EFFECTS OF NUTRIENT SUPPLY AND COOLING ON GROWTH, FLOWER BUD DIFFERENTIATION, AND PROPAGATION OF THE NOBILE DENDROBIUM ORCHID

A Thesis

by

CHRISTINE YUNG-TING YEN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2008

Major Subject: Horticulture

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ABSTRACT

Effects of Nutrient Supply and Cooling on Growth, Flower Bud Differentiation, and Propagation of the Nobile Dendrobium Orchid. (August 2008)

Christine Yung-Ting Yen, B.S., National Taiwan University

Co-Chairs of Advisory Committee: Dr. Yin-Tung Wang
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Studies of *Dendrobium* Sea Mary 'Snow King' investigated the effect of nutrient termination (1 Aug., 1 Sept., or 1 Oct.) and reapplication [at the beginning, in the middle, immediately after, or 2 weeks after (relative to cooling), or no nutrient reapplication] on growth and flowering, quantified cooling requirements (10, 13, 15, or 18 °C for 2 to 6 weeks) for flowering, and determined optimum nutrient termination (on the three above dates) and nutrient rate (0.33, 0.67, or 1.33 g•L^{-1} 15N-2.3P-12.9K) for producing single-node cuttings. Regardless of reapplication stages, nutrient termination on 1 Oct. caused taller plants with more nodes, more leaves, more flowering nodes, more total flowers, and fewer aborted flowers than those being terminated earlier. Only buds protruding above 2 mm from pseudobulb surface showed differentiated floral structures. Plants with 1 Aug. nutrient termination had larger flower primordia than those with 1 Oct., indicating flower differentiated earlier or faster with an earlier nutrient termination. No reversion of reproductive to vegetative buds arose due to either late nutrient termination or resumption of

nutrients during cooling. Interactions between temperature and cooling duration were significant on time required for anthesis and full flowering, recorded from either beginning or completion of cooling, average flower number per flowering node, and flower diameter. Increasing cooling duration from 2 to 6 weeks led plants to reach anthesis and full flowering faster after cooling; however, the increasing cooling duration actually extended total time for producing flowering crops. Increasing temperature from 10 to 15 °C accelerated flowering after cooling. Plants had more flowering nodes and total flowers when cooled at 10 to 15 °C than at 18 °C. The results suggest that 3 weeks of cooling at 13 or 15 °C produce quality flowering plants that require less time to reach flowering. Plants fertilized at 0.67 or 1.33 $g \cdot L^{-1}$ were taller with 18% more nodes and more leaves than those receiving 0.33 $g \cdot L^{-1}$. Increasing nutrient rate with prolonged supply to the plants caused more single-node cuttings to grow into vegetative shoots for propagation, fewer cuttings to transition to flowering nodes, and less flower abortion to occur.

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CHAPTER I

INTRODUCTION

Orchids have long been attractive plants to many people because of their wide variety of shapes and patterns, exotic colors, long-lasting flowers, and their adaptability to diverse habitats. The USDA reported that in the United States, the wholesale value of potted orchids increased from $47 million in 1996 to $139 million in 2005 (USDA, 2007). The world's production of pot orchids was forecasted to continue increasing at a steady pace to reach a total of 305 million pots by 2014 (Wang, 2004). Research is needed to support this fast expanding and profitable industry.

Among the genera and species in the family Orchidaceae, few have been studied extensively to make their year-round production possible. The most common genera being commercially produced are *Phalaenopsis*, *Dendrobium*, *Oncidium*, *Cymbidium*, and *Cattleya*. *Dendrobium* was reported to be the second most valued orchid genus sold in Japan in 2002, which had a market share of 20%, only behind *Phalaenopsis* (Laws, 2004; Wang, 2004). *Dendrobium* has also been reported to be the most economically important flowering pot orchid genus sold in Hawaii with a wholesale value of $6 million in

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both 2005 and 2006 (USDA, 2007).

D. nobile Lindl. is one of the most commonly cultivated *Dendrobium* species (Baker and Baker, 1996) and has been grown for decades. It is native to habitats ranging from the Himalayas to Southeast Asia and much of southern China. The average temperature of the habitat in the spring is 30 to 31 °C during the day and 12 to 19 °C at night, whereas the average temperature in the summer is 26 to 28 °C during the day and 19 to 20 °C at night (Baker and Baker, 1996). However, *D. nobile* must be exposed to moderately low temperatures to induce flowering (Arditti, 1966; Goh et al., 1982; Rotor Jr., 1952, 1959), which usually occurs in the fall or following sudden rainstorms in its habitat. This species has been observed to grow and flower well in the warm extreme south Texas when managed properly.

D. nobile is a sympodial epiphyte with pseudobulbs 60 to 90 cm in length (Baker and Baker, 1996). The lateral buds that are produced during the current season remain quiescent or dormant until the next year. Upon flower initiation, bud primordia develop on the alternate axillary nodes of pseudobulbs. Inflorescences emerge and flowers open simultaneously from leaf nodes of the long pseudobulbs that matured the previous year (Ichihashi, 1997; Rotor Jr., 1952, 1959; Wood, 2006).

Although *D. nobile* has been grown for years, its hybrids, the nobile-type dendrobiums, are relatively new, commercially mass-produced orchids having a

high market potential. Scientific research on this kind of orchids thus far has rarely been published for growers to refer to.

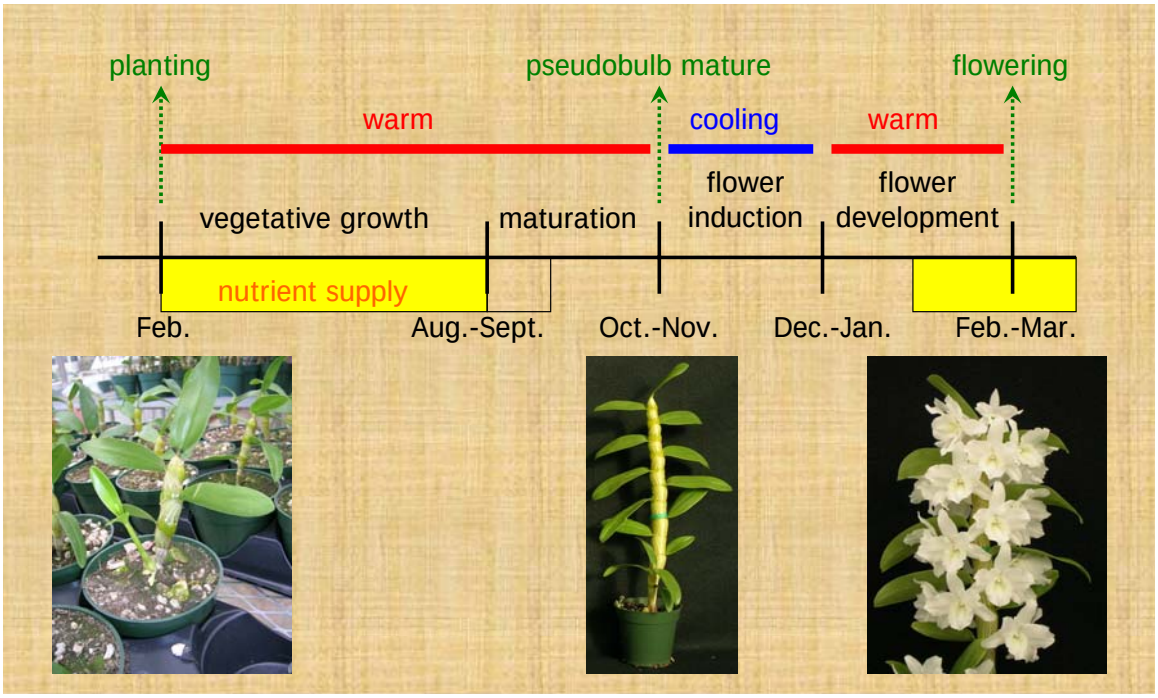

Fig. 1 The culture schedule for growing nobile dendrobiums into two-year-old flowering pot plants in commercial production.

Based on the nature of nobile dendrobium, a culture schedule for growing nobile dendrobiums into two-year-old flowering pot plants has been used in commercial production (Fig. 1). One-year-old liners are transplanted in February for the production of the current year's new pseudobulbs. When grown under a warm environment, new shoots emerge from the base of the liners, and maintain vegetative growth until August or September when they stop producing new leaves. Then, pseudobulbs begin to swell as photoassimilates start to

accumulate until they are fully matured in October or November. Plants with matured pseudobulbs are then subjected to cooling treatment for flower induction. After cooling, plants are again grown under a warm environment for flower development, finally reaching anthesis in February or March. Flower longevity in the nobile-type dendrobium varies among cultivars. The average flower longevity among thirty-one cultivars tested was 28.6 d, ranging from 15 to 47 d (Sakai et al., 1998).

To grow healthy plants with high flower quality, commercial growers apply nutrients early in the growing season, then reduce the rate or stop applying mineral nutrients completely in the fall to avoid potential flower buds from becoming aerial shoots (Yamamoto Dendrobiums, 2007). However, resumption of nutrient supply is possibly needed at a later time for flower development since reproductive growth may represent a strong nutrient sink.

It is essential to understand the factors that control each stage of growth and development to maintain a steady production of excellent quality plants. Adequate thermal, trophic, and light sequences are necessary for the acceleration of plants' growth and development (Tran Thanh Van, 1974). Since limited research has been conducted to characterize the factors that affect the nobile-type dendrobium's propagation, growth, and flowering, we can only begin the investigation based on the experiences of growers and what we know about culturing the progenitor, *D. nobile.* Referring to the similar growth habits of other orchid genera may help to clarify the confusion in growing nobile dendrobiums.

Studies in *D*. Red Emperor 'Prince', a nobile-type dendrobium, revealed that proper nutrient application helped to reduce defoliation during the cooling period and to produce more flowers on plants, resulting in more attractive flowering pot plants (Bichsel et al., 2008). Furthermore, higher nutrient rates, particularly N, resulted in more nodes per pseudobulb at maturation, giving potentially more single-node cuttings per plant for propagation. Therefore, my current study was conducted to solidify and supplement what was found previously.

The cultivar *D.* Sea Mary 'Snow King' was used in the current study. The objectives were to 1) investigate the effect of nutrient termination time and reapplication stage on growth, flower initiation, and flowering, and determine if nutrient reapplication would cause aerial shoot formation; 2) quantify the cooling requirements for flowering; and, 3) determine the optimum nutrient level for producing the highest amount of single-node cuttings and how the growth of new shoots would be affected by various nodal positions of the cuttings.

CHAPTER II

LITERATURE REVIEW

The nobile-type dendrobiums are cultivars bred mainly from the species *Dendrobium nobile* Lindl. (Ichihashi, 1997). They are still a relatively new mass-produced commercial crop for the floriculture industry. To this day, the scientific reports on nobile dendrobiums have been limited. The information from the existing reports is relatively out-of-date, contradictory, and in some cases, even disagreeable with the cultural practices that commercial growers have been using for some time. Recent scientific publications in *Dendrobium* within the last five years focused primarily on tissue culture, gene expression, and bioengineering. These projects require a long time to have the results becoming applicable to the flowering potted orchid industry. Studies to determine the cultural requirements are needed to improve the cultivation system and to make possible year-round production of flowering nobile dendrobiums.

Nutrient Application in Relation to Growth and Flowering

Nutrient rates. Nutrient application is important to plant growth and flowering. When nutrient requirements are not met or are only partially met, plants exhibit characteristic deficiency symptoms that, if severe enough, result in plant death (Hopkins, 1999; Wang, 2007a). As in other plants, orchids require mineral nutrients for optimum plant growth. Sufficient nutrient supply helps to

establish robust plants, including vigorous vegetative shoot formation, tall plant height, high leaf counts, high node number, etc., with a maximum reproductive performance in flowering nodes, flower counts, flower diameter, etc.

Several studies of various *Cymbidium* cultivars all showed that high N supply, compared to low N, increased vegetative shoot formation (Arnold Bik and Van den Berg, 1983; De Kreij and Van den Berg, 1990; Lunt and Kofranek, 1961; Powell et al., 1988). In *D.* Red Emperor 'Prince', a nobile-type dendrobium, plants receiving nutrients containing increasing N or K rates from 0 to 0.1 g•L^{-1} at every irrigation were increasingly taller with more nodes (Bichsel et al., 2008). In addition, applying nutrients containing N from 0.05 to 0.4 g•L^{-1} reduced defoliation during the cooling period. Nutrient supply containing K from 0 to 0.2 g•L^{-1} also resulted in increasingly less defoliation (Bichsel et al., 2008). In a white-flowered *Phalaenopsis* hybrid (*P. amabilis* Blume x *P.* Mount Kaala 'Elegance'), increasing fertility from 0.25 to 1.0 g•L^{-1} using 20N-8.6P-16.6K increased leaf production following flowering (Wang and Gregg, 1994).

It is noteworthy that improvement in leaf retention by nutrient supply may benefit orchid production, especially to the nobile-type dendrobiums. It is a recent trend that consumers have a preference toward nobile dendrobiums having green leaves rather than those without foliage at the time of flowering. However, cooling for flower initiation often causes the leaves to turn yellow and abscise. Consequently, in nature, inflorescences of several *Dendrobium* species develop on defoliated pseudobulbs (Wood, 2006). If there is a

possibility to establish a good culture system combining optimum nutrient application with cooling that effectively makes the nobile dendrobium flower with foliage, a huge potential market may be successfully developed.

Higher fertility rates have shown to improve reproductive growth. Increasing the fertility from 0.25 to 1.0 g•L^{-1} with 20N-8.6P-16.6K fertilizer increased flower count and stalk diameter of the white-flowered *Phalaenopsis* hybrid for two consecutive flowering seasons (Wang and Gregg, 1994). Furthermore, Wang (1995a) found that constant fertilization resulted in more inflorescences and flowers than intermittent fertilization in *D.* Linnapa 'No. 3', a phalaenopsis-type dendrobium. In *D.* Red Emperor 'Prince', plants had more flowers when receiving nutrients containing P between 0.025 and 0.02 g•L^{-1}, or containing K between 0.1 and 0.4 g•L^{-1}, compared to those with lower nutrient rates (Bichsel et al., 2008). All of these studies pointed out that, with a high rate or frequency of nutrient supply, plants had more flowers or inflorescence spikes when compared to lower rates or frequencies.

However, studies in various *Cymbidium* cultivars showed adverse effects of nutrient application on reproductive growth. High N rates reduced inflorescence/shoot ratio, as a result of low inflorescence production and vigorous vegetative shoot formation (Arnold Bik and Van den Berg, 1983; De Kreij and Van den Berg, 1990; Lunt and Kofranek, 1961; Powell et al., 1988). In *Cymbidium* Mary Pinchess 'Del Rey', high electrical conductivity (EC) associated with high N concentration in the nutrient supply, as compared with low EC,

resulted in more inflorescences per unit of greenhouse area (De Kreij and Van den Berg, 1990). However, this increase was due to the increased number of vegetative shoots that produced inflorescences, not to the promotion of reproductive growth. In addition, *Cymbidium* cv. 'Pendragon Sikkim' plants that were given increasing N rates from 0.06 to 0.11 $g \cdot L^{-1}$ had decreased spike length and fresh weight of inflorescences and delayed flowering (Arnold Bik and Van den Berg, 1983). These findings showed that high N rate was relatively detrimental to reproductive growth but beneficial to vegetative growth.

Nutrient termination. Commercial growers apply mineral nutrients to the nobile dendrobiums early in the growing season once liners are transplanted in February, then reduce the rate or stop applying nutrients completely, especially N, in late July or August, to avoid potential flower buds turning into aerial shoots (Yamamoto Dendrobiums, 2007). In a commercial nursery, mature plants that produce aerial shoots instead of flowers are unmarketable and become a total financial loss to the grower. Wood (2006) recommended withholding N after 1 Aug. in the Northern Hemisphere for *D. nobile* and its hybids. Baker and Baker (1996) suggested using a high P fertilizer in late summer and autumn in *D. nobile*; then, reducing watering after new growth matures in autumn and withholding the nutrients until the following spring. However, none of these claims were supported by solid research data or explained the cause and effect in detail.

Bichsel (2006) investigated how various rates of nitrogen (N), phosphorus (P), and potassium (K) and nutrient termination times (1 Sept., 1 Oct., or 1 Nov. 2005) affected growth and flowering of *D.* Red Emperor 'Prince' in a warm climate. It was found that terminating nutrient application on 1 Oct. resulted in the best vegetative and reproductive characteristics. Regardless of P rates, plants were taller with more nodes, leaves, and flowers when nutrient application was terminated on 1 Oct. than 1 Sept. or 1 Nov. For N effects on reproductive growth, when given 0.1 $g \cdot L^{-1}$ N, plants produced more flowers with 1 Oct. nutrient termination time than the earlier or later time. When N rate was 0.2 or 0.4 $g \cdot L^{-1}$, more flowers were produced with 1 Sept. or 1 Oct. than the later nutrient termination time. Plants with various N rates and nutrient termination times took similar time to reach anthesis, except for a delay when terminating nutrients containing 0.2 or 0.4 $g \cdot L^{-1}$ N on 1 Nov.

In one study on the nobile dendrobium (cultivar unspecified in the English abstract), nutrients (a fertilizer providing 0.0125 g N per pot every month) were terminated at various times at two-month increments, starting with thirteen months before cooling treatment began (Sakai et al., 1982). It was found that terminating nutrient application from one to seven months before cooling, as compared with nine to thirteen months before cooling, promoted growth and flowering, resulting in taller plants, more flowering nodes, and increased flower count.

Terminating or reducing nutrient application has also been reported for other orchid genera. Arnold Bik and Van den Berg (1983) investigated whether regulating nutrient application time would increase floral spikes of *Cymbidium* cv. 'Pendragon Sikkim'. They found that withholding nutrient application during flower initiation in May and June in the Northern Hemisphere resulted in reduced total shoot formation when recorded in July of the same year, but a higher inflorescence/shoot ratio along with earlier flowering, as compared with a continuous nutrient supply. A similar fertilization regimen is used in Dutch commercial horticulture where *Cymbidium* is given low fertilization of 0.4 $dS \cdot m^{-1}$ EC from April to July at the time of flower initiation, and high fertilization of 1.0 $dS \cdot m^{-1}$ EC for the remainder of the year (De Kreij and Van den Berg, 1990).

In *Phalaenopsis* TAM Butterfly, terminating nutrient application on 1 Sept., 29 Sept., or 27 Oct. caused fewer flowers on plants than those being terminated nutrients on 24 Nov. or continuously fed, indicating that maintaining N fertilization until near the completion of floral bud initiation is crucial to sustaining the initiation of floral primordia for high flower count (Wang, 2000). In addition, flower longevity was reduced by 12 d when nutrient supply was terminated on 1 Sept. Terminating nutrients completely on any date had no impact on the date of spiking and anthesis or on flower size (Wang, 2000).

From the above studies in various orchid genera, optimum timing of nutrient termination for quality flowering varies. For *Cymbidium* cv. 'Pendragon Sikkim', nutrient application was suggested to be terminated before flower

initiation began; for *Phalaenopsis* TAM Butterfly, maintaining N fertilization until the completion of flower initiation was preferred. The best time to terminate nutrient application in nobile dendrobiums remains to be defined.

Nutrient reapplication. Since orchids are perennial crops, once nutrient application has been terminated, nutrients are expected to be resumed at a certain time and then be terminated again in the next growing season. However, research pertinent to timing of nutrient reapplication in orchids has rarely been documented. Sakai et al. (1982) conducted research on various periods of nutrient application, January to April, April to July, July to October, or October to January, with a fertilizer providing 0.0375 g N per pot every month to evaluate growth and flowering of two-year-old nobile dendrobiums (cultivar unspecified in the English abstract). Among treatments, the most effective nutrient application period for increased flower count (plants flowered in late January) of the second year's growth was from October to January, which corresponded to the time of new vegetative growth for the next growing season. The result indicated that the new vegetative growth was a strong sink that drew the newly applied mineral nutrients. It also implied that if not given nutrients, flower differentiation and development could be constrained by the depleted nutrients. Therefore, in orchid production, once the floral primordia have been initiated, it seems that resumption of nutrient supply is needed for subsequent flower differentiation and development. An ideal nutrient reapplication time that optimizes flowering still needs to be investigated.

Bud reversion. Goh et al. (1982) indicated that once floral primordia have been initiated, flower buds do not always develop into mature flowers. Young inflorescences of *Aranda* Christine 130 had been observed to change to vegetative shoots after high N (nutrient rate unspecified) application (Hew and Yong, 2004). Since growers have claimed that aerial shoots may arise from the potential floral buds if growing conditions are not favorable to flowering (Yamamoto Dendrobiums, 2007), it is possible that a similar reversion from reproductive to vegetative growth due to excessive nutrient supply may exist in the nobile dendrobiums.

Reversion from reproductive back to vegetative growth in several other *Aranda* cultivars, *D.* Louisae 'Dark' (a phalaenopsis-type dendrobium), and *Phalaenopsis* have also been reported (Goh, 1975, 1976, 1977a,b, 1979; Hew and Yong, 2004; Rotor Jr., 1952, 1959; Tran Thanh Van, 1974). However, once *A.* Deborah flower buds had reached a length of 2 mm, they continued to develop and reach maturity (Goh, 1977a,b; Goh et al., 1982).

Histology. In order to observe if the bud reversion occurs in nobile dendrobiums, it is necessary to identify histological differences between vegetative and reproductive primordia so developmental changes may be defined. Rotor Jr. (1952) pioneered the histological studies in orchids and described that comparatively inactive apical meristems in buds showed slightly convex form; apical meristems of differentiating vegetative buds were more rounded, while those of initiated floral buds became quite angular and pointed.

A scanning electron microscope study in *D*. Snowflake 'Red Star', a nobile-type dendrobium, showed that the initiated flower buds developed into a dome-shape structure when air temperature was below 10 °C (Chae, 2002). Ferreira et al. (2006) reported that growing shoots obtained from micropropagated plants at 26 °C, the vegetative shoot apex of *D*. Second Love before flower induction showed the typical tunica-corpus organization in histology and changed into a mantle-core structure after 10 d in the flowering inductive medium, which was an indication of a transition to floral differentiation. The cells in the central core of the floral apex had larger vacuoles in contrast to the smaller and more densely stained cells which formed the mantle of the floral apex.

Flower Induction by Low Temperature

As in many other plants, flowering in orchids is controlled by genetic and environmental factors. Orchid genera or species respond to temperature and/or light differently in regulating flowering. Some orchids can be induced to flower by photoperiod and thereby are classified as long-day, short-day, or day-neutral plants. Examples of short-day plants in orchids are *Cattleya labiata* Lindl. and *Cattleya trianae* Linden & Rchb. f., whereas *D. nobile* and *Paphiopedilum insigne* Lindl. are day-neutral plants and their flowering is not affected by daylength (Goh and Arditti, 1985; Goh et al., 1982; Rotor Jr., 1952, 1959). Reviewed by Goh and Arditti (1985), several West African orchid species have been speculated to be long-day plants, but no scientific reports validated the

presumption. Flowering in some other orchids requires a period of fluctuating or relatively low temperatures, such as several *Dendrobium* and *Phalaenopsis* species (Goh and Arditti, 1985; Goh et al., 1982; Rotor Jr., 1952, 1959).

Low-temperature induced flowering. Low temperature and its duration that induce flowering vary with different orchid genera and species. Two major types of dendrobiums, the phalaenopsis-type and nobile-type, that are grown commercially have been reported to initiate flowers with low temperatures (Rotor Jr., 1952, 1959). Flower initiation in *D. phalaenopsis* Fitzg., the progenitor of phalaenopsis dendrobium hybrids, was induced by low temperature of 13 °C regardless of daylength (Rotor Jr., 1952, 1959). From evaluation made over a period of five years and monthly minimum temperatures varying from 15 to 19 °C, minimum temperature in the fifth month before every harvest (twice monthly throughout the year) was significantly and negatively correlated with spike yield per plant, number of flowers per spike, and spike length in *D.* Jaquelyn Thomas, a phalaenopsis-type dendrobium (Paull et al., 1995). The lower the minimum temperature was, the higher spike yield and number of flowers were. The result implied that flowering was promoted by the monthly low temperature about five months before a harvestable spike was produced.

For the nobile-type dendrobiums, Rotor Jr. (1952, 1959) reported that *D. nobile* plants exposed to 13 °C produced flowers regardless of photoperiod. Regardless of low night temperature treatments of 7.5 to 15 °C, day temperature at 20 °C for *D.* Snowflake ‘Red Star’ and day temperature at either 20 or 25 °C

for *D.* Hinode 'Toutenkou' caused a relatively similar performance in time required for floral bud emergence, flowering date, and percentage of flowering nodes, suggesting that the former variety required a lower cooling temperature than the latter for flower initiation (Sinoda et al., 1988). To achieve the best flowering performance, the optimum temperature for flower initiation in these two nobile dendrobium cultivars was below 20 °C in the daytime and 10 to 12.5 °C at night (Sinoda et al., 1988).

Besides the above two types of dendrobiums, *D. crumenatum* Sw. was also reported to require low temperatures for flowering (Goh et al., 1982; Hew and Yong, 2004). In nature, the inflorescence of *D. crumenatum* produces flower buds that grow until the anther is almost fully developed and all other floral parts are formed and then undergo dormancy. Development resumes in the dormant flower buds after a sudden 5 °C temperature drop (exact temperature unspecified), which is often provided by rainstorms in Southeast Asia (Goh et al., 1982; Hew and Yong, 2004). From all the studies above, it is obvious that the recommended temperature for flower induction differs among *Dendrobium* hybrids and species.

Other orchids besides *Dendrobium* that require relatively low temperatures for flowering include some species in genera *Cymbidium*, *Paphiopedilum, Miltonia*, *Doritis*, and *Phalaenopsis*. However, due to lack of strictly controlled experimental information, whether the promoted flowering response by low

temperatures was a result of triggering flower initiation itself or a result of triggering the initiated floral buds to develop into flowers could not be determined.

Regardless of daylength, several *Cymbidium* hybrids (*C.* Madeleine, *C.* Doreen, *C.* Zebra, and *C.* No. 2212) and *Paphiopedilum insigne* flowered at low temperature of 13 °C (Rotor Jr., 1952, 1959). Tran Thanh Van (1974) reported that temperatures close to 17 °C were favorable for abundant flowering in *Miltonia* (species unspecified). In *Doritaenopsis* 'Lava Glow' (*Phalaenopsis* Buddha's Treasure x *Doritis pulcherrima* Lindl.), among the set of growing day/night (12 h each daily) temperatures of 30/25, 25/30, 25/20, or 20/25 °C, or another set of 30/20, 20/30, 25/15, or 15/25 °C, the cool day/warm night temperature regimens of 20/25 °C and 15/25 °C markedly restricted vegetative growth but were able to trigger flowering effectively (Wang, 2007b). Nishimura et al. (1976) reported that the transition from vegetative growth to flower development in *Phalaenopsis* Sea Mist required relatively low temperatures of 15 to 18 °C. The inflorescence of *Phalaenopsis amabilis* was induced by temperature below 25 °C for more than 12 h daily (Sakanishi et al., 1980). Two to 5 weeks of night temperature between 12 and 17 °C and day temperature not over 27 °C for flower initiation in *P. amabilis* and *Phalaenopsis schilleriana* Rchb. f. was reported by Tran Thanh Van (1974). Therefore, it can be seen that the low temperatures needed to induce flowering vary among orchid genera, ranging from 10 to 25 °C, and even differ from one species to another within the same genera.

Low-temperature-induced flowering affected by light. Flowering of plants could be affected by light intensity and quality, and/or photoperiod (Arditti, 1961). Some orchids that are induced to flower by low temperatures were reported to be affected also by light (photoperiod or intensity), such as some species or hybrids of *Dendrobium*, *Miltonia*, *Cymbidium,* and *Phalaenopsis*.

Flower initiation of *D. phalaenopsis* was induced by short days (9 h) regardless of temperatures or by low temperature of 13 °C regardless of daylength (Rotor Jr., 1952, 1959). For *D. nobile*, although flower initiation by low temperature induction was reported not to be affected by photoperiod, it was found that daylength altered the speed of subsequent flower development (Rotor Jr., 1952, 1959). Rotor Jr. (1952) reported that long days (16 h) hastened flower development by 1 to 4 weeks, possibly due to the effect of accumulated high energy provided by extended duration of light.

For *Miltoniopsis* Augres 'Trinity' (*Miltonia* Pam-pam x *Miltonia* Alger), among treatments of a 9-h or 16-h photoperiod before vernalization with a temperature of 8, 11, 14, 17, 20, or 23 °C, flowering percentage was the highest (75% or above) when plants were exposed to the 9-h photoperiod and then vernalized at temperatures of 11 or 14 °C (Lopez and Runkle, 2006). Vernalization at the lowest 8 °C appeared to cause adverse effect on flower development. The enhanced flowering percentage by short photoperiod, however, might be a result of either shortened juvenile phase of the plants to

possess flowering capability or promoted flower initiation. No evidence showed the direct effect of photoperiod on flower initiation.

For *Cymbidium* hybrids (*C.* Madeleine, *C.* Doreen, *C.* Zebra, and *C.* No. 2212), light intensity affected the degree of response to low temperature (Rotor Jr., 1952, 1959). Flowering was promoted when the *Cymbidium* hybrids were grown outdoors without shading than being slightly shaded (degree of shading unspecified) in a greenhouse in summer. However, due to lack of detailed information on growing conditions of the experiment setup, it is uncertain that the flowering response was truly affected by the light intensity or by other environmental factors that differed from indoor to outdoor.

Flower inhibition. In contrast to flower induction, relatively high temperatures or specific light conditions may be unfavorable to flowering. Several *Cymbidium* hybrids, *Paphiopedilum insigne,* and *D. nobile* plants held at 18 °C, compared to 13 °C, remained vegetative (Rotor Jr., 1952, 1959). Flowering of *Phalaenopsis amabilis* was inhibited when plants were grown under high temperatures (30 °C day/23 °C night), compared to low temperatures at 25 °C day/20 °C night (Chen et al., 1994). The inflorescence of *P. amabilis* did not emerge if the temperature was over 28 °C (Sakanishi et al., 1980). In addition, when day temperature was maintained at 28 or 30 °C, certain *Phalaenopsis* hybrids remained vegetative even when night temperature was dropped to 19 or 14 °C, respectively (Wang et al., 2006).

Research in *Phalaenopsis* Joseph Hampton 'Diane' revealed that plants responded to cool temperature for spiking only when light intensity was above a certain level (Wang, 1995b, 1997). Plants exposed to 160 or 60 μmol•m^{-2}•s^{-1} *PPF* for 12 h daily at 20 °C day/15 °C night spiked in 4 and 5 weeks, respectively. Those under 8 μmol•m^{-2}•s^{-1} or in complete darkness for 6 weeks did not spike, despite being exposed to the ideal spiking temperatures (Wang, 1995b). Furthermore, plants produced no flowers for two years under 10 or 20 μmol•m^{-2}•s^{-1} for 12 h daily, while those under 30 or 50 μmol•m^{-2}•s^{-1} flowered (Wang, 1997). Growing several *Phalaenopsis* hybrids with night temperature reaching as low as 8 to 9 °C, nearly all plants subjected to complete darkness for 5 d weekly by covering with a woven black polypropylene groundcover remained vegetative over a three-month (October to December) treatment period, whereas plants that were exposed to light daily in a greenhouse spiked normally (Wang et al., 2006).

Flowering control practices. By understanding the environmental factors that regulate flower initiation and development, programming flowering to meet a specific market time for high profitability can be expected. Hew and Yong (2004) listed considerations for developing techniques for flower induction in commercial production: 1) the method must be simple, economical and give reproducible results; 2) the quantity and quality of flowers must not be affected; 3) there should not be any adverse effects on the plant or on subsequent flowering. Therefore, if the threshold temperature for initiating flowers,

promoting the best flower quality, or that adversely affects plant growth is known, applications for flower induction with the above considerations in mind can be applied to production systems successfully.

From the findings discussed for *D. nobile* and *Paphiopedilum insigne*, therefore, practices for controlling flowering were described (Rotor Jr., 1952, 1959). Plants grown continuously at a minimum of 18 °C remain vegetative until such time as it is desired to initiate flowering by dropping the temperature to 13 °C. It takes four months for *D. nobile* and six months for *P. insigne* to reach anthesis from flower initiation. This period can be shortened by raising the growing temperature for both species, or by providing long days for *D. nobile* after the floral buds are well-formed to hasten flower development (Rotor Jr., 1952, 1959).

For *Phalaenopsis*, relatively low temperatures of 15 to 25 °C are required for flower initiation (Goh et al., 1982; Nishimura et al., 1976; Rotor Jr., 1952, 1959; Sakanishi et al., 1980; Tran Thanh Van, 1974). The temperature and the duration required from flower initiation to anthesis vary slightly among *Phalaenopsis* species and hybrids. Day temperature over 27 °C hinders flower initiation, so plants remain vegetative (Chen et al., 1994; Goh et al., 1982; Sakanishi et al., 1980; Tran Thanh Van, 1974; Wang et al., 2006). However, the effective, low-cost alternative to high temperature for inhibiting spiking in *Phalaenopsis* by heavy shading could thereby be used to program flowering

when heating is extremely expensive and not easy to maintain during the winter months (Wang et al., 2006).

Propagation through Single-node Cuttings

Nobile-type dendrobiums for pot-plant production are propagated mainly through single-node stem cuttings (Ichihashi, 1997). Experiments conducted by Bichsel et al. (2008) showed that nutrient rates of 0.1 to 0.2 g•L^{-1} N resulted in more nodes per pseudobulb at maturation in *D.* Red Emperor 'Prince', as compared with nutrient rates of 0, 0.05, or 0.4 g•L^{-1} N. From several studies in the nobile-type and phalaenopsis-type dendrobiums, results showed that plants had taller pseudobulbs with more nodes as the level of N in the nutrient supply increased (Miwa and Ozaki, 1975; Sakai et al., 1982; Uesato et al., 1987). Bhattacharjee (1981) also found that the vegetative growth of *D. moschatum* Wall., including number and height of pseudobulbs and number of leaf nodes, increased markedly with increasing N or P_2O_5 from 0 to 1 g•L^{-1}. Consequently, if nutrients are properly applied to obtain longer pseudobulbs, many more cuttings can be obtained in a given greenhouse bench area, resulting in lower cost of cutting production. However, it is not known how the nutrient rates during the previous growing season may affect the new growth and, possibly, subsequent flowering from the cuttings the following year.

Rotor Jr. (1952) indicated that the inflorescence primordia at the upper part and those nearest the basal part of the pseudobulb usually develop smaller

inflorescences, while the lower-most bud may not develop flower primordia at all. Goh (1975, 1977a,b, 1979) also reported that for several *Aranda* hybrids (*A.* Deborah, *A.* Hilda Galistan, *A.* Lucy Laycock, *A.* Mei Ling, and *A.* Nancy) and *D*. Louisae 'Dark', a growth and flowering gradient existed that the developmental fates or growth rates of the nodes varied along the pseudobulbs. Buds near the apex developed into inflorescences or intermediate structures between inflorescence and vegetative shoots, while those situated further away from the apex developed into vegetative shoots. For the nobile dendrobiums, the single-node cuttings taken from the middle-part of the pseudobulbs for propagation may be more vigorous and therefore produce stronger plantlets than those taken from other parts.

CHAPTER III

EFFECT OF NUTRIENT TERMINATION TIME AND REAPPLICATION STAGE ON GROWTH, FLOWER INITIATION, AND FLOWERING

Introduction

Orchids have long been attractive plants to many people because of their wide variety of shapes and patterns, exotic colors, long-lasting flowers, and their adaptability to diverse habitats. The USDA reported that in the United States, the wholesale value of potted orchids increased from $47 million in 1996 to $139 million in 2005 (USDA, 2007). *Dendrobium* was reported to be the second most valued orchid genus sold in Japan in 2002, which had a market share of 20%, only behind *Phalaenopsis* (Laws, 2004; Wang, 2004). *Dendrobium* has also been reported to be the most economically important flowering pot orchid genus sold in Hawaii with a wholesale value of $6 million in both 2005 and 2006 (USDA, 2007).

D. nobile Lindl. is one of the most commonly cultivated *Dendrobium* species (Baker and Baker, 1996) and has been grown for decades. It is native to habitats ranging from the Himalayas to Southeast Asia and much of southern China. This sympodial epiphyte produces lateral flower buds (Baker and Baker, 1996). Flowers open simultaneously from leaf nodes of the long pseudobulbs

that matured the previous year (Ichihashi, 1997; Rotor Jr., 1952, 1959; Wood, 2006).

Although *D. nobile* has been grown for years, its hybrids, the nobile-type dendrobiums, are relatively new, commercially mass-produced orchids having a high market potential. Commercial growers apply nutrients to one-year-old nobile dendrobium liners soon after they have been planted, then reduce the rate or stop applying mineral nutrients completely in late July or August to avoid potential flower buds turning into aerial shoots (Yamamoto Dendrobiums, 2007). Wood (2006) recommended withholding N after 1 Aug. in the Northern Hemisphere for *D. nobile* and its hybids. Baker and Baker (1996) suggested reducing watering after new growth matures in autumn and withholding nutrients until the following spring. However, none of these claims were supported by solid research data or explained the cause and effect in detail.

Bichsel (2006) investigated how various rates of nitrogen (N), phosphorus (P), or potassium (K) and nutrient termination times (1 Sept., 1 Oct., or 1 Nov. 2005) affected growth and flowering of *D.* Red Emperor 'Prince' in a warm climate. It was found that terminating nutrient application on 1 Oct. resulted in the best vegetative and reproductive characteristics. Regardless of P rates, plants were taller with more nodes, leaves, and flowers when nutrient application was terminated on 1 Oct. than 1 Sept. or 1 Nov. For N effects on reproductive growth, when given 0.1 $g \cdot L^{-1}$ N, plants produced more flowers with 1 Oct. nutrient termination time than the earlier or later time. When N rate was 0.2 or

0.4 g•L^{-1}, more flowers were produced with 1 Sept. or 1 Oct. than the later nutrient termination time. Plants with various N rates and nutrient termination times took similar time to reach anthesis, except for a delay when terminating nutrients containing 0.2 or 0.4 g•L^{-1} N on 1 Nov.

Arnold Bik and Van den Berg (1983) investigated whether regulating nutrient application time would increase floral spikes of *Cymbidium* cv. 'Pendragon Sikkim'. They found that withholding nutrient application during flower initiation in May and June in the Northern Hemisphere resulted in reduced total shoot formation when recorded in July of the same year, but a higher inflorescence/shoot ratio along with earlier flowering, as compared with a continuous nutrient supply. In *Phalaenopsis* TAM Butterfly, terminating nutrient application on 1 Sept., 29 Sept., or 27 Oct. caused fewer flowers on plants than those being terminated nutrients on 24 Nov. or continuously fed, indicating that maintaining nutrient application until near the completion of floral bud initiation is crucial to sustaining the initiation of floral primordia for high flower count (Wang, 2000).

From the above studies in various orchid genera, optimum timing of nutrient termination for quality flowering varies. For *Cymbidium* cv. 'Pendragon Sikkim', nutrient application was suggested to be terminated before flower initiation began; for *Phalaenopsis* TAM Butterfly, maintaining N fertilization until the completion of flower initiation was preferred. The best time to terminate nutrient application in nobile dendrobiums remains to be defined.

Resumption of nutrient supply is possibly needed at a later time for flower development since reproductive growth may represent a strong nutrient sink. However, research pertinent to timing of nutrient reapplication in orchids has rarely been documented. An ideal nutrient reapplication time that supports flowering still needs to be investigated. The objectives of this study were to investigate the effect of nutrient termination time and reapplication stage on growth, flower initiation, and flowering, and to determine if nutrient reapplication would cause aerial shoot formation.

Materials and Methods

Plant material and growing conditions. A *Dendrobium nobile* Lindl. hybrid, *Dendrobium* Sea Mary 'Snow King', was used. One-year-old liners, propagated from single-node cuttings and planted in sphagnum moss with an average pseudobulb (i.e., a thickened portion of the stem in orchids functioning as a water and food storage device) height of 7 to 10 cm, were shipped from Yamamoto Dendrobiums in Mountain View, Hawaii. Plants arrived at Texas A&M University, College Station on 15 Feb. 2006 and were potted into 10.2 cm (top diameter, 414 mL vol.) standard green plastic pots on 16 to 18 Feb. and placed in a glass wall and polycarbonate roof greenhouse. The root substrate consisted of two parts of coarse peat (Sunshine Peat; Sun Gro Horticulture, Bellevue, Wash.), one part coarse perlite, and one part no. 3 grade diatomite (Diatomite USA, Elma, N.Y.), amended with powdered Micromax (Scotts,

Marysville, Ohio) at 1 g•L^{-1} as a source of micronutrients and powdered dolomitic limestone at 5 g•L^{-1}. A wetting agent, Aqua Gro 2000 G (Scotts, Marysville, Ohio), was added to the medium at 0.5 g•L^{-1}.

Plants were potted with the root substrate packed tightly to secure them in place when lifted up only by the upper portion of the pseudobulbs. Immediately after potting, plants were irrigated with reverse osmosis (RO) water containing a fungicide (Banrot 40% WP; Scotts, Marysville, Ohio) at 0.6 g•L^{-1} to prevent root rot. Plants were spaced pot-to-pot in 30.8 x 51.4 cm molded carrying trays (4.00 Transport Tray (15); Landmark Plastic Corporation, Akron, Ohio) on the greenhouse bench with the plant leaves orienting east and west to best capture sunlight. The newly grown pseudobulbs were staked upright with bamboo sticks (Bamboo Supply, Lakeland, Fla.) to minimize mutual shading. Whenever an undesirable second new pseudobulb emerged, it was removed to keep only one new pseudobulb per pot.

Greenhouse light level and air temperature at plant canopy level were recorded hourly with data loggers [HOBO (Onset Computer Co., Bourne, Mass.), WatchDog (Spectrum Technologies, Plainfield, Ill.), and Apogee line quantum sensors (Apogee Instruments, Logan, Utah)] (Appendix 1). Plants were grown in a warm environment (average 25.3 °C day/23.0 °C night) under an average 4.9 mol•m^{-2}•d^{-1} daily light integral (*DLI*) until the pseudobulbs matured. Pots were irrigated as needed with RO water containing a 15N-2.2P-12.5K (Peters Excel 15-5-15 Cal-Mag; Scotts, Marysville, Ohio) water-soluble fertilizer at 0.67

g•L^{-1} (100 mg•L^{-1} N). Plain RO water was used after nutrient application was terminated. Pesticides [*Bacillus thuringiensis israelensis* (Gnatrol), Valent BioSciences Coroperation, Libertyville, Ill.; Azadirachtin (Azatin) and Cyfluthrin (Decathlon), Olympic Horticulture Products, Mainland, Pa.; Imidacloprid (Marathon II), Olympic Horticulture Products, Mainland, Pa.] were applied when necessary to control fungus gnats, caterpillars, and mealy bugs, respectively.

Plants were subjected to a 6-week cooling treatment at 15 °C in a growth chamber starting on 13 Nov. 2006. The growth chamber was maintained at 65% RH and a 12-h photoperiod with 350 μmol•m^{-2}•s^{-1} photosynthetic photon flux (*PPF*) provided by both fluorescent and incandescent lamps. The average air temperature recorded was 14.5 °C with a fluctuation between 14.2 to 14.9 °C. Frequency of watering or fertilization was reduced from the beginning of cooling until full flowering, allowing the medium to dry somewhat between waterings. Plants were moved back to the warm greenhouse set at 25 °C day/20 °C night after the completion of cooling for flower development. The average air temperatures and *DLI* from the end of cooling until full flowering were 24.3 °C day/21.9 °C night and 6.1 mol•m^{-2}•d^{-1}, respectively.

Experimental design. The experiment was a 3 x 5 factorial with three nutrient termination times and five nutrient reapplication stages. A randomized complete block design with twelve replications was used. A single plant in a pot with one new pseudobulb constituted an experimental unit. A total of one hundred and eighty vegetatively propagated nobile dendrobium plants were

used. The nutrient termination times were 1 Aug., 1 Sept., and 1 Oct. 2006. The five nutrient reapplication stages were 1) at the beginning of cooling, 2) in the middle of cooling, 3) immediately following cooling, 4) 2 weeks after the completion of cooling, and 5) no nutrient reapplication. Twelve plants served as an observational control group (i.e., not included in statistical analysis) and continued receiving nutrients at 0.67 $g \cdot L^{-1}$ (100 $mg \cdot L^{-1}$ N) throughout the experimental period and remained in the greenhouse without cooling.

Data collection. Weekly photographs were taken to compare and record the visual differences among treatments. Data including plant height (measured from the medium surface to the tip of the pseudobulb), number of nodes, number of remaining leaves on the pseudobulb before anthesis, pseudobulb width (the diameter of the widest point from side to side) and thickness (the diameter of the thickest point from front to back), time to anthesis (the first flower bud on a plant cracked to open), time to full flowering (all flowers on a plant fully opened), the number of aerial shoots on a pseudobulb, aborted buds and flowering nodes, total flower number, and flower diameter were collected. For the two variables, time to anthesis and full flowering, the numbers of days were recorded from plants being removed from the cooling treatments. Flower diameter was determined by averaging those of two flowers, one on each of the two middle nodes of a plant. Average flower number per flowering node was calculated by dividing total flower number by flowering node number.

Statistical analysis. All the data collected except for those from the observational control group were analyzed with analysis of variance (ANOVA) and Duncan's multiple range test for significant differences, all at $P \leq 0.05$. Percentage data were arcsine-transformed to normalize distribution of variance before subjected to statistical analysis. All analysis was performed by SAS 9.1.3 statistical software (SAS Institute, Cary, N.C.).

Histology. For each of the treatments and control group, two plants were randomly selected when lateral buds started to swell. Buds protruding 2 mm from the pseudobulb surface were sampled to study the histological changes and to determine the possible inter-conversion between vegetative and reproductive buds. All microscopic studies were performed at the Microscopy and Imaging Center, Texas A&M University, College Station.

One of the middle lateral buds on each selected plant was excised from the pseudobulb. Tissue was transferred into freshly prepared fixative after the outer bracts were removed as much as possible under water using a dissecting microscope without damaging the bud primordia. Every 10 mL of the fixative was made by mixing 2.5 mL formaldehyde solution (heating 0.8 g paraformaldehyde, in 10 mL of distilled water and a pellet of NaOH in a flask until the solution cleared and allowed to cool), 5 mL 0.2 M phosphate buffer (mixing 72 mL of 0.2 M Na_2HPO_4 and 28 mL of 0.2 M NaH_2PO_4, pH 7.2), 0.5 mL 50% (w/v) glutaraldehyde, 0.15 mL dimethyl sulfoxide (DMSO), and 1.85 mL

distilled water. The tissue in the fixative was vacuumed for 1 min to remove air from the tissue and expedite the penetration of the fixative.

After sitting at room temperature for 30 min, the tissue was cold-microwaved for 6 min with a 30-s on-and-off vacuum cycle at 178 W power and 20 °C by using the PELCO BioWave Pro Laboratory Tissue Processing System (Ted Pella, Redding, Calif.) that equipped with the ColdSpotT temperature control system. Then, the fixative was replaced by a phosphate buffer and microwaved with a setting of 30-s on-and-off vacuum cycle for 1 min at 250 W power and 20 °C. The setting was used thereafter when microwaving was needed. The phosphate buffer was replaced again and the microwave treatment was repeated for a total of four buffer washes. The buffer was decanted and 1% (w/v) osmium tetroxide aqueous solution was added for post-fixation. Then, a 1-min microwaving was applied to the specimens before the eppendorf vials were sealed with parafilm and placed at 4 °C overnight.

The following day, the osmium tetroxide solution was decanted and water was added for a rinse. The methanol/water dehydration series at 5% methanol v/v increments (from 5% to 95%) was used by substitution with the next graded methanol/water solution followed by a 1-min microwaving treatment. After the 95% methanol step, three consecutive steps with 100% methanol were performed. The tissue was then transferred to glass vials, immersed in propylene oxide under a chemical hood, and placed on a rotator while preparing resin mixture for infiltration.

Every 10 g (excluding the weight of DER 736 resin and BDMA) resin mixture was made by fully mixing 2.18 g ERL 4221 resin, 1.39 g Quetol 651 resin, 1.43g DER 736 resin, 6.43 g nonenyl succinic anhydride (NSA), and 0.2 mL benzyldimethylamine (BDMA) in order. A volume of the resin mixture was added to the sample vials to have 50% (v/v) resin concentration and placed on a rotator for 2 h. Another volume of resin was then added to give 75% resin concentration and left rotating overnight. The next day, the tissue was vacuumed for 1 min. Propylene oxide/resin mixture was replaced with newly mixed resin, and sample vials were rotated overnight. Vacuum and replacement of newly mixed resin for rotating overnight were repeated four times. Then, a last batch of resin was mixed for embedding. Specimens were transferred into molds in the desired orientation, and the molds were filled up with resin mixture. Molds were placed in an incubator at 55 °C overnight for resin polymerization. After the molds were removed from the incubator to cool the next day, the polymerized resin blocks were ready for sectioning.

Longitudinal sections of bud primordia were cut at approximately 0.5 μm thickness by using the Reichert Ultracut microtome (Leica Microsystems, Wetzlar, Germany) with glass knives, mounted on glass slides, stained with aqueous toluidine-blue-borax solution (both 1%, w/v) for the visualization of histology, and examined under the Zeiss Axiophot (Carl Zeiss, Oberkochen, Germany) light microscope with brightfield illumination to determine if reproductive or vegetative primordia had developed. Images were acquired by a

monochrome CCD camera (DXM 1200; Nikon, Melville, N.Y.) and processed with both MetaVue 7 imaging system and ImageJ 1.37 image processing and analysis program. Images were contrast enhanced and sharpened when needed.

Results

Interaction between nutrient termination time and reapplication stage on growth and flowering of *Dendrobium* Sea Mary 'Snow King' was non-significant (Tables 1, 2). Timing of nutrient termination had more impact on vegetative growth than reapplication stage. Plants were taller with more nodes when nutrient application was terminated on 1 Aug. or 1 Oct. than 1 Sept. (Fig. 2). Late nutrient termination on 1 Oct. resulted in more leaves remaining on pseudobulbs than those being terminated of nutrients on 1 Aug. or 1 Sept. The nutrient termination time on leaf number showed even more prominently when viewed as percentage of leaves remaining on the nodes of a pseudobulb (Table 3). Based on the fact that each node accompanies a leaf on a pseudobulb, this difference indicated plants that stopped receiving nutrients at an earlier stage tended to defoliate more severely. Plants that were terminated of nutrients on 1 Oct. had higher leaf retention (82%) on the pseudobulbs than those terminated earlier (74%). On the other hand, reapplication stage appeared to affect the number of leaves remaining following cooling (Fig. 3, Table 1). Plants had more leaves when nutrient application resumed at the beginning of cooling or

Table 1. ANOVA for the effect of nutrient termination time and reapplication stage on vegetative data of *Dendrobium* Sea Mary 'Snow King'.

	Plant height	Node number	Leaves remaining		Pseudobulb	
Treatment	(cm)	(no.)	(no.)	(%)	Width (cm)	Thickness (cm)
Termination time (T)	*	**	***	***	NS	NS
Reapplication stage (R)	NS	NS	*	***	NS	NS
T x R	NS	NS	NS	NS	NS	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

Table 2. ANOVA for the effect of nutrient termination time and reapplication stage on flowering data of *Dendrobium* Sea Mary 'Snow King'.

	Time to anthesis after cooling	Time to full flowering after	Time from anthesis to full flowering	Nodes with flowers		Nodes with aborted buds	Total flower number	Average flower no. per flowering	Flower diameter
Treatment	(d)	cooling (d)	(d)	(no.)	(%)	(no.)	(no.)	node (no.)	(cm)
Termination time (T)	NS	***	***	**	NS	*	***	***	NS
Reapplication stage (R)	NS	NS	NS	NS	NS	NS	NS	NS	*
T x R	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

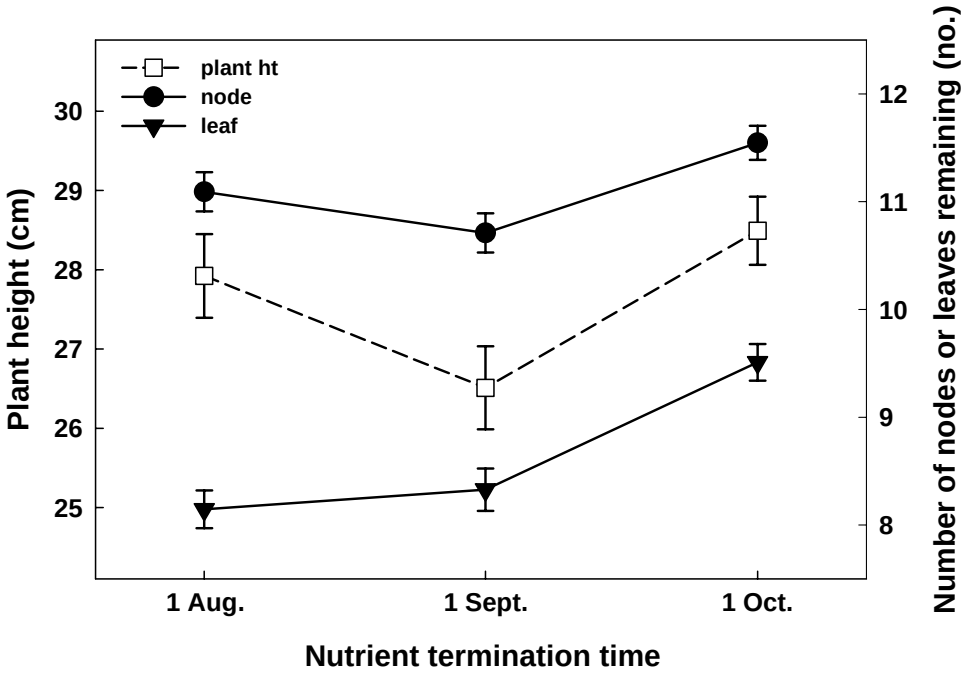

Fig. 2 Effect of nutrient termination time regardless of nutrient reapplication stage on plant height, node number, and leaves remaining in *Dendrobium* Sea Mary 'Snow King'. Bars indicate ± SE of the mean.

Table 3. Effect of nutrient termination time regardless of nutrient reapplication stage on plant height, node number, and leaves remaining of *Dendrobium* Sea Mary 'Snow King'.

Nutrient termination time	Plant height (cm)	Node number (no.)	Leaves remaining (no.)	Leaves remaining (%)
1 Aug.	27.9 ab[z]	11.1 ab	8.1 b	73.5 c
1 Sept.	26.5 b	10.7 b	8.3 b	77.5 b
1 Oct.	28.5 a	11.5 a	9.5 a	82.2 a

[z]Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

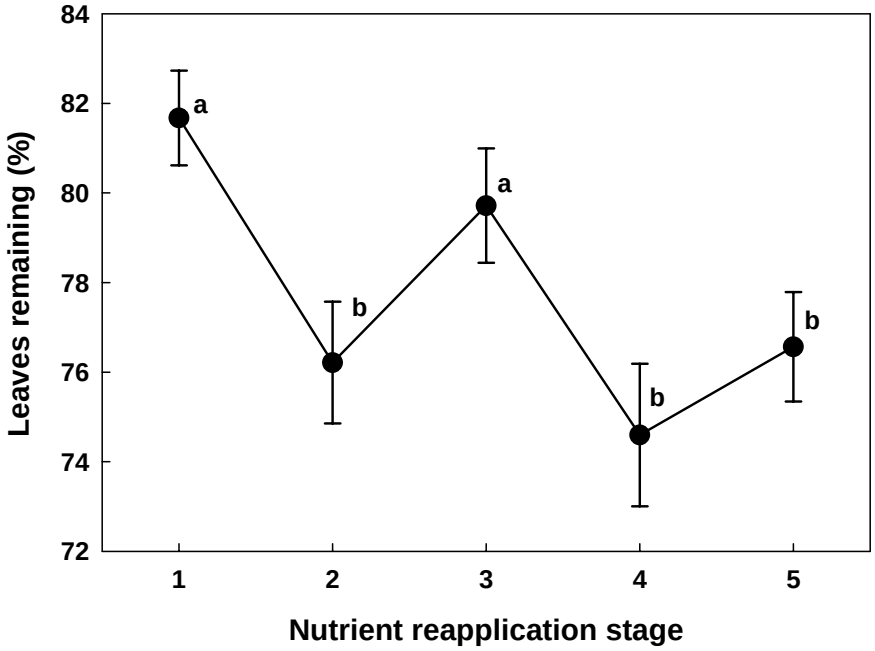

Fig. 3 Effect of nutrient reapplication stage regardless of nutrient termination time on percentage of leaves remaining in *Dendrobium* Sea Mary 'Snow King'. Five nutrient reapplication stages: 1 = at the beginning of cooling, 2 = in the middle of cooling, 3 = immediately following cooling, 4 = 2 weeks after cooling, and 5 = no reapplication. Lowercase letters represent mean separation by Duncan's multiple range test at $P \leq 0.05$. Bars indicate ± SE of the mean.

immediately following cooling than at other reapplication stages. The overall trend revealed that the earlier the nutrients were reapplied, the higher potential for plants to retain their leaves.

Flowering among nutrient termination and reapplication treatments were not different by visual observation. Nearly all plants started to flower simultaneously and they were in full flower within one week from 26 Jan. 2007 (Fig. 4). The visualized similarity of the flowering plants among treatments

suggests that the treatments in this experiment had no influence on flowering performance except for some minor effect on time to full flowering and total flower number. Prolonged nutrient supply during vegetative growth did not lead to differences in time required for anthesis after the completion of cooling treatment, whereas extended nutrient supply slightly extended the time that plants needed to reach full flowering after cooling (Fig. 5). Full flowering on plants, that were terminated nutrient application on 1 Oct., was delayed for about 1.5 d than those terminated of nutrients on 1 Aug. (from 37.3 to 38.7 d, Table 4), resulting in slightly less uniformed flowering. None of the treatments resulted in the formation of aerial plants on pseudobulbs.

Except for the slightly slower flower development from anthesis to full flowering, flower quality was improved by prolonged nutrient supply with more flowering nodes, more total flower number, and fewer aborted floral buds on a plant (Table 4). A positive relationship between average flower number per node and length of nutrient application before cooling treatment was also observed. Plants that received nutrients until 1 Sept. or 1 Oct. had more flowers on each flowering node than those terminated of nutrients early on 1 Aug.

Flower diameter was not affected by nutrient termination time, but was affected by nutrient reapplication stage. Plants without nutrient reapplication produced bigger flowers than plants receiving nutrients at the beginning or 2 weeks after cooling (Fig. 6). However, stage of reapplying nutrients had limited visual effect on flower diameter.

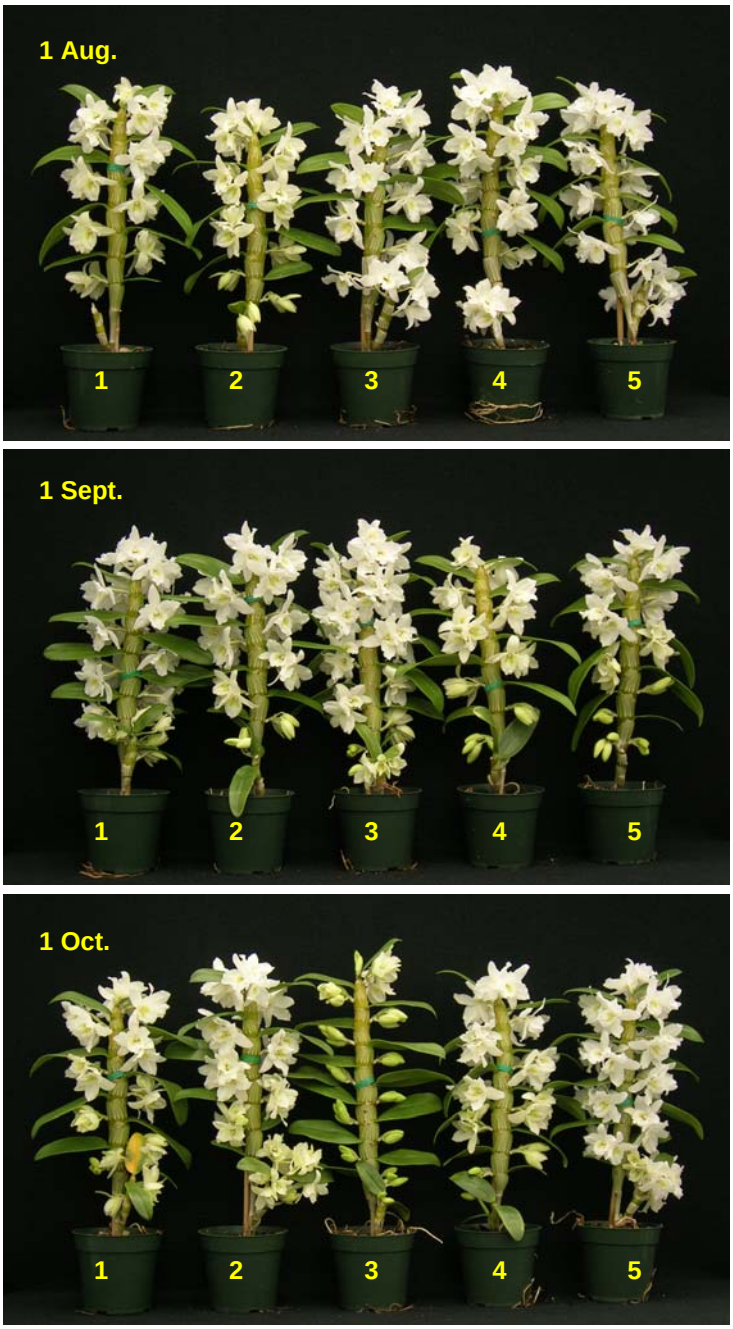

Fig. 4 Similar visual performance on *Dendrobium* Sea Mary 'Snow King' with various nutrient termination times and reapplication stages. Nutrient termination times are marked in upper left corner. Five nutrient reapplication stages: 1 = at the beginning of cooling; 2 = in the middle of cooling; 3 = immediately following cooling; 4 = 2 weeks after cooling; and, 5 = no reapplication. Photopraphs were taken on 30 Jan. 2007. The third plant in the third photograph was not well-representative for the experimental treatment.

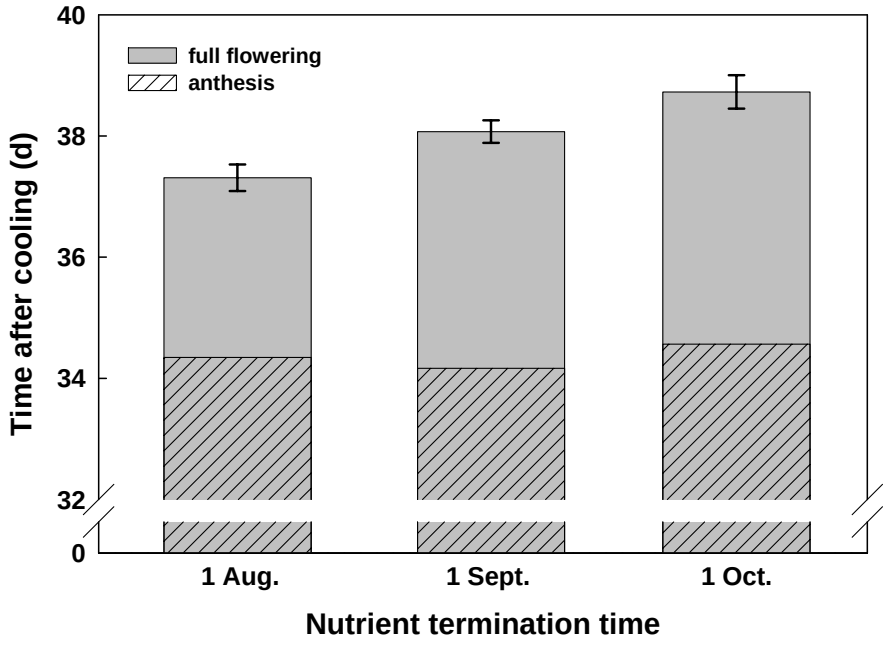

Fig. 5 Effect of nutrient termination time regardless of nutrient reapplication stage on time required to reach anthesis and full flowering after cooling treatment in *Dendrobium* Sea Mary 'Snow King'. Bars indicate ± SE of the mean.

Table 4. Effect of nutrient termination time regardless of nutrient reapplication stage on flowering data of *Dendrobium* Sea Mary 'Snow King'.

Nutrient termination time	Time to full flowering after cooling (d)	Time from anthesis to full flowering (d)	Nodes with flowers (no.)	Nodes with aborted buds (no.)	Total flower number (no.)	Average flower no. per flowering node (no.)
1 Aug.	37.3 c[z]	3.0 b	8.5 b	0.47 a	24.6 b	2.9 b
1 Sept.	38.1 b	3.9 a	8.3 b	0.33 ab	26.5 b	3.2 a
1 Oct.	38.7 a	4.2 a	9.2 a	0.13 b	30.3 a	3.3 a

[z]Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

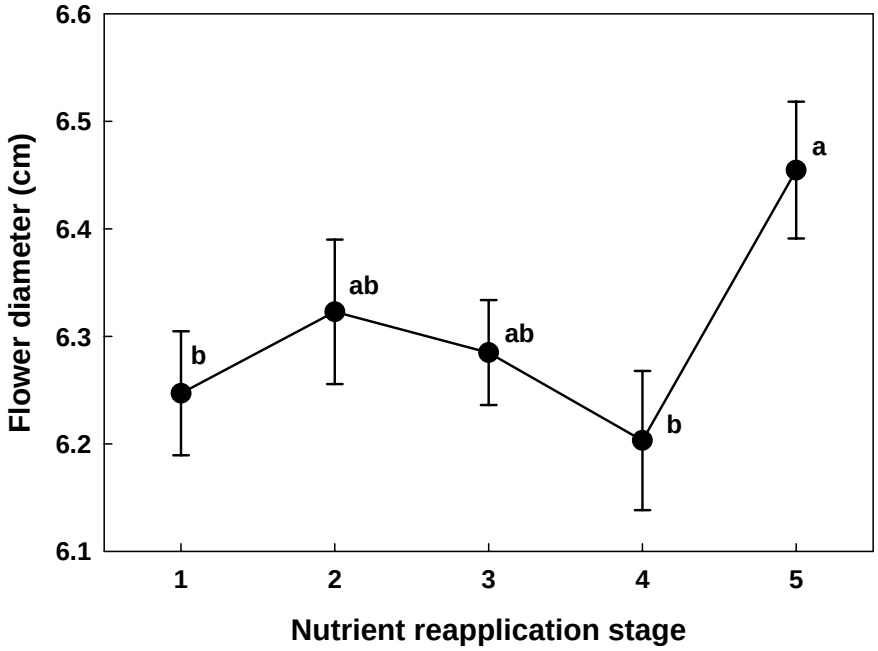

Fig. 6 Effect of nutrient reapplication stage regardless of nutrient termination time on flower size of *Dendrobium* Sea Mary 'Snow King'. Five nutrient reapplication stages: 1 = at the beginning of cooling, 2 = in the middle of cooling, 3 = immediately following cooling, 4 = 2 weeks after cooling, and 5 = no reapplication. Lowercase letters represent mean separation by Duncan's multiple range test at $P \leq 0.05$. Bars indicate ± SE of the mean.

Histology. Whether a plant had been exposed to cooling or not, flower differentiation was not observed in the flat axillary buds. In thin longitudinal sections, each of these flat buds only showed a single dome-shape apical meristem in the center with several layers of leaf primordia embracing it (Fig. 7). Cells in the apical meristem had dense cytoplasmic contents and chromosomes

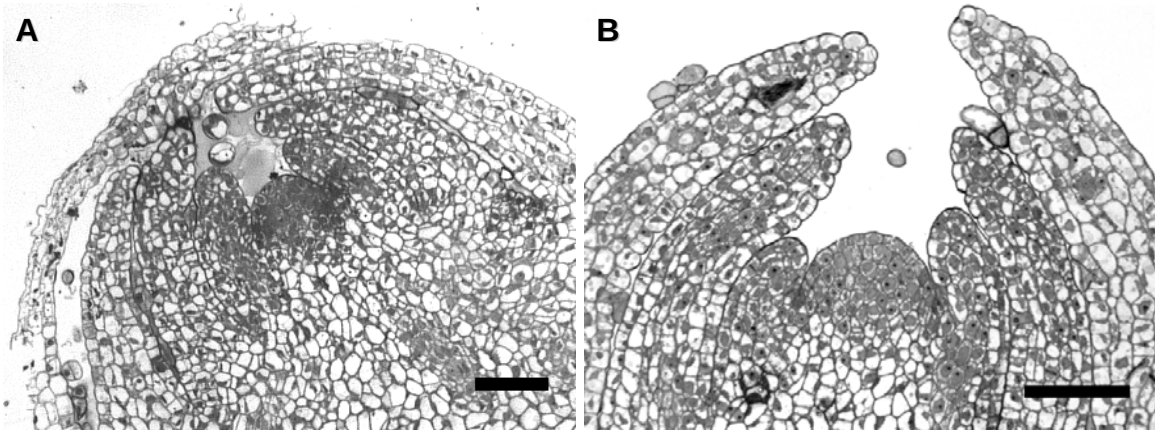

Fig. 7 Vegetative apices of *Dendrobium* Sea Mary 'Snow King' in longitudinal thin sections. One single dome-shape apical meristem in the center with several layers of leaf primordia embracing it can be seen. Bars = 100 μm. (A) Bud was sampled before protruding from pseudobulb surface. (B) Bud was sampled when less than 1 mm protruding from pseudobulb surface.

were condensed. The histological structure corresponded to a vegetative shoot primordium or, perhaps, a reproductive primordium with flower formation already being induced but primordia not yet differentiated. Therefore, to investigate whether flower primordia had been differentiated, axillary buds were sampled only after they had protruded 2 mm from the pseudobulb surface. These buds were then made into microscopic slides.

In the middle of cooling (after 3 weeks) at 15 °C, lateral buds on plants in all treatments had protruded slightly from the pseudobulb surface. This indicated that buds may have fulfilled their cooling requirement for flower initiation, so they started to develop and protrude from the pseudobulbs before being removed from the cooling treatment. Regardless of nutrient termination

Table 5. Histological results for axillary buds of *Dendrobium* Sea Mary 'Snow King' with various nutrient termination times and reapplication stages[z].

Nutrient reapplication stage (relative to cooling)	Date reapplied	Nutrient termination time		
		1 Aug.	1 Sept.	1 Oct.
1 (beginning)	13 Nov. 2006	R[x]	R	R
2 (middle)	4 Dec. 2006	R	R	R
3 (immediately after)	25 Dec. 2006	R	R	R
4 (2 weeks after)[y]	8 Jan. 2007			
5 (no reapplication)[y]				

[z]Buds were sampled on 14 Dec. 2006.

[y]Buds were unnecessary to be sampled because plants had been in identical growing conditions to those resumed nutrient in stage 3.

[x]'R' denotes the lateral buds of plants in the treatment were examined and recognized as reproductive primordia.

time and reapplication stage treatments, all buds were sampled when having reached 2 mm on 14 Dec. 2006 for histological examination. Since only the first three nutrient reapplication stages (at the beginning, in the middle, and immediately after cooling) had begun, lateral buds from plants in only nine treatments (Table 5) and the observational control group were sampled. Lateral buds on plants that resumed nutrients 2 weeks after the completion of cooling were not sampled.

All the buds, except for those from the observational control group (Fig. 8A), had entered the reproductive stage, with flower primordia differentiated. They contained multiple, less-than-perfect dome-shape floral meristems that were each embraced by bract primodium (Fig. 8B-D, Fig. 9, and Fig. 10). Actively

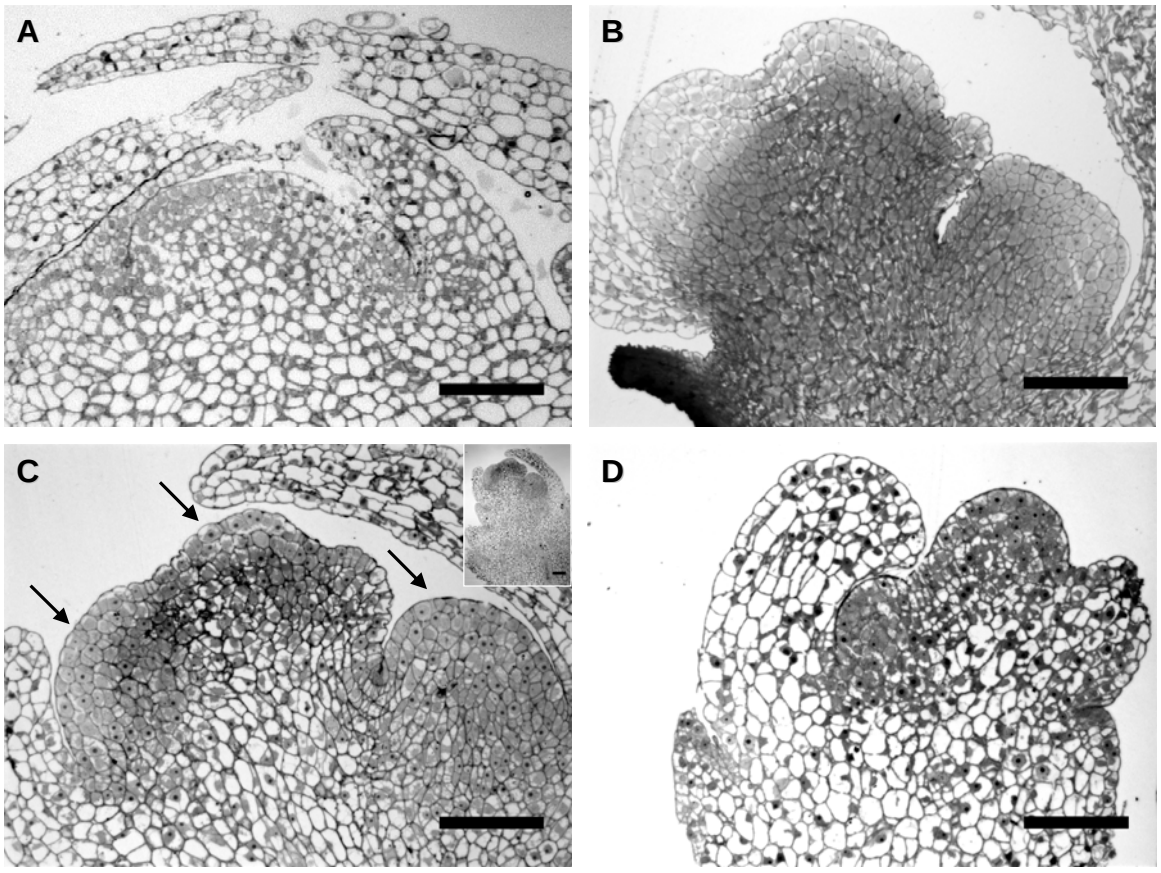

Fig. 8 Comparison of vegetative apex with differentiated reproductive primordia from *Dendrobium* Sea Mary 'Snow King' plants with various nutrient termination times and stage 1 nutrient reapplication. (A) Vegetative apex from a plant in the observational control group, without cooling. Nutrient termination times were 1 Aug. (B), 1 Sept. (C), and 1 Oct. (D). Buds (B), (C), and (D) were all from plants with stage 1 nutrient reapplication: resuming nutrient application at the beginning of cooling. Arrows indicate active meristematic tissue to form individual flowers. A broad view of the bud (C) is inlayed in its upper right corner. Bars = 100 μm.

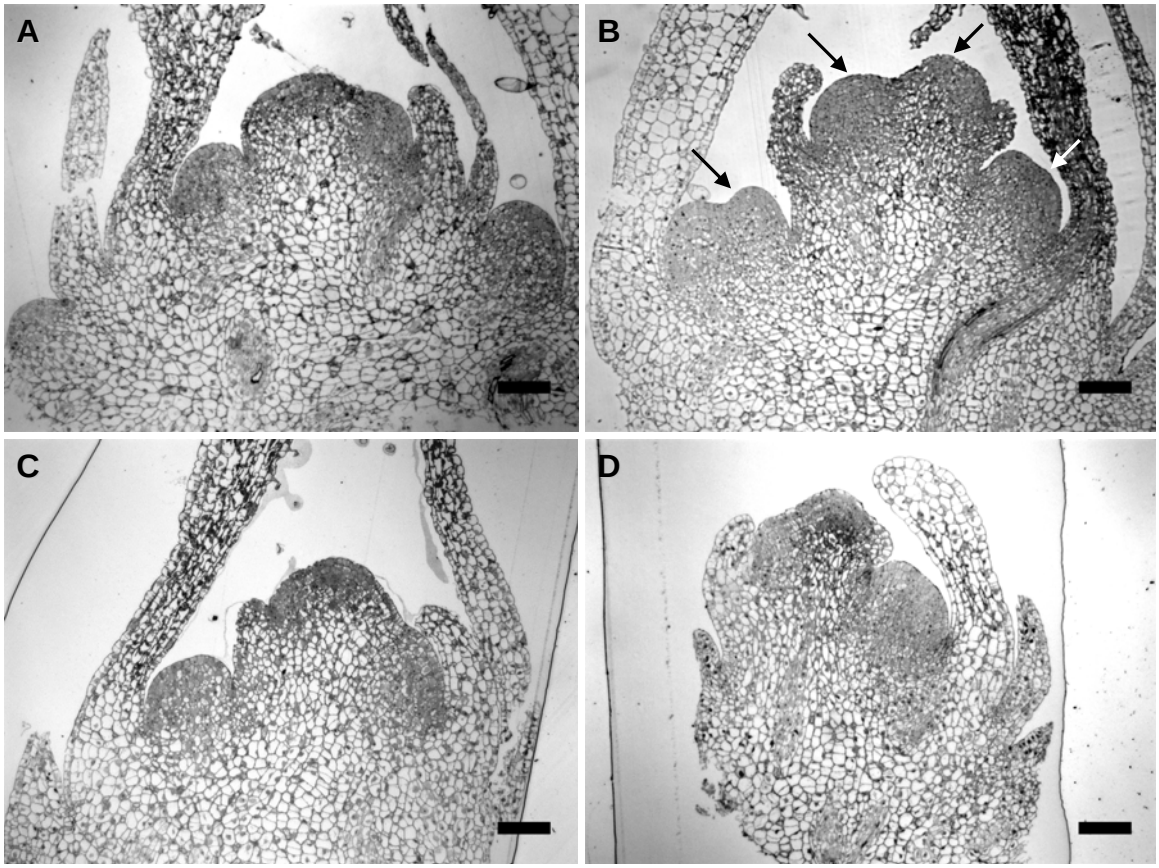

Fig. 9 Longitudinal sections of differentiated reproductive primordia from *Dendrobium* Sea Mary 'Snow King' plants with various nutrient termination times and stage 2 nutrient reapplication. Nutrient termination times were 1 Aug. (A) and (B), 1 Sept. (C), and 1 Oct. (D). All buds were from plants with stage 2 nutrient reapplication: resuming nutrient application after 3 weeks of cooling. Arrows indicate active meristematic tissue to form individual flowers. Bars = 100 μm.

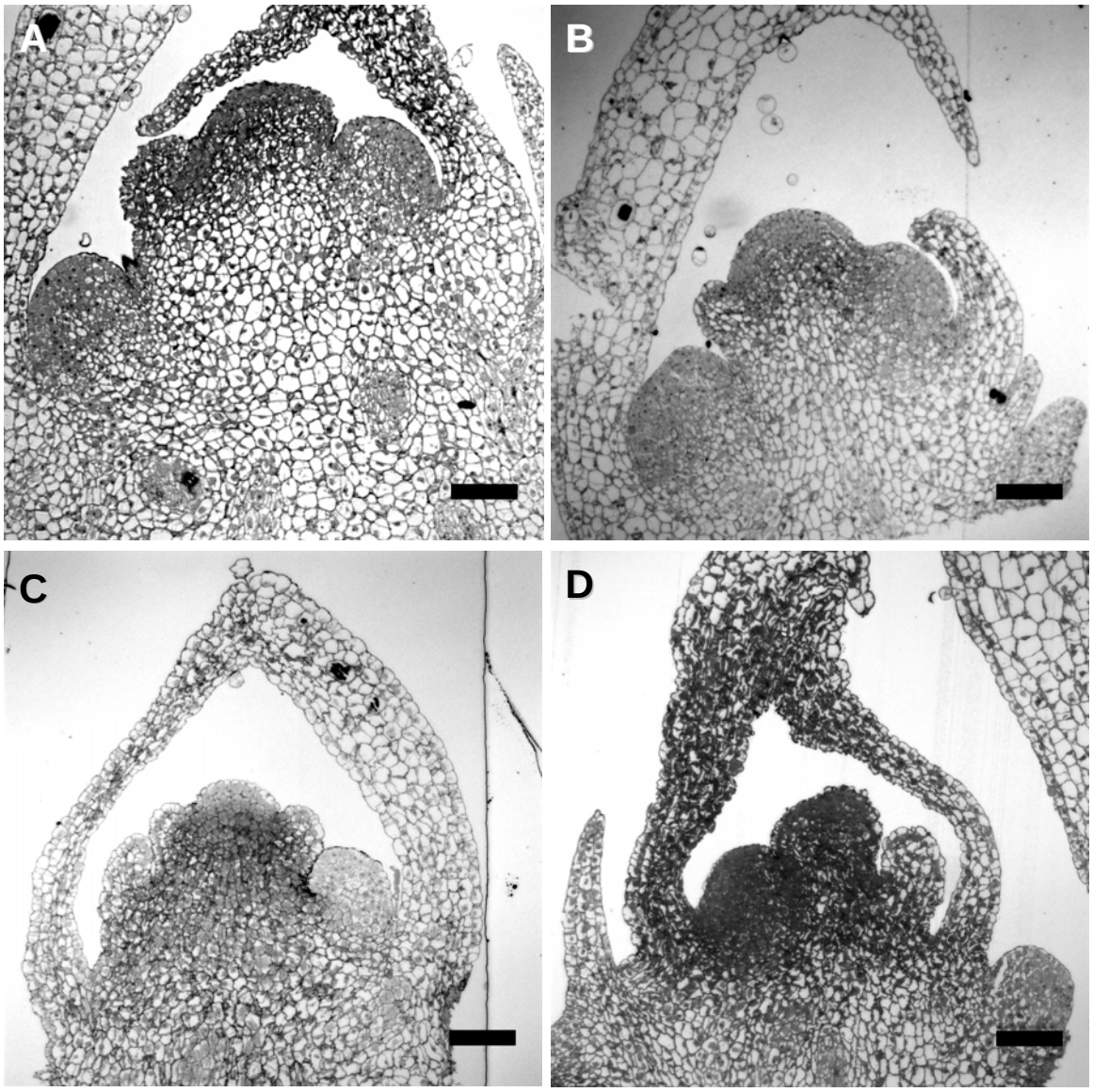

Fig. 10 Longitudinal sections of differentiated reproductive primordia from *Dendrobium* Sea Mary 'Snow King' plants with various nutrient termination times and stage 3 nutrient reapplication. Nutrient termination dates were 1 Aug. (A), 1 Sept. (B), and 1 Oct. (C) and (D). All buds were from plants with stage 3 nutrient reapplication: resuming nutrient application immediately after the completion of cooling for 6 weeks. Bars = 100 μm.

Fig. 11 Conjunction of the flowers to a node on a pseudobulb of *Dendrobium* Sea Mary 'Snow King'. The structure showed similar arrangement of the meristematic tissue in reproductive primordia. Note that a bracteole formed at the base of each pedicel.

differentiating tissues in these apices could be easily identified. Cells in these tissues had thin, smooth cell walls and were full of cytoplasm instead of vacuoles. Chromosomes in the nuclei were condensed for vigorous cell division. The reproductive buds' large histological differences in structure and comparatively larger size than the vegetative apex (Fig. 8A) assured that they were differentiating flower primordia. For all of the reproductive buds, three to four meristematic zones co-existed and were separated by cells that were less in cytoplasmic contents. The number of vivid cell clusters in each lateral bud was consistent with the average flower number per flowering node that was observed later in the greenhouse following anthesis (Fig. 11). Thus, it was further verified

that the meristematic zones represented individual flowers on a node, whereas the tissue between the zones would develop into the bracteoles born at the base of each flower pedicel. Distinguishable flower promordia in Fig. 9B can be compared with the flowers in similar positions in Fig. 11.

Few histological differences were noticed among nutrient termination or reapplication stage treatments. Fig. 8, Fig. 9, and Fig. 10 show results for the first three nutrient reapplication stages sampled, respectively. The only distinction among them appeared to be in the nutrient termination time. Plants in the first nutrient termination time, i.e., 1 Aug., had larger flower primordia than those that were terminated of nutrients on 1 Oct. Differentiating primordia from early nutrient termination treatment were about 550-800 μm wide in the longitudinal sections (Fig. 8B, Fig. 9A-B, and Fig. 10A), whereas those from second termination time were about 500-600 μm wide (Fig. 8C, Fig. 9C, and Fig. 10B), and 300-550 μm wide from the 1 Oct. termination (Fig. 8D, Fig. 9D, and Fig. 10C-D). Although the differences were not drastic, they still imply that flower differentiation commenced slightly earlier or faster when nutrient supply was terminated at an earlier time.

To sum up the results in this experiment, all the buds examined except for the observational control group had entered the stage of differentiating reproductive primordia because of the inductive cooling treatment, and all the plants except for some plants in the observational control group flowered profusely without producing any aerial shoots (Fig. 12). That is to say no

reversion of reproductive to vegetative buds occurred due to either late nutrient termination or resumption of nutrient application at the stages of cooling examined. However, timing of nutrient termination had greater effects than reapplication stage on growth and flowering to produce high quality plants.

Discussion

Nutrient availability. Ample supply of nutrients during vigorous vegetative growth is important not only to producing robust shoots but also to subsequent flowering in orchids. The emerging vegetative shoots that grow into matured

Fig. 12 Aerial shoot formation on a *Dendrobium* Sea Mary 'Snow King' plant from the observational control group continuously receiving nutrients without cooling. Picture was taken in Feb. 2007.

pseudobulbs and the newly differentiated inflorescences, both strong sinks, demand large amounts of mineral nutrients and photoassimilates for their development. Mineral nutrients from the growing media and fertilization and, often, those stored in other parts of the plant body are the main sources to support the rapid growth of these strong sinks. Research on a nobile dendrobium, *D.* Red Emperor 'Prince' (Bichsel, 2006), *D. moschatum* Wall. (Bhattacharjee, 1981), and a white-flowered *Phalaenopsis* hybrid (*P. amabilis* Blume x *P.* Mount Kaala 'Elegance') (Wang and Gregg, 1994) showed that adequate rates of nutrient supply promoted vegetative growth and thereby enhanced the subsequent flowering performance, including taller plants with more nodes or leaves, increased flower counts or size, or a faster growth rate of inflorescences. Although in the current study, nutrient supply varied by application durations rather than nutrient rates, providing optimal nutrients resulted in improved growth and flowering.

A proper timing and duration of nutrient supply can help to produce desirable flowering pot orchids. To improve bud initiation and the number of developed buds in monopodial and sympodial orchids, promoting vigorous vegetative growth by proper nutrient application is highly desirable, including proper timing and nutrient application of high rates of N, P, or K (Hew and Yong, 2004). Ng and Hew (2000) also recommended paying special attention to the fertilization regimen (i.e., nutrient rate, application time, and duration), especially during the period of new shoot development.

Nutrient termination. In this present experiment, the optimum nutrient supply was evaluated by termination times and reapplication stages. Either a later nutrient termination time or an earlier reapplication stage provided higher availability of nutrients to the plants, hence, improved growth and flowering were expected. However, vegetative growth and flowering were more affected by nutrient termination time than reapplication stage. Even when nutrients were reapplied during the period of flower initiation and differentiation, slightly delayed flowering, increased flowering nodes, and increased flower count were still caused by nutrient termination time rather than reapplication stage. The data suggest that flower development benefits more from the nutrients accumulated in mature pseudobulbs before nutrient supply is terminated, rather than from those being taken directly from fertilization while approaching flowering. It is not known if nutrients that were reapplied during cooling and the flowering process would have contributed markedly to an enhanced flowering performance to plants that were not fertilized properly during vegetative growth.

Since flowering benefited mostly from the nutrients accumulated in the pseudobulbs before termination, remobilization of nutrients from the current mature pseudobulbs to the inflorescences may be required. Remobilization of nutrients and photoassimilates from pseudobulbs for inflorescences development has been reported for *Oncidium* Goldiana (Hew and Ng, 1996; Yong and Hew, 1995). By evaluating changes of dry matter and total mineral contents in various plant parts, it was found that part of the minerals and

carbohydrates accumulated in mature pseudobulbs were later allocated to developing inflorescences and axillary buds. The active accumulation of mineral nutrients and carbohydrates during pseudobulb development constituted an important source of reserves for the subsequent development of inflorescences and new shoots (Hew and Ng, 1996). As a result, to support a high flowering capacity with optimum quality in nobile dendrobiums, nutrient supply should not be terminated until the current year's pseudobulbs have swollen and matured to establish robust plants (i.e., long pseudobulbs with a high node number and high leaf retention), so the accumulated reserves can be later utilized for inflorescence development.

Properly extended nutrient supply before being terminated has shown to benefit orchid production. Wang (2000) found that discontinuing fertilization prior to late November caused reduced flower count and loss of lower leaves in *Phalaenopsis* TAM Butterfly. For a one-year-old nobile-type dendrobium (cultivar unspecified in the English abstract) started in spring 1978, prolonged nutrient supply until one to seven month(s) before cooling treatment started on 1 Sept. 1979 resulted in better second year's growth and flowering than those being terminated at earlier times (Sakai et al., 1982). For *D.* Red Emperor 'Prince', another nobile-type dendrobium, terminating nutrients on 1 Oct. rather than earlier or later was recommended because plants produced more flowers without adverse effects on flowering, such as reduced flower size and delayed anthesis that were caused by 1 Nov. nutrient termination (Bichsel, 2006). In the

current study, termination of nutrients on 1 Oct. caused the highest leaf number and flower count and the fewest aborted floral buds among treatments. The result suggests that termination of nutrients at a later time in the fall for plants to accumulate enough reserves in the matured pseudobulbs is beneficial to growth and subsequent flowering of nobile dendrobiums. The suggestions by Wood (2006) and Yamamoto Dendrobiums (2007) to terminate nutrient application as early as August without giving specific information on developmental stage of the plants or cultivation latitude are thus questionable.

Although there are many benefits derived from the prolonged nutrient supply, it may delay flowering slightly. Termination of nutrient supply on 1 Oct. delayed time to reach full flowering for 1.5 d compared to being terminated on 1 Aug. However, the slight delay would not be a main concern in commercial production. Flowering pot plants are purchased by consumers before flowers open, so consumers may enjoy the process of flowering. The delay may even be beneficial to growers and consumers because plants have a better postharvest quality if shipped with flowers in bud than with fully opened flowers to avoid damage. Consumers would also have additional time to enjoy the flowers opening. Therefore, as long as plants reach anthesis simultaneously to produce a uniform crop to meet a specific market time, growers still can disregard the slight delay in full flowering and terminate the nutrient supply at a later time to ensure a better plant quality.

Nutrient reapplication. Although nutrient reapplication appeared to have limited effect on growth and flowering in this experiment, nutrient reapplication may be necessary in growing nobile dendrobiums under certain circumstances. For example, if other strong sinks such as new vegetative buds coexist and compete with flower development, then, nutrient reapplication at the time of new growth emergence may be beneficial to both the current and the next season's flowering. While the nutrient reserves in the matured pseudobulbs can be devoted to flower development, the reapplied mineral nutrients would largely support the active vegetative growth. Sakai et al. (1982) reported that to maximize flower count for the coming flowering season (late January) in a nobile-type dendrobium (cultivar unspecified in the English abstract), the most effective time to apply nutrients was from October to January right before flowering, which corresponded with the time of new vegetative growth for the next growing season. Therefore, reapplication of nutrients may be regarded as the main source of nutrient supply for the following year's vegetative growth when emerging shoots exist.

In contrast, nutrient reapplication should be omitted when no vigorous vegetative growth is to take place. Growers often remove newly emerged basal vegetative shoots before flowering in order to obtain two-year-old flowering pot plants with optimum flowering quality. In the current study, all basal axillary shoots on the pseudobulbs being studied were removed to exclude interferences by the new vegetative growth. Therefore, the effect of nutrient reapplication on a

subsequent third year's growth was not investigated but still cannot be neglected. The effect of nutrient reapplication to the overall flowering of two-year-old pot plants was relatively not significant. Consequently, without the strong direct uptake of nutrients by new vegetative shoots, reapplying nutrients seems to be unnecessary. Cost of production can thereby be reduced by not reapplying nutrients when growing two-year-old flowering pot plants.

Leaf retention. Optimal nutrient supply during production serves to prevent the undesirable leaf abscission. When nutrient supply is restrained, leaves are the organs prone to be affected by nutrient deficiency. *D. phalaenopsis* Fitzg. was severely affected by the omission of N, P, K, Ca, or Mg in the nutrient solution and leaf abscission occurred before deficiency symptoms appeared (Hew and Yong, 2004). Bichsel (2006) found that without N supply, leaf loss in *D*. Red Emperor 'Prince' was greater, having only 38% leaf retention, compared to those supplied with N. For *Phalaenopsis* TAM Butterfly that spiked in late September and reached anthesis in mid-January, plants with the earlier nutrient termination times (1 Sept., 29 Sept., and 27 Oct., as compared with the later 24 Nov. or continuous fertilization) had fewer leaves, mainly as a result of abscission of lower leaves (Wang, 2000). In the current study, plants supplied with nutrients until 1 Oct. had significantly higher leaf retention (82%) than those with the earlier nutrient termination times. Therefore, sufficient nutrient duration to support vegetative growth and flowering seems crucial for leaf retention in orchids.

Bud reversion. It was suspected that factors favoring vegetative growth of the nobile dendrobiums might trigger the reversion of floral primordia to vegetative shoots. In two nobile-type dendrobium cultivars, *D*. Snowflake 'Red Star' and *D*. Hinode 'Toutenkou', although the actual reversion from an initiated floral bud to a vegetative shoot was not observed, following 16 h of cooling, exposure to a high day temperature of 30 °C during the rest of the cooling period for only 2 h daily for 40 d decreased the number of flowers and promoted aerial shoot formation (Sinoda et al., 1988). Therefore, it is possible that a high temperature treatment during cooling period might be a stimulus to trigger the reversion of buds or to prevent floral initiation in nobile dendrobiums. Reversion of reproductive to vegetative growth by lack of cytokinin stimulation in *D.* Louisae 'Dark', a phalaenopsis-type dendrobium (Goh, 1979), by decapitation (Goh, 1975, 1976, 1977a,b) or high N application (Hew and Yong, 2004) in *Aranda,* and by either high temperature (Tran Thanh Van, 1974) or long days (Rotor Jr., 1952, 1959) in *Phalaenopsis* have been reported.

Reversion of buds was not observed in *D.* Sea Mary 'Snow King'. In nobile dendrobiums, it has been speculated that excessive nutrient supply, which is in favor of vigorous vegetative growth, might cause aerial shoot formation and perhaps, might trigger floral primordia to revert to vegetative primordia. However, in the current and a previous study in *D.* Red Emperor 'Prince' (Bichsel et al., 2008), no aerial shoots formed despite nutrient termination time as late as 1 Oct. or 1 Nov. or nutrient reapplication that spanned an 8-week time

period from stage 1 to 4. Plants had visible protruding lateral floral buds before removal from the growth chambers in Dec. 2006 and started to flower in late Jan. 2007. The prolonged nutrient supply examined in the experiment appeared not to trigger aerial shoot formation.

On the contrary, plants in the observational control group that continued receiving nutrients without cooling produced aerial shoots. In addition, these plants still had many quiescent flat nodes on the pseudobulbs by late Apr. 2007, which was already three months after plants in all other treatments were in full flower. It implied that plants in the observational control group had not been grown under optimum conditions for flowering.

Comparing the aerial shoot formation in the observational control group with no aerial shoot formation in all of the other treatments, both/either the 6 weeks of cooling and/or the gap without nutrient supply between the last termination (1 Oct.) and the earliest reapplication (13 Nov.) might have prevented the formation of aerial shoots. Whether one or both of these factors that caused aerial shoot formation could not be determined through this experiment. Bichsel et al. (2008) showed that applying nutrients until flowering resulted in no aerial shoot formation. Therefore, it is possible that the formation of aerial shoots was enhanced by extended nutrient supply when cooling requirement for flowering was not fulfilled. In the current cooling experiment (Expt. 2), cooling plants for 2 weeks at 18 °C was already able to induce less-than-optimal flowering. Six weeks at 15 °C may be more than the degree of

cooling that is needed for optimum flower induction in *D*. Sea Mary ‘Snow King’. Consequently, all plants in this experiment, except for the observational control group, did not produce aerial shoots. Aerial shoot formation might only be caused by excessive nutrient supply when cooling is insufficient or suboptimal to initiate flowering.

In conclusion, to produce healthy two-year-old quality flowering pot nobile dendrobiums, plants should be grown with ample nutrient supply during vigorous vegetative growth until pseudobulb maturation. Prolonged nutrient supply until 1 Oct. helps to establish robust plants with high leaf retention and to accumulate enough reserves in the mature pseudobulbs for subsequent flower development. Prolonged nutrient supply lasting after 1 Oct. might lead to adverse effects in flowering, such as aerial shoot formation, when cooling treatment is marginal for flower initiation. Nutrient reapplication around flowering can be omitted to save production cost, especially when no new vegetative growth coexists with flower development.

CHAPTER IV

EFFECT OF COOLING TEMPERATURE AND DURATION ON FLOWERING

Introduction

Dendrobium was reported to be the second most valued orchid genus sold in Japan in 2002, which had a market share of 20%, only behind *Phalaenopsis* (Laws, 2004; Wang, 2004). *Dendrobium* has also been reported to be the most economically important flowering pot orchid genus sold in Hawaii with a wholesale value of $6 million in both 2005 and 2006 (USDA, 2007). However, the nobile-type dendrobiums, cultivars bred mainly from the species *Dendrobium nobile* Lindl. (Ichihashi, 1997), are still a relatively new mass-produced commercial crop having a high market potential. Studies to determine the cultural requirements are needed to improve the cultivation system and to make possible year-round production of flowering nobile dendrobiums.

D. nobile, the progenitor of nobile-type dendrobium hybrids, is native to habitats ranging from the Himalayas to Southeast Asia and much of southern China (Baker and Baker, 1996). Plants must be exposed to moderately low temperatures to induce flowering (Arditti, 1966; Goh et al., 1982; Rotor Jr., 1952, 1959), which usually occurs in the fall or following sudden rainstorms in its habitat. Rotor Jr. (1952, 1959) reported that *D. nobile* plants exposed to 13 °C produced flowers regardless of photoperiod. To achieve the best flowering

performance in two nobile dendrobium cultivars, *D.* Snowflake 'Red Star' and *D.* Hinode 'Toutenkou', the optimum temperature for flower initiation was below 20 °C in the daytime and 10 to 12.5 °C at night (Sinoda et al., 1988).

Besides the nobile-type dendrobiums, several other orchid genera and species also require low temperatures for flower induction. The low temperature and its duration that induce flowering vary with different orchid genera and species. Flower initiation in *D. phalaenopsis* Fitzg. was induced by low temperature of 13 °C regardless of daylength (Rotor Jr., 1952, 1959). In nature, the inflorescence of *D. crumenatum* Sw. produces flower buds that grow until the anther is almost fully developed and all other floral parts are formed and then undergo dormancy. Development resumes in the dormant flower buds after a sudden 5 °C temperature drop (exact temperature unspecified), which is often provided by rainstorms in Southeast Asia (Goh et al., 1982; Hew and Yong, 2004).

Regardless of daylength, several *Cymbidium* hybrids (*C.* Madeleine, *C.* Doreen, *C.* Zebra, and *C.* No. 2212) and *Paphiopedilum insigne* Lindl. flowered at low temperature of 13 °C (Rotor Jr., 1952, 1959). In *Doritaenopsis* 'Lava Glow' (*Phalaenopsis* Buddha's Treasure x *Doritis pulcherrima* Lindl.), among the set of growing day/night (12 h each daily) temperatures of 30/25, 25/30, 25/20, or 20/25 °C, or another set of 30/20, 20/30, 25/15, or 15/25 °C, the cool day/warm night temperature regimens of 20/25 °C and 15/25 °C markedly restricted vegetative growth but were able to trigger flowering effectively,

whereas plants at other temperature regimens did not (Wang, 2007b). Nishimura et al. (1976) reported that the transition from vegetative growth to flower development in *Phalaenopsis* Sea Mist required relatively low temperatures of 15 to 18 °C. The inflorescence of *Phalaenopsis amabilis* Blume was induced by temperature below 25 °C for more than 12 h daily (Sakanishi et al., 1980). Two to 5 weeks of night temperature between 12 and 17 °C and day temperature not over 27 °C for flower initiation in *P. amabilis* and *P. schilleriana* Rchb. f. was reported by Tran Thanh Van (1974). Therefore, it can be seen that the low temperatures needed to induce flowering vary among orchid genera, ranging from 10 to 25 °C, and even differ from one species to another within the same genera.

In contrast to flower induction, relatively high temperatures may be unfavorable to flowering. Several *Cymbidium* hybrids, *Paphiopedilum insigne,* and *D. nobile* plants held at 18 °C, compared to 13 °C, remained vegetative (Rotor Jr., 1952, 1959). Flowering of *P. amabilis* was inhibited when plants were grown under high temperatures (30 °C day/23 °C night), compared to low temperatures at 25 °C day/20 °C night (Chen et al., 1994). The inflorescence of *P. amabilis* did not emerge if the temperature was over 28 °C (Sakanishi et al., 1980). In addition, when day temperature was maintained at 28 or 30 °C, certain *Phalaenopsis* hybrids remained vegetative even when night temperature was dropped to 19 or 14 °C, respectively (Wang et al., 2006).

Therefore, if the threshold temperature for initiating flowers, promoting the best flower quality, or that adversely affects plant growth or inhibits flowering is known, flowering can be programmed to meet a specific market time in production. The objectives of this study were to quantify the cooling requirement for flower initiation and to define an optimum cooling treatment for producing quality two-year-old flowering nobile dendrobiums.

Materials and Methods

Plant material and growing conditions. A *Dendrobium nobile* Lindl. hybrid, *Dendrobium* Sea Mary 'Snow King', was used. One-year-old liners, propagated from single-node cuttings and planted in sphagnum moss with an average pseudobulb (i.e., a thickened portion of the stem in orchids functioning as a water and food storage device) height of 7 to 10 cm, were shipped from Yamamoto Dendrobiums in Mountain View, Hawaii. Plants arrived at Texas A&M University, College Station on 15 Feb. 2006 and were potted into 10.2 cm (top diameter, 414 mL vol.) standard green plastic pots on 16 to 18 Feb. and placed in a glass wall and polycarbonate roof greenhouse. The root substrate consisted of two parts of coarse peat (Sunshine Peat; Sun Gro Horticulture, Bellevue, Wash.), one part coarse perlite, and one part no. 3 grade diatomitic (Diatomite USA, Elma, N.Y.), amended with powdered Micromax (Scotts, Marysville, Ohio) at 1 g•L^{-1} as a source of micronutrients and powdered dolomitic

limestone at 5 g•L^{-1}. A wetting agent, Aqua Gro 2000 G (Scotts, Marysville, Ohio), was added to the medium at 0.5 g•L^{-1}.

Plants were potted with the root substrate packed tightly to secure them in place when lifted up only by the upper portion of the pseudobulbs. Immediately after potting, plants were irrigated with reverse osmosis (RO) water containing a fungicide (Banrot 40% WP; Scotts, Marysville, Ohio) at 0.6 g•L^{-1} to prevent root rot. Plants were spaced pot-to-pot in 30.8 x 51.4 cm molded carrying trays (4.00 Transport Tray (15); Landmark Plastic Corporation, Akron, Ohio) on the greenhouse bench with the plant leaves orienting east and west to best capture sunlight. The newly grown pseudobulbs were staked upright with bamboo sticks (Bamboo Supply, Lakeland, Fla.) to minimize mutual shading. Whenever an undesirable second new pseudobulb emerged, it was removed to keep only one new pseudobulb per pot.

Greenhouse light level and air temperature at plant canopy level were recorded hourly with data loggers [HOBO (Onset Computer Co., Bourne, Mass.), WatchDog (Spectrum Technologies, Plainfield, Ill.), and Apogee line quantum sensors (Apogee Instruments, Logan, Utah)] (Appendix 1). Plants were grown in a warm environment (average 25.3 °C day/23.0 °C night) under an average 4.9 mol•m^{-2}•d^{-1} daily light integral (*DLI*) until the pseudobulbs matured. Pots were irrigated as needed with RO water containing a 15N-2.2P-12.5K (Peters Excel 15-5-15 Cal-Mag; Scotts, Marysville, Ohio) water-soluble fertilizer at 0.67 g•L^{-1} (100 mg•L^{-1} N). Plain RO water was used after nutrient application was

terminated on 1 Sept. 2006. Pesticides [*Bacillus thuringiensis israelensis* (Gnatrol), Valent BioSciences Coroperation, Libertyville, Ill.; Azadirachtin (Azatin) and Cyfluthrin (Decathlon), Olympic Horticulture Products, Mainland, Pa.; Imidacloprid (Marathon II), Olympic Horticulture Products, Mainland, Pa.] were applied when necessary to control fungus gnats, caterpillars, and mealy bugs, respectively.

Plants were subjected to cooling treatments in growth chambers starting on 13 Nov. 2006. The growth chambers were maintained at 65% RH and a 12-h photoperiod with 350 μmol•m^{-2}•s^{-1} *PPF* provided by both fluorescent and incandescent lamps. Frequency of watering or fertilization was reduced from the beginning of cooling until full flowering, allowing the medium to dry somewhat between waterings. Plants were moved back to the warm greenhouse set at 25 °C day/20 °C night after the completion of cooling for flower development. The average air temperatures and *DLI* from the end of cooling until full flowering were 24.3 °C day/21.9 °C night and 6.1 mol•m^{-2}•d^{-1}, respectively.

Experimental design. The experiment was a 4 x 5 factorial with four cooling temperatures (10, 13, 15, and 18 °C) and five cooling durations (2, 3, 4, 5, and 6 weeks). A randomized complete block design with twelve replications was used. A single plant in a pot with one new pseudobulb constituted an experimental unit. A total of two hundred and forty vegetatively propagated nobile dendrobium plants were used. The average air temperatures recorded in the growth chambers were 9.8 (fluctuation between 9.5 to 10.6 °C), 13.3 (fluctuation

between 12.8 to 13.9 °C), 14.5 (fluctuation between 14.2 to 14.9 °C), and 18.0 °C (fluctuation between 17.1 to 19.3 °C), respectively. Twelve plants served as an observational control group (i.e., not included in statistical analysis) and continued receiving nutrients at 0.67 $g \cdot L^{-1}$ (100 $mg \cdot L^{-1}$ N) throughout the experimental period and remained in the greenhouse without cooling.

Data collection. Weekly photographs were taken to compare and record the visual differences among treatments. Data including plant height (measured from the medium surface to the tip of the pseudobulb), number of nodes, number of remaining leaves on the pseudobulb before anthesis, pseudobulb width (the diameter of the widest point from side to side) and thickness (the diameter of the thickest point from front to back), time to anthesis (the first flower bud on a plant cracked to open), time to full flowering (all flowers on a plant fully opened), the number of aerial shoots on a pseudobulb, aborted buds and flowering nodes, total flower number, and flower diameter were collected. For the two variables, time to anthesis and full flowering, the numbers of days were recorded from completion of the cooling treatments. In addition, the numbers of days from beginning of the cooling treatments were also calculated to show total time required from cooling to flowering. Flower diameter was determined by averaging those of two flowers, one on each of the two middle nodes of a plant. Average flower number per flowering node was calculated by dividing total flower number by flowering node number.

Statistical analysis. All the data collected except for those from the observational control group were analyzed with analysis of variance (ANOVA), Duncan's multiple range test for significant differences, and regression analysis when necessary, all at $P \leq 0.05$. Percentage data were arcsine-transformed to normalize distribution of variance before subjected to statistical analysis. All statistical analysis was performed by SAS 9.1.3 statistical software (SAS Institute, Cary, N.C.).

Results

Interactions between temperature and cooling duration were significant on time to anthesis and full flowering, recorded from either the beginning or the completion of cooling, average flower number per flowering node, and flower diameter (Tables 6, 7). Changes in time to full flowering from completion of cooling followed the same trend as time to anthesis due to the same flower development rate among treatments after anthesis (Fig. 13). Regardless of cooling temperature, increasing cooling duration from 2 to 6 weeks decreased the average time needed to reach anthesis (49 to 37 d) and to full flowering (53 to 41 d) after plants had been moved from growth chambers to a warm greenhouse (Fig. 14). The time required from anthesis to full flowering, 4 d on average, was found not significantly different among treatments. The differences in time required to reach anthesis or full flowering were therefore proven to be caused mainly by the speed of flower differentiation and faster

Table 6. ANOVA for the effect of cooling temperature and duration on time required for flowering in *Dendrobium* Sea Mary 'Snow King'.

Treatment	Time to anthesis from completion of cooling (d)	Time to full flowering from completion of cooling (d)	Time to anthesis from beginning of cooling (d)	Time to full flowering from beginning of cooling (d)	Time from anthesis to full flowering (d)
Temperature (T)	***	***	***	***	NS
Duration (D)	***	***	***	***	NS
T x D	***	***	***	***	NS

NS, *** Nonsignificant or significant at $P \leq 0.001$.

Table 7. ANOVA for the effect of cooling temperature and duration on flowering data of *Dendrobium* Sea Mary 'Snow King'.

Treatment	Nodes with flowers		Nodes with aerial shoots	Nodes with aborted buds	Total flower number	Average flower no. per flowering node	Flower diameter
	(no.)	(%)	(no.)	(no.)	(no.)	(no.)	(cm)
Temperature (T)	***	***	NS	NS	***	***	NS
Duration (D)	NS	NS	NS	NS	NS	**	**
T x D	NS	NS	NS	NS	NS	**	***

NS, **, *** Nonsignificant or significant at $P \leq 0.01$, 0.001, respectively.

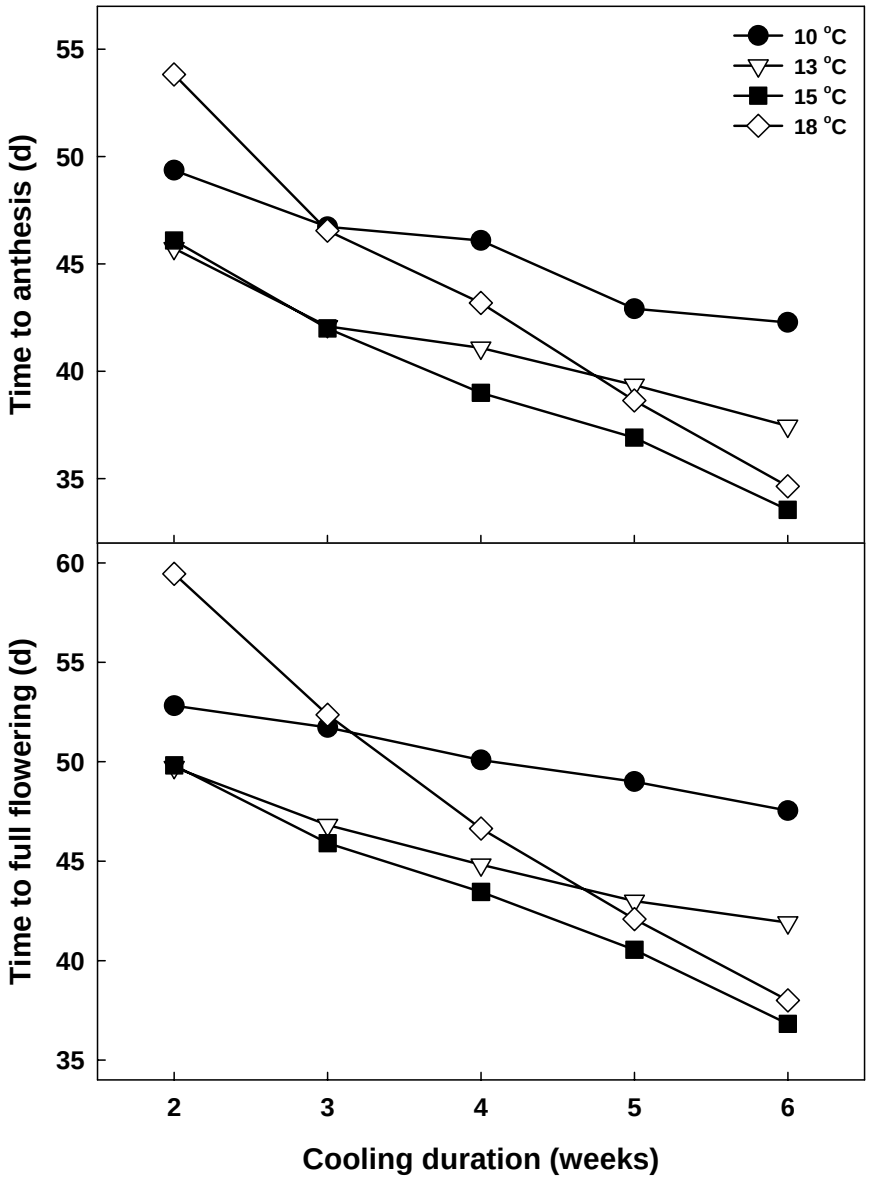

Fig. 13 Interaction for the effect of cooling temperature and duration on time required to reach anthesis and full flowering from completion of cooling in *Dendrobium* Sea Mary 'Snow King'.

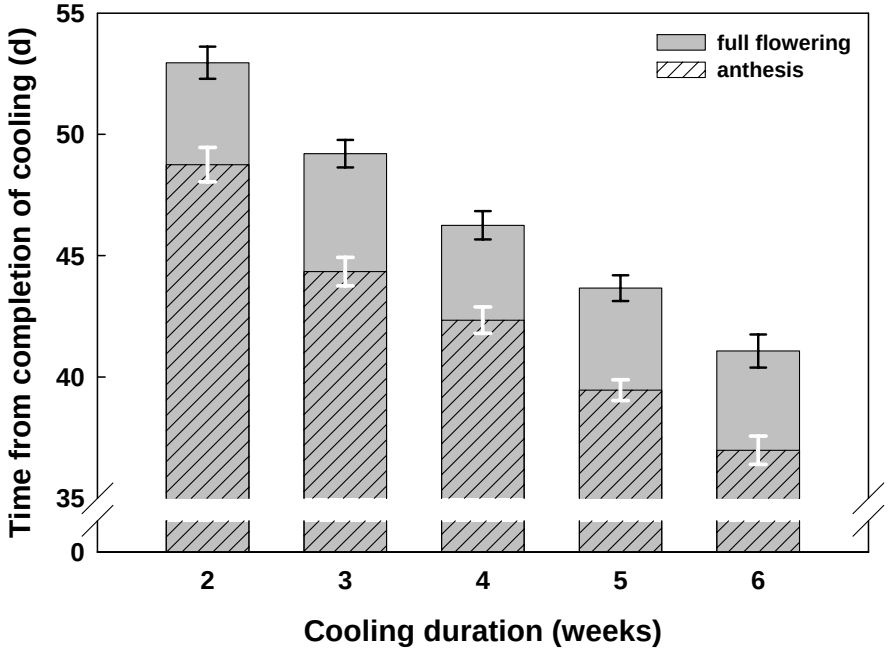

Fig. 14 Time required to reach anthesis and full flowering from completion of cooling treatment in *Dendrobium* Sea Mary 'Snow King' in response to cooling duration. Bars indicate ± SE of the mean.

flower development before anthesis, not at the final development stage from anthesis to full flowering.

As cooling temperature increased from 10 to 18 °C, the difference in time required to reach anthesis and full flowering from completion of cooling widened as cooling duration lengthened from 2 to 6 weeks (Table 8). For example, time to full flowering from completion of cooling, plants that were cooled at 10, 13, 15, or 18 °C had a range of 5 d (from 48 to 53 d), 8 d (from 42 to 50 d), 13 d (from 37 to 50 d), or 22 d (from 38 to 60 d), respectively, when cooling duration

Table 8. Interaction for the effect of cooling temperature and duration on flowering data of *Dendrobium* Sea Mary 'Snow King'.

Cooling duration (weeks)	Cooling temperature (°C)											
	10			13			15			18		
Time to anthesis from completion of cooling (d)												
2	49.4	b[z]	A	45.7	c	A	46.1	c	A	53.8	a	A
3	46.7	a	B	42.1	b	B	42.0	b	B	46.5	a	B
4	46.1	a	B	41.1	bc	C	39.0	c	C	43.2	b	B
5	42.9	a	C	39.4	b	D	36.9	c	D	38.6	b	C
6	42.3	a	C	37.5	b	E	33.5	c	E	34.6	c	C
Time to full flowering from completion of cooling (d)												
2	52.8	b	A	49.7	c	A	49.8	c	A	59.5	a	A
3	51.7	a	A	46.8	b	B	45.9	b	B	52.4	a	B
4	50.1	a	B	44.8	bc	C	43.5	c	C	46.6	b	C
5	49.0	a	B	43.0	b	D	40.5	c	D	42.1	b	D
6	47.5	a	C	41.9	b	D	36.8	c	E	38.0	c	E
Time to anthesis from beginning of cooling (d)												
2	63.4	b	E	59.7	c	E	60.1	c	E	67.8	a	C
3	67.7	a	D	63.1	b	D	63.0	b	D	67.5	a	C
4	74.1	a	C	69.1	bc	C	67.0	c	B	71.2	b	BC
5	77.9	a	B	74.4	b	B	71.9	c	C	73.6	b	AB
6	84.3	a	A	79.5	b	A	75.5	c	A	76.6	c	A

Table 8. Continued,

Cooling duration (weeks)	Cooling temperature (°C)											
	10			13			15			18		
Time to full flowering from beginning of cooling (d)												
2	66.8	b	E	63.7	c	E	63.8	c	E	73.5	a	C
3	72.7	a	D	67.8	b	D	66.9	b	D	73.4	a	C
4	78.1	a	C	72.8	bc	C	71.5	c	C	74.6	b	BC
5	84.0	a	B	78.0	b	B	75.5	c	B	77.1	b	B
6	89.5	a	A	83.9	b	A	78.8	c	A	80.0	c	A
Average flower no. per flowering node (no.)												
2	2.8	ab	B	3.1	a		2.8	ab	B	2.5	b	C
3	3.2	a	A	3.0	a		3.1	a	A	2.6	b	BC
4	2.8		B	3.0			3.1		A	2.9		A
5	3.2	a	A	3.1	ab		3.0	ab	AB	2.8	b	A
6	2.8	b	B	3.2	a		3.1	a	A	2.8	b	AB
Flower diameter (cm)												
2	6.5		A	6.7		A	6.9		A	6.8		AB
3	6.2	b	B	6.6	ab	A	6.8	a	A	7.0	a	A
4	6.6		A	6.4		AB	6.5		B	6.4		B
5	6.8	a	A	6.2	b	B	6.5	ab	B	6.3	b	B
6	6.8	a	A	6.6	ab	A	6.4	b	B	6.6	ab	AB

[z]Mean separation by Duncan's multiple range test at $P \leq 0.05$. Lowercase within rows; uppercase within columns.

Table 9. Linear regression analysis on time required for flowering in response to cooling temperature in *Dendrobium* Sea Mary 'Snow King'.

Cooling temperature (°C)	Equation	Significance level	R^2
Time to anthesis from completion of cooling			
10	Y = 52.7 – 1.8 X	***	0.79
13	Y = 48.9 – 1.9 X	***	0.84
15	Y = 51.6 – 3.0 X	***	0.93
18	Y = 61.9 – 4.6 X	***	0.65
Time to full flowering from completion of cooling			
10	Y = 55.5 – 1.3 X	***	0.58
13	Y = 53.0 – 1.9 X	***	0.73
15	Y = 55.9 – 3.1 X	***	0.86
18	Y = 69.0 – 5.3 X	***	0.85
Time to anthesis from beginning of cooling			
10	Y = 52.7 + 5.2 X	***	0.97
13	Y = 48.9 + 5.1 X	***	0.97
15	Y = 51.6 + 4.0 X	***	0.96
18	Y = 61.9 + 2.4 X	***	0.32
Time to full flowering from beginning of cooling			
10	Y = 55.5 + 5.7 X	***	0.96
13	Y = 53.0 + 5.1 X	***	0.95
15	Y = 55.9 + 3.9 X	***	0.90
18	Y = 69.0 + 1.9 X	***	0.37

***Significant at $P \leq 0.001$.

increased from 2 to 6 weeks. The positive relationship can be seen by the slopes of the lines in Fig. 13, which were analyzed by linear regression (Table 9).

The coefficients of X (representing slopes) in regression equations for cooling treatments at 10, 13, 15, and 18 °C changed from -1.8, -1.9, -3.0 to -4.6 for time to anthesis from completion of cooling, and from -1.3, -1.9, -3.1 to -5.3 for time to full flowering from completion of cooling, respectively. The higher the cooling temperature was, the steeper the slope became. In addition, more time was needed to reach anthesis and full flowering from completion of cooling as cooling temperature decreased from 15 to 10 °C (Fig. 13).

For cooling durations of 2 to 4 weeks, plants that were cooled at 13 or 15 °C required less amount of time to reach anthesis and full flowering than 10 or 18 °C after the completion of cooling (Fig. 13). For 5 or 6 weeks of cooling, plants reached anthesis and full flowering faster following treatment at 15 or 18 °C than at 10 or 13 °C.

It was worthy to note that there was much greater variation within treatments in time required to reach anthesis from completion of cooling at 18 °C than at all the other cooling temperatures (data not shown). The SE for time to anthesis from completion of cooling at 18 °C for each duration was approximately four times that at 10, 13, or 15 °C.

When cooling duration was extended from 2 to 6 weeks, time required to reach anthesis and full flowering from beginning of cooling treatments increased (Fig. 15), contrary to the time required to reach anthesis from completion of cooling. With cooling duration lengthened from 2 to 6 weeks, there was an increase of 16 d in time required to reach either anthesis (63 to 79 d on average)

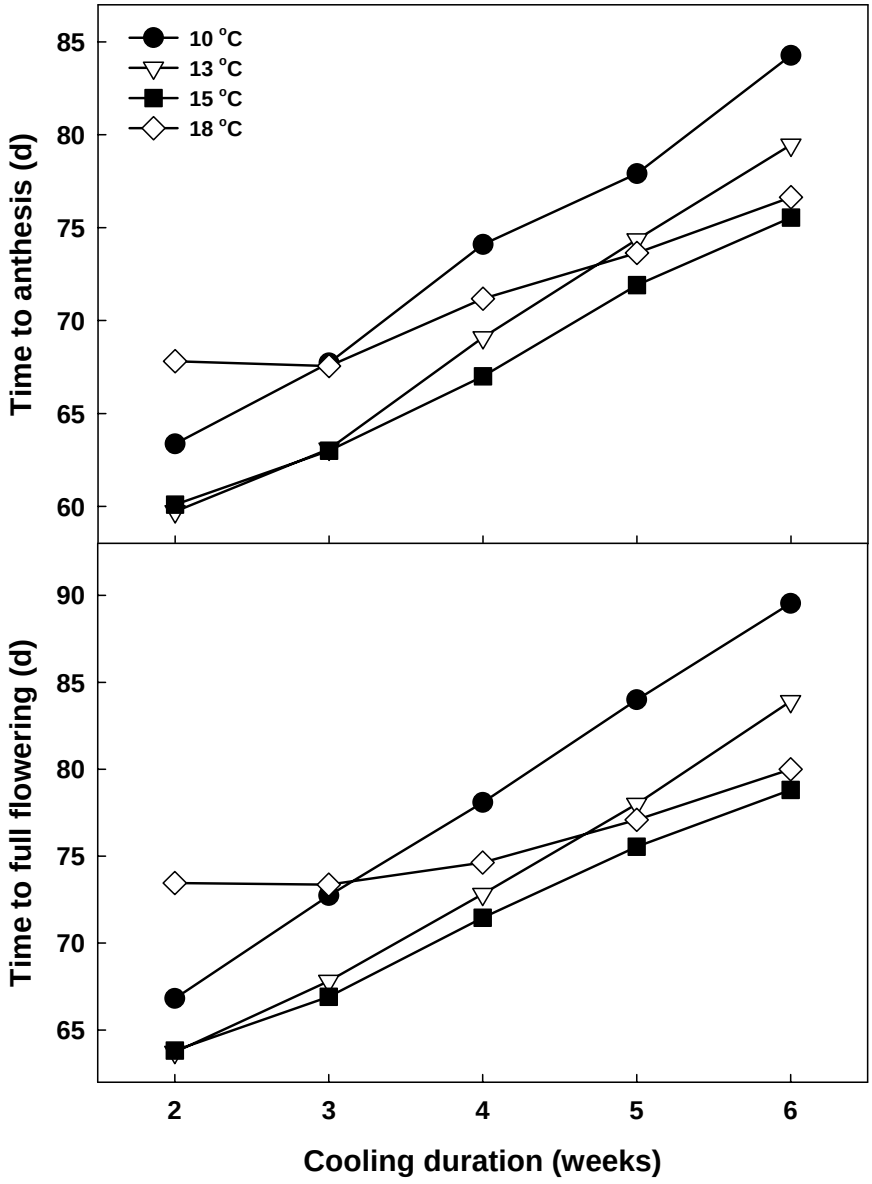

Fig. 15 Interaction for the effect of cooling temperature and duration on time required to reach anthesis and full flowering from beginning of cooling in *Dendrobium* Sea Mary 'Snow King'.

or full flowering (67 to 83 d on average) from beginning of cooling treatments (Table 8).

Plants cooled at 10, 13, or 15 °C had more drastic changes in time to anthesis and full flowering from beginning of cooling with increasing cooling duration than those cooled at 18 °C (Fig. 15). For example, in time to full flowering from beginning of cooling, when cooling duration increased from 2 to 6 weeks, plants that were cooled at 10, 13, 15, or 18 °C had an increase of 23 d (from 67 to 90 d), 20 d (from 64 to 84 d), 15 d (from 64 to 79 d), or 6 d (from 74 to 80 d), respectively (Table 8). Adverse changes in steepness of the linear slopes were observed in time required to reach anthesis and full flowering from beginning of cooling in response to cooling temperatures (Table 9). The coefficients of X in regression equations for cooling treatments at 10, 13, 15, and 18 °C changed from 5.2, 5.1, 4.0 to 2.4 for time to anthesis from beginning of cooling, and from 5.7, 5.1, 3.9 to 1.9 for time to full flowering from beginning of cooling, respectively.

Under various cooling temperatures, the linear regression model for each of the four variables, time to anthesis or full flowering from completion of cooling, or time to anthesis or full flowering from beginning of cooling, fitted very well with $P < 0.001$ (Table 9). Coefficients of determination (R^2), presenting the strength of the regression predictability, were high for all the models, except those for time to anthesis from completion of cooling at 18 °C, time to full flowering from completion of cooling at 10 °C, and time to anthesis and full flowering from

beginning of cooling at 18 °C. The high significance level with high R^2 value indicated that appropriate models were established for future prediction in time to flower.

Generally, increasing cooling duration from 2 to 6 weeks led plants to reach anthesis and full flowering faster from completion of cooling; however, the increasing cooling duration actually extended the total time required from the beginning of cooling to the stages of anthesis and full flowering.

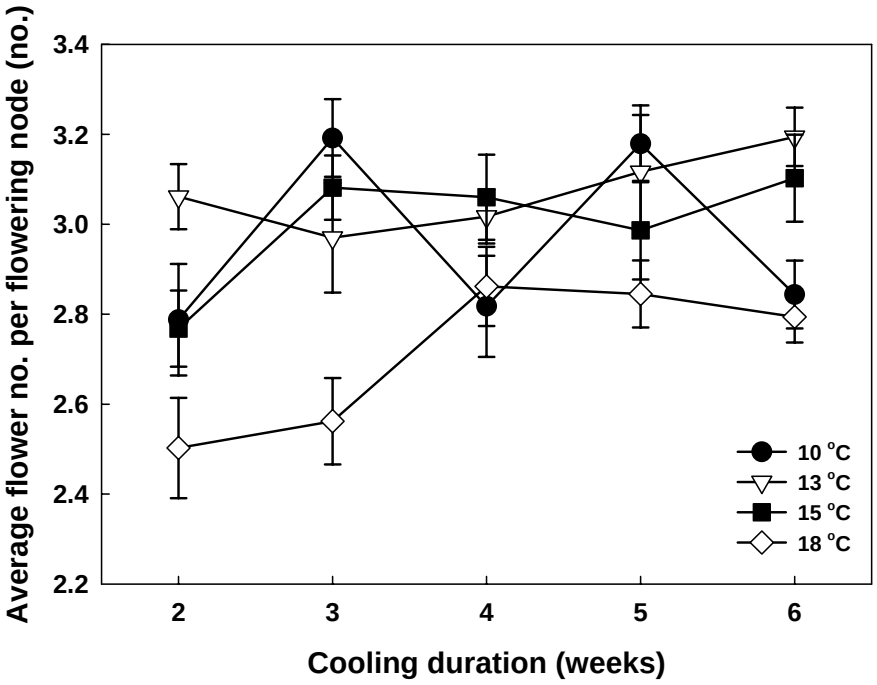

Fig. 16 Interaction for the effect of cooling temperature and duration on average flower number per flowering node in *Dendrobium* Sea Mary 'Snow King'. Bars indicate ± SE of the mean.

Flower number per flowering node stayed relatively stable around 3.0 as weeks of cooling increased at all temperatures except for 18 °C (Fig. 16). At 18 °C, 2 to 3 weeks of cooling resulted in a lower flower count of 2.5 per flowering node. The flower diameter ranged from 6.2 to 7.0 cm among treatments (Fig. 17). No prominent consistent trend could be observed except at 15 °C where 4 to 6 weeks of cooling resulted in reduced flower diameter than those being exposed to 2 to 3 weeks of cooling. In addition, 3 weeks of cooling at 10 °C led to a decrease in flower diameter.

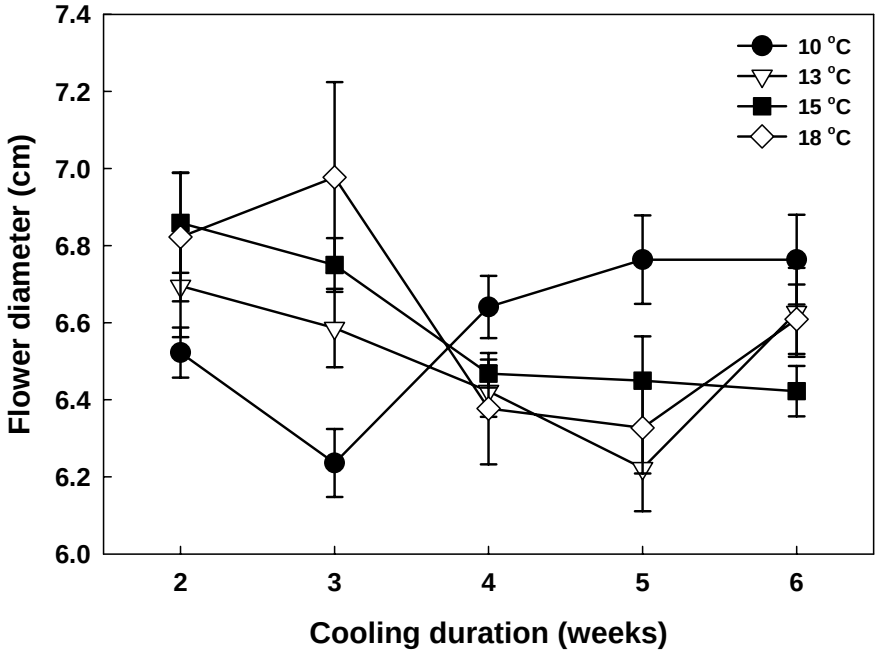

Fig. 17 Interaction for the effect of cooling temperature and duration on flower diameter in *Dendrobium* Sea Mary 'Snow King'. Bars indicate ± SE of the mean.

All plants in this cooling experiment flowered without producing any aerial shoots, and even the least amount of cooling, 2 weeks at 18 °C, was enough to trigger flower initiation. Plants that were removed from treatments after 2 weeks of cooling at all temperatures had obvious (more than 2 mm) protruding buds developing during the ensuing week. However, total flower number per plant was slightly affected by the least cooling treatments (Table 10). Plants subjected to 2 or 3 weeks of cooling at 18 °C had lower flower counts (around 20) per plant than those in all other treatments (more than 25 flowers).

Regardless of cooling duration, decreasing cooling temperature from 18 to 10 °C improved flower counts. Plants that were cooled at 10, 13, or 15 °C had more flowering nodes, percentage of nodes with flowers, and total flower number than those cooled at 18 °C (Table 11). Three sets of photographs,

Table 10. Total flower number per plant in response to cooling temperature and duration in *Dendrobium* Sea Mary 'Snow King'.

Cooling duration (weeks)	Cooling temperature (°C)								
	10			13		15		18		
Total flower number (no.)									
2	24.7	bc[z]	B	30.2	a	26.0	ab	20.4	c	BC
3	30.7	a	AB	29.7	a	27.9	a	19.5	b	C
4	25.2		B	27.2		29.5		24.5		ABC
5	31.8	a	A	29.0	ab	25.9	b	25.7	b	A
6	28.4	ab	AB	32.2	a	28.0	ab	24.8	b	AB

[z]Mean separation by Duncan's multiple range test at $P \leq 0.05$. Lowercase within rows; uppercase within columns.

Table 11. Effect of cooling temperature regardless of cooling duration on nodes with flowers and total flower number of *Dendrobium* Sea Mary 'Snow King'.

Cooling temperature (°C)	Nodes with flowers (no.)	Nodes with flowers (%)	Total flower number (no.)
10	9.4 a[z]	84.1 a	28.2 a
13	9.6 a	83.8 a	29.7 a
15	9.1 a	80.8 a	27.5 a
18	8.4 b	74.9 b	23.0 b

[z]Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

taken a week apart, are shown in Figs. 18-20.

The effect of either cooling temperature or cooling duration on vegetative parameters was non-significant, except for the percentage of leaves remaining on pseudobulbs (Table 12). Plants subjected to longer periods of cooling had a tendency to defoliate slightly more than those with shorter durations, but the slight difference was not commercially important (Table 13).

To summarize, this experiment validated that optimum cooling assured best flowering quality. Higher cooling temperature of 18 °C resulted in less desirable flowering performance, such as fewer flowering nodes and total flowers. Furthermore, as cooling temperature increased from 10 to 15 °C, flowering was accelerated after completion of cooling with decreasing uniformity in flowering speed in response to cooling durations (Fig. 13). Cooling duration from 2 to 6 weeks markedly accelerated the early stages of flower development

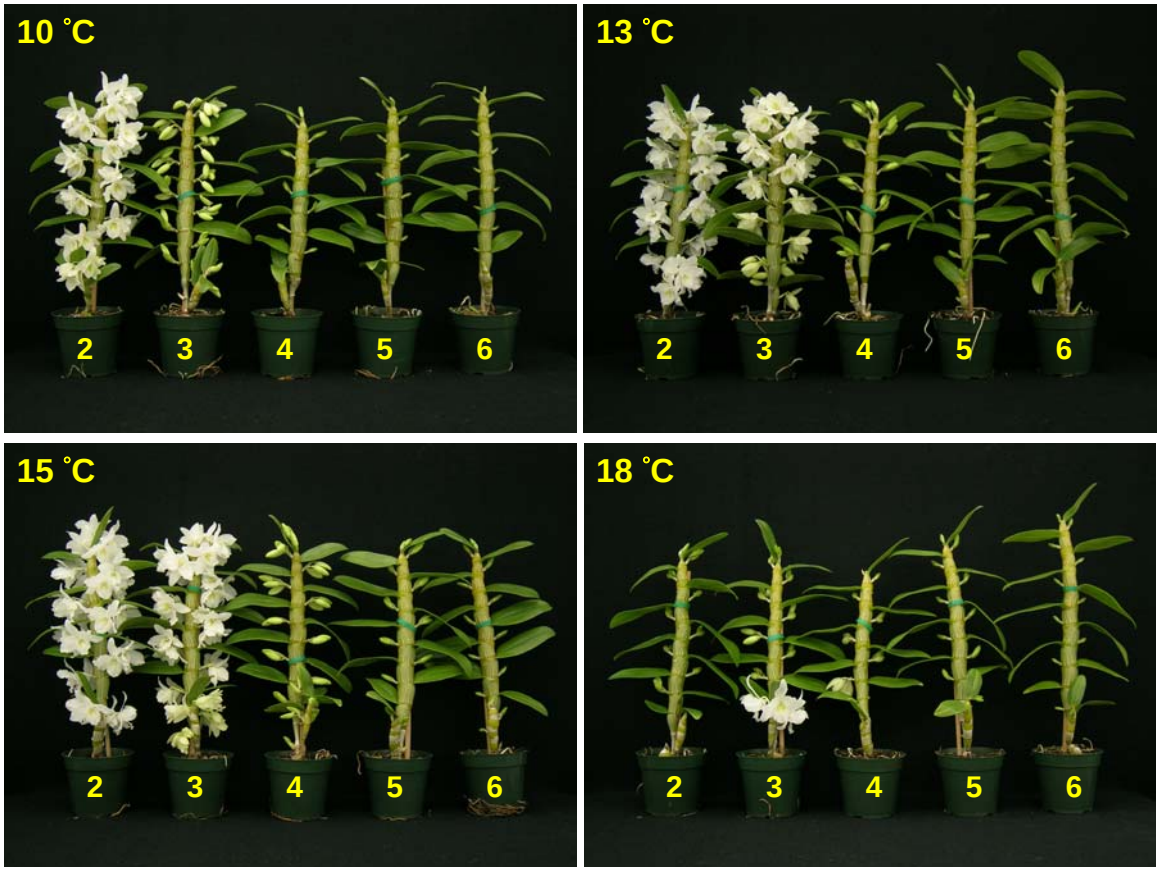

Fig. 18 Visual performance on 16 Jan. 2007 of *Dendrobium* Sea Mary 'Snow King' with various cooling temperatures and durations. Five cooling durations began on 13 Nov. 2006 are numbered as 2 to 6 weeks.

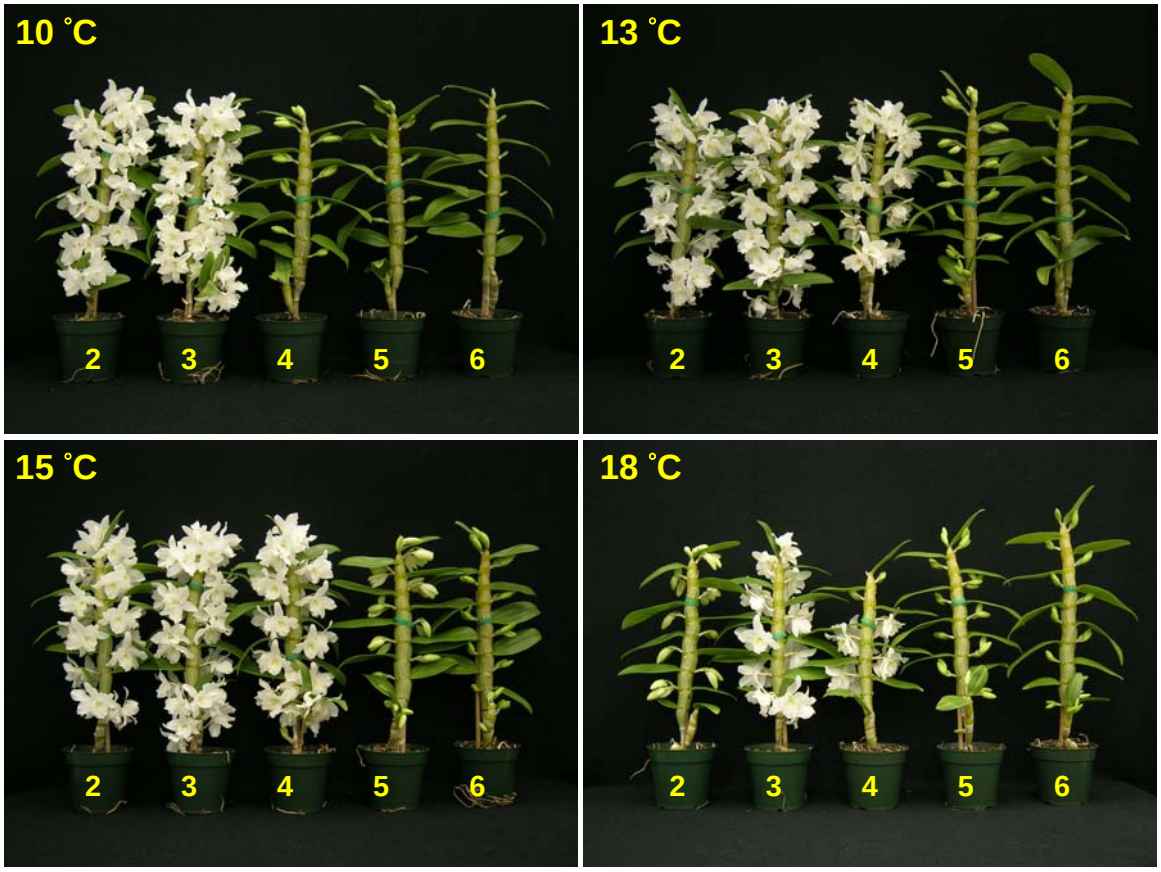

Fig. 19 Visual performance on 23 Jan. 2007 of *Dendrobium* Sea Mary 'Snow King' with various cooling temperatures and durations. Five cooling durations began on 13 Nov. 2006 are numbered as 2 to 6 weeks.

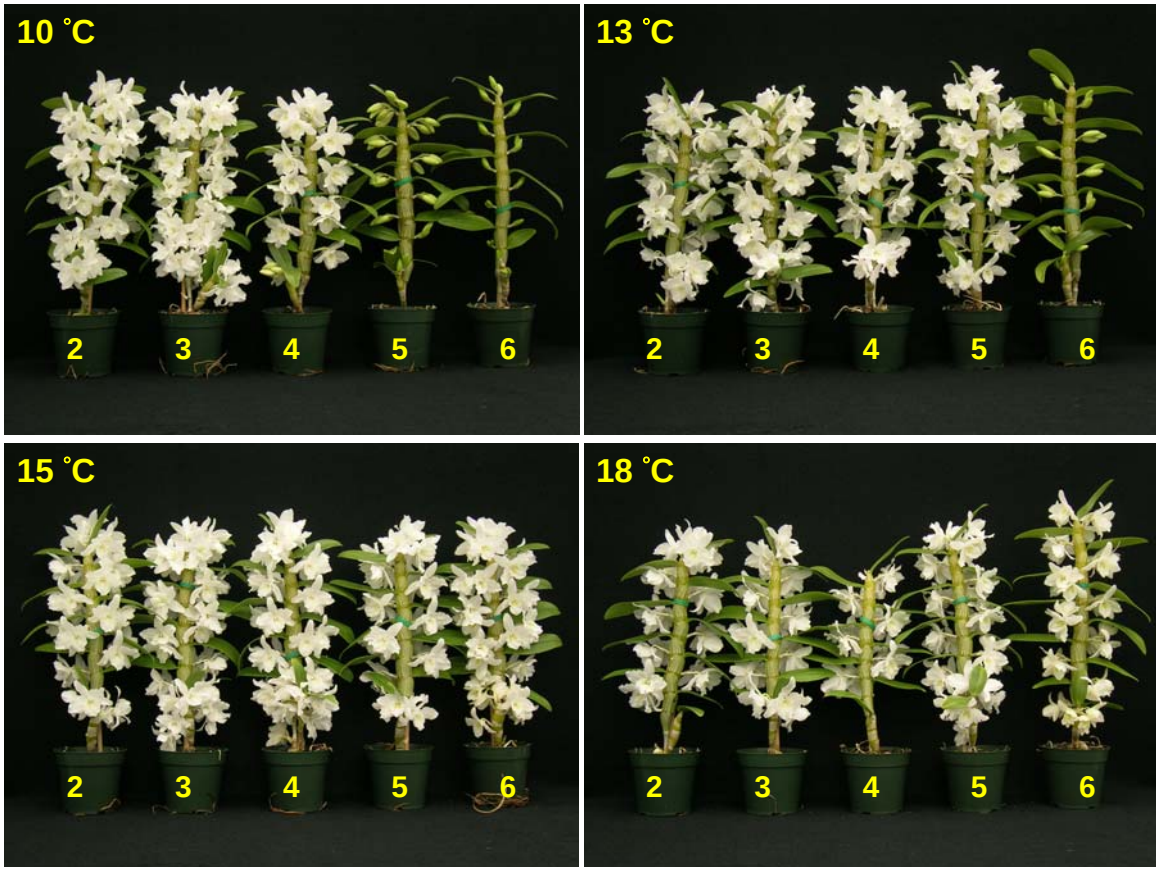

Fig. 20 Visual performance on 30 Jan. 2007 of *Dendrobium* Sea Mary 'Snow King' with various cooling temperatures and durations. Five cooling durations began on 13 Nov. 2006 are numbered as 2 to 6 weeks.

Table 12. ANOVA for the effect of cooling temperature and duration on vegetative data of *Dendrobium* Sea Mary 'Snow King'.

	Plant	Node			Pseudobulb	
	height	number	Leaves remaining		Width	Thickness
Treatment	(cm)	(no.)	(no.)	(%)	(cm)	(cm)
Temperature (T)	NS	NS	NS	NS	NS	NS
Duration (D)	NS	NS	NS	**	NS	NS
T x D	NS	NS	NS	NS	NS	NS

NS, ** Nonsignificant or significant at $P \leq 0.01$.

Table 13. Effect of cooling duration regardless of cooling temperature on percentage of leaves remaining on pseudobulbs in *Dendrobium* Sea Mary 'Snow King'.

Cooling duration (weeks)	Percentage of leaves remaining (%)
2	81.8 ab[z]
3	82.5 a
4	79.5 b
5	79.0 b
6	79.6 b

[z]Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

(Fig. 14); therefore, plants required less time to reach anthesis after the completion of cooling.

Discussion

Nobile dendrobiums require a period of cooling to trigger flower differentiation. Flowering in *D. nobile*, the progenitor of the nobile-type

dendrobiums, was shown to be promoted with low temperatures ranging from 13 to 16 °C and not above 18 °C (Rotor Jr., 1952, 1959). A closely related descendant, *D.* Sea Mary 'Snow King', also needed to be subjected to a specific amount of cooling for flowering.

The specific amount of cooling constitutes the threshold to initiate flowering of nobile dendrobiums. Below the threshold, plants may fail to initiate flowers and aerial shoots may arise. Above the threshold, flowering occurs but flower or plant quality and flowering speed would vary with the amount of cooling. Sinoda et al. (1988) reported that aerial shoots were formed when buds were subjected to insufficient cooling (above 20 °C) in *D*. Snowflake 'Red Star'. In the current study, observational control plants without cooling failed to flower and produced aerial shoots. From this result in contrast to the plants among the cooling treatments that flowered without aerial shoot formation, it indicated that the least cooling treatment, 2 weeks at 18 °C, was above the threshold for triggering flower initiation in *D.* Sea Mary 'Snow King'.

Various amounts of cooling above the threshold may affect plant quality. Temperatures below 25 °C day/ 10 °C night caused leaf yellowing and defoliation of the nobile-type dendrobiums (Ichihashi, 1997). In *D.* Hinode 'Toutenkou' and *D.* Snowflake 'Red Star', when cooling with low temperatures of 7.5 to 15 °C at night, high temperatures above 20 °C at daytime hindered flower development and reduced the flowering nodes (Sinoda et al., 1988). In the current study, plants treated with shorter durations of cooling at 18 °C had fewer

flowering nodes and total flowers. Regardless of temperature, cooling plants for an increasingly longer duration from 2 to 6 weeks resulted in lower leaf retention. Therefore, cooling temperatures below 18 °C for less than 6 weeks are suggested for producing nobile dendrobiums with an optimum quality.

Flowering speed may be altered by varying temperatures and durations of cooling. The temperature used for flower initiation can affect the speed of subsequent flower bud development in nobile dendrobiums (Ichihashi, 1997) if left under such conditions once the cold requirement has been fulfilled, possibly because temperature generally affects the metabolic activity and the balance of respiration and photosynthesis (Hopkins, 1999). In the present study, regardless of cooling temperatures, plants treated with longer cooling durations required less time to reach anthesis and full flowering after the completion of cooling. The cooling treatments affected the rate of flower development before anthesis without markedly changing the rate from anthesis to full flowering. Interestingly, the nutrient application that was studied in Expt. 1 affected the flower development differently, in which the nutrient termination time did change the rate of flower development between anthesis and full flowering, but not the rate before anthesis. To summarize briefly, increasing amount of cooling alters the speed of flower development before anthesis, whereas prolonged nutrient supply until pseudobulb maturation slightly retards the flower development from anthesis to full flowering.

On the other hand, excessive amount (i.e., temperature and/or duration) of cooling delays the time to flowering of the crop. Once the flowers have been initiated, the growing temperature affects the rate of subsequent growth and development of inflorescences. Anthesis could be advanced by increasing temperatures during inflorescence development (Rotor Jr., 1952, 1959). Therefore, once the cooling requirement for flower initiation is saturated and floral primordia have been differentiated, subjecting plants to higher temperatures accelerates the development of flower buds. In the current study, plants cooled for 5 or 6 weeks flowered earlier at the higher cooling temperatures and slower at the lower temperatures. This result indicated that 5 or 6 weeks of cooling was much more than the optimum cooling requirement for flower initiation and the excessive amount of cooling retarded subsequent flower development.

A cooling treatment that precisely meets the saturated cooling for flowering helps to save the production cost in cooling without retarding flower development. When cooling has not been saturated, the stronger inducing cooling treatments (i.e., lower temperatures) accelerate flower initiation and differentiation, so flowers begin to develop and reach anthesis at an earlier time. When cooling has been saturated, continuously exposing the plants to low temperatures retards subsequent flower development. Therefore, by comparing the flowering speed among treatments, a saturated cooling for achieving the

best flowering performance (i.e., fast flower development with reasonable high flower count) was defined as follows.

From the time required to reach anthesis from completion of cooling, plants with various cooling durations all had delayed flowering at the lower 10 °C than 13 °C on one hand, but delayed flowering at the higher 18 °C than 15 °C on the other hand. This result shows that the shortest cooling duration at 10 °C had exceeded the optimum cooling. In addition, all durations at 18 °C did not reach the optimum cooling for fast flowering with high flower count, although 2 weeks at 18 °C was already beyond the threshold to trigger flower initiation. Comparing treatments at 13 or 15 °C, slightly less time (0.4 d) was needed for anthesis after 2 weeks of cooling at 13 °C than 15 °C. It indicates that the optimum cooling had not been saturated. Adversely, plants cooled at 13 °C took slightly more time (0.1 or 1.1 d) to reach anthesis after 3 or 4 weeks of cooling, and significantly more time (2.5 or 4.0 d) after 5 or 6 weeks of cooling than those cooled at 15 °C. It implies that 3 weeks at 13 °C had just saturated the optimum cooling and the excessive amount of cooling afterwards delayed the time to reach anthesis. Furthermore, since there was no significant difference in the total flower number, flowering nodes, or flower diameter when comparing the plants cooled at 13 or 15 °C for 3 weeks, a decision to use either 13 or 15 °C as the cooling temperature can be made according to minimizing the production cost in temperature control. As a result, 3 weeks at 13 or 15 °C is a

recommended cooling treatment that saves production cost without retarding flower development.

Manipulating temperature could be used to program flowering. Rotor Jr. (1952, 1959) found that *D. nobile* plants remained vegetative at 18 °C and flowers were produced when plants were at 13 °C regardless of daylength. Therefore, it was reported that flowering may be delayed by growing the plants at 18 °C and maintaining this temperature until about four months before the desired flowering date, and then the temperature should be dropped for flower initiation. Flower bud development may be hastened by using a higher temperature after the buds have been initiated (Rotor Jr., 1952, 1959). Such programming approach simply by using the inductive or inhibitive temperatures for flowering is easy to execute, but continuously maintaining a high temperature long after pseudobulb maturation to prevent flower initiation may cause matured axillary buds to form aerial shoots. It has been reported that high day temperature above 25 °C promotes aerial shoot formation in *D*. Snowflake 'Red Star' (Sinoda et al., 1988). From the current study in *D.* Sea Mary 'Snow King', using various temperatures from 10 to 18 °C effectively extended the time to reach anthesis for a period of 20 d. Consequently, using various temperatures from 10 to 18 °C that delay or accelerate flower development after initiation may be another way to program flowering. Such approach may be especially useful when commercial growers manage to meet a period of sustained market with a crop.

Results from regression analysis help to estimate an ideal cooling temperature and duration to manage the desired flowering dates. For example, if pseudobulbs of the plants are fully matured on 15 Oct. and growers expect to meet the market in 60 d for Christmas holiday, then the linear equations for the variable, time required to reach anthesis from beginning of cooling, should be substituted by $Y_T = 60$ (T = cooling temperature in °C) as following (X_T = cooling duration in weeks).

For 10 °C, $Y_{10} = 60 = 52.7 + 5.2\ X_{10}$, then $X_{10} = 1.4$ (weeks);

For 13 °C, $Y_{13} = 60 = 48.9 + 5.1\ X_{13}$, then $X_{13} = 2.2$ (weeks);

For 15 °C, $Y_{15} = 60 = 51.6 + 4.0\ X_{15}$, then $X_{15} = 2.1$ (weeks);

For 18 °C, $Y_{18} = 60 = 61.9 + 2.4\ X_{18}$, then $X_{18} = -0.8$ (weeks), not applicable. Consequently, growers can choose from the above cooling treatments at 10, 13, or 15 °C by evaluating the cost, availability of cooling space, and flowering quality to program flowering. However, it is worthy to note that growth and development likely will vary depending on cultivar, greenhouse environment, fertilization, season, etc.

From the results of this experiment, all cooling treatments were able to initiate flowering. The higher 18 °C cooling treatments caused a less desirable flowering quality with fewer flowering nodes and fewer total flowers, while the prolonged cooling durations resulted in slightly lower leaf retention. Three weeks at 13 or 15 °C is a recommended cooling treatment that saves production cost without retarding flower development in *D.* Sea Mary ‘Snow King’. However,

more or less amount of cooling that delays or accelerates flowering may help to program flowering to meet a specific market time.

CHAPTER V

EFFECT OF NUTRIENT RATE AND TERMINATION TIME ON GROWTH OF CUTTINGS FROM VARIOUS NODAL POSITIONS

Introduction

Nobile dendrobiums for pot-plant production are propagated mainly through single-node stem cuttings (Ichihashi, 1997). Experiments conducted by Bichsel et al. (2008) showed that nutrient rates of 0.1 to 0.2 g•L^{-1} N resulted in more nodes per pseudobulb at maturation in *D.* Red Emperor 'Prince', as compared with nutrient rates of 0, 0.05, or 0.4 g•L^{-1} N. From several studies in the nobile-type and phalaenopsis-type dendrobiums, results showed that plants had taller pseudobulbs with more nodes as the level of N in the nutrient supply increased (Miwa and Ozaki, 1975; Sakai et al., 1982; Uesato et al., 1987). Bhattacharjee (1981) also found that the vegetative growth of *D. moschatum* Wall., including number and height of pseudobulbs and number of leaf nodes, increased markedly with increasing N or P_2O_5 from 0 to 1 g•L^{-1}. Consequently, if nutrients are properly applied to obtain longer pseudobulbs, many more cuttings can be obtained in a given greenhouse bench area, resulting in lower cost of cutting production. However, it is not known how the nutrient rates during the previous growing season may affect the new growth and, possibly, subsequent flowering from the cuttings the following year.

Rotor Jr. (1952) indicated that the inflorescence primordia at the upper part and those nearest the basal part of the pseudobulb usually develop smaller inflorescences, while the lower-most bud may not develop flower primordia at all. Goh (1975, 1977a,b, 1979) also reported that for several *Aranda* hybrids (*A.* Deborah, *A.* Hilda Galistan, *A.* Lucy Laycock, *A.* Mei Ling, and *A.* Nancy) and *D*. Louisae 'Dark', a growth and flowering gradient existed that the developmental fates or growth rates of the nodes varied along the pseudobulbs. Buds near the apex developed into inflorescences or intermediate structures between inflorescence and vegetative shoots, while those situated further away from the apex developed into vegetative shoots. For the nobile dendrobiums, the single-node cuttings taken from the middle-part of the pseudobulbs for propagation may be more vigorous and therefore produce stronger plantlets than those taken from other parts. From previous experiments on *D.* Sea Mary 'Snow King' (Expts. 1, 2), the widest and thickest points of pseudobulbs were always measured at the middle-part of the pseudobulbs, averaging 2.3 cm in width and 1.8 cm in thickness (data not shown). Better understanding the behavior of cuttings from various nodal positions on nobile dendrobium pseudobulbs would help to grow uniform and quality crops for the best commercial value. The objectives of this study were to determine the optimum nutrient level for producing the highest amount of single-node cuttings and to investigate how the growth of new shoots would be affected by various nodal positions of the cuttings.

Materials and Methods

Plant material and growing conditions. A *Dendrobium nobile* Lindl. hybrid, *Dendrobium* Sea Mary 'Snow King', was used. One-year-old liners, propagated from single-node cuttings and planted in sphagnum moss with an average pseudobulb (i.e., a thickened portion of the stem in orchids functioning as a water and food storage device) height of 7 to 10 cm, were shipped from Yamamoto Dendrobiums in Mountain View, Hawaii. Plants arrived at Texas A&M University, College Station on 15 Feb. 2006 and were potted into 10.2 cm (top diameter, 414 mL vol.) standard green plastic pots on 16 to 18 Feb. and placed in a glass wall and polycarbonate roof greenhouse. The root substrate consisted of two parts of coarse peat (Sunshine Peat; Sun Gro Horticulture, Bellevue, Wash.), one part coarse perlite, and one part no. 3 grade diatomitic (Diatomite USA, Elma, N.Y.), amended with powdered Micromax (Scotts, Marysville, Ohio) at 1 $g \cdot L^{-1}$ as a source of micronutrients and powdered dolomite limestone at 5 $g \cdot L^{-1}$. A wetting agent, Aqua Gro 2000 G (Scotts, Marysville, Ohio), was added to the medium at 0.5 $g \cdot L^{-1}$.

Plants were potted with the root substrate packed tightly to secure them in place when lifted up only by the upper portion of the pseudobulbs. Immediately after potting, plants were irrigated with reverse osmosis (RO) water containing a fungicide (Banrot 40% WP; Scotts, Marysville, Ohio) at 0.6 $g \cdot L^{-1}$ to prevent root rot. Plants were spaced pot-to-pot in 30.8 x 51.4 cm molded carrying trays (4.00 Transport Tray (15); Landmark Plastic Corporation, Akron, Ohio) on the

greenhouse bench with the plant leaves orienting east and west to best capture sunlight. The newly grown pseudobulbs were staked upright with bamboo sticks (Bamboo Supply, Lakeland, Fla.) to minimize mutual shading. Whenever an undesirable second new pseudobulb emerged, it was removed to keep only one new pseudobulb per pot.

Greenhouse light level and air temperature at plant canopy level were recorded hourly with data loggers [HOBO (Onset Computer Co., Bourne, Mass.), WatchDog (Spectrum Technologies, Plainfield, Ill.), and Apogee line quantum sensors (Apogee Instruments, Logan, Utah)] (Appendix 1). Plants were grown in a warm environment (average 25.3 °C day/23.0 °C night) under an average 4.9 $mol \bullet m^{-2} \bullet d^{-1}$ daily light integral (*DLI*) until the pseudobulbs matured. Pots were irrigated as needed with RO water containing a 15N-2.2P-12.5K (Peters Excel 15-5-15 Cal-Mag; Scotts, Marysville, Ohio) water-soluble fertilizer at rates designated for each treatment. Plain RO water was used after nutrient application was terminated. Pesticides [*Bacillus thuringiensis israelensis* (Gnatrol), Valent BioSciences Coroperation, Libertyville, Ill.; Azadirachtin (Azatin) and Cyfluthrin (Decathlon), Olympic Horticulture Products, Mainland, Pa.; Imidacloprid (Marathon II), Olympic Horticulture Products, Mainland, Pa.] were applied when necessary to control fungus gnats, caterpillars, and mealy bugs, respectively.

Single-node cuttings from mature pseudobulbs were taken on 4 to 6 Dec. 2006 and immediately planted in Chilean sphagnum moss in 98-cell square plug

trays of 5-cm depth. Cuttings were planted in every other cell, wetted with plain RO water, and kept under a warm environment to prevent the lateral buds on cuttings from producing flowers and to promote vegetative growth. Since cuttings were propagated, the day/night air temperature was set at 25/20 °C and averaged 24.6/21.4 °C. The average recorded *DLI* was 5.3 $mol \cdot m^{-2} \cdot d^{-1}$.

Experimental design. The experiment was a 3 x 3 x 3 factorial with three nutrient rates, three nutrient termination times, and three nodal positions of the cuttings. A randomized complete block design with twelve replications was used. A single plant in a pot with one new pseudobulb constituted an experimental unit. A total of one hundred and eight vegetatively propagated nobile dendrobium plants were used. The nutrient termination times were 1 Aug., 1 Sept., and 1 Oct. 2006. The three nutrient rates were 0.33, 0.67, and 1.33 $g \cdot L^{-1}$ (50, 100, and 200 $mg \cdot L^{-1}$ N). The three nodal positions were the upper one-thirds, the middle one-thirds, and the lower one-thirds of the pseudobulbs.

Data collection. Data including plant height (measured from the medium surface to the tip of the pseudobulb), number of nodes, and number of remaining leaves on the pseudobulb were collected before propagation. New growth from the cuttings was then evaluated on 6 Mar. 2007. All cuttings were then destroyed.

Statistical analysis. All the data collected were analyzed with analysis of variance (ANOVA) and Duncan's multiple range test for significant differences,

all at $P \leq 0.05$. All statistical analysis was performed by SAS 9.1.3 statistical software (SAS Institute, Cary, N.C.).

Results

For the vegetative parameters collected before propagating cuttings, preliminary analysis could be used to predict the best nutrient application based on the fact that more nodes on pseudobulbs provided more usable single-node cuttings (Fig. 21). There was no significant interaction between nutrient rates and termination times to affect plant height, number of nodes and leaves (Table 14). When the 15N-2.2P-13K fertilizer was used at 0.33, 0.67 or 1.33 $g \cdot L^{-1}$ (N rate of 50, 100, or 200 $mg \cdot L^{-1}$, respectively), higher nutrient levels enhanced plant height, node number, and leaf number; whereas time of nutrient termination did not affect these variables in this experiment (Fig. 22). Plants that received nutrients at 0.67 or 1.33 $g \cdot L^{-1}$ produced two more nodes (an 18% increase) than those receiving 0.33 $g \cdot L^{-1}$. Therefore, nutrient supply at 0.67 $g \cdot L^{-1}$ could make the best use of a given bench area without wasting fertilizer in producing an increased number of single-node cuttings.

During this investigation, it was attempted to maintain the air temperature in the greenhouse above 25 °C day/20 °C night to avoid flower initiation. However, several cold fronts and ice storms caused difficulties in maintaining the desired temperatures. Unfortunately, brief periods of low temperature between Dec. 2006 and Feb. 2007 were critical to the morphogenesis of axillary buds, so a

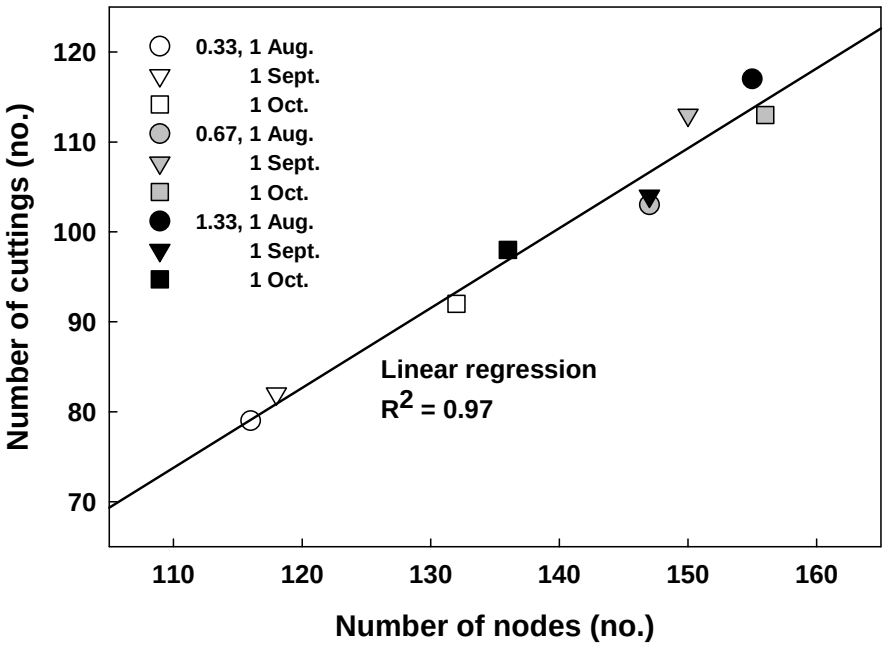

Fig. 21 Positive relationship between number of nodes on pseudobulbs and number of usable single-node cuttings obtained for propagation in *Dendrobium* Sea Mary 'Snow King'. Data points were for the total number of nodes or cuttings within various nutrient rate ($g \cdot L^{-1}$) and termination time treatments.

Table 14. ANOVA for the effect of nutrient rate and termination time on vegetative data of *Dendrobium* Sea Mary 'Snow King'.

Treatment	Plant height (cm)	Node number (no.)	Leaves remaining (no.)
Termination time (T)	NS	NS	NS
Rate (R)	***	***	***
T x R	NS	NS	NS

NS, *** Nonsignificant or significant at $P \leq 0.001$.

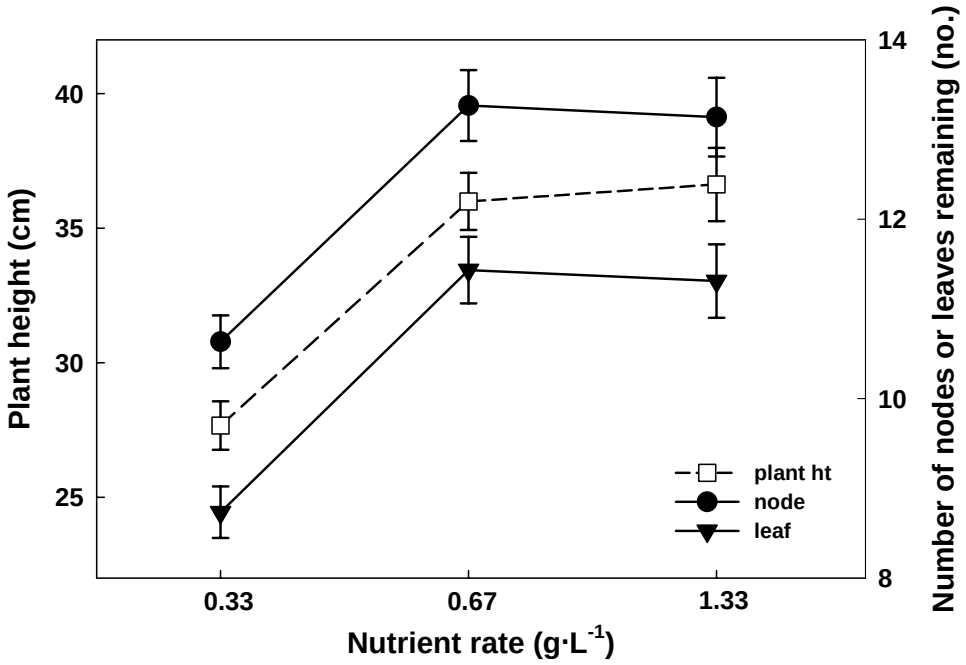

Fig. 22 Effect of nutrient rate regardless of nutrient termination time on plant height, number of nodes, and leaves remaining on pseudobulbs before single-node cutting propagation in *Dendrobium* Sea Mary 'Snow King'. Bars indicate ± SE of the mean.

significant portion of the buds on the cuttings failed to develop into desired vegetative shoots and produced flowers directly on the nodes instead.

On 6 Mar. 2007, four types of growth and development were observed on the cuttings including those producing vegetative shoots, opened flowers, aborted flowers, or without new growth (Fig. 23). The results showed that the longer the nutrients were supplied to the plant from juvenile to mature phase, the more single-node cuttings obtained from the plant grew into a vegetative shoot for propagation (8.0% to 20.8%; Fig. 24), the fewer cuttings transitioned to a

flowering node (35.5% to 20.5%), and the less flower abortion occurred (12.0% to 6.9%).

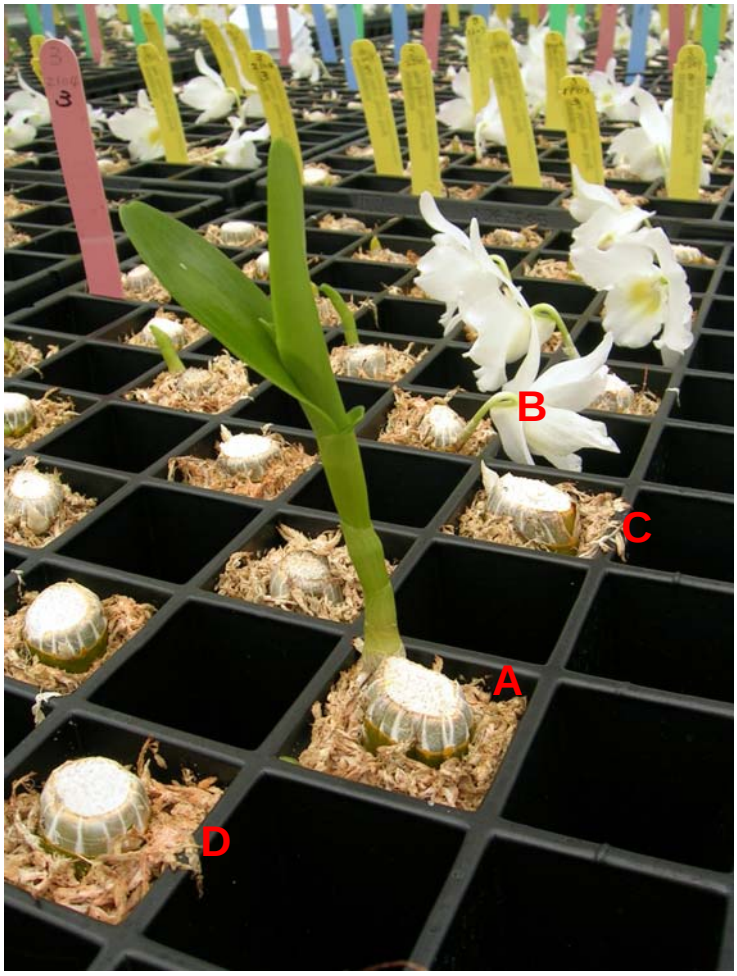

Fig. 23 Propagation by single-node cuttings of *Dendrobium* Sea Mary 'Snow King'. Four types, cuttings producing vegetative shoots (A), cuttings with opened flowers (B), cuttings with aborted flowers (C), and undetermined cuttings without new growth (D), are illustrated with uppercase letters. Photograph was taken in Mar. 2007.

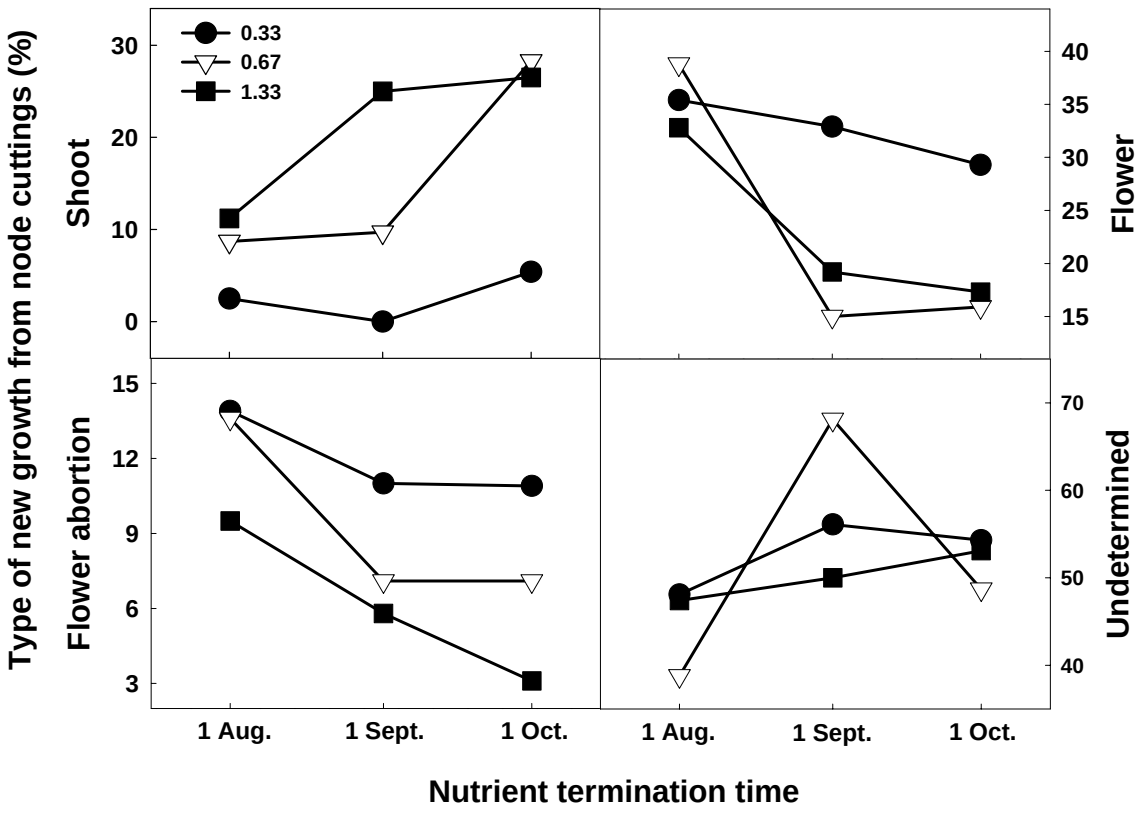

Fig. 24 Effect of nutrient rate and nutrient termination time on propagating single-node cuttings in *Dendrobium* Sea Mary 'Snow King'. Four types, cuttings producing vegetative shoots, cuttings with opened flowers, cuttings with aborted flowers, and undetermined cuttings without new growth, were recorded on 6 Mar. 2007 and interpreted in percentage of the node cuttings within each designated treatments. Nutrient rates ($g \cdot L^{-1}$) are denoted in the upper left corner.

Performance of the cuttings was also influenced by the nutrient rate. Cuttings obtained from plants fertilized at 0.33 $g \cdot L^{-1}$ grew into fewer vegetative shoots, produced more opened flowers, and had more aborted flowers than those obtained from plants receiving nutrients at 0.67 or 1.33 $g \cdot L^{-1}$. In general,

higher nutrient rate with prolonged supply facilitated the liner propagation. However, the above observation was concluded from only half of the cuttings because the other half abnormally stayed quiescent for three months without any new growth. The new growth may have been suppressed by the undesirable propagation environment, i.e., lower than optimal temperatures. Further statistical analysis thus could not be applied to the incomplete data.

Discussion

The growing temperature is critical in single-node cutting propagation. To effectively produce liners, each axillary bud on the single-node cuttings is expected to grow into a vegetative shoot. However, this goal can be achieved only if the propagation environment is strictly controlled to maintain a temperature above that which flower initiation would take place. In the previous experiment (Expt. 2), 2 weeks at 18 °C was enough to trigger the axillary buds of *D.* Sea Mary 'Snow King' to differentiate floral primordia. Therefore, in order to propagate cuttings successfully, the ambient temperature must be always kept high above 18 °C.

Adversely, Suto et al. (1984) disagreed with keeping cuttings always under high temperatures to promote vegetative shoot emergence. For three nobile dendrobium cultivars, *D.* Malones 'Fantasy', *D.* Golden Blossom 'Kogane', and *D.* Snowflake 'Red Star', the sprouting and mature plantlet ratios in stem cuttings were highest when the night temperature was 16.5 °C compared to 14, 19, or

22.5 °C with a day temperature at 25 °C (12 h each for day and night) during the propagation period (Suto et al., 1984). However, with daily temperatures of these treatments averaged 19.5, 21, 22, and 24 °C, it was possible that even if fluctuating temperature was as low as 16.5 °C, successful sprouting still could be achieved as long as the average daily temperature was kept high enough. The uncertainty on propagation temperatures should be clarified by further experiments.

Although our results were complicated by difficulties in maintaining the desired temperatures, the preliminary data still revealed some effect of nutrient rate and nutrient termination time on propagation. Nutrient supply has been shown to affect the growth of nodes on pseudobulbs. Bichsel (2006) reported that plants were taller and had more nodes when they were supplied with 0.1 or 0.2 g•L^{-1} N than those with lower or higher N rates. It has also been reported that N levels above 0.1 g•L^{-1} delayed the emergence of the last leaf in *D.* Malones 'Fantasy' and *D.* Snowflake 'Red Star' (Suto et al., 1984), so plants have longer time to produce more nodes on the pseudobulbs. Several other reports in *Dendrobium* also showed that increasing nutrient rates caused more leaf or node production (Bhattacharjee, 1981; Miwa and Ozaki, 1975; Sakai et al., 1982; Uesato, 1987). In the current study, plants that received nutrients at 0.67 or 1.33 g•L^{-1} produced more nodes than those receiving 0.33 g•L^{-1}. Therefore, more single-node cuttings can be obtained when growing plants with high rates of nutrient supply.

However, duration of nutrient supply until termination in this experiment did not affect the vegetative growth for subsequent propagation, which is different from the previous nutrient experiment (Expt. 1). The inconsistent results between the two experiments suggest that further investigation is required to verify the effect of nutrient termination time.

New growth from cuttings was also markedly affected by the nutrient supply as those emerge on intact plants. In *D.* Red Emperor 'Prince', plants receiving nutrients with a high N rate at 0.4 g•L^{-1} N until 1 Nov. had a high occurrence of aerial shoots in place of flower buds, resulting in 70% of the plants in the treatment having aerial shoots (Bichsel, 2006). A high N concentration inhibited the development of *Phalaenopsis* Pink Leopard 'Petra' floral buds in vitro (Duan and Yazawa, 1995). In *D.* Lim Hepa, a phalaenopsis-type dendrobium, longer stems or delayed flowering were also obtained with increasing N rates from 0 to 0.3 g•L^{-1} (Uesato et al., 1987). Furthermore, applying high N rate at 1 g•L^{-1} to *D. nobile* resulted in aerial shoot formation and a decrease in the percentage of flowering nodes (Miwa and Ozaki, 1975). These studies all pointed out that nutrient supply with high N content favored vegetative growth and adversely affected reproductive growth. In the current study, cuttings obtained from plants with increasing nutrient rate from 0.33 to 1.33 g•L^{-1} of 15N-2.2P-12.5K or prolonged nutrient supply produced more vegetative shoots, fewer flowers, and fewer aborted floral buds. Consequently, to successfully and efficiently produce

liners through single-node cuttings, it is necessary to provide sufficient nutrients, especially N, to grow the plants for propagation.

Many orchids exhibit a growth and flowering gradient of the axillary buds along the vegetative stem. Most of these gradients showed a higher potency of vegetative growth at the lower part of the stems. Goh (1975, 1977a,b) found that buds near the apex developed into inflorescences while those situated further away from the apex developed into vegetative shoots in *Aranda* hybrids, and the rate of development at the higher nodes was faster than lower nodes (Goh, 1977a). In *D.* Louisae 'Dark', a phalaenopsis-type dendrobium, the basal buds invariably developed into vegetative shoots, and the apical buds developed into inflorescences, vegetative shoots, or intermediate structures under certain conditions (Goh, 1979). In *Cymbidium* Astronaut 'Rajah', 73% of the vegetative buds grew from the bottom two nodes of the pseudobulb while only 36% of reproductive buds did so (Powell et al., 1988). Additionally, Rotor Jr. (1952) also reported that the inflorescence primordia in *Dendrobium* at the apical and basal part of the pseudobulb usually developed smaller inflorescences than those at the middle, while the lower-most bud primordia may not develop at all. It is possible that a similar development gradient may also exist in vegetative primordia as was seen in the flower primordia. However, if the propagation temperature would have been ideally controlled for stem cuttings to successfully produce new growth, the fact that new growth follows a particular pattern based on their nodal positions may possibly have been observed.

To summarize, high rate of nutrient supply at 1.33 $g \cdot L^{-1}$ helped to grow plants with more nodes for single-node cutting propagation and promoted vegetative growth from the cuttings. However, since the uncertainty on using high or low propagation temperatures still needs to be clarified, effect of temperature for cutting propagation should be re-examined. Inconsistent effects of nutrient termination on the vegetative growth between the Expt. 1 and 3 also suggest that various nutrient termination times should be included in future studies. Further well-structured experiments are definitely needed to better understand the growth of cutting propagation in nobile dendrobiums.

CHAPTER VI

SUMMARY OF FINDINGS

- Interaction between nutrient termination time and reapplication stage on growth and flowering was non-significant, with more significant effect of nutrient termination time than reapplication stage.
- Regardless of nutrient reapplication stages, nutrient termination on 1 Oct. caused taller plants with more nodes, more leaves remaining on pseudobulbs, more flowering nodes, more total flowers, and fewer aborted floral buds than those being terminated on 1 Aug. or 1 Sept.
- Plants that were terminated of nutrients on 1 Oct. had higher leaf retention (82%) on the pseudobulbs than those terminated earlier (74%) at the time of flowering, indicating that terminating nutrient supply at an earlier stage caused more defoliation. Plants subjected to longer periods of cooling also had a tendency to defoliate slightly more than those with shorter durations.
- Prolonged nutrient supply during vegetative growth did not lead to differences in time required for anthesis after the completion of cooling treatment, whereas extending nutrient supply slightly extended the time that plants needed to reach full flowering after cooling.
- Only buds protruding more than 2 mm from the pseudobulb surface showed differentiated floral structures: three to four less-than-perfect dome-shape floral meristems were each embraced by bract primodium, and cells in the

actively differentiating tissues had thin, smooth cell walls and were full of cytoplasm instead of vacuoles. Flat axillary buds only showed a single dome-shape vegetative apical meristem in the center with several layers of leaf primordia embracing it.

- Plants with 1 Aug. nutrient termination time had larger flower primordia than those with 1 Oct., indicating flower differentiation commenced earlier or faster at an earlier nutrient termination time.
- No reversion of reproductive to vegetative buds arose due to either late nutrient termination or resumption of nutrient supply during the early stages of cooling.
- Interactions between temperature and cooling duration were significant on time to anthesis and full flowering, recorded from either beginning or completion of cooling, average flower number per flowering node, and flower diameter.
- Increasing cooling duration from 2 to 6 weeks led plants to reach anthesis and full flowering faster from completion of cooling; however, the increasing cooling duration actually extended total time required from the beginning of cooling to anthesis and full flowering. The differences in time required to reach anthesis or full flowering were caused by the speed of flower differentiation and faster flower development before anthesis, not at the final stage from anthesis to full flowering.

- For cooling durations of 2 to 4 weeks, plants that were cooled at 13 or 15 °C required the least amount of time to reach anthesis and full flowering than 10 or 18 °C upon the completion of cooling. For 5 or 6 weeks of cooling, plants reached anthesis and full flowering faster following treatment at 15 or 18 °C than at 10 or 13 °C.
- The well-fitted linear regression model for each of the four variables, time to anthesis or full flowering from completion of cooling, or time to anthesis or full flowering from beginning of cooling, can be used for future prediction in time to flower and thereby programming flowering.
- All plants in the nutrient application experiment and the cooling experiment except for the plants in the observational control group were able to flower without producing any aerial shoots.
- The least amount of cooling, 2 weeks at 18 °C, was enough to trigger flower initiation.
- Increasing temperature from 10 to 15 °C accelerated flowering after cooling with decreasing uniformity in flowering speed.
- Plants had more flowering nodes and total flowers when cooled at 10, 13, or 15 °C than at 18 °C.
- To produce high quality flowering plants that require less time to reach flowering, terminating of nutrients on 1 Oct. and subjecting plants to 3 weeks of cooling at 13 or 15 °C are recommended.

- Plants fertilized with 15N-2.2P-13K at 0.67 or 1.33 g•L^{-1} had more nodes (an 18% increase), more leaves, and taller plant height than those receiving 0.33 g•L^{-1}. Therefore, nutrient supply at 0.67 g•L^{-1} could make the best use of a given greenhouse bench area without wasting fertilizer in producing an increased number of single-node cuttings.
- High nutrient rate with prolonged supply to the plants from juvenile to mature phase caused more single-node cuttings obtained from the plants to grow into vegetative shoots for propagation, fewer cuttings to transition to flowering nodes, and less flower abortion to occur.

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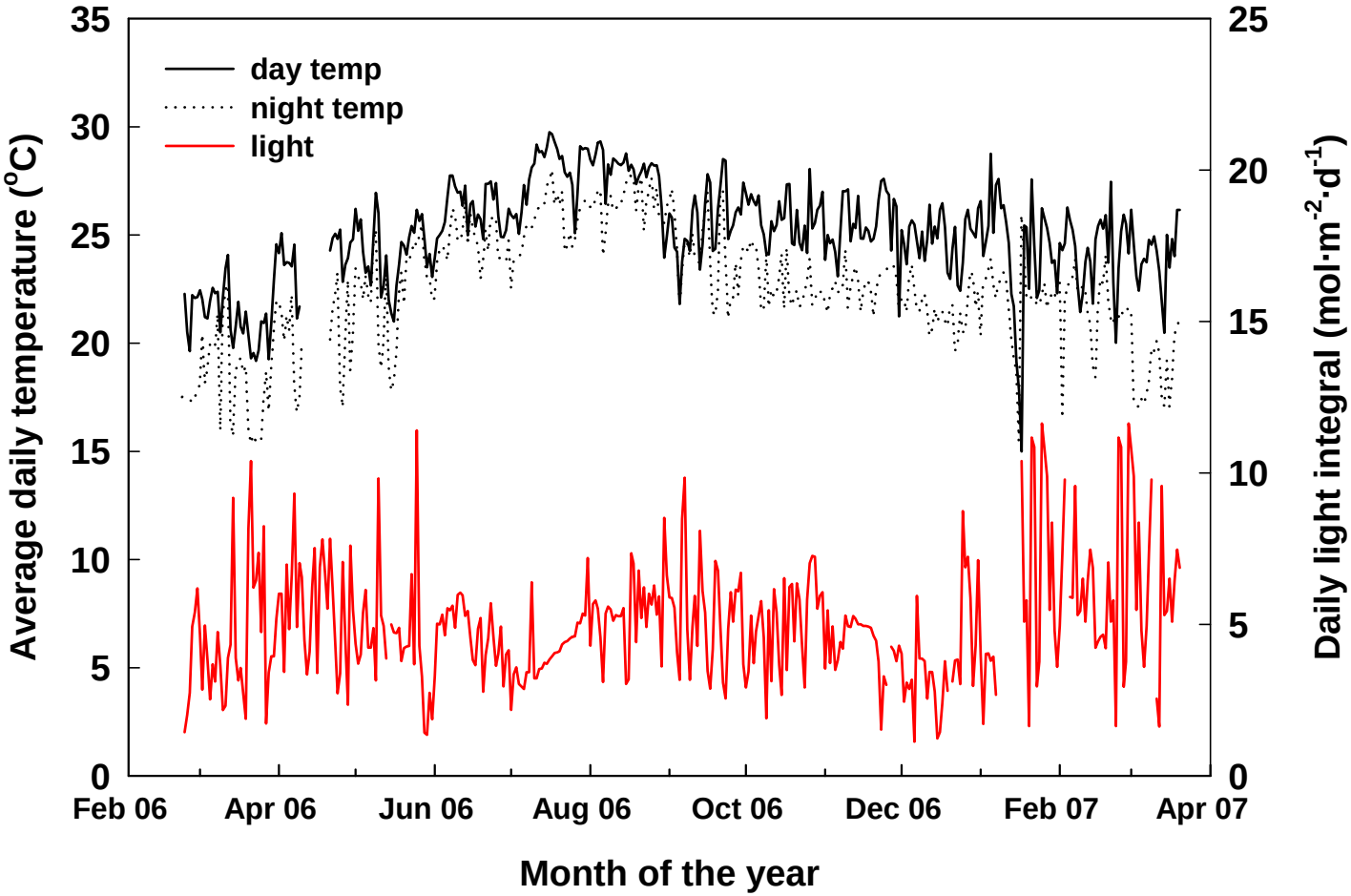
APPENDIX



A-1. Daily light and air temperature data recorded in the greenhouse throughout the research time period.

A-2. Effect of nutrient reapplication stage regardless of nutrient termination time on leaves remaining of *Dendrobium* Sea Mary 'Snow King'.

Nutrient reapplication stage (relative to cooling)	Leaves remaining (no.)
1 (beginning)	9.1 a[z]
2 (middle)	8.3 b
3 (immediately after)	9.0 a
4 (2 weeks after)	8.2 b
5 (no reapplication)	8.6 ab

[z]Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

A-3. Effect of nutrient rate and termination time on plants producing single-node cuttings in *Dendrobium* Sea Mary 'Snow King'.

		Type of new growth from cuttings								
Nutrient rate ($g \cdot L^{-1}$)	Nutrient termination time	Shoot		Flower		Flower abortion		Undetermined		total
		no.	%	no.	%	no.	%	no.	%	no.
0.33	1 Aug.	2	2.5	28	35.4	11	13.9	38	48.1	79
	1 Sept.	0	0.0	27	32.9	9	11.0	46	56.1	82
	1 Oct.	5	5.4	27	29.3	10	10.9	50	54.3	92
0.67	1 Aug.	9	8.7	40	38.8	14	13.6	40	38.8	103
	1 Sept.	11	9.7	17	15.0	8	7.1	77	68.1	113
	1 Oct.	32	28.3	18	15.9	8	7.1	55	48.7	113
1.33	1 Aug.	13	11.2	38	32.8	11	9.5	55	47.4	117
	1 Sept.	26	25.0	20	19.2	6	5.8	52	50.0	104
	1 Oct.[z]	26	26.5	17	17.3	3	3.1	52	53.1	98

[z]Cuttings were obtained from twelve plants in each treatments with an exception of eleven plants in the denoted treatment.

A-4. Effect of nutrient rate regardless of nutrient termination time on plant height, node number, and leaves remaining on pseudobulbs before single-node cutting propagation of *Dendrobium* Sea Mary 'Snow King'

Nutrient rate ($g \cdot L^{-1}$)	Plant height (cm)	Node number (no.)	Leaves remaining (no.)
0.33	27.7 b[z]	10.6 b	8.7 b
0.67	36.0 a	13.3 a	11.4 a
1.33	36.6 a	13.1 a	11.3 a

[z]Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

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