were chilled overnight had significant declines ($P<0.05$) in the diurnal mean of stomatal conductance values and rate of CO_2 assimilation on day 2 compared with day 1 and on day 3 compared with day 1. The diurnal mean of intercellular CO_2 was significantly higher ($P<0.05$) on each day for 4^0C plants which were shaded, and was significantly higher ($P<0.05$) for chilled plants in full sun on day 3 compared with day 1. Plants which were kept overnight at 20^0C and

placed into shaded conditions maintained the same level of assimilation rate and stomatal conductance throughout the experiment, while C_i values for these plants were the same on day 2 compared with day 1 but significantly higher ($P<0.05$) on day 3 compared with day 1. Non-chilled plants placed into full sun positions had significantly lower ($P<0.05$) diurnal mean values of stomatal conductance and assimilation rate and a significantly higher ($P<0.05$) diurnal mean value of C_i on day 3 compared with day 1.

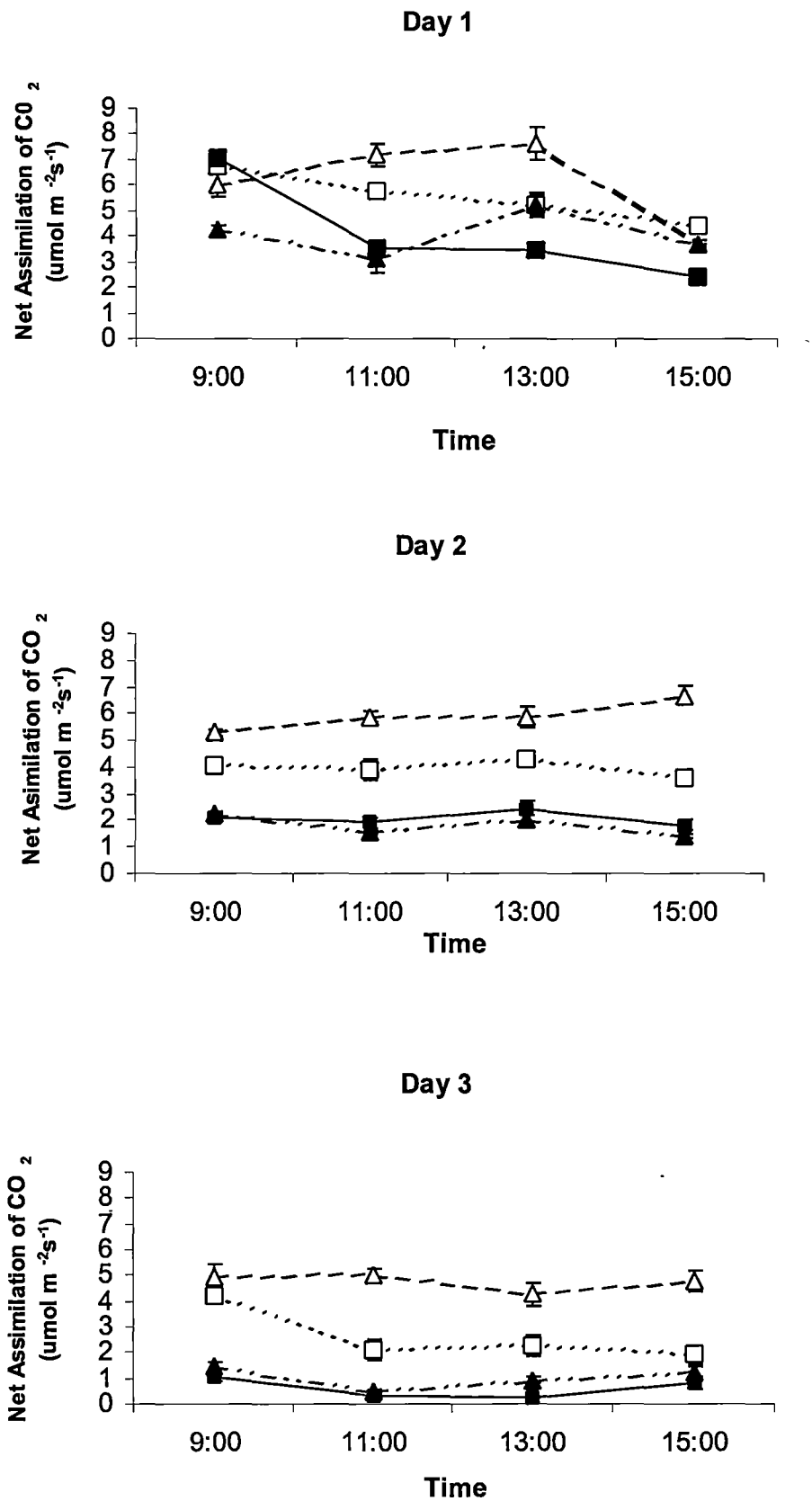


Figure 11: The response of net assimilation rate of CO_2 in myoga leaves exposed to full sun with a 20°C night ( ), full sun with a 4°C night ( ), shaded with a 20°C night (Δ) and shaded with a 4°C night ( ) over a period of three days. Bars show SE (n=4). Where bars are not visible SE was very small.

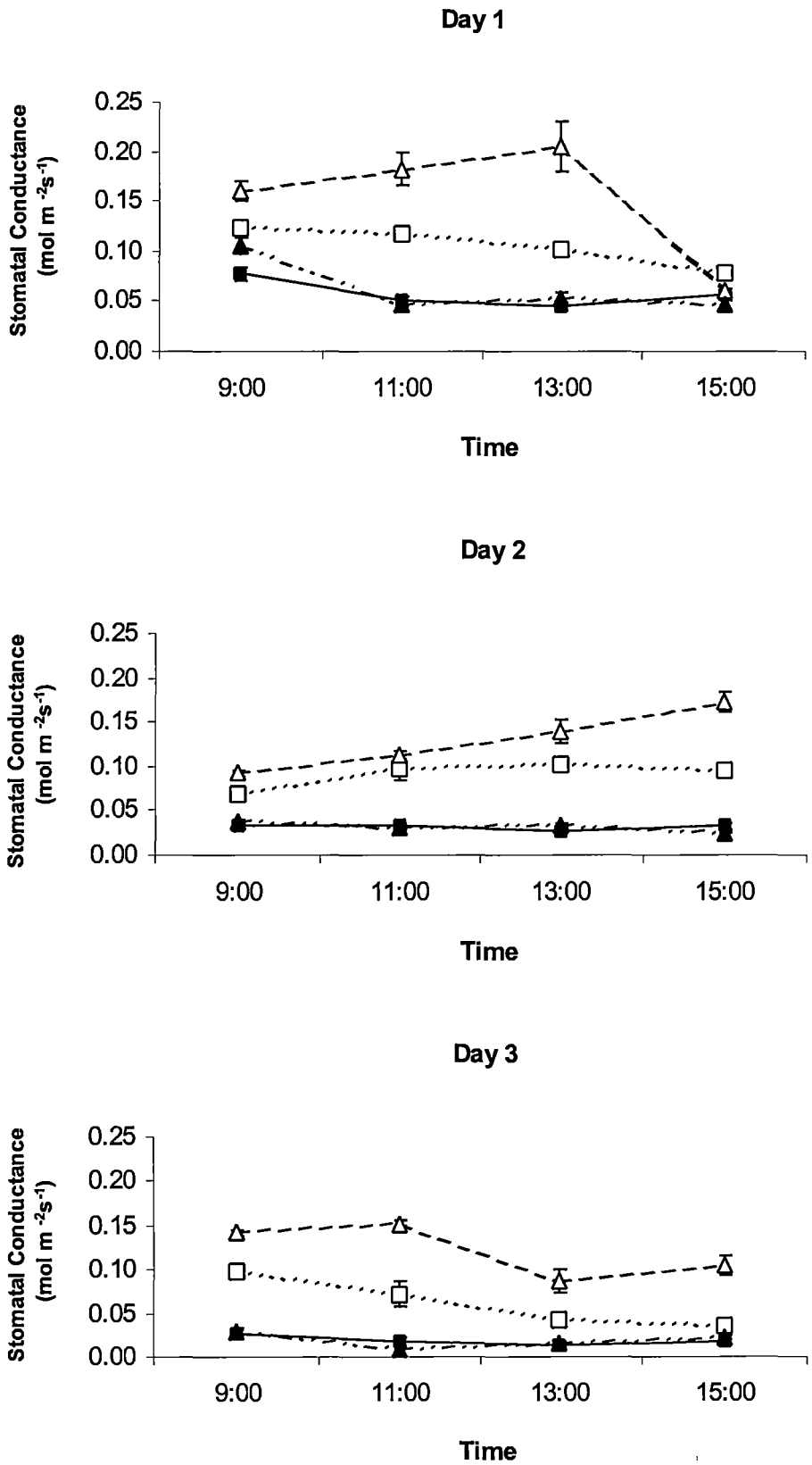


Figure 5: The response of stomatal conductance rates in myoga leaves exposed to full sun with a 20°C night ( ), full sun with a 4°C night ( ), shaded with a 20°C night (Δ) and shaded with a 4°C night ( ) over a period of three days. Bars show SE (n=4). Where bars are not visible, SE was very small.

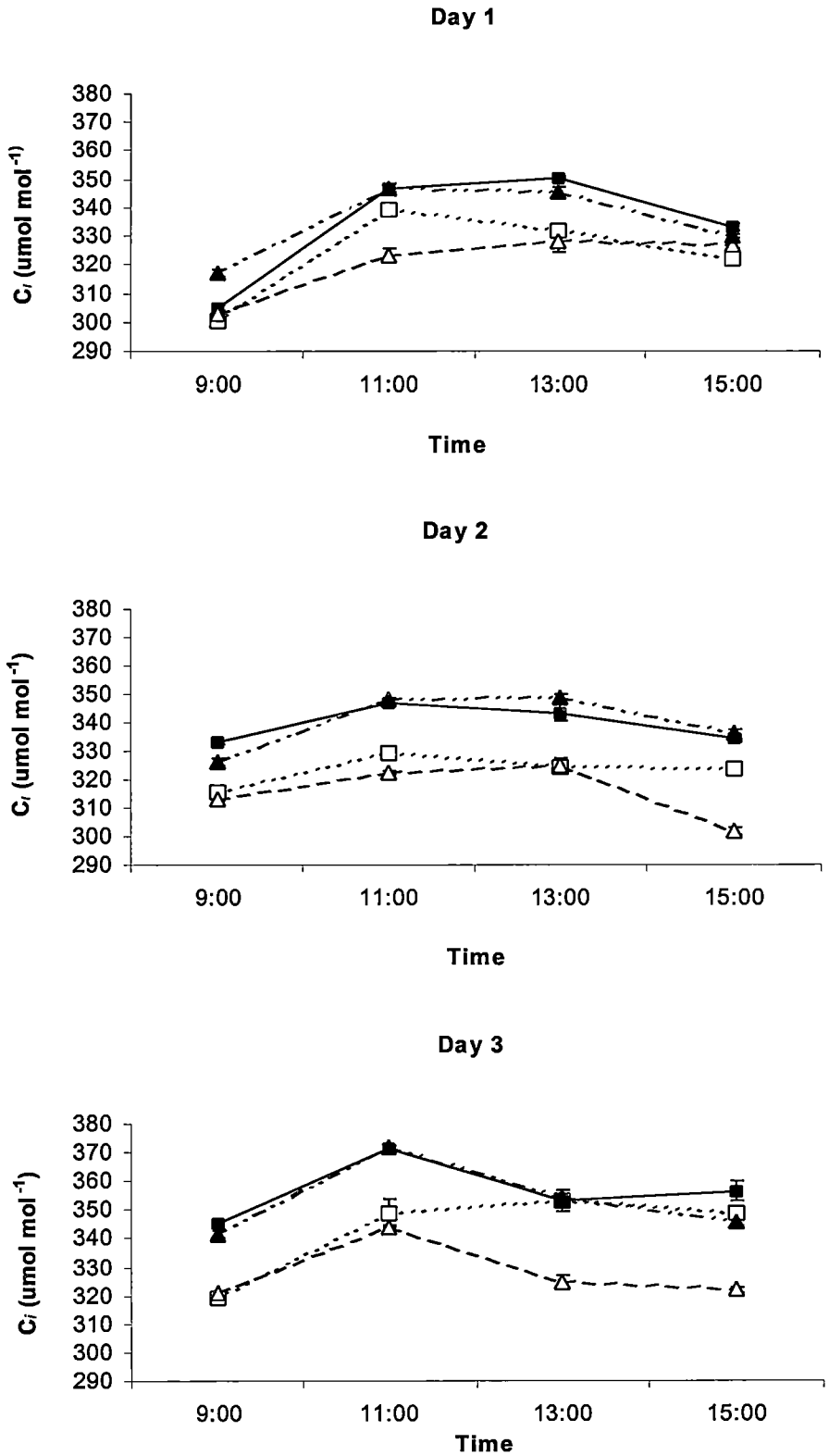


Figure 6: The response of intercellular CO_2 levels in myoga leaves exposed to full sun with a 20°C night ( ), full sun with a 4°C night ( ), shaded with a 20°C night (Δ) and shaded with a 4°C night ( ) over a period of three days. Bars show SE (n=4). Where bars are not visible SE, was very small.

Dark-adapted chlorophyll fluorescence measurements

There was a significant ($P<0.05$) decline in pre-dawn Fv/Fm values for all treatment plants from day 1 through to day 3 (table 1). This pattern continued on day 4, except for shaded plants kept overnight at 20^0C, which had no further significant decline. Plants in both light treatments with a 20^0C night temperature showed an increase in pre-dawn Fv/Fm values on the fourth day, while plants in both light treatments kept overnight at 4^0C continued to decline. There was no significant difference between all treatment plants in pre-dawn Fv/Fm values on day 2. On day 3 shade plants from both temperature treatments had similar pre-dawn Fv/Fm values, which were significantly higher ($P<0.05$) than those values from plants in full sun positions. Plants in sun positions had similar Fv/Fm values irrespective of the overnight temperature. On day 4 shade plants still had similar pre-dawn Fv/Fm values however due to the increased Fv/Fm value for plants in full sun positions with a night temperature of 20^0C, plants in the high light treatment had significantly different Fv/Fm values.

Day	Treatment	Chlorophyll fluorescence measurements		
		Fv/Fm	Fo	Fm
1	shade20	0.73 ± 0.01	470.25 ± 0.88	1773.38 ± 7.80
	shade4	0.69 ± 0.00	508.38 ± 0.93	1660.75 ± 2.08
	sun20	0.69 ± 0.01	560.50 ± 5.48	1764.25 ± 8.76
	sun4	0.71 ± 0.00	508.50 ± 0.57	1739.00 ± 3.95
2	shade20	0.72 ± 0.01	434.13 ± 0.91	1545.25 ± 6.31
	shade4	0.66 ± 0.01	481.63 ± 0.76	1443.13 ± 3.43
	sun20	0.57 ± 0.04	460.75 ± 0.53	1217.88 ± 10.00
	sun4	0.62 ± 0.02	457.38 ± 0.83	1265.75 ± 9.37
3	shade20	0.66 ± 0.01	450.25 ± 0.95	1342.38 ± 6.66
	shade4	0.64 ± 0.01	467.25 ± 0.69	1298.50 ± 3.15
	sun20	0.52 ± 0.02	511.75 ± 1.69	1086.50 ± 4.22
	sun4	0.50 ± 0.02	480.75 ± 0.98	1000.25 ± 7.28
4	shade20	0.69 ± 0.01	437.63 ± 1.42	1438.38 ± 8.00
	shade4	0.58 ± 0.01	446.50 ± 0.95	1080.88 ± 4.47
	sun20	0.53 ± 0.02	502.88 ± 1.38	1135.38 ± 6.55
	sun4	0.42 ± 0.02	435.75 ± 2.12	811.38 ± 8.12

Table 1: Maximum quantum yield of PSII (Fv/Fm), minimal fluorescence (Fo) and maximal fluorescence (Fm) of myoga plants exposed to various night temperatures and light intensities over a period of four days. Each value is the mean ± standard error of eight replicates.

Discussion

Quantum yield values depict the proportion of the light absorbed by chlorophyll associated with PS II that is used in photochemistry and thus gives an indication of overall photosynthesis (Maxwell & Johnson 2000). All the plants in this trial showed some degree of photoinhibition of photosynthesis, identified by the decrease in quantum yield values over the course of the day. The degree of photoinhibition depended on the intensity of sunlight received and the temperature of the previous night. Plants placed into full sun had the greatest decreases in quantum yield during the light periods of the experiment. The decline from 0.7 to 0.1 exhibited on the first day of exposure by plants in full sun indicated that very little light energy was being utilised photochemically and that these plants were severely photoinhibited. Plants under shade conditions also exhibited reduced quantum yield values over the period of the day and the mean quantum yield decreased from day 1 through to day 3. The preceding night temperature was observed to have a significant effect on the photosynthetic ability of plants placed under shadecloth, since those maintained at 4^0C during the dark period had a significantly greater reduction in photosynthetic efficiency than those kept at 20^0C.

The increased NPQ values observed in chilled myoga plants during this experiment is indicative of the increased efficiency at which excess light energy is being dissipated as heat. In response to such stressful conditions the photoprotective mechanisms within the plant could be expected to have increased the efficiency at which light energy is dissipated as heat while decreasing the amount of energy entering photochemistry, resulting in a decrease in the quantum yield of PS II. The significant difference in NPQ levels between temperature treatments for plants in both full sun and shaded positions was due most likely to plants at low overnight temperatures experiencing excessive excitation of PS II, as a result of low temperature-induced inhibition of the dark reactions of photosynthesis (Huner et al. 1993; Öquist et al. 1993a). Additional amounts of light energy would then have to be dissipated as heat through the xanthophyll cycle in an attempt to prevent photodamage occurring. The high NPQ levels observed in plants in shaded conditions with a night temperature of 4^0C at the beginning of day 2 and 3 of the

experiment, clearly showed that no relaxation of non-photochemical quenching was occurring over the dark period. The sustained high level of non-photochemical quenching and decreased PS II efficiency observed on day 2 and 3 is suggestive of the overnight retention of the xanthophyll carotenoids, zeaxanthin and antheraxanthin (Adams et al. 2002). The sustained engagement of these carotenoids in a state primed for energy dissipation is indicative of increased photoprotective activity in these plants aimed at preventing damage such as photo-oxidation of pigments and membranes.

The susceptibility of leaves to photoinhibition is known to be enhanced by additional stress factors which coincide with light (Nir 1997). By chilling plants in the dark before exposing them to high light there is an even greater chance that photoinhibition will occur. Very little difference in the degree of photoinhibition experienced by plants kept at cool and warm night temperatures was observed in myoga plants placed into full sun. It would appear that even without the stress of a preceding low night temperature the light intensity experienced by plants in full sun was sufficient to cause a severe reduction in photosynthetic efficiency of myoga leaves. The reduced light intensity experienced by plants under shadecloth allowed an interaction between the preceding night temperature and the light intensity of the following day to be observed. Under shade, plants which were chilled before exposure to light exhibited a greater reduction in photosynthetic efficiency compared with those plants that had not been chilled.

For chilling-sensitive herbaceous species, low temperature during the dark period can cause severe reduction in both $C0_2$ fixation and stomatal conductance on the day following the chill (Izhar et al. 1967; Pasternak & Wilson 1972; Crookston et al. 1974). During this investigation myoga plants kept at 4^0C the previous night had lower rates of CO_2 assimilation and reduced stomatal conductance the following day compared with plants kept at 20^0C. Reduced stomatal conductance of chilled plants is often observed in conjunction with lowered photosynthetic rates. The closure of stomates in response to chilling has previously been thought to be the cause of reduced leaf photosynthesis, due to the associated lowering of the

intercellular concentration of CO_2 (Izhar et al. 1967; Pasternak & Wilson 1972; Crookston et al. 1974). However on the second and third day of this experiment the intercellular CO_2 concentration in chilled myoga leaves increased rather than decreased despite the fact that stomatal conductance was reduced. Therefore the limitations to CO_2 uptake within the leaf due to impairment of biochemical functions were much greater than the limitations imposed by the decreased stomatal conductance. Stomatal limitations due to pre-chilling of myoga plants is unlikely therefore to be the primary cause of decreased photosynthetic performance.

The reduction of carbon uptake, while still absorbing high levels of radiation would be expected to lead to excess excitation energy. Absorption of excess excitation energy that cannot be utilised for photosynthesis has the potential to produce reactive oxygen species which damage pigments and membranes. This damage can be pre-empted by down-regulating photochemical processes and increasing levels of photoprotection. Dark-adapted Fv/Fm values are a sensitive indicator of the photosynthetic efficiency of a plant; representing the fraction of solar energy absorbed in PS II antennae that can be utilized in PS II photochemistry. Any perturbation in the photosynthetic apparatus due to stress will result in a lowering of Fv/Fm values. On the morning of the second, third and fourth day sustained decreases in Fv/Fm values were observed in plants from all treatment conditions. The sustained depressions in Fv/Fm values were possibly due to a combination of PSII core degradation and a high potential for zeaxanthin-dependent thermal energy dissipation (Adams et al. 2002).

The high sustained levels of non-photochemical quenching, while decreasing PSII efficiency, would have protected myoga plants from the formation of damaging reactive oxygen species. Myoga plants kept at a night temperature of 4^0C and placed into full sun positions over the subsequent light periods exhibited large sustained decreases in pre-dawn Fv/Fm values over the course of the experiment. Plants in this treatment would be susceptible to severe photoinhibition from the first day onwards as the first cool night temperature probably resulted in excessive excitation of PS II due to an inhibition of the dark reactions of photosynthesis. Over

subsequent dark periods, a reduction in the efficiency of D1 protein repair due to the low temperature would mean that even less light energy could be utilised in photochemistry resulting in increased levels of photoinhibition. However upon analysis of the dark-adapted chlorophyll fluorescence measurements, the minimal fluorescence (Fo) value was observed to decrease in conjunction with the lowering of maximal fluorescence (Fm). Chlorophyll fluorescence measurements can help to distinguish between photoprotective mechanisms and photoinhibitory damage. The photoprotective component of NPQ often termed qE, results in a decrease of both Fo and Fm, whereas photodamage normally increases the Fo level while decreasing the Fm level (Gilmore et al. 1996). Therefore although constant decreases in the fluorescence ratio was observed for plants in the most stressful treatment, the failure of the minimal fluorescence (Fo) value to rise indicated that the high level of NPQ observed consisted of the qE component which protects PSII against short-term high light conditions (Müller et al. 2001).

During this trial myoga plants were shown to be susceptible to photoinhibition when placed into light intensities greater than approximately 700 μmol $m^{-2}s^{-1}$. The induced photoinhibition of photosynthesis was indicated by decreases in photochemical yield and increased rates of non-photochemical quenching over the light period. In addition the constant decreases of pre-dawn Fv/Fm values from all treatment plants indicated that the stressful light conditions had a significant effect on the capacity of photosystem II. The addition of chilling temperature during the night period increased the susceptibility of myoga to excessive light absorption resulting in a greater degree of photoinhibition. Plants which experienced low night temperatures exhibited lower rates of CO_2 assimilation and stomatal conductance the following day. Increased intercellular concentration of CO_2 in the same plants suggested that the stomata closed as a result of an increased C_i, when the demand for CO_2 decreased because of low-temperature inhibition of the biochemical processes of photosynthesis. Therefore it is most likely that the primary loss of photosynthetic activity arose from direct impairment of biochemical functions as observed in plants chilled in the light (Kee et al. 1986). The consistent decreases in dark-adapted Fv/Fm values and the severity of diurnal quantum yield depressions potentially indicated that photoprotective functions within myoga plants were

exhausted and unable to prevent photodamage from occurring. However the high level of photoprotective NPQ sustained throughout the experiment and the observed decrease of minimal fluorescence in conjunction with maximal fluorescence suggested that photoprotective processes were increased so that photoxidation of membranes and pigments could be avoided.

Myoga plants grown in areas with night temperatures below 10-12^{0}C are likely to become severely photoinhibited upon exposure to bright, sunny days. Although myoga plants proved capable of managing photosynthetic function under such stressful conditions, the investigations were of a short-term nature. Long-term exposure to this type of conditions could prove photodamaging to the plants resulting in bleaching and necrosis of vegetation with severe reduction of yield and potentially senescence. For this reason it is advisable that myoga plants cultivated in areas with cool night temperatures be protected by shadecloth to reduce incident photosynthetically active radiation. Use of shadecloth for cultivation of myoga comes at some cost both in terms of economics and plant performance. The level of shading chosen for cultivation should be done with careful consideration as over-shading of myoga plants will result in a decrease in photosynthetic performance and flower bud yield (Gracie et al. *in press*).

SECTION 2: LOW-TEMPERATURE INDUCED PHOTOINHIBTION – *COLD HARDENING*

Introduction

Results from previous investigation of the susceptibility of myoga to photoinhibition in high light conditions preceded by cool night temperatures indicated that the photoinhibition experienced was most likely dynamic in nature. That is, the down regulation of photosynthesis observed was serving as a protective measure from permanent damage to PS II occurring. In this trial myoga plants were exposed to ambient conditions at the Horticultural Research Centre, University of Tasmania which consisted of a low night temperature followed by a high light day. Chlorophyll fluorescence measurements were conducted after plants had been left for 17 hours in conditions conducive to recovery. Measurements were delayed in order to determine if the photoinhibition observed was dynamic or chronic. To confirm that the responses observed in previous investigations which had utilised controlled environment cabinets to achieve set temperatures would also occur in normal field cultivation, this experiment was conducted using ambient night conditions in an exposed position at the University of Tasmania.

This experiment while confirming the nature of low temperature-induced photoinhibition in myoga plants also sought to examine the possibility that myoga plants could become acclimated to cold temperatures. Plants such as spinach (Boese and Huner, 1990), rye (Lapointe et al., 1991) and wheat (Hurry and Huner, 1992) have all exhibited the capacity to increase tolerance to photoinhibition when acclimated to cold temperatures. In this experiment myoga plants were grown in environments with various levels of shading: while two of the conditions included moderate night temperatures and warm diurnal periods the third set of plants were cultivated outside and exposed to low night temperatures and moderate diurnal periods. Chlorophyll fluorescence measurements were conducted on plants from each of the three environments subsequent to exposure to a low night temperature followed by a high light day.

Materials and Methods

Planting Material (For details refer to section materials and methods)

Plant Growth Conditions

Twenty plants were grown in a shadehouse at the Horticultural Research Centre, University of Tasmania. Average day/night ambient temperatures in the shadehouse were 17/8^{0}C. Level of shading in the shadehouse was approximately 60 %. A further twenty plants were grown in a glasshouse at the Horticultural Research Centre, University of Tasmania. Temperatures in the glasshouse were maintained at day/night temperatures of 25/15^{0}C. Half the plants in the glasshouse were grown under shadecloth and the combined level of shading (glasshouse plus shadecloth) was approximately 80%. The other ten plants were grown inside the glasshouse without shadecloth. Compared with outside light levels, the glasshouse reduced light levels by approximately 50%. Measurements commenced on plants approximately three to four months after planting.

Photoinhibition Treatment

Dark-adapted and steady-state (illuminated) chlorophyll fluorescence measurements were conducted on the three most recently expanded leaves of three plants from each growth environment before they were moved at 1600 h to an area outside. These measurements were used as an indication of the pre-exposed relaxed state of leaves from each environment. Outside plants were positioned so that they received full sun and were not shaded by any structures. The plants remained outside overnight and at 16:00h the following day were placed inside a large darkened room maintained at approximately 17^{0}C where they equilibrated overnight. The following morning dark-adapted chlorophyll fluorescence measurements were conducted inside the darkened room. Following dark-adapted measurements, plants were moved outside for steady-state (illuminated) measurements. Measurements began after plants had been left to equilibrate for 1 hour. Temperature was measured and logged on a tiny-tag recorder, light intensity was measured and logged using a LI-COR quantum sensor.

Chlorophyll Fluorescence Measurements (refer to section materials and methods)

Statistical Analysis

The effect of growth environment and exposure to photoinhibiting conditions on the photosynthetic performance of myoga plants was determined using a 2 x 3 factorial experimental design. Experimental units were arranged in a completely randomised layout. Chlorophyll fluorescence parameters were analysed using analysis of variance.

Results

After exposure to ambient conditions outside, plants grown in the glasshouse displayed a significant (P=0.0001) decrease in Fv/Fm values (figure 1). Plants grown in the glasshouse under shade (GHS) had the largest decrease of 34%, followed by plants grown in the glasshouse without shade (GHNS) with 21%. Shadehouse plants retained the same Fv/Fm value after overnight exposure. The decrease in Fv/Fm was largely due to decreases in Fm (figure 2) since minimal fluorescence (F_0) levels remained almost the same before and after exposure (figure 3).

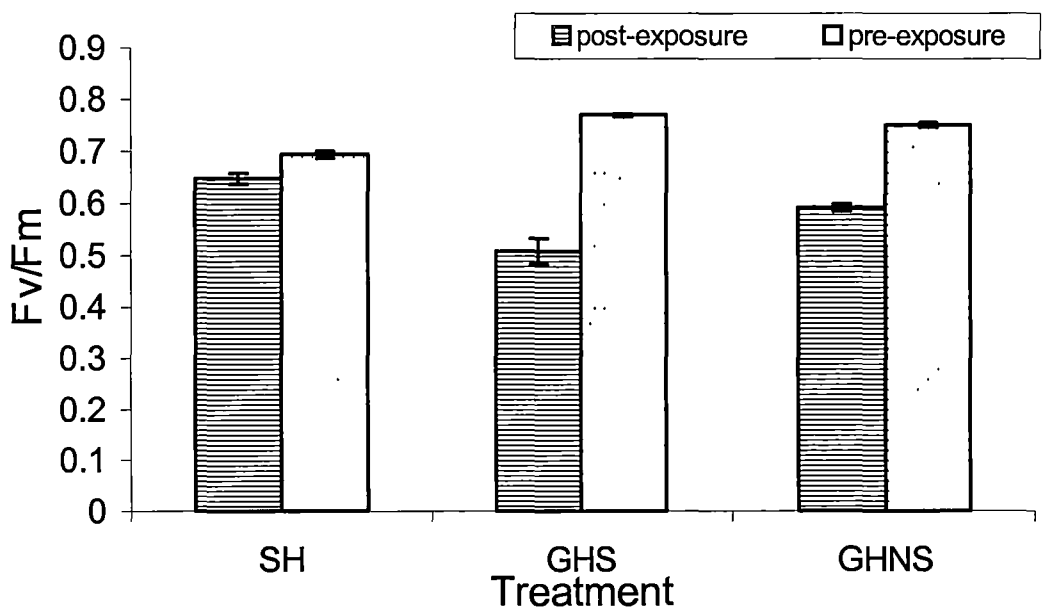


Figure 3: Maximum quantum yield of PSII values for plants from the following environments: shadehouse, glasshouse and glasshouse+shade. Units are dimensionless. Bars show SE (n=3).

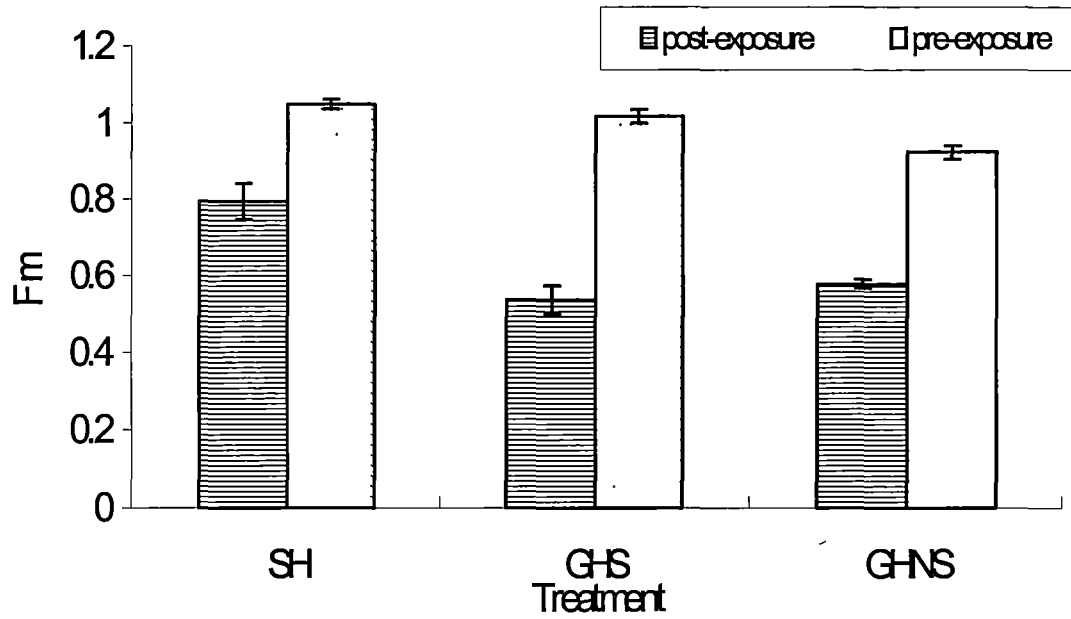


Figure 2: Maximal fluorescence values for plants from the following environments: shadehouse, glasshouse and glasshouse+shade. Units are dimensionless. Bars show SE (n=3).

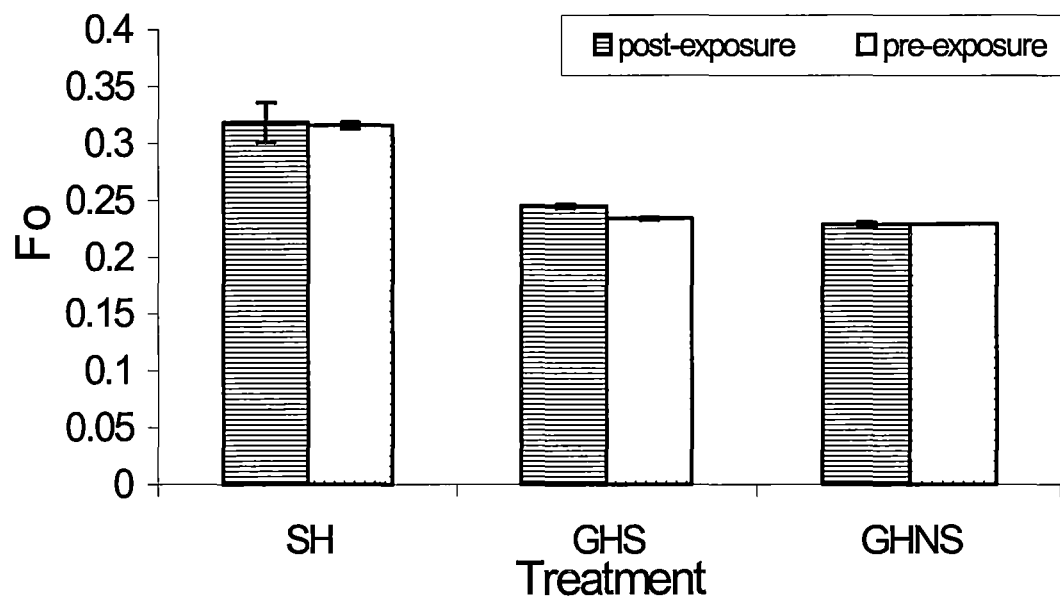


Figure 3: Minimal fluorescence values for plants from the following environments: shadehouse, glasshouse and glasshouse+shade. Units are dimensionless. Bars show SE (n=3).

Glasshouse plants grown without shade had a significantly ($P<0.05$) higher initial quantum yield value than GHS and SH plants (figure 4). Although shadehouse plants maintained their initial yield value after exposure, plants grown in the glasshouse had significant ($P<0.05$) decreases in yield after exposure. GHS plants had a 19% decrease in the quantum yield of PSII, which was significantly ($P<0.05$) lower than SH and GHNS plants. Glasshouse plants grown without shade exhibited a 10% decrease following overnight exposure.

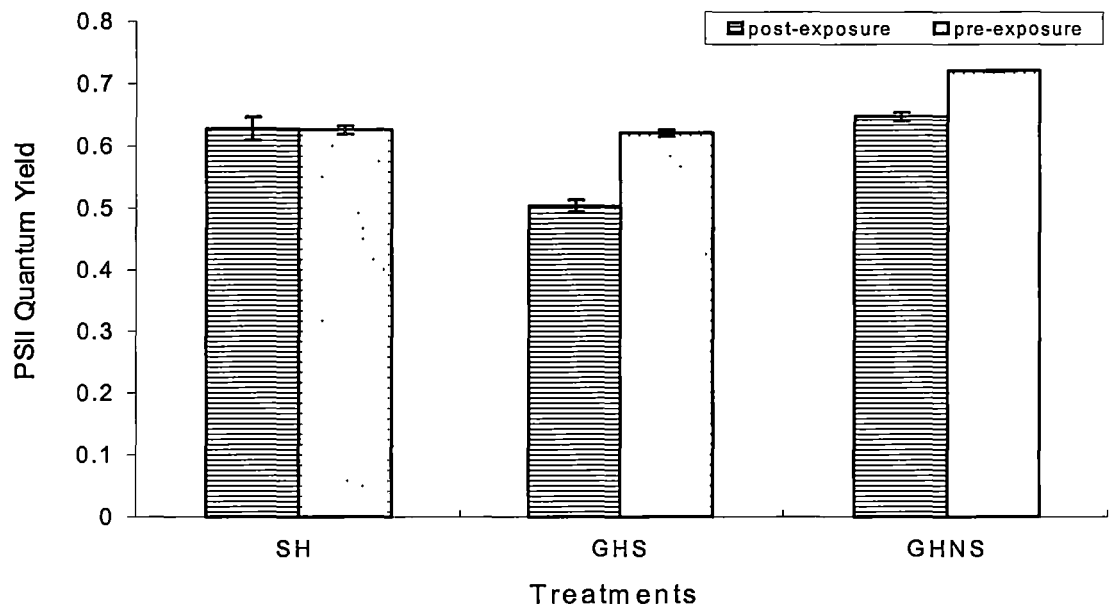


Figure 4: Quantum yield of PSII values for plants from the following environments: shadehouse, glasshouse and glasshouse+shade. Units are dimensionless. Bars show SE (n=3).

The number of closed reaction centres is indicated by the fluorescence parameter 1-qP, which approximates the reduction state of Q_A (Bilger and Bjorkman, 1990). Plants from all treatments showed no significant difference in the degree of PSII reaction centre closure as a result of overnight exposure (figure 5). Prior to overnight exposure plants from all environment conditions had the same number of reaction centres closed.

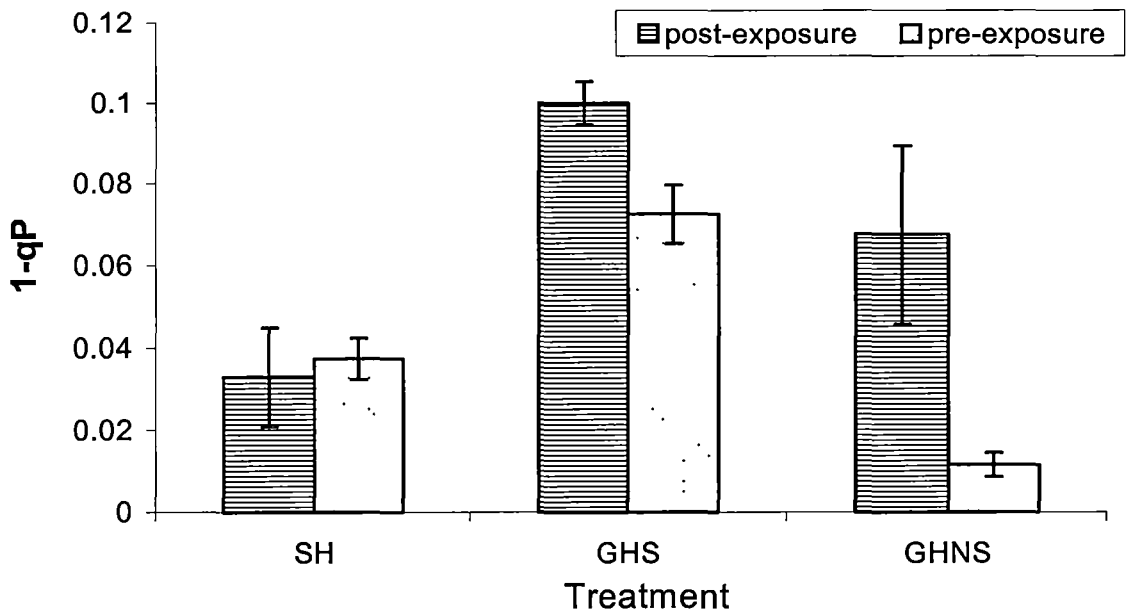


Figure 5: 1-qP values for plants from the following environments: shadehouse, glasshouse and glasshouse+shade. Units are dimensionless. Bars show SE (n=3).

The intrinsic photochemical efficiency of open centres in light-adapted leaves is calculated as Fv'/Fm' (Warren et al., 1998). It gives an indication of the efficiency of those reaction centres that are open, that is, the efficiency with which the reaction centres transfer electrons through the electron transport chain to photosystem I. Reaction centres within SH and GHNS plants retained their initial efficiency levels after exposure. Glasshouse plants grown under shade displayed a significant ($P<0.05$) decrease of 17% in reaction centre efficiency after exposure (figure 6). Prior to overnight exposure, the level of reaction centre efficiency was the same in plants from the shadehouse and those grown in the glasshouse with shade. Plants grown in the glasshouse without shade had a significantly ($P<0.05$) higher level of reaction centre efficiency.

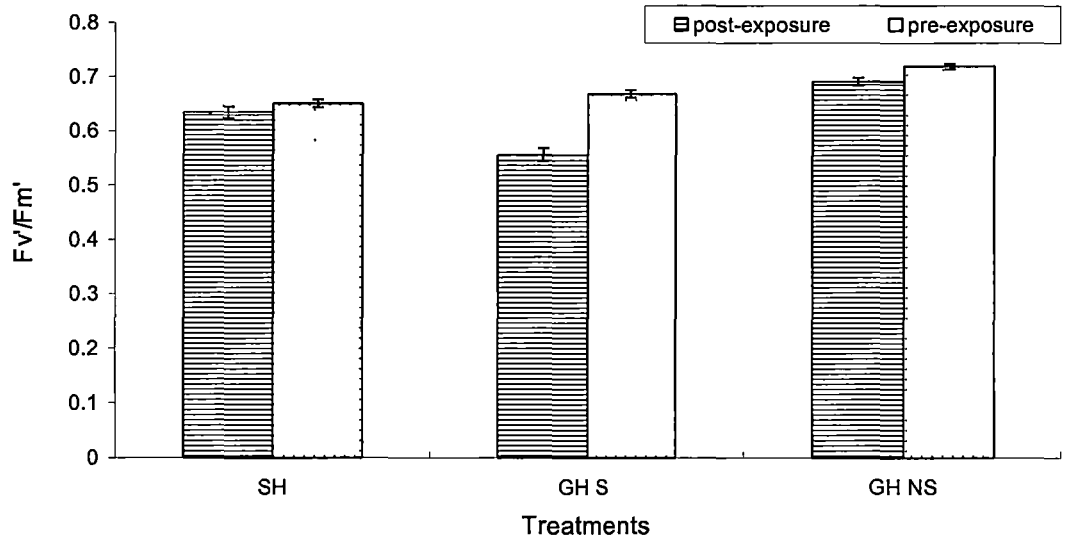


Figure 6: Fv'/Fm'values for plants from the following environments: shadehouse, glasshouse and glasshouse+shade. Units are dimensionless. Bars show SE (n=3).

Plants from each growth environment have increased levels of NPQ after exposure to ambient conditions outside (figure 7). The largest increase of 62 % occurred in plants grown in the glasshouse without shade ($P<0.0001$). Plants grown in the glasshouse with shade had an increase of 51 % while those grown in the shadehouse had an increase in NPQ of 30 %. Before exposure GHS plants had the highest levels of NPQ followed by SH control plants. Plants from the glasshouse grown without shade had very low initial levels of NPQ.

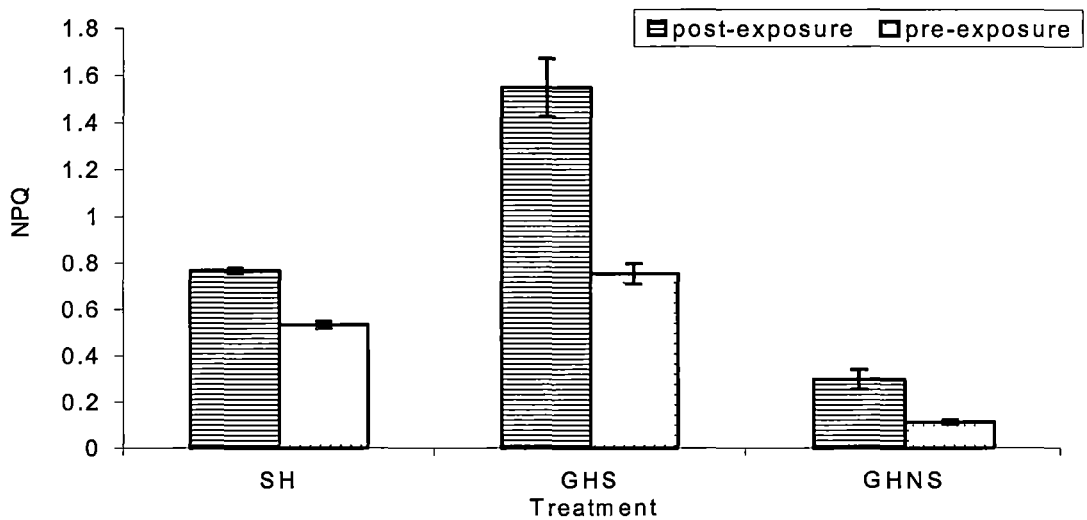


Figure 7: Non-photochemical quenching values for plants from the following environments: shadehouse, glasshouse and glasshouse+shade Units are dimensionless. Bars show SE (n=3).

Night temperatures experienced by the plants during exposure were between 5 and 10^0C. The temperature gradually increased during the morning and from approximately 09:00 to 16:00 h the average ambient temperature was 22^0C (figure 8). The light intensity during the light period of exposure from approximately 09:00 to 16:00 h averaged 900 μmol $m^{-2}s^{-1}$ (figure 9).

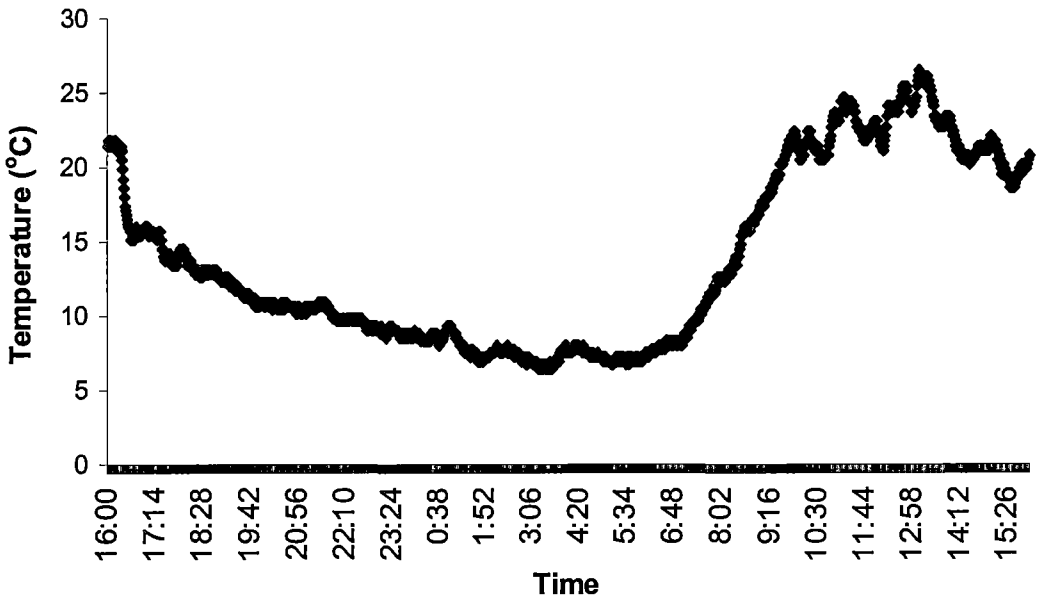


Figure 8: Ambient temperature at HRC, University of Tasmania from 16:00 h (10/12/01) to 16:00 h (11/12/01).

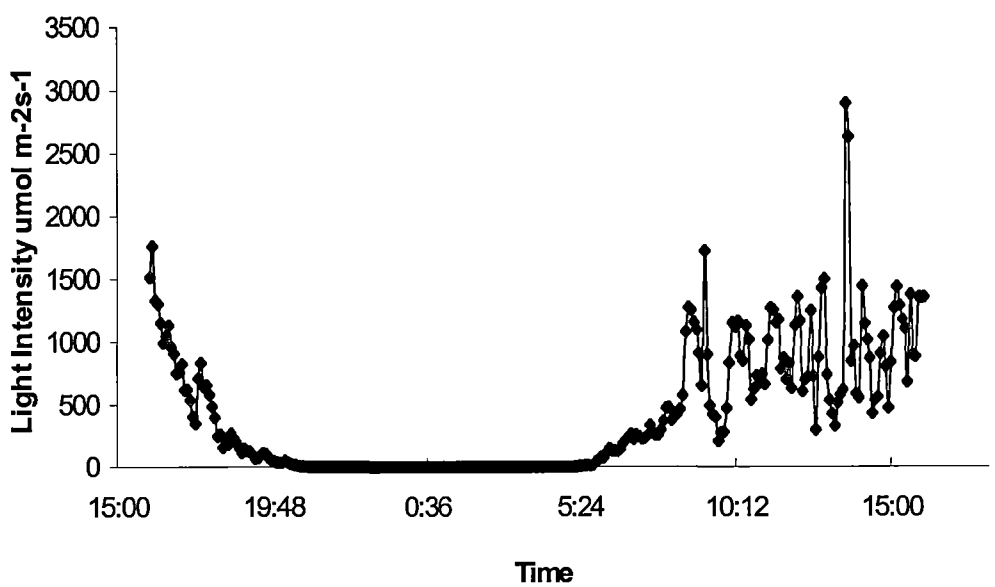


Figure 9: Ambient light intensity at the HRC, University of Tasmania from 16:00 h (10/12/01) through to 16:00 h (11/12/01).

Discussion

The reduction in dark-adapted Fv/Fm values in glasshouse plants after exposure revealed a decrease in the efficiency of excitation energy capture by open photosystem II (PSII) reaction centres indicating that photoinhibition had occurred (Butler and Kitajima, 1975). A decrease in Fv/Fm values accompanied by a decrease in maximal fluorescence (Fm) could result from damage to PS II or from sustained high rates of energy dissipation. Although the decrease in Fv/Fm may indicate photodamage, it is also consistent with the presence of sustained rates of photo-protective non-photochemical quenching (Naidu & Elucia, 1997).

Quantum yield measures the proportion of the light absorbed by chlorophyll associated with PS II that is used in photochemistry. As such, it can give a measure of the rate of linear electron transport and so an indication of overall photosynthesis (Maxwell & Johnson, 2000). Glasshouse plants grown under shade had the greatest reduction in the proportion of absorbed energy being used in photochemistry. These plants would have been the most susceptible to the photoinhibiting conditions since they were grown and therefore acclimated to, the lowest light intensities and warmest night temperatures. Therefore in response to the stressful conditions experienced during exposure they were unable to maintain the same level of photosynthesis. The decrease in yield occurred due to a decrease in efficiency of open reaction centres. Plants grown in the glasshouse without shade had a smaller decrease in overall photosynthesis compared with those grown under shade. Reaction centre closure although not significant was the highest amongst all the treatment plants and it may be due to this that a decrease in yield was observed.

In a healthy leaf exposed to photon flux densities similar to those encountered in the field most of the overall quenching in the steady state is photochemical. On the other hand leaves in which energy conversion is suppressed tend to show preferential quenching by non-photochemical mechanisms (Bolhar-Nordenkampf, 1989). The decreases in yield observed in the glasshouse plants mean that light energy must be utilised by other mechanisms if damage to the photosystem is to be prevented. To avoid damage, light energy is dissipated by non-photochemical

quenching, as evidenced by increases in NPQ in response to overnight exposure. Plants grown in the shaded glasshouse conditions which were likely to be the most sensitive to cold temperatures and high light conditions approximately doubled their levels of NPQ. Plants from GHNS conditions had the highest increase of 62 %. These plants had very low initial levels of NPQ, which could be why they displayed the greatest increase in non-photochemical quenching in response to overnight exposure.

The dark-adapted chlorophyll fluorescence parameters displayed here were obtained from measurements taken after plants had been kept approximately 17 hours in warm, dark conditions. This substantial period of recovery should have been sufficient to return Fv/Fm values to pre-exposure levels. The fact that Fv/Fm values were still lower in glasshouse plants following the recovery period indicates that these plants were severely stressed as confirmed by the decreased yield values and increased levels of NPQ. The maintenance of minimal fluorescence levels at the same level before and after exposure indicates that the non-photochemical quenching was successfully dissipating the excess light energy not utilised in photochemical processes and permanent damage to PS II had not occurred.

Plants that had been grown in the shadehouse were exposed to ambient temperature conditions and had therefore experienced some degree of cold-hardening compared with those plants grown in the glasshouse. There are several different mechanisms cold-acclimated plants may use to increase resistance to photoinhibition. Authors such as Oquist et al. (1993 a,b), view the effect on carbon metabolism as the main reason for the increased resistance to photoinhibition. They found that cold-acclimated leaves of winter cereals were shown to possess an increased capacity for photosynthetic carbon metabolism at low temperatures and, therefore, are more capable of maintaining the PS II electron acceptor Q_A in the oxidised state. Scavenger systems for active oxygen species in chloroplasts are also known to increase upon cold-acclimation (Krause,1994). There also appears to be a number of changes in the xanthophyll cycle in response to growth at low temperatures. In cold-acclimated spinach, the total xanthophyll content was increased (Shoner and

Krause, 1990), and cold-grown winter and spring wheat contained much greater amounts of violaxanthin and zeaxanthin than leaves grown at 20^0C (Hurry and Huner, 1992).

From the results observed here, it would appear that the increased resistance to photoinhibition achieved by plants grown in the shadehouse was due to an increased capacity for photosynthetic carbon metabolism at low temperatures. This is shown in figure 5, where shadehouse plants had decreased numbers of closed reaction centres after exposure compared with plants grown in the glasshouse, which had increased numbers of closed reaction centres. An increase in the number of open reaction centres indicates that the PS II electron acceptor Q_A is being kept in an oxidised state and more energy can be utilised in photochemistry. In addition there were no significant decreases in the level of overall photosynthesis occurring, as indicated by unchanging yield values and the efficiency of open reaction centres remained the same.

A number of differences in fluorescence parameters can be observed between plants grown in the shadehouse and those grown in the glasshouse. Shadehouse plants had no decrease in Fv/Fm after exposure indicating that severe photoinhibition did not occur in these plants. Shadehouse plants also had the lowest Fv/Fm value before exposure suggesting that the proportion of efficiently working PS II units in the total PS II population was lower during development of the plant. The minimal fluorescence level (Fo) was significantly higher both before and after exposure compared with glasshouse plants. The minimal fluorescence reflects the effectiveness of coupling between antennae chlorophyll and reaction centres of PS II (Shabala, *in press*). Fo often increases under stress conditions and indicates that less energy is reaching the reaction centres and is therefore unavailable for use in photochemistry. Therefore although shadehouse plants have effectively increased tolerance to photoinhibition in response to stressful conditions, the general level of photosynthetic activity while in protected environments may be lowered, reducing plant growth and yield.

Chronic photoinhibition usually predominates in shade-grown plants and occurs following sustained exposure of the photosynthetic apparatus to light intensities greater than that normally experienced during growth. Alternatively it may occur at light intensities normally experienced during growth, but under environmental conditions which themselves impair photosynthetic function such as low temperatures (Osmond, 1994). The results from this experiment show that although chronic photoinhibition did not occur in glasshouse plants in response to a single overnight exposure to cold temperatures followed by a sunny day, repeated exposure to these conditions may result in chronic photoinhibition developing.

The resistance to severe photoinhibition displayed by shadehouse plants can be attributed to cold-acclimation rather than an adaptation to high light intensities. Plants grown at similar or slightly higher light intensities (i.e. GHNS plants) did not have an increased resistance to photoinhibition as displayed by the decrease in Fv/Fm values. If previous light acclimation afforded some protection from photoinhibition it would be expected that plants from the glasshouse grown without shade would be less susceptible to photoinhibition than plants from the shadehouse. As this was not the case protection from photoinhibition during exposure to low temperatures combined with full light appeared to be a result of cold-hardening rather than previous growth at higher light intensities. Field grown crops in temperate climates are quite likely to experience cold overnight temperatures followed by sunny days. When this happens photoinhibition is likely to occur. Whether the photoinhibition is permanently damaging to PS II or simply a down-regulation of photosynthesis will depend on a number of factors. These include the sensitivity of the plant, the degree of acclimation to cold temperatures and whether environmental conditions continue to be stressful or conducive to recovery.

Conclusions

The predisposition of myoga plants to shaded conditions was observed throughout these experiments. At a moderate light intensity of 750 μmol $m^{-2}s^{-1}$ the photochemical capacity within myoga plants was reduced forcing the plants to engage non-photochemical mechanisms to dissipate excess light energy. Although myoga could be safely classified as a shade plant, the successful prevention of photodamage during the short-term stressful conditions of the previous experiments indicates that myoga plants are capable of withstanding higher levels of light than they would normally be exposed to in their natural environment. However the point at which photoprotective mechanisms become exhausted and damage begins has not been established and it is likely that long-term exposure to high light intensities would result in the oxidation of pigments and membranes.

Susceptibility to chilling temperatures in myoga plants has shown an increased predisposition to photoinhibition, with lower light intensities being tolerated. Chilling in conjunction with light exposure of myoga plants resulted in light intensities as low as 250μmol $m^{-2}s^{-1}$ becoming photoinhibitory. When plants were chilled in the dark and subsequently placed into warm photoperiods, photosynthesis became depressed primarily through limitations of biochemical functions within PS II which lowered stomatal conductance. Myoga plants were successfully able to prevent photodamage occurring over a three day period of stressful conditions, most likely due to sustained engagement of the xanthophyll cycle carotenoids in a state primed for light energy dissipation.

Acclimation to cold temperatures can occur to a certain degree in a range of chilling-sensitive plants and can provide an increased level of protection from photoinhibition. Myoga plants which were grown in cooler ambient temperatures displayed a decreased susceptibility to photoinhibition when placed into stressful light conditions, preceded by a cool night. Prevention of photoinhibition was thought to be due to an increased capacity for photosynthetic carbon metabolism at low temperatures which maintained the PS II electron acceptor Q_A in the oxidised state.

Industry Implications

The preference of myoga plants for shaded conditions and warm temperatures during growth was confirmed during this series of trials. The ability of myoga plants to maintain flower bud yields when grown without shade in Rockhampton (Section 3), would therefore appear to be a result of the warm night temperatures experienced in this region. Although these plants are likely to be photoinhibited during field cultivation in this area, the down-regulation of photosynthesis may serve to protect these plants from permanent damage. The minor amount of leaf scorching and necrosis observed in plants grown under these conditions did not appear to reduce flower bud yield.

Myoga plants grown in areas with night temperatures below 10-12^{0}C are likely to become severely photoinhibited upon exposure to bright, sunny days. Although myoga plants proved capable of managing photosynthetic functions under such stressful conditions, the investigations were of a short-term nature. Long-term exposure to this type of conditions could prove photodamaging to the plants resulting in bleaching and necrosis of vegetation with severe reduction of yield and potentially senescence. For this reason it is advisable that myoga plants cultivated in areas with cool night temperatures be protected by shadecloth to reduce incident photosynthetically active radiation. Use of shadecloth for cultivation of myoga comes at some cost both in terms of economics and plant performance. The level of shading chosen for cultivation should be done with careful consideration as over-shading of myoga plants will result in a decrease in photosynthetic performance and flower bud yield (Gracie et al., *in press*).

Acclimation of myoga plants to cooler temperatures was observed during this investigation. Therefore it is possible that myoga plants given sufficient exposure to low temperatures could decrease susceptibility to low temperature-induced photoinhibition. However the geophytic nature of this crop results in plant vegetation having little time to become acclimated to low temperatures. Vegetative growth occurs over a short six month period, with all vegetation developed, directly impacting on the yield of flower buds. Therefore myoga plants can not afford to have damaged photosynthetic tissue, without adversely affecting flower bud yields.

SECTION 3: INVESTIGATION OF CULTURAL FACTORS AFFECTING COMMERCIAL CULTIVATION OF MYOGA - *INTRODUCTION*

During the first two years of commercial cultivation of myoga plants a number of production issues became apparent. The correct management of this crop required information on several aspects of plant growth and development affecting the production of flower buds. Previous trials which examined some of these issues had been conducted in controlled environment conditions and it was not known whether the same results would be achieved in a commercial situation. For this reason several trials were designed within the commercial planting or in environmental conditions which closely resembled those that would be found in future commercial plantings. This would enable any results to be quickly adopted as industry practice.

The first four trials examining cultural factors affecting flower bud yield were conducted within the commercial planting at Albion Park, NSW. These trials were primarily aimed at investigating whether myoga could be viably cultivated as a perennial crop or whether one year rotations would be more productive. A production trial was also conducted at Rockhampton, QLD so that the results which had been obtained during controlled environment trials in Hobart, TAS could be tested under environmental conditions which would be similar to potential new commercial plantings.

SECTION 3: INVESTIGATION OF CULTURAL FACTORS AFFECTING COMMERCIAL CULTIVATION OF MYOGA – *SECTION MATERIALS AND METHODS*

Albion Park Trials

Plant Material

Plant material was propagated vegetatively from myoga plants grown in the field at New Norfolk, Tasmania. The rhizome material was soaked in a fungicide treatment consisting of 100 ml of Previcur®, 200 ml of Baviston®, 100 ml of Sumisclex® and 200g of Kocide® per 100 L of water. The rhizome material was packed into boxes and transported to Albion Park, NSW where approximately 4800, 100g pieces were planted into the ground in September 1999. No additional chilling treatment was given to the rhizome material before planting as the material had already over-wintered in the ground at New Norfolk. Irrigation was by hand and done according to visual measure. In the first year of growth one base application of a N:P:K (8:4:10) mix was applied at a rate of 400kg/hectare and 34% ammonium nitrate was applied twice at a rate of 100kg/hectare. In the second year of growth there were two more applications of 34% ammonium nitrate at a rate of 100kg/hectare.

Albion Park Field Site

The commercial production site at Albion Park consisted of a 2000 m^2 area covered by 50% shadecloth (Alumni-net, thermal screen). The shadecloth extended along all four sides of the planting and was covered by a retractable roof at an approximate height of 3m. Rhizome material was planted in 24 raised beds which were 1.2 m wide and 45m long. Two rows of rhizome material were planted per bed with 0.4m spacing within and between rows so that plants were diagonally staggered. Each raised bed was bordered by timber 6cm high and 2cm wide. The soil contained within the beds was sourced

from Albion Park, while the mulch placed on top of the soil to aid with flower bud colour development consisted of pine shavings sourced from Wollongong.

Grading of flower buds

Flower buds harvested from field trials conducted at Albion Park were graded according to a chart used for grading flower buds in Japan (plate 1). Flower buds were visually assessed for colouration and designated to either A, B or C grade. If flower buds had reached anthesis by the time of harvesting they were considered no longer suitable for commercial sale.

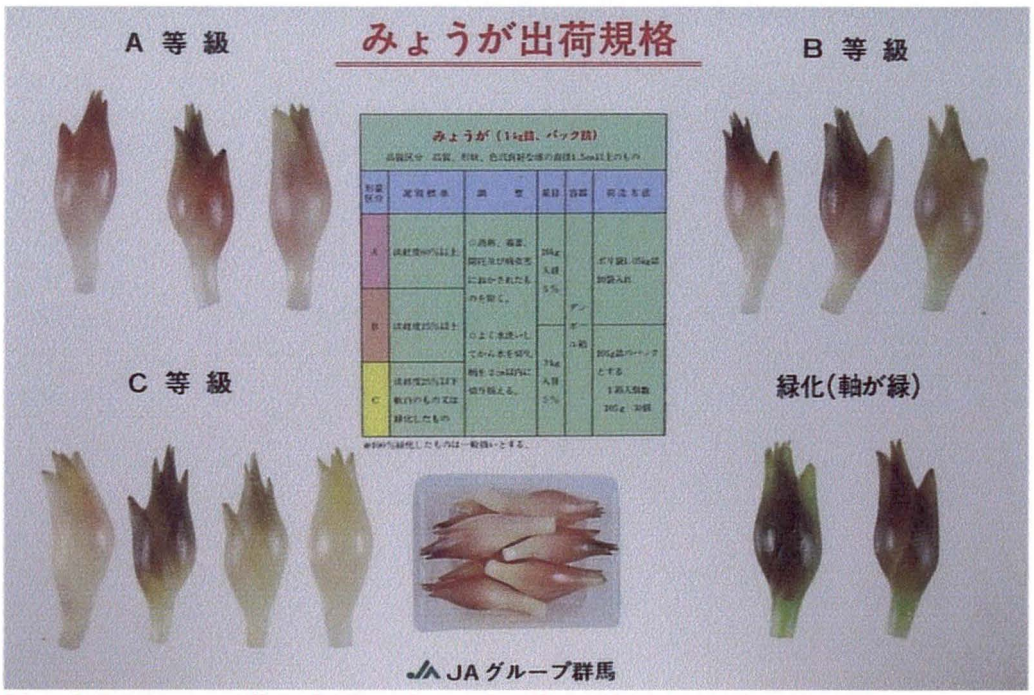


Plate 1: Japanese grading chart used to assign flower buds to A, B or C grade.

SECTION 3: INVESTIGATION OF CULTURAL FACTORS AFFECTING COMMERCIAL CULTIVATION OF MYOGA – *DETERMINATION OF CHILL REQUIREMENT FOR COMMERCIALLY CULTIVATED MYOGA*

Introduction

Myoga exhibits a distinct periodicity of growth. Under natural conditions, growth commences after a period of dormancy or rest over winter months. Pseudostems (vegetative shoots) grow from the original rhizome segments in spring followed by a flush of flower buds which develop from the terminal meristem of further branched rhizomes, in mid summer to early autumn. Rhizome and tuberous root development continues during the later stages of flower bud development, followed by foliage senescence and abscission in late autumn (Gracie et al., 2000).

Rhizome dormancy in myoga is of approximately six weeks duration, with the loss of dormancy not requiring a period of chilling (Gracie, et al. 2000). Chilling does however affect the rate at which pseudostems emerge when replanted, with longer periods of chilling resulting in decreased time to shoot emergence (Follett, 1991; Gracie, 2000). Longer storage periods before planting and increased duration of chilling treatments decrease the variability in the sprouting response of rhizome segments indicating that variability exists in the physiological state of buds on rhizomes when rhizomes are first divided (Gracie et al. 2000).

Maeda (1994) reported that dormancy could be broken by chilling and that chilling for periods of time greater than 60 days promoted new pseudostem development from buds on the rhizome. Plants that did not receive any chilling failed to develop new pseudostems. Researchers in New Zealand also determined that storage of rhizomes at cool temperatures affected subsequent emergence of pseudostems. Follett (1991) showed that 90% of rhizomes which had been subjected to six or more weeks of chilling at 4^0C would develop pseudostems within three months of being replanted. Whereas only 45% of non-chilled rhizomes developed pseudostems. These results conflict with those obtained by Gracie et al. (2000) who detected no difference in the

total number and growth rate of pseudostems produced from chilled and non-chilled plants.

Yield results from commercial field production within Australia have to this point been based on one year old plants, whose dormancy period has been spent within the ground at New Norfolk, Tasmania. With respect to climatic conditions in Japan, myoga was initially produced in Tasmania due to the expectation that a period of winter chilling was required. It was thought that the winter dormancy period might also be satisfying a vernalization requirement for subsequent flower bud initiation (Clark, 2001). The proposal that myoga plants remain in the ground in Albion Park, NSW during the dormant winter period caused concern that the chilling requirement would not be satisfied. Without the chilling period poor pseudostem emergence and low flower bud yields might occur.

This trial aimed to determine if additional chilling was required for plants that remained in Albion Park for periods greater than one year. Furthermore if additional chilling was required, it was desirable to establish what the most effective length of chilling would be.

Materials and Methods

Plant Material

Rhizome material originally planted in September, 1999 at Albion Park was used in this trial. For details refer to section materials and methods. Harvesting of flower buds finished in March, 2000 after which time senescence of above ground plant material occurred in response to the colder temperatures. Treatments were imposed from July through to September, 2000. Plants produced vegetative growth from November through to January, 2001 at which point flower buds began to emerge at the ground surface.

Treatments

Plants were lifted from the ground, washed and packed in wax boxes with moist sacking to avoid desiccation. The boxes were placed in a cool store in Wollongong maintained at an average temperature of 4^0C. After the required amount of chilling had been received, the rhizome material was brought back to Albion Park, trimmed to 100 g pieces and replanted using 0.4 x 0.4 m spacing. Plants in treatment 1 were lifted, washed, trimmed to 100 g pieces and replanted on the same day and therefore received no artificial chilling (Table 1).

Treatment	*Length of Chilling*	*Date Lifted*	*Date replanted*
1	Zero weeks	4th of September	4th of September
2	Two weeks	24th of August	4th of September
3	Four weeks	7th of August	4th of September
4	Six weeks	24th of July	4th of September

Table 1: Lifting and replanting schedule used to achieve chilling treatments of rhizome material.

A bed within the commercial planting (for details refer to section materials and methods) was divided into plots which were 2.4 m long by 1.2 m wide. Each plot, containing ten plants, was randomly assigned a treatment. Each treatment was replicated three times. Each plot was marked out by digging down through the mulch to soil level and placing a block of wood in between each plot. This prevented rhizomes from plants of different treatments growing together.

Harvest

Half the plants from each treatment plot were regularly harvested of commercially mature flower buds, while the other half were left until the end of the harvesting period when a single, final harvest was performed at 208 DAP. The first seven sequential harvests were performed every three to five days, while the last two harvests were conducted seven to fourteen days after the previous harvest. The final sequential harvest was performed at the same time as the single, final harvest. All flower bud material was removed from the plots at this time. At each harvest, flower buds from each treatment plot were counted, weighed and examined for anthesis. A grading chart

similar to that used for commercially cultivated flower buds in Japan was used to assess the quality of the flower buds and determine if they were acceptable for commercial sale (plate 1; section materials and methods).

Statistical Analysis

The experiment was established using a split-plot design. The length of chilling given to rhizome pieces was the main plot effect, while the sub-plot effect was the type of harvesting method used. Results were analysed using ANOVA with assumption of equal variance, normal distribution of underlying population and random sampling. Least significant differences (LSD) were calculated for treatment effects to aid interpretation of results.

Results

Sequential Harvest

Harvest weight and number of flower buds produced by plants from all treatments increased gradually from 155 to 194 DAP. A large increase in yield was observed after 194 DAP most likely due to the increased duration between harvests and the removal of all grades of flower bud material at the final harvest (figures 1 & 2). There was no significant difference in the number or total harvest weight of flower buds produced from plants that were given different lengths of chilling from 0 to 6 weeks (figures 1, 2, 3 & 4). The mean individual bud weight was significantly ($P<0.05$) lower in control plants (0 weeks chilling) compared to plants that were given chilling periods of 2 and 6 weeks (figure 5).

Single Harvest

Plants that received no chilling (0 weeks chilling) produced significantly higher ($P<0.05$) numbers of flower buds and had a higher total harvest weight than those plants that were chilled for 2, 4 and 6 weeks (figures 3 & 4). The individual mean weight of flower buds remained the same regardless of the length of chilling (figure 5).

Comparison of harvesting methods

There was no difference between the number of flower buds produced and the total harvest weight of buds when plants were sequentially harvested as compared to when there was only one harvest. The individual mean bud weight was however much lower across all chilling treatments when plants were sequentially harvested (figures 3, 4 & 5).

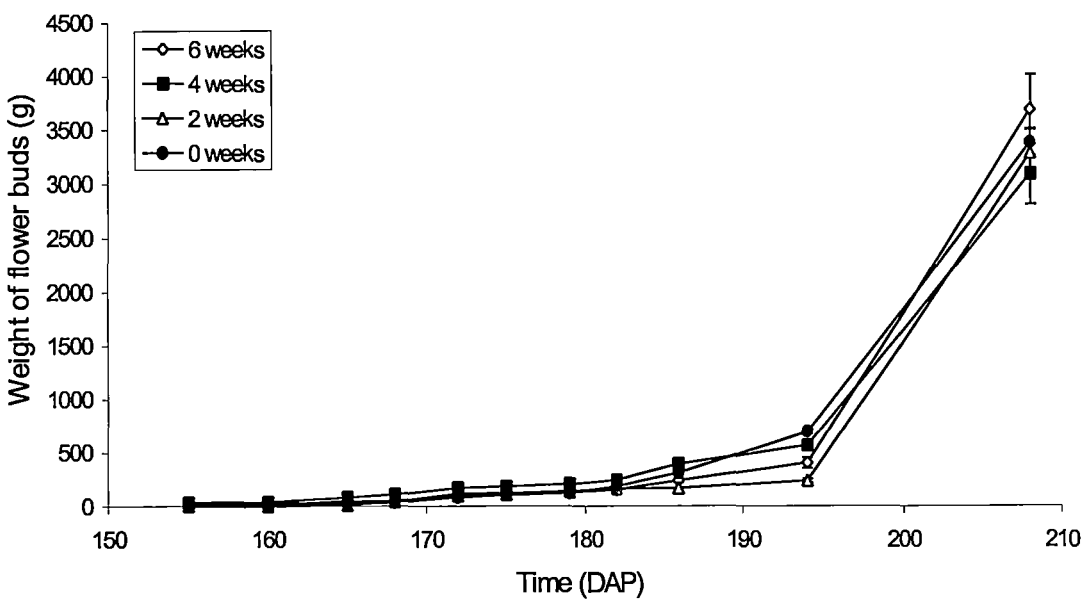


Figure 1: Cumulative harvest weight of flower buds produced by all treatment plants from 155 to 210 DAP, using a sequential harvesting method. Bars show SE (n=3).

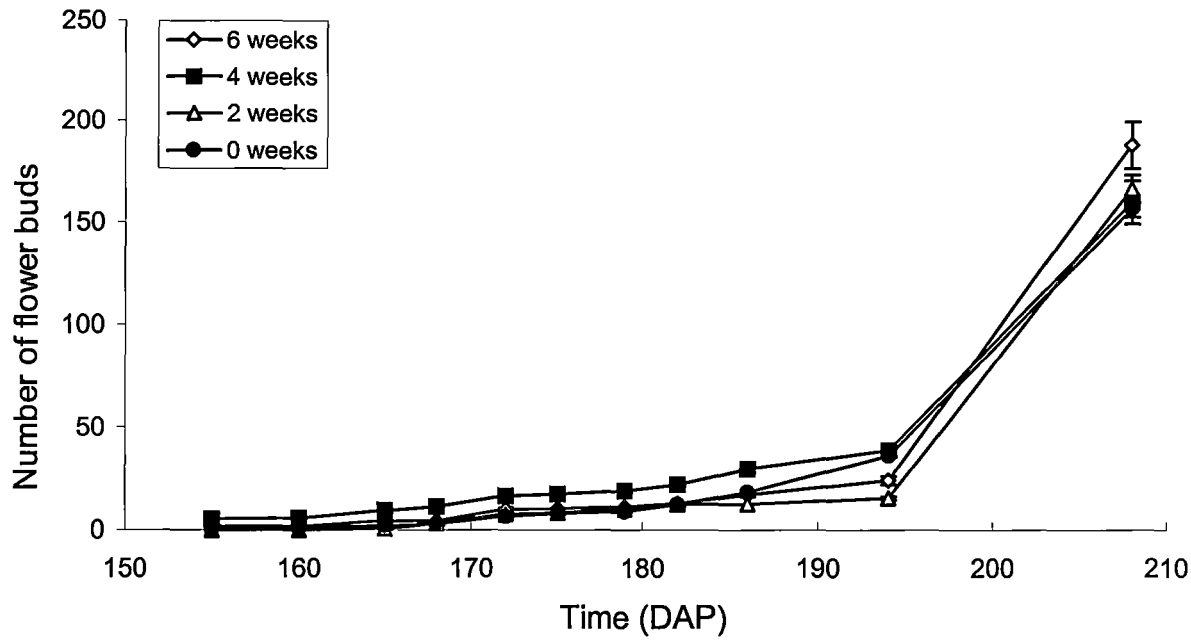


Figure 2: Cumulative number of flower buds produced by all treatment plants from 155 to 210 DAP, using a sequential harvesting method. Bars show SE (n=3).

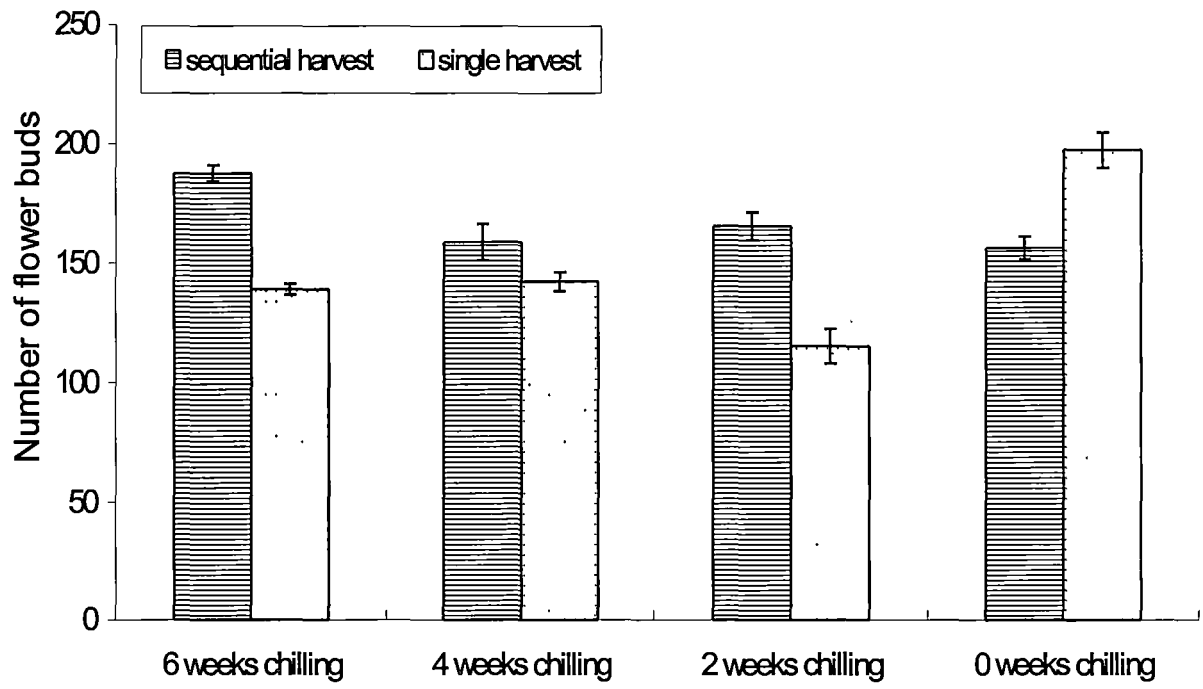


Figure 3: Total number of flower buds produced for all treatment plants using both harvesting methods. Bars show SE (n=3).

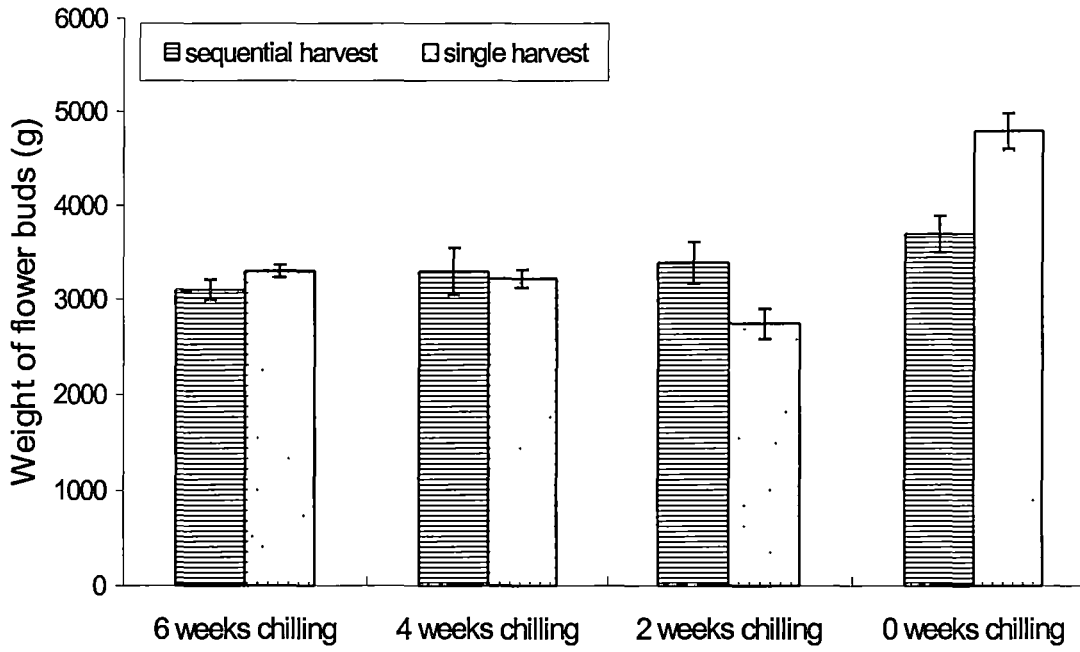


Figure 4: Total harvest weight of flower buds produced by all treatment plants, using both harvesting methods. Bars show SE (n=3).

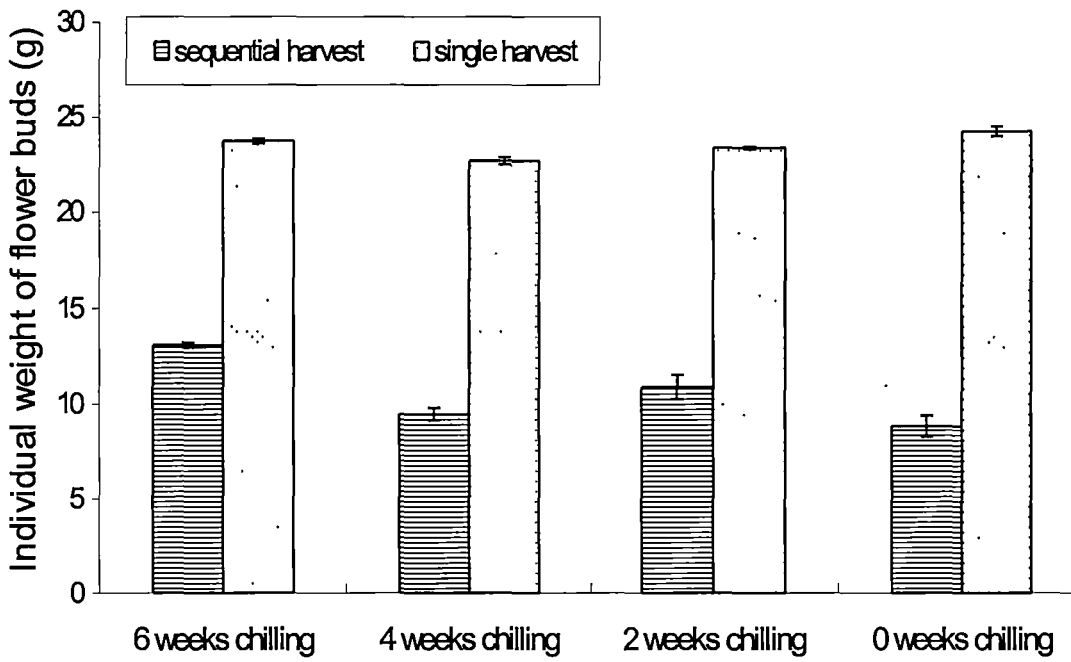


Figure 5: Mean weight of individual flower buds from all treatment plants using both harvesting methods. Bars show SE (n=3).

Main plot	*Sub plot*	*Average flower bud weight*	*Number of flower buds*	*Total harvest weight*
0 weeks chill	Sing. harvest	A	A	A
0 weeks chill	Seq. harvest	B	ABC	AB
2 weeks chill	Sing. harvest	A	C	B
2 weeks chill	Seq. harvest	C	ABC	B
4 weeks chill	Sing. harvest	A	BC	B
4 weeks chill	Seq. harvest	BC	ABC	B
6 weeks chill	Sing. harvest	A	BC	B
6 weeks chill	Seq. harvest	C	AB	AB

Table 2: Treatment LSD's for mean flower bud weight, total harvest weight and number of flower buds produced. Treatments with the same letter are not significantly different ($P<0.05$).

Discussion

Under glasshouse conditions, myoga was shown to have a rhizome dormancy of approximately 6 weeks (Gracie et al., 2000). However when grown in field conditions myoga plants remained dormant for a period of approximately 6 months. The observed extended dormancy is due to an exodormic mechanism, most likely being low temperature (Gracie et al., 2000). This conclusion conflicts with Maeda (1994) who concluded that rhizome dormancy in myoga was endogenously controlled.

The length of chilling at 4^0C did not affect the number and mean weight of flower buds produced. This finding was supported by Gracie et al., (2000) who determined that flower bud number in myoga was not affected by increasing durations of chilling from 0 to 8 weeks. The amount of ambient chilling received by rhizome pieces that remained in the ground at Albion Park over the winter period would appear adequate to promote pseudostem and flower bud production the following spring/summer, with additional chilling at 4^0C unnecessary. This was possibly due to rhizome material in the ground at Albion Park from March to July, receiving enough chilling due to ambient temperatures, to satisfy any vernalisation requirement. Average minimum temperatures in the area of Albion Park ranged from 15.9^0C in March to 7.9^0C in July. The optimum temperature range for vernalization lies between about 1 and 7^0C, with the effective temperature ranging from just below freezing to about 10^0C (Thomas & Vince-Prue, 1997). It is possible therefore that during the months of June (8.9^0C) and July (7.9^0C)

when ambient minimum temperatures dropped below 10^0C that the vernalization requirement of myoga was satisfied. However these temperatures would be considered marginal for vernalization.

The effect of low temperature increases with the duration of exposure until the response is saturated at a duration which varies widely with species and cultivar (<10 to >100 days). Vernalization is a progressive process and the effect becomes increasingly stable as the duration of cold is increased (Thomas & Vince-Prue, 1997). If the vernalization requirement for flower bud initiation in myoga plants had been saturated by the low temperatures experienced in the field at Albion Park during the months of June and July, differences in the yield of plants which spent additional durations of time at 4^0C would not be expected. However if the effect of low temperature was not saturated during the period spent in the field, increases in the number of flower buds produced by plants that spent increasing periods of time at 4^0C would be anticipated. Since no significant differences were observed in flower bud yield, it can be assumed that if myoga plants do have a vernalization requirement, it had been previously satisfied by ambient temperatures in the field and no further chilling was required. Further research investigating the required length of cold treatment and the optimum temperature range for vernalization in myoga would be required to confirm this. However based on the results from the controlled environment trial conducted by Gracie et al., 2000, and the results achieved during this trial it appears more likely that field grown myoga does not require a period of low temperature to produce flower buds.

Although the results from this trial concur with those of Gracie et al., (2000), they differ significantly from those of Maeda (1994) and Follett (1991) where pseudostem development was markedly reduced or completely ceased without prior rhizome chilling. This difference may be due to a genetic or physiological difference between the materials used in each study. The rhizome material used in this study was the same as that used by Gracie et al. (2000) however it is unknown what cultivar was used by Maeda (1994) and Follett (1991). Genetic variability in the pattern of growth of myoga has been previously described by Maeda (1994) in an examination of early and late

flowering varieties, and similar variability in dormancy periods may explain inconsistencies between studies.

Chilling at low temperatures can be used to break rhizome dormancy. Gracie et al., 2000 showed that with increasing length of chilling at 4^0C, plants displayed reduced time to emergence and reduced variability in emergence. Although chilling could be used in the field to shorten the dormancy period, such procedures would not be beneficial at Albion Park as ambient temperatures would not support pseudostem growth until October or November. In areas such as Queensland where ambient temperatures are suitable year round for growth, it may be beneficial to provide rhizome pieces with additional chilling. This would enable commercial production to occur year round with dormant periods of only three weeks.

SECTION 3: INVESTIGATION OF CULTURAL FACTORS AFFECTING COMMERCIAL CULTIVATION OF MYOGA - *STUDY OF RHIZOME MATERIAL USED FOR PROPAGATION OF COMMERICAL MYOGA CROPS*

Introduction

Myoga plants are propagated vegetatively as they are sterile pentaploids with the presence of seed unknown (Palmer, 1984). Propagation is conducted using dormant pieces of rhizome lifted from the ground during or after winter. These are replanted during spring and in response to warmer temperatures, produce new pseudostem growth. It was planned that the commercial crop of myoga at Albion Park, NSW should be established using 100 g pieces of rhizome material. This weight of propagation material had been used in previous production and glasshouse trials and appeared to provide adequate reserves for successful pseudostem emergence and development (Gracie et al., 2000). However in the first season of growth, the commercial crop displayed reduced vegetative and flower bud development. Based on anecdotal evidence it was hypothesized that this effect was due to the use of a number of smaller rhizome pieces being grouped together and used as propagation material rather than whole 100 g 'plantlets'. The 100 g 'plantlets' as used in initial production trials consisted of a whole 100 g rhizome piece usually containing the residual primary pseudostem from the previous season of growth.

The type and size of rhizome material used for propagating myoga is obviously an important consideration in commercial cultivation. Previous published reports have already shown that the weight of rhizome material used for propagation of myoga plants will have a significant effect on the number of pseudostems and flower buds produced in the first season of growth (Clark, 2001). Production trials in New Zealand reported poor flower bud yields in the first year of growth, most likely due to an insufficient weight of rhizome being used as propagation material (Palmer, 1984; Douglas and Follett, 1992). However, commercial production trials conducted in Australia have indicated that very good flower bud yields are achievable in the first

season of growth, provided rhizome material of sufficient weight is used as propagation material. To examine the effect of size of rhizome pieces used for propagation material on subsequent flower bud yield, several weights of rhizomes were used to constitute 100 g lots.

Materials and Methods

Plant Material

Plant material was sourced from mature, dormant plants that had over-wintered at New Norfolk, Tasmania. Rhizome material was lifted and transported to the University of Tasmania where it was washed and cut up into pieces of varying weight. All three treatments in this trial involved planting rhizome material, which weighed a total of 100g. Treatment 1 consisted of a whole 100g piece; treatment 2 consisted of two 50g pieces and treatment three consisted of four 25g pieces. The material was dipped in a fungicide treatment consisting of 100ml of Previcur®, 200ml of Baviston®, 100ml of Sumisclex® and 200g of Kocide® per 100L of water. It was then packed into seed trays filled with a moistened mix of 50% perlite, 25% coarse sand and 25% peat and given a standard chilling treatment of three weeks at 4^0C (Gracie et al, 2000). At the completion of the chilling treatment the rhizome material was packed into boxes and freighted to Albion Park.

A bed within the commercial planting (for details refer to section materials and methods) was divided into plots, which were 2.4m long by 1.2 m wide. Each plot, containing ten plants, was randomly assigned a treatment. Each treatment consisted of three separate plots. Each plot was marked out by digging down through the mulch to soil level and placing a block of wood in between each plot. This prevented rhizomes from plants of different treatments growing together.

Harvest

Half the plants from each treatment plot were regularly harvested of commercially mature flower buds, while the other half were left until the end of the harvesting period

when a single, final harvest was performed at 208 DAP. The first seven sequential harvests were performed every three to five days, while the last two harvests were conducted seven to fourteen days after the previous harvest. The final sequential harvest was performed at the same time as the single, final harvest. All flower bud material was removed from the plots at this time. At each harvest, flower buds from each treatment plot were counted, weighed and examined for anthesis. A grading chart similar to that used for commercially cultivated flower buds in Japan was used to assess the quality of the flower buds and determine if they were acceptable for commercial sale (plate 1; section materials and methods).

Statistical Analysis

The experiment was established using a split-plot design. The size of rhizome pieces used to constitute the 100 g lots was the main plot effect, while the sub-plot effect was the type of harvesting method used. Results were analysed using ANOVA with assumption of equal variance, normal distribution of underlying population and random sampling. Least significant differences (LSD) were calculated for treatment effects to aid interpretation of results.

Results

The vegetative development of treatment plants at 64 DAP can be observed in plate 1.

Sequential Harvest

Harvest weight and number of flower buds produced by plants from all treatments increased steadily from 155 to 194 DAP. A large increase in yield was observed after 194 DAP most likely due to the increased duration between harvests and the removal of all grades of flower bud material at the final harvest (figures 1 & 2). There were no significant differences in total harvest weight, mean flower bud weight and number of flower buds produced from all treatment plants when buds were sequentially harvested (figures 3, 4 & 5).

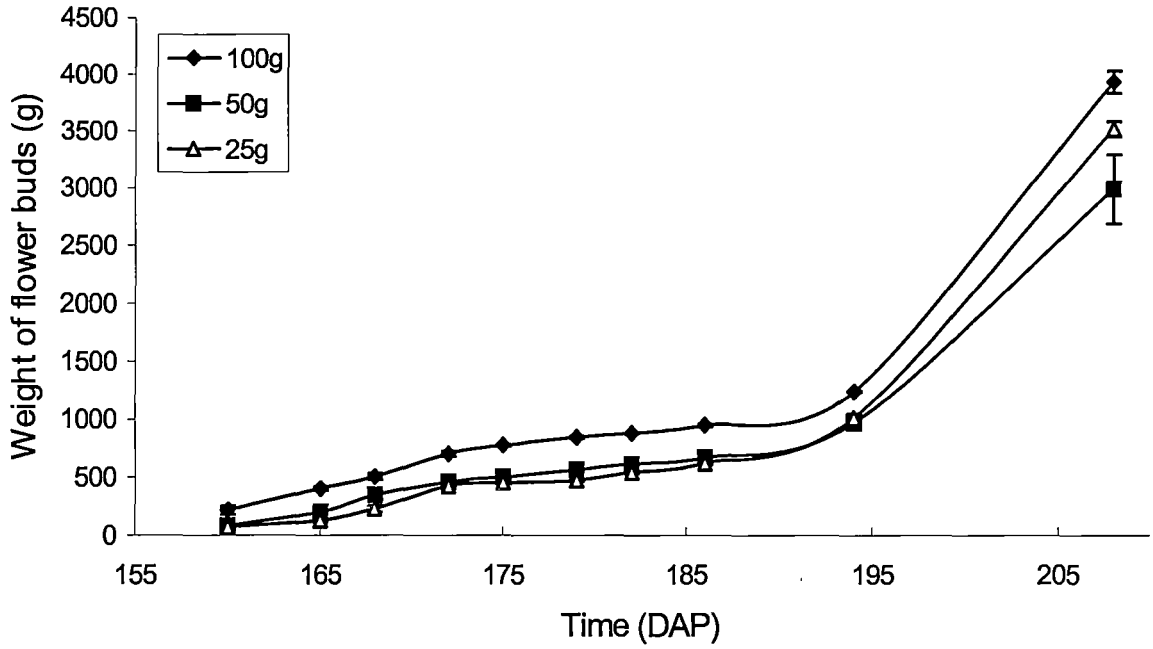


Figure 1: Cumulative harvest weight of flower buds produced by all treatment plants from 155 to 210 DAP, using a sequential harvesting method. Bars show SE (n=3).

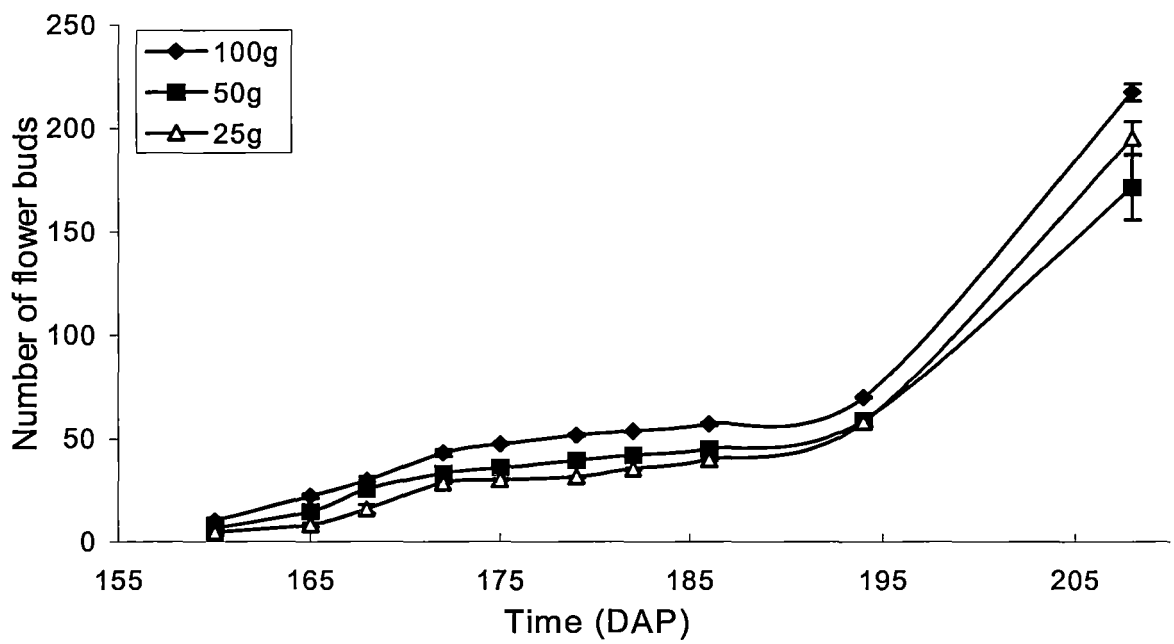


Figure 2: Cumulative number of flower buds produced by all treatment plants from 155 to 210 DAP, using a sequential harvesting method. Bars show SE (n=3).

Single Harvest

There were no significant differences in total harvest weight, mean flower bud weight and number of flower buds produced from all treatment plants when buds were harvested only once (figures 3, 4 & 5).

Comparison of harvest methods

The total harvest weight and number of flower buds produced by all treatment plants was the same using both harvesting methods. Flower buds produced by plants which were propagated using 50 and 25g rhizome pieces were significantly heavier when harvested only once (figure 5). Flower buds from plants propagated using 100g pieces were of the same weight when harvested once and sequentially.

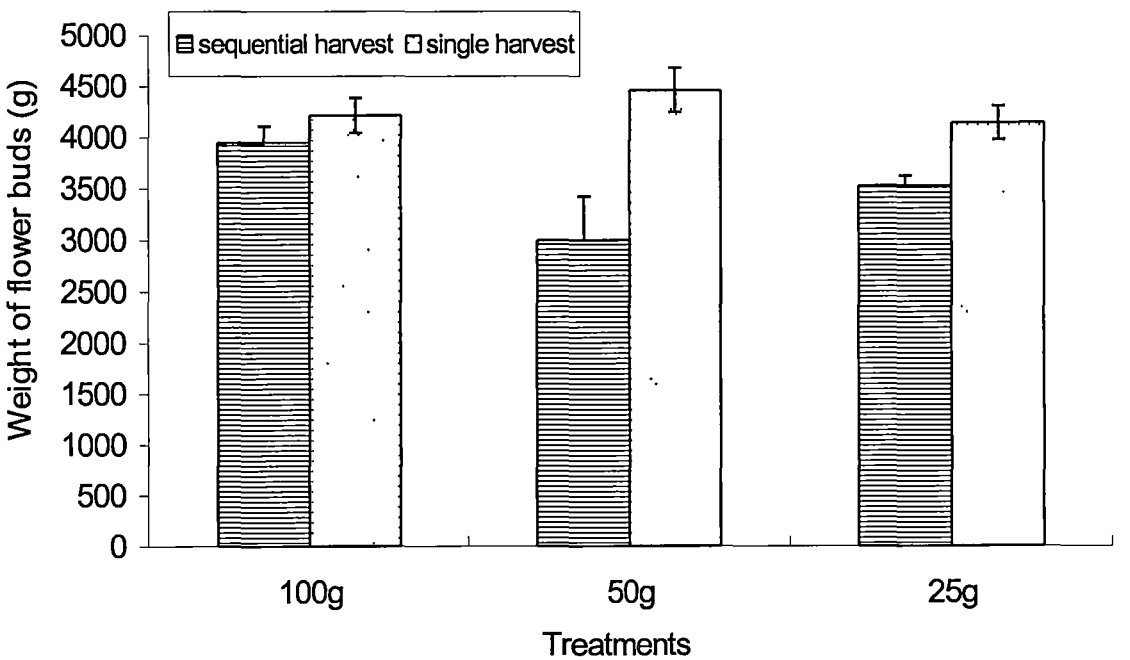


Figure 3: Total harvest weight for all treatments using both harvesting methods. Bars show SE (n=3).

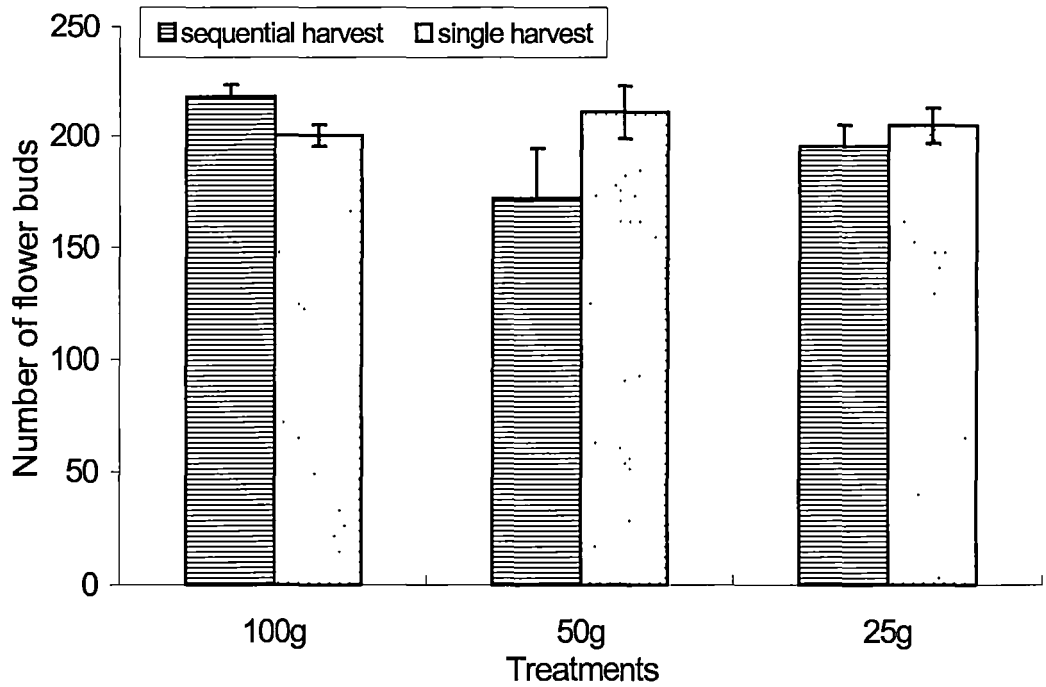


Figure 4: Total number of flower buds produced for all treatments using both harvesting methods. Bars show SE (n=3).

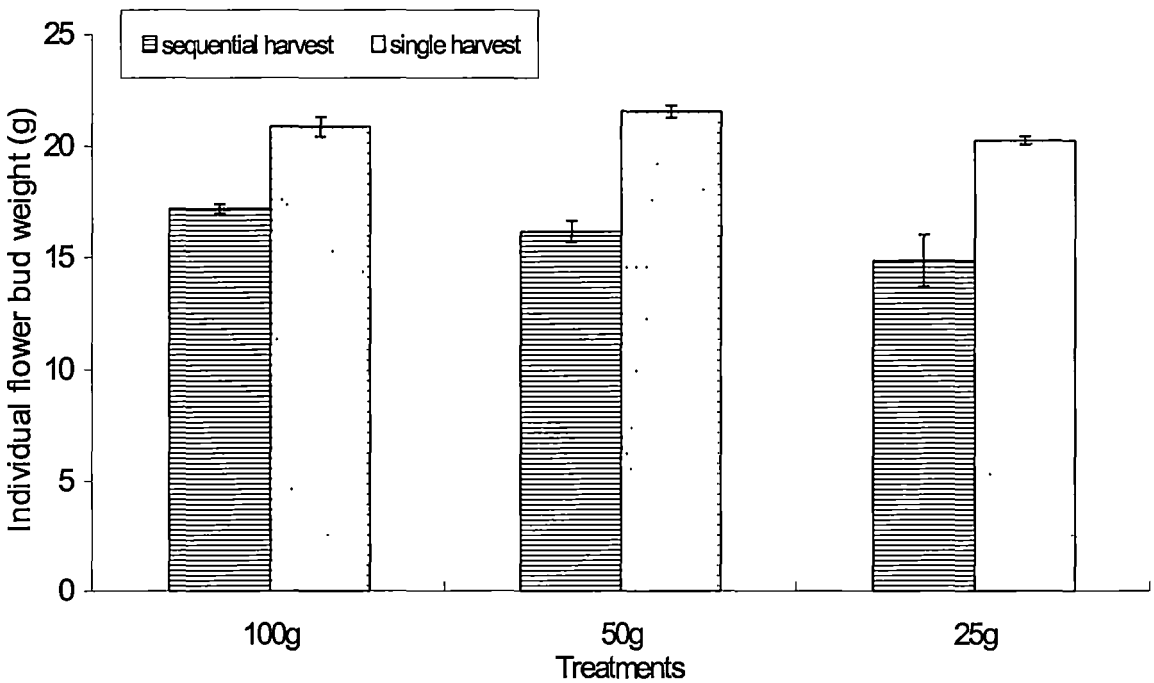


Figure 5: Mean individual flower bud weight of all treatment plants using both harvesting methods. Bars show SE (n=3).

Main Plot	*Sub Plot*	*Average flower bud weight*	*Total harvest weight*	*Number of flower buds*
100g	Sing. harvest	AB	A	A
100g	Seq. harvest	BCD	A	A
50g	Sing. harvest	A	A	A
50g	Seq. harvest	CD	A	A
25g	Sing. harvest	ABC	A	A
25g	Seq. harvest	D	A	A

Table 1: Treatment LSD's for mean flower bud weight, total harvest weight and number of flower buds produced. Treatments with the same letter are not significantly different ($P<0.05$).

Plate 1: Vegetative development of treatment plants at 64 DAP.

Discussion

Based on the results of this trial it would appear that 100g lots of propagation material constituted by either one whole piece or several pieces is suitable for commercial flower bud production. The size and number of rhizome pieces used to constitute a 100g lot had little effect on the number of flower buds produced by each plant and when harvested either once or sequentially there was no significant difference in mean

flower bud weight. If the reduced vegetative and reproductive growth observed during the first season of the commercial crop was due to the propagation material used, the pieces used to compose the 100g lots must have been very small, i.e. less than 25g in weight.

The initial size of a rhizome piece is likely to have an effect on the subsequent vegetative and reproductive development of myoga plants Extremely small rhizome pieces containing only one or several nodes may contain only minor reserves available for the development of a primary pseudostem. Poor initial pseudostem development may affect subsequent vegetative and reproductive growth resulting in small, poor-yielding plants. This effect was observed in Gracie et al. *(in press)*, where the pattern of plant development was shifted further towards floral development under conditions which favoured increased plant growth rate. This indicated that photoassimilate production in myoga may be linked to the rate of flower bud initiation and development as has been proposed for a number of other plant species (Kinet 1993).

Clark (2001) conducted trials examining the effect of different weight of propagation material on subsequent pseudostem and flower bud development. The results indicated that although larger rhizome pieces (120 – 300g) typically produced more pseudostems and flower buds than smaller pieces of rhizome; the smaller rhizome sections (30 – 80g) were more vigorous and produced more pseudostems per gram of planted rhizome than larger pieces. Smaller segments also had a higher flower bud to pseudostem ratio indicating that the pseudostems may have been more effective at assimilating resources for flower bud initiation and development (Clark, 2001). It may be possible that the smaller 25g segments used in this trial were more vigorous in vegetative and reproductive development and were therefore able to more effectively channel the available resources to drive photoassimilate supply towards reproductive development than the larger rhizome pieces.

SECTION 3: INVESTIGATION OF CULTURAL FACTORS AFFECTING COMMERCIAL CULTIVATION OF MYOGA – *THE EFFECT OF RHIZOME TRIMMING ON FLOWER BUD YIELD FROM ESTABLISHED PLANTINGS OF COMMERCIALLY CULTIVATED MYOGA*

Introduction

As a perennial crop, myoga plants can remain in the same location and produce flower buds for several years before the plants need to be removed or replaced with new material. However the continuous rhizomatous spread of the crop between and during growing seasons raises a number of challenging issues relating to how best to establish and manage this crop to optimize production. At Albion Park initial rhizome pieces were planted at a spacing of 0.4 x 0.4 m, which provided more than enough space for plants in their first year of growth. Observation of myoga crop development in New Zealand led to the hypothesis that this planting density may be too high, resulting in insufficient space for plants in their second year of growth. Douglas and Follett (1992) observed the spread of a single rhizome to an area of 0.3 x 0.5 m in three seasons. Furthermore rhizomes planted in rows 0.4 m apart gave continuous rows 0.5 m wide after two seasons of growth. In addition to effects on crop yield, density changes exert significant influences on individual plant morphology. As density increases, plant height increases and pseudostem strength decreases. This increases the risk of lodging in high density plantings. Under field crop conditions intense interplant competition is created in high density plantings, resulting in high yielding crops being communities of suppressed plants (Donald, 1968).

If myoga plants are to be grown as a perennial crop it may be necessary to reduce density by rhizome trimming between the first and second year, and subsequent years to avoid overcrowding and decreased crop performance. One potential method of density control is to remove rhizomes from the exterior of the raised beds while the plants are dormant. In the following trials this rhizome removal is referred to as trimming and was imposed to determine the viability of this method and its effect on flower bud yield and vegetative growth.

Materials and Methods

Plant Material

One-year-old myoga plants from which flower buds had previously been harvested during January, February and March, 2000 were used in this trial. Following the harvesting period, senescence of plant material was allowed to occur during the colder months of May, June and July. Trimming of the below-ground material was carried out on the 24th of August 2000. The plants were then left to produce new vegetative growth during the following spring and summer months.

A bed within the commercial planting (for details refer to section materials and methods) was divided into plots, which were 2.4m long by 1.2 m wide. Each plot, containing ten plants, was randomly assigned a treatment. Each treatment consisted of three separate plots. Each plot was marked out by digging down through the mulch to soil level and placing a block of wood in between each plot. This prevented rhizomes from plants of different treatments growing together.

Trimming of below-ground rhizome material consisted a 0.72 m^2 region on the outside edges of each plot being completely removed of rhizome material.

Harvest

Half the plants from each treatment plot were regularly harvested of commercially mature flower buds, while the other half were left until the end of the harvesting period when a single, final harvest was performed at 208 DAP. The first seven sequential harvests were performed every three to five days, while the last two harvests were conducted seven to fourteen days after the previous harvest. The final sequential harvest was performed at the same time as the single, final harvest. All flower bud material was removed from the plots at this time. At each harvest, flower buds from each treatment plot were counted, weighed and examined for anthesis. A grading chart similar to that used in Japan was used to assess the quality of the flower buds and

determine if they were acceptable for commercial sale (plate 1; General materials and methods).

Statistical Analysis

The experiment was established using a split-plot design. The modification of below-ground rhizome material was the main plot effect, while the sub-plot effect was the type of harvesting method used. Results were analysed using ANOVA with assumption of equal variance, normal distribution of underlying population and random sampling. Least significant differences (LSD) were calculated for treatment effects to aid interpretation of results.

Results

The reduction in vegetative growth of trimmed plants can be observed at 64 DAP in plate 1.

Sequential Harvest

There was no significant difference in the total weight or number of flower buds produced from trimmed and untrimmed plants when sequentially harvested from 160 to 208 DAP (figures 1 & 2). Although the number of flower buds produced from plants steadily increased from 160 DAP, the majority of flower buds were produced from plants following 195 DAP. The harvested weight of flower buds from both untrimmed and trimmed plants follows the same pattern with the highest yields harvested after 195 DAP. The individual mean weight of flower buds was the same from both treatments (figure 5).

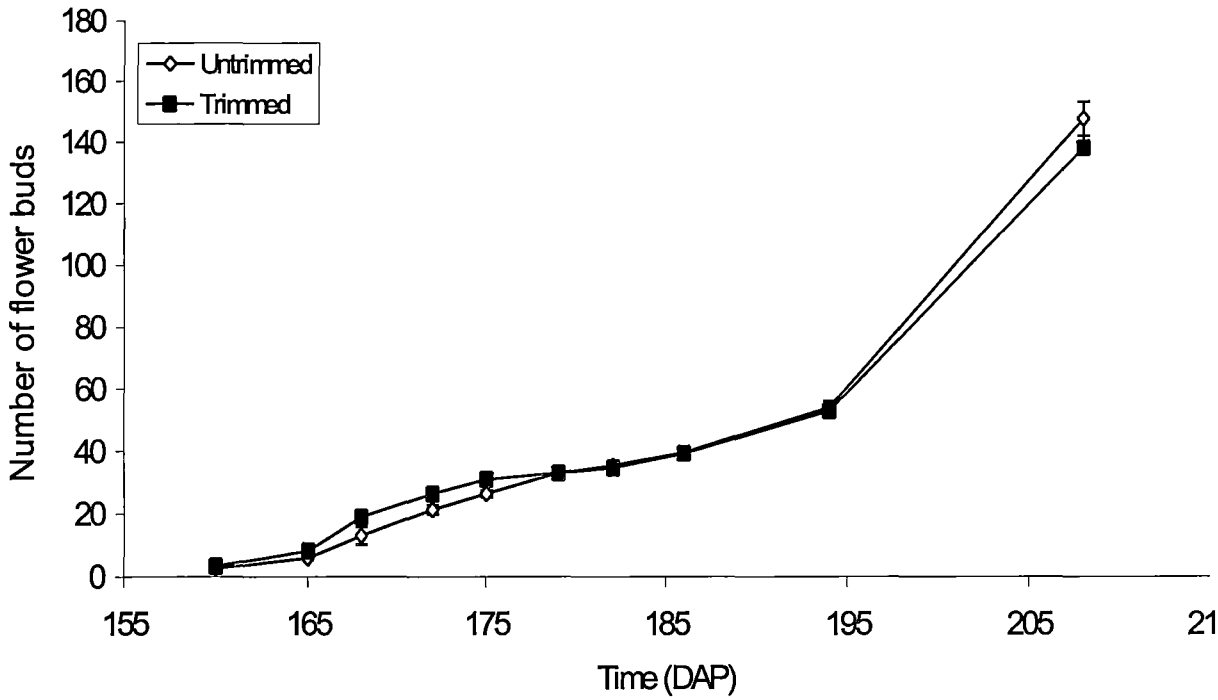


Figure 1: Cumulative number of flower buds produced by all treatment plants from 155 to 210 DAP, using a sequential harvesting method. Bars show SE (n=3).

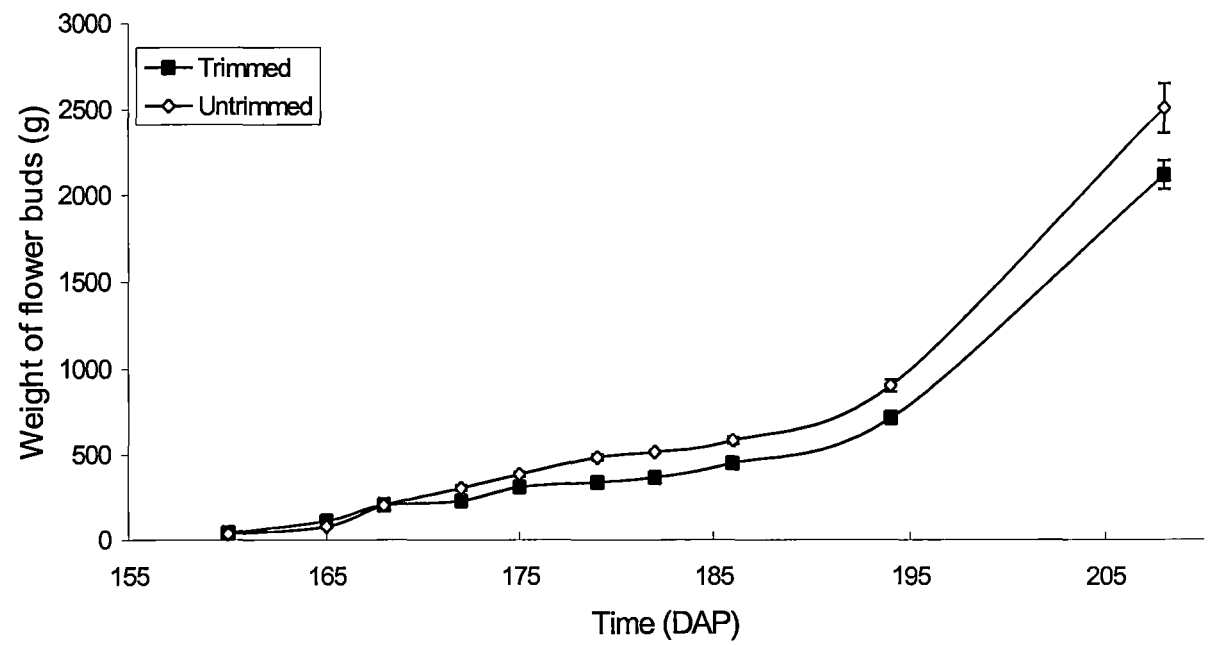


Figure 2: Cumulative harvest weight of flower buds produced by all treatment plants from 155 to 210 DAP, using a sequential harvesting method. Bars show SE (n=3).

Single Harvest

There was a significantly ($P<0.05$) higher number and total weight of flower buds harvested from untrimmed plants compared with trimmed plants when flower buds were harvested from plants in a final, single harvest (figures 3 & 4). The individual mean weight of flower buds was the same for both treatments.

Comparison of harvesting methods

There was no significant difference in the total weight and number of flower buds produced from trimmed plants subjected to the different harvesting methods. Although untrimmed plants showed no significant differences in the number of flower buds produced under either harvest regimes, there was a significant difference ($P<0.05$) in the total weight of flower buds produced. Untrimmed plants that were only harvested once produced a higher total weight of flower buds than those sequentially harvested. This was due to the significantly higher average weight of individual flower buds from these plants (figure 5).

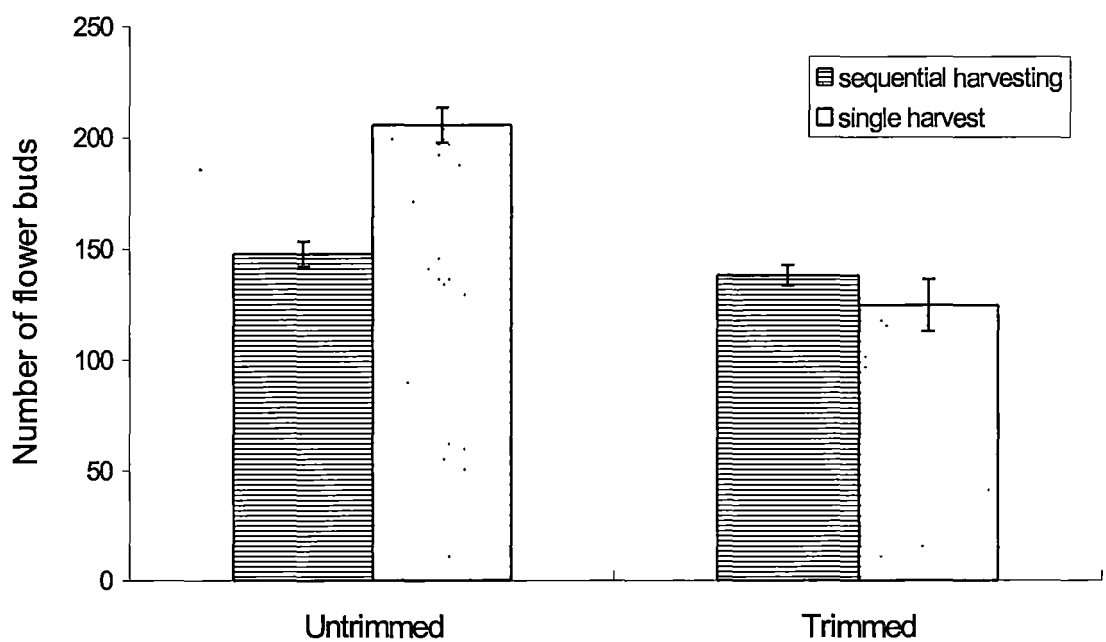


Figure 3: Total number of flower buds produced from all treatment plants using both harvesting methods. Bars show SE (n=3).

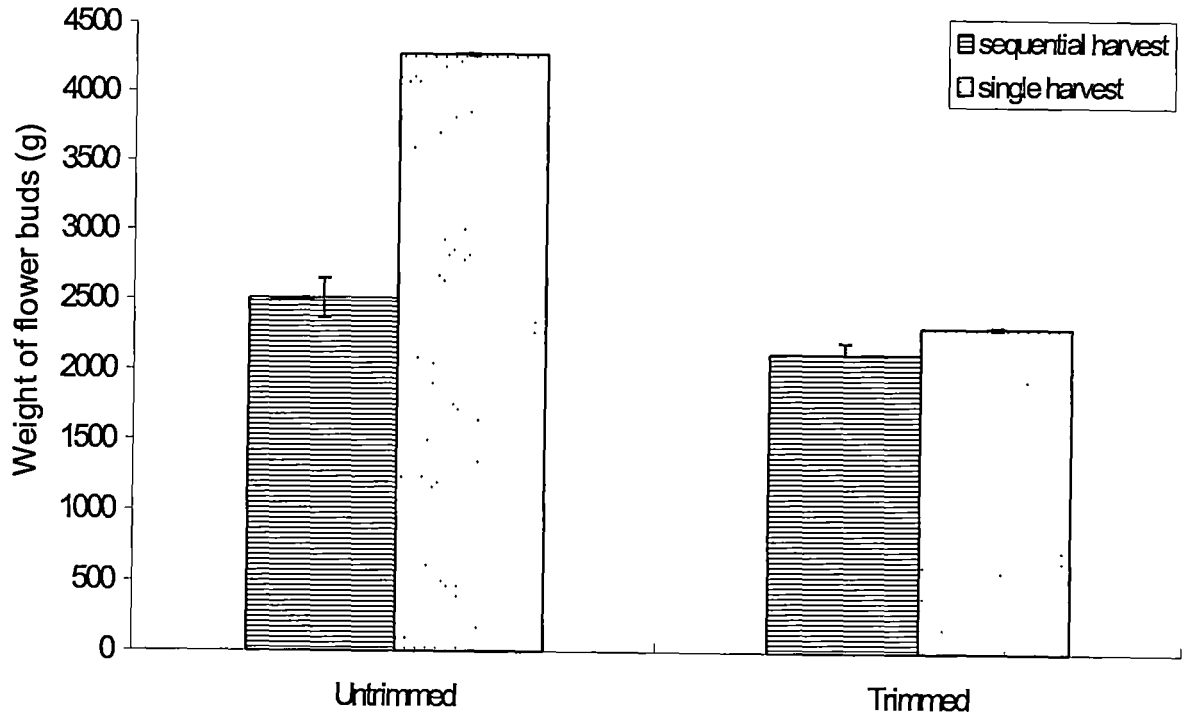


Fig. 4: Weight of flower buds produced from trimmed and untrimmed plants under both harvesting methods. Bars show SE (n=3).

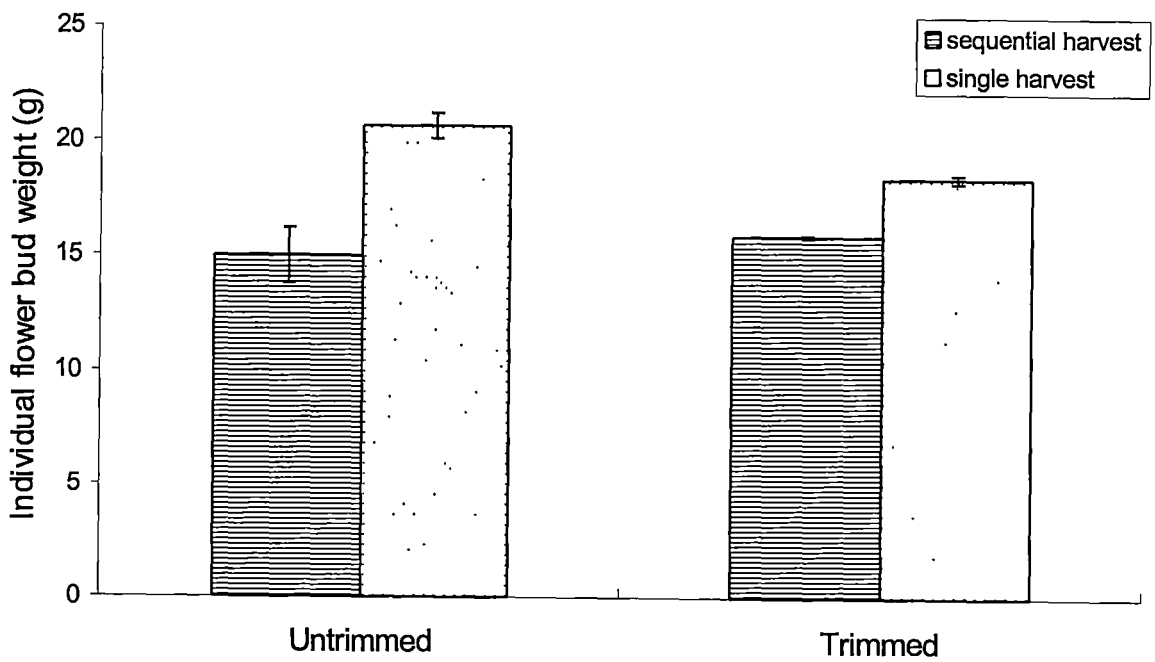


Fig. 5: Mean weight of individual flower buds from trimmed and untrimmed plants using both harvesting methods. Bars show SE (n=3).

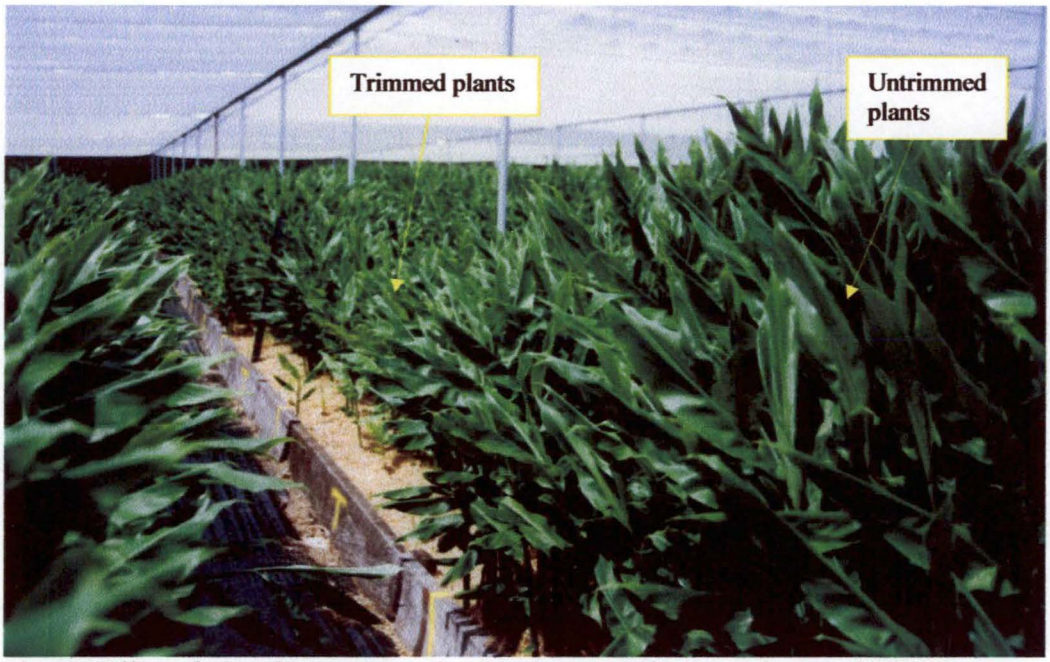


Plate 1: Effect of trimming on vegetative development of myoga plants at 64 DAP.

Main Plot	*Sub Plot*	*Flower bud number*	*Total harvest weight*	*Average bud weight*
Trimmed	Sing. harvest	B	B	AB
Trimmed	Seq. harvest	B	B	B
Control	Sing. harvest	A	A	A
Control	Seq. harvest	AB	B	B

Table 1: Treatment LSD's for mean flower bud weight, total harvest weight and number of flower buds produced. Treatments with the same letter are not significantly different ($P<0.05$).

Discussion

Trimming of rhizome material from myoga plants during the winter period reduced the potential number of flower buds available for harvest, as indicated by the number of flower buds harvested from both treatment plants at the single harvest. However using a sequential harvesting method, which is employed during commercial cultivation, both treatment plants produced a similar yield of flower buds. The differences in flower bud yield observed between trimmed and untrimmed plants at the single harvest occurred due to untrimmed plants producing higher numbers of flower buds which were also of a heavier individual weight. Therefore trimming reduced the potential number of flower

buds and deferring harvest until the end of the flowering period allowed flower buds to increase in weight.

Two year old myoga plants which were not trimmed produced excessive levels of vegetation under protected cultivation at Albion Park. Consequently large amounts of lodging occurred when plants were unable to support this level of vegetative growth and resulted in a number of undesirable outcomes in regards to flower bud yield. Hand harvesting flower buds became difficult and time-consuming as buds were obscured under vast quantities of vegetation. The number of buds harvested would have been reduced as pickers found it difficult to locate flower buds under the dense vegetation. This is reflected in the number and weight of flower buds produced by trimmed and untrimmed plants using both harvesting methods. During sequential harvesting, pickers had to be more careful with the vegetation and ensure that developing buds were not damaged. This made it difficult to locate buds that were covered by the dense vegetation. Therefore the yield of flower buds from untrimmed plants which were sequentially harvested would have been decreased.

Plants which had undergone rhizome trimming were smaller plants and did not produce the same excessive level of vegetation. Although the potential number of flower buds produced by these plants was reduced as a result of the rhizome trimming, the flower buds which were produced were easier to locate and therefore easier for the pickers to harvest. As a result of these two factors, sequential harvesting produced similar yields of flower buds from both trimmed and untrimmed plants. This was confirmed by the yield of flower buds produced by trimmed and untrimmed plants when harvested only once, which allowed the potential yield of these plants to be more fully realized. At the final harvest at the end of the season, the pickers were able to more thoroughly search through vegetation for flower buds as damage to the plant was no longer a concern. As a result they were able to locate the majority of flower buds produced by all plants. Under this situation plants which were not trimmed of rhizome material were clearly shown to yield higher levels of flower buds.

The quality of the flower buds was also affected by the trimming treatment. During this experiment, it was clearly observed that flower buds harvested from plants which had not been trimmed were often of a poorer quality than buds from trimmed plants. The main reason for this was that many flower buds from untrimmed plants were harvested at an over-mature stage, as it was difficult to detect buds through dense vegetation. In addition the level of light that reached flower buds was decreased, affecting anthocyanin development and colouration of the buds. Susceptibility to fungal attack was also increased as air circulation around the buds was restricted.

If rhizomes were to remain in the ground for longer than one production season, the trimming of rhizome material during the senescent winter period would appear to be a viable solution for the prevention of excessive levels of vegetation. Although the trimming of rhizome material resulted in a reduction in the potential number of flower buds produced, this difference in yield is unlikely to be realized in a commercial situation where sequential harvesting is used. The labour required to remove rhizomes from bed exteriors during winter months is substantial if done manually, however if mechanical equipment could be adapted to perform this task it would certainly seem feasible for a commercial operation.

There are a number of other methods which could be employed in a commercial situation to control vegetation levels. Strategic application of a hormonal spray to inhibit gibberellin production in myoga may be one such alternative. Anti- GA sprays control crop vegetation by decreasing inter-node length, therefore pseudostems would be shorter and the risk of lodging would be reduced. Anti-GA sprays have also been shown to increase flower bud number and advance time to flowering in a number of flowering plants (Shanks, 1981). Strategic application of anti-GA sprays in commercial myoga crops may therefore be of benefit in reducing vegetative vigour in two-year old plants. Obviously this would need to be tested in future production trials before application in commercial crops, as the effect of hormonal sprays on flowering varies widely according to the species of plant being treated.

SECTION 3: INVESTIGATION OF CULTURAL FACTORS AFFECTING COMMERCIAL CULTIVATION OF MYOGA – *EFFECT OF PROPAGATION MATERIAL AND CHILLING ON GROWTH AND FLOWER BUD YIELD*

Introduction

In Japan and New Zealand myoga has been cultivated as a perennial crop. Low flower bud yields are expected in the first growing season, followed by much higher yields in the second and third year of growth (Follett, 1986). In Australia, commercially grown myoga plantings have yielded reasonable levels of flower buds in their first harvest season. As in New Zealand, it was anticipated that flower bud yield would be even higher for second year plants, however the continuous spread of rhizomes and increased vegetative vigour in second and third year crops in Australia have presented a number of management challenges. These management challenges may potentially limit flower bud yield in more mature crops.

Determination of the potential yielding capacity of both one and two year old myoga crops was deemed necessary in order to determine the potential loss of flower bud yield due to cultivation as an annual crop rather than a perennial crop. Flower bud yields from myoga plants grown in New Zealand are only available for two year old plants and from crops grown in different years with different planting densities. It is therefore difficult to determine what a properly managed myoga crop should be yielding in both its first and second year of growth. Similarly, commercial cultivation of myoga in Australia has provided only a few years of yield data with climatic factors differing markedly from one growing season to the next. To assist the future management of myoga production in Australia it was deemed necessary to determine how mature myoga plants yielded in comparison to those that were in their first season of growth.

The difficulty in managing two year olds crops has prompted commercial growers to consider producing myoga as an annual crop in NSW. Given previous research both by other authors and observations from previous production trials, it was recognised that

issues such as plant size, chilling, establishment and other cultural factors would need to be addressed if annual crop production was to be considered. Consequently, the following field trial was conducted within a commercial myoga planting in NSW.

Materials and Methods

Plant Material

This experiment was conducted at the commercial site in Albion Park where growers had previously recorded high yields from first year plantings. Two plant ages were included; namely first and second year plants. First year plants, were considered to be rhizome material which had been removed from the ground and trimmed into the commercially accepted weight for propagation, which was 100 g. Two year old plants were considered to be rhizome material which had been left *in situ* without any modifications such as trimming or artificial chilling. In addition, since earlier work pointed to a potential issue with the adequacy of winter chill at the commercial site at Albion Park and earlier high yielding first year plant material at Albion Park was sourced from Tasmania after receiving winter chill, a further treatment was included; namely one year old plants from Tasmania.

Treatment 1: This treatment consisted of plants from the commercial planting at Albion Park. These plants were left in the ground following the first flower bud harvesting season. Subsequent to harvest, all above ground plant material would have senesced. These plants were not lifted, treated with fungicide, chilled or modified in any way. For the purposes of this experiment, these plants were labelled '2 year old, NSW plants – no chilling' (2 yo, NSW (NC)).

Treatment 2: Rhizome material for this treatment was sourced from field grown plants in Tasmania. Rhizomes were cut into approximately 100g pieces (not always one whole piece) and then soaked in a fungicide treatment consisting of 100ml of Previcur®, 200ml of Baviston®, 100ml of Sumisclex® and 200g of Kocide® per 100L

of water. They were then packed into seed trays filled with a moistened mix of 50% perlite, 25% coarse sand and 25% peat and given a standard chilling treatment of three weeks at 4^0C (Gracie *et al*, 2000). At the completion of the chilling treatment the rhizome material was packed into boxes and freighted to Albion Park where it was planted into the ground using 0.4 x 0.4m spacing on the 23/24th of August. This rhizome material had received 3 months post senescence chilling under Tasmanian conditions plus 3 weeks cool room chilling at 4^0C. These plants were labelled ' 1 year old, TAS plants – chilled' (1 yo, TAS (C)).

Treatment 3: Rhizome material for treatment 3 plants was removed from the ground at Albion Park, washed and stored at 4^0C for 2.5 weeks. It was then trimmed into 100g lots and replanted into the ground using 0.4 x 0.4 m spacing on the 23/24th August. These plants were labelled '1 year old, NSW plants – chilled' (1 yo, NSW (C)).

A bed within the commercial planting (for details refer to section materials and methods) was divided into plots, which were 2.4m long by 1.2 m wide. Each plot, containing ten plants, was randomly assigned a treatment. Each treatment consisted of three separate plots. Each plot was marked out by digging down through the mulch to soil level and placing a block of wood in between each plot. This prevented rhizomes from plants of different treatments growing together.

Harvest

Half the plants from each treatment plot were regularly harvested of commercially mature flower buds, while the other half were left until the end of the harvesting period when a single, final harvest was performed at 208 DAP. The first seven sequential harvests were performed every three to five days, while the last two harvests were conducted seven to fourteen days after the previous harvest. The final sequential harvest was performed at the same time as the single, final harvest. All flower bud material was removed from the plots at this time. At each harvest, flower buds from each treatment plot were counted, weighed and examined for anthesis. A grading chart similar to that used in Japan was used to assess the quality of the flower buds and

determine if they were acceptable for commercial sale (plate 1; section materials and methods).

Statistical Analysis

The experiment was established using a split-plot design. The age and modification of below-ground rhizome material was the main plot effect, while the sub-plot effect was the type of harvesting method used. Results were analysed using ANOVA with assumption of equal variance, normal distribution of underlying population and random sampling. Least significant differences (LSD) were calculated for treatment effects to aid interpretation of results.

Results

The differences in level of vegetative growth between one year old and two year old plants can be observed in plate 1.

Sequential Harvest

Harvest weight and number of flower buds produced by plants from all treatments increased steadily from 155 to 194 DAP. A large increase in yield was observed after 194 DAP most likely due to the increased duration between harvests and the removal of all grades of flower bud material at the final harvest (figures 1 & 2). There was no significant difference ($P<0.05$) in the number of flower buds produced from all treatment plants (figure 1). Average flower bud weight and total harvest weight were also not significantly different between treatments (figures 2 & 5).

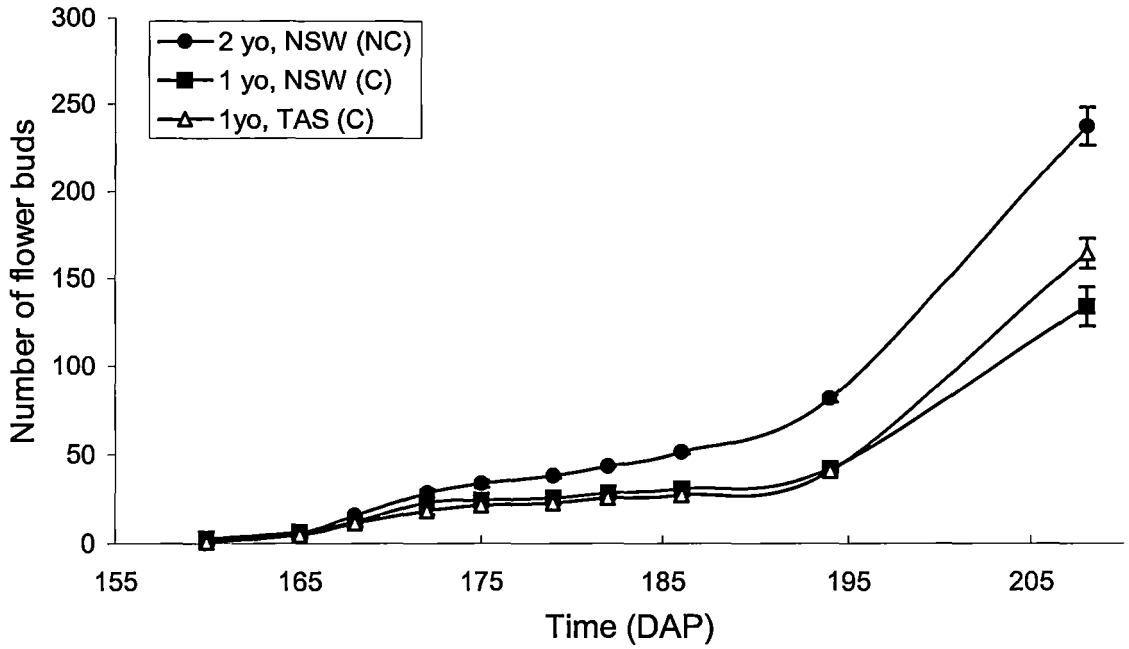


Figure 1: Cumulative number of flower buds produced by all treatment plants from 155 to 210 DAP, using a sequential harvesting method. Bars show SE (n=3).

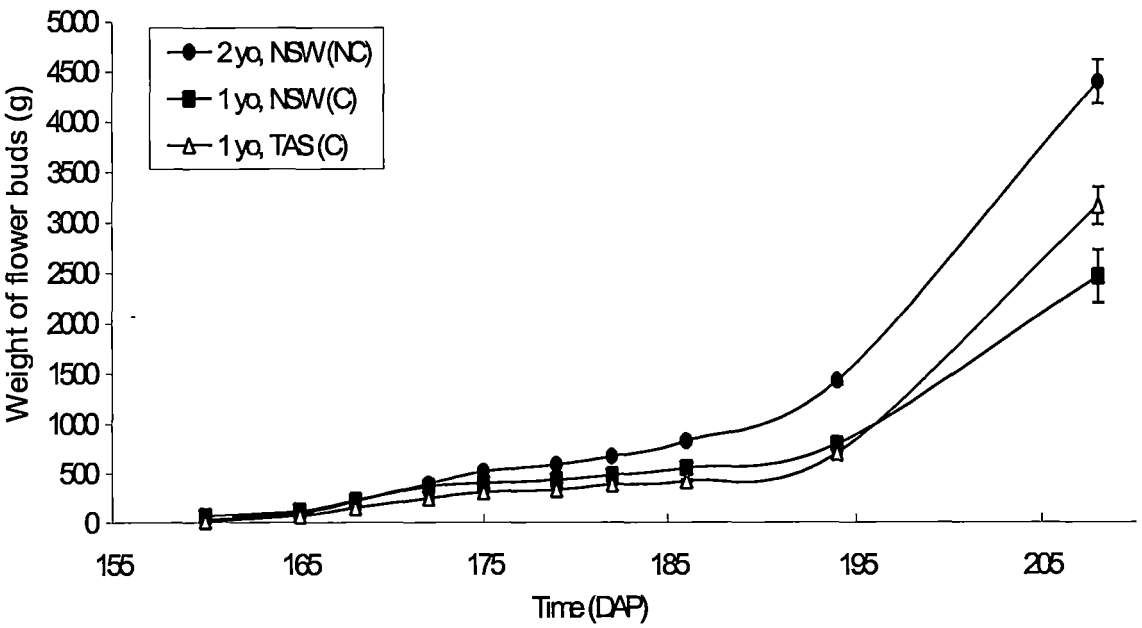


Figure 2: Cumulative harvest weight of flower buds produced by all treatment plants from 155 to 210 DAP, using a sequential harvesting method. Bars show SE (n=3).

Single Harvest

Plants from all treatments produced significantly different numbers of flower buds (figure 3). Two year old plants from NSW, which were not chilled produced the highest number of flower buds followed by one year old flower buds from NSW, that were chilled. One year old plants from TAS, that were chilled produced the lowest number of flower buds. Both one and two year old plants from NSW produced a higher harvest weight of flower buds than one-year old plants from TAS (figure 4). The average weight of individual flower buds was significantly higher in one year old plants from NSW, compared with two year old plants in NSW (figure 5). The higher average individual bud weight but lower number of flower buds produced from one year old NSW plants resulted in the same harvest weight of flower buds being produced by these plants as two year old plants from NSW.

Comparison of harvest methods

One year old plants from NSW, that were chilled produced the same number of flower buds when harvested under both methods, however the average flower bud weight was higher when flower buds were harvested only once and therefore the total harvest weight was also significantly higher ($P<0.05$) using this harvesting method (figures 3, 4 & 5). Non-chilled two-year old plants had similar average flower bud weights under both methods however a significantly higher number of flower buds and total harvest weight was produced when plants were harvested only once. One-year old plants from TAS produced the same number of flower buds and total harvest weight under both harvesting regimes

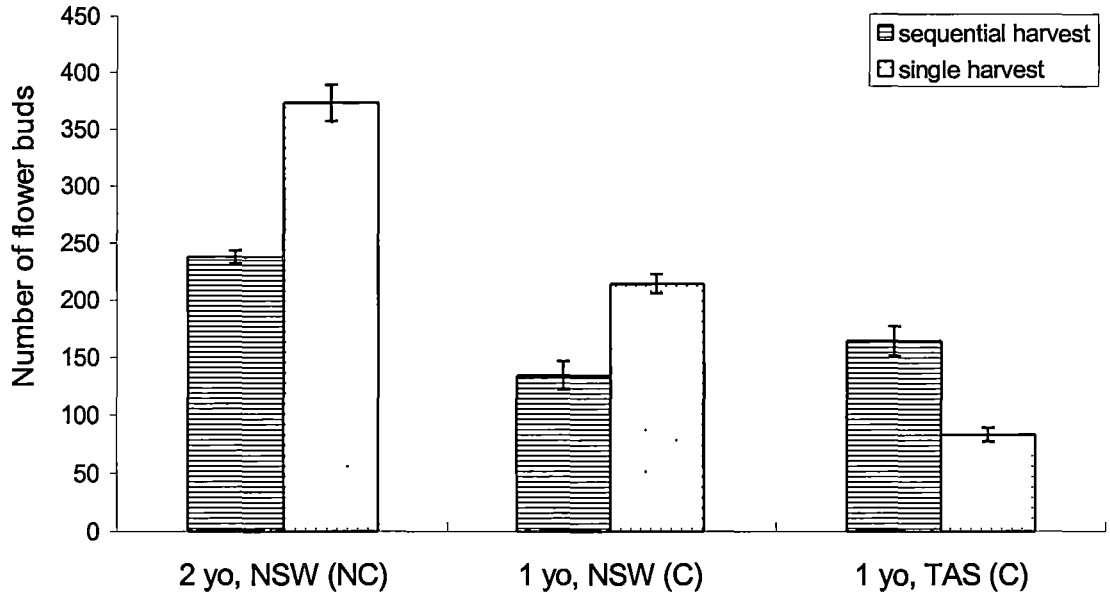


Figure 3: Number of flower buds produced by all treatment plants using both harvesting methods. Bars show SE (n=3).

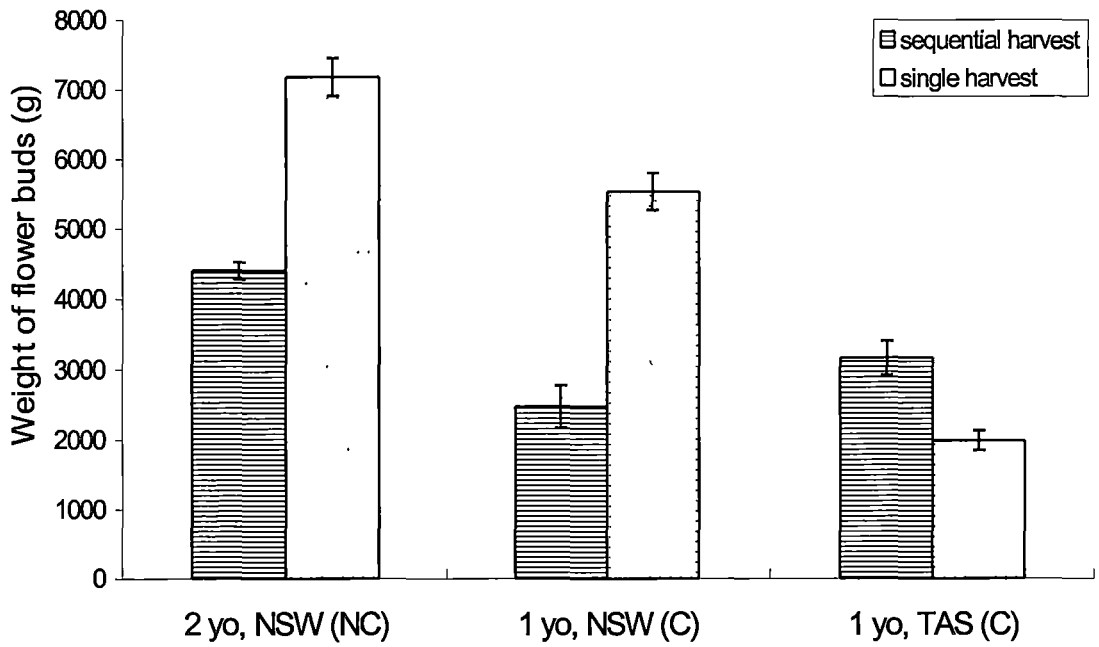


Figure 4: Weight of flower buds produced by all treatment plants using both harvesting methods. Bars show SE (n=3).

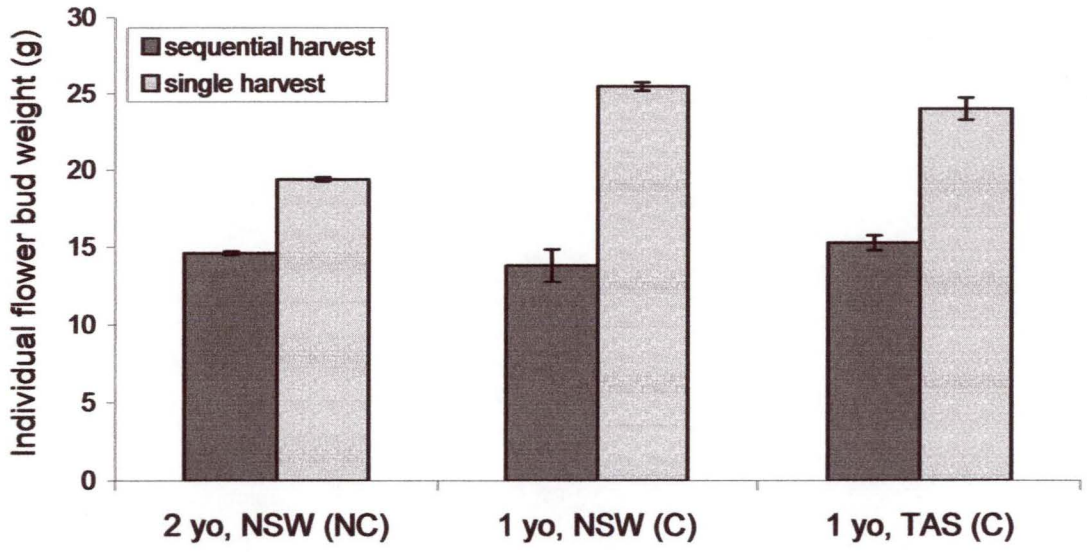


Fig. 5: Mean weight of individual flower buds produced by all treatment plants under both harvesting regimes. Bars show SE (n=3).

Plate 1: Treatment effect on vegetative development of myoga plants at 64 DAP.

Main Plot	*Sub Plot*	*Mean flower bud weight*	*Total harvest weight*	*Number of flower buds*
1 yo, NSW (C)	Seq. harvest	CD	CD	BC
1 yo, NSW (C)	Sing. harvest	A	AB	B
2 yo, NSW (NC)	Seq. harvest	CD	BC	B
2 yo, NSW (NC)	Sing. harvest	BC	A	A
1 yo, TAS (C)	Seq. harvest	D	CD	BC
1 yo, TAS (C)	Sing. harvest	AB	D	C

Table 1: Treatment LSD's for mean flower bud weight, total harvest weight and number of flower buds produced. Treatments with the same letter are not significantly different ($P<0.05$)

Discussion

When sequentially harvested, both one and two-year old myoga plants produced the same flower bud yields indicating that annual planting of myoga would not result in a potential decrease in yield. The flower buds harvested sequentially during this trial were picked when they were visually assessed as having reached a certain maturity level and were commonly of an A grade standard. The results achieved here support yield data obtained from the commercially cultivated crop at Albion Park where very similar A grade flower bud yields were achieved from both one and two year old plants. It appears therefore that at Albion Park similar A grade flower bud yields are achieved from both one and two year old plants. This result should be interpreted with some caution however as two year plants may have produced higher flower bud yields if vegetative vigour had been reduced allowing for greater ease of hand harvesting. A number of A grade buds may not have been located in time by harvesters and were consequently left to develop to an over mature stage.

When plants were harvested only once, two-year old plants produced significantly higher numbers of flower buds than one-year old plants. It is likely that this is the true producing potential of one and two year old myoga plants, however due to the large amounts of vegetation that accompanied the higher production of flower buds in two year old plants, this number was not observed in two year old plants that were sequentially harvest. The higher number of flower buds produced at the single harvest

would have been due to the increased ease with which pickers could harvest buds in comparison to the sequential harvest method where greater care was required.

Although two year old NSW plants produced higher numbers of flower buds than one year old NSW plants at the single harvest, the total harvest weight from the two treatments was the same. This was due to one year old plants producing heavier flower buds which resulted in the same harvest weight. The reasons for this are not known. It is possible that flower buds from two year old plants did not attain the same weight due to competition for resources from the large amount of vegetative growth.

This trial also confirms the results of previous chilling trials, where myoga plants appear to receive adequate chilling or a sufficient dormancy period while in the ground at Albion Park. The artificial chilling period received by one year old, NSW plants appeared to have no promotive effect on the number of flower buds produced. In addition the extended period of chilling received by Tasmanian plants before planting at Albion Park did not appear to promote higher numbers of flower buds to be produced in comparison with those plants that had not received any chilling. These results conflict with those from New Zealand research trials where it was reported that flower initiation without chilling was random and erratic (Follet, 1994).

Flower buds harvested from plants in the final single harvest were of variable quality with both A and B grade flower buds present. Currently the yield of A grade flower buds achieved in a commercial situation is only a small proportion of the total yield, with a large percentage of flower buds consigned to B grade. Flower buds which are classed as B grade are either too-mature, with the presence of opened flowers or have developed green colouration in response to excessive light exposure. Both of these problems can be resolved by harvesting buds at the correct stage and ensuring adequate levels of mulch exist. Overcoming these current management issues would ensure that a greater percentage of flower buds from the total harvest would be of premium quality and could be sold in the fresh produce market. It is therefore likely that with correct crop management, two and three year old plants could provide much higher flower bud

yields than one year plants and therefore myoga should be cultivated as a perennial crop rather than as an annual.

SECTION 3: INVESTIGATION OF CULTURAL FACTORS AFFECTING COMMERCIAL CULTIVATION OF MYOGA – *EFFECT OF TEMPERATURE AND DAYLENGTH ON FLOWER BUD YIELD OF MYOGA GROWN IN SUB-TROPICAL CONDITIONS*

Introduction

One of the major issues facing commercial production of myoga in Australia is the current length of the harvesting period. Ideally an extended period of flower bud production of at least six months is required so that consumer awareness of the product can be increased. Furthermore, to obtain shelf space in supermarkets consistency and adequate length of product supply is expected. The current harvesting period of flower buds at the commercial production site in NSW extends from six to eight weeks. In order to address the continuity of supply issue it has been suggested that production be extended from its present location at Albion Park, NSW where the crop growing season is from September to March to more tropical areas such as Southern Queensland. The major limiting factor for growth at Albion Park is temperature, with maximum temperatures during the winter months not being high enough to sustain adequate growth.

From glasshouse trials conducted in Tasmania and commercial cultivation in Japan it appears that optimum myoga growth occurs at daytime temperatures between 20 and 30^0C. At temperatures lower than this vegetative growth is delayed and the time from planting to harvesting increased. Ambient temperatures experienced at Rockhampton, Qld do not generally drop below 20^0C and therefore myoga production could occur in this location all year round, in particular during the winter months when cultivation is not possible further south.

The other major environmental factor controlling flower bud production in myoga is daylength. Although temperature conditions during the majority of the year in Rockhampton are suitable for myoga growth, the length of day falls below that which is considered necessary for flower bud development to occur. Night break lighting can

be used in commercial situations so that long day plants (LDP) can be grown in short day (SD) conditions, usually achieved by breaking the normally long night into two short dark periods. Using this method it was proposed that myoga could be grown during winter months in Rockhampton even though photoperiod conditions were thought to be unsuitable.

Although myoga grows naturally as an understorey plant in Japan, in certain prefectures it is cultivated successfully in open fields. Based on recommendations from production trials conducted in New Zealand myoga is cultivated under shade cloth in Australia. When exposed to full sunlight myoga pseudostems are reported to suffer sunburn resulting in poor or non-existent flower bud production. Successful cultivation therefore requires 30-50% shade to prevent 'sunburn' or leaf scorching (Gracie et al., *in press*; Douglas & Follett, 1992). Since myoga had not been previously grown in sub-tropical regions of Australia it was not known if shade would be required to successfully produce flower buds.

To determine if myoga could be grown in sub-tropical conditions such as those at Rockhampton, plants were grown under four different environmental conditions designed to investigate a number of production issues. The environmental conditions were;

- 50% shade protection with shortened dark period,
- glasshouse conditions with shortened dark period,
- ambient Rockhampton conditions with shortened dark period,
- ambient Rockhampton conditions with normal dark period.

Although a number of these issues had previously been integrated into glasshouse trials it was aimed to investigate these environmental factors in a field trial where conditions would be similar to that of a commercial situation. The successful production of flower buds under these conditions would allow industry to adopt the results and incorporate them into commercial production protocols.

Materials and Methods

Plant Material

Propagation material was sourced from dormant plants at New Norfolk, Tasmania. Rhizome material was dug up and treated with a fungicide treatment consisting of 100ml of Previcur®, 200ml of Bavinston®, 100ml of Sumisclex® and 200g of Kocide® per 100L of water. Rhizomes received winter chill in the ground over the Tasmanian winter. In addition, rhizomes received a standard chilling treatment at 4^0C for three weeks at the University of Tasmania (Gracie *et al.*, 2000). All rhizome material was then air-freighted to the Central Queensland University at Rockhampton. The rhizomes were stored in a 10^0C cool room and on the 02/09/2000 were cut into 100g (+/-10g) pieces and planted into lay-flat black, plastic potting bags. Each bag contained one 100 g rhizome piece.

The rhizome was planted inside of the bag on top of 10 cm of potting mix and then covered with a further 20 cm of potting mix. The potting mix consisted of 70% composted pine bark, 20% coarse sand and 10% peat. Added to this soil mix was 10.5kg of dolomite, 10.5kg of $CaCO_4$, 21kg of Osmocote Plus and 1.75kg of $FeSO_4$. The potting mix had a pH of 5.5-6.0. On top of this a further 10cm of composted pine bark, mulch material was added. After planting, all bags were spray painted white to prevent the development of excess temperatures within the bags and were then randomly allocated to a treatment condition.

Treatments

Each treatment consisted of 25 plants. Treatment conditions were:

- Plants were grown outside, under natural Rockhampton conditions with a 30 minute night light break, every night at midnight (NNLB).
- Plants were grown outside under a 50% shade cloth with a 30 minute night light break, every night at midnight (SNLB).

- Plants were grown in a glasshouse (maintained at 26^0C) with a 30 minute night light break, every night at midnight (GNLB).
- Plants grown outside, under natural Rockhampton conditions without a night light break (NCO).

Lighting

Three 2 x 3m timber frames were built to support six, 60w light bulbs, in each of the three night break treatments. Timers were used to turn the lights on, for 30 minutes at midnight. The three lighting frames were placed in the centre of the glasshouse + NLB plot, the shade cloth + NLB plot and the natural conditions + NLB plot. Timers and bulbs were tested on a regular basis.

Watering

Based on data from previous trials in Tasmania each plant was given 1.7 litres of water per day for the first 10 weeks and thereafter a total of 3.4 litres per day, delivered in two separate applications. Delivery was through a dripper system, governed by a solenoid set-up that (after the first ten weeks) delivered 1.7 litres at 06:30 and 17:30 hrs, to each pot.

Harvesting

On a fortnightly basis the number of pseudostems per plant, height of pseudostems and date of first pseudostem emergence were recorded. Once flower bud development commenced, flower buds were harvested every four to eight days and the date of first flower bud emergence noted as well as the height of pseudostems at time of flower bud emergence. The number, weight and width of flower buds produced per plant was also recorded.

Statistical Analysis

The four treatment factors NNLB, SNLB, GNLB and NCO were analysed using one-way analysis of variance, with LSD tables created to sort significant differences between treatments. Experimental units within treatment factors were arranged in a completely randomised design.

Additional Data

A portable IRGA (Infrared Gas Analysis) was used to measure the net assimilation rate of CO_2 in five randomly selected plants from each treatment.

Technical Support

A collaborative relationship was established between researchers at the Central Queensland University at Rockhampton and the University of Tasmania at Hobart to aid with the collection of experimental data. The development of the aim and experimental design of this trial, in addition to all analysis and interpretation of results was conducted at Hobart. Implementation of the experimental design plus collection and submission of the data was performed by Sarah Purdy, at the Central Queensland University.

Results

Number of Pseudostems

Pseudostem emergence was first noted six days after planting (DAP) in plants grown under glasshouse conditions while plants grown under the other three treatment conditions did not begin producing pseudostems until approximately 16 DAP (figure 1). At 16 DAP, 42 out of the total 100 plants had produced at least one pseudostem. Plants grown in the glasshouse had developed the highest number of pseudostems with pseudostems noted in the majority of pots. Plants grown outside had approximately 50% emergence at this stage, while only 2 of the 25 pots in the shade house had developed pseudostems (figure 1). There were no significant differences in the number of pseudostems produced in plots NNLB, NCO, GNLB and SNLB, 5 weeks after planting.

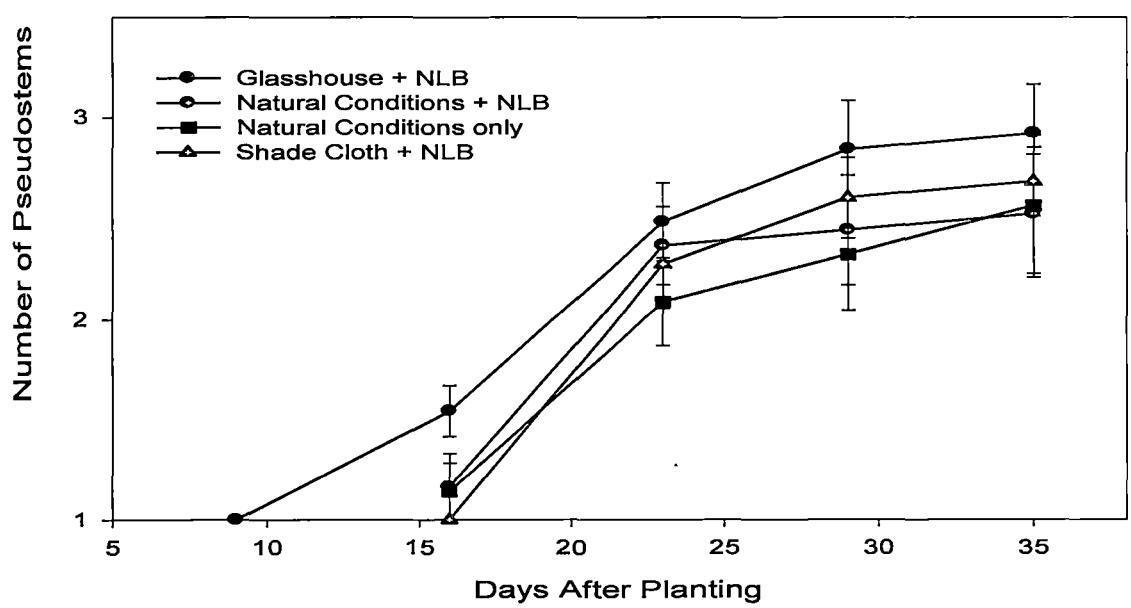


Figure 1: Pseudostem emergence per pot of all treatment plants. Bars show SE (n=25).

Pseudostem Height

Plants grown under glasshouse conditions produced significantly taller ($P<0.05$) pseudostems than plants from the other three treatments at 35 DAP (figure 2). At 16 DAP plants from all environment conditions displayed pseudostems of a similar height. However, subsequent to this, pseudostems of plants in glasshouse conditions were taller than pseudostems of plants in all other conditions as can be observed in figure 2. There was no significant difference in pseudostem height of plants from treatments NNLB, NCO and SNLB at 35 DAP.

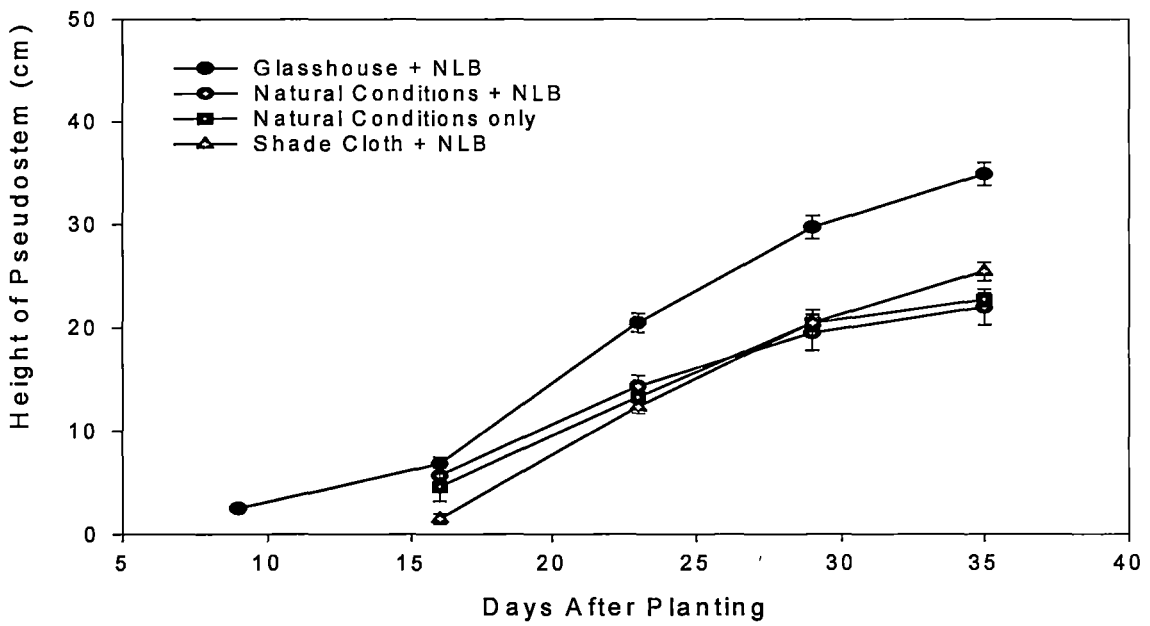


Figure 2: Pseudostem height of all treatment plants from 0 to 35 DAP. Bars show SE (n=25).

Flower Bud Number

Flower buds first emerged above the mulch surface 94 DAP. The size and shape of these buds varied greatly between plots and plants. Plants grown in glasshouse conditions produced a significantly higher ($P<0.05$) total number of flower buds at the completion of the harvesting period than plants from any other treatment. Plants from NNLB, SNLB and NCO plots all produced a similar number of flower buds at the completion of harvesting (figure 3).

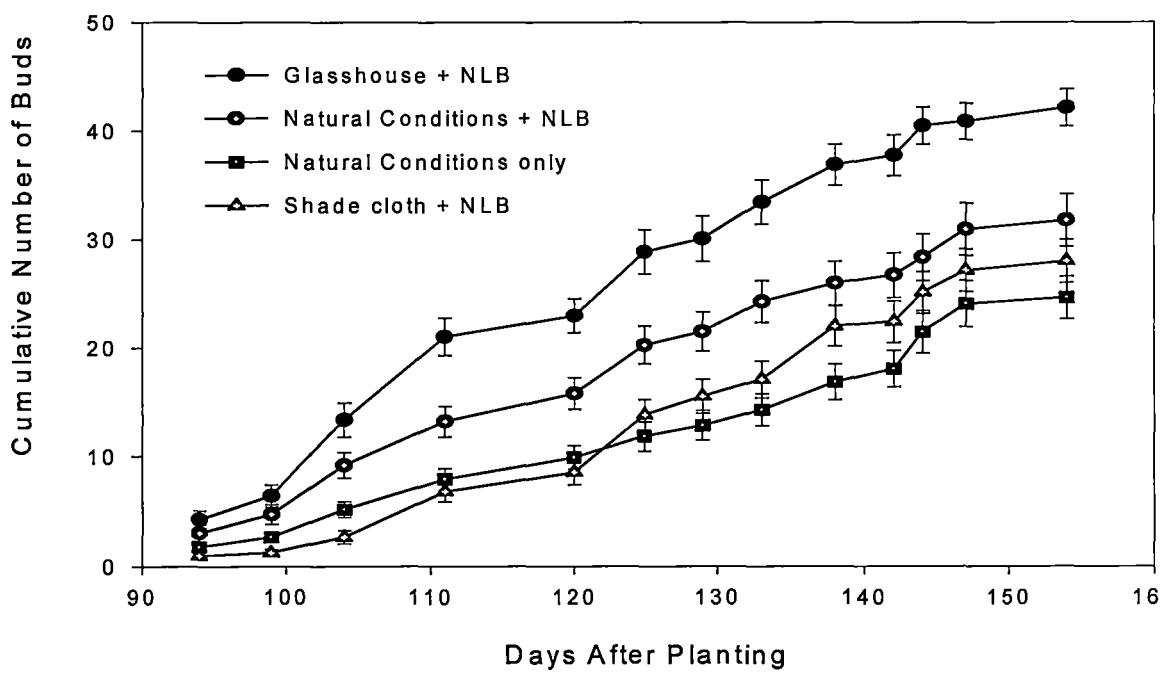


Figure 3: Cumulative number of flower buds harvested from all treatment plants. Bars show SE (n=25).

Flower Bud Weight

GNLB plants produced a significantly higher ($P<0.05$) total harvest weight of flower buds than plants from other treatments (figure 6). There were no significant differences in the weight of flower buds harvested from plants in treatments NNLB, NCO and SNLB.

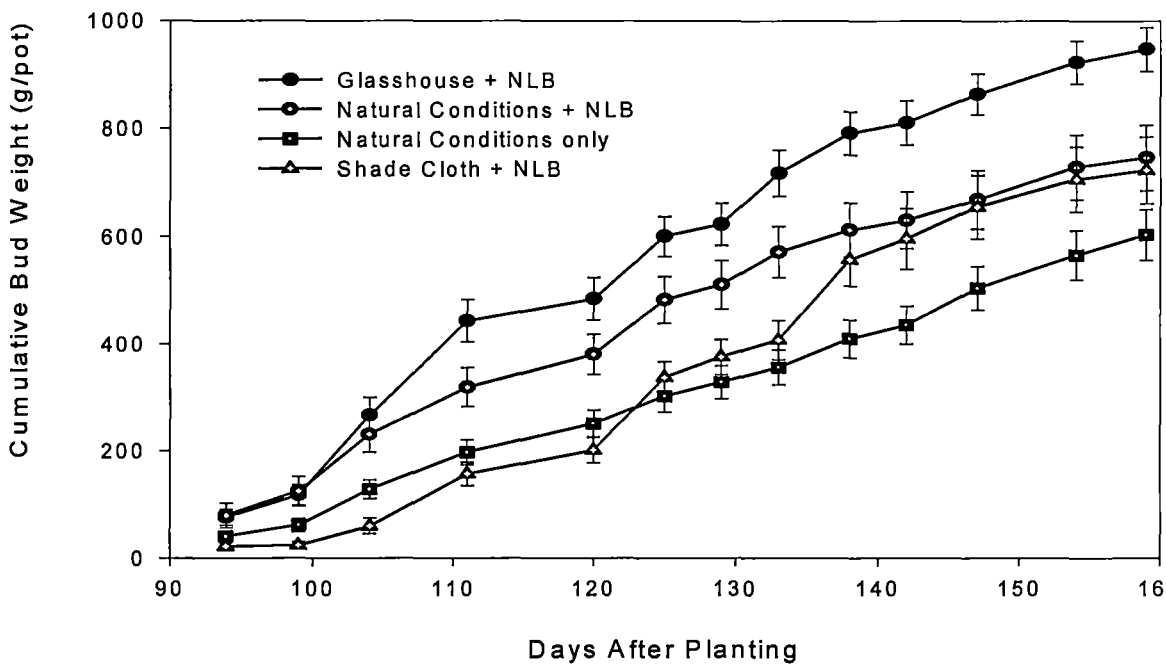


Figure 6: Cumulative weight of flower buds harvested from all treatment plants. Bars show SE (n=25).

There was no significant difference in the mean individual weight of flower buds produced by myoga plants from all treatment plots (table 1).

Treatments	*Glasshouse + NLB*	*Natural conditions + NLB*	*Natural Conditions Only*	*Shadecloth + NLB*
Mean individual flower bud weight (g)	21.179 ± 0.96	23.470 ± 0.75	23.441 ± 0.54	23.427 ± 0.92

Table 1: Mean weight of flower buds produced from plants in all treatment conditions. Each value is the mean ± standard error of 25 replicates.

The flower buds harvested from all treatment plants during this trial were of exceptional quality (plate 2). When compared with flower buds obtained during production trials at Albion Park and glasshouse trials in Tasmania, the colouration and general flower bud size was regarded as superior. Grading according to a chart used for commercial cultivation of myoga flower buds in Japan confirms that these buds easily satisfy requirements for classification as A grade flower buds.

Plate 2: Size and quality of myoga flower buds harvested at Rockhampton. Photo courtesy of Mr. Richard Warner.

Net Assimilation of CO_2

Photosynthetically Active Radiation (PAR) readings give an indication of the general light levels that plants were exposed to in each treatment condition. At the time of data collection plants grown outside without shade (NNLB and NCO treatments) were showing signs of severe scorching. These plants had a similar assimilation rate to plants grown outside under shade cloth, even though the PAR was much lower for these shaded plants. Relative to GNLB plants, plants grown outside had lower assimilation rates. It is likely that plants under shade had a reduced net assimilation rate because

they received lower PAR and plants grown outside without shade had a lowered net assimilation rate due to excessive light and observed sunburn. Plants grown in the glasshouse assimilated CO_2 at twice the rate of plants grown in other treatment conditions and this is reflected in higher biomass of these plants.

Treatment	*P.A.R* ($\mu mol\ m^{-2}s^{-1}$)	*CO_2 Net Assimilation Rate* ($\mu mol\ m^{-2}s^{-1}$)
GNLB	1675	4.558
NCO	1943.4	2.278
NNLB	2247.6	2.73
SNLB	876.4	2.724

Table 1: CO_2 Net Assimilation Rate and Photosynthetically Active Radiation readings of plants from all treatment conditions.

Discussion

Overall, myoga plants performed very well when grown in the sub-tropical environment of Rockhampton. The number and average weight of flower buds produced by myoga plants exceeded that produced by plants under commercial cultivation in Albion Park, NSW and was comparable to that produced by plants grown in glasshouse conditions in Hobart, TAS. Obviously plants grown in pots cannot strictly be compared with those under field cultivation, as the constraints placed on the plant when grown in pots are quite different to those experienced in field conditions. However the results obtained during this trial indicate that plants grown in ambient conditions similar to those at Rockhampton would provide high flower bud yields. The quality of flower buds produced during this trial was also of a superior standard. The majority of buds harvested were of an acceptable size and many surpassed that usually obtained during field cultivation in Wollongong. Furthermore the pink colouration of these flower buds was excellent and easily satisfied the requirements placed on myoga flower buds in the Japanese market.

The length of time from planting to harvest is obviously an important aspect of production. Plants grown in Albion Park usually take six months to produce flower

buds, most likely due to the cooler temperatures at the beginning of vegetative growth in August, September and October. Plants grown in Rockhampton began producing flower buds three months after they were planted. This substantial decrease in the time taken from planting to flower bud production would enable several crops of flower buds to be grown in one year rather than only one. It is also apparent from the results of this trial that there would be no reduction in yield resulting from a decreased period of vegetative development. Furthermore the length of harvesting period at Rockhampton was approximately 9 weeks, three weeks longer than that normally experienced at Albion Park. Obviously this has important implications for the potential yielding capacity of the plant.

When this trial was initially established, preliminary research conducted at the University of Tasmania had determined that myoga was a photoperiodic plant and that the plant most likely had a qualitative long day requirement for flower bud production. However the critical daylength, i.e. that daylength below which myoga will not flower had yet to be established. Night break lighting was included in this trial as the daylength at the time of year that the trial was conducted was suspected to be below the critical daylength (CDL) for flower bud production. However plants grown outside without an additional night light break still produced flower buds indicating that the daylength during this time of year was sufficient to enable flower bud development to occur. This result and information from other trials conducted in Tasmania place the CDL for flower bud development in myoga in the region of 12:15 to 13:15 hours.

Plants grown in shaded conditions were initially slower to develop pseudostems and flower buds. For successful cultivation of myoga in Southern Australia and New Zealand, shade has been identified as a necessary requirement. However over shading can result in a decrease in photosynthetic performance and ultimately decrease flower bud yield. Shade trials conducted in Tasmania indicated that when myoga plants were grown under warm, glasshouse conditions those at a 30% level of shading produced greater flower bud yields than those grown at higher levels of shading (Clark, 2001).

Based on these results it would appear that myoga plants grown in Rockhampton should not be cultivated under shade levels greater than 30%.

While excessive shading may decrease flower bud production because the plant does not perform at optimum photosynthetic capacity, excessive amounts of light can also result in problems. Myoga plants grown outside in Rockhampton began suffering sun damage soon after pseudostem emergence. Typical symptoms included development of chlorosis, leaf folding from the midrib during the middle of the day, necrosis around the edges of the leaves and stunting of vegetation. However while the severity of sun damage to plants in Southern Australia resulted in no development of flower buds and eventual necrosis of pseudostems, in Rockhampton, myoga plants were able to maintain healthy levels of growth and produce similar numbers of flower buds as those plants grown under 50% shade conditions.

The main climatic difference between Rockhampton and production areas in Southern Australia and New Zealand is temperature. Not only are average day temperatures much higher but night temperatures in Rockhampton are higher as well. The level of photosynthetically active radiation (PAR) incident on myoga plants in Rockhampton is comparable to that experienced by plants in Southern Australia and New Zealand therefore it does not seem likely that increased levels of PAR in Southern Australia are responsible for the observed plant damage and eventual death. However the combination of low night temperatures and strong light conditions experienced by plants in Southern Australia may be causing the observed symptoms of photodamage. Therefore the difference in plant performance when myoga is grown outside in Southern and Northern Australia may be due to low temperature-induced photoinhibition. Plants grown in both areas of Australia are probably subject to dynamic photoinhibition; a down-regulation mechanism which protects the photosynthetic apparatus of the plant. However plants grown in Southern Australia may also be subject to chronic photoinhibition induced by low night temperatures preceding high light conditions. Chronic photoinhibition results in permanent damage occurring

to photosystem II and can lead to photo-oxidative process and eventually death (Atwell, 1999).

The most productive plants, in terms of highest number and total weight of flower buds were plants grown in plot GNLB. Plants in this treatment received slightly less PAR than those grown outside. The temperature of the glasshouse was maintained at 26^0C, which was generally cooler than external day temperatures. Plants from NNLB, SNLB and NCO treatments produced the same number and total weight of flower buds. Although flower bud yield from these plants was lower it was still considered superior to those yields obtained from commercially cultivated plants in Albion Park. As mentioned previously estimations of flower bud yield from this trial should be viewed cautiously with respect to field cultivation. As observed during this and other trials in Tasmania, potting bags exert a large influence on rhizome and flower bud development. Rhizome development within the potting bags is limited and a dense mass is formed often hampering the upward movement of flower bud bearing rhizomes. This results in many flower buds residing within the sides of the bag and their size and shape is modified. The exact effect of this on flower bud yield is not yet known. However the modifying force of potting bags on rhizome development is likely to have some detrimental effect on final flower bud yield.

Based on the results of this trial, production of myoga flower buds in sub-tropical regions of Australia, such as Rockhampton is not only possible but also highly advantageous. The current harvesting season in Australia could be extended by the production of flower buds in Rockhampton over the winter months followed by production of flower buds over the summer months in Albion Park. Furthermore the potential yield of flower buds from each planting and the number of plantings possible in one year surpass that currently achieved in Albion Park. Management techniques identified during this trial such as the use of night light breaking to manipulate daylength, the use of 20-30% shade cloth to afford minimal protection from excessive sunlight, correct water scheduling and rigorous pest and disease monitoring and control can be used to achieve premium flower bud yields from field cultivated myoga plants.

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Conclusions and Industry Implications

High yields of flower buds can be achieved from first year crops provided the propagation material used is of sufficient weight. Rhizome material weighing approximately 100 g has provided good flower bud yields, with the number of pieces used to constitute this weight not likely to have an effect, provided the pieces weigh 25 g or more. From the results of these trials it appears however that even higher yields could be obtained from older plants. With correct management protocols at Albion Park, myoga could be effectively managed as a two year crop with potentially higher flower bud yields than one year crops. This conclusion is supported by research conducted in New Zealand where older crops were found to be more productive than first year crops (Follett, 1991; Palmer, 1984).

The degree of chilling experienced by myoga plants which remained in the ground over the winter months at Albion Park was shown to be sufficient for normal plant growth and development to occur the following season. Additional chilling was not necessary and did not promote reproductive development over those plants which had not received additional chilling. Therefore myoga plants could be over-wintered in the ground at Albion Park following the first growing season without adverse effects on flower bud production in the second season, due to an adequate winter chill.

The level of vegetative growth produced by second year plants was found to create harvesting problems, which reduced flower bud yield. For the full potential of two year plants to be realized the density of vegetation above ground would have to be controlled. Modification of below-ground rhizome material during the winter period helped reduce pseudostem density and when plants were sequentially harvested no reduction in flower bud yield was apparent.

Two harvesting methods were included in the four cultural trials conducted at Albion Park. It was hypothesised that continuous harvesting of flower buds from myoga plants during the period of active production might be required in order for new flower buds to develop. This occurs in asparagus crops where spears must be harvested regularly to

ensure that the maximum number of marketable spears are produced (Robb, 1984). If this hypothesis was correct it was expected that the number of flower bud produced from plants that were sequentially harvested would be far higher than those plants that were only harvested once. Since flower bud yields were generally higher from plants that were harvested only once it can be assumed that this was not the case. This result confirms research by Palmer (1984) who also concluded that cutting of myoga flower buds did not stimulate new rhizome formation. Differences in the pattern of apical dominance may explain the observed difference in plant performance. In asparagus apical buds exert dominance over lateral buds throughout the season, with lateral buds developing into spears only when an apical spear has been removed (Robb, 1984). In myoga plants, although apical buds initially exert dominance over lateral buds, this dominance is lost when pseudostems begin to export assimilates back down into the rhizome piece, shortly after flower bud initiation commences (Gracie et al., *in press*). As new flower bud development in myoga plants is not as apically dominated, the removal of apical flower buds is not likely to be necessary for subsequent flower bud development. It would appear therefore that continued development of new flower buds in myoga is more likely to be controlled by factors other than harvesting. In conclusion, although flower buds should be regularly harvested from myoga plants to ensure good quality, it does not appear necessary in order to stimulate new flower bud production.

The production trial conducted at Rockhampton clearly illustrated the benefits of cultivation of myoga in a sub-tropical environment. The quality and weight of flower buds produced by plants in this area surpassed that currently achieved with plants at Albion Park, NSW. The rate of plant development was also much higher in Rockhampton than at Albion Park, with flower bud harvesting commencing 90 DAP at Rockhampton compared with 120 or greater DAP required before flower bud emergence at Albion Park. The current harvesting season in Australia could be extended by the production of flower buds in Rockhampton over the winter months followed by production of flower buds over the summer months at Albion Park, NSW. Alternatively production of flower buds could occur continuously in Rockhampton. This could be achieved by implementing an artificial chilling period for approximately

six weeks in between production seasons and the use of night break lighting during winter months to create long-day photoperiod conditions.

GENERAL CONCLUSION

The research conducted during this project has contributed significantly to the development of myoga as a new vegetable crop in Australia. Increased scientific knowledge on the effect of environmental and cultural factors on flower bud production will aid the future development of this product, as informed decisions can now be made utilizing a better understanding of the factors affecting the vegetative and reproductive development of myoga. In addition, detailed information on the optimum temperature and photperiod conditions required will enable growers to schedule production of this crop throughout the year thus maintaining a sustained presence in domestic and potential export markets.

The identification of photoperiod as a major factor affecting flower bud initiation and development in myoga has not only greatly increased knowledge on the factors controlling flowering in myoga but has also greatly impacted on industry development. Myoga was found to have a quantitative short day requirement for flower bud initiation while successful flower bud development would only occur when plants were grown in strict long day conditions. Much of the research conducted during this project has indicated that myoga can be successfully produced across wide geographical areas of Australia. With the results of the photoperiod trials, scheduling of crops along the eastern seaboard of Australia utilizing the environmental conditions to maximize yearly production, can now occur. However due to this wide geographical range ambient temperatures will also vary greatly, therefore it was deemed necessary to investigate the interactive effects of temperature with photoperiod. Night temperature was found to modify photoperiod responses in cultivar S plants, with plants grown at low night temperatures developing flower buds at a daylength not permissive for flower bud development at higher night temperatures. This result may mean that in warmer areas such as Rockhampton, daylengths will need to be longer for successful flower bud development than that required in cooler areas such as Tasmania.

The previously unexplainable premature senescence of some field grown myoga plants has puzzled researchers and growers in New Zealand and Australia. During these

photoperiod trials a number of plants grown under short day conditions that were unable to successfully develop flower buds were also observed to prematurely senesce. While this was observed in both cultivar S and I plants, it was more apparent in cultivar I plants, most likely due to these plants having a longer critical daylength requirement for flower bud development. This conclusion was supported by the results of a number of trials where it was established that cultivar S plants had a critical daylength requirement of approximately 13 hours for flower bud development, while cultivar I plants had a longer daylength requirement of approximately 14 hours. The premature senescence of myoga plants in the field was usually observed after one season of growth. Older plants would probably be initiating flower buds at an earlier stage than younger plants and at a time when photoperiods were shorter. During a reciprocal transfer experiment it was shown, that if myoga plants which had initiated flower buds remained in short day conditions for too long, the plant would cease to initiate new flower buds even if the daylength was subsequently extended.

Myoga plants cultivated in different climatic regions were observed to have differing tolerance levels to ambient light. Traditional field cultivation of myoga in Japan is done in the open with no shade protection. Similarly, myoga plants grown in the open in Rockhampton, QLD were observed to produce comparable flower bud yields to those grown under shade. In New Zealand and Southern Australia however, shade is required to protect plants from excessive scorching of vegetation and eventual plant necrosis. The observed difference in light tolerance of myoga plants when grown in field conditions with warm night conditions as opposed to cool night temperatures indicated that these plants were likely to be susceptible to low-temperature induced photoinhibition. Growth cabinet experiments enabled the parameters of light sensitivity in myoga plants to be defined. While further experimentation concluded that preceding low night temperatures did increase the susceptibility of myoga plants to photoinhibition, resulting in a down-regulation of photosynthesis at light intensities that would not normally have been inhibiting. These results indicated that in sub-tropical areas such as Queensland myoga plants would only require minimal shading to prevent photodamage, while in temperate areas such as Tasmania much higher levels of

shading would be required. In terms of crop productivity it was important to define the shading requirements of myoga plants in response to the ambient environmental conditions. Excessive levels of shading can result in a decrease in potential photosynthetic performance, which would also affect flower bud production. Therefore, although it is important to prevent photodamage occurring to vegetative growth, it is also important not to decrease the potential photosynthetic efficiency of the plant through over shading.

A number of issues regarding the commercial cultivation of this crop at Albion Park, NSW became apparent during the first two years of production. Information on the best way to manage this crop was required, particularly in the transition from the first season of growth through to the second season. Due to the unexpected vigour of two year old myoga plants and the suspected need for chilling over the winter period, it was thought that myoga would need to be cultivated at this site as an annual plant. However the results of on-farm trials showed that with correct management of vegetation, excellent flower bud yields could be attained from two year old plants. In addition, it was shown that the period of chilling received by myoga plants remaining in the ground over winter was more than adequate to satisfy any vernalization requirement.

The production trial conducted at Rockhampton, QLD confirmed that myoga could be successfully produced in a sub-tropical environment. The shorter time period from planting to the start of harvesting, extended period of flower bud harvesting and the quality of flower buds indicated that with management of photoperiod during the winter period, myoga could cultivated in this area throughout the year and with potentially better yields achieved than those currently attained at Albion Park, NSW.

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