VARIETY COMPARISON AND MODELLING FLOWERING OF *LIMONIUM PEREZII* (Stapf) Hubb. × *LIMONIUM SINUATUM* (L.) Mill. 'LSLP4'

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Abstract

A series of new inter-specific hybrids have been derived between *L. perezii* and *L. sinuatum*. One of the selections 'LSLP4' offers potential as a cut flower. Precise knowledge on quality, yield and timing of these selections, as well as scheduling 'LSLP4' accurately in commercial production were required. To address these needs, this project comprised both a variety trial and an experiment to model the time to flower.

Plants of 'LSLP4', 'LSLP5' (a sibling of 'LSLP4'), *L. perezii* and *L. sinuatum* derived from tissue culture were grown in a temperature-controlled (daily mean temperature around 20°C) greenhouse and long-day photoperiod. With the exception of the inferior wing characteristic, the yield, timing, and quality as well as the consistency of yield and quality of 'LSLP4' were intermediate or superior to *L. sinuatum* and *L. perezii*. The potential of 'LSLP5' as a cut flower could not be assessed due to its failure to flower during the variety trial.

To develop a predictive model for time to flower of 'LSLP4', 7 sequential plantings were conducted from autumn through to late spring, utilizing one of two light regimes (50% shaded and no-shade). This resulted in 11 treatments of average daily light integral (DLI). Duration from transplanting to first visible flower bud (DTV) was correlated with average DLI, with the response being saturated above 15 $mol \cdot m^{-2} \cdot d^{-1}$. This relationship between DTV and average DLI is the foundation of a 'pre-planting' predictive model for 'LSLP4'. DTV was also correlated with leaf number accumulation rate (LNAR) and ground cover index increase rate (GCIR). The

combination of average DLI and LNAR together as predictors of DTV improved the r^2 of the model over that using DLI alone from 88% to 92%, which subsequently formed the basis of a 'post-planting' predictive model. It was recommended that growers of 'LSLP4' for cut flowers use the 'pre-planting' model to schedule planting dates and predict flowering time according to historical DLI data. Once planting occurs, and actual DLI and LNAR are collected, the prediction of DTV can be refined by the post-planting model.

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List of Abbreviations and Units of Measurement

CDLI	cumulative daily light integral	$mol \cdot m^{-2}$
DLI	daily light integral	$mol \cdot m^{-2} \cdot d^{-1}$
DTFH	duration from transplanting to first harvest	days
DTH	duration from first visible flower bud to harvest	days
DTV	duration from transplanting to first visible flower bud	days
GCI	ground cover index	$cm^2 \cdot cm^{-2}$
GCIR	ground cover index increase rate	$cm^2 \cdot cm^{-2} \cdot d^{-1}$
GDD	growing degree days	$°C \cdot d$
LNAR	leaf number accumulation rate	$leaves \cdot d^{-1}$
MLN	number of leaves below the first visible flower bud	leaves
NLN	number of leaves presented between transplanting and first visible flower bud	leaves
PRSS	predictive residual sum of squares	--
PTR	photothermal ratio	$mol \cdot m^{-2} \cdot °C^{-1} \cdot d^{-1}$
RD	rosette diameter	cm
SE	Standard error	--
T_a	daily mean temperature	°C
VIA	visual impact area	cm^2

Chapter 1 General Introduction

1.1 Overview of the New Zealand Cut Flower Industry

The New Zealand cut flower industry has developed well in the last two decades. Exports of cut flowers have increased from $8 million in 1985 to $48 million in 2002 (Kerr et al., 2002). Combined domestic and export earnings from cut flowers currently contribute approximately $125 million to the New Zealand economy, with "new" cut flower selections representing 22% of this value.

The floriculture industry in New Zealand has been successful in developing novel cut flowers for export, from species and cultivars of *Cymbidium* Swartz., *Zantedeschia* Spreng and *Sandersonia* Hook. These successes have encouraged the New Zealand cut flower industry to focus on developing new cut flower varieties so as to ensure survival in the international cut flower market.

Crop & Food Research Ltd. is one of New Zealand's Crown Research Institutes and has a programme that specializes in introducing and breeding novel cut flowers. They have successfully developed a series of inter-specific hybrids within the genus *Limonium*. One of the hybrids has been commercialized as 'Chorus Magenta', and exported from New Zealand as both planting material and flowers. With ongoing breeding, more new *Limonium* selections have been identified with potential as cut flower crops.

1.2 *Limonium* Species Grown as Cut Flower Crops

Limonium is a well-known genus in the international cut flower market, and was ranked 19th in cut flower sales through Dutch auctions in 1999 (VBN, 1999). The popularity of *Limonium* is not only because of their wide range of adaptation within tropical and temperate zones, but also their attractive florets and long lasting calyces. The flowers of most *Limonium* species can be air-dried, which further extends their use and marketing opportunities.

Several *Limonium* species are grown as cut flowers. The best known species are *L. sinuatum* and the free-flowering statice hybrids between *L. latifolium* (Sm.) Kuntze. and *L. bellidifolium* (Gouan) Dumort. (Armitage, 2003). As *Limonium* became popular in the international flower market, more species were selected as cut flowers, such as *L. perezii*, *L. tetragonum* (Thunb.) Bullock., *L. suworowii* (Reg.) Kuntze. and *L. perigrinum* (Bergius) R.A.

There are more than 150 species in the *Limonium* genus (Baker, 1948). These display a range of morphological characteristics, which provides many opportunities to develop new selections through inter-specific hybridisation (Burge et al., 1995). Breeding to incorporate desirable traits (e.g. long flower stem) from different *Limonium* species into new selections has been demonstrated. For example, 'Chorus Magenta' is a selection from crosses between *L. perigrinum* and *L. purpuratum* L. (Morgan et al., 2001). The long stem characteristic is an attribute from *L. purpuratum*, which is not grown commercially due to its less attractive inflorescence.

1.3 *Limonium* 'LSLP4' and 'LSLP5'

A series of inter-specific hybrids have been developed between *L. perezii* and *L. sinuatum* using embryo rescue techniques (Morgan et al., 2001; Morgan et al., 1998). The objective of this breeding was to produce new forms of *Limonium* which retain inflorescence characteristics from *L. perezii*, e.g. long and smooth stem (i.e. no wings or wing extensions) and a large panicle, but include the range of flower colours evident in *L. sinuatum*.

L. sinuatum is one of the most common *Limonium* species in the international cut flower market. It is usually grown as an annual. The inflorescence is particularly valued for the dense and bright colours from long lasting calyces. Breeding of *L. sinuatum* has provided numerous hybrids of various colours, ranging from the pure white 'Iceberg' through the clear pink 'Pacific Twilight' and the aptly named 'Sunset' mixtures, to deep blues and violets (Huxley et al., 1992). There are however some characteristics of *L. sinuatum* that reduce the ornamental value of cut stems. *L. sinuatum* has angular stems with 0.5-0.6 cm wings and 2-3 cm wing extensions. The wings and wing extensions easily become yellow in the vase shortening the vase life (Steinitz and Cohen, 1982). The stem length (40 cm) is shorter than some other species, e.g. *L. perezii* (60 to 90 cm), and the panicle is small (Armitage, 2003; Huxley et al., 1992). Thus, breeding aims for *L. sinuatum* are to increase stem length, reduce wings and wing extensions, and enlarge the panicle (Ed Morgan, per. comm.).

L. perezii is also grown as a commercial cut flower though only a few cultivars are available. 'Violet' was selected for its deep colour, earliness to flower

and high production (Harada, 1992). 'Atlantis' has dark blue flowers and 60 to 90 cm stems (Armitage, 2003). This species is considered attractive with its long stem length, large panicle, and smooth stem without any wings and wing extensions, but the colour range in this species is limited. It is mainly blue. Therefore, one of the breeding aims for this species is to broaden the colour range (Ed Morgan, per. comm.).

The initial inter-specific hybrids between *L. perezii* and *L. sinuatum* were sterile and the fertility was restored by doubling chromosome numbers of the hybrids (Morgan et al., 2001). A blue tetraploid was back-crossed to *L perezii* to produce a range of back-cross selections designated as 'LSLP1' to 'LSLP7'. 'LSLP4' was the first of these selections to produce flowers. 'LSLP5' was the last (Ed Morgan, per. comm.).

Preliminary visual observation by the breeder has identified that these selections, in particular 'LSLP4' (Fig. 1-1), included some improved characteristics from its parents and might have potential as a commercial cut flower. For example, the inflorescence of 'LSLP4' retained the form of *L. perezii*, i.e. larger panicle and longer stem length, while the wings and wing extensions were considered less frequent than in *L. sinuatum* (Ed Morgan, per. comm.). When the flowers within the inflorescence of 'LSLP4' reach maturity, the funnel-like calyces open acropetally and expose a white corolla. The corolla abscises 2-3 days after anthesis while the calyces remain open, a feature that also occurs in both *L. sinuatum* and *L. perezii*. The calyx colour of 'LSLP4' is deep purple-blue, and was different to the blue of *L. perezii* (Ed Morgan, per. comm.). The preceding information was only based on visual observation. Therefore, a more detailed and accurate study was required to further

quantify the morphological characteristics of 'LSLP4' through variety trials. Furthermore, to replace or supplement existing species or cultivars for horticultural use, new selections should display a number of features including: early flowering after planting, compactness of flowering over time, high flower yield, and consistent quality of product (Funnell et al., 2003). To date no evaluation of the selections of *L. sinuatum* and *L. perezii* through variety trials has been carried out and, therefore, this forms the basis of the research reported in Chapter 2.

Fig. 1-1. Inflorescence of *Limonium* 'LSLP4' showing stem length, leaf, and panicle (left) as well as close-up of flowers (right).

The commercial introduction of any new cultivars of cut flowers not only requires the validation from variety trials that their quality, yield and timing are similar or superior to that of the industry standard cultivars, but also need to provide growers with the knowledge that allows growing and scheduling of the new cultivars accurately. No research has been published investigating the response of 'LSLP4' to

light intensity, temperature, and photoperiod. Hence no data was available to develop a model for flowering prediction and scheduling plantings. This therefore forms the foundation of the research reported in Chapter 3.

1.4 Goals and aims of this study

The goals of this research were to provide horticulturists with some useful information for selecting *Limonium* selections, and also some crop scheduling strategies of 'LSLP4'. Within these goals the aims were:

1. To compare the quality, yield and timing of 'LSLP4'and 'LSLP5' to the industry standards of *L. sinuatum* and *L. perezii* through a variety trial
2. To develop and validate a model to predict time to flower of 'LSLP4'

Chapter 2 Evaluation of new *Limonium* selections as cut flowers

2.1 Introduction

2.1.1 Variety Trial for 'LSLP4' and 'LSLP5'

Variety trials may be defined as the studies in which species or selections are evaluated for comparative performance (Osborne and Simonne, 2002). They are used by horticulturists to develop and update variety recommendations. A variety trial, therefore, was desired to evaluate 'LSLP4' through comparing the selected attributes with one of its siblings, i.e. 'LSLP5', as well as its parents, i.e. *L. perezii* and *L. sinuatum*.

Variety trials have been conducted to evaluate horticultural crops, such as, *Trachelium caeruleum* L. (Liang and Harbaugh, 2001), cabbages [*Brassica oleracea* Group Capitata] (Morales-Payan and Stall, 2004), Marianna rootstocks [*Prunus cerasifera* Ehrh.×*P. munsoniana* Wight & Hedr.] (Southwick et al., 1999) and lemons [*Citrus limon* (L.) Burm. f.] (Fallahi et al., 1990). Although the objectives and methodology of these variety trials varied, three basic aspects were included in most trials. As explored below in more detail, these are: 1) selecting desirable crop attributes for variety comparison; 2) including reference varieties (current industry standards); 3) growing crops under standard or representative conditions for data collection.

2.1.1.1 Attribute Selection

Not only should attributes of crops measured in variety trials be able to differentiate varieties, but they should also be of interest to consumers, growers, industry representatives, and other professional horticulturists (Osborne and Simonne, 2002). For example, a variety trial for lemon fruits may focus primarily on fruit quality including: fruit size, juice content and rind thickness, because consumers are concerned about these qualities when they buy lemons (Fallahi et al., 1990). At the same time, fruit yield, tree growth and disease resistance, which were also recorded in the trial, are important attributes that the growers of lemons are interested in.

In variety trials attributes can be grouped under the three broad categories of quality, yield or timing (Table 2-1). As discussed in more detail below, quality, comprising a wide range of attributes, has been a main focus in variety trials. While typically being reported as a single attribute, yield is recorded as the quantity of cumulative-harvested-saleable parts of crops within a certain period. Timing involves the days required to produce crops from sowing or transplanting, but has not always been reported in variety trials.

2.1.1.1.1 Quality

Quality includes both sensory factors that are readily perceived by human senses (e.g. appearance) and hidden factors (e.g. disease resistance, flavour, nutritional value, and vase life) (Shewfelt, 1999). These factors are crop-specific. For example when rootstocks of prune were chosen, at least in part, it was based on their disease or pest resistance/susceptibility (Southwick et al., 1999). In contrast, flavour

and nutritional value are primary qualities for edible crops, i.e. vegetable and fruits. For cut flowers however, appearance and post harvest quality are key quality factors that affect their marketability and determine the acceptability by consumers.

Table 2-1. List of attributes measured in the variety trials of selected-published articles.

Crop	Measured attributes	Reference
Trachelium cut flower	Flower colour (Q*) Stem length, diameter and weight (Q) Inflorescence diameter (Q) Vase life (Q) Days to harvest (T) Disease resistance (Q)	Liang and Harbaugh (2001)
Rootstocks for prune	Leaf potassium and nitrogen (Q) Fruit weight and size (Q) Tree root suckers (Q) Dry fruit yield (Y) Disease resistance (Q)	Southwick et al. (1999)
Cabbage hybrids	Head weight, diameter and length (Q) Core diameter and weight (Q) Marketable yield (Y) Days to harvest after transplanting (T)	Morales-Payan and Stall (2004)
White sweet corn varieties	Ear characteristics (Q) Appearance (Q) Sweetness (Q) Flavour (Q) Yield (Y)	Simonne et al. (1999)

*Q: Quality; Y: Yield; T: Timing.

2.1.1.1.1.1 Appearance

Quality factors included in appearance of cut flowers are: size and shape of the inflorescence, colour, stem length and stem strength (Grower Books, 1980). The size and shape of an inflorescence can be quantified by flower diameter, petal length, the number of flower buds per stem, and product or ratio of panicle width and height (Harbaugh and Scott, 2003; Liang and Harbaugh, 2001; Sachs et al., 1976). For *Limonium* species, which are normally used as fillers for flower arrangements,

appearance is one of the main qualities that customers and florists are interested in. The appearance of *Limonium* is not dependent on individual florets, but a whole view of the panicle. In addition, the presence of stem wings and wing extensions, stem length and calyx colour also contribute. The cut stems of some *Limonium* species, e.g. *L. perezii*, which have a large panicle, can easily make a saleable bunch with an adequate visual impact of colour using 3 to 6 stems, while 12 or more stems are required for *L. sinuatum* to make a bunch with a similar amount of visual impact of colour (Armitage, 2003). Therefore, measuring calyx colour, panicle size (i.e. visual impact area), stem length and presence of stem wings and wing extensions would be an optimal way to evaluate and distinguish the appearance of 'LSLP4', 'LSLP5' and their parents.

2.1.1.1.1.2 Post harvest Quality

When consumers buy cut flowers they not only consider the appearance of the products, but also the behaviour in the vase, which is defined as post harvest quality (e.g. vase life and flower bud opening). A high quality cut flower has a long vase life and develops flowers while in the vase.

Previous studies have identified that stem degradation (e.g., stem yellowing, branch bending and drying) and cessation of flower bud opening were two primary factors determining the vase life of many *Limonium* selections, e.g. *L. sinuatum* (Steinitz and Cohen, 1982), *L. perigrinum* (Lewis and Borst, 1993), and 'Chorus Magenta' (Burge et al., 1998). Although the decorative value of the stems is maintained by the open calyces long after the wilting of the petals in these *Limonium*

selections, stem degradation soon after harvest noticeably reduced their ornamental value. The post harvest performance of 'LSLP4' and 'LSLP5' is unknown. However, considering the genetic similarity between *L. sinuatum*, 'LSLP4', and 'LSLP5', it was hypothesized that the stem degradation and cessation of flower bud opening would also be major limitations for long vase life of 'LSLP4' and 'LSLP5'. Therefore in this variety trial, stem degradation and flower bud opening, as determinants of vase life, were considered to be appropriate to evaluate the four selections.

The Hunter colour meter has been used to record colour change of horticultural crops, such as tomato fruit (Arias et al., 2000) and the spathe of *Zantedeschia* (Funnell and Downs, 1987). The L*a*b colour system closely represents human sensitivity to colour. L* presents the lightness factors of colour, while the a*/b* ratio corresponds to the magnitude of green through to yellow colour. The a*/b* ratio can, therefore, be a potential parameter to quantify the degree of stem yellowing during the vase life of *Limonium* species.

The maturity at which stems are harvested affects flower bud opening during vase life in many *Limonium* species. Industry experience suggests that *L. perezii* can be harvested with at least 25% of the flower buds open, while stems do not continue to open adequately if harvested with less than 11% buds open (Hebditch, 1985). Few (< 5%) flower buds on stems of *L. perigrinum* 'Ballerina Rose' opened after being harvested with 40% flowers open (Lewis and Borst, 1993). Current commercial recommendations suggest *L. sinuatum* can be harvested when the flower calyces are mostly (≥ 80%) open (Armitage, 2003), with about 80% open for *L. perezii*. It was assumed in this study that the optimal maturity for the harvest of 'LSLP4' and

'LSLP5' could also be when about 80% of the calyces were open. This assumption avoided the need to conduct separate investigations into the influence of inflorescence maturity at harvest.

2.1.1.1.2 Yield

Yield is an important attribute because it directly determines growers' market returns. Cultivars with higher yields are more desirable to growers. Accordingly, yield is a vital component measured in variety trials.

Yield varies greatly in different species and cultivars of *Limonium*. Commercial reports on the yield of *L. sinuatum* showed that the plant can produce about 20 stems per plant in 7 months. For *Limonium* hybrids of the 'Misty' series, yields of up to 20 stems per plant were recorded after 4 months from planting, while only 5 stems were harvested per plant for *L. perezii* in the first year (Armitage, 2003). Yield even varied between the cultivars of *L. sinuatum*. The number of harvested stems of *L. sinuatum* 'Fortress Yellow' (35 stems per plant) after 4 months were 13, 22 and 27 stems per plant more than that of 'Fortress rose', 'Fortress Apricot', and 'Dark Blue', respectively (Whipker and Hammer, 1994). Considering that yield as an attribute has the potential of differentiating *Limonium* selections, and is of interest to growers, it was, therefore, selected as an attribute in this study.

2.1.1.1.3 Timing

Timing, defined as the time required to produce a harvestable product from sowing or transplanting, is one of the main attributes used by growers to schedule

crop production and target specific market periods. By knowing the timing of a crop, growers can arrange their production in the right seasons to avoid extreme environmental conditions, e.g. drought, frost and the cold of winter. For cut flowers, whose prices fluctuate between seasons, and particularly at specific festivals (e.g. Valentine's Day and Mother's Day), scheduling them to flower at these times creates the potential for increased financial returns for growers.

Timing of *Limonium* varies greatly due to growing conditions and genetic variance. *L. sinuatum* flowers naturally in spring and summer in Mediterranean conditions, while in the tropical highlands, which are characterized by consistently warm-temperate conditions, it may bloom all year round (Shillo and Zamski, 1985). Commercial reports on the timing of *L. sinuatum* indicated that the first harvest occured in the field in Southern America approximately 3-5 months after sowing, while anywhere between four and seven months were required in North America (Armitage, 2003; Wilfret et al., 1974). However, information on the timing of the new selections investigated here was not available. This, therefore, also forms the basis of the research on modelling flowering time of 'LSLP4' using environmental factors (e.g. temperature and light intensity) presented in Chapter 3.

2.1.1.1.4 Consistency of quality, yield and timing over time

The consistent quality, yield and timing of product determine a grower's production efficiency and financial returns. This therefore has been of concern to horticulturists when selecting superior cultivars. The variability in quality and yield of individual plants, or the same plant, over the whole harvest season has previously

been reported in *Limonium* (Funnell et al., 2003; Whipker and Hammer, 1994). The stem length of 'Chorus Magenta' varied depending on the type of planting material, and there was a difference in average stem fresh weight for *L. sinuatum* over the harvest season. The corolla of *L. sinuatum* was noticeably smaller in later harvest seasons, which reduced the quality of the stems. In the current study, we therefore examined the variability in quality and yield of 'LSLP4', 'LSLP5', *L. sinuatum*, and *L. perezii*, as affected by harvest seasons.

2.1.1.2 Summary of the Selected Attributes in the Variety Trial

In summary, the attributes assessed in this variety trial included:

- quality (calyx colour, visual impact area (VIA), stem length, presence of wings and wing extensions, and post harvest quality)
- yield
- duration from transplanting to first harvest (DTFH)
- Consistency of quality and yield.

2.1.1.3 Reference Plants

To ensure the variety trial is scientifically sound, a reference variety is required. Typically the reference can be a current industry standard variety, which is commonly grown and recognized by growers. The parents of both 'LSLP4' and 'LSLP5', *L. sinuatum* and *L. perezii* are well known cut flower species, and the production of them as commercial cut flowers is well established (Armitage, 2003).

Thus, *L. sinuatum* and *L. perezii* were desirable references for 'LSLP4' and 'LSLP5' in this study.

2.1.1.4 Growing Conditions for 'LSLP4' and 'LSLP5'

Several studies have been conducted on the production protocol and environmental response of some *Limonium* species, particularly *L. sinuatum* (Krizek and Semeniuk, 1972; Semeniuk and Krizek, 1972; Semeniuk and Krizek, 1973; Shillo and Zamski, 1985). Temperature has the most pronounced effect on growth, flower initiation, and flower development of *L. sinuatum*. Mild night temperature (i.e. < 15°C) promoted flower initiation of *L. sinuatum* at the seedling stage (i.e. 5 leaves), but was not an obligate requirement (Semeniuk and Krizek, 1973). Hence a facultative vernalization response does appear to be evident for cultivars of this species. Subsequent development of flowers was favoured by higher temperatures (22-27°C /12-16°C day/night).

The vernalization requirements vary greatly between different *Limonium* species, even between the cultivars of the same species. *L. perezii* is referred to as being a "free-flowering *Limonium*" (Harada, 1992), which is taken to mean that vernalization is not requirement for flowering in this species. 'LSLP4' and 'LSLP5' have both been successfully grown to flower in greenhouses maintained at temperatures ranging between 15°C and 20°C, without vernalization during the seedling stage (E. Morgan, per. comm.). Currently there is no quantified information available concerning the vernalization requirements for 'LSLP4' and 'LSLP5', or the temperature response during the subsequent flower development period. It was

therefore assumed in this study that 15-20°C would be a suitable temperature range for the growth and development to flowering of 'LSLP4' and 'LSLP5', and would not preclude flowering of *L. sinuatum* and *L. perezii*. It would also avoid the need to examine differing vernalization requirements for each selection under investigation.

Photoperiod has been shown to influence flower initiation and development in *Limonium*. A photoperiod greater than 13–h resulted in: earlier flowering, a greater percentage of flowering plants, and higher yields, of vernalized plants of *L. sinuatum* (Semeniuk and Krizek, 1972; Shillo and Zamski, 1985). While not substantiated by data, it is considered likely that long days promote flowering of *L. perezii*, i.e. similar to *L. sinuatum* (Armitage, 2003). The response of 'LSLP4' and 'LSLP5' to photoperiod is unknown. Given the genetic similarity of the selections involved in the current experiment, we assumed that a photoperiodic response for flowering was likely in the selections under examination. Natural photoperiods at Palmerston North (40°20'S) range between 9-h and 15-h, hence 4-h of night-interaction lighting would ensure plant receive ≥13-h photoperiod. So as to avoid confounding effects of photoperiod on flowering, a long-day environment (>13–h) was created within the greenhouse used for both the variety trial and the experiment modelling the time to flower.

Supplemental photosynthetic lighting applied to the seedlings of *L. sinuatum* affected flower production (Vardar et al., 1975). The higher the irradiance, the greater the number of flower stems produced by the plants, particular in winter plantings. Accordingly, cool-white fluorescent lighting on the seedlings of *L. sinuatum* for 12–h daily, or natural light for 6–h every morning, is recommended during vernalization in

the commercial production for *L. sinuatum* (Shillo and Zamski, 1985). Clearly flower yield of *L. sinuatum* may be optimised with supplemental photosynthetic lighting. Given the genetic linkage of *L. sinuatum* to the other selections under evaluation in the current variety trial, the possibility of a similar response to light levels can not be ignored. Conducting the variety trial under the high light levels of summer was seen as a suitable strategy to grow the plants under standard or representative conditions for data collection.

In summary therefore, the growing conditions comprising temperature of 15 to 20°C, a photoperiod greater than 13–h, and summer light levels, were seen as being suitable for the growth and development of *L. sinuatum*, *L. perezii* and their hybrids.

2.2 Objectives

Some of the new inter-specific selections of *L. sinuatum* and *L. perezii*, in particular 'LSLP4', have been preliminarily identified with the potential to be used as commercial cut flowers. To date, no evaluation of these selections has been reported. A variety trial is, therefore, required to compare the quality, yield and timing, as well as the consistent quality and yield over time, of 'LSLP4'and 'LSLP5' to the industry standards, i.e. *L. sinuatum* and *L. perezii*. With this information it is hoped to provide horticulturists with an informed basis on the selection of *Limonium* selections.

2.3 Materials and Methods

2.3.1 Experiment 1: appearance quality, yield and timing

2.3.1.1 General

Plants of 'LSLP4', 'LSLP5', *L. sinuatum*, and *L. perezii* produced by tissue culture were provided by Crop & Food Research Ltd, N.Z. Once deflasked, the plants were grown in a greenhouse in 50-cell trays (85 ml cell volume) for between 5 and 8 weeks to reach transplant size. Cell trays contained a soil-less medium (bark: pumice, 50: 50) plus 4.3 kg/m^3 Osmocote (16N-3.5P-10K+1.2mg), 5 kg/m^3 dolomite, 1 kg/m^3 superphosphate, 0.2 kg/m^3 calcium ammonium nitrate, 0.2 kg/m^3 FTE, 0.3 kg/m^3 iron, 0.5 kg/m^3 potassium sulphate, and 0.1 kg/m^3 terrazole. On 4 November 2003 when the majority of transplants reached transplant size, they were repotted into 1.5-litre plastic pots containing a 50: 30: 20 bark: peat: pumice mixture, plus 2.0 kg/m^3 each of agricultural lime and dolomite, 1.0 kg/m^3 gypsum, and 3.0 kg/m^3 Osmocote 16N-3.5P-10K (Grace-Sierra International, Netherlands). The plants were subsequently transferred to a greenhouse at the Plant Growth Unit, Massey University (Palmerston North, New Zealand; 40°20'S). There were ten blocks, each measuring 1.2×3.6 m, with six plots in each block (Appendix 1). All the plants were placed next to each other in a plot or between plots in a block, resulting in plants being at centers of about 20 cm. The blocks were 50 cm apart.

During the course of the experiment plants were irrigated using capillary matting. The matting was kept moist at all times through automatic watering from drippers three times per day for 5 minutes in winter and spring, and for 7 minutes in

summer. The plants were also overhead watered by hand once per week. The greenhouses (both that used during initial growth until transplant and that for the experiment) were heated at 15 °C, ventilated at 20 °C, and received natural sunlight and photoperiod (9 to 15-h). Additionally, there was 4-h of night lighting from 2200 to 0200 HR using incandescent lamps providing 2.4 $\mu mol \cdot m^{2} \cdot s^{-1}$ at the plant canopy height.

2.3.1.2 Treatments

Treatments comprised the four selections, i.e., 'LSLP4', 'LSLP5', *L. sinuatum* and *L. perezii*. While originally arranged as a completely randomized block design, the unavailability of equal numbers of plants in each selection resulted in an unequal number of replicate plots per treatment, i.e., 11 plots for 'LSLP4', 1 plot for 'LSLP5', 11 plots for *L. sinuatum* and 7 plots for *L. perezii*. Each replicate plot contained 8 plants with 16 guard plants surrounding the plot. Replicate plots of all four selections were randomly distributed across 10 blocks (Appendix 1).

2.3.1.3 Data collection

Data collection occurred twice per week over a six-month period from the date of transplanting. For individual stems of the four selections, flowering (i.e. harvest maturity) was defined as the stage of development when about 80% of calyces were open, being the stage most commonly used to signify commercial harvest maturity (refer Section 2.1.1.1.1.2). The inflorescence of 'LSLP4' and *L. perezii* typically bear 400 to 600 florets making it impractical to count the exact number of open florets on

each inflorescence. A visual method of estimation was developed and used to judge this stage of maturity (Appendix 2).

Three stems of each treatment, from those harvested on 26 March 2004, were randomly chosen for measurement of flower calyx colour. The calyx colours were identified under natural lighting following standard protocols (Royal Horticultural Society, 1966).

To maximise potential stem length, harvested stems were cut as close to the stem base as possible. The width and height of the panicle, stem length (from the top of panicle to the bottom of the stem), and presence of wings and wing extensions were recorded for each stem at harvest. The product of panicle width and height (cm^2) was subsequently calculated as a representation of the visible impact of flower colour, i.e. VIA. The appearance of wings was categorized as either '1' (less than 1 cm), '2' (less than 2 cm but more than 1 cm), or '3' (more than 2 cm). Wing extensions were categorized as '1' (present) or '0' (absent).

The date when the first stem flowered (80% of calyces open), and was harvested, was recorded for each plant. DTFH was calculated as the number of days from transplanting to the date of first harvest of a plant.

Yield was calculated as the total number of the stems harvested from an individual plant over the six months after transplanting.

2.3.1.4 Data analysis

Data were analyzed using GENSTAT 6 (VSN International Ltd., UK). The data were tested first for block and plot effects, and for the presence of a linear trend across the blocks and plots in the greenhouse. Data were then subjected to an analysis of variance. When necessary, data were log transformed to stabilise the variance. Means reported are based on the back-transformed data and were separated using least significant difference ($\alpha = 0.05$).

2.3.2 Experiment 2: postharvest quality evaluation

2.3.2.1 General

'LSLP5' failed to flower within the six-month period of the experiment. Thus, only *L. sinuatum*, *L. perezii* and 'LSLP4' stems were available for assessment. Stems with 80% of calyces open were harvested between 0900 HR and 1000 HR on 18 June 2004, and trimmed to 40 cm for *L. sinuatum* and 70 cm for both *L. perezii* and 'LSLP4'. The stems were then transferred to a vase life room (20 ± 1 °C, 70-90% relative humidity, and 12-h (0600 HR – 1800 HR) photoperiod with 25μmol·m^{-2}·s^{-1} at bench height provided by cool-white fluorescent tubes. The stems were held in distilled water during the assessment period.

The experiment was designed as a randomized block with three treatments of *L. sinuatum*, *L. perezii* and 'LSLP4' and three blocks. Each block comprised 10 stems, including three or four stems of each treatment.

2.3.2.2 Data collection

Data were recorded every two days for a 24-day period. Within a panicle the youngest branch (i.e. the lowest branch) in each stem was chosen to record the number of buds (petals) continuing to open during vase life. The open flower buds were counted then removed. The date when no more buds opened was recorded for each stem. The period over which the flower buds continued to open was then calculated.

The end of vase life was defined as the time when stems showed symptoms of unacceptable quality, i.e., $\geq$ 10 cm in stem length of wings and 8 wing extensions showing obvious yellowing, a branch having dried out and becoming bent, or no more buds opening.

Stem colour was measured using a Minolta Chroma Meter CR-200 (Minolta Camera Co. Ltd., Osaka, Japan) consisting of a head with an 8 mm diameter measuring area. Three points along the main stem were randomly sampled and a*, b* and L* values were measured on each of these three points every two days.

2.3.2.3 Data analysis

Data were subjected to ANOVA using the general linear model procedure of SAS (SAS Institute Inc., 1999), and means were separated by using Duncan's multiple range test at $P \leq 0.05$.

2.4 Results and Discussion

'LSLP5' failed to flower during the six months of cultivation. In contrast all plants of 'LSLP4', *L. sinuatum* and *L. perezii* flowered within the period of experiment with the first harvest commencing on 5 January 2004. Hence only the data for 'LSLP4', *L. sinuatum* and *L. perezii* are presented. The potential of 'LSLP5' as a cut flower could not be assessed due to its failure to flower.

2.4.1 ANOVA

A likelihood ratio test indicated that the effects from blocks and plots were not significant ($P = 0.262$). Similarly Wald tests showed that the linear trends across the blocks and plots in the greenhouse were also not significant ($P = 0.951$). Therefore, the data were pooled for a one-way ANOVA (Table 2-2). Log transformation was required to stabilise the residual variance of all attributes.

Table 2-2. Summary of ANOVA for all attributes of 'LSLP4', *L. sinuatum*, and *L. perezii* grown at Palmerston North, New Zealand from Nov. 2003 to May 2004.

Attribute	Mean square error	F ratio	Significance
Ln[z] (Yield)	0.1164	931.42	$P < 0.001$
Ln (DTFH)[y]	0.01297	760.97	$P < 0.001$
Ln (Stem length)	0.008664	529.74	$P < 0.001$
Ln (VIA)[x]	0.1112	1519.28	$P < 0.001$

[z]Data were log transformed
[y]Duration from transplanting to the first harvest.
[x]Visual impact area

2.4.2 Yield

The yield of 'LSLP4' in the four-month-harvest period was 2 stems per plant greater than that achieved by *L. perezii* ($P < 0.05$), and 10 stems per plant lower than *L. sinuatum* ($P < 0.05$; Table 2-3), suggesting that the yield of 'LSLP4' was closer to that of *L. perezii*. The yield of *L. sinuatum* and *L. perezii* reported here differed from data available from other published sources, i.e., 20 stems per plant per year for *L. sinuatum* and 5 stems per plant in the first year trial of *L. perezii* (Armitage, 2003). Starman et al. (1995) also reported that the yield of *L. sinuatum* 'Pastel Shades' grown in the field was 17 stems per plant over a three month period, while they recorded no stems harvested over this period for *L. perezii*. These differences in yield are likely to result from differences compared with this experiment in: data-collection period, growing environmental conditions, and selections evaluated.

2.4.2.1 Yield distribution

The yield distribution of the three selections differed in the 19-week harvest period (from 5 January to 14 May 2004). For 'LSLP4', the number of cut stems remained relatively evenly distributed from the 4th week to 19th week, with 47% of yield occurring in the first 8 weeks of harvest for this selection (Fig. 2-1). Whilst 71% yield of *L. sinuatum* and 93% yield of *L. perezii* were concentrated in the first 8 weeks of their respective harvest periods. Once flowering commenced, there was no week in which 'LSLP4' did not have flowers harvested, but in *L. sinuatum* and *L. perezii* one and four weeks of no yield was recorded, respectively. The concentrated-yield distribution of *L. sinuatum* reported here is in accordance with previous reports that

the number of the stems cut over the harvest season was focused in the first 8 weeks for yellow and rose cultivars of *L. sinuatum* (Whipker and Hammer, 1994).

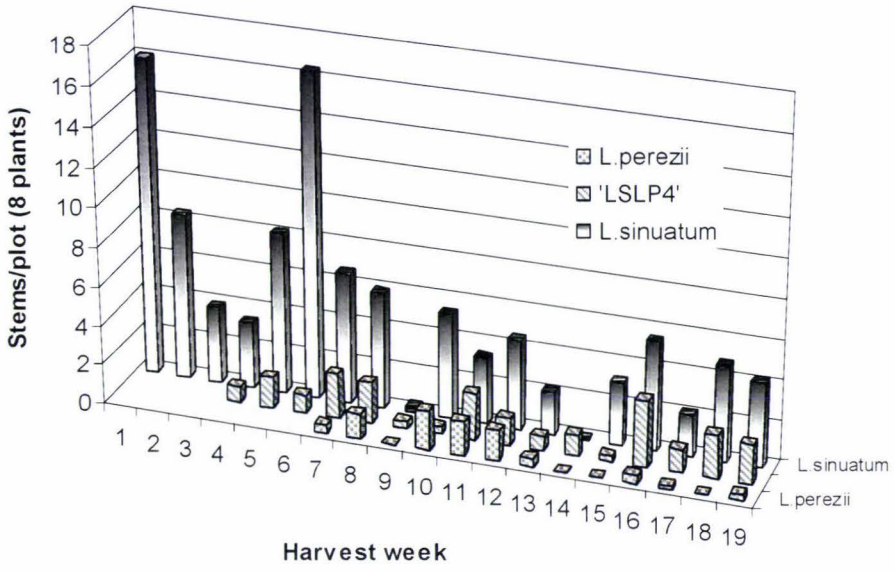

Fig. 2-1. Number of stems per plot (8 plants) harvested weekly (week 1 began at 5 Jan. 2004) of *L. sinuatum*, 'LSLP4', and *L. perezii*.

Table 2-3. Stem yield, stem length, visual impact area, and duration from transplanting to the first harvest (DTFH) of 'LSLP4', *L. sinuatum*, and *L. perezii* grown in Palmerston North, New Zealand between Nov. 2003 to May 2004.

Variety	Stem Length (cm)	Visual impact area (cm^2)	Yield (stem/ plant)	DTFH (days)	Frequency of wing appearance		Frequency of wing extension appearance		Vase life (days)	Frequency of reason for vase life termination		
					'1' [y]	'2' [y]	'1' [x]	'0' [x]		Stem yellowing	Branch bending	Bud opening
'LSLP4'	73	481	3	107	95%	5%	100%	0%	9.2	13%	63%	50%
L. sinuatum	48	45	13	64	100%	0%	100%	0%	8.9	80%	0%	40%
L .perezii	74	711	1	131	0%	0%	0%	100%	8.6	33%	22%	67%
LSD	1.0	1.1	1.1	1.1	--	--	--	--	2.7	--	--	--

[z] total number of the plants

[y] '1': wing less than 1 cm; '2': wing less than 2 cm but more than 1 cm

[x] '1': presence of wing extension; '0': absence of wing extensions

2.4.3 Timing

'LSLP4' required an average 107 days from transplanting to reach first harvest, which was 24 days earlier than *L. perezii*, but 43 days longer than *L. sinuatum* (Table 2-3). This indicates that the time required to the first harvest of 'LSLP4' was also intermediate between that for *L. sinuatum* and *L. perezii*.

2.4.4 Quality

2.4.4.1 Appearance

2.4.4.1.1 Colour

The calyx colour of 'LSLP4' was deep purple (93C; RHS, 1966), which was different from the blue (91B) in *L. perezii* and pink (78C) in *L. sinuatum* (Appendix 2). 'LSLP4' therefore has met one of the breeding goals for *Limonium*, i.e. broadening the colour range of *L. perezii*.

2.4.4.1.2 Stem length and visual impact area

The inflorescence form of 'LSLP4' was more like that of *L. perezii* than *L. sinuatum*. The average stem length of 'LSLP4' over the harvest season was not different from *L. perezii* ($P > 0.05$), but was 25 cm longer ($P < 0.05$) compared with *L. sinuatum* (Table 2-3). Although the VIA of 'LSLP4' was intermediate between its parents, VIA of 'LSLP4' (480 cm^2) was closer to *L. perezii* (711 cm^2) than to *L. sinuatum* (45 cm^2). Hence, 'LSLP4' appears to have retained the form of *L. perezii* in terms of both stem length and VIA, which was desired in the *Limonium* breeding

objectives. The similarities between 'LSLP4' and *L perezii* reflect the back-cross lineage in the breeding of 'LSLP4', i.e. [*L. perezii* × *L. sinuatum*] × *L. perezii*.

2.4.4.1.3 Consistency of stem length and visual impact area

The stem length of the three selections varied over the 19 weeks of flowering (from 5 Jan to 14 May 2004). The stem length of 'LSLP4' increased up to the 9th harvest week and then remained relatively constant until the end of the harvest period (Fig. 2-2). *L. perezii* also had increasing stem length up to the 13th harvest week, while the stem length of *L. sinuatum* decreased up to the 11th week and then increased noticeably from 40 to 64 cm until the 19th week. As a measure of variability of stem length over time, the standard deviation for 'LSLP4', *L. sinuatum* and *L. perezii* were 4.0, 6.2 and 6.7 cm, respectively. The stem length of 'LSLP4' was, therefore, more consistent than for *L. perezii* and *L. sinuatum* over the whole harvest period. As consistency is a desirable feature of any new selections, this outcome is considered to be an improvement over the reference selections.

The inconsistency of the stem length over the harvest season might result from the variation of environmental factors (e.g. irradiance). Reduction of irradiance has increased the stem length of many species, such as *Sandersonia aurantiaca* Hook. (Davies et al., 2002), and *Angelonia augustifolia* Benth. (Miller and Armitage, 2002). During the current study the weekly daily light integral (DLI) dropped from 23 to 15 $mol \cdot m^{-2} \cdot d^{-1}$ between 30 January and 3 March 2004, which might partly explain the increase in stem length of 'LSLP4' over this period (i.e., 4th to 9th week of harvest). A further decrease of DLI from 15 to 5 $mol \cdot m^{-2} \cdot d^{-1}$ between March and May 2004, had

no effect on the stem length of 'LSLP4'. However, for *L. sinuatum*, the reduction in stem length up to the 11th week, and the notable increase again after that, did not correspond with the decreasing DLI during that period. So as to maintain and improve stem quality over the seasons, more research on the factors affecting stem length in *Limonium* is, therefore, recommended for the future.

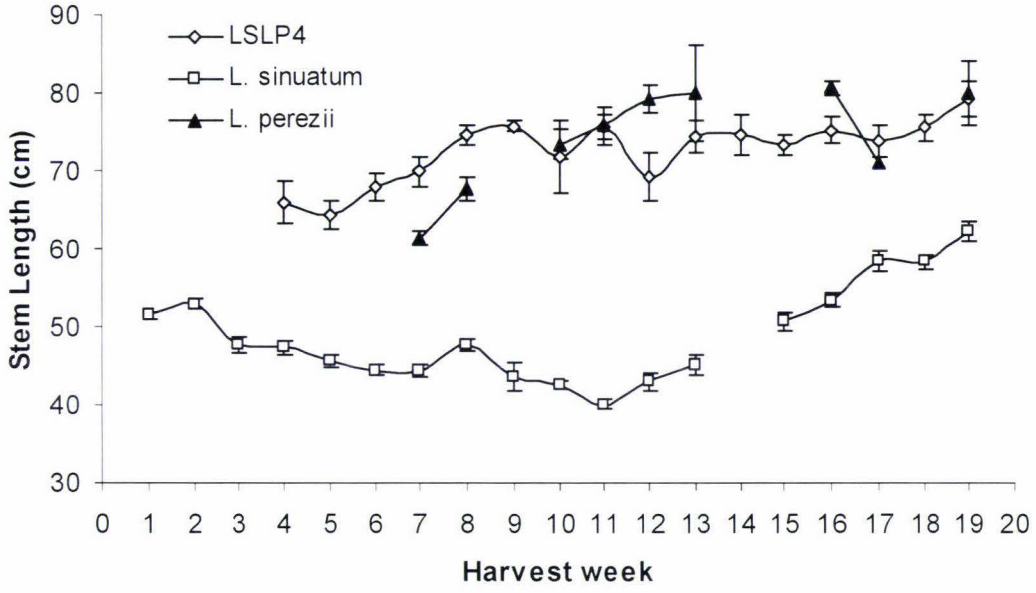

Fig. 2-2. Average stem length harvested weekly (week 1 began at 5 Jan. 2004) of 'LSLP4', *L. sinuatum*, and *L. perezii*. Vertical bars are the SE of the stems harvested in the same week.

The VIA of the three selections also varied over the harvest period. The VIA of 'LSLP4' and *L. perezii* remained relatively stable over the entire harvest period, while VIA of *L. sinuatum* was larger in the first two weeks than subsequently, and declined significantly from the 10th to 13th week (Fig. 2-3). During the entire period of flower harvesting the standard deviation of VIA after log transforming for 'LSLP4', *L. sinuatum*, and *L. perezii*, were 0.3, 0.4 and 0.2, respectively. Hence, the consistency of VIA exhibited by 'LSLP4' is intermediate to that of its parents.

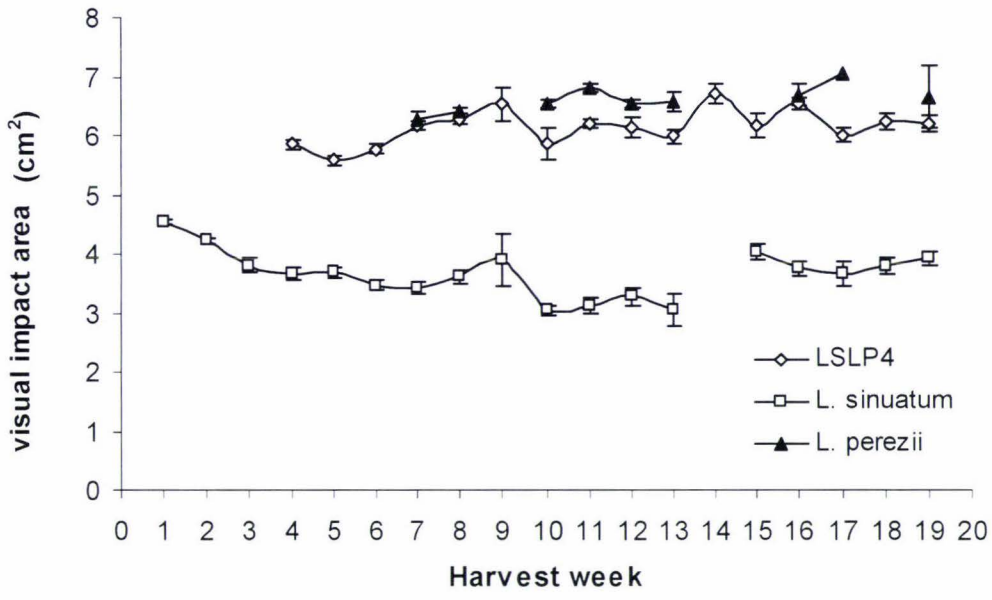

Fig. 2-3. Visual impact area (log-transformed) of stems of 'LSLP4', *L. sinuatum*, and *L. perezii*, harvested weekly (week 1 began at 5 Jan. 2004). Vertical bars are the SE of the stems harvested in the same week.

2.4.4.1.4 Wing and wing extensions

Wings and wing extensions were present on stems of 'LSLP4' (Fig. 2-6 A). Wings of 'LSLP4' were categorized as either '1' or '2', though the frequency of category '1' wings was 90% higher than '2' (Table 2-3). All stems of *L. sinuatum* were categorized as '1' for the presence of wings and *L. perezii* had no wings on any stems. In addition, wing extensions were present on all stems of 'LSLP4' and *L. sinuatum*, but not on stems of *L. perezii*.

With a desire to minimise the presence of wings and wing extensions, the appearance quality of 'LSLP4' in this regard was, therefore, inferior compared to that of both its parents. It was interesting to note however, that the 5% of 'LSLP4' stems which achieved category '2' wings, all occurred in the later harvest weeks (i.e. from

14th to 19th week) (Fig. 2-4). The reason for the appearance of stems with category '2' wings being concentrated later in the season is unknown. It would be interesting and valuable to investigate in future research what causes the change in wing development of 'LSLP4' as the harvest season progresses.

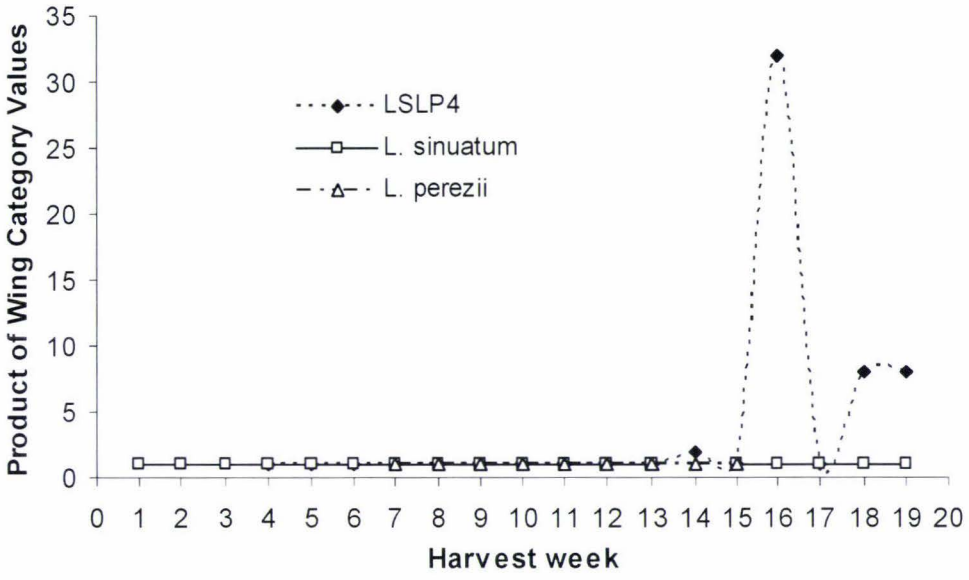

Fig. 2-4. The product of wing category values (equal to number of stems harvested weekly multiplied by wing category, i.e., '1', '2' and '3') of 'LSLP4', *L. sinuatum*, and *L. perezii* (week 1 began at 5 Jan. 2004).

2.4.4.1.5 Summary of appearance quality

In terms of pre-harvest appearance-quality, 'LSLP4' has retained the form of *L. perezii*, i.e. long stem length and large panicle, whilst it has retained the inferior wing characteristic of *L. sinuatum*. The colour of 'LSLP4' was different from that of *L. sinuatum* and *L. perezii*. The consistency of stem length in 'LSLP4' over the harvest period was higher than that of *L. sinuatum* and *L. perezii*, while VIA was intermediate to that achieved by its parents.

2.4.4.2 Post harvest quality

The vase life of 'LSLP4', *L. sinuatum* and *L. perezii*, as determined by stem yellowing, branch bending, or completion of bud opening, were not significantly different (Table 2-3). However the highest frequency for the reason vase life was terminated differed between selections. Branch bending was the main reason for termination of the vase life in 'LSLP4', compared with stem yellowing in *L. sinuatum*, and completion of bud opening in *L. perezii*.

The a*/b* ratio of 'LSLP4' and *L. sinuatum* remained relatively constant over the first five days, and increased noticeably after that (Fig. 2-5). In contrast, the a*/b* ratio of *L. perezii* decreased over the first five days and subsequently increased over the remaining period of vase life assessment. The rapid increase of the a*/b* ratio in *L. sinuatum* and 'LSLP4' corresponded with visible yellowing of the stems (Fig. 2-6). At the termination of vase life of *L. sinuatum*, which was most frequently attributed to stem yellowing, the a*/b* ratio reached a value of -0.48. As yellowing of stems progressed, i.e. a*/b* values greater than -0.48, the stem colour was not considered to be acceptable. Hence, an a*/b* ratio of -0.48 can be used as a threshold value for acceptable stem colour in *Limonium*. During the period of vase life assessment, the a*/b* ratio of *L. perezii* was significantly lower than for 'LSLP4' and *L. sinuatum* ($P < 0.05$), and also not greater than -0.48. This corroborates the suggestion that an a*/b* ratio of -0.48 can be used as a threshold value for acceptable stem colour in *Limonium*, and also that stem yellowing is not a key issue for vase life termination of *L. perezii*. The a*/b* ratio of 'LSLP4' was also significantly less than that recorded for *L. sinuatum* from day 5 to 10 of the vase life period ($P < 0.05$), and reached -0.48 at day

12. Collectively this suggests that stem yellowing of 'LSLP4' was more similar in nature to *L. sinuatum* than *L. perezii*.

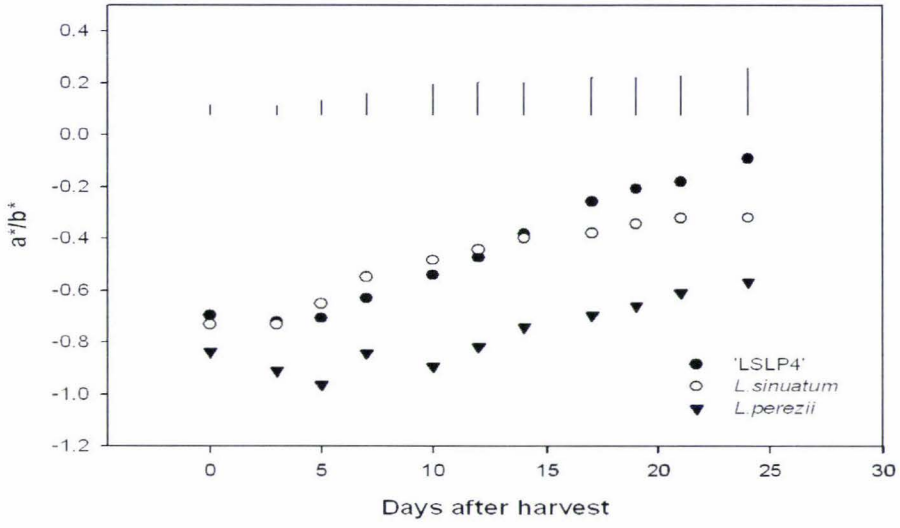

Fig. 2-5. Chromaticity a*/b* ratio of 'LSLP4', *L. perezii* and *L. sinuatum* during 24 days of vase life evaluation. Bars are LSD 5% (d.f.=23) on days with a significant difference ($P < 0.05$) between treatments.

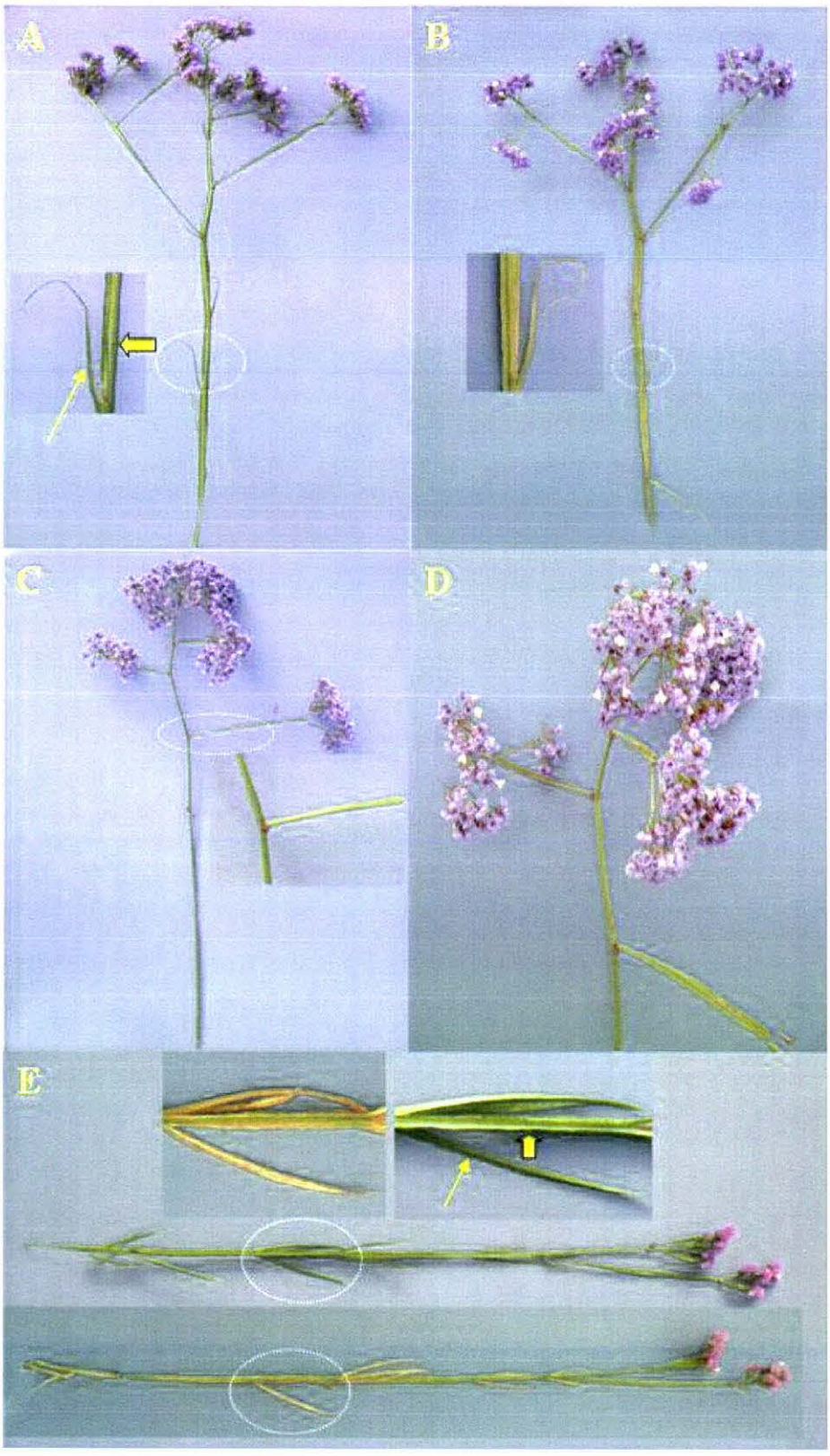

Fig. 2-6. Inflorescence of *Limonium* 'LSLP4' (A and B), *L. perezii* (C and D), *L. sinuatum* (E), showing differences between stems at harvest and 9 days later. Inserted images are magnified regions of stems contained within the ellipse. ↑ indicates wing extension and ⇦ indicates wing.

2.5 Conclusion

The potential of 'LSLP5' as a cut flower could not be assessed due to its failure to flower during the variety trial. In contrast, with exception to the inferior wing characteristic, the yield, timing, and quality, as well as the consistency of quality and yield of 'LSLP4' were intermediate or superior to *L. sinuatum* and *L. perezii*. Hence 'LSLP4' can be considered a worthwhile selection for consideration by cut flower growers, offering some improvements over those used as a reference.

Chapter 3 Modelling duration to flower of *Limonium* 'LSLP4'

3.1 Introduction

Limonium 'LSLP4' has been identified as a potential commercial cut flower (refer Chapter 2). To be adopted by commercial growers as a new cut flower crop, information needs to be available on the timing of flowering after planting (Funnell et al., 2003). No information exists for this crop on the basic topics such as the response to changes in temperature, photoperiod, and light intensity. Hence no information is available for growers to cultivate 'LSLP4' and ensure accurate timing. Being a novel inter-specific hybrid, with parents of divergent environmental responses, these basic environmental responses can not safely be assumed. So as to construct a reliable timing model, investigation into the effects of temperature, photoperiod, and light intensity on time to flower of 'LSLP4' was, therefore, required.

The effects of temperature, light intensity and photoperiod on time to flower has been reported for many horticultural crops, such as, petunia (*Petunia* × *hybrida* Vilm; Adams et al., 1998), raspberry (*Rubus idaeus* L.; Carew et al., 2003), and *Thalictrum delavayi* Franch. (Huang et al., 1999). A timing model has then been developed for these crops to predict flowering time and schedule plantings according to actual environmental conditions during commercial production. A brief review on this research will be included in the following section, with extension of this to discuss the potential relevance to 'LSLP4' and, therefore, the research reported here.

3.1.1 Thermal energy and duration to flower

Thermal energy (i.e. temperature) is one of the most important environmental factors that determine the rate of plant developmental processes, including progress to flower. Temperature may affect time to flower in three different ways: vernalization for flower initiation; hastening progress to flower with increasing temperature up to an optimum temperature; and delaying time to flower at supra-optimum temperature (Roberts and Summerfield, 1987). In the absence of evidence to suggest that an obligate requirement for vernalization exists for 'LSLP4' (refer Section 2.1.1.4), only the latter two temperature responses will be discussed here.

3.1.1.1 Effective temperatures

For a diverse range of plant species, such as pansy [*Viola×wittrockiana* Gams.], raspberry and chrysanthemum [*Dendranthema grandiflora* Tzvelev.], the rate of progress to flower increased linearly with increasing temperature up to an optimum temperature (Adams et al., 1997; Carew et al., 2003; Pearson et al., 1993). This linear relationship can be described as a simple function Eq. [3-1] as:

$$1/f = a + b\,T_e \qquad \textbf{[3-1]}$$

where 1/f is the rate of progress to flower (reciprocal of time to flower), a and b are genotypic-specific constants, and T_e is the effective temperature. T_e can be estimated Eq. [3-2] as:

$$T_e = T_o - |T_o - T_a| \quad (T_b < T_a < T_c) \qquad \textbf{[3-2]}$$

where T_o is the optimum temperature at which progress to flower is maximal; T_a is the actual daily mean temperature; T_b and T_c are the base and ceiling temperatures below and above which progress to flower is zero (Pearson et al., 1993). This technique assumes that the rate of progress to flower is similar but opposite above and below T_o. The base, optimum and ceiling temperatures differ between plant species and cultivars. For example, the optimum temperature for progress to flower of raspberry was 24 °C, 19 °C for the chrysanthemum cv Snapper and 22 °C for the chrysanthemum cv Westland (Carew et al., 2003; Pearson et al., 1993). A base temperature from 0 to 5 °C was generally adequate for all cultivars of pea [*Pisum sativum* L.] (Bourgeois et al., 2000), while a 6 °C base temperature was determined for sweet corn [*Zea mays* L.] (Brooking and McPherson, 1989).

3.1.1.2 Growing degree days

The linear relationship between rate of progress to flower and effective temperatures enables the progress to flower to be monitored and forecasted under conditions of fluctuating temperatures, through temperature sum, heat units or growing-degree-day (GDD) models, in units of °C·d. This GDD model has been widely used to monitor and schedule successive plantings in many crops, such as: sweet corn, summer squash [*Cucurbita pepo* L.], *Thalictrum delavayi*, pea, and Asiatic lilies [*Lilium* spp]. GDD has been recognized as an improved model for prediction of events such as flowering, as compared to the model using calendar days (Brooking and McPherson, 1989; Huang et al., 1999; NeSmith and Hoogenboom, 1994; Roberts and Summerfield, 1987; Steininger and Pasian, 2003).

Although GDD has been used extensively as a predictor of flowering time for horticultural crops, a single model does not always apply in all circumstances. There is variation in GDD to flower between cultivars, or within the same cultivar but when grown under various conditions, e.g. differing levels of vernalization, light intensity or photoperiod. As illustrations of such differences, the GDD requirement for flowering varied about 20 °C·d between five summer squash cultivars (NeSmith and Hoogenboom, 1994). *Thalictrum delavayi* accumulated 3338 °C·d to reach anthesis without previous vernalization, whilst only 2848 °C·d to flower after 6 weeks at 8 °C vernalization (Huang et al., 1999). The predicted GDD to first flowering of raspberry decreased from 2451 to 1743 °C·d as daily light integral (DLI) increased from 9.4 to 19.4 $mol \cdot m^{-2} \cdot d^{-1}$ (Carew et al. 2003). To improve the validity of some GDD models, when used in commercial horticultural scenarios, adding into the model the effects on time to flower from other environmental factors, such as DLI, photoperiod, and vernalization is, therefore, required.

3.1.1.3 Potential effect of temperature on flowering of 'LSLP4'

Limited information is available concerning the temperature effect on time to flower of *L. sinuatum* and *L. perezii*, and none is available for 'LSLP4'. Previous studies have reported that temperature had a pronounced effect on flower initiation and flower development of *L. sinuatum*, with development of flowers after initiation being favoured by higher temperatures (22-27°C /12-16°C day/night; Semeniuk and Krizek, 1973; refer Section 2.1.1.4). In addition, commercial reports noted that warm temperatures promoted flowering of *L. perezii*, and temperatures lower than 5 °C can damage the plants (Armitage, 2003; Harada, 1992). As discussed already (refer

Section 2.1.1.4) given the genetic similarity between 'LSLP4' and both *L. sinuatum* and *L. perezii*, it has been assumed in this study that 15-20°C would be a suitable temperature range for the growth and development to flowering of 'LSLP4'.

Due to time limitation for a Master's thesis, temperature was not chosen as an environmental factor to be investigated in this research. Maintaining the temperatures around 15 to 20 °C in the greenhouse over the whole period of the experiment allowed for investigating the influence of other factors (e.g. DLI) on time to flower of 'LSLP4'. This strategy was not intended to ignore or deny the potential influence of temperature on 'LSLP4', but merely reflects a situation of limited resources.

3.1.2 Daily light integral and duration to flower

Plant species show considerable phenotypic acclimation to the light environment and may respond to the quantity, quality, and duration of radiation being intercepted (Carew et al., 2003; Erickson et al., 1980; Loehrlein and Craig, 2004; Warrington and Norton, 1991). Photosynthetic radiation (400 to 700 nm) drives plant photosynthesis and dry-weight accumulation, and consequently plant development. This radiation can be integrated as daily light integral (DLI) in units of $mol \cdot m^{-2} \cdot d^{-1}$ or cumulative DLI (CDLI) in units of $mol \cdot m^{-2}$ over the duration of different development phases, e.g. vegetative and reproductive phases.

3.1.2.1 DLI

The influence of DLI on time to flower has been well documented for a wide range of species. The time to flower was progressively decreased by between 4 to 21 days as DLI increased from 4.1 to 17 $mol \cdot m^{-2} \cdot d^{-1}$ for geraniums [*Pelargonium* × *hortorum* Bailey] (White and Warrington, 1988), pansy (Niu et al., 2000), and petunia [*Petunia* × *hybrida*] (Kaczperski et al., 1991). However, DLI has no significant effects on time to flower when it is out of an effective range. There was no significant reduction in time to flower of geraniums, when DLI was above 17 $mol \cdot m^{-2} \cdot d^{-1}$, and flowering did not even occur below a DLI of 3.3 $mol \cdot m^{-2} \cdot d^{-1}$ (White and Warrington, 1988). Similarly, there was only a marginal decrease in time to flower of pansy when DLI increased above 10.6 $mol \cdot m^{-2} \cdot d^{-1}$ (Niu et al., 2000). These results indicate that time to flower responds to DLI only within a certain effective range, and that this range is crop specific.

There is little previous information published on the flowering response of *Limonium* species to light (refer section 2.1.1.4). *L. sinuatum* was recently classified as an 'irradiance indifferent' plant (Mattson and Erwin, 2005). This was based on the fact that there was no significant difference in time to flower and leaf number below the first flower, as DLI increased from 15.3 to 27.6 $mol \cdot m^{-2} \cdot d^{-1}$. However, the response of *L. sinuatum* to a DLI out of this range has not been previously examined, and, similarly, how DLI determines time to flower of 'LSLP4' was unknown.

In a commercial horticultural environment, DLI fluctuates daily and with changes in seasons. In the open in New Zealand, DLI ranges naturally from 55 $mol \cdot m^{-}$

$^{2}\cdot d^{-1}$ (clear day in midsummer) to 5 $mol\cdot m^{-2}\cdot d^{-1}$ (typical day in winter; per. comm. Prof. Ian Warrington). Hence, sequential plantings of 'LSLP4' over a period of seasonal changes of DLI would result in the plantings experiencing a wide range of DLI. This, therefore, formed the basis of our logic for this experiment, so as to enable us to investigate how 'LSLP4' responded to the natural seasonal progression of DLI, and to determine what DLI would be effective for early flowering.

3.1.2.2 Cumulative DLI

While the majority of published research has focused on determining the effects of DLI when held constant, only a few studies have evaluated the influence of CDLI during specific developmental phases. A CDLI of about 975 $mol\cdot m^{-2}$ was necessary for earliest flower initiation of geraniums grown at 18°C, and values above 1000 $mol\cdot m^{-2}$ had no further reduction (White and Warrington, 1988). In contrast a CDLI of 652 $mol\cdot m^{-2}$ significantly delayed flower bud development. This suggests that accumulating a minimum CDLI of > 652 and ≤ 975 $mol\cdot m^{-2}$ is required for rapid flower initiation of geraniums. The minimum CDLI however varies, depending on changes of other environmental factors, e.g. temperature and photoperiod. Erickson et al. (1980) found that both the duration (days) and CDLI until flowering of geranium [*Pelargonium* × *hortorum* Bailey] were greater for plants sown in February than those in April. The minimum CDLI for earliest flowering from a February sowing date was 472 $mol\cdot m^{-2}$, whilst 308 $mol\cdot m^{-2}$ was required for an April sowing. This variation might be partly explained by the increasing temperature and photoperiod in the late spring season reducing the requirement for CDLI. Therefore, not only does this highlight the need for further investigations into the combination effects of CDLI,

temperature and photoperiod on time to flower, but underlines why, for the experiment reported here, temperature and photoperiod were kept relatively constant.

3.1.3 Photoperiod and duration to flower

Plants have mechanisms for flower initiation that involve responding to photoperiod as an environmental signal. There are three main categories of these responses in different species: photoperiod-insensitive or day-neutral plants (DNPs), and two types of photoperiod-sensitive plants, i.e. short-day plants (SDPs) and long-day plants (LDPs) (Roberts and Summerfield, 1987). LDPs initiate flowers when the duration of darkness is below a certain minimum length, whilst SDPs require the duration of darkness above a certain critical length for flower initiation. Within both SDPs and LDPs, there are plants with qualitative or quantitative responses to photoperiod.

The photoperiod requirement of 'LSLP4' for flowering has not been investigated, but 'LSLP4' has been successfully cultivated under heated greenhouse conditions (minimum temperature 15 °C) with growth to flower occurring under natural long days. A quantitative long day requirement has been reported in some *Limonium* species, e.g. *L. gmelinii* and *L. sinuatum* (Enrico et al., 2000; Shillo and Zamski, 1985). While not substantiated by data, it is considered likely that long days also promotes flowering of *L. perezii,* similar to that as with *L. sinuatum* (Armitage, 2003). Based on this previous experience and the genetic similarity between 'LSLP4', *L. sinuatum* and *L. perezii,* we hypothesized that 'LSLP4' may also exhibit a LDP response for flowering. Growing 'LSLP4' in a constant long-day environment

(approximate 13 to 18-h day length) over the entire duration of the experiment reported here would, therefore, reduce the potential confounding effects of photoperiod on flowering.

3.1.4 Combination effects of thermal energy, radiant energy and photoperiod on duration to flower

Progress to flower is not determined by thermal energy, radiant energy, or photoperiod alone, but by combinated effects of two or more of these three factors. For example, time to the macrobud stage of geraniums was increased by 3 days when T_a decreased from 22.5 °C to 18°C at a DLI of 28 $mol \cdot m^{-2} \cdot d^{-1}$ compared with 9 days at a DLI of 17 $mol \cdot m^{-2} \cdot d^{-1}$ (White and Warrington, 1988). Hence changes of temperature have a stronger effect on time to flower at low than that at high DLI. Furthermore, photoperiod and DLI also interact to affect time to flower. Warner and Erwin (2003) found that DLI had no influence on time to flower of *Hibiscus trionum* L. under a 16-h photoperiod, while under a 9-h photoperiod increasing DLI from 9.5 to 16.4 $mol \cdot m^{-2} \cdot d^{-1}$ nearly halved the time to flower from 95 to 57 days.

To quantify these combination effects, temperature, DLI and/or photoperiod have been integrated into predictive models to describe time to flower of geranium, pansy, raspberry and chrysanthemum (Adams et al., 1998; Adams et al., 1997; Carew et al., 2003; Pearson et al., 1993), taking the basic form (Eq. [3-3]) of:

$$1/f = a + b\,T_e + c\,P + d\,M \qquad \textbf{[3-3]}$$

where f is the time to flower, a, b, c, and d are genotype-specific constants, T_e is the effective temperature, P is the photoperiod and M is DLI. When this basic form of model was applied for the prediction of time to flower, it explained 94% of the variation for pansy and 91% for raspberry.

Photothermal ratio (PTR) has also been used to quantify the interaction and combination effects of light intensity and temperature on plant development and quality (Liu and Heins, 1997; Liu and Heins, 2002). For any period of growth PTR can be defined as the ratio of DLI to T_a (daily mean temperature) with units of $mol \cdot m^{-2} \cdot °C^{-1} \cdot d^{-1}$. The dry weight of poinsettia [*Euphorbia pulcherrima* Willd.] increased as PTR was increased in both the reproductive and vegetative phases of growth. Enhancing PTR in the reproductive phase of poinsettia also increased the size of bracts and cyathia. No influence of PTR on time to flower has been reported, but in the context of the current study, was considered worthy of further examination for possible inclusion in a predictive model.

3.1.5 Plant growth parameters as predictors of duration to flower

Flowering of plants is not only driven by the plant's external factors (e.g. temperature and DLI), but also is strongly associated with the internal factors, e.g. vegetative growth and structural development. For example, in plants with a determinate shoot growth habit like *L. sinuatum*, the morphological change of the younger emerging leaves from a horizontal to vertical position indicates the transition from the vegetative to the reproductive stages (Shillo and Zamski, 1985). Therefore, tracking vegetative development can be an indication of physiological maturity and

also a tool of use by growers for decision support for flowering prediction in some species.

3.1.5.1 Leaf number

Leaf number accumulation is an obvious indicator of plant vegetative growth. In many species with a determinate shoot growth habit, a certain number of leaves is required before floral initiation under the conditions optimal for flowering. With *Hibiscus radiatus* Cav., regardless of changes in DLI 9 leaves were initiated below the first flower when grown under a long-day environment (Warner and Erwin, 2003). Similarly in self-inductive-flowering species, such as roses [*Rosa hybrida* L.], at any one time of the year, the transition of their shoot apices from vegetative to floral stages requires a fixed number of leaves to have appeared on the shoot (Zieslin and Moe, 1985). Thus, in these species, assuming that the leaf number accumulation rate (LNAR) is constant, the fixed number of leaves required before flowering can be used as a predictor of flowering time. Our preliminary investigations indicate that 'LSLP4' has a determinate shoot growth habit, hence the number of leaves on a shoot can be used as an indicator of both the transition from vegetative growth to flower and, it is hoped, LNAR might be useful as a predictor of time to flower.

3.1.5.2 Leaf area and rosette diameter

Vegetative growth of plants can be viewed not only from the perspective of leaf number accumulation, but also leaf area enlargement. The flowering of plants has been proposed to occur when the capacity of a photosynthetic leaf area is sufficient to sustain this procedure (Bernier et al., 1981). This infers that some measurement of

changes in leaf area could be useful as a predictor of time to flower. This hypothesis has been supported by the research with *Bougainvillea* Comm. and geranium, where leaf area and flowering time were significantly correlated (Armitage, 1984; Ramina et al., 1979).

Percentage of ground covered by leaf (i.e., ground cover index; GCI) has been widely used for the estimation of vegetation cover of land surfaces (Cyr et al., 1995; Olmstead et al., 2004), and has also been correlated with leaf area index (Shimomura et al., 2003). In contrast to LAI, GCI can be readily measured using computer-assisted digital image analysis, in which there is no need to utilize extremely large and expensive experiments so as to enable destructive harvesting of plants for measurement. Similarly the diameter of leaf area presented by a plant, i.e. rosette diameter in the case of 'LSLP4', also provides a coarse but non-destructive measure of changes in plant leaf area. As potential parameters of plant growth that can now be readily recorded, the changes in both rosette diameter and GCI, and any relationship with time to flower of 'LSLP4', were investigated in this experiment.

3.1.5.3 Application of plant growth parameters as a flowering predictor

Plant growth parameters have only been incorporated infrequently into models for prediction of flowering (Armitage, 1984; Faust and Heins, 1994; Healy and Wilkins, 1984; Heins et al., 2000). For example, with African violet [*Saintpaulia ionantha* Wendl.] the appearance of a visible flower bud in a leaf axil was correlated with the growth of the subtending leaf blade (Faust and Heins, 1994). A polynomial model was developed to describe duration to visible flower buds as a function of both

temperature and leaf blade length. Thus, in the current study, attempts were made to examine the combination of both plant growth parameters and environmental factors for prediction of flowering in 'LSLP4'.

3.2 Summary

The time to flower is affected by both environmental factors and plant growth parameters. These relationships can be quantified through a timing model, i.e. a mathematical equation (e.g. Eq. [3-1] and [3-2]). The responsive variable in the equation could be any phase of flowering, e.g. in the current experiment from transplanting to the first visible flower bud (phase 1) or from the first visible flower bud to harvest (phase 2). The predictive variables may include DLI, CDLI, temperature, GDD, photoperiod, and/or plant growth parameters. Once developed and validated, the timing model could be used as a basis for accurate crop scheduling in horticultural production.

The objective of this study was to develop and validate a model to predict time to flower (i.e. phase 1 and phase 2) of 'LSLP4' based on environmental factors (e.g. DLI and temperature) and/or various plant growth parameters (e.g. leaf number and GCI).

3.3 Materials and Methods

3.3.1 General

Plants of *L.* 'LSLP4' were derived from tissue culture, as supplied by Crop & Food Research Ltd, N.Z. The environmental conditions in the greenhouse and general methods of cultivation were as noted in Chapter 2 (refer Section 2.3.1.1).

At each date of transplanting individual plants were selected for even size and development. This was validated by destructively harvesting 5 sample plants at each planting date and recording leaf number, leaf area, rosette diameter, and plant dry weight (Table 3-1).

Table 3-1. Initial leaf number, leaf area, and plant dry weight of 5 plants sampled from planting dates between May and October 2003.

Planting month	Leaf number	Leaf area (cm^2)	Plant dry weight (g)
May	12.2a[Z]	38.8a	0.32c
June	9.2b	40.9a	0.34bc
July	10.2ab	42.3a	0.19d
August	10.4ab	26.3b	0.27cd
September	10.6ab	36.6ab	0.43ab
October	12.6a	46.3a	0.47a
L.S.D	2.84	11.8	0.11

[Z] Within columns data followed by the same letter are not significantly different ($P < 0.05$).

There were 10 sample plants with 18 guard plants surrounding each plot. All plants were placed next to each other in a plot or between plots in a block, resulting in plants being at centers of 17 cm (Fig. 3-1).

Fig. 3-1. Experimental greenhouse after initial plantings illustrating basic layout of blocks (defined by each metal frame area), plant spacing, and 'shaded' vs. 'no-shade' treatments.

3.3.2 Treatments

Treatments comprised seven planting dates from May to Nov. 2003 (Table 3-2). At most planting dates, two light levels were achieved by covering half the number of plots with spectrally-neutral woven polypropylene shade cloth of nominal 50% density (Fig. 3-1). The combination of planting dates and light levels (i.e. shaded or no-shade) resulted in treatments differing in DLI over the period from transplanting to flowering. Since a 50% reduction in light level during the months of low natural irradiance, i.e. June and July, was considered to be in excess of what would be expected to be encountered in commercial horticultural situations, shading treatments were not used in these two months. Thus, there were 11 treatment combinations (planting date × shade level) with three replicates (plots) for each treatment. With the exception of the last planting in November, each replicate comprised 10 individual plants. Due to limited plant supply the November planting treatments comprised 8 plants for each of the 3 replicates.

Table 3-2. Experimental treatments showing average DLI and daily average air temperature (T_a) of each treatment and replicate from transplanting to the first visible flower bud (phase 1) and from the first visible flower bud to harvest (phase 2).

Treatment label	Transplant Date	Shade	Plot (Rep.)	DLI (phase 1) ($mol \cdot m^{-2} \cdot d^{-1}$)	T_a (phase 1) (°C)	DLI (phase 2) ($mol \cdot m^{-2} \cdot d^{-1}$)	T_a (phase 2) (°C)
May_HL[Z]	7/05	-	1	5.7	20.9	13.0	18.8
May_HL		-	2	6.0	20.8	13.8	18.8
May_HL		-	3	6.4	20.6	14.8	18.9
May_LL		+	1	4.2	19.5	4.4	18.7
May_LL		+	2	4.0	19.5	5.7	18.6
May_LL		+	3	4.3	19.4	4.2	18.9
Jun_HL	24/06	-	1	9.0	19.9	15.8	18.9
Jun_HL		-	2	7.5	20.2	14.9	18.9
Jun_HL		-	3	8.0	20.1	15.6	18.9
Jul_HL	22/07	-	1	11.5	19.3	18.6	19.2
Jul_HL		-	2	11.4	19.2	18.1	19.2
Jul_HL		-	3	11.2	19.3	17.3	20.2
Aug_HL	4/09	-	1	14.5	19.2	19.3	19.3
Aug_HL		-	2	14.6	19.2	19.5	19.3
Aug_HL		-	3	14.8	19.1	19.8	19.5
Aug_LL		+	1	7.7	18.6	10.0	19.3
Aug_LL		+	2	7.8	18.6	10.1	19.5
Aug_LL		+	3	7.8	18.6	10.0	19.5
Sep_HL	23/09	-	1	16.1	19.2	20.3	20.7
Sep_HL		-	2	15.8	19.2	20.3	20.3
Sep_HL		-	3	16.0	19.2	20.4	20.9
Sep_LL		+	1	8.4	18.8	8.9	18.0
Sep_LL		+	2	8.2	18.8	10.2	20.5
Sep_LL		+	3	8.3	18.8	10.2	20.7
Oct_HL	7/10	-	1	17.4	19.3	20.5	21.1
Oct_HL		-	2	17.3	19.2	20.4	21.1
Oct_HL		-	3	17.3	19.3	20.6	21.2
Oct_LL		+	1	9.0	19.1	9.8	20.6
Oct_LL		+	2	8.8	19.0	10.1	20.7
Oct_LL		+	3	8.8	19.0	10.2	20.7
Nov_HL	4/11	-	1	19.7	19.8	20.0	21.7
Nov_HL		-	2	19.9	20.0	19.4	21.5
Nov_HL		-	3	19.8	20.1	19.9	21.6

[Z] Planting month and light level (HL = High Light (no-shade), LL = Low Light (shaded))

3.3.3 Data collection

3.3.3.1 Environmental parameters

3.3.3.1.1 Temperature

Greenhouse air temperatures were measured at 10 min intervals with a shaded sensor in each block at plant height, and data were recorded using a Squirrel 1200 Digital Meter/Logger (Grant Instruments Ltd., Barrington, Cambridge, U.K.). One sensor was placed in each block and, in addition, one sensor within one of the shaded plots. T_a in the greenhouse and under shade was used for the calculation of daily PTR and GDD. A linear GDD model (Roberts and Summerfield, 1987) with a base temperature 0°C was used. GDDs in units of °C·d during both phase 1 and phase 2 were calculated as Eq. [3-4].

$$GDD = T_{a1} + T_{a2} + \ldots\ldots T_{an} \quad \textbf{[3-4]}$$

where n is the duration (days) from transplanting to the first visible flower bud (phase 1) or from the first visible flower bud to harvest (phase 2). Average T_a in both phase 1 and phase 2 were calculated for each plant as Eq. [3-5].

$$\text{Average } T_a = (T_{a1} + T_{a2} + \ldots\ldots T_{an}) / n \quad \textbf{[3-5]}$$

3.3.3.1.2 DLI

Photosynthetic photon flux (PPF) was measured every 5 min using a quantum sensor (LI 190S; LI-COR Inc., Lincoln, Nebraska, USA) placed at leaf canopy level in a single shaded plot and a second sensor in a representative no-shade plot. The sensors were linked to a light meter (LI-1000; LI-COR Inc., Lincoln, Nebraska, USA). Recordings of PPF over a day were integrated to calculate DLI.

CDLI in units of $mol \cdot m^{-2}$ during both phase 1 and phase 2 were calculated by summing the DLI values as Eq. [3-6].

$$CDLI = DLI_1 + DLI_2 + \ldots\ldots DLI_n \quad \textbf{[3-6]}$$

where n is the duration (days) from transplanting to the first visible flower bud (phase 1) or from the first visible flower bud to harvest (phase 2). Average DLI in units of $mol \cdot m^{-2} \cdot d^{-1}$ during both phase 1 and phase 2 was calculated for each plant (Eq. [3-7]).

$$\text{Average DLI} = (DLI_1 + DLI_2 + \ldots\ldots DLI_n) / n \quad \textbf{[3-7]}$$

3.3.3.1.3 PTR

For each day, PTR was calculated as Eq. [3-8] in units of $mol \cdot m^{-2} \cdot {}^{\circ}C^{-1} \cdot d^{-1}$.

$$PTR = DLI / T_a \quad \textbf{[3-8]}$$

Cumulative PTR (CPTR) in units of $mol \cdot m^{-2} \cdot °C^{-1}$ during phase 1 was calculated for each plant (Eq [3-9]).

$$CPTR = DLI_1 / T_{a1} + DLI_2 / T_{a2} + \ldots\ldots DLI_n / T_{an} \quad \textbf{[3-9]}$$

where n is the duration (days) from transplanting to the first visible flower bud. Average PTR in phase 1 was also estimated for each plant (Eq. [3-10]).

$$\text{Average PTR} = (DLI_1 / T_{a1} + DLI_2 / T_{a2} + \ldots\ldots DLI_n / T_{an}) / n \quad \textbf{[3-10]}$$

3.3.3.2 Plant growth parameters

3.3.3.2.1 Leaf number

Once transferred to the greenhouse, accumulated leaf number on the main shoot of each plant was counted once per week until there was no change in leaf number for a further 7 weeks. The leaves were marked using a waterproof marker after being counted. The new leaves were counted once they expanded to a width of 1 cm. The average maximum leaf number (MLN) and new leaf number (NLN) below the first visible flower bud, was calculated for each plot of 10 plants.

LNAR ($leaves \cdot d^{-1}$) during phase 1 was derived from simultaneously fitting two straight-line equations to the changes in average leaf number per plot over time for the period covering both phases 1 and 2. Predicting by fitting straight-line equations was found to approximate the data more closely than other methods (Appendix 3).

3.3.3.2.2 Ground cover index

GCI, i.e. the proportion of ground in a plot covered by leaf area, was recorded throughout the period of phase 1. Each plot was photographed weekly with a digital camera (Fuji 2100, Japan) from directly above and included a 1 cm^2 object as a scale (Fig. 3-2 A). To calculate GCI, the images were processed using two software packages, Corel Photo-Paint (Corel Corporation, USA) and SigmaScan Pro4 (SPSS, USA). Each image of a plot was initially cropped to the position of guard plants. The images of visible leaves of sample plants were digitally converted to a black colour leaving bare ground white (Fig. 3-2 B). SigmaScan Pro4 software was subsequently used to measure the visible leaf area and ground area in the processed black and white image, utilizing the 1 cm^2 scale for calibration. On any single date of measurement, GCI in each plot was calculated as Eq. [3-11].

$$GCI_n = \text{Visible leaf area / Ground area} \qquad \textbf{[3-11]}$$

where 'n' is any day of measurement.

For each plot, changes of GCI with time were described using a Gompertz function (Eq. [3-12]). This function was chosen over other sigmoid growth functions due to the derivation of more biologically relevant parameters and its wide acceptability by other researchers (Causton and Venus, 1981; Hunt, 1982).

$$\mathbf{GCI = A + C\,e^{(-e^{(B(M-t)})}} \qquad \textbf{[3-12]}$$

where:

A = lower asymptote of GCI ($cm^2 \cdot cm^{-2}$)

C = upper asymptote of GCI ($cm^2 \cdot cm^{-2}$)

B = GCI increase rate (GCIR) over time ($cm^2 \cdot cm^{-2} \cdot d^{-1}$)

t = time (days)

M = value of t at the point of inflection (i.e. when increase of GCI occurs)

GCIR was chosen as a potential predictor of DTV for further data analysis.

A

B

Fig. 3-2. Example of digital images containing 6 sample plants of *Limonium* 'LSLP4' from one plot before image processing (A); highlighting leaf area and 1 cm^2 scale converted to black colour (B).

3.3.3.2.3 Rosette diameter

Rosette diameter (RD) of individual plants was measured from digital photographs taken weekly using SigmaScan Pro4 software. The rosette diameter of each plant was calculated from the average of 2 measurements taken at a right angle to each other.

3.3.3.2.4 Floral development

The floral development of 'LSLP4' was divided into two phases, i.e., phase 1 from transplanting to the first visible flower bud, and phase 2 from the first visible flower bud to harvest. The date when the first flower bud visibly appeared on the main stem was recorded for each plant (Fig. 3-3). Duration from transplanting to the first visible flower bud (DTV) of each plant was calculated.

Harvest maturity of 'LSLP4' was defined as when 80% of calyces were open (Appendix 2). The first harvest date of each plant was recorded. Duration to harvest (DTH) was calculated as the difference between the date of the first visible flower bud and the date of the harvest of each plant.

Fig. 3-3. The first visible flower bud (highlighted by ellipse) appeared at the apex of the main stem of 'LSLP4'.

3.3.4 Experimental design and data analysis

The 11 treatments were randomly allocated within the ten blocks. Each treatment had three replicates (plots). Each plot comprised 10 plants. Across all treatments, 17 out of 324 plants had abnormal multiple apexes with slim and weak leaves and inflorescence. Data from these plants were excluded from further analysis

Linear or exponential regression analysis was performed on treatment means using GENSTAT 7 (VSN International Ltd., UK). An exponential curve was fitted to data that presented a plateau typical of biological saturation responses. Regression lines were presented only when the correlation was significant.

After an initial screening for parameters showing correlation with DTV, average DLI, average T_a, LNAR, GCIR, MLN, DTV and DTH were used in further regression analysis. Parameters with a significant correlation to DTV were then

subjected to multiple regression analysis to determine if the initial predictive model could be improved.

3.3.5 Model validation

The regression models were validated using cross validation (Draper and Smith, 1981). In summary a treatment was excluded from the data set and a model was constructed based on the data from the other 10 treatments. The model was then used to predict the data of the excluded treatment. This procedure was completed for each treatment in turn, and the predictive residual sum of squares (PRSS) was used to compare the models. The lower the value of PRSS, the higher the predictive power.

3.4 Results

Plants from all treatments flowered and produced a flower stem during the assessment period. DTV ranged between 52 to 165 days, while DTH varied from 47 to 68 days.

3.4.1 Environmental parameters

DLI ranged from 0.4 to 36 $mol \cdot m^{-2} \cdot d^{-1}$ over the whole period from 7 May 2003 to 24 Feb. 2004 (Fig. 3-4). DLI was relatively stable (< 10 $mol \cdot m^{-2} \cdot d^{-1}$) in May, June and July, while increasing significantly from August through to February. Across all treatments, the average DLI in phase 1 ranged from 4 to 19.9 $mol \cdot m^{-2} \cdot d^{-1}$, and from 4.2 to 20.6 $mol \cdot m^{-2} \cdot d^{-1}$ in phase 2 (Table 3-2).

T_a in the greenhouse rarely exceeded 24°C or was lower than 15°C (Fig. 3-5). For all treatments, the average T_a remained relatively constant during the course of the experiment, varying from 18.6 to 20.9 °C in phase 1 and 18 to 21.7 °C in phase 2 (Table 3-2).

As with the change of DLI and T_a, average PTR changed between 0.21 and 1.02 $mol \cdot m^{-2} \cdot °C^{-1} \cdot d^{-1}$ in phase 1 and between 0.22 to 1.01 $mol \cdot m^{-2} \cdot °C^{-1} \cdot d^{-1}$ in phase 2.

Initial screening of the environmental parameters, for their ability to predict time to flower, resulted in a refined list of those originally presented in Section 3.3.3. Presentation of treatment differences in the following section on plant growth parameters therefore, utilizes those environmental parameters as predictors that were also used in developing the predictive models.

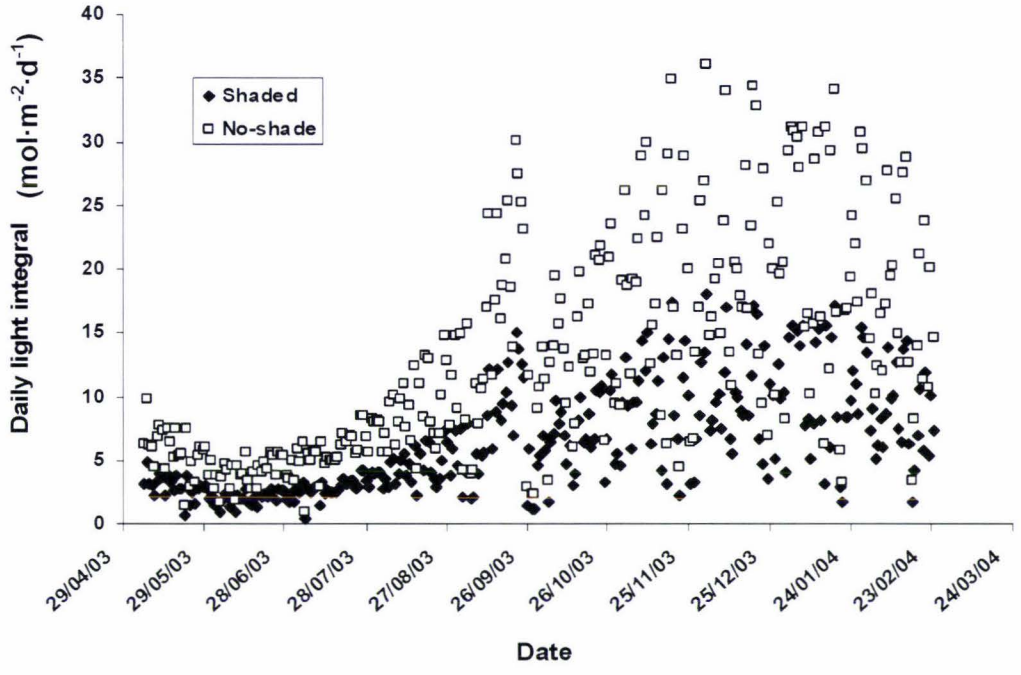

Fig. 3-4. Daily light integrals of shaded and no-shade treatments from 7 May 2003 to 24 Feb. 2004 in the greenhouse at the Plant Growth Unit, Massey University, Palmerston North, N.Z.

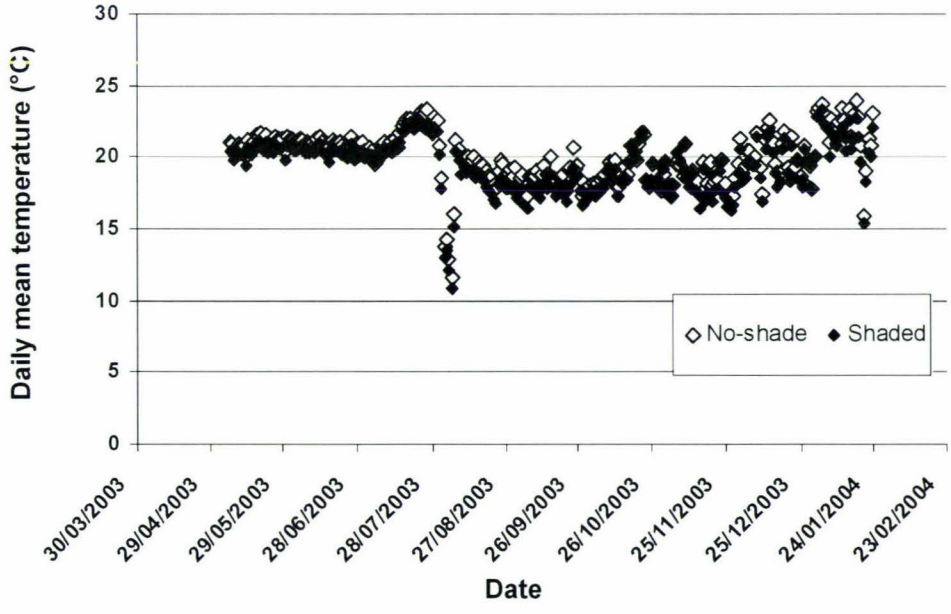

Fig. 3-5. Daily mean temperature (T_a) of shaded and no-shade treatments from 7 May 2003 to 24 Feb. 2004 in the greenhouse at the Plant Growth Unit, Massey University, Palmerston North, N.Z.

3.4.2 Plant growth parameters

3.4.2.1 Maximum leaf number below the first visible flower bud

With the exception of the May_HL and May_LL treatments, plants in all treatments produced a relatively consistent MLN of 29±2 leaves (Table 3-3). Both treatments planted during May had significantly greater MLN than all other treatments. The May_LL treatment had the highest MLN, which was 4 leaves greater than May_HL treatment ($P < 0.05$) and 10 leaves greater ($P < 0.05$) than the average of other treatments (Table 3-3).

MLN declined exponentially with increasing average DLI ($P < 0.0001$; Fig. 3-6 A). The predicted MLN decreased by 10 leaves as average DLI increased from 4 to 9 $mol \cdot m^{-2} \cdot d^{-1}$, while the predicted MLN remained relatively constant between 28 and 29 leaves when average DLI increased from 9 to 20 $mol \cdot m^{-2} \cdot d^{-1}$. The new leaf number (NLN, i.e., the difference between MLN and initial leaf number at transplanting) also declined exponentially with increasing average DLI ($P < 0.0001$; Fig. 3-6 B), with no significant improvement in predictive accuracy or treatment response over that achieved for MLN.

Table 3-3. Maximum Leaf number below the first visible flower bud (MLN) of 'LSLP4' for all treatments.

Treatment Label	MLN	SE	
May_LL	39	0.24	A#
May_HL	35	0.46	B
Jul_HL	31	0.61	C
Sep_LL	30	0.6	DC
Jun_HL	29	0.34	DCE
Aug_LL	29	0.75	DCE
Nov_HL	29	0.53	DE
Sep_HL	29	0.41	DE
Oct_HL	28	0.38	DFE
Oct_LL	28	0.59	FE
Aug_HL	27	0.83	F
$LSD_{0.05}$	2		

Treatments followed by the same letter are not significantly different ($P < 0.05$).

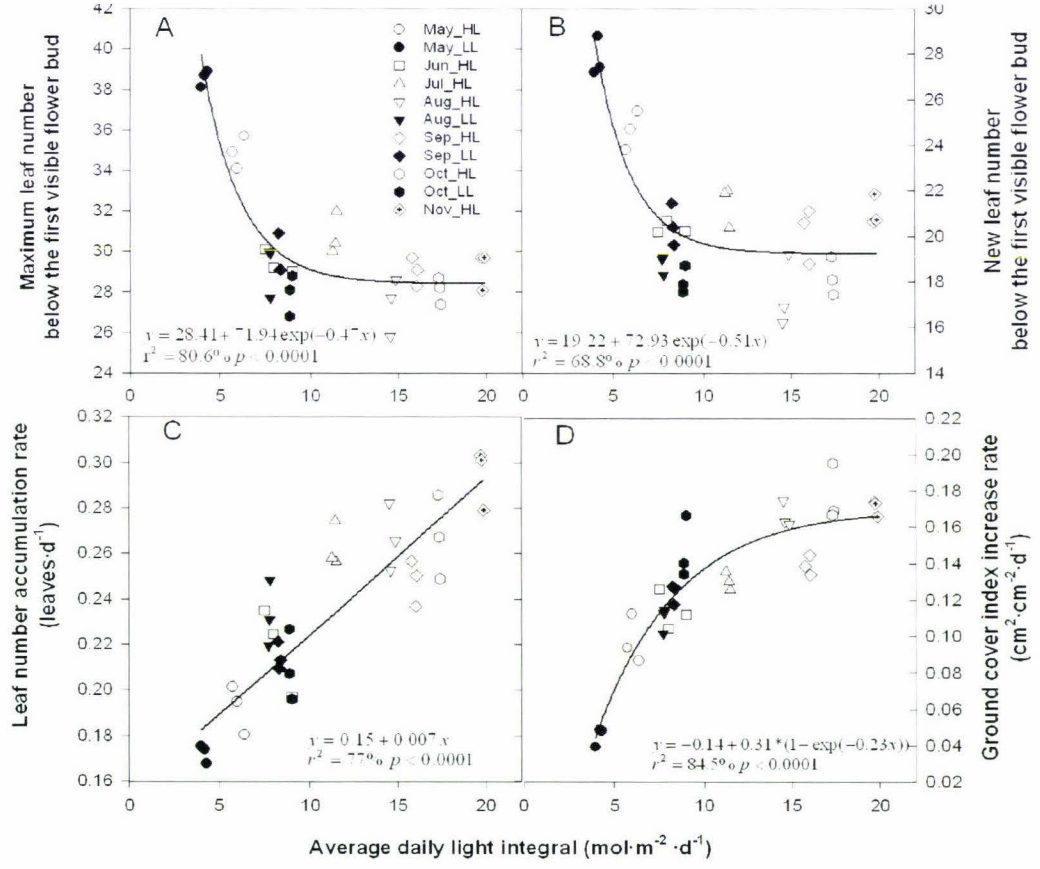

Fig. 3-6. Influence of daily light integral on maximum leaf number (A) and new leaf number below the first visible flower bud (B), leaf number accumulation rate (C), and ground cover index increase rate over time (D). Each data point is the average value for 10 plants in a plot except for 8 plants in a plot for the Nov_HL treatment.

3.4.2.2 Leaf number accumulation rate and ground cover index increase rate

Leaf number accumulation over time was described closely by fitting two-straight-line equations (i.e. Broken Stick Model; Appendix 3), while the change of GCI over time was suitably described by fitting a Gompertz Curve (Fig. 3-7). LNAR and GCIR were derived from these two models, respectively.

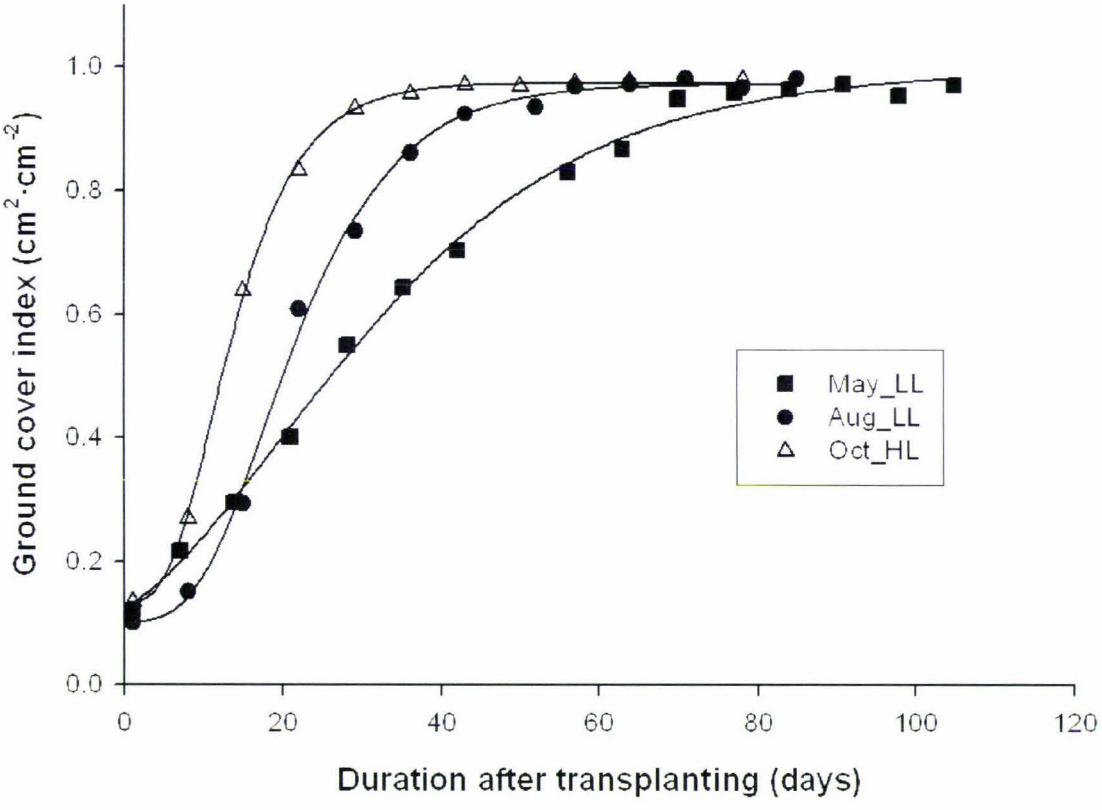

Fig. 3-7. Example curves describing the change in ground cover index over time for May_LL, Aug_LL and Oct_HL treatments. Across all treatments MSE values for the fitted curves ranged between 0.0001 and 0.0004 with r^2 values ranging between 99.7% and 99.9%.

During phase 1, LNAR increased linearly as average DLI increased from 4 to 20 mol·m^{-2}·d^{-1}, and explained 77% of the variation ($P < 0.0001$; Fig. 3-6 C). For phase 1 GCIR increased exponentially with increasing DLI from 4 to 20 mol·m^{-2}·d^{-1} and explained 84.5% of the variation ($P < 0.0001$; Fig. 3-6 D).

3.4.2.3 Rosette diameter

The initial measurements of changes in RD after transplanting for plants from May_HL, May_LL, Jun_HL and Jul_HL treatments in phase 1 were not notably different compared to that illustrated by changes in leaf number and GCI. Given that measuring and calculating RD was also time consuming for each individual plant, RD measurements were discontinued for the other treatments, and are not presented here for further analysis.

3.4.3 Duration from transplanting to first visible flower bud

3.4.3.1 Environmental parameters as predictors

The duration from transplanting to first visible flower bud (DTV) decreased exponentially with increasing average DLI and, by itself, explained in excess of 88% of the variation ($P < 0.0001$; Fig. 3-8 A). CDLI had no significant correlation with DTV ($P = 0.79$; Fig. 3-8 B). For the plants under the shaded treatments, with average DLI ranging from 4 to 9 $mol \cdot m^{-2} \cdot d^{-1}$, CDLI values for DTV were, however, relatively constant (i.e. 587 to 695 $mol \cdot m^{-2}$). In contrast, for plants under the no-shade treatments (i.e. average DLI ranging from 6 to 20 $mol \cdot m^{-2} \cdot d^{-1}$) CDLI for DTV varied widely (i.e. 578 to 1245 $mol \cdot m^{-2}$).

While a significant linear relationship was found between GDD and DTV ($P < 0.0001$; Fig. 3-8 D), there was no clear correlation between DTV and average temperature (Fig. 3-8 C).

As reported for average DLI, DTV also declined exponentially with increasing average PTR, and explained over 88% of the variation in DTV ($P < 0.0001$; Fig. 3-8 E). As reported for CDLI, CPTR also had no significant correlation with DTV ($P = 0.8$; Fig. 3-8 F).

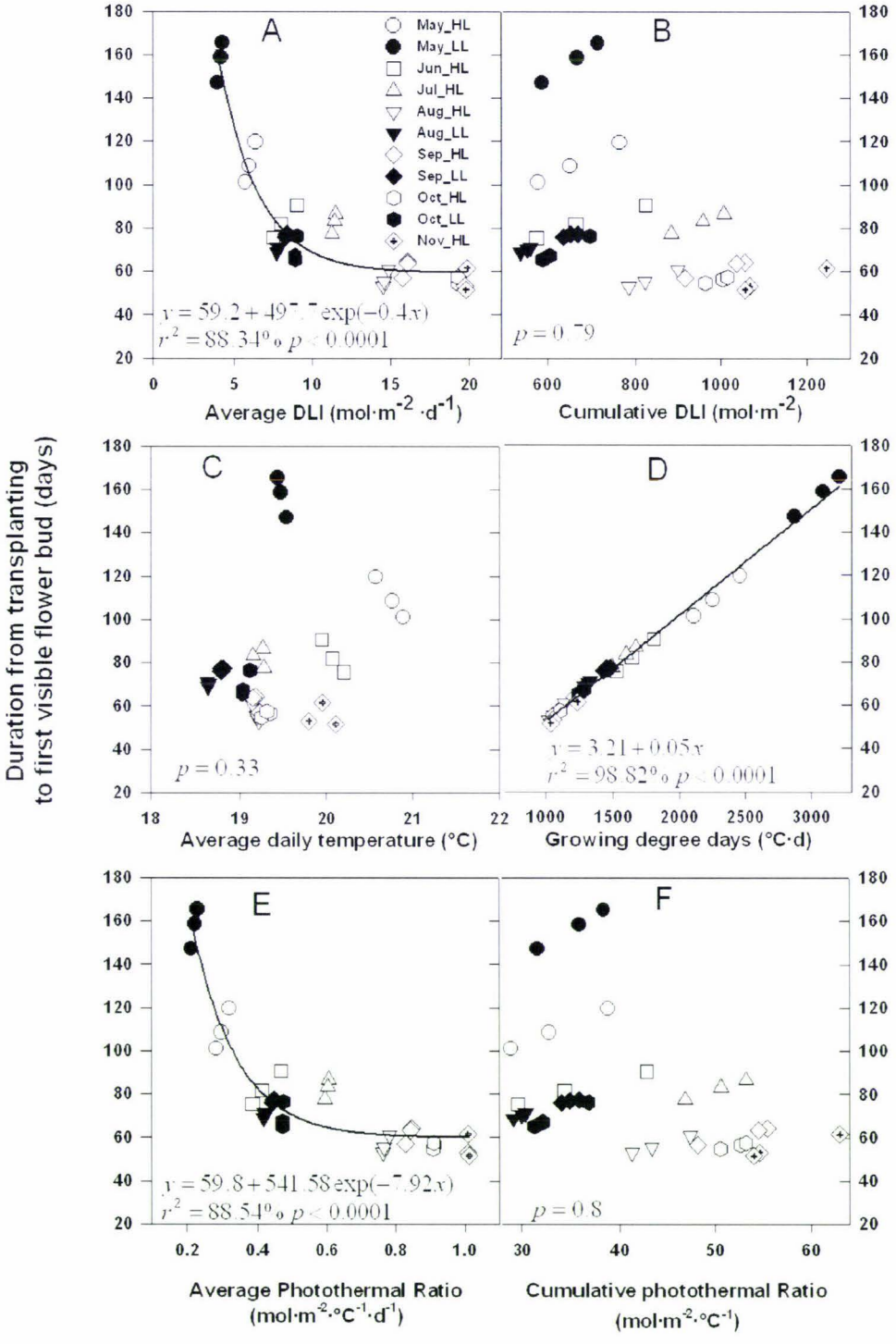

Fig. 3-8. Duration from transplanting to the first visible bud of 'LSLP4' as a function of average daily light integral (A), cumulative daily light integral (B), average daily temperature (C), growing degree days (D) , average photothermal ratio (E) and cumulative photothermal ratio (F). Each data point is the average value for 10 plants in a plot except for 8 plants in a plot for the Nov_HL treatment.

3.4.3.2 Plant parameters as predictors

DTV decreased exponentially as LNAR increased ($P < 0.0001$; Fig. 3-9 A). The predicted DTV decreased by about 90 days as LNAR increased from 0.16 to 0.22 leaves·d^{-1}, whilst the further increase of LNAR from 0.22 to 0.3 leaves·d^{-1} only reduced the predictive DTV by 30 days, i.e., the plateau appeared within this range.

GCIR and DTV were correlated ($P < 0.0001$). The predicted DTV decreased exponentially from 165 to 50 days as GCIR increased from 0.04 to 0.2 (cm^2·cm^{-2}·d^{-1}) (Fig. 3-9 B). In contrast to LNAR, there was no plateau on the curve of DTV against GCIR within the data range examined.

DTV increased exponentially with increasing values of MLN and NLN ($P < 0.0001$; Fig. 3-9 C, D).

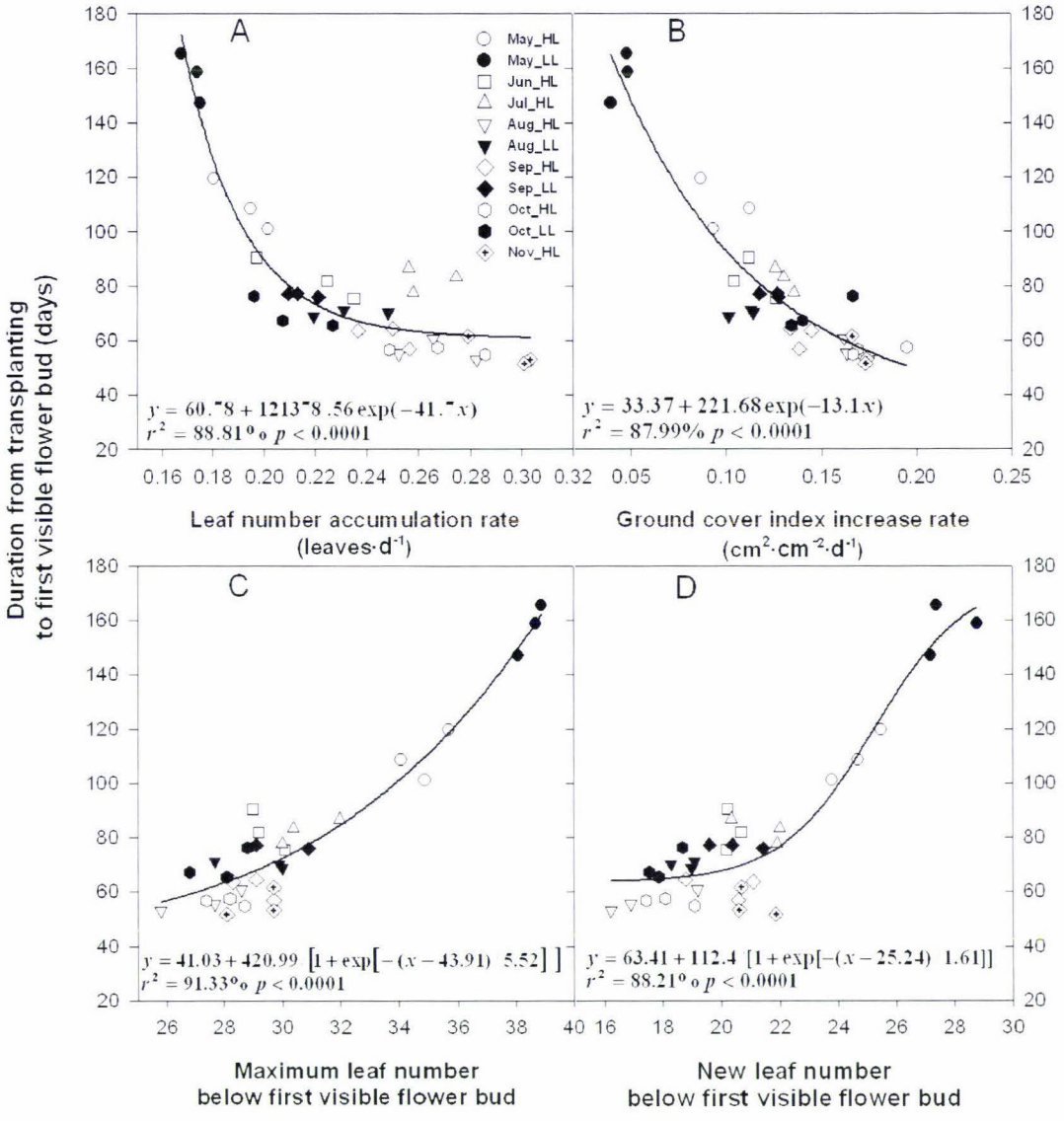

Fig. 3-9. Duration from transplanting to the first visible bud of 'LSLP4' as a function of leaf number accumulation rate (A), ground cover index increase rate (B), maximum leaf number (C), and new leaf number below the first visible bud (D). Each data point is the average value for 10 plants in a plot except for 8 plants in a plot of Nov_HL treatment.

3.4.3.3 Rate of progress to first visible flower bud

The rate of progress to the first visible flower bud (1/DTV) increased exponentially with increasing average DLI from 4 to 20 mol·m^{-2}·d^{-1}, and linearly with both LNAR and GCIR ($P < 0.0001$; Fig. 3-10 A, C, D). There was no significant

correlation between 1/DTV and average T_a over the narrow range experienced in this study (P = 0.57; Fig. 3-10 B)

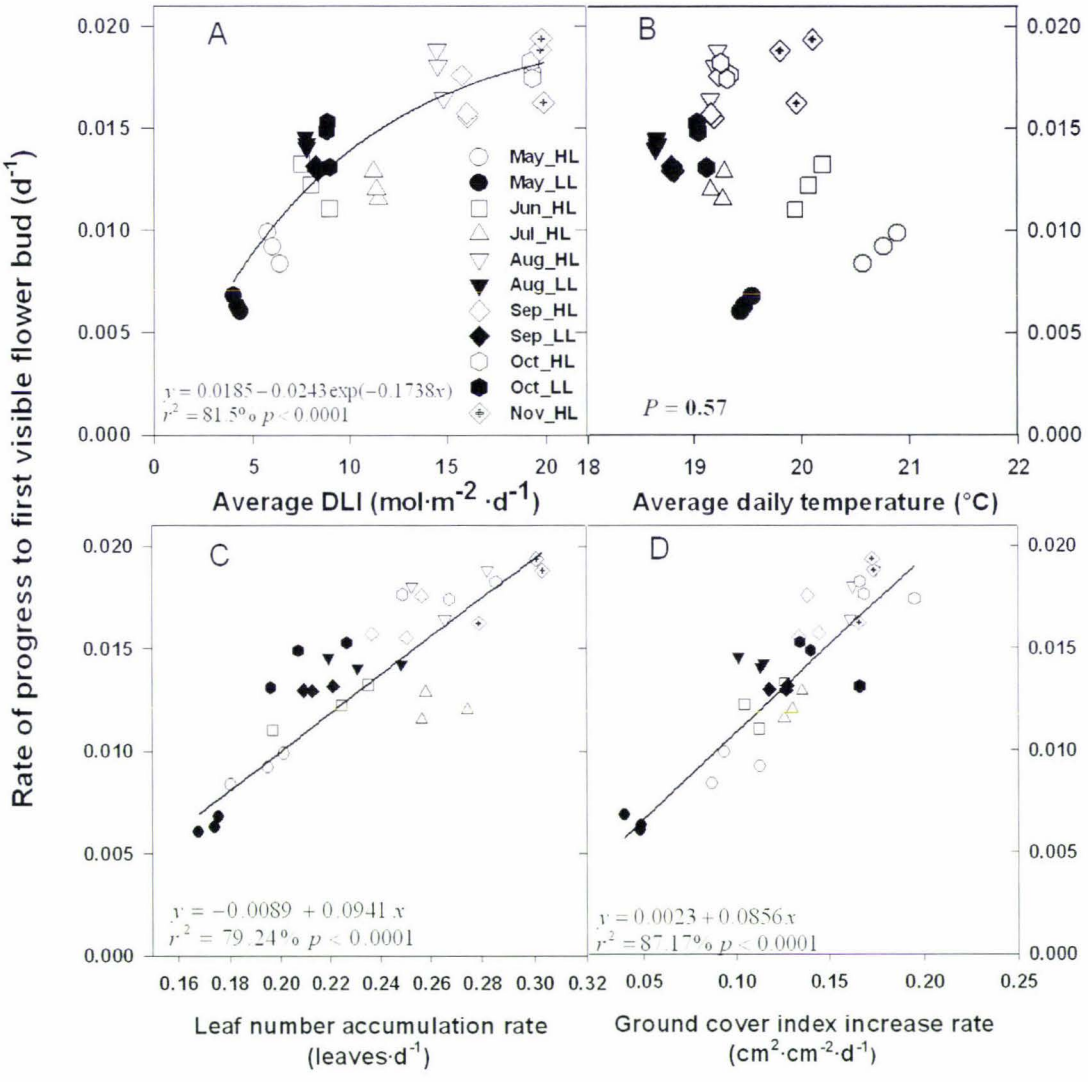

Fig. 3-10. Rate of progress to the first visible bud of 'LSLP4' as a function of average daily light integral (A), average daily mean temperature (B), leaf number accumulation rate (C), and ground cover index increase rate (D). Each data point is the average value for 10 plants in a plot except for 8 plants in a plot of Nov_HL treatment.

3.4.3.4 Multiple regression model predicting duration from transplanting to first visible flower bud

The individual predictors that significantly correlated with DTV (e.g. DLI, LNAR and GCIR) were evaluated as predictors using multiple regression. Models using both a plant parameter (i.e., LNAR or GCIR) and the environmental parameter (i.e. average DLI) as predictors, accounted for more variation in DTV than any of the single-predictor models (Table 3-4). However the probability for adding both DLI and GCIR into the model was not significant for the coefficient associated with DLI (P = 0.21). The model with the highest r^2 (i.e. 92.5%) incorporated both DLI and LNAR as predictors (Fig. 3-11), with probabilities for the coefficients involving both DLI and LNAR being significant (Table 3-4).

Table 3-4. Regression models predicting duration from transplanting to the first visible bud (DTV) using daily light integral (DLI), leaf number accumulation rate (LNAR), and/or ground cover index increase rate (GCIR) as predictors.

Predictors	Model	r^2	Predictor probability	
DLI	DTV=59.2+497.7*Exp(-0.4*DLI)	88.34%	$P < 0.0001$	
LNAR	DTV=60.78+121378.56*Exp(-41.7* LNAR)	88.81%	$P < 0.0001$	
GCIR	DTV=33.37+221.68*Exp(-13.1*GCIR)	87.99%	$P < 0.0001$	
DLI, LNAR	DTV=59.01+6578*Exp(-0.1891*DLI-19.82*LNAR)	92.5%	$P < 0.01$	$P < 0.001$
DLI, GCIR	DTV=49.55+262.4*Exp(-0.0966*DLI-11.13*GCIR)	89.3%	$P = 0.21$	$P < 0.001$

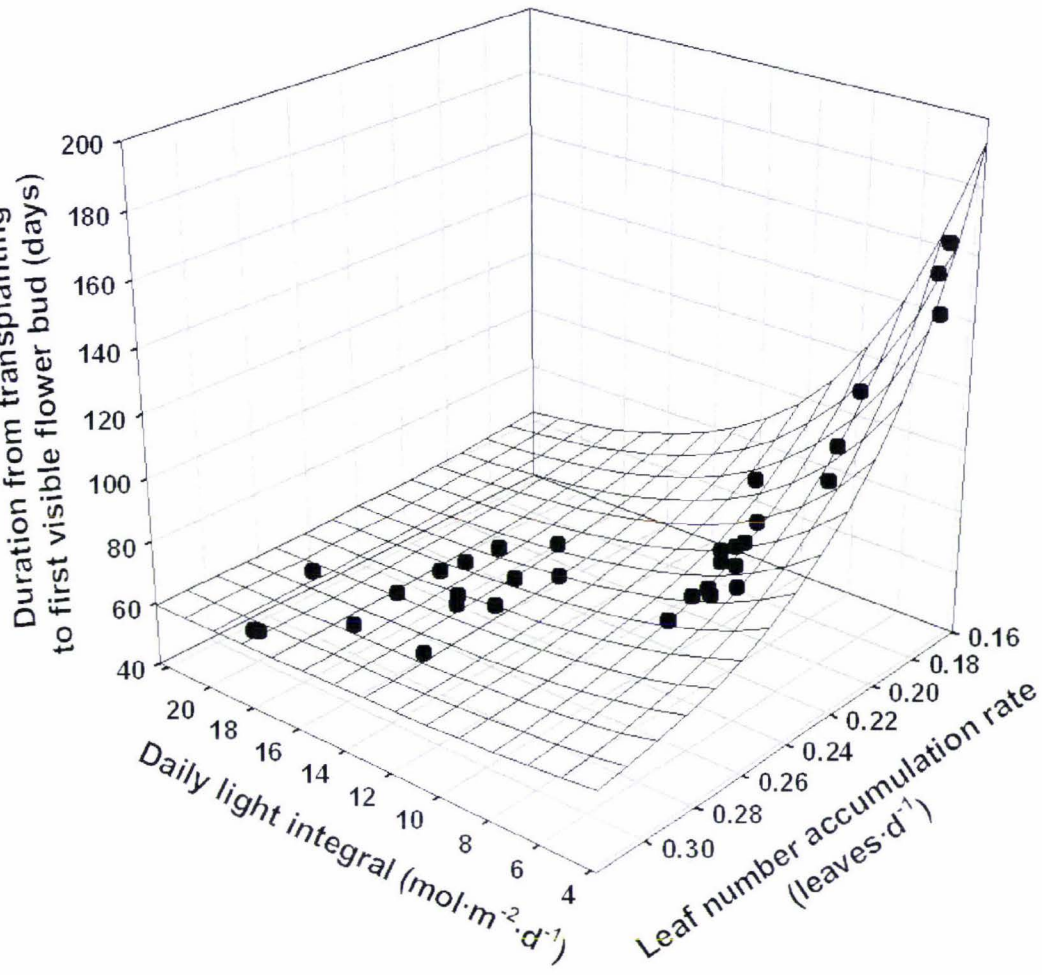

Fig. 3-11. The relationship between daily light integral, leaf number accumulation rate, and duration from transplanting to the first visible flower bud of *Limonium* 'LSLP4'. The response surface was fitted by regression analysis; DTV=59.01+6578*Exp(-0.1891*DLI-19.82*LNAR), r^2=92.5%.

3.4.3.5 Multiple regression model for predicting rate of progress to first visible flower bud

As with DTV, the significant individual predictors of 1/DTV were examined using multiple regression analysis. Combining LNAR with DLI as predictors for 1/DTV resulted in significant contributions for both coefficients, and also greater r^2, than when each parameter was used alone (Table 3-5; Fig. 3-12). As occurred when

predicting DTV, the model incorporating both GCIR and DLI as predictors was not significant for DLI ($P = 0.21$).

Table 3-5. Regression models predicting rate of progress to the first visible bud (1/DTV) using daily light integral (DLI), leaf number accumulation rate (LNAR), and/or ground cover index increase rate (GCIR) as predictors.

Predictors	Model	r^2	Predictors probability	
DLI	1/DTV=0.0185-0.0243*Exp(-0.1738*DLI)	81.5%	$P < 0.0001$	
LNAR	1/DTV=-0.0089+0.0941*LNAR	79.24%	$P < 0.0001$	
GCIR	1/DTV=0.0023+0.0856*GCIR	87.17%	$P < 0.0001$	
DLI+LNAR	1/DTV=0.0192-0.0528*Exp(-0.1007*DLI-5.84*LNAR)	82.6%	$P = 0.04$	$P = 0.05$
DLI+GCIR	1/DTV=0.02457-0.02518*Exp(-0.0408*DLI-3.46*GCIR)	84.3%	$P = 0.21$	$P = 0.03$

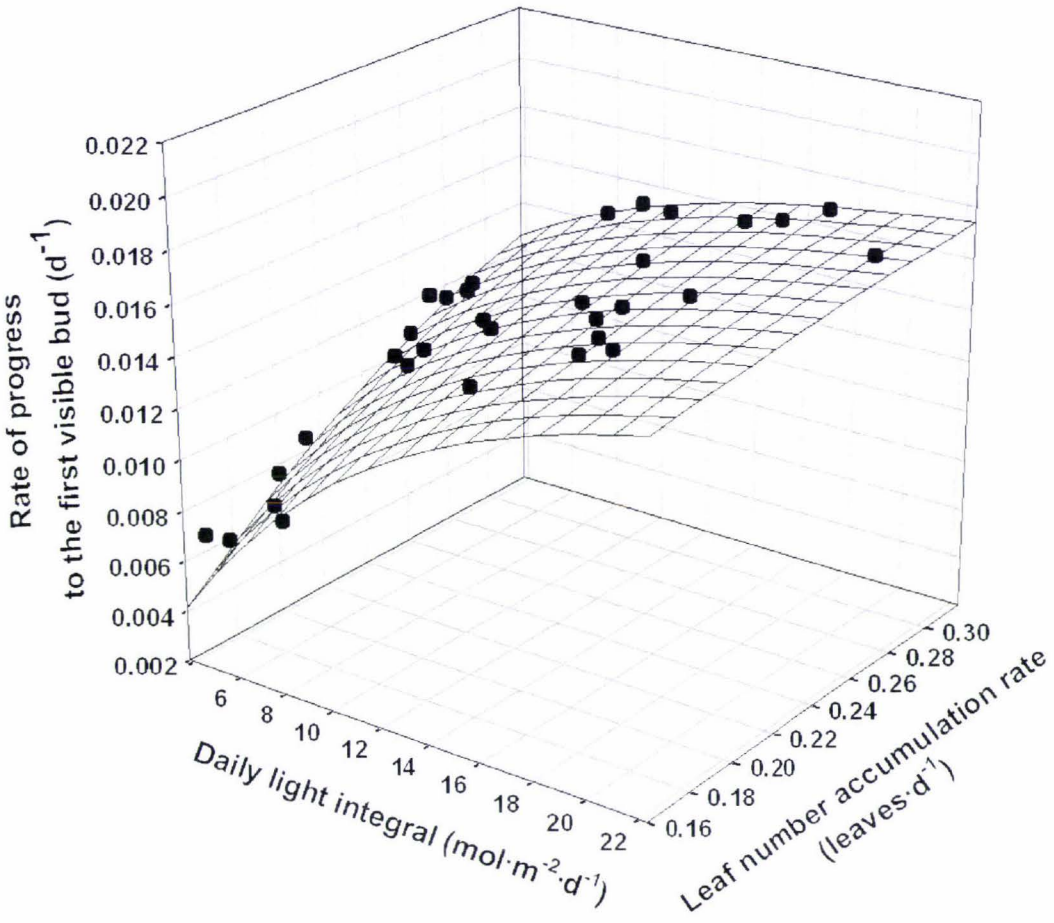

Fig. 3-12. The relationship between daily light integral, leaf number accumulation rate, and rate of progress to the first visible flower bud of *Limonium* 'LSLP4'. The response surface was fitted by regression analysis; 1/DTV=0.0192-0.0528*Exp(-0.1007*DLI-5.84*LNAR), r^2=82.6%.

3.4.3.6 Model validation

Cross validation of the predictive models for DTV using DLI only, or both DLI and LNAR resulted in PRSS values of 4578 and 3970, respectively. With the model containing both DLI and LNAR having the lower PRSS value, this validated the model and confirmed it had the greatest predictive power.

3.4.4 Duration from first visible flower bud to harvest

Across all treatments DTH varied from 47 to 68 days (Fig. 3-13 A). The shortest DTH was recorded from the Nov_HL treatment (i.e. the treatment with the highest average DLI of 20 $mol \cdot m^{-2} \cdot d^{-1}$; Table 3-2). The longest DTH was recorded from the May_LL treatment (i.e. the treatment with the lowest average DLI of 4 $mol \cdot m^{-2} \cdot d^{-1}$). Because of the substantial variation in DTH, especially for those treatments resulting in higher average DLI values (14 days), there was no significant correlation between DTH and average DLI. There was also no significant correlation between DTH and cumulative DLI ($P = 0.88$; Fig. 3-13 B).

DTH had no significant correlation with the narrow range of average T_a encountered in this study ($P = 0.84$), although there was a general trend towards shorter DTH with increasing temperature (Fig. 3-13 C). GDD accumulated during DTH varied from 1022 to 1324 °C·d (Fig. 3-13 D), which was less variation than that accumulated for DTV (i.e. ranging from 1021 to 3213 °C·d).

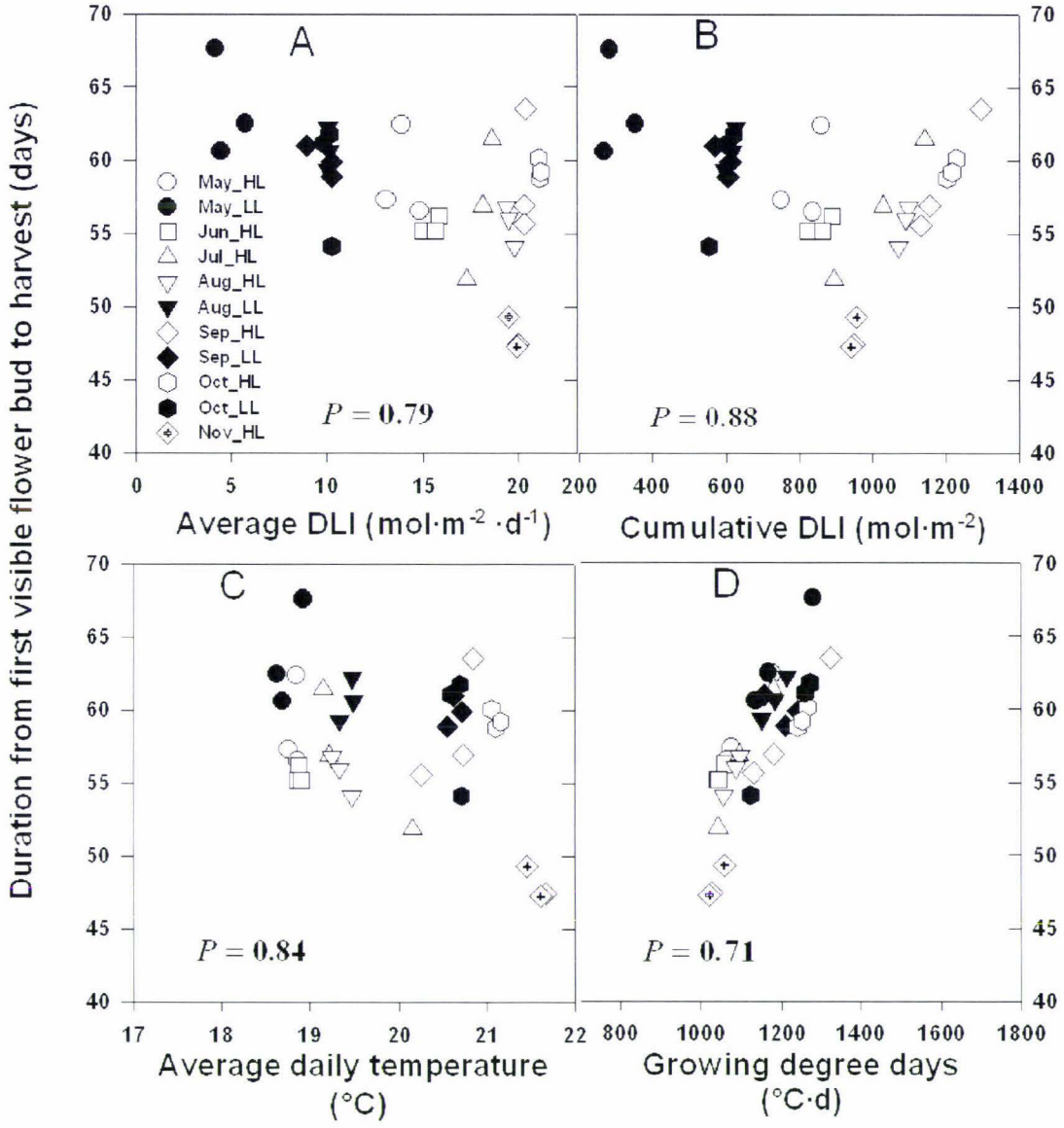

Fig. 3-13. Duration from the first visible flower bud to harvest of *Limonium* 'LSLP4' as a function of average daily light integral (A), cumulative daily light integral (B), average daily mean temperature (C), and growing degree days (D). Each data point is the average value for 10 plants in a plot except for 8 plants in a plot of Nov_HL treatment.

3.5 Discussion

3.5.1 DLI as a predictor of duration or rate of progress to first visible flower bud

DLI is the main factor that influenced DTV of 'LSLP4' and, by itself, explained in excess of 88% of the variation within the current experiment. Flowering occurred in all plants under average DLI as low as 4 $mol \cdot m^{-2} \cdot d^{-1}$, which means that if there is a minimum average DLI requirement for flower initiation of 'LSLP4', it is less than 4 $mol \cdot m^{-2} \cdot d^{-1}$. As average DLI increased from 4 to 10 $mol \cdot m^{-2} \cdot d^{-1}$, DTV was reduced by about 90 days, which was significantly greater than the 8 days reduction with pansy (Niu et al., 2000), and 3 days with *Achillea* × *millefolium* L. (Fausey et al., 2005), for the comparable increase in DLI. However, for 'LSLP4', the response of DTV to average DLI was saturated above 15 $mol \cdot m^{-2} \cdot d^{-1}$. In the current experiment the increase of DLI from 15 to 20 $mol \cdot m^{-2} \cdot d^{-1}$ resulted in no further reduction in DTV (Fig. 3-8 A). Similar to the finding here, there was no difference in days to first flower of *L. sinuatum* when grown above this saturation value of DLI, i.e. 15.3 to 27.6 $mol \cdot m^{-2} \cdot d^{-1}$ in Mattson and Erwin's (2005) study. Considering the genetic similarity between 'LSLP4' and *L. sinuatum*, and the significant response of DTV to DLI from 4 to 15 $mol \cdot m^{-2} \cdot d^{-1}$ experienced in the current study, classifying *L. sinuatum* as an irradiance indifferent plant must be considered to be arbitrary. Further investigations into the response of *L. sinuatum* to DLI levels < 15 $mol \cdot m^{-2} \cdot d^{-1}$ are warranted before this can be proved or disproved.

Within a defined 'effective range', a linear relationship between rate of progress to flower and DLI has been shown with; chrysanthemum, for DLI ranging from 2 to 10 $mol \cdot m^{-2} \cdot d^{-1}$ (Pearson et al., 1993), and pansy, for DLI ranging from 4.5 to 12.7 $mol \cdot m^{-2} \cdot d^{-1}$ (Adams et al., 1997). In contrast, a curvilinear relationship was determined with raspberry, for DLI ranging from 9.4 to 19.4 $mol \cdot m^{-2} \cdot d^{-1}$ (Carew et al., 2003). In the current study, a curvilinear (i.e. exponential) relationship was also determined between 1/DTV and average DLI ranging from 4 to 20 $mol \cdot m^{-2} \cdot d^{-1}$ (Fig. 3-10 A). The increase in 1/DTV was greatest (almost linear) with the increase of average DLI from 4 to 15 $mol \cdot m^{-2} \cdot d^{-1}$, while the rate of increase in 1/DTV declined when DLI exceeded 15 $mol \cdot m^{-2} \cdot d^{-1}$. Although appearing to be linear, attempting to fit a linear curve between 1/DTV and DLI values between 4 and 15 $mol \cdot m^{-2} \cdot d^{-1}$, resulted in an $r^2 = 78\%$, while the probability of the coefficient was 0.21 (i.e. not significant). Rather than refuting the hypothesis that a linear relationship exists between 1/DTV and DLI between 4 and 15 $mol \cdot m^{-2} \cdot d^{-1}$, this may highlight the need for more research within this range of DLI.

3.5.2 Influence of DLI on plant vegetative growth

The increase in average DLI from 4 to 20 $mol \cdot m^{-2} \cdot d^{-1}$ not only accelerated the flowering of 'LSLP4', but also the rate of vegetative growth, quantified here as LNAR and GCIR. As well as a reduced DTV, plants under higher average DLI had both a faster rate of leaf appearance (Fig. 3-6 C) and expansion of leaf area (Fig. 3-6 D). This finding supports a hypothesis that average DLI levels supporting the most rapid rate of vegetative growth of 'LSLP4' result in optimal or fast flower initiation. More specifically, that some of the shortest durations to flower initiation in 'LSLP4'

will occur at average DLI of ≥ 15 mol·m^{-2}·d^{-1}, a GCIR of ≥ 0.16 cm^2·cm^{-2}·d^{-1}, and/or a LNAR ≥ 0.26 leaves·d^{-1}.

The LNAR of *Primula vulgaris* L. also increased linearly with increasing DLI, with a maximum 0.36 leaves·d^{-1} at 18 mol·m^{-2}·d^{-1} and average T_a of 20 °C (Karlsson, 2002). This rate is higher than the LNAR (i.e. 0.28 leaves·d^{-1}) of 'LSLP4' under the average DLI of 18 mol·m^{-2}·d^{-1}, and also the maximum LNAR observed in the current study, i.e. 0.29 leaves·d^{-1} at 20 mol·m^{-2}·d^{-1} and average T_a between 18.6 and 20.9 °C. Differences noted can be resolved by recognising: 1) the response of leaf number accumulation to DLI is genotype-specific; 2) the response saturation of LNAR to DLI is possibly higher in 'LSLP4' than *Primula*.

3.5.3 Effect of DLI varies in different development stages.

The influence of DLI varied in different development stages of 'LSLP4', which, in the current study, has been classified into phase 1 (from transplanting to first visible flower bud) and phase 2 (from first visible flower bud to harvest). Average DLI significantly affected the rate of vegetative growth, and DTV in phase 1 (Fig. 3-6 C, D; Fig. 3-8 A), while it had no evident correlation with DTH in phase 2 (Fig. 3-13 A). Although the range of DLI tested in these two stages were similar, i.e. from 4 to 20 mol·m^{-2}·d^{-1}, the variation in DTV (about 100 days) was significantly greater than for DTH (about 20 days). DLI is, therefore, more influential in the process of vegetative growth through flower initiation and appearance, than in the subsequent stage of flower bud development to harvest. This finding is in accordance with the results reported for geraniums (Armitage et al., 1981) and on *Rosa hybrida* L. (Pasian

and Lieth, 1994), where time required from visible bud to anthesis was correlated with temperature while DLI had no effect.

3.5.4 Cumulative DLI as a poor predictor of duration from transplanting to first visible flower bud

A correlation between CDLI and time of flowering has been shown in geranium (Erickson et al., 1980; White and Warrington, 1988) and *Pelargonium* ×*domesticum* L. H. Bailey (Loehrlein and Craig, 2004). The transition of the meristem from vegetative to reproductive stage in *Pelargonium* ×*domesticum* 'Duchess' could be predicted by a CDLI between 200 and 250 $mol \cdot m^{-2}$. However, in the current study no significant correlation was detected between CDLI and DTV for 'LSLP4'. This is despite the fact that plants under the shade treatments (average DLI between 4 and 9 $mol \cdot m^{-2} \cdot d^{-1}$) had minimal variation in CDLI (i.e. between 538 and 695 $mol \cdot m^{-2}$), compared with the wider ranging 578 to 1245 $mol \cdot m^{-2}$ recorded under the no-shade treatments (Fig. 3-8 B).

The poor correlation of CDLI and DTV might result from the method used to calculate CDLI in this study, i.e. simply integrating DLI values from the date of transplanting to the first visible flower bud, for each individual plant. This calculation is based on the assumption that the integrated DLI values are all within an effective range, where rate of progress to visible flower bud increases linearly with increasing values of DLI. Time to flower of plants responds to DLI within a certain effective range (Niu et al., 2000; Pearson et al., 1993; White and Warrington, 1988). In the current study the response between average DLI and DTV was saturated at average

DLI values ≥ 15 $mol \cdot m^{-2} \cdot d^{-1}$, which might be the upper limit of the effective DLI. However, DLI fluctuated notably over the course of the experiment, and some DLI values exceeded this threshold. This was especially evident within the no-shade treatments (Fig. 3-4). Consequently, simply adding the DLI values during phase 1 to calculate CDLI is not valid without knowing the effective DLI responsive range for DTV. This concept of only calculating CDLI when DLI values are within the effective range is similar to using the concept of calculating GDD with prior knowledge of the base and ceiling temperatures. In other words, by integrating the DLI values that exceeded the upper limitation of the effective range, CDLI was overestimated in some no-shade treatments, but not in shaded treatments. This might partly explain why the plants under shaded treatments had relatively constant CDLI to first visible flower bud. To more precisely identify the effective DLI range for 'LSLP4' (i.e. linear response range), future research should utilize controlled environment chamber experiments where DLI can be more accurately controlled.

3.5.5 Effect of temperature

During the current experiment, T_a in the greenhouse was restricted to the range of 18 and 21.7 °C. No correlation was detected between average T_a and DTV. Given the established influence of temperature on rate of development to flowering of other crops (Adams et al., 1997; Carew et al., 2003; Pearson et al., 1993), the lack of correlation in the current study further confirms that any confounding effect of T_a on DTV was removed by keeping T_a controlled to such a narrow range. In addition, as a result of uniformity of the temperatures between the successive plantings, GDD and

DTV were linearly correlated (Fig. 3-8 D). This relationship is a mathematical artifact and, therefore, has no biological application.

Average T_a also had no clear correlation with DTH, though there was a trend where plants grown at relatively higher average T_a resulted in smaller values of DTH (Fig. 3-13 C). Regardless of DLI, DTH had less variation than DTV. This may relate in part to the uniformity of T_a throughout the experiment, because past research found that temperature was more important in determining the rate of flower development to harvest than the initial flower initiation and appearance (Armitage et al., 1981; Pasian and Lieth, 1994). Experiments utilizing a wider range of temperatures and DLI are, therefore, required to provide a more accurate conclusion to the relative effects of temperatures at various stages of growth and flowering of 'LSLP4'.

3.5.6 Combination effect of DLI and temperature on DTV

The combination of effect of DLI and temperature can be quantified as PTR (Liu and Heins, 2002; Niu et al., 2000). In the current study, average PTR had a significant correlation with DTV, and explained 88% of the variation (Fig. 3-8 E). However given the methodology of calculation of average PTR and the minimal variation in T_a experienced, the similarity of the response between average PTR and average DLI is not surprising (Fig. 3-8 A, E). Not only was the general shape of the response the same, but also the order of treatments from low to high values of either average DLI or PTR. Hence rather than determining a true biological response between average PTR and DTV, this is most likely an artefact of the experimental design.

Although the range of average PTR experienced in this study (i.e. 0.2 to 1 $mol \cdot m^{-2} \cdot °C^{-1} \cdot d^{-1}$) was similar to that reported for pansy (Niu et al., 2000), the range of average T_a values tested for 'LSLP4' in phase 1 (i.e. from 18.6 to 20.9 °C), was 10 °C narrower than that used in the study of pansy. This uniformity of T_a might be the reason for the similarity between the response of DTV to average PTR, and that for DTV against average DLI. In addition, due to the uniformity of the average T_a, the relationship between average PTR and DTV did not provide information of use on the potential combination effect of both DLI and temperature. Therefore, in conjunction with the range of DLI values used in the current experiment, examination of a wider range of temperatures is still required to determine the influence of PTR on time to flower of 'LSLP4'.

3.5.7 Is a specific leaf number required for flower initiation of 'LSLP4'?

A specific or minimum leaf number has been suggested as a requirement for flower initiation in a number of plant species (Mattson and Erwin, 2005; Warner and Erwin, 2003). In this study, the maximum leaf number below the inflorescence varied from 27 to 39 leaves, but was highly dependent on average DLI (Fig. 3-6 A). Plants grown under a DLI of ≥ 9 $mol \cdot m^{-2} \cdot d^{-1}$ achieved a more consistent leaf number of 28 or 29 leaves. This infers that under favorable growing conditions, (i.e., DLI ≥ 9 $mol \cdot m^{-2} \cdot d^{-1}$, T_a 20 °C, and long photoperiods) 'LSLP4' accumulates a specific number of leaves before flower initiation. This finding is in agreement with the conclusions of Mattson and Erwin (2005), where there was no difference in leaf number for *L. sinuatum* grown under long days with DLI ranging from 15.3 to 27.6 $mol \cdot m^{-2} \cdot d^{-1}$. In

the current study however, at DLI < 9 $mol \cdot m^{-2} \cdot d^{-1}$, the relationship with leaf number does not hold, and a significant increase of maximum leaf number was found as the decrease of DLI from 9 to 4 $mol \cdot m^{-2} \cdot d^{-1}$. This increased maximum leaf number corresponded with the significant delay of DTV under DLI < 9 $mol \cdot m^{-2} \cdot d^{-1}$. Thus, it is possible that the presence of some promoters for flower initiation in 'LSLP4' appeared to be inhibited or postponed under DLI < 9 $mol \cdot m^{-2} \cdot d^{-1}$, which resulted in the continuation of vegetative growth, i.e., leaf number accumulation, and greater maximum leaf number. Future research might also investigate this as a hypothesis.

The consistency of the leaf number before flower initiation of 'LSLP4' can be used as a predictor of DTV under growing conditions where average DLI is > 9 $mol \cdot m^{-2} \cdot d^{-1}$. However, this prediction can only be accurate under average DLI values ranging from 9 to 15 $mol \cdot m^{-2} \cdot d^{-1}$, since above 15 $mol \cdot m^{-2} \cdot d^{-1}$, no further reduction in DTV occurs, regardless of the increase of LNAR. This suggests that the combination of plant growth parameters (e.g. LNAR) with DLI might be a more precise way to explain the variation of DTV.

3.5.8 Correlation between rates of both vegetative growth and visible flower bud

The rate of vegetative growth, i.e. LNAR and GCIR, were highly correlated with both DTV and 1/DTV for 'LSLP4'. The faster the rate of leaf appearance, and expansion of leaf area, the shorter the DTV and/or increased 1/DTV (Fig. 3-9 A, B; Fig. 3-10 C, D). This suggests that DTV or 1/DTV of 'LSLP4' can be predicted by monitoring leaf number or leaf area in actual growing conditions. The significant

association between DTV or 1/DTV with LNAR or GCIR is contrary to findings for *P. vulgaris* (Karlsson, 2002) and *P ×domesticum* (Loehrlein and Craig, 2004) where faster leaf unfolding or leaf area enlargement did not result in earlier flower initiation. For *P. vulgaris* and *P ×domesticum*, these plant growth parameters have limited application for predicting such critical stages of physiological development.

In the current study, data from the Jul_HL treatment was an obvious outlier from the trend in the curve for both DTV and 1/DTV against LNAR (Fig. 3-9 A; Fig. 3-10 C). However data from this treatment was not considered to be an outlier when plotted against GCIR (Fig. 3-9 B; Fig. 3-10 D). The plants in the Jul_HL treatment also had relatively high values of MLN (Table 3-3) and low initial plant dry weight (Table 3-1). It is possible that some internal promoter could have triggered the faster leaf initiation in the Jul_HL treatment, since there were five plants identified to have abnormal multiple apexes with slim and weak leaves in this treatment (N.B. data from such plants were excluded from any analysis). On the other hand, the dramatic drop in T_a below 15 °C in the greenhouse between 3 and 5 Aug. 2003 (Fig. 3-5), might be an external reason for the unusual leaf accumulation.

3.5.9 Models for predicting DTV

One of the key objectives in this study was to develop a model to predict DTV for 'LSLP4'. The sensitivity of 'LSLP4' to environmental factors, especially DLI, should allow growers to utilize a range of planting dates and predict DTV based on the historical data of DLI (e.g. 30-year-average monthly DLI data). The decay-

exponential relationship between DLI and average DTV can therefore, form the basis of a decision support tool for growers of 'LSLP4', i.e. Eq. [3-13].

$$DTV = 59.2 + 497.7*Exp(-0.4 * DLI) \quad \textbf{[3-13]}$$

In addition, once planted, the combination of both observed DLI and LNAR data, can be used by growers as a post-planting decision support tool to further refine the accuracy of prediction as the growing period progresses, i.e. Eq. [3-14].

$$DTV=59.01+6578*Exp(-0.1891*DLI-19.82*LNAR) \quad \textbf{[3-14]}$$

3.5.9.1 Application of the pre-planting model

The pre-planting model based on DLI explained about 88% of the variation in DTV when the temperature was controlled at around 20 °C and the photoperiod was greater than 13 h. This model has considerable value as a decision support tool for crop scheduling and lighting management for growers of 'LSLP4' anywhere around the world. For example, in the northern United States of America, if grown in a heated and no-shade greenhouse, the optimal planting months for the earliest flowering of 'LSLP4' as a cut flower are from February to October. This is when the average DLI is greater than 15 $mol \cdot m^{-2} \cdot d^{-1}$ (Korczynski et al., 2002). However, to avoid a notable delay in flowering date, supplementary photosynthetic lighting would be necessary if planting in December, where the average DLI ranges from 5 to 10 $mol \cdot m^{-2} \cdot d^{-1}$. A planting in November and January, when DLI ranges from 10 to 15 $mol \cdot m^{-2} \cdot d^{-1}$, should result in a maximum 8-day delay in DTV, compared with that in the optimal

seasons. If the use of supplementary lighting is not provided for a planting in December (due to e.g. high power cost), this pre-planting model may still be used to predict DTV, albeit it would be later than in a planting season with DLI greater 15 $mol \cdot m^{-2} \cdot d^{-1}$.

The pre-planting model can also be applied to schedule 'LSLP4' production in New Zealand, based on historical data of DLI. The New Zealand Meteorological Service provides data on the monthly average solar radiation in different areas. While presented in units of $MJ \cdot m^{-2} \cdot d^{-1}$, this can be converted into the relevant units ($mol \cdot m^{-2} \cdot d^{-1}$) using the approximate conversion factor 1.96 $mol \cdot m^{-2} \cdot d^{-1}$ per 1 $MJ \cdot m^{-2} \cdot d^{-1}$ (Korczynski et al., 2002; Thimijan and Heins, 1983).

From August through to April, the monthly average DLI in a greenhouse located at either Ohakea, Gisborne, or Christchurch (New Zealand), are all greater than 15 $mol \cdot m^{-2} \cdot d^{-1}$ (New Zealand Meteorological Service, 1983)Fig. 3-14). The exception to this is for August at Christchurch, which only achieves 14.3 $mol \cdot m^{-2} \cdot d^{-1}$. Hence, the optimal planting months for the earliest flowering of 'LSLP4' are from August to April at Ohakea and Gisborne, and from September to April at Christchurch. In winter (May to July), DLI ranges from 10 to 15 $mol \cdot m^{-2} \cdot d^{-1}$ at Ohakea and Gisborne, which would result in a maximum 8-day delay of DTV. Hence, supplementary photosynthetic lighting is not necessary for year-around production of 'LSLP4' at Ohakea and Gisborne, if an 8-day delay of DTV is acceptable. However, since the monthly mean DLI at Christchurch is less than 10 $mol \cdot m^{-2} \cdot d^{-1}$, supplementary photosynthetic lighting is required in these months to avoid a significant delay in DTV.

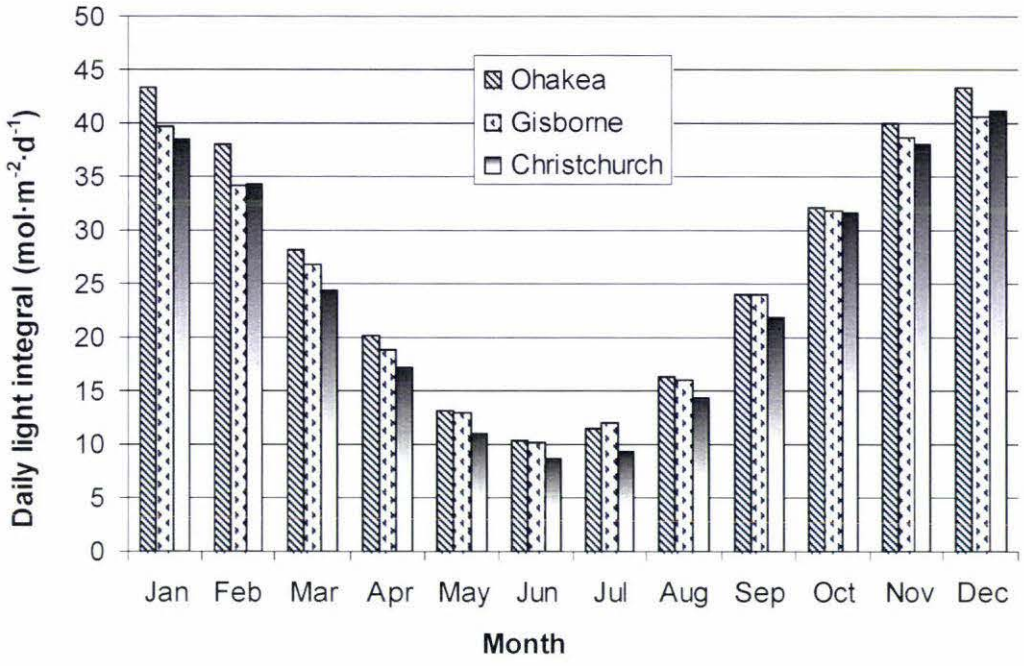

Fig. 3-14. Monthly average of daily light integral inside a greenhouse at Ohakea, Gisborne or, Christchurch (New Zealand) from 1969 to 1980 (New Zealand Meteorological Service, 1983).

3.5.9.2 Application of the post-planting model

The application of post-planting models, i.e. 'graphical tracking', has been developed for Easter lily and chrysanthemum, utilizing the monitoring of leaf number or stem length during production (Fisher and Heins, 1996; Karlsson and Heins, 1994). These models allow growers to compare observed, i.e. actual plant performance (e.g. leaf number or stem height), with target, i.e. desired values, so as to allow decisions on changes in crop management and timing of maturity. Similarly, therefore, for growers of 'LSLP4' the post-planting model including both DLI and LNAR as predictors (i.e. Eq. [3-14]), can be used to refine the prediction of DTV.

Having used Eq. [3-13] to establish a predicted DTV prior to planting, predicted DTV can be further adjusted once planting has occurred, and the actual data

for DLI and LNAR in the first period of growth have been collected by growers. For example, for a November planting at Ohakea, where the historical record of average DLI in November is 24 $mol \cdot m^{-2} \cdot d^{-1}$, an estimated DTV would be 59 days (i.e. according to the pre-planting model, Eq. [3-13]). If the actual average DLI in the first month of the planting collected by growers is 18 $mol \cdot m^{-2} \cdot d^{-1}$, and accumulated leaf number is 6 (i.e., LNAR = 0.2 $leaves \cdot d^{-1}$), the adjusted prediction of DTV would now be 63 days.

3.5.9.3 Limitation of the pre-planting and post-planting models

It has been well established that temperature and photoperiod also significantly influence time to flower of other plants (Causton and Venus, 1981; Karlsson, 2002; Roberts and Summerfield, 1987). However, the input from these two parameters on DTV of 'LSLP4' has not been investigated and included into the pre- and post- planting models presented here. Hence the predictive accuracy of both the pre- and post-planting models may be reduced under conditions, where T_a can not be controlled to around 20 °C, and photoperiod is shorter than 13 h. Further research is, therefore, required to construct more comprehensive models that integrate the effects of temperature, photoperiod, and DLI.

The pre- and post-planting models can be used to predict DTV of 'LSLP4', but not DTH. Since no evident correlation was detected between DLI and DTH, the data in phase 2 has not been combined with the data in phase 1 to construct such models. However, DTH remained relatively constant, varying from 47 to 68 days, with an average of 58 days. Thus, at this current point in time the prediction of days

from transplanting to actual harvest maturity can be achieved by adding 58 days to the predicted DTV from either of the pre- or post-planting models presented here.

The pre- and post-planting models have not been validated using the data from a separate experiment, though the post-planting model was validated using a statistical method, i.e. cross validation. A further experiment utilizing multiple planting dates is, therefore, required for the pre- and post-planting models to be more thoroughly validated.

3.6 Conclusion

DTV was significantly correlated with average DLI, with the response of DTV being saturated above 15 $mol \cdot m^{-2} \cdot d^{-1}$. The decay-exponential relationship between DTV and average DLI explained > 88% of the variation, which formed the basis of a predictive model that can be used prior to planting.

Regardless of DLI, DTH remained relatively constant at an average 58 days, reflecting the uniformity of T_a over the experimental period. In contrast, despite the uniformity of T_a, DTV varied from 52 to 165 days dependent on DLI. Hence, DLI appears to be more influential in the process of plant vegetative growth of 'LSLP4' through flower initiation and appearance, than in the subsequent stage of flower bud development to harvest. In contrast, T_a was more important in the phase of flower bud development to harvest. Experiments using a wider range of T_a and more accurately controlled DLI are required in future to investigate differential effects of temperature and DLI at various developmental stages of 'LSLP4'.

CDLI was a poor predictor of DTV in the current study. This might partly result from the method used to estimate CDLI, i.e. simply integrating DLI values in phase 1 without considering the effective range of DLI. Some of the natural DLI value fluctuations during phase 1 exceeded the response saturation of DTV to DLI, i.e. 15 $mol \cdot m^{-2} \cdot d^{-1}$. Therefore, controlled environment chamber experiments where DLI can be accurately controlled are recommended in future research to more precisely identify the effective DLI range (i.e., linear response range) for 'LSLP4'.

Under favorable growing conditions, (i.e., DLI $\geq$ 9 $mol \cdot m^{-2} \cdot d^{-1}$, T_a 20 °C, and long photoperiods), a specific leaf number (28 or 29 leaves) was accumulated by 'LSLP4' before flower initiation. This specific leaf number can be used to predict DTV of 'LSLP4' grown under the optimal conditions. Furthermore, plant growth parameters (i.e. LNAR and GCIR) were significantly correlated with DTV, each explaining >88% of the variation. The incorporation of LNAR, but not GCIR, together with DLI as the predictors of DTV, improved the predictive power of the original model using DLI alone. This formed the basis of a post-planting model.

Within the confines of temperatures and photoperiod used in the current study, the pre-planting model (i.e. DTV = 59.2 + 497.7*Exp (-0.4 * DLI)) can be used by growers to schedule planting dates and predict flowering time of 'LSLP4' anywhere around the world, based on historical DLI data. Once planted the prediction of flowering time can be further improved using the post-planting model (i.e. DTV=59.01+6578*Exp(-0.1891*DLI-19.82*LNAR)).

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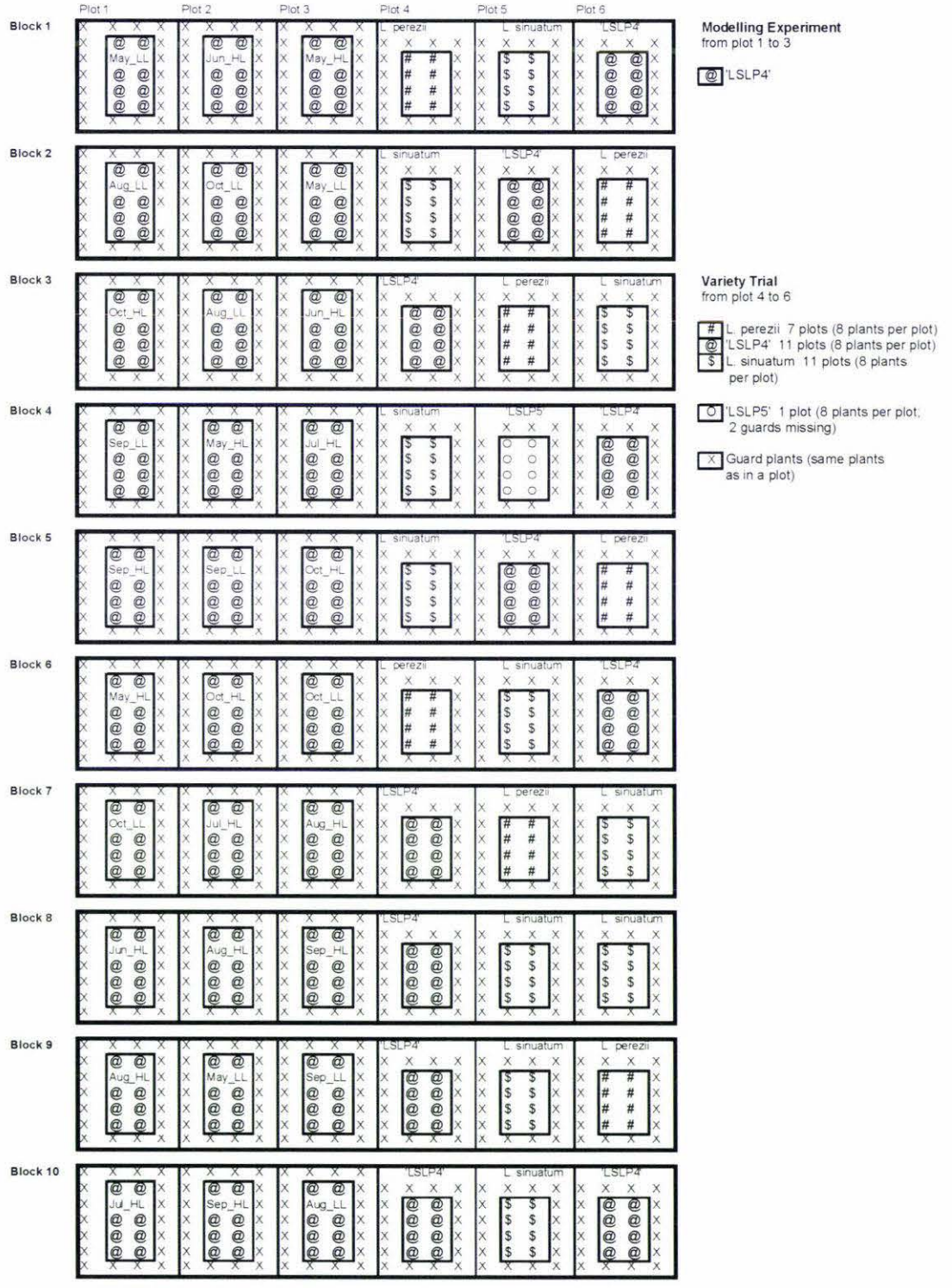
Appendix 1 Experiment design



Appendix 2 Harvest stage

Initial observations found that more than 50% of the open calyxes in the youngest branch (i.e. the lowest branch) corresponded with when about 80% of the calyces in the whole inflorescence were open. Therefore, during the main experiment harvest stage of 'LSLP4' and *L. perezii* was estimated by inspecting the youngest branch, i.e. when more than 50% of calyces in the youngest branch were open (Fig. I; Fig. III).

L. sinuatum bears 80 to 100 florets in an inflorescence, which is 300 to 500 less than that of *L. perezii* and 'LSLP4'. Hence, the harvest stage of *L. sinuatum*, i.e. 80% of calyces open, can be directly estimated by eye (Fig. II)

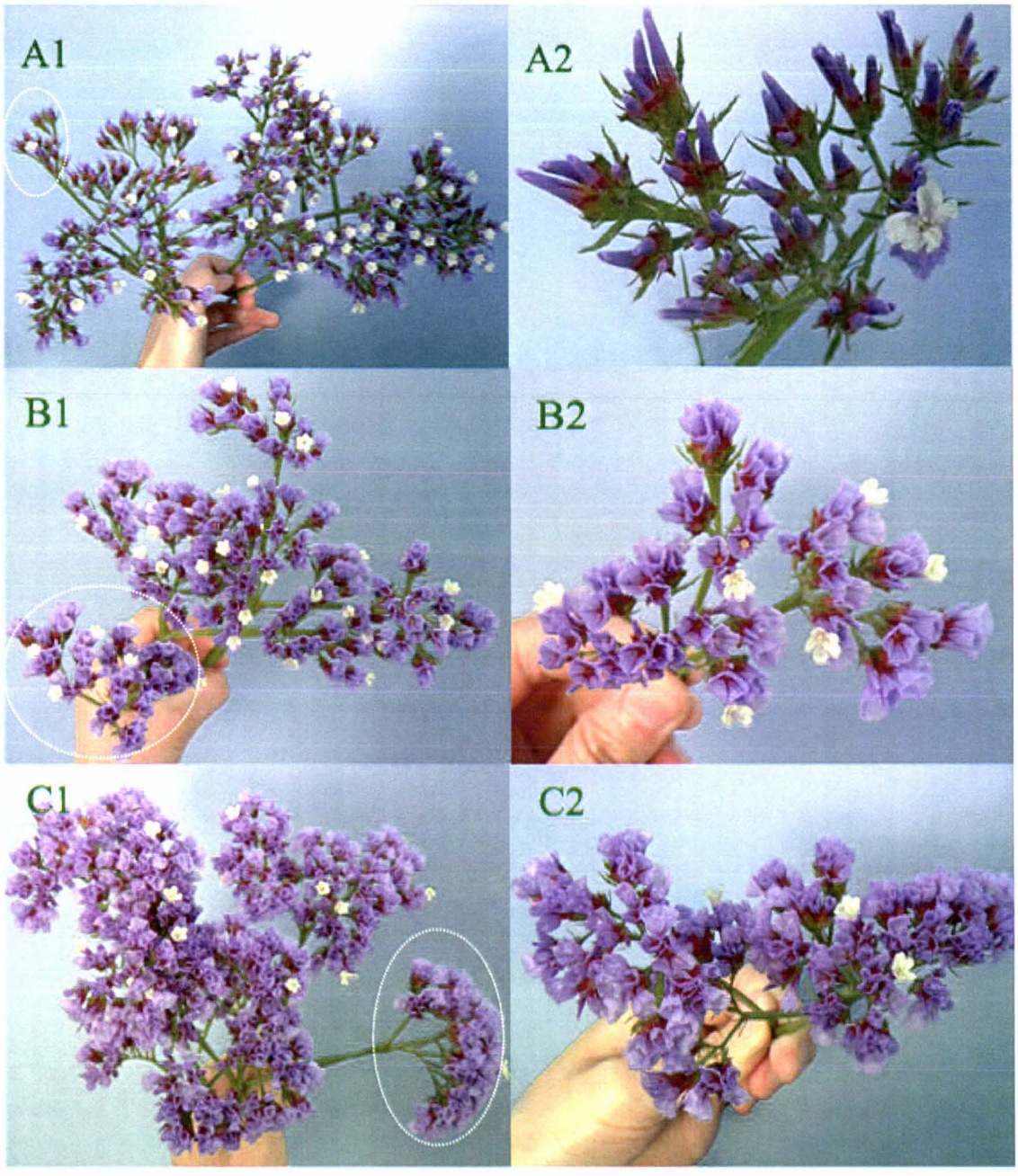


Fig. I Inflorescences of 'LSLP4' when about 30% (A1), 50% (B1) and 80% (C1) of all calyces were open. The youngest branch (highlighted by white ellipse) of the inflorescence in the three stages is enlarged in A2, B2 and C2, respectively.

Fig. II. Inflorescences of *L. sinuatum* when about 50% (A1) and 80% (B1) of calyces were open. For each of the two stages, the youngest portion (highlighted by white ellipse) of the inflorescence is enlarged in A2 and B2, respectively.

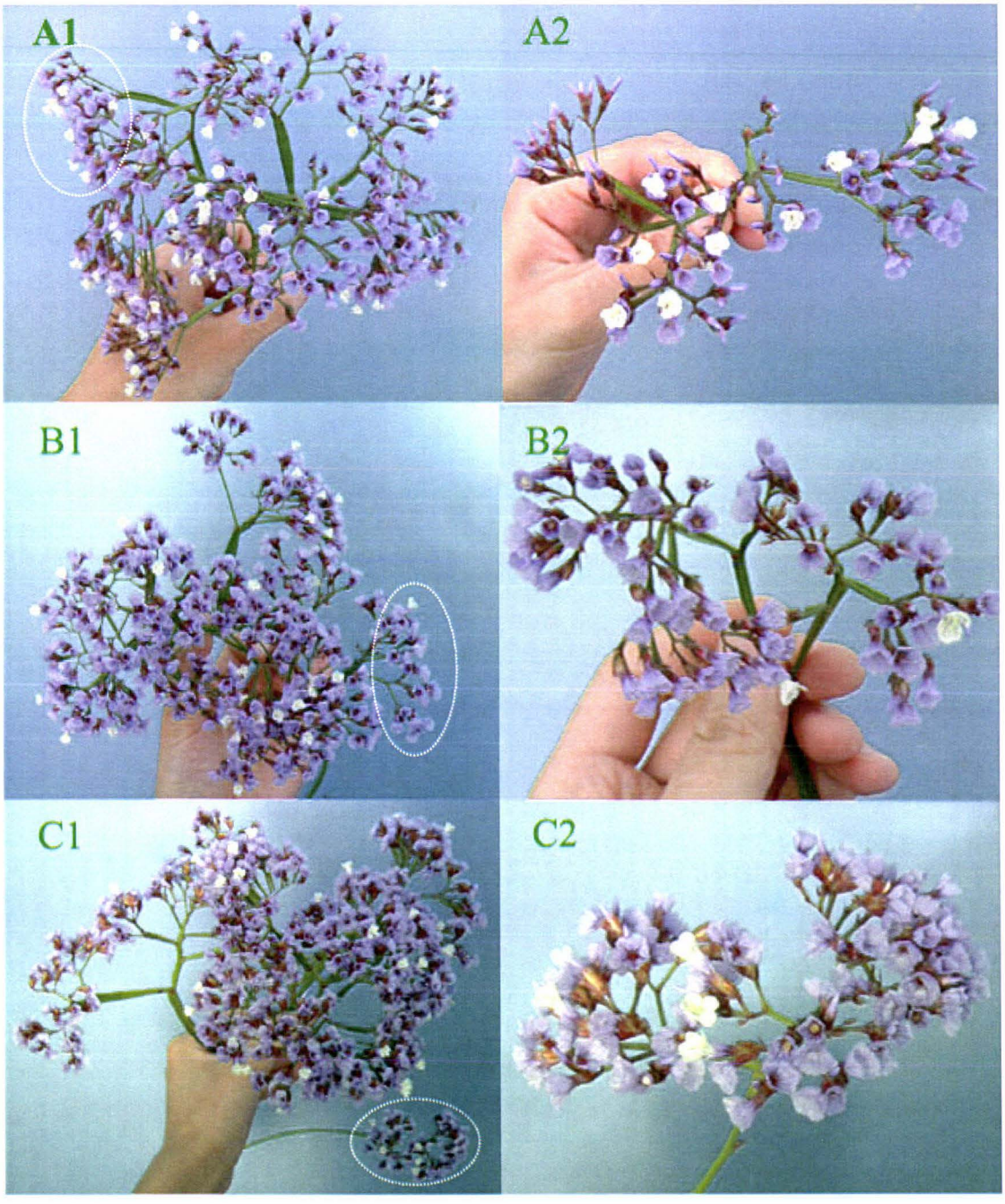


Fig. III Inflorescences of *L. perezii* when about 30% (A1), 50% (B1) and 80% (C1) of calyces are open. For each of the three stages, the youngest branch (highlighted by white ellipse) of the inflorescence is enlarged in A2, B2 and C2, respectively.

Appendix 3 Suitability of 'Broken Stick Model' and Gompertz Curve to Describe Changes in Leaf Number of *Limonium* 'LSLP4'

Introduction

Preliminary observation has identified that *Limonium* 'LSLP4' has a determinate shoot growth habit, i.e. shoot apex terminates in an inflorescence, and no new leaves are produced from the apex during the reproductive stage. For any single plant, leaf number accumulation therefore relates directly to the onset of reproductive growth. To summarize some potential predictors for time to flower of 'LSLP4', we desired to fit an empirical model to the repeating record of leaf number over time.

The data randomly chosen from the August planting showed that the leaf number of 'LSLP4' increased almost linearly till it reached the maximum. Hence, simultaneously fitting two straight lines (i.e., one for the linear increase phase and one for the maximum or horizontal phase; defined here as 'Broken Stick Model') to the data, might be suitable to explain leaf number accumulation over time.

Sigmoid curves (e.g. Gompertz Curve) has been found to be more appropriate than other empirical models, such as the exponential curve and polynomials, to describe plant development showing a determinate shoot growth pattern (Causton and Venus, 1981; Hunt, 1982). The Gompertz Curve is, therefore, one of the most likely

options to illustrate changes in leaf number accumulation of 'LSLP4'. The Gompertz Curve is a non-symmetric sigmoid function, which can be written in Eq. [1].

$$\mathbf{f(t) = A + C\, e^{(-e^{(B(M-t))})}} \qquad [1]$$

where:

A = lower asymptote

C = upper asymptote

B = slope parameter

M = value of t at a point of inflexion

t = time

To save time and accommodate variation between individual plants, development of the predictive models for DTV were based on per-plot data (i.e. average of 10 plants in a plot), not per-plant data. However, our initial attempts at plotting the per-plot data of leaf number against time created a curvature near the breakpoint, which was not representative of the data. This curvature also appeared to be absent when fitted to the per-plant data. Attempts therefore were made to determine the potential effect of this curvature on the estimate of leaf number accumulation rate (LNAR) and maximum leaf number (MLN).

Therefore, the objective of this study was to compare the suitability of the Broken Stick Model and Gompertz Curve in their ability to describe leaf number accumulation of 'LSLP4' over time, and whether this was more accurately described using per-plot or per plant data.

Materials and Methods

Broken Stick Model vs. Gompertz Curve

Data of leaf number over time from six individual plants of 'LSLP4' were sampled from the plantings that occurred from May through to August. Attempts were then made to fit the data to both the Broken Stick Model and Gompertz Curve using GENSTAT 7 (VSN International Ltd., UK). The percentage of accountability and mean square error of these two models were compared using T-tests.

Per plot vs. per plant data

Data from five replicate plots of leaf number over time were randomly sampled from the 11 treatments. The Broken Stick Model was fitted to both per-plant and per-plot data. The estimates of LNAR and MLN of per-plot data were compared through T-test with that derived from fitting to the per-plant data.

Results and Discussion

Broken Stick Model vs. Gompertz Curve

Both the Broken Stick Model and Gompertz Curve accounted for more than 98% of the variation (Table I). Although there was no difference ($P > 0.05$) between fitting the Broken Stick Model and Gompertz Curve in either r^2 and mean square error achieved, the Gompertz Curve did not accurately describe the upper asymptote (i.e. MLN of the actual data), underestimating the MLN by about 8 leaves ($P < 0.05$; Fig. IV; Table I).

There was no difference in the estimated MLN when using the Broken Stick Model compared with that recorded ($P > 0.05$; Table I). Thus the Broken Stick Model was more suitable than the Gompertz Curve to describe the changes in leaf number change over time, and to estimate MLN.

Table I Comparison of r^2 and mean square error between fitting the Broken Stick Model (Broken) and Gompertz Curve (Gompertz) to changes in leaf number of 'LSLP4' over time. The data were randomly chosen from the individual plants of plantings made in May through to August.

Plant No.	r^2 (%)		Mean square error		Estimated MLN		Recorded MLN
	Broken	Gompertz	Broken	Gompertz	Broken	Gompertz	
1	98.7	99.8	0.709	0.1479	35	25	35
2	99.8	99.6	0.1109	0.3491	35	33	35
3	99.4	99.4	0.2445	0.3088	29	23	29
4	98.5	99.5	1.194	0.5374	32	27	32
5	98.8	99.4	0.5483	0.2512	29	18	29
6	99.1	99.1	0.3985	0.4655	29	20	29
Mean	99.05	99.47	0.53	0.34	32	24	32
T-test	N.S[z]		N.S[z]		N.S[y]	*[x]	

[z] Not significant between 'Broken' and 'Gompertz'
[y] Not significant different from recorded MLN
[x] Significant different from recorded MLN ($P < 0.05$)

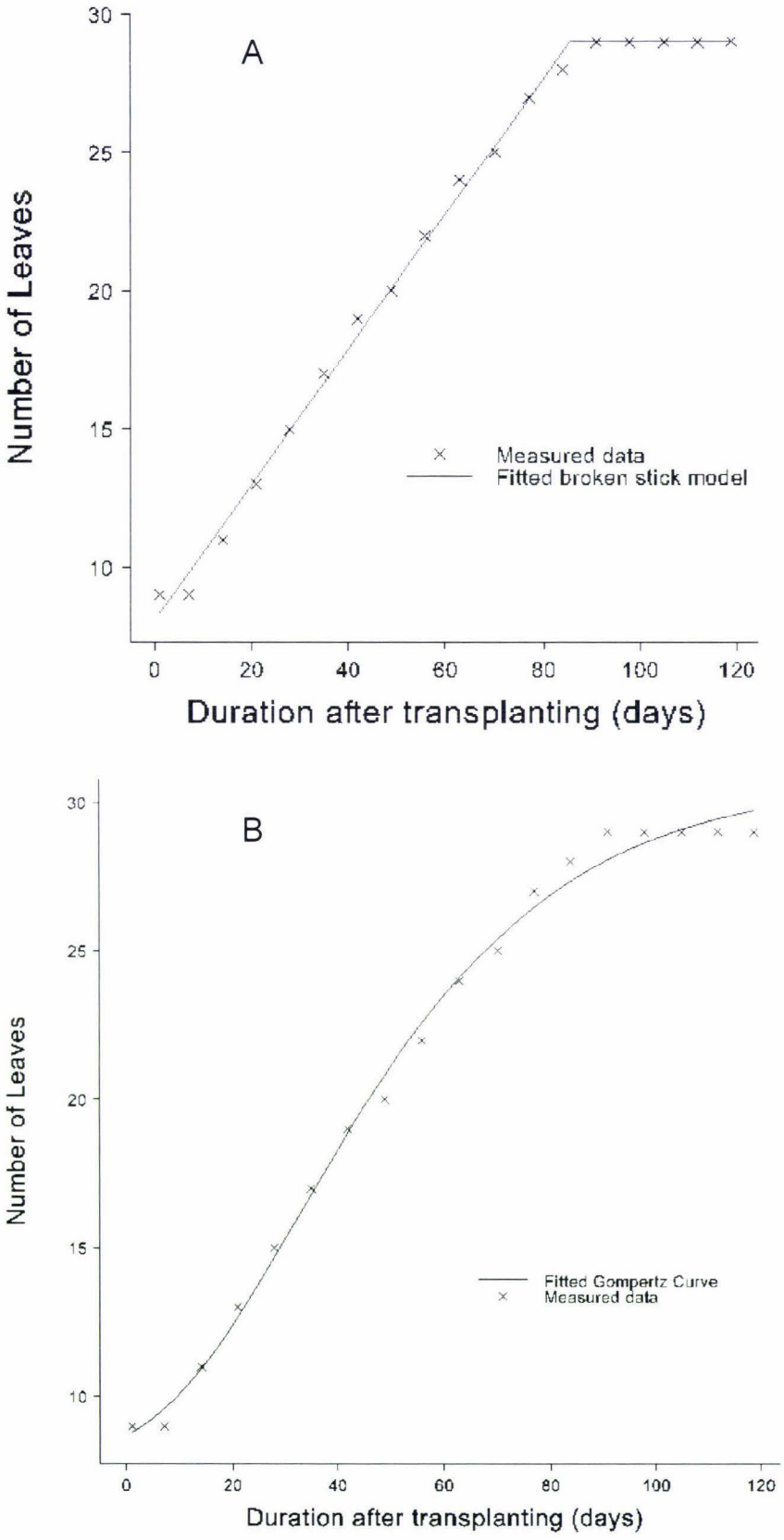


Fig. IV Example of actual data and fitted curves using the Broken Stick Model (A) and Gompertz Curve (B), describing the change in leaf number per plant over time. Data was sampled from the Jun_HL treatment.

Per plot vs. per plant data

There was no difference ($P > 0.05$) between the per-plot and the average of per-plant data, in their ability to estimate LNAR and MLN (Table II). However, the MLN was consistently less by about 0.3 leaves ($P < 0.05$) when estimated from the fitted curve of per-plot data than the actual recorded per-plot MLN. This might be partly explained by the curvature at the breakpoint of the per-plot data pulling down the position of the fitted horizontal line (i.e., estimated per-plot MLN), but not affecting the slope of the first phase (i.e., LNAR; Fig. V). Therefore, while the curvature does not affect the accuracy of estimating LNAR, it does affect the estimate of MLN. In such cases the recorded per-plot MLN is more accurate.

Because the curvature appearing in the per-plot data is just a mathematical artefact of averaging the per-plant data, and the estimate of LNAR is not affected, fitting the Broken Stick Model for per-plot data and ignoring the curvature was considered suitable for estimating the parameters required.

Table II Comparison between the per-plot and average of per-plant data on leaf number accumulation rate (LNAR) and maximum leaf number (MLN), derived from the broken stick model.

Plot No.	LNAR		Estimated MLN		Recorded MLN
	Per plant	Per plot	Per plant	Per plot	Per plot
1	0.20	0.19	33.9	33.83	34.1
2	0.18	0.17	38.5	38.63	38.7
3	0.31	0.31	29.7	29.83	30.4
4	0.26	0.25	28.8	28.84	29.1
5	0.21	0.21	28.8	28.81	29.1
Mean	0.23	0.23	32.0	32.0	32.3
T test	N.S[z]		N.S		*[y]

[z] Not significant

[y] Estimated per-plot MLN and recorded MLN had significant different at $P < 0.05$

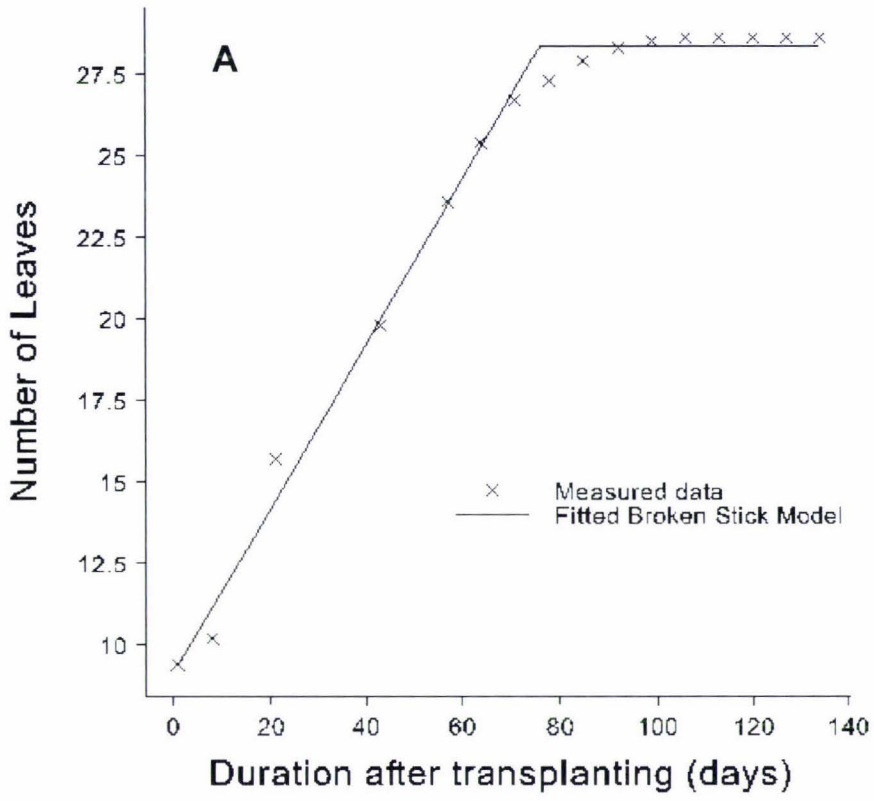


Fig. V The relationship between duration after transplanting and average number of leaves of 10 plants of 'LSLP4' in a plot sampled from the Aug_HL treatment.

Conclusion

In summary, the Broken Stick Model was better than the Gompertz Curve in describing changes in leaf number over time of 'LSLP4', and estimating both MLN and LNAR. When fitting the Broken Stick Model to per-plot data of leaf number over time the curvature created at the breakpoint influenced the estimate of MLN, but not LNAR. Therefore, the actual data recorded per-plot of MLN and estimated LNAR, from fitting the Broken Stick Model to per-plot data, were chosen for further modelling of flower.