EFFECTS OF VERNALIZATION DURATION,

LIGHT INTENSITY DURING VERNALIZATION

AND LOW TEMPERATURE HOLDING AFTER VERNALIZATION

ON FLOWERING OF NOBILE DENDROBIUM HYBRIDS

A Thesis

by

MIN LIN

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

May 2011

Major Subject: Horticulture

Effects of Vernalization Duration, Light Intensity during Vernalization and Low Temperature Holding after Vernalization on Flowering of Nobile Dendrobium Hybrids

EFFECTS OF VERNALIZATION DURATION,

LIGHT INTENSITY DURING VERNALIZATION

AND LOW TEMPERATURE HOLDING AFTER VERNALIZATION

ON FLOWERING OF NOBILE DENDROBIUM HYBRIDS

A Thesis

by

MIN LIN

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

Approved by:

Chair of Committee,	Terri W. Starman
Committee Members,	Yin-Tung Wang
	Genhua Niu
	Tom Cothren
Head of Department,	Tim Davis

May 2011

Major Subject: Horticulture

ABSTRACT

Effects of Vernalization Duration, Light Intensity during Vernalization and Low Temperature Holding after Vernalization on Flowering of Nobile Dendrobium Hybrids. (May 2011)

Min Lin, B.S., Beijing Normal University

Chair of Advisory Committee: Dr. Terri W. Starman

Flowering time and flower quality of three nobile dendrobium hybrids in relation to vernalization duration and light intensity during vernalization were studied in the first experiment. Mature *Dendrobium* Red Emperor ‘Prince’, *Dendrobium* Sea Mary ‘Snow King’, and *Dendrobium* Love Memory ‘Fizz’ were cooled at 10 °C with 300 to 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux (*PPF*) (12-h photoperiod) or darkness, each with four cooling durations (2, 4, 6, or 8 weeks). Plants were forced in a greenhouse after vernalization. At least 4 weeks of 10 °C cooling in light was needed for flower initiation of Red Emperor ‘Prince’; whereas Sea Mary ‘Snow King’ and Love Memory ‘Fizz’ only needed 2 weeks of 10 °C cooling regardless of light. Darkness during vernalization slightly delayed flowering and resulted in fewer but larger flowers. Longer cooling duration delayed flowering, decreased the flower longevity, and produced larger and more flowers. In the second experiment, Love Memory ‘Fizz’ were cooled at 15 °C for 4 weeks with *PPF* of 0, 50, 100, or 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (12-h photoperiod). Compared to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, low *PPF* of 50 or 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ did not affect flowering time or flower quality; however, darkness delayed flowering and reduced flower quality. The third experiment was aimed at developing a strategy to defer flowering of nobile dendrobium orchids by holding them under low temperature. Mature *Den.* Red Emperor 'Prince' and *Den.* Sea Mary 'Snow King' were held at 10 °C for various durations (0, 4, 8, 12 or 16 weeks) after vernalization (4 weeks at 10 °C). Plants were forced in a greenhouse after holding. Time to flowering, flower differentiation and flower quality were determined. Increase of low temperature holding duration from 0 to 16 weeks extended time to flowering up to 3 months and did not affect parameters of flower except producing larger flowers and reducing flower number per flowering node for *Den.* Red Emperor 'Prince'. Notably, the flower longevity was not adversely affected. Defoliation was aggravated in *Den.* Red Emperor 'Prince' by longer duration of cooling and was considered a detrimental effect of low temperature holding.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the members of my thesis committee.

Dr. Terri Starman, you have been such a wonderful advisor, helping me through several difficult decisions, almost every presentation, and of course, the writing and defense of this thesis. Thanks for accepting me into this program; your encouragement, support and understanding at the beginning of my studies gave me the time I needed to get familiar with an area which was completely new for me. I enjoyed every course you teach. They were so interesting and broadened my vision, and I really wish I could take them again.

Dr. Yin-Tung Wang, you have been most encouraging during my adventures in horticulture. You had all the answers for my questions, but you always led me to find them by myself. Thank you for sharing all of your knowledge with me about taking plant pictures, making slides and improving my written and spoken English. Visiting Matsui Nursery and seeing how you apply research findings to commercial production was the most exciting experience for me. Your way of guidance showed me not just how well you know and love orchids, but how much you care for training your students. The experience I gained under your guidance will benefit me throughout my career.

Dr. Genhua Niu, you have been so patient and helpful, and you are more like a friend to me. You provided great advice and always made time for me. Thanks for all the encouragement, the thorough review of my manuscripts and thesis, and the constructive criticism. Your expertise and helpful advice have been invaluable for me.

Dr. J. Tom Cothren, you have been a joyful member of this committee. Thank you very much for recommending great classes for me, for always giving an encouraging word and for coming all the way to the Horticulture building for all the meetings.

My sincere gratitude also goes to Kristen Eixmann. You have been always willing to help me on greenhouse and growth chamber procedures, statistics and everything else. It would be impossible to make it through without your help.

Special thanks to Christine Yung-Ting Yen. I would never be able to transfer to this amazing major without you.

I would like to express my deepest gratitude to my parents and my husband who have been so incredibly encouraging and supportive during my entire graduate study. Without your love and support, I am not sure how I would be fairing at the moment. I love you all!

TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Orchids have become the most valuable potted flowering crop in the United States with a reported wholesale value of $160 million in 2009, up 26 percent from the previous year. About 19.5 million potted orchids were sold in 2009 with a mean wholesale price of $8.20 per pot (USDA, 2010). Although *Phalaenopsis* remains the most popular potted orchid sold, the types of orchids on the market are becoming more diversified, and commercial growers are looking for other orchids that have consumer appeal, can be grown and sold at mass markets and programmed into flower for specific markets dates.

Dendrobium Nobile

Dendrobium nobile is an Asiatic orchid species that is native to the seasonal deciduous forest at Burma, India, Thailand, and Indochina. Plants grow epiphytically on trees from the lowlands all the way up to the cool highlands of the Himalayas at elevations of 1,200 m, often in full sun (SOC, 2009b; Yamamoto, 2010c). There are two seasons in these areas – the dry and the rainy; and the *Dendrobium nobile* survives

This thesis follows the style of Journal of the American Society for Horticultural Science.

during the dry season although there is virtually no rain for more than four months (Yamamoto, 2010c). *Dendrobium nobile* is tolerant of warm, downright hot or enduring freezing temperature (SOC, 2009b; Yamamoto, 2010c). This species has pseudobulbs which can be up to 60 cm long and swollen at the nodes. They can be divided into two groups based on the size of the pseudobulb: tropical type (big pseudobulb, tetraploid) and winter type (small pseudobulb, diploid). Strap-shaped leaves are alternate along the pseudobulb, and are deciduous, dropping some or all of their leaves in the winter. An outstanding characteristic of this particular species is that flowers are produced at each node alternately on opposite sides of the pseudobulb almost simultaneously. The waxy flowers have sweet fragrance but their natural color spectrum is meager (Fulcher, 2003; Lavarack et al., 2000; SOC, 2009a) .

In nature, vegetative growth of *Dendrobium nobile* begins between December and January of each year, producing a new pseudobulb at the base of the old pseudobulb. The new pseudobulb matures following one year of growth. Different from most other tropical and subtropical orchids, *Dendrobium nobile* must be exposed to moderately low temperature to induce flowering. After going through adequate cooling, which usually occurs in the fall, winter or following sudden rainstorms, *Dendrobium nobile* normally flower in late winter or spring (Lavarack et al., 2000; Rotor, 1952). During cooling, if kept dry, this species can survive winter temperatures of 3 to 4 ºC and flower in April. If temperatures are maintained at 17 to 18 ºC as soon as buds appear, plants will flower in January or February (Yamamoto, 2010b).

Dendrobium Nobile Hybrids (the Nobile Dendrobium/the Nobile-Type Dendrobium)

The nobile dendrobium has been grown for many years in the history of orchid cultivation. The amount of production by commercial growers around the world has increased greatly in recent years (Wang and Starman, 2008). Bred mainly from *Dendrobium nobile*, the nobile dendrobium was developed by the European and Japanese orchid breeders during the last 100 years. As a result, the nobile dendrobium today has all the good characteristics of color, color combination, flower shape and size, plant height and strong stems (Yamamoto, 2010c). Flowers vary in color from white, yellow and pink, to many shades and bi-colored combinations in between, and have become one of the most colorful orchid in the world (SOC, 2009b). A dozen or more sprays of flowers can open on a plant at once and they are fragrant (Fulcher, 2003).

Since 1957, Jiro Yamamoto began his personal challenge to improve the quality of *Dendrobium nobile* by hybridizing. He observed that the tetraploids were healthy, fast growing plants that were resistant to insects and diseases and most of them produce larger blooms (Teoh, 2005). Working with polyploid parents, he created about 4000 kinds of nobile dendrobium over 50 years, and The Royal Horticulture Society called his nobile hybrids “the Yamamoto type”. Nowadays, Yamamoto dendrobiums are famous throughout the world as a class of their own. These hybrids are vigorous and easy to grow. They can simultaneously produce 40 to 50 thick-textured and brightly colored flowers on one plant and the flowers can last from one to two months in cool temperatures. (SOC, 2009a; Yamamoto, 2010a)

Most of the commercially produced nobile dendrobiums come from Yamamoto Dendrobiums in Hawaii. A schedule for growing two-year-old potted flowering nobile dendrobium has been used in commercial production based on the natural growth pattern of *Dendrobium nobile* (Fig. 1). In February, one-year-old liners are transplanted and grown under a warm environment to produce new pseudobulbs. One or two new shoots emerge from the base of the liners and grow into new pseudobulbs until August or September when a terminal leaf forms at the apical node. After terminal leaf formation, pseudobulbs stop elongation and begin to swell (increase in diameter) until fully mature (when tips of pseudobulb become round and hard) in October or November. Mature pseudobulbs are then subjected to cooling for flower induction (vernalization). After adequate cooling, plants are forced under warm conditions and reach flowering in February or March (Yen, 2008).

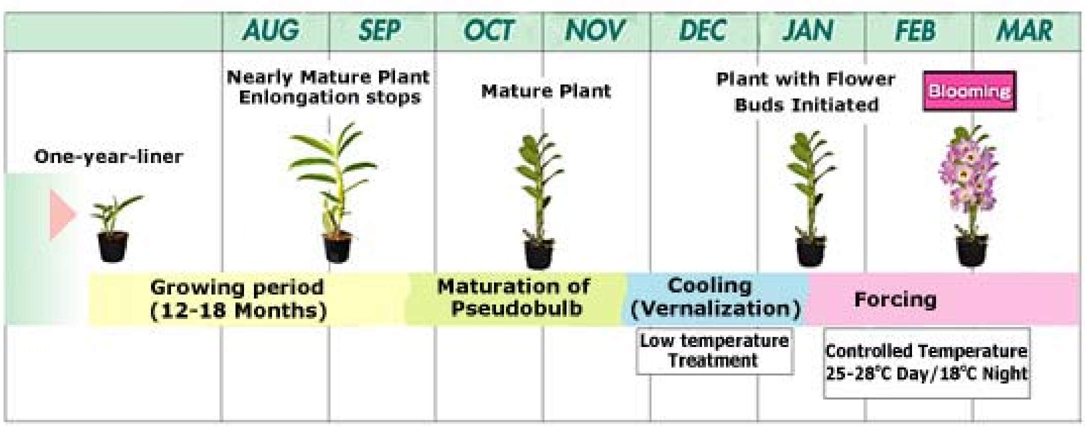

Fig. 1. Culture cycle for nobile dendrobium (Yamamoto Dendrobiums, 2010).

Presently, production of young orchid plants is dominated by the Asian countries of Taiwan and Thailand, while finished plant production is concentrated in China, Europe, Japan, Taiwan, and the United States. In most areas of Europe, Japan and the U.S., winters are too cold for the production cycle of most orchids and require heating during the winter that results in high cost for producing orchid. However, as the hybrids from Asian wild tropical plants and temperate plants (such as Japanese wild orchids), some nobile dendrobium are hardy enough to withstand extremely cold but not freezing temperatures and can also grow in warm to hot climates. They can survive in temperatures down to 0 ºC if the potting medium is dry. Since they need about one month of cooling for flower initiation, the grower can keep them in the greenhouse during winter with lower heating cost. In addition, the plants grow upright and require less space, so more plants can be grown in a given area which lowers the cost of production. (SOC, 2009a; Yamamoto, 2010c)

Considerable progress has been achieved in the understanding of flowering in orchids. Most of the published reports focused on flower induction for *Phalaenopsis* and *Cymbidium*. Some research on nobile-type dendrobium was conducted in Japan more than 30 years ago, and cultivation and flowering control may have been studied in some commercial nurseries, but the findings are usually not published. Thus, more extensive research is needed before we can develop a commercially sound and viable method for control flowering of the nobile dendrobium.

Cultivation of the *Dendrobium Nobile* Hybrids

The most common problem with the nobile dendrobium is poor flowering and the appearance of aerial shoots (keikies) in spite of robust growth (Yamamoto, 2010b). Precautions should be taken to avoid the problem.

Light and air movement

During the pseudobulb thickening phase, the nobile dendrobium should be grown in full sun with good air ciculation to produce strong pseudobulbs (Zou and Liu, 2010). As tropical orchid hybrids, the nobile dendrobium is indifferent to daylength (Hew and Yong, 2004). Photoperiodism does not seem to exist in *Den.* Nodoka, and high light intensities during flower initiation were not necessary for *Den.* Nodoka and *Den.* Snowflake ‘Red Star’. (Higuchi et al., 1974; Sinoda et al., 1984) During the flowering season, if ventilation is inadequate, buds may be damaged, and flowering will be poor. Therefore, Yamamoto Dendrobiums in Hawaii (2010b) recommendes 30%-40% shade from the time flower buds appear until the end of the flowering season, if the temperature is high and sunlight is strong.

Temperature (vernalization)

The nobile dendrobium must be exposed to low temperature for flower differentiation. In Japan, the nobile-type dendrobiums are moved to the mountains after summer to provide chilling below 15 ºC for early flower bud differentiation. Lack of a low temperature period causes blind buds and no flower formation (Ichihashi, 1997). High temperatures inhibite flower bud development, reduce the number of flower buds, and induce the formation of aerial shoots (Sinoda and Suto, 1988). The critical and optimum temperature for flower bud induction varies among cultivars (Ichihashi, 1997), but Sinoda et al. reported that day temperatures above 25 ºC inhibited flower initiation for all cultivars (1984; 1985). In addition, the length of time required for vernalization differs depending on cooling temperature and cultivar. In order to produce 3 mm long flower buds, which indicating the termination of flower induction, *Den.* Snowflake ‘Red Star’ required 60 d of chilling in June or July; however, in September, only 40 d of chilling is enough (Sinoda et al., 1984). Yen et al. (2008b) found that two weeks at a constant 10, 13, 15 or 18 ºC was enough for complete flower initiation for *Den.* Sea Mary ‘Snow King’; however, some nodes failed to initiate flowers and the flower buds on some nodes aborted when cooled at 21 ºC. If the cooling temperature becomes high, a longer cooling duration may be needed to induce complete flower initiation (Yen et al., 2008b).

Pseudobulb maturity

The nobile dendrobium has a juvenile phase during which flowering cannot be induced by any treatment(Hew and Yong, 2004). Thus, cooling is effective for mature pseudobulbs. If cooling started from the beginning of August, only primary shoots (the liners) flowered; however, both primary and secondary shoots (new pseudobulbs) flower if cooled after September (Higuchi and Hara, 1973; Suto and Tutui, 1980). Sakanishi and Fuziwara (1982) found that buds on immature pseudobulbs stayed dormant and did not respond to low temperature. Longer periods of growth after terminal leaf formation, which produced more mature pseudobulb, had a favorable effect on flower bud differentiation. However, recent study found that single foliar application of 1000 $mg \cdot L^{-1}$

6-Benzylaminopurine (BA) on immature pseudobulbs of *Den.* Red Emperor 'Prince' and *Den.* Oriental Smile 'Fantasy' induced lateral buds to swell, elongate, and develop into advanced flower buds at 30 ºC day/ 25 ºC night, which is completely non-inductive for flowering (Wang et al., 2010).

Nutrient application

Commercial growers apply nutrients early in the growing season. Bichsel et al. (2008) reported that *Den.* Red Emperor 'Prince' needed 100 $mg \cdot L^{-1}$ of nitrogen (N), no more than 25 $mg \cdot L^{-1}$ of phosphorus (P), and 100 $mg \cdot L^{-1}$ of potassium (K) during the period of active vegetative growth for best flowering. The most common cause of poor flowering in nobile dendrobium was said to be the accumulation of too much N, thus, fertilizers with a low percentage of N are recommended and slow-release fertilizers (e.g., Osmocote) should be avoided (Yamamoto, 2010b). N higher than 200 $mg \cdot L^{-1}$ promoted formation of aerial shoot and delayed terminal leaf formation (Ichihashi, 1997). It is generally believed that extended application of N promotes the formation of aerial shoots instead of flower buds, and results in total loss in commercial production. Thus, Yamamoto Dendrobiums Hawaii (2010b) suggested that nitrogen should be given only at the beginning of new shoot growth, and growers should stop application of any fertilizer after plant maturation to avoid aerial shoots formation. However, Bichsel et al. (2008) found that if the nobile dendrobium received adequate cooling, non-stop and continuous applications of nitrogen through flowering did not result in the formation of aerial shoots. Growers believed that resumption of nutrient supply is needed at a later time for flower development since reproductive growth may present a strong nutrient sink. However, Yen et al. (2008a) reported that re-applying fertilizer during cooling or during flower bud development is not necessary because it does not improve flower number or size.

Control of Flowering

An important aspect of commercial orchid production is to have coincide with the market demand, which can increase their profitability (Hew and Yong, 2004). In Japan, the nobile dendrobiums are sold mostly in December, January, and February (Yamamoto, 2010c). However, in the U.S., it would be beneficial if the flowering season coincided with holidays such as Christmas, New Year, Valentine's Day or Mother's Day. Most nobile dendrobium hybrids bloom in February or March, and become limited in supply by May when market demand for spring and Mother's Day is strong. Usually, mature nobile dendrobium need one month of cooling for flower initiation and an additional two to three months for complete flowering (floral development) depending on the temperature plants are exposed to. The whole process spans three to four months, which could be manipulated in commercial production (SOC, 2009a).

Flowering of orchids can be separated into two processes, flower induction (flower initiation) and floral development. Flower induction is usually influenced by genetic, physiological or environmental factors, such as low temperature or water stress; whereas, the subsequent growth of flower buds (floral development) mainly depends on the supply of photoassimilates (Hew and Yong, 2004) and air temperature. From a practical point of view, environmental control of flowering holds considerable potential.

Vernalization—Response to low temperature

Some species of orchids need a period of vernalization after shoot maturation to induce flowering. During the period of vernalization, growers can manipulate the temperature, the time of initiation, or its duration, as well as the difference in day/night temperature to program flower initiation.

The fact that flowering can be induced by low temperature has contributed significantly to the large-scale production of *Phalaenopsis* potted plants in Taiwan and Japan. Lin and Lee (1984) showed that day/night air at 25/20 ºC or 20/15 ºC triggered 100% spiking of *Phalaenopsis* in 4 and 5 weeks, respectively. However, spiking and flowering did not occurred if plants were maintained at ≥28 ºC daily (Yoneda et al., 1991). Wang et al. (2006) reported that various day/night temperature combinations might inhibit spiking of *Phalaenopsis* effectively; however, it is cultivar dependent. For example, 12/12 hr (12-hr day/12-hr night) 30/14 ºC day/night temperature inhibited spiking of *Phalaenopsis* Joseph Hampton 'Diane'. *Phalaenopsis* 'B79-23' did not spike when kept at 12/12 hr 28/16 ºC day/night temperature. 12/12 hr 28/23 ºC day/night temperature was effective in complete inhibition of spiking of *Phalaenopsis* Taisuco Firebird 'DL18'. In commercial production, spiking in *Phalaenopsis* needs to be deferred from early fall to January or February or even later to have plants in flower for the Mother's Day and summer market (Wang et al., 2006). Thus growers in Japan and Taiwan heat their greenhouses to 28 ºC or higher to inhibit spiking in fall (Sinoda, 1994), regardless of the fact that energy costs are considerably high during this period of time.

Higuchi and Hara (1973) found that artificial chilling (which usually occurs naturally after September) from the beginning of August advanced the flowering season of the nobile dendrobium by more than 30 days. In order to advance the flowering time of nobile dendrobium to New Year's or Chinese New year (January to February), the Chinese growers usually start to cool nobile dendrobium from late September or late October with night temperature of 9 to 13 ºC, to accumulate at least 400 hours for complete flower induction (Li and Wang, 2005). Cooled below 13 ºC, *Dendrobium nobile* flowered in February or March, too early for Easter. Plants kept under 18 ºC for 4 months prior to being induced to initiate flower buds, could be delayed to the desired dates (Rotor, 1952). Yen et al. (2008b) reported that lower temperature and shorter induction durations (two weeks) accelerated flowering. For instance, counting from the day cooling starts, *Den.* Sea Mary 'Snow King' cooled at 18 ºC for three weeks took 93 days whereas those cooled at 15 ºC for the same duration needed only 80 days to reach flowering. Plants that were cooled at 15 ºC for six weeks took 75 days and those cooled for two weeks needed only 60 days to reach flowering.

Light intensity

Response to low temperature in orchid is further complicated by an interaction between temperature and light, thus light intensity can also be used to manipulate the time of flowering.

Wang (1995) reported that *Phalaenopsis* required photosynthetic photon flux (*PPF)* exceeding a threshold level (some level above 8 and below 60 $\mu mol \bullet m^{-2} \bullet s^{-1}$) for spiking while they were subjected to inductive temperatures. However, plants that were exposed to low *PPF* (8μmol•m^{-2}•s^{-1}) or in complete darkness did not spike under otherwise similar conditions, but remained healthy. In another experiment, after being subjected to inductive temperature with complete darkness for 2, 4, or 6 weeks and the following 6 weeks of 160 μmol•m^{-2}•s^{-1} *PPF*, spiking and anthesis of *Phalaenopsis* were deferred by exactly the length of time that plants were held in darkness. Wang (1998) also found that alternating darkness (4 d) and light (3 d) in weekly cycles completely inhibited spiking of *Phalaenopsis* TAM Butterfly, and was able to defer spiking from late September to mid-March. A large-scale test in three commercial nurseries in Taiwan showed that heavy shading (5 d) alternating with light (2 d) was an effective alternative to heating for inhibiting *Phalaenopsis* spiking in commercial operations (Wang et al., 2006). Compared to heating, this new method was more low-cost and not cultivar dependent.

Kubota et al. (2005) and Yamaguchi et al. (1977) found that low light intensity decreased the flower bud formation of *Odontioda* and *Cymbidium*. Rotor (1952) found that the degree of flower response of cymbidiums to low temperature (21 ºC) was determined by light intensity; however, low temperature induction of flowering in *Dendrobium nobile* was not affected by light.

Besides flowering time, light intensity during flower initiation affected the flower or plant quality. McConnell et al. (1990) observed a correlation between light intensity and flowering in *Dendrobium* Jaquelyn Thomas with more flowers produced in high irradiance. *Vanda* Miss Joaaquim and *Arachnis* Maggie Oei required extended periods of full sunlight to flower (McConnell et al., 1990), while *Oncidium* Goldiana showed reduced flowering under high irradiance (Ding et al., 1980).

Plant Growth Regulators (PGR)

Application of 400 mg•L^{-1} BA to nobile dendrobium at the beginning of flower induction increased the number of flower buds and reduced the inhibitory effects of high temperature (Sinoda et al., 1985). Under warm night temperature (18 to 22 ºC) in south China, 200 mg•L^{-1} paclobutrazol (PP_{333}) + 200 mg•L^{-1} thiazolidinediones (TDZ) media drench caused 84% flower bud formation rate on *Den.* Wardianum Warner, 30% higher than that of the control group. Also, the plants that received the PGR treatment flowered 20 d earlier than the control (Wang et al., 2008). Qian et al. (2009) reported that application of 1000 mg•L^{-1} 6-BA on *Den.* Pretty Ribbon 'Loura' promoted flower bud formation and flower development and at the same time increased flower bud number. Various concentrations of 6-BA were applied separately on *Den.* 'White Christmas' during the pseudobulb thickening stage, 400 mg•L^{-1} resulted in 70% flower bud formation rate without vernalization compared to only 35% on the control group (Li et al., 2009). Since the control had 35% flowering rate, some degree of vernalization had occurred. Therefore the effect of BA is not ideal in this case because a near 100% flowering is needed for commercial operation. Wang et al. (2010) reported that a single foliar application of BA at 1000 mg•L^{-1} induced flowering of *Den.* Red Emperor 'Prince' and *Den.* Oriental Smile 'Fantasy' at 30 °C day/25 °C night and a single application of BA in the range of 500 to 1000 mg•L^{-1} triggers multiple basal shoots to form.

Floral development after vernalization

Temperature controls the rate of plant development, including time for an inflorescence to develop into open flowers. Based on Lopez and Runkle's (2004) model, the rate of flower development of *Zygopetalum* Redvale 'Fire Kiss' was accelerated by high forcing temperature in a certain temperature rage (12-26 ºC). Besides, temperature could be adjusted using this model to help meet specific finishing dates. Rotor (1952) also reported that flower bud development of *Dendrobium* nobile was hastened by using a higher temperature after the buds have been initiated.

Lowering the temperature reduces respiration and other biochemical activities, as well as floral development (Hew and Yong, 2004). This has contributed to the production of bulbous plants. In the Netherlands, tulip bulbs are planted, frozen, and then thawed out to extend the forcing period and lead to a year-round production (de Jong et al., 1990). Easter lilies at the "puffy white" bud stage can be placed in dark coolers at 1-4°C for a week or less to delay the progress of flowering without adverse effects (Kessler, 2001). However, ill effects are usually brought by long period of cooling during floral development. At the "puffy white" bud stage, increasing cooling time beyond a week increased foliar chlorosis and decreased postharvest floral longevity of Easter lilies (Prince et al., 1987; Staby and Erwin, 1977). After flower initiation in Asiatic hybrid lilies, as the duration of bulb freezing increased, the number of blasted primary and aborted secondary and tertiary buds increased (Roh, 1990).

Flowering control challenges

Leaves and pseudobulbs Sinoda et al. (1984; 1985) reported that for *Den.* Snowflake 'Red Star', low day temperatures caused leaf yellowing, defoliation and reduction in growth rate. Higuchi et al. (1974) also reported that the dehydration of pseudobulbs occurred under low light intensity for *Den*. Nodoka. *Phalaenopsis* cooled in complete darkness for six weeks dropped one leaf per plant, while leaf abscission rarely occurred for those cooled under light (Wang, 1995).

In Japan, the nobile dendrobium plants are cooled at low temperatures (8-10 ºC) to for the purpose of defoliating plant because Japanese consumers prefer flowering plants without foliage. However, the green foliage is required on flowering nobile dendrobium plants in Europe and the U.S. (Wang and Starman, 2008). Thus, maintaining proper nutrient application to retain the foliage in a healthy and attractive condition is very important to obtaining products that are preferred by the American and European consumers.

Aerial shoots Aerial shoots formed in nobile dendrobium when pseudobulbs were subjected to insufficient cooling (Sinoda and Suto, 1988). In this situation, some of the lateral buds develop as flower buds or aerial shoots, and the rest remain dormant (Ichihashi, 1997). From spring to early summer, aerial shoots may appear on the upper nodes of the pseudobulb due to damage to the new shoot by mechanical breakage or by slugs (Yamamoto, 2010b).

Flower longevity Orchid flowers are well known for their longevity. Many of the economically important tropical orchid flowers last for several months when placed in a postharvest environment. When in full bloom, flowers will last longer when the plant is placed in a cool, dry spot away from any draft and out of direct sunlight. A night temperature of 5-10 ºC is ideal for maintaining shelf life of nobile dendrobium (Yamamoto, 2010b). The flower longevity of potted *Rhipsalidopsis* decreased from 43 to 26 d as temperature increased from 18 to 24 ºC (Hartley et al., 1995). As temperature increased from 14 to 29 ºC, longevity of the first open flower of *Zygopetalum* decreased from 37 d to 13 d (Lopez and Runkle, 2004).

Growers have the requirements of getting cultivation of nobile dendrobium down to a science. Hew and Yong (2004) listed three criteria for flower induction to be commercially viable: (1) the method must be simple, economical and give reproducible results; (2) the quantity and quality of flowers must not be affected; and, (3) there should not be any adverse effects on the plant or on subsequent flowering. Therefore, further studies are needed to improve the cultivation and flowering control system of potted orchids. For instance, by combining use of low temperature, light intensity and PGRs to make year-round production of nobile dendrobium possible and at the same time, keep production costs as low as possible, as well as eliminate or limit the potential ill effects such as formation of aerial shoots, leaf yellowing or abscission, and shortening of flower longevity, etc.

CHAPTER II

VERNALIZATION DURATION AND LIGHT INTENSITY INFLUENCE FLOWERING OF THREE NOBILE DENDROBIUM HYBRIDS

Introduction

As a relatively new mass-produced commercial crop, the nobile dendrobium has a high market potential because it produces large amounts of flowers and inflorescences simultaneously (Rotor, 1952). A mature pseudobulb of nobile dendrobium may have 10 to 15 nodes each with a leaf and an axillary inflorescence which may develop into two to six flowers.

Nobile dendrobiums need a period of vernalization after maturation to induce flowering. Ichihashi (1997) reported that 15 ºC chilling was required for flower bud differentiation of nobile type dendrobium; lack of chilling caused blind buds, aerial shoots or no flower formation. Thus, temperature is the most important factor to control flowering of nobile dendrobium. In China, growers start cooling (9 to 13 ºC night temperature) from late September or October to advance the flowering time of nobile dendrobium to make the New Year or Chinese Spring Festival market time (Li and Wang, 2005). Rotor (1952) reported that cooling under 18 ºC for 4 months delayed flowering of *Dendrobium nobile* until Easter, however, plants kept under 13 ºC started to flower in February or March. Yen et al. (2008b) determined that 3 weeks at constant 13 °C saturates the cooling requirement for *Den.* Sea Mary ‘Snow King’. Higher temperature during vernalization and longer cooling durations (up to six weeks) were reported to delay flowering of nobile dendrobium (Yen et al., 2008b). For example, counting from the day cooling starts, *Den.* Sea Mary 'Snow King' cooled at 18 ºC for three weeks took 93 days and those cooled at 15 ºC for the same duration only needed 80 days to flower. Plants cooled at 15 ºC for six weeks took 75 days and those cooled for two weeks under same temperature needed only 60 days to reach flowering.

Response to low temperature in orchid is further complicated by an interaction between temperature and light, thus light intensity can also be used to manipulate the timing of flowering. Wang (1995) reported that the number of days in darkness or at a low *PPF* delayed inflorescence initiation (spiking) of *Phalaenopsis* orchids by that duration, even when plants were exposed to temperatures that induced spiking in light. A large-scale test conducted in Taiwan showed that 5 d of heavy shading (complete darkness or $PPF \leq 20$ μmol·m^{-2}·s^{-1}) alternating with 2 d in light is an effective and inexpensive alternative to heating to a constant 28 °C for inhibiting *Phalaenopsis* spiking in commercial operations (Wang et al., 2006). Rotor (1952) found that the degree of cymbidiums flower response to low temperature (21 ºC) is determined by light intensity; however, response to low temperature induction in *Dendrobium nobile* was not affected by light.

The results from a preliminary study (Yin-Tung Wang, personal communication), suggested that unlike *Phalaenopsis* which requires adequate light while being cooled to induce spiking, the nobile dendrobium can be cooled in complete darkness to induce flower initiation. In practice, heavy shade may be used in nobile dendrobium cooling greenhouses to better achieve the lowest possible temperatures during the warmer months for timely flower induction (Wang and Starman, 2008). Additional research is needed to obtain more data to study if flower induction in darkness or under low light would negatively impact flower quality of the nobile dendrobium. The objective of this study was to determine the effect of light intensity during vernalization and duration of vernalization on the subsequent flower development and flower quality of nobile dendrobium.

Materials and Methods

Experiment 1. Light and cooling duration

Mature plants of three cultivars (Love Memory 'Fizz', Red Emperor 'Prince', and Sea Mary 'Snow King') with the average of 14, 11, and 13 total nodes respectively, potted in standard green plastic pots (10.2 cm top diameter, 414 mL vol.), were shipped from Matsui Nursery in Salinas, CA. Sea Mary 'Snow King' and Red Emperor 'Prince' plants arrived at Texas A&M University, College Station on 12 Sept. 2008 (2 Jan. 2009 for Love Memory 'Fizz') and were immediately placed in a greenhouse having glass walls and a polycarbonate roof. The root substrate consisted of nine parts of bark mix (95% pine bark and 5% ground sphagnum moss, Bas Van Buuren B.V., De Lier, The Netherlands) to one part long fiber peat (Pindstrup Mosebrug A/S, Ryomgaard, Denmark).

Plants were irrigated with reverse osmosis (RO) water and spaced in every other hole in 30.8 × 51.4-cm molded polypropylene carrying trays [4.00 Transport Tray (15); Landmark Plastic Corporation, Akron, OH] on the greenhouse bench with leaves orienting east and west to best capture sunlight. To maintain a single pseudobulb per pot, any undesirable secondary shoots were removed when emerged.

Greenhouse irradiance and air temperature at plant canopy level were recorded every 30 min with a Quantum Light 3 Sensor Bar (Spectrum Technologies, Plainfield, IL) and a WatchDog Data Logger Model 450 (Spectrum Technologies). Plants were grown in a warm greenhouse (Fig. 2) with mean daily temperature that ranged from 20 to 25 °C and mean photosynthetic daily light integral (DLI) of 12.0 $mol•m^{-2}•d^{-1}$. The automatic thermal screen system was set to be pulled as needed to reduce light levels and prevent severe temperature increase in the greenhouse. Pots were irrigated every fourth watering with a nutrient solution made with RO water and a 15N–2.2P–12.5K (Peters Excel 15-5-15 Cal-Mag; Scotts, Marysville, OH) water-soluble fertilizer at 0.33 $g•L^{-1}$. Pesticides azoxystrobin (Heritage, Syngenta Crop Protection, Greensboro, NC) and chlorfenapyr (Pylon, OHP, Mainland, PA) were applied when necessary to control fungus and spider mites, respectively.

The experiment was a 2 × 4 factorial with two light intensities during cooling (darkness or 300 - 350 $\mu mol•m^{-2}•s^{-1}$ *PPF*) and four cooling durations (2, 4, 6, or 8 weeks). Plants were subjected to cooling treatments in a growth chamber with the mean relative humidity of 75%, starting on 15 Sept. 2008 (15 Jan. 2009 for Love Memory ‘Fizz’). One of the growth chambers was totally dark, and the other one had a 12-h photoperiod of 300-350 $\mu mol•m^{-2}•s^{-1}$ *PPF* provided by both fluorescent and incandescent lamps. Plants in each growth chamber were completely randomized for cooling duration, with 7 or 10 replicates (depending on cultivar) per treatment. The mean air temperatures recorded in the growth chambers were 10.0 ± 0.2 °C.

Plants were moved back to the greenhouse after the completion of cooling treatments. The mean DLI from the end of cooling until full flowering was 9.0 mol•m^{-2}•d^{-1} (13.4 mol•m^{-2}•d^{-1} for Love Memory 'Fizz').

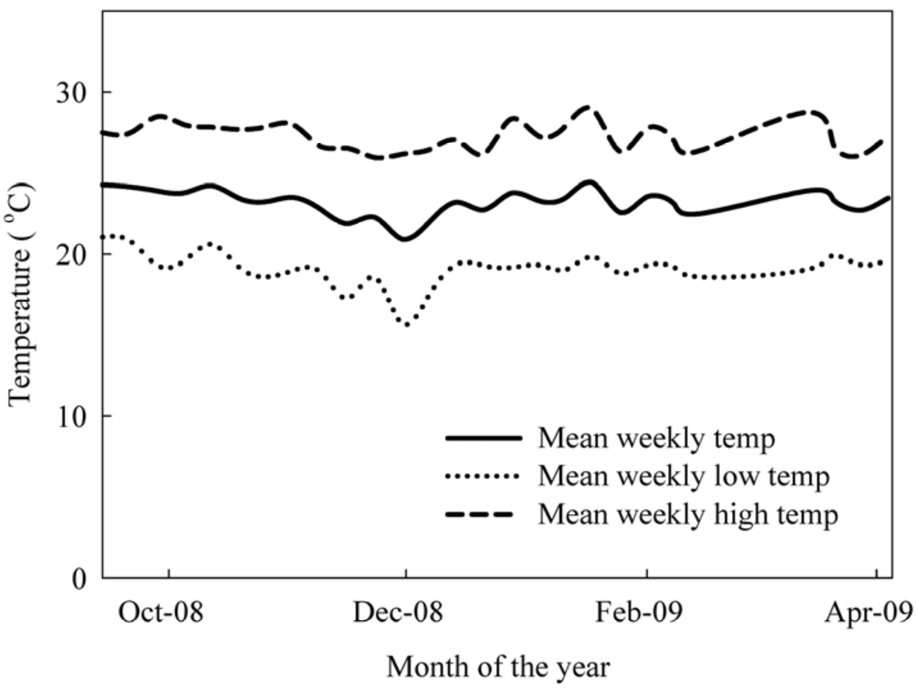

Fig. 2. Mean weekly air temperature in the greenhouse through the experimental period (from October, 2008 to August, 2009) in College Station, TX.

Experiment 2. Effect of light intensity during vernalization

Love Memory 'Fizz' plants in the same 10.2-cm pots arrived at Texas A&M University, College Station on 2 Jan. 2009. Growing conditions in the greenhouse before vernalization were the same as those of Expt. 1. The plants were then moved on 15 Jan. 2009 to growth chambers (15°C/10°C day/night, 12-h photoperiod) to induce flower initiation. Four light intensities were given: (1) 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$; (2) 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, covered with one layer of ARMEX Polypropylene Shade Cloth 50% (XS Smith, Red Bank, NJ); (3) 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, covered with two layers of ARMEX Polypropylene Shade Cloth 50%; and (4) darkness, covered with one layer Nav-Black Mum Cloth (XS Smith). Cooling was terminated after 4 weeks, and plants were transferred into a greenhouse to flower.

Data collection

Date to flowering (when the first flower bud opened) and full flowering (when all the flower buds opened), flowering node percentage (flowering node number / total nodes), total flower number, flower diameter (mean diameter of the three largest flowers), flower longevity (duration from the first flower bud opened until this flower wilted), flower number per flowering node, aerial shoot percentage (aerial shoot number / total nodes), remaining leaf percentage and number of nodes without flowers on the bottom or top of the pseudobulb were collected. The time to flowering was presented from the beginning and the end of cold treatment.

Statistical analysis

All data were first tested on additivity and normal distribution, then subjected to analysis by GLM and Duncan's multiple range test for comparing the treatment means, all at $P \leq 0.05$. Statistical analysis was performed by SAS 9.2 statistical software (SAS Institute, Cary, NC).

Results

Experiment 1. Light and cooling duration

Regardless of cooling duration, only 45% of Red Emperor ‘Prince’ plants vernalized under darkness flowered, compared to 98% of those cooled in light. However, the flowering percentage of both Sea Mary ‘Snow King’ and Love Memory ‘Fizz’ was 100% either under light or in darkness (data not shown).

For Red Emperor ‘Prince’, significant interactions between light and cooling duration were found on time to flowering. For those cooled under light, longer cooling durations increased the time to flowering from the beginning of cooling, indicating that extending cooling duration could defer flowering (Fig. 3a). The time to flowering from the end of cold treatment decreased with increased cooling duration (Fig. 3b). Plants that were cooled in darkness needed an extra 5 to 7 d to reach flowering than plants that were exposed to light, except for those cooled two weeks in darkness which required an especially longer time to flower (Fig. 3a and 3b). The same interaction was found to affect the time to flowering of Love Memory ‘Fizz’. Extending cooling duration deferred flowering (Fig. 3c) and reduced the time to reach flowering when cooling duration was not counted (Fig. 3d). Regardless of how long the cooling duration was, plants cooled in darkness always required a longer time (3 to 6 d more) to reach flowering compared to those exposed to light (Fig. 3c and 3d). There was no interaction between light and cooling duration for time to flowering of Sea Mary 'Snow King'. However, the same trends were found as the other two cultivars: darkness during vernalization delayed flowering (by 6 d) for this cultivar (Fig. 4). With the increase of cooling duration, time to flowering, including the cooling duration, was extended; however time to flowering, excluding the cooling duration, was shortened (Table 1, Fig. 4).

Light and cooling duration interactively affected the total flower number of Red Emperor 'Prince'. Plants that were cooled under light always produced more flowers than those cooled in darkness. Plants cooled for two weeks in light or darkness produced fewer than five flowers per plant (Fig. 5). Regardless of light treatment, Sea Mary 'Snow King' cooled for two weeks produced fewer but larger flowers compared to those that received other cooling durations (Table 1, Fig. 4). For both Sea Mary 'Snow King' and Love Memory 'Fizz', the presence of light during vernalization increased the flowering node percentage by at least 22, producing more but slightly smaller flowers. In addition, plants exposed to darkness during cooling had more non-flowering nodes on both ends of the pseudobulb (Table 2, Fig. 4).

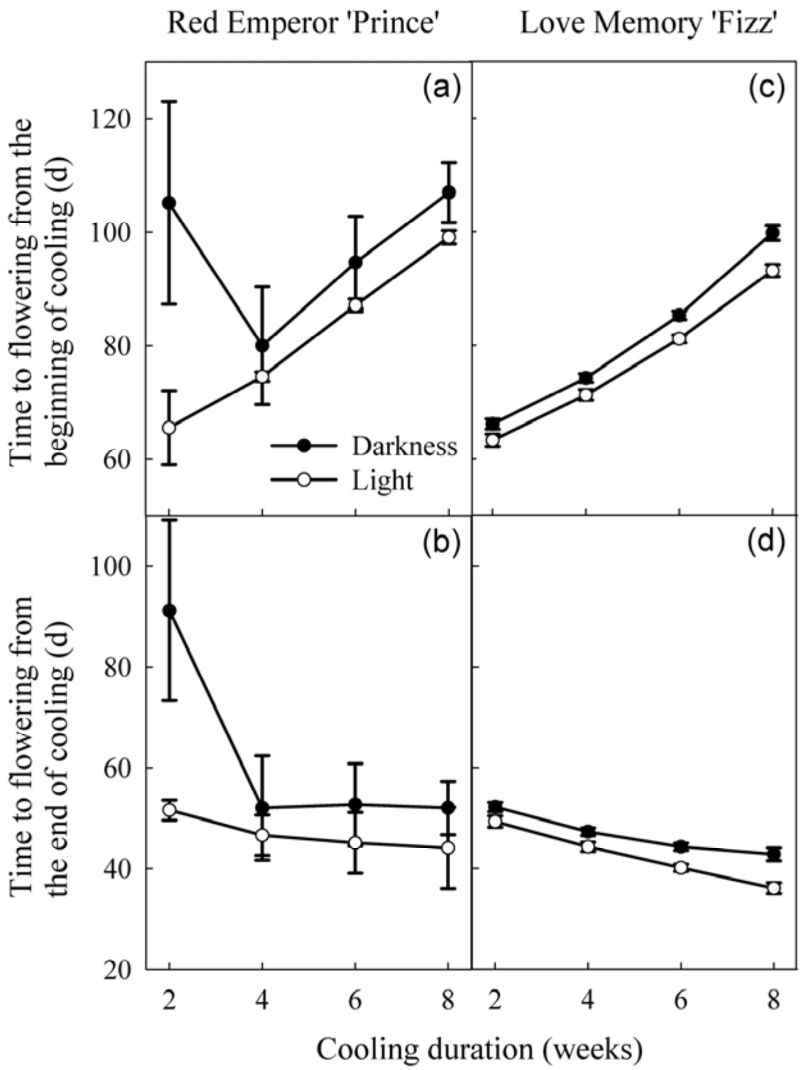

Fig. 3. Effect of light and cooling duration on time required to reach flowering from the beginning or the end of cooling of *Dendrobium* Red Emperor 'Prince' (a and b) and *Den.* Love Memory 'Fizz' (c and d). Vertical bars represent 95% confidence intervals.

Table 1. Effect of cooling duration, regardless of light, on time to flowering from the beginning of cooling, time to flowering from the end of cooling, total flower number, and flower diameter of *D.* Sea Mary 'Snow King'.

Cooling duration (weeks)	Time to flowering from the beginning of cooling (d)	Time to flowering from the end of cooling (d)	Total flower number	Flower diameter (cm)
2	67.8 d[Z]	53.8 a	19.2 b	7.8 a
4	76.3 c	48.3 b	27.1 a	7.5 ab
6	89.2 b	47.2 b	24.8 a	7.5 ab
8	101.5 a	46.5 b	26.3 a	7.4 b

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

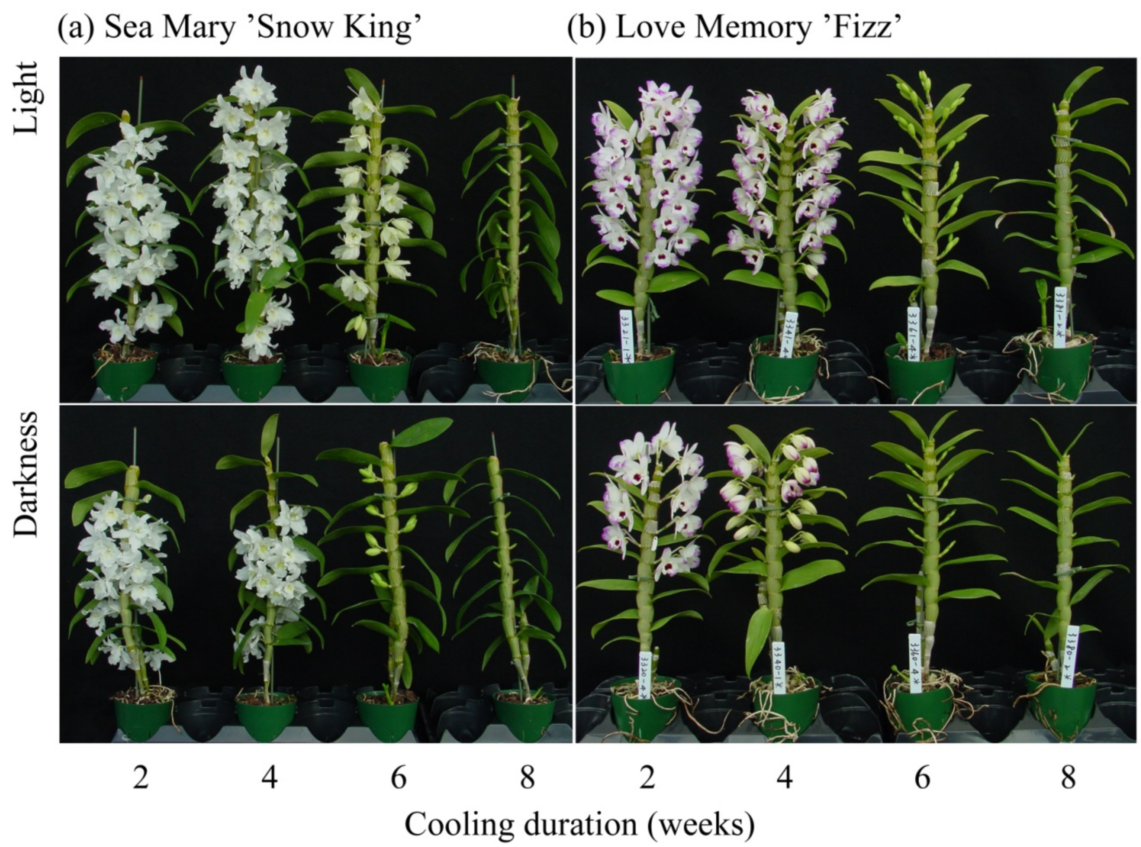

Fig. 4. Visual performance with various cooling durations and light conditions (a) of *Den.* Sea Mary ‘Snow King’, on 14 Dec. 2008, 13 weeks after initiation of vernalization and, (b) of *Den.* Love Memory ‘Fizz’, on 30 March 2009, 10 weeks after initiation of vernalization.

Table 2. Effect of light, regardless of cooling duration, on flowering node percentage, total flower number, flower diameter, number of nodes without flower on top, and bottom of pseudobulbs of *Den.* Sea Mary ‘Snow King’ and *Den.* Love Memory ‘Fizz’.

Dendrobium cultivar	Light treatment	Flowering node percentage (%)	Total flower number	Flower diameter (cm)	Node w/o flower (top)	Node w/o flower (bottom)
Sea Mary	Light	74.9 a[Z]	29.7 a	7.4 b	1.2 b	2.0 b
‘Snow King’	Darkness	48.0 b	19.0 b	7.7 a	2.8 a	3.3 a
Love Memory	Light	71.4 a[Z]	37.0 a	6.1 b	0.4 b	3.8 b
‘Fizz’	Darkness	48.7 b	23.7 b	6.5 a	1.0 a	6.0 a

[Z]Mean separation in columns within cultivars by Duncan’s multiple range test at $P \leq 0.05$.

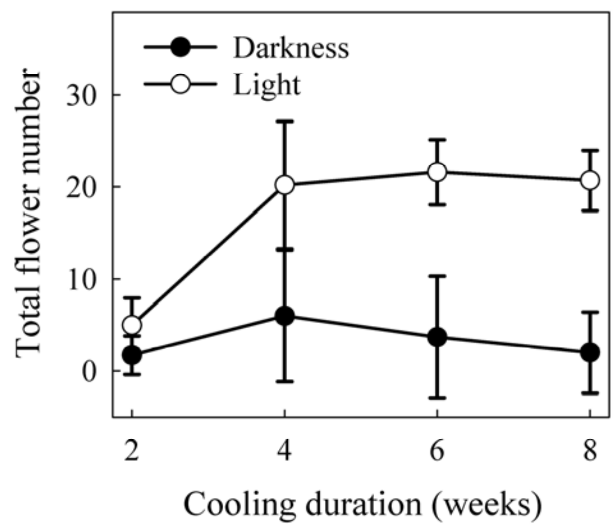

Fig. 5. Effect of light and cooling duration on total flower number of *Den.* Red Emperor 'Prince'. Vertical bars represent 95% confidence intervals.

Table 3. Effect of cooling duration, regardless of light, on flower longevity of *Den.*Red Emperor 'Prince', *Den.* Sea Mary 'Snow King', and *Den.* Love Memory 'Fizz'.

	Flower longevity (d)		
Cooling duration (week)	Red Emperor 'Prince'	Sea Mary 'Snow King'	Love Memory 'Fizz'
2	38.3 a[z]	39.1 a	61.4 a
4	32.6 b	37.3 a	54.0 a
6	26.2 c	30.8 b	41.6 b
8	27.2 c	28.6 b	37.3 b

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

Longer cooling durations reduced the flower longevity for all three cultivars. Flowers of Red Emperor 'Prince' and Sea Mary 'Snow King' lasted 10 d longer when cooled for two weeks compared to those cooled for eight weeks; Love Memory 'Fizz' had longer flower longevity than the other two cultivars, flowers cooled for two weeks lasted more than 24 d longer compared to those receiving eight weeks cooling treatment (Table 3).

For Red Emperor 'Prince' and Love Memory 'Fizz', regardless of light, the percentage of remaining leaves decreased while the cooling duration increased (Fig. 6a). For Red Emperor 'Prince' and Sea Mary 'Snow' King, regardless of cooling duration, darkness treatment increased the leaf drop (Fig. 6b). In addition, Red Emperor 'Prince' was more susceptible to losing leaves compared to the other two cultivars (Fig. 6a and 6b).

Experiment 2. Effect of light intensity during vernalization

Compared to 200 and 100 $\mu mol•m^{-2}•s^{-1}$, 50 $\mu mol•m^{-2}•s^{-1}$ slightly increased flower diameter. However, the various light intensities, darkness excluded, did not affect time to flowering, time to full flowering, and flower qualities including flowering node percentage, total flower number, and flower longevity. Consistent with Expt. 1 (Table 2), darkness treatment deferred time to flowering, resulted in lower flowering node percentage, reduced total flower number, and increased flower diameter. In addition, plants exposed to darkness during vernalization in this experiment needed 10 more days to reach full flowering, and had about 8 d shorter flower longevity (Table 4, Fig. 7).

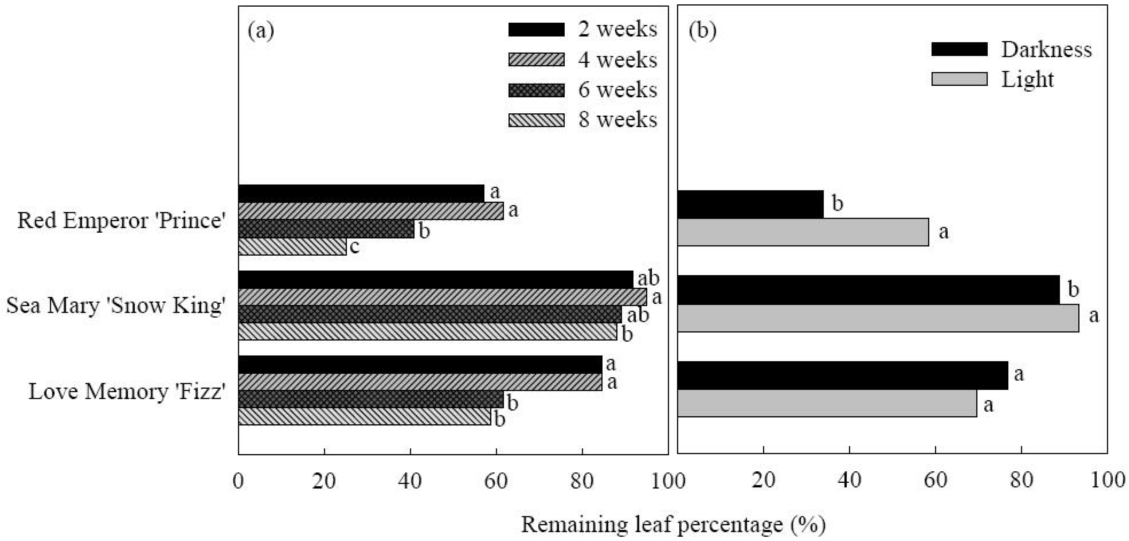

Fig. 6. Remaining leaf percentage of *Den.* Red Emperor 'Prince', *Den.* Sea Mary 'Snow King', and *Den.* Love Memory 'Fizz'. (a) Effect of cooling duration regardless of light and (b) Effect of light regardless of cooling duration.

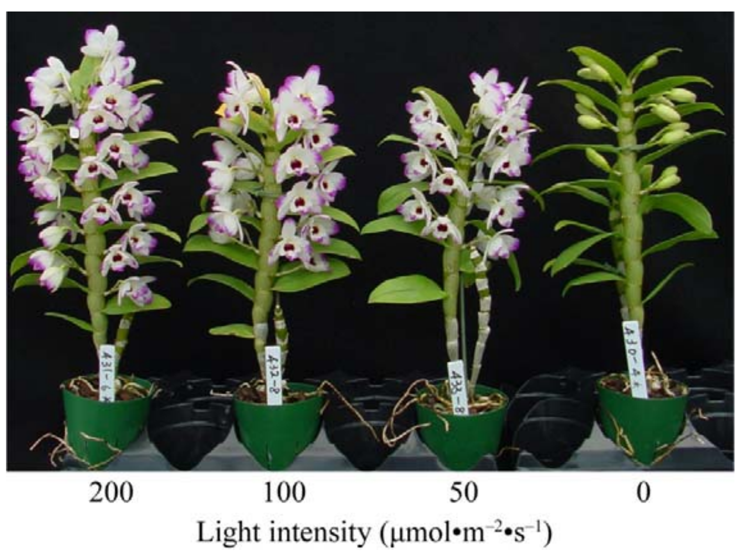

Fig. 7. Visual performance with various light intensity of *Den.* Love Memory 'Fizz' on 30 March, 2009, 6 weeks and 4 days after the completion of vernalization.

Table 4. Effect of light intensity during vernalization on time to flowering from the end of cooling, time to full flowering, flowering node percentage, total flower number, flower diameter, and flower longevity of *Den.* Love Memory 'Fizz'.

Light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Time to flowering from the end of cooling (d)	Time to full flowering (d)	Flowering node percentage (%)	Total flower number	Flower diameter (cm)	Flower longevity (d)
0 (Darkness)	52.5 a	15.3 a	38.8 b	15.6 b	7.6 a	41.8 b
50	45.5 b	5.5 b	61.8 a	27.4 a	6.9 b	50.1 a
100	45.6 b	6.0 b	65.8 a	32.5 a	6.4 c	52.0 a
200	43.8 b[Z]	5.8 b	71.5 a	31.1 a	6.4 c	50.9 a

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

Discussion

Nobile dendrobium requires a critical amount of thermal time (degree days) for a vernalization response. In this study, we used three nobile dendrobium cultivars and cooled them for 2 and 4 weeks to span the 3 weeks exposure which was reported to be the critical vernalization period for *Den.* Sea Mary 'Snow King' cooled at 13 ºC by Yen et al. (2008b). For *Den.* Sea Mary 'Snow King', aerial shoots or aborted buds were produced instead of flower buds when the amount of cooling was below the critical amount. Above the critical amount, flowers were produced but higher cooling amounts delayed flowering time and affected flower quality (Yen et al., 2008b). Our results differed in that we did not encounter the formation of aerial shoots among cooling treatments. Wang and Starman (2008) suggested that tetraploid varieties may require longer durations of cooling to induce flower initiation. We found that tetraploid Red Emperor 'Prince' produced fewer flowers per plant than the other two cultivars (Sea Mary 'Snow King' and Love Memory 'Fizz', which are both diploid) when cooled at 10 °C for two weeks under light, suggesting that this cultivar has a higher cooling threshold for flower induction than for the other two cultivars.

Rotor's (1952) research on *Dendrobium nobile* suggested that light did not affect the flower induction effect of low temperature. However, in this study, we found that in addition to the amount of cooling, light intensity during vernalization is shown to be another factor that influences flower quality and timing of the nobile dendrobium, and this effect is cultivar dependent. Without light during vernalization, very few flower buds initiated for Red Emperor 'Prince'. For Sea Mary 'Snow King' and Love Memory

'Fizz', 25% fewer flower buds were produced when cooled in darkness compared to those receiving light treatments, indicating that the light requirement for flower initiation for these two cultivars is facultative not obligate. Consequently, light was not required for flower induction in the three dendrobium nobile cultivars used in this study, but may be needed for more complete flower development and more uniform flowering, especially for Red Emperor 'Prince'. Higuchi et al. (1974) reported that the shriveling of pseudobulbs occurred under low light intensity for *Den*. Nodoka. We found the same shriveling of pseudobulbs on some Red Emperor 'Prince', suggesting that carbohydrate loss could be a reason that flower initiation of this cultivar was not complete under darkness. We recommend at least 4 weeks of 10 °C cooling in light for Red Emperor 'Prince', and at least 2 weeks of 10 °C cooling regardless of light for Sea Mary 'Snow King' or Love Memory 'Fizz' to achieve complete vernalization. From a practical point of view, vernalization of *Den.* Sea Mary 'Snow King' and Love Memory 'Fizz' in the dark would be simpler and less expensive for growers.

Dendrobium Hinde 'Toutenkou' had fewer flowers on the upper nodes under inducing temperature. Ichihashi suggested (1997) that the upper nodes which were not fully mature did not respond to low temperatures. We found that when cooled in darkness, flowers were mainly concentrated in the middle of the pseudobulb for Sea Mary 'Snow King' and Love Memory 'Fizz', indicating that bud maturity could be one of the factors that affect flower initiation. Compared to the less mature (top) buds, the more mature buds (in the middle of the pseudobulb) were easier to be induced by low temperature under darkness. Plants cooled in darkness produced slightly larger flowers than those in light, which could be attributed to fewer flowers and relatively more nutrients being allocated to each flower.

As long as flower buds are initiated, the subsequent floral development is mainly affected by temperature. Lowering the temperature reduces respiration and other biochemical activities, as well as floral development (Hew and Yong, 2004).This could explain our results that longer cooling duration deferred flowering, which is consistent with the findings of Yen et al. (2008b). Thus, we recommend that growers adjust cooling duration to defer flowering of nobile dendrobium from Valentine's to Mother's Day to increase their marketing window. Rotor (1952) found that flower bud development may be hastened by the use of artificial lights to supplement natural daylight, and Ichihashi (1997) reported that high light and longer irradiation promote flower bud development . In this study, darkness during vernalization slightly delayed flowering of Sea Mary 'Snow King' and Love Memory 'Fizz', suggesting that covering the greenhouse by black cloth could be another strategy for growers to defer flowering of this cultivar.

Shorter flower longevity under longer cooling duration in this study probably attributes to the stress under low temperature that the plants underwent. However, compared to the potential market reward obtained by flowering control, slightly shorter longevity could be an acceptable sacrifice. Further experimentation could investigate how to optimize the cooling duration after flower initiation to delay flowering without reducing flower longevity. Ketsa et al. (2001) reported that carbohydrate levels affected the life of open florets, and compared to the tetraploid line, the shorter vase life of cut *Den.* 'Caesar' (diploid) was due to their high respiration rate. However, in our study, Red Emperor 'Prince' (tetraploid) had similar flower longevity to Sea Mary 'Snow King' (diploid), and much shorter flower longevity than Love Memory 'Fizz' (diploid). Since potted plants were used in this study, carbohydrate level may not be the limiting factor for flower senescence as it is with cut flowers.

The American Orchid Society (2002) reported that temperatures below 10 °C may cause leaf abscission in some orchid species. Low day temperature caused leaf yellowing and defoliation of *Den.* Snowflake 'Red Star' (Sinoda et al., 1984; 1985). Wang (1995) reported that *Phalaenopsis* cooled in complete darkness for six weeks dropped an average of one leaf per plant compared to those cooled under light. These results were in agreement with our findings of leaf abscission on Red Emperor 'Prince'. Consumers in the Europe and U.S. prefer green foliage on flowering nobile dendrobium plants (Wang and Starman, 2008). As a result, keeping proper nutrients application rates to retain the foliage in a healthy and attractive condition is very important to obtaining products that are preferred by the consumers.

Our study shows that, light intensity during vernalization does not substantially affect flowering time and most of the flower qualities of these dendrobium nobile cultivars. Thus, for the growers in cooler or mild climates, such as in Salinas, CA, vernalization requirement of dendrobium nobile can be achieved in a shaded greenhouse without the cost for coolers.

CHAPTER III

DEFERRING FLOWERING OF TWO NOBILE DENDROBIUM HYBRIDS BY HOLDING PLANTS UNDER LOW TEMPERATURE AFTER VERNALIZATION

Introduction

In 2009, the U.S. Department of Agriculture (USDA) reported that 19.5 million potted orchids, with a total wholesale value of $160 million, were sold and orchids have become the most valued flowering potted crop in the United States (USDA, 2010). An estimated 85% to 90% of the potted orchids sold in the United States are *Phalaenopsis* and related genera (Nash, 2003). Commercial growers are now looking for other orchids that have consumer appeal and can be grown and sold in mass markets.

The nobile dendrobium has been grown for many years in the history of orchid cultivation. Its commercial production around the world has much increased in recent years (Wang and Starman, 2008). The outstanding characteristic of this orchid is that the bright and lightly fragrant flowers are produced on each node of the pseudobulb and they nearly open simultaneously. The native *Dendrobium nobile* blooms after passing the winter drought and experiencing the low temperature in its natural habitat (SOC, 2009a). *Dendrobium* Sea Mary ‘Snow King’ needs to be subjected to a specific amount of cooling for flowering (Yen et al., 2008b). Lin et al. (2011) found that 2 weeks of 10 °C satisfied the vernalization requirement for *Den.* Sea Mary ‘Snow King’ and *Den.* Love

Memory 'Fizz'; however, at least 4 weeks of 10 °C cooling was needed for *Den.* Red Emperor 'Prince' to flower best.

The ordinary prime blooming period of most nobile dendrobium hybrids is from February to March. However, plants to be sold for Mother's Day must start to bloom no earlier than late April for safe shipping (Wang, 1998). As a consequence, growers are looking for ways to produce quality, blooming nobile dendrobium from May through July for the Mother's Day and summer markets.

For species that require low temperatures for flower induction (vernalization), one way to defer flowering is to expose plants to high temperatures. Heating the greenhouse to ≥ 28 °C can keep *Phalaenopsis* from spiking i.e. flowering and they remain vegetative (Sinoda, 1994). However, in temperate climates, this is an expensive cultural practice because of the large energy input. Since spiking induction of *Phalaenopsis* is light dependant, an inexpensive alternative for inhibiting spiking in *Phalaenopsis* is to alternate 5 d of heavy shading (darkness) with 2 d of light (Wang, 1998; Wang et al., 2006) on weekly cycles. Different from *Phalaenopsis*, light does not affect flower induction of three dendrobium nobile hybrids (Lin et al., 2011), so darkness may not be an alternative to heating for flowering control of the nobile dendrobium.

Another strategy to defer flowering of vernalization requiring plants in temperate climates is to hold plants under low temperature to slow the growth and development process. Tulip (*Tulipa gesneriaana*) growers in the Netherlands schedule planting that is followed by freezing and subsequent thawing of tulip bulbs to extend the forcing period and lead to year-round tulip production (Roh, 1990). Easter lilies (*Lilium longiflorum*) at the “puffy white” bud stage can be kept in dark coolers at 1-4 ºC for up to one week to delay flower development without adverse effects (Kessler, 2001). Increasing cooling duration (up to 8 weeks) deferred flowering of three nobile dendrobium hybrids (Lin et al., 2011; Yen et al., 2008b).

However, adverse impacts on plant quality could occur with long periods of cooling. More than one week of cooling during the “puffy white” bud stage increased foliar chlorosis and decreased postharvest flower longevity of Easter lilies (Prince et al., 1987; Staby and Erwin, 1977). After flower initiation of Asiatic hybrid lilies, the number of blasted primary and aborted secondary and tertiary buds increased as the bulb freezing duration increased (Roh, 1990). Low day temperatures caused leaf yellowing, defoliation and reduction of growth rate on *Den.* Snowflake ‘Red Star’ (Sinoda et al., 1984; Sinoda et al., 1985).

The objective of this experiment was to develop a strategy to defer flowering of nobile dendrobium by holding them under low temperature after vernalization and to determine whether long duration of holding at low temperature would negatively impact flower quality.

Materials and Methods

Plant materials and growing conditions

Mature plants of two cultivars (the diploid *Den.* Sea Mary ‘Snow King’ and tetraploid *Den.* Red Emperor ‘Prince’) with an average of 14 and 11 total nodes respectively, and potted in standard green plastic pots (10.2 cm top diameter, 414 mL vol.), were shipped from Matsui Nursery in Salinas, CA. Plants arrived at Texas A&M University, College Station on 12 Sept. 2008 and were immediately placed in a greenhouse having glass walls and a polycarbonate roof. The root substrate consisted of nine parts of bark mix (95% pine bark and 5% ground sphagnum moss, Bas Van Buuren B.V., De Lier, The Netherlands) to one part long fiber peat (Pindstrup Mosebrug A/S, Ryomgaard, Denmark).

Plants were irrigated with reverse osmosis (RO) water and spaced in every other hole in 30.8 51.4-cm molded polypropylene carrying trays [4.00 Transport Tray (15); Landmark Plastic Corporation, Akron, OH] on the greenhouse bench with leaves orienting east and west to best capture the sunlight. To maintain a single pseudobulb per pot, all undesirable secondary shoots were removed when emerged.

Greenhouse irradiance and air temperature at plant canopy level were recorded every 30 min with a Quantum Light 3 Sensor Bar (Spectrum Technologies, Plainfield, IL) and a WatchDog Data Logger Model 450 (Spectrum Technologies). Plants were grown in a warm greenhouse (Fig. 8) with mean daily temperature ranging from 20 to 25 °C and a mean photosynthetic daily light integral (DLI) of 9.5 $mol{\cdot}m^{-2}{\cdot}d^{-1}$ (Fig. 8). The automatic thermal screen system was set to be pulled as needed to control the light level and prevent severe temperature increase in the greenhouse. Pots were irrigated every fourth watering with a nutrient solution made with RO water and a 15N–2.2P–12.5K (Peters Excel 15-5-15 Cal-Mag; Scotts, Marysville, OH) water-soluble fertilizer at 0.33 g/L. Pesticides azoxystrobin (Heritage, Syngenta Crop Protection, Greensboro, NC) and chlorfenapyr (Pylon, OHP, Mainland, PA) were applied when necessary to control fungus and spider mites, respectively.

On 15 Sept. 2008, vernalization commenced by placing plants in a growth chamber with mean air temperature of 10.0 ± 0.2 °C and mean relative humidity of 75% for four weeks. The growth chamber had a 12-h photoperiod of 300-350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* (13-15 $mol \cdot m^{-2} \cdot d^{-1}$ of photosynthetic photons) provided by both fluorescent and incandescent lamps. Following vernalization, cooling with five durations (0, 4, 8, 12 or 16 weeks) was conducted in the same growth chamber. Plants were moved back to the greenhouse after the completion of each cooling treatment. A completely randomized design with 10 single plant replicates for each treatment was used.

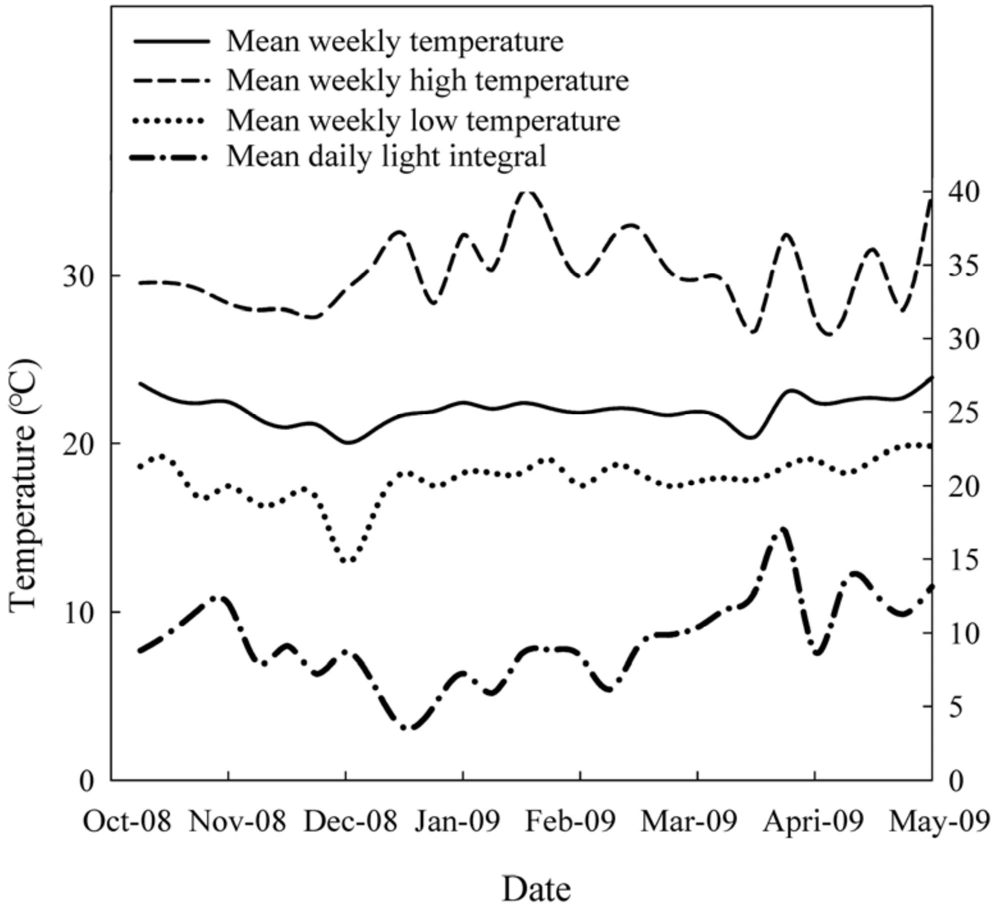

Fig. 8. Mean weekly air temperature and photosynthetic daily light integral (DLI) in the greenhouse throughout the experimental period (from October, 2008 to May, 2009) in College Station, TX.

Data collection

Date to flowering (when the first flower bud opened); flowering node percentage (flowering node number/total nodes); number of aerial shoots, aborted buds, and total flowers; flower diameter (mean diameter of the three largest flowers); flower number per flowering node; time to full flowering (duration from the first flower bud opened until all flowers on the pseudobulb opened); flower longevity (duration from the first flower bud opened until this flower wilted); and remaining leaf percentage were recorded. The time to flowering was presented from the beginning and the end of the low temperature treatment after vernalization.

Statistical analysis

All data were first tested on additivity and normal distribution, and then subjected to analysis by GLM and Duncan's multiple range tests for comparing the treatment means, all at $P \leq 0.05$. Statistical analysis was performed by SAS 9.2 statistical software (SAS Institute, Cary, NC). The correlation between time to flowering and low temperature holding duration, and the correlation between remaining leaf percentage and low temperature holding duration were investigated by linear regression. Differences of two lines were analyzed using GLM procedure by comparing the slopes and intercepts.

Results

Time to flowering

For both cultivars, the time to flowering (from the beginning of low temperature holding, or from the end of low temperature holding) was a linear function of low temperature holding duration, and there was no significant difference in regression slope between cultivars (Fig. 9.). As the duration of low temperature holding increased from 0 to 16 weeks, the time to flowering from the beginning of low temperature holding increased (from 78 to 172 d for *Den.* Red Emperor ‘Prince’ and from 75 to 167 d for *Den.* Sea Mary ‘Snow King’), clearly indicating the delay of flowering due to the increase of low temperature holding duration. However, the time to flowering from the end of low temperature holding decreased (from 50 to 34 d for *Den.* Red Emperor ‘Prince’and from 47 to 30 d for *Den.* Sea Mary ‘Snow King’) (Fig. 9). There was a sequence of flowering with increasing low temperature holding duration. For example, when the plants that received the16-week of holding treatment had 3-5 cm flower buds on the pseudobulb, the flowers already had began to wilt on the plants in the 0 and 4-week group; plants that received the 8-week holding treatment reached full flowering at this point; and flowers had just started to open in the 12-week holding group (Fig. 10). The duration of low temperature holding after vernalization had no influence on time to full flowering (counting from the first flower bud open) in both nobile dendrobium cultivars (data not presented).

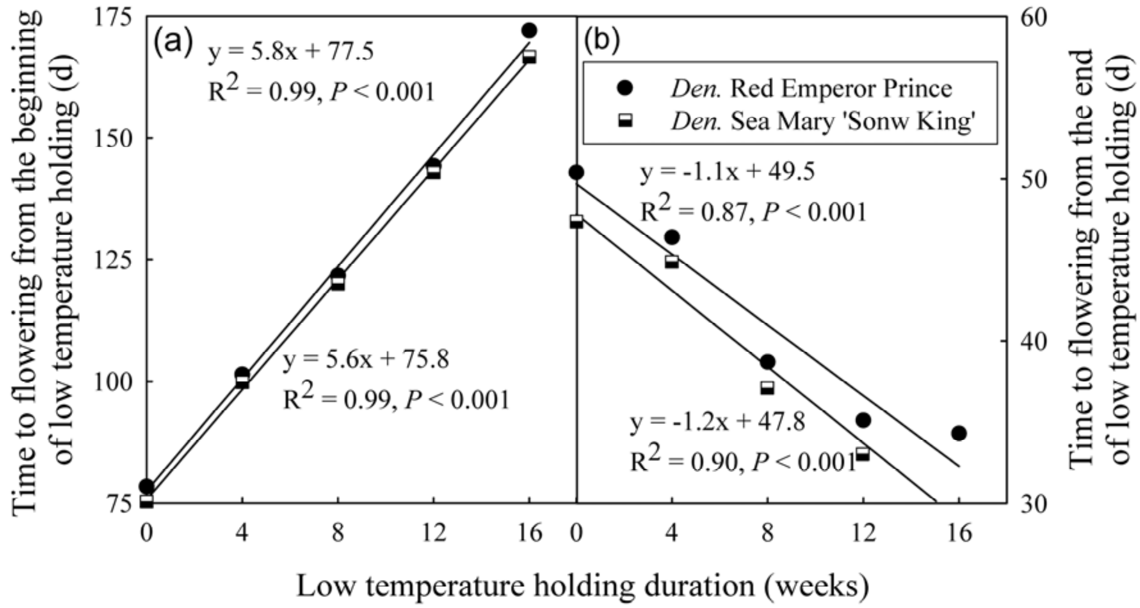

Fig. 9. Effect of low temperature holding duration after vernalization on time to flowering from the beginning (a) or the end (b) of low temperature holding of *Dendrobium* Red Emperor 'Prince' and *Den.* Sea Mary 'Snow King'. Mean±S.E. of the average values. Equations and determination coefficient (R^2) were determined by linear regression.

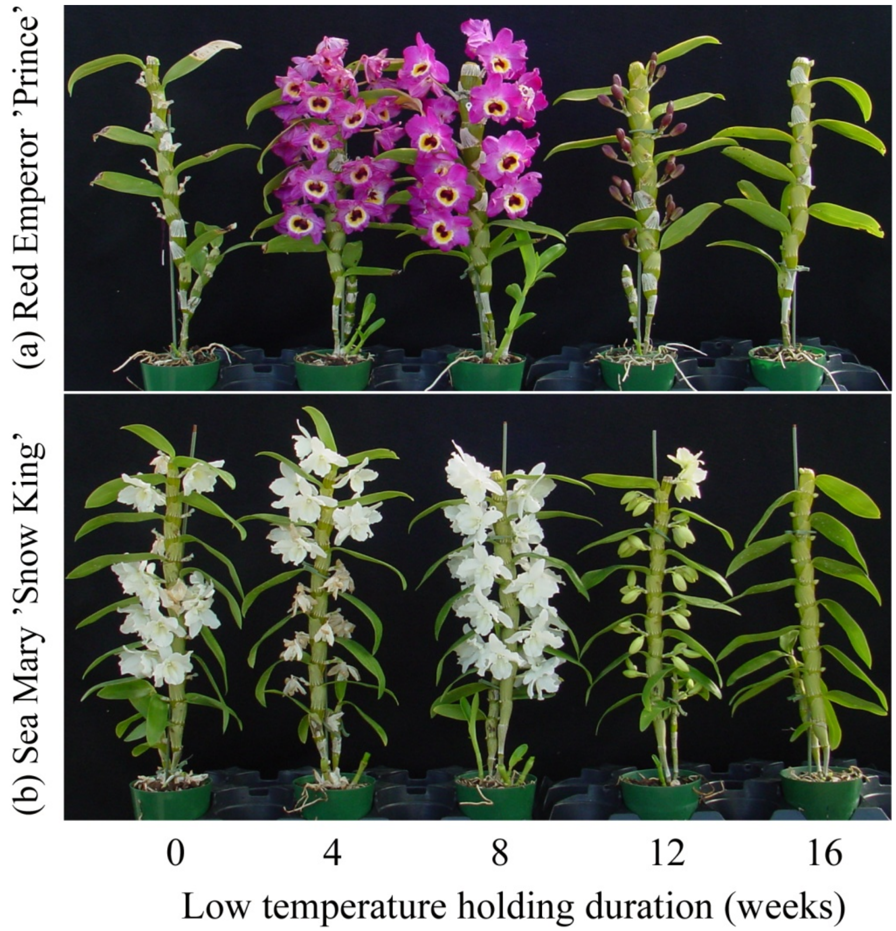

Fig. 10. Visual performance with various durations of low temperature holding after vernalization of (a) *Den.* Red Emperor ‘Prince’ and (b) *Den.* Sea Mary ‘Snow King’ on 6 Feb. 2009, 17 weeks after the completion of vernalization.

Table 5. Effect of low temperature holding duration after vernalization on node differentiation of *Dendrobium* Red Emperor 'Prince' and *D.* Sea Mary 'Snow King'.

Low temperature holding duration (week)	Flowering node percentage (%)	Aerial shoot	Aborted bud
		D. Red Emperor 'Prince'	
0	66	0.0	0.7
4	68	0.2	0.7
8	71	0.0	0.1
12	71	0.0	0.5
16	72	0.1	0.6
Significance	NS	NS	NS
		D. Sea Mary 'Snow King'	
0	80	0.1	1.3 a[Z]
4	85	0.0	1.0 a
8	82	0.0	0.3 b
12	82	0.1	0.0 b
16	85	0.0	0.2 b
Significance	NS	NS	**

[Z] Means within columns followed by different letters are different
** Significant at $P \leq 0.01$, NS No significant.

Flower differentiation

Once flowers had been initiated (after four-weeks of vernalization), all of the plants were able to flower regardless of the various low temperature holding durations after vernalization (Fig. 10). There were no differences among treatments in the flowering node percentage, and means were 70 ± 3% and 83 ± 3% for *Den.* Red Emperor 'Prince' and *Den.* Sea Mary 'Snow King', respectively. Very few aerial shoots and aborted buds occurred in any treatment, except for *Den.* Sea Mary 'Snow King', a significant difference was found among treatments in the number of aborted bud (Table 5).

Flower and leaf quality

For both cultivars, the total flower number was not affected by treatment. For *Den.* Red Emperor 'Prince', as the duration of low temperature holding increased from 0 to 16 weeks, flower diameter generally increased, but the flower number per flower node decreased. Low temperature holding duration had no influence on any flower quality parameter of *Den.* Sea Mary 'Snow King', especially on the flower longevity, however, its effect on flower longevity of *Den.* Red Emperor 'Prince' had no clear trend (Table 6). An increase in low temperature holding duration from 0 to 16 weeks caused more severe leaf abscission in *Den.* Red Emperor 'Prince' from 80% to 32% of the leaves remaining (Fig. 11), but not in *Den.* Sea Mary 'Snow King' (data not shown).

Table 6. Effect of low temperature holding duration after vernalization on flower quality of *Den.* Red Emperor 'Prince' and *Den.* Sea Mary 'Snow King'.

Low temperature holding duration (week)	Total Flower number	Flower diameter (cm)	Flower number per flowering node	Flower longevity (d)
		D. Red Emperor 'Prince'		
0	19	7.1 b	2.7 a	34.8 ab
4	24	7.2 b	3.0 a	29.5 c
8	21	7.6 a	2.7 a	32.0 bc
12	21	7.8 a	2.8 a	35.2 ab
16	19	7.6 a	2.4 b	38.1 a
Significance	NS	**	**	**
		D. Sea Mary 'Snow King'		
0	33	6.9	3.2	35.4
4	35	6.7	3.1	37.7
8	32	6.9	3.0	39.9
12	32	7.0	2.9	35.2
16	30	6.6	2.8	31.6
Significance	NS	NS	NS	NS

[z] Means within columns followed by different letters are different

** Significant at $P \leq 0.01$, * Significant at $P \leq 0.05$, NS No significant.

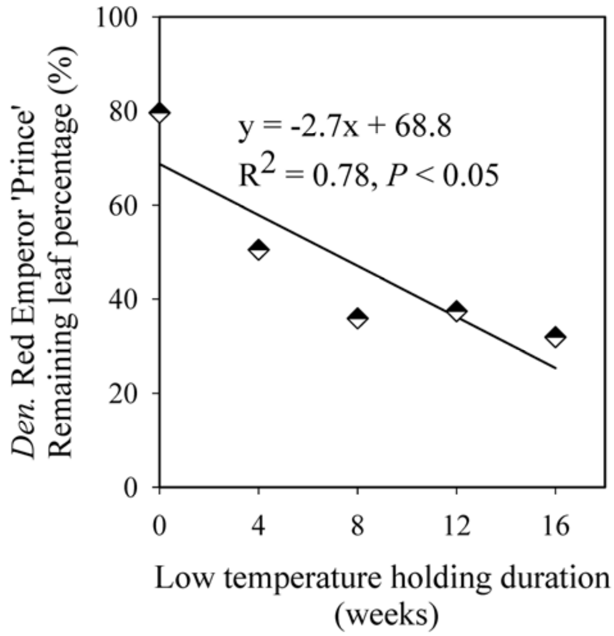

Fig. 11. Effect of low temperature holding duration after vernalization on remaining leaf percentage of *Den.* Red Emperor ‘Prince’. Mean±S.E. of the average values. Equations and determination coefficient (R^2) were determined by linear regression.

Discussion

The results of this study showed that increasing low temperature holding duration after vernalization could defer time to flowering for both nobile dendrobium hybrids. The observations are consistent with the study by Yen et al. (2008b) and Lin et al. (2011) that the time to flowering of nobile dendrobium is delayed by longer vernalization duration. However, in this experiment, much longer low temperature holding durations were applied. Plants without low temperature holding (0-week treatment) started to flower from Dec. 2008, and those that received 16-weeks cooling flowered from early

March. Through various durations of cooling after vernalization, flowering time was extended by more than three months (95 d). Since most nobile dendrobium hybrids naturally bloom in February or March, by keeping plants under low temperature after vernalization, growers would be able to defer flowering to May or June to supply the Mother's Day and early summer market.

Deleterious effects are usually brought about by long periods of cooling during floral development. The postharvest floral longevity of Easter lilies decreased after receiving more than a week of cooling (Prince et al., 1987; Staby and Erwin, 1977). For Asiatic hybrid lilies, more blasted and aborted buds resulted from the increased duration of bulb freezing (Roh, 1990). Lin et al. (2011) reported that increased vernalization duration (from 2 to 8 weeks) reduced flower longevity of three nobile dendrobium hybrids. However, no significant ill effects were found under long cooling duration after vernalization in this study. These conflicting results could be attributed to the difference of greenhouse environment during the flowering period subsequent to cooling. For both cultivars, complete and uniform flowering occurred regardless of the duration of low temperature holding. In addition, very few aerial shoots and aborted buds occurred in any treatment, indicating that once flower buds have been initiated, the differentiated nodes would not be affected by the following low temperature holding.

Den. Red Emperor 'Prince' produced larger flowers when exposed to longer low temperature holding. However, the flower number per node for this cultivar slightly decreased with the increasing low temperature holding duration, probably because extensive low temperature holding resulted in the failure of development of some flower buds. On the other hand, the total flower number was not affected by treatments. Duration of low temperature holding had no influence on any flower quality parameter of *Den.* Sea Mary 'Snow King'. Notably, there was no adverse effect on the postharvest flower longevity.

The data showed that the longer *Den.* Red Emperor 'Prince' was held in low temperature, the more leaves abscised. However, the low temperature holding duration did not affect the leaf number of *Den.* Sea Mary 'Snow King', suggesting that the defoliation caused by low temperature is cultivar dependant and *Den.* Red Emperor 'Prince' is less tolerant to extended chilling compared to *Den.* Sea Mary 'Snow King'. This is a potential detrimental effect of holding *Den.* Red Emperor 'Prince' under 10 ºC for long durations. Green and healthy foliage on nobile dendrobium is preferred by the consumers in Europe and the U.S. (Wang and Starman, 2008). Further research is needed to prevent leaf abscission caused by low temperature and to retain the foliage in a healthy and attractive condition. Growers may maintain a proper feeding program (Wang and Starman, 2008) by fertilizing every 3 to 4 weeks, especially when approaching flowering. The application of benzylaminopurine and gibberellins could be tested to determine if it would prevent leaf yellowing of nobile dendrobium as it works on potted asiflorum lilies (Funnel and Heins, 1998).

In summary, low temperature holding after vernalization is an effective and inexpensive way to extend flowering time of nobile dendrobium orchids in temperate climate areas and open the marketing window. Compared to the potential market reward obtained by coinciding the flowering time with Mother's Day and the summer market, slight leaf abscission and slightly fewer flower number per node could be an acceptable forfeiture.

CHAPTER IV

SUMMARY OF FINDINGS

- Significant interactions were found between light and vernalization duration on time to flowering of Red Emperor ‘Prince’ and Love Memory ‘Fizz’, but not on that of Sea Mary ‘Snow King.
- Regardless of the cooling duration, the flowering rate of Red Emperor ‘Prince’ was 45% when vernalized in darkness and 98% in light. However, Sea Mary ‘Snow King’ and Love Memory ‘Fizz’ got 100% flowering rate regardless of being vernalized in darkness or light.
- Darkness slightly delayed flower opening for all three cultivars for any given vernalization duration.
- Regardless of light, for all three cultivars, the increase of vernalization duration deferred flowering, but decreased the time to flowering from the completion of cooling.
- Light and vernalization duration interactively affected the total flower number of Red Emperor ‘Prince’.
- For all three cultivars, light treatment during vernalization resulted in higher flowering node percentage and more but slightly smaller flowers.
- Sea Mary ‘Snow King’ and Love Memory ‘Fizz’, that were exposed to darkness during cooling, had more non-flowering nodes on both ends (top and bottom) of the pseudobulb.

- Regardless of light, compared to plants cooled for 2 weeks, those cooled for 4 to 8 weeks produced more but smaller flowers.
- Four to 8 weeks of cooling with light increased the flowering node percentage of Red Emperor ‘Prince’ compared to 2 weeks of cooling, but for Sea Mary ‘Snow King’ and Love Memory ‘Fizz’, the flowering node percentage was not affected by cooling duration.
- Regardless of light, longer cooling durations reduced the flower longevity for all the three cultivars in the first two experiments.
- Darkness during vernalization resulted in partial defoliation of Red Emperor ‘Prince’ and Sea Mary ‘Snow King’.
- Longer vernalization leaded to defoliation of Red Emperor ‘Prince’ and Love Memory ‘Fizz’.
- For Love Memory ‘Fizz’, different light intensity treatments during cooling, excluding 0 µmol•m–2•s–1 (darkness), did not affect time to flowering, node differentiation or flower quality, except that 50 µmol•m–2•s–1 treatment slightly increased flower diameter compared to 200 and 100 µmol•m–2•s–1.
- For Love Memory ‘Fizz’, 0 µmol•m–2•s–1 (darkness) treatment increased time to flowering as well as time to full flowering. It also lowered flowering node percentage and total flower number, increased flower diameter and shortened flower longevity.
- Increasing low temperature holding duration after vernalization from 0 to 16 weeks extended the time to reach flowering of Sea Mary ‘Snow King’ and Red

Emperor ‘Prince’ up to 3 months (59 d).

- Longer low temperature holding duration deferred flowering, but shortened the time to flowering from the completion of cooling.
- Increasing low temperature holding duration after vernalization had no effect on the node differentiation of Red Emperor ‘Prince’ or Sea Mary ‘Snow King’, except slightly reducing the aborted bud number of Sea Mary ‘Snow King’.
- For Red Emperor ‘Prince’, longer low temperature holding duration slightly increased the flower diameter, slightly decreased the flower number per flowering node, affected flower longevity without a clear trend, and had no impact on total flower number.
- Longer low temperature holding duration after vernalization did not affect any flower quality parameter (especially no reverse impact on flower longevity) of Sea Mary ‘Snow King’.
- Increasing low temperature holding duration led to leaf yellowing and abscission of Red Emperor ‘Prince’, but not for Sea Mary ‘Snow King’.

LITERATURE CITED

The American Orchid Society. 2002. *Dendrobium:* The American Orchid Society culture sheets. 5 Apr. 2010. http://www.mikes-orchids.com/dendrobe.pdf.

Bichsel, R.G., T.W. Starman, and Y.T. Wang. 2008. Nitrogen, phosphorus, and potassium requirements for optimizing growth and flowering of the nobile dendrobium as a potted orchid. HortScience 43:328-332.

de Jong, K.Y., F.T.h. de Greef, A.J. Dop, and N.A.A. Hof. 1990. Year-round culture of tulips. Acta Hort. 266:109-114.

Ding, T.H., H.T. Ong, and H.C. Yong. 1980. Factors affecting flower development and production of Golden Shower (*Oncidium* Goldiana). Proc. of the Third ASEAN Orchid Congress. p: 65-78.

Fulcher, M., 2003. The enchanted orchid. Cooroy Gardens Pty Ltd, Queensland, Australia.

Funnel, K.A. and R.D. Heins. 1998. Plant growth regulators reduce postproduction leaf yellowing of potted asiflorum lilies. HortScience 33:1036-1037.

Hartley, G., T.A. Nell, R.T. Leonard, J.E. Barrett, and T.H. Boyle. 1995. Effects of interior light and temperature on longevity of *Rhipsalidopsis*. Acta Hort. 405:164-169.

Hew, C.S. and J.W.H. Yong, 2004. The physiology of tropical orchids in relation to the industry. 2nd ed. World Scientific Publishing Co. Pte. Ltd., Singapore.

Higuchi, H. and M. Hara. 1973. Effects of low temperature and light on flowering of *Dendrobium*. Abstr. Jpn. Soc. Hort. Sci. Autumn Meet. p: 290-291 (in Japanese).

Higuchi, H., M. Hara, and U. Katano. 1974. Effects of low temperature and light on flowering of *Dendrobium*. (2) Effects of low temperature treatment, light quality and light intensity on flowering respose. Abstr. Jpn. Soc. Hort. Sci. Spring Meet. p: 284-285 (in Japanese).

Ichihashi, S. 1997. Orchid production and research in Japan, p. 171-212. In: J. Arditti and A. M. Pridgeon (eds.), Orchid biology: reviews and perspectives, VII. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Kessler, J.R. 2001. Easter lily: commercial greenhouse production. 26, Apr. 2010. http://www.ag.auburn.edu/hort/landscape/Elily.htm.

Ketsa, S., A. Uthairatanakij, and A. Prayurawong. 2001. Senescence of diploid and tetraploid cut inflorescences of *Dendrobium* 'Caesar'. Scientia Hort. 91:133-141.

Kubota, S., J. Yamamoto, Y. Takazawa, H. Sakasai, K. Watanabe, K. Yoneda, and N. Matsui. 2005. Effects of light intensity and temperature on growth, flowering, and single-leaf CO_2 assimilation in *Odontioda* orchid. J. Jpn. Soc. Hort. Sci. 74:330-336.

Lavarack, P.S., W. Harris, and G. Stocker, 2000. Dendrobium and its relatives. Timber Press, Portland, OR.

Li, Z. and Y. Wang. 2005. The flowering control of nobile-type dendrobium. China Flowers and Hort. 8:20-24 (in Chinese).

Li, Z., Y. Wang, Z. Peng, C. Wang, K. Miao, and Y. Yu. 2009. Physiological effects of 6-BA and GA_3 on flower bud differentiation of nobile type dendrogium. Subtropical Plant Sci. 38:15-18.

Lin, G.M. and N. Lee. 1984. Effect of temperature on growth and flowering of *Phalaenopsis* white hybrid. J. Chinese Soc. Hort. Sci. 30:223-131.

Lin, M., T. Starman, Y.T. Wang, and G. Niu. 2011. Vernalization duration and light intensity influence flowering of three hybrid nobile dendrobium cultivars. HortScience 46:406-410.

Lopez, R.G. and E.S. Runkle. 2004. The effect of temperature on leaf and flower development and flower longevity of *Zygopetalum* Redvale 'Fire Kiss' orchid. HortScience 39:1630-1634.

McConnell, J., Guerrero, R. Leon, and J. Mafnas. 1990. Environmental factors affecting flowering in some vandas and dendrobiums in the tropics. Proc. Nagoya Intl. Orchid Show. p: 174-175.

Nash, N. 2003. *Phalaenopsis* primer: a beginner's guide to growing moth orchids. Orchids 72:906-913.

Prince, T.A., M.S. Cunningham, and J.S. Peary. 1987. Floral and foliar quality of potted Easter lilies after STS or phenidone application, refrigerated storage, and simulated shipping. J. Amer. Soc. Hort. Sci. 112:469-473.

Qian, H., Y. Liu, Y. Zheng, J. Yu, and W. Fan. 2009. Effects of applying 6-BA on the Nobile-type Dendrobium flower bud differentiation and changes of hormones. J. of Beijing Forestry Univ. 31:27-31.

Roh, M.S. 1990. Bub abnormalities during year-round forcing of Asiatic hybrid lilies. Acta Hort. 266:147-154.

Rotor, G.B.J. 1952. Daylength and temperature in relation to growth and flowering of orchids. Cornell Agr. Expt. Sta. Bul. 885:1-47.

Sakanishi, Y. and K. Fuziwara. 1982. Effects of temperature on shoots and lateral buds development of *Dendrobium.* Abstr. Jpn. Soc. Hort. Sci. Autumn Meet. p: 422-423 (in Japanese).

Sinoda, K. 1994. Orchid, p. 161-165. In: K. Konishi, S. Iwahori, H. Kitagawa and T. Yakuwa (eds.), Hort. in Japan. Asakura Publ. Co., Ltd., Tokyo, Japan.

Sinoda, K., M. Hara, and M. Aoki. 1984. Growth and flowering control in *Dendrobium.* (4) Cold treatments and flowering. Abstr. Jpn. Soc. Hort. Sci. Spring Meet. p: 364-365 (in Japanese).

Sinoda, K., M. Hara, and M. Aoki. 1985. Growth and flowering control in *Dendrobium*. (5) The effects of day and night temperature during cold treatment on development of lateral buds. Abstr. Jpn. Soc. Hort. Sci. Autumn Meet. p: 396-397 (in Japanese).

Sinoda, K. and K. Suto. 1988. Growth and flowering control in *Dendrobium.* (7) Influence of duration of low and high temperature on flower bud initiation. . Abstr. Jpn. Soc. Hort. Sci. Autumn Meet. p: 542-543 (in Japanese).

Siam Orchid Culture Co., Ltd. (SOC). 2009a. About Nobile type: The investment of dendrobium orchid hybird. 15 Sept.
2010. http://www.siamnobile.com/aboutnobile.html.

Siam Orchid Culture Co., Ltd. (SOC). 2009b. History: Our *Dendrobium* nobile. 15 Sept. 2010. http://www.siamnobile.com/history/history.html.

Staby, G.L. and T.D. Erwin. 1977. The storage of Easter lilies. Florists' Review 161:38.

Suto, K. and K. Tutui. 1980. Flower induction of *Dendrobium*. Abstr. Jpn. Soc. Hort. Sci. Spring Meet:438-439 (in Japanese).

Teoh, E.S., 2005. Orchids of Asia. Times Editions- Marshall Cavendish, Singapore.

U.S. Department of Agriculture (USDA). 2010. Floriculture crops 2009 summary. U.S. Dept. Agr., Washington, D.C.

Wang, Y.T. 1995. *Phalaenopsis* orchid light requirement during the induction of spiking. HortScience 30:59-61.

Wang, Y.T. 1998. Deferring flowering of greenhouse-grown *Phalaenopsis* orchids by alternating dark and light. J. Amer. Soc. Hort. Sci. 123:56-60.

Wang, Y.T. and T. Starman. 2008. The Nobile Dendrobium. ICOGO Bul. 2:12-13.

Wang, Y.T., T.W. Starman, R.G. Bichsel, C.Y. Yen, and M. Lin. 2010. The commercial production of the nobile dendrobium as a pot plant - a scientific and practical approach. Proc. 2010 Taiwan Intl. Orchid Symp. p: 98-143.

Wang, Y.T., W.T. Tsai, and T.E. Dai. 2006. Heavy shading - An effective, low-cost alternative to high temperature for inhibiting spiking in *Phalaenopsis*. Proc. 2006 Taiwan Intl. Orchid Symp. p: 164-173.

Wang, Z., G. Zhu, M. Ou, and B. Wang. 2008. Induction of plant growth regulators on early in vivo flowering of Nobile-type Dendrobium. Guangdong Agri. Sciences 10:37-39.

Yamaguchi, S., T. Kataoka, and T. Nakano. 1977. Studies on the flowering of *Cymbidium*. 4. Bull. Mie Agric. Tech. Center 6:67-72.

Yamamoto Dendrobiums Hawaii. 2010a. About Yamamoto Dendrobiums. 6 Sept. 2010. http://www.yamamotodendrobiums.com/html/About%20Us.html.

Yamamoto Dendrobiums Hawaii. 2010b. General Care. 6 Sept. 2010. http://www.yamamotodendrobiums.com/html/general_care.html.

Yamamoto Dendrobiums Hawaii. 2010c. Hints. 6 Sept. 2010. http://www.yamamotodendrobiums.com/html/hints.html.

Yen, C.Y.T. 2008. Effects of nutrient supply and cooling on growth, flower bud differentiation, and propagation of the nobile dendrobium orchid. MS Thesis, Texas A&M Univ., College Station.

Yen, C.Y.T., T.W. Starman, Y.T. Wang, A. Holzenburg, and G. Niu. 2008a. Timing of nutrient termination and reapplication for growth, flower initiation, and fowering of the nobile dendrobium orchid. J. Amer. Soc. Hort. Sci. 133:501-507.

Yen, C.Y.T., T.W. Starman, Y.T. Wang, and G. Niu. 2008b. Effect of cooling temperature and duration on flowering of the nobile dendrobium orchid. HortScience 43:1765-1179.

Yoneda, K., H. Momose, and S. Kubota. 1991. Effect of daylength and temperature in juvenile and adult *Phalaenopsis* plants. J. Jpn. Soc. Hort. Sci. 60:651-657.

Zou, C.Y. and Y. Liu. 2010. Research advances in *Dendrobium* research in China. J. Anhui Agr. Sci. 38:6164-6166 (in Chinese).

APPENDIX A

REPEATED EXPERIMENT: EFFECT OF LIGHT AND COOLING DURATION ON FLOWERING OF DENDROBIUM RED EMPEROR ‘PRINCE’ (JAN. 2009 – May. 2010)

In this experiment, *Den.* Red Emperor 'Prince' plants had received some degree of vernalization before the cooling treatment started. In addition, plants cooled in darkness for 8 weeks (14.3% flowering rate) were infected by fungus and got very sick (Fig. A1), resulting in a low flowering percentage (Fig. A2).

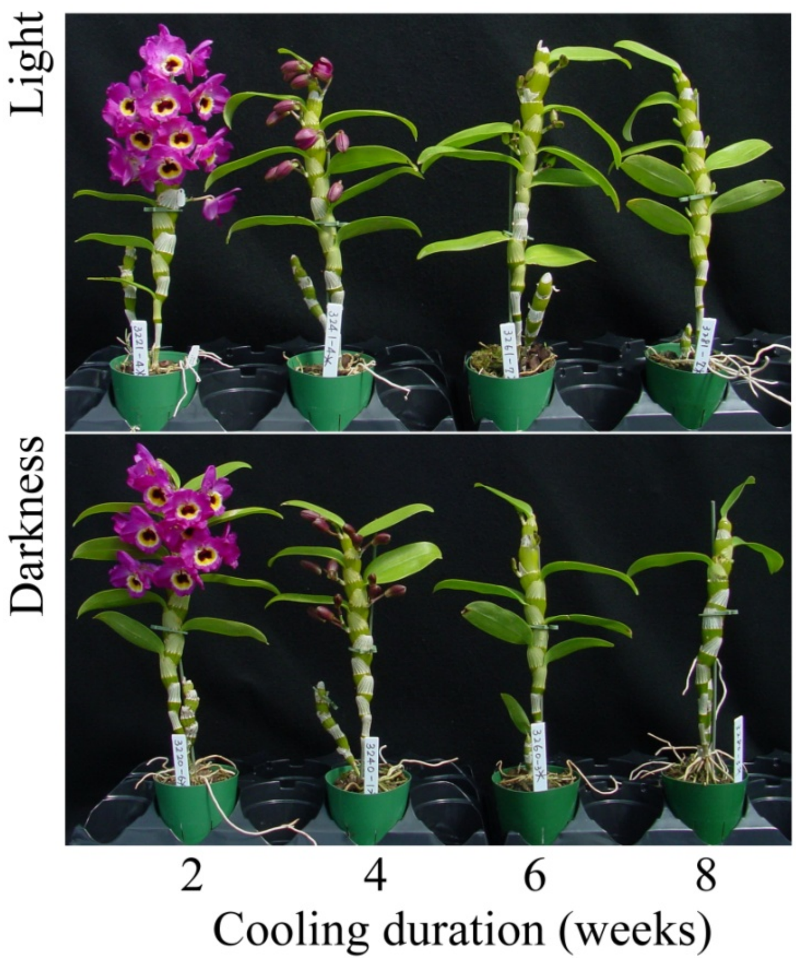

Fig. A1. Visual performance with various cooling durations and light conditionsof *Den.* Sea Mary 'Snow King', on 30 March. 2009, 10 weeks and 4 days after initiation of vernalization

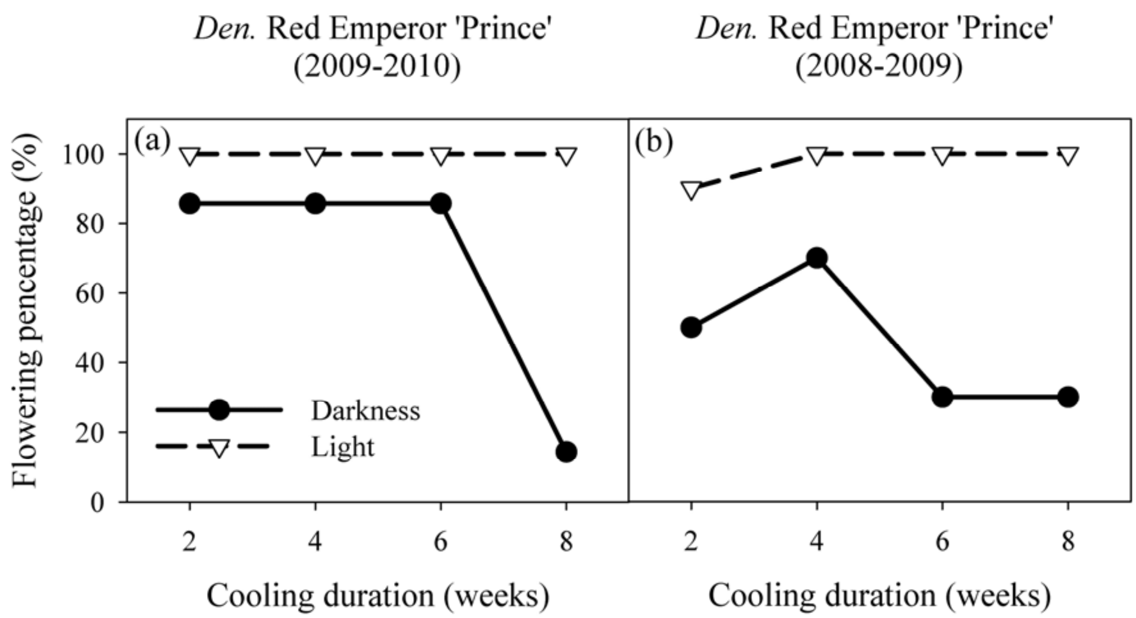

Fig. A2. Effects of various cooling duration on flowering percentage of *Den.* Red Emperor 'Prince' in two experiments: (a) experiment conducted during 2009 to 2010; (b) experiment conducted during 2008 to 2009 (Chapter II).

Table A1. Effect of cooling duration regardless of light intensity on time to flowering of *Den.* Red Emperor 'Prince'.

Vernalization duration (week)	Time to flowering from the beginning of cooling (d)	Time to flowering from the end of cooling (d)	Time to full flowering (d)
2	61.5 d[Z]	47.5 a	3.2 a
4	75.7 c	48.7 a	1.7 b
6	85.4 b	44.4 b	1.6 b
8	98.6 d	41.6 c	1.5 b
Significance	**	**	**

[Z] Means within columns followed by different letters are different
** Significant at $P \leq 0.01$, NS No significant.

Table A2. Effect of light regardless of vernalization duration on time to flowering of *Den.* Red Emperor 'Prince'.

Light during vernalization	Time to flowering from the beginning of cooling (d)	Time to flowering from the end of cooling (d)	Time to full flowering (d)
Light	78.9 a[Z]	44.1 b	1.8
Darkness	77.6 b	48.7 a	2.4
Significance	**	**	NS

[Z] Means within columns followed by different letters are different
** Significant at $P \leq 0.01$, NS No significant.

Table A3. Interaction of vernalization duration and light during vernalization on time to flowering of *Den.* Red Emperor 'Prince'.

Light × Cooling duration	Time to flowering from the beginning of cooling (d)	Time to flowering from the end of cooling (d)	Time to full flowering (d)
Significance	**	**	NS

** Significant at $P \leq 0.01$, NS No significant.

Table A4. Effect of cooling duration regardless of light intensity on node differentiation of *Den.* Red Emperor 'Prince'.

Vernalization duration (week)	Flower node percentage (%)	Aerial shoots number	Aborted flower bud number
2	40.5	0.0 b^{Z}	0.0
4	38.2	0.0 b	0.1
6	38.2	0.0 b	0.2
8	41.6	0.1 a	0.5
Significance	NS	**	NS

^{Z} Means within columns followed by different letters are different
** Significant at $P \leq 0.01$, NS No significant.

Table A5. Effect of light regardless of vernalization duration on node differentiation of *Den.* Red Emperor 'Prince'.

Light during vernalization	Flowering plant percentage (%)	Flower node percentage (%)	Aerial shoots number	Aborted flower bud number
Light	100 a	44.2 a	0 b	0.3
Darkness	68.9 b	32.4 b	0.1 a	0.0
Significance	**	**	**	NS

[Z] Means within columns followed by different letters are different

** Significant at $P \leq 0.01$, NS No significant.

Table A6. Interaction of vernalization duration and light during vernalization on node differentiation of *Den.* Red Emperor 'Prince'.

Vernalization duration × Light	Flower node percentage (%)	Aerial shoots number	Aborted flower bud number
Significance	NS	**	NS

** Significant at $P \leq 0.01$, NS No significant.

Table A7. Effect of cooling duration regardless of light intensity on flower quality and remaining leaf percentage of *Den.* Red Emperor 'Prince'.

Vernalization duration (week)	Total flower number	Flower diameter (cm)	Flower number per flowering node	Flower longevity (d)	Node w/o flower (top)	Node w/o flower (bottom)	Remaining leaf percentage (%)
2	10.4	6.3	2.4	26.6	0.8	5.6	49.0
4	10.5	6.6	2.5	23.5	0.8	6.0	53.1
6	9.9	6.3	2.4	22.1	0.9	5.8	45.5
8	11.6	6.6	2.3	20.5	1.0	5.8	50.5
Significance	NS	NS	NS	NS	NS	NS	NS

** Significant at $P \leq 0.01$, NS No significant.

Table A8. Effect of light regardless of vernalization duration on flower quality and remaining leaf percentage of *Den.* Red Emperor 'Prince'.

Vernalization duration (week)	Total flower number	Flower diameter (cm)	Flower number per flowering node	Flower longevity (d)	Node w/o flower (top)	Node w/o flower (bottom)	Remaining leaf percentage (%)
Light	11.8 a	6.2 b	2.3 b	22.0	0.7 b	5.7	44.7
Darkness	8.6 b[Z]	6.7 a	2.5 a	25.6	1.1 a	5.9	56.0
Significance	**	**	*	NS	**	NS	NS

[Z] Means within columns followed by different letters are different

* Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$, NS No significant.

Table A9. Interaction of vernalization duration and light during vernalization on flower quality and remaining leaf percentage of *Den.* Red Emperor 'Prince'.

Vernalization duration (week)	Total flower number	Flower diameter (cm)	Flower number per flowering node	Flower longevity (d)	Node w/o flower (top)	Node w/o flower (bottom)	Remaining leaf percentage (%)
Significance	NS	**	NS	NS	**	NS	NS

** Significant at $P \leq 0.01$, NS No significant.

APPENDIX B

REPEATED EXPERIMENT: EFFECT OF LIGHT INTENSITY DURING VERNALIZATION ON FLOWERING OF DEN. RED EMPEROR ‘PRINCE’ AND DEN. LOVE MEMORY ‘FIZZ’ (AUG. 2009 – DEC. 2010)

In this experiment, plants had received some degree of vernalization before the cooling treatment started. The flowering rate of the plants cooled in darkness was almost 0 (Fig. B1), which conflicted with the results of previous experiments (Chapter II). Besides, for *Den.* Love Memory 'Fizz', 2 to 4 aerial shoots occurred in each pseudobulb, compare to 0 aerial shoots produced in the previous experiment (Chapter II).

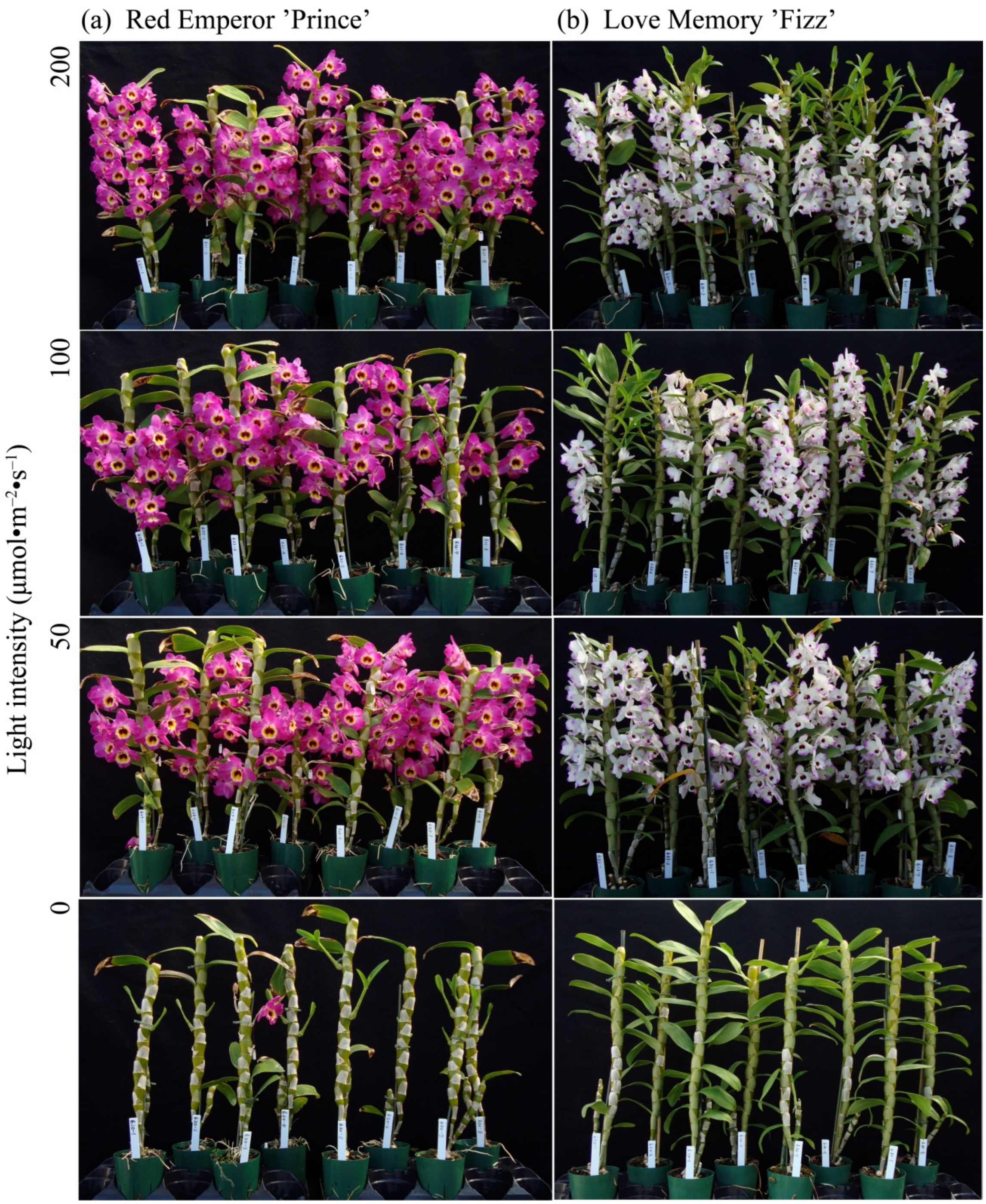

Fig. B1. Visual performance with light intensity during vernalization of (a) *Den.* Red Emperor 'Prince' and (b) *Den.* Love Memory 'Fizz' on 6 Nov. 2009, 8 weeks and 2 days after the completion of vernalization.

Table B1. Effect of light intensity during vernalization on time to flowering and node differentiation of *Den.* Red Emperor 'Prince' and *Den.* Love Memory 'Fizz'.

Light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Time to flowering (d)	Time to full flowering (d)	Flowering plants percentage (%)	Flowering nodes percentage (%)	Aerial shoot number	Aborted bud number
			D. Red Emperor 'Prince'			
0 (darkness)	N/A	N/A	12.5 b[Z]	N/A	1.8 a	N/A
50	72.1	2.1	100 a	46.5 ab	0.3 b	0.3 b
100	71.7	2.6	87.5 a	40.1 b	0.4 b	1.1 a
200	70.5	1.3	100 a	63.0 a	0 b	0.4 b
Significance	NS	NS	**	*	**	*
			D. Love Memory 'Fizz'			
0 (darkness)	N/A	N/A	0.0 b	N/A	0.6 b	N/A
50	68.4	5.6	87.5 a	52.3	2.0 ab	1.6
100	68.8	3.9	87.5 a	48.1	3.0 a	0.3
200	67.4	3.1	100 a	44.5	3.8 a	0.3
Significance	NS	NS	**	NS	*	NS

[Z] Means within columns followed by different letters are different

** Significant at $P \leq 0.01$, * Significant at $P \leq 0.05$, NS No significant.

Table B2. Effect of light intensity during vernalization on flower quality and remaining leaf percentage of *Den.* Red Emperor 'Prince' and *Den.* Love Memory 'Fizz'.

Light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Total flower number	Flower diameter (cm)	Flower number per flowering node	Flower longevity (d)	Remaining leaf percentage (%)
			D. Red Emperor 'Prince'		
0 (darkness)	N/A	N/A	N/A	N/A	15.6 b
50	15.4 b^{Z}	8.1	2.7	34.5 b	56.0 a
100	14.6 b	8.0	2.9	44.1 a	48.9 a
200	22.1 a	7.6	2.8	39.8 ab	46.6 a
Significance	*	NS	NS	*	**
			D. Love Memory 'Fizz'		
0 (darkness)	N/A	N/A	N/A	N/A	77.0 b
50	33.0	6.9	3.7	39.4 ab	87.6 ab
100	27.6	7.2	3.5	30.5 b	93.1 b
200	25.3	6.9	3.5	43.4 a	87.6 ab
Significance	NS	NS	NS	*	*

^{Z} Means within columns followed by different letters are different

** Significant at $P \leq 0.01$, * Significant at $P \leq 0.05$, NS No significant.

APPENDIX C

REPEATED EXPERIMENT: EFFECT OF LOW TEMPERATURE HOLDING DURATION AFTER VERNALIZATION ON FLOWERING OF DEN. RED EMPEROR ‘PRINCE’ AND DEN. LOVE MEMORY ‘FIZZ’ (DEC. 2009 – APRIL 2010)

During the period of this experiment, the greenhouse air temperature was inconsistent and fluctuated from 27℃ to 15℃ (Fig.C1), which could affect the results of the experiment.

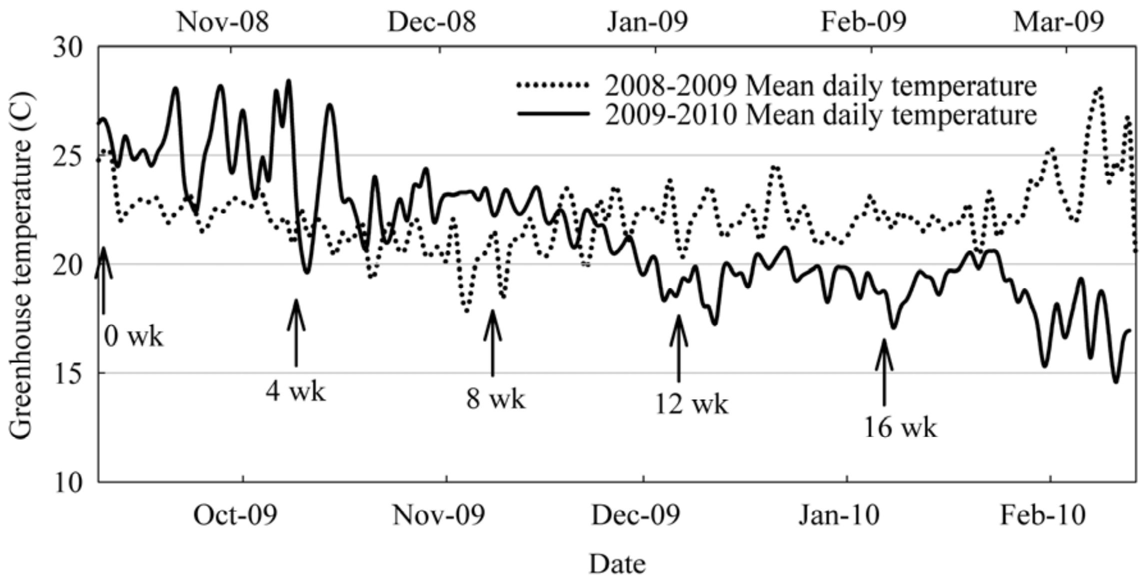

Fig. C1. Mean daily air temperatures in the greenhouse throughout the experimental period of two experiments (Experiment in Chapter III: Oct, 2008 – March, 2009; Repeated experiment: Sept, 2009 – Feb, 2010) in College Station, TX. Arrows indicate the time when plants finished cooling and entered the greenhouse.

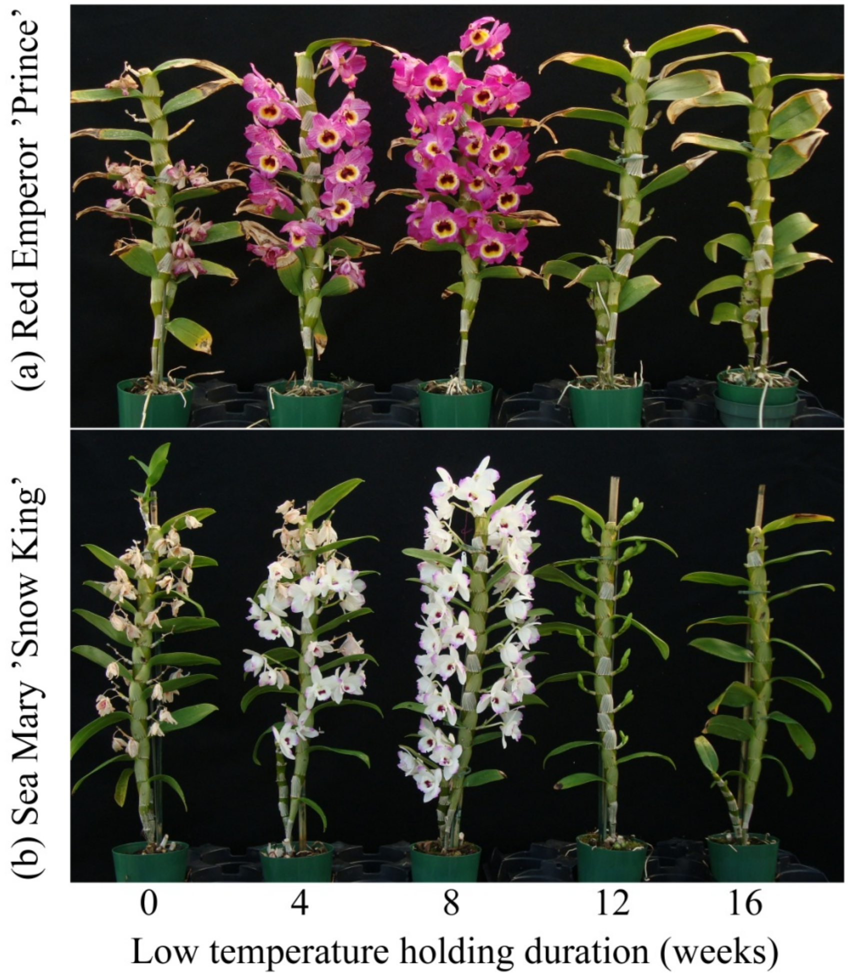

Fig. C2. Visual performance with various durations of low temperature holding after vernalization of (a) *Den.* Red Emperor 'Prince' and (b) *Den.* Love Memory 'Fizz' on 5 Jan. 2010, 17 weeks after the completion of vernalization.

Table C1. Effect of low temperature holding duration after vernalization on node time to flowering of *Den.* Red Emperor 'Prince' and *Den.* Love Memory 'Fizz'.

Low temperature holding duration (week)	Time to flowering from the beginning of cooling (d)	Time to flowering from the end of cooling (d)	Time to full flowering (d)
		D. Red Emperor 'Prince'	
0	70.9 e[Z]	43.9 c	1.5 b
4	97.0 d	42.0 d	2.3 b
8	129.4 c	47.4 b	1.9 b
12	157.5 b	47.5 b	3.8 a
16	187.8 a	49.8 a	3.8 a
Significance	**	**	**
		D. Love Memory 'Fizz'	
0	66.0 e	39.0 b	2.6
4	93.8 d	38.8 b	4.9
8	125.1 c	43.1 a	4.3
12	153.1 b	43.1 a	8.0
16	182.3 a	44.3 a	6.4
Significance	**	**	NS

[Z] Means within columns followed by different letters are different

** Significant at $P \leq 0.01$, NS No significant.

Table C2. Effect of low temperature holding duration after vernalization on node differentiation of *Den.* Red Emperor 'Prince' and *Den.* Love Memory 'Fizz'.

Low temperature holding duration (week)	Flowering node percentage (%)	Aerial shoot	Aborted bud
		D. Red Emperor 'Prince'	
0	58.3 b[Z]	0.3	0.3
4	68.5 ab	0.0	0.1
8	76.7 a	0.0	0.1
12	70.5 a	0.0	0.3
16	68.8 ab	0.0	0.9
Significance	*	NS	NS
		D. Love Memory 'Fizz'	
0	64.0 c	2.2	0.4 ab
4	74.9 ab	1.6	0.8 a
8	80.7 a	1.9	0 b
12	74.8 ab	1.9	0.5 ab
16	72.0 b	0.6	0.1 b
Significance	**	NS	*

[Z] Means within columns followed by different letters are different

** Significant at $P \leq 0.01$, * Significant at $P \leq 0.05$, NS No significant.

Table C3. Effect of low temperature holding duration after vernalization on flower quality and remaining leaf percentage of *Den.* Red Emperor 'Prince' and *Den.* Love Memory 'Fizz'.

Low temperature holding duration (week)	Total Flower number	Flower diameter (cm)	Flower number per flower node	Flower longevity (d)	Remaining leaf (%)
			D. Red Emperor 'Prince'		
0	19.1 c[Z]	7.5 c	2.7 a	37.0 c	47.9
4	24.5 a	7.6 bc	2.7 a	46.3 ab	38.9
8	24.0 ab	7.7 bc	2.5 a	41.0 bc	38.7
12	20.0 bc	7.9 b	2.2 b	50.5 a	31.7
16	15.6 c	8.6 a	1.9 c	44.9 ab	37.4
Significance	**	**	**	**	NS
			D. Love Memory 'Fizz'		
0	32.9	6.9	3.2 a	35.4	85.1 a
4	35.4	6.7	3.1 ab	37.7	73.5 ab
8	31.6	6.9	3.0 ab	39.9	76.3 ab
12	32.2	7.0	2.9 ab	35.2	48.1 c
16	30.3	6.6	2.8 b	31.6	60.3 bc
Significance	NS	NS	*	NS	**

[Z] Means within columns followed by different letters are different

** Significant at $P \leq 0.01$, * Significant at $P \leq 0.05$, NS No significant.

VITA

Name: Min Lin

Address: Department of Horticultural Sciences,
Room 202 Horticulture/Forest Science Bldg., 2133 TAMU,
College Station, TX 77843-2133

Email Address: limitmm@tamu.edu

Education: B.S., Biological Sciences, Beijing Normal University, 2007
M.S., Horticulture, Texas A&M University, 2011

Experience: Graduate Teaching / Research Assistant. August 2008 – present.
Department of Horticultural Sciences, Texas A&M University,
College Station, TX.

Graduate Research Assistant, August 2007 – August 2008.
College of Medicine, Texas A&M System Health Science Center,
College Station, TX

Undergraduate Research Assistant, April 2006 – June 2007.
Key Laboratory for Cell Proliferation and Regulation Biology,
Beijing Normal University, Beijing, China

Publications: Lin, M., T. Starman, Y.T. Wang, and G. Niu. 2011. Vernalization duration and light intensity influence flowering of three hybrid nobile dendrobium cultivars. HortScience 46:406-410

Wang, Y.T., T.W. Starman, R.G. Bichsel, C.Y. Yen, and M. Lin. 2010. The commercial production of the nobile dendrobium as a pot plant – a scientific and practical approach. Proc. 2010 Taiwan Intl. Orchid Symp. p: 98-143.

Presentations: Lin, M., T. Starman, Y.T. Wang, and G. Niu. 2010. Deferring flowering of two hybrid nobile dendrobium cultivars by holding plants under low temperature after vernalization. Poster presentation at the conference of the American Society for Horticultural Science, Aug 2010, Palm Desert, CA

Lin, M., T. Starman, Y.T. Wang, and G. Niu. 2009. Light and cooling requirements for vernalization of two hybrid nobile dendrobium Orchids. Oral presentation at the conference of the American Society for Horticultural Science, July 2009, St. Louis, MI