EFFECTS OF PLANT GROWTH REGULATORS AND TEMPERATURE ON FLORAL INDUCTION AND DEVELOPMENT OF *EXACUM* STYER GROUP

by

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Abstract

Plants of *Exacum* Styer Group, interspecific hybrids from five Sri Lankan taxa from the Gentian family, have significant horticultural potential as flowering potted plants, bedding plants and cut flowers. However, a better understanding of the requirements for floral induction and flower development is needed before commercialization.

Experiments examining the impact of plant growth regulators (PGRs) on floral induction and subsequent plant development consisted of seven treatments: ethephon (500 and 1000 PPM), daminozide (2500 and 3500 PPM), gibberellin (GA_{4+7} at 10 and 15 PPM), and a control of water plus surfactant. Analysis of data collected on weeks to anthesis resulted in a significant interaction between treatment and genotype indicating that genotypes did not behave uniformly across treatments. Overall, the effect of PGR treatments on flowering of *E.* Styer Group is impacted more by genotype than by chemical with late flowering genotypes less affected by chemical than earlier flowering genotypes. Nonetheless, daminozide effectively reduced stem length without dramatic negative impact on flowering and can be considered safe for commercial production.

Experiments to evaluate the effect of temperature on floral induction and subsequent plant development consisted of four temperature treatments over two seasons (one treatment repeated in both seasons). The two most extreme treatments prevented flowering for some or all genotypes (35° C DT/31° C NT and 16° C DT/12 ° C NT, respectively). Intermediate treatments (23° C DT/19° C NT and 30° C/26° C NT) were optimal for fastest flowering depending on genotype. As with the response to PGR treatments, significant genotypic variation was observed in response to temperature indicating production schemes must be developed empirically for each individual genotype. However, the optimal temperature for fast flowering and attractive plant conformation is likely between 21° and 28° C average daily temperature for most *E.* Styer

Group genotypes and should be used as a starting point for future studies. In addition, a cultivar series of *E*. Styer Group should be selected based on common production responses, not based on genetic relationships.

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List of Acronyms

ADT	Average daily temperature
AN	Anthesis
ANOVA	Analysis of variance
BA	Benzyladenine
C	Celsius
DIF	Differences between day and night temperatures
DT	Day temperature
DTF	Days to flower
EC	Electrical conductivity
FR	Far red
GA	Gibberellic acid
LD	Long day
MB	Macrobud
MS	Mean square
NAA	Naphthaleneacetic acid
NT	Night temperature
PGR(s)	Plant growth regulator(s)
PPM	Parts per million
R	Red
SD	Short day
SS	Sums of squares
UBC	University of British Columbia, Vancouver
VB	Visible bud

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Chapter 1 Introduction and Literature Review

1.1 Introduction

The genus *Exacum* L. is a member of the Gentianaceae family and contains approximately 65 species (Klackenberg, 1985). Only one species, *Exacum affine*, is currently grown and sold for the horticulture industry. However, selection and interspecific breeding of five Sri Lankan taxa, *E. pedunculatum* L., *E. macranthum* Arn., *E. pallidum* Trimen., *E. trinervium* (L.) Druce, and *E. trinervium* ssp. *ritigalensis* (Willis) Cramer, has resulted in the creation of a new taxon, *Exacum* Styer Group (Riseman et al. 2005) that has significant horticultural potential as flowering potted plants, bedding plants and cut flowers. Their appeal is based in part on their glossy green foliage and contrasting large blue flowers and bright yellow anthers. These traits, among others, make these plants very appealing to both commercial growers as well as home-owners as they add to the limited selection of crops with blue flowers. However, before this new crop can reach its commercial potential, fundamental information related to production requirements is needed. Specifically, a better understanding of the requirements for floral induction and flower development is needed to create commercially viable production protocols.

1.2 Research Objectives

The broad objective of my research project is to generate information related to the best management practices for commercial production of *E.* Styer Group. I investigated factors (i.e., plant growth regulators and temperature) that affect floral induction and subsequent development. The long term objective of this research program is to develop an effective scheme for commercial production that includes accurate prediction of flowering time.

The specific objectives of this research were:

1. To evaluate the effect of PGR treatments on plant and flower development of ten genotypes of *E.* Styer Group;
2. To evaluate the effect of temperature treatments on plant and flower development of six genotypes of *E.* Styer Group.

1.3 Literature Review

1.3.1 Flowering of Ornamental Crops

The complexity of control mechanisms associated with flowering is enormous (Erwin 2006). Transition from vegetative phase to reproductive phase can be stimulated by a multitude of internal and/or external factors acting either independently or in conjunction with each other. Internal cues affecting flower induction include plant maturity (physiological ability to reproduce) or size, whereas external cues include environmental stimuli such as light and/or temperature. Even in the presence of a floral inductive factor, either internal or external, other conditions may inhibit floral induction through interaction. For example, an obligate short day plant that is mature enough to flower (internal) will not flower until it has been exposed to the correct photoperiod (external). In addition, many plants have evolved flowering control mechanisms that have "redundancy" to ensure that a number of different stimuli can promote flowering to compensate for environmental fluctuations (Erwin 2006). For example, plant maturity (e.g., internal) is significant since a juvenile plant does not have the resources required for reproduction. However, once a plant has reached maturity, flowering may or may not occur depending on whether additional stimuli from other factors are required. In addition, horticultural plants which have predictable internal controls sufficient for floral induction, such as maturity, often do not require external manipulation for commercial production. For example, *Cyclamen persicum* Mill. flower buds initiate in the axil of the sixth leaf, making it easy for

growers to predict flowering. However, for horticultural plants lacking such strong internal controls, manipulation of external factors is often the most efficient technique to control reproduction. Accordingly, horticultural manipulation for flowering generally involves control of photoperiod, temperature and/or irradiance (Erwin 2006).

1.3.2 Photoperiod, Temperature, and Irradiance

Research focused on photoperiod, temperature, and irradiance has greatly increased our understanding of these control components in floral induction. Many common floriculture crops have been identified as photoperiodic or needing a vernalization period to achieve flowering. Furthermore, the affect of photoperiod or vernalization is often influenced by other factors such as mean temperature or daily light integral. For example, research on *Petunia* x *hybrida* Vilm (petunia) 'Express Blush Pink' revealed that both photoperiod and temperature affect the rate of progress to flowering (estimated as the reciprocal of time to flowering) (Adams et al. 1998). They found that the time to flowering decreased linearly with increasing photoperiod up to a critical photoperiod of 14.4 ± 0.6 hours a day; extending the photoperiod beyond this resulted in no further hastening to flowering. This study also determined the optimum temperature for flowering increased linearly with increasing photoperiod from 20.7° C at 8 $h \cdot d^{-1}$, up to 24.3° C at the critical photoperiod of 14.4 $h \cdot d^{-1}$. No significant multiplicative effects between temperature and photoperiod were detected, indicating the additive effects of temperature and photoperiod on the time to flower (Adams et al. 1998). These results support the idea that interactions between temperature and photoperiod on flowering time may be present and need to be thoroughly evaluated. However, before interactions among external factors can be evaluated, base-line values for plant responses to individual factors must first be established.

Average daily temperature (ADT, i.e., constant or diurnal) has been shown to influence time to flower in many floriculture crops. For example, under constant temperature treatments,

flowering of *Antirrhinum majus* L. (snapdragon) 'Rocket Rose', *Calendula officinalis* L. (calendula) 'Calypso Orange' and *Torenia fournieri* Linden ex E. Fourn (torenia) 'Clown Burgandy' was developmentally delayed, as evidenced by increased node number below the first flower, at 32˚ compared to 20˚ C (Warner and Erwin 2005). In another study designed to evaluate diurnal temperature effects on flowering of *Pharbitis nil* Chois. (L.) 'Violet' (Japanese morning glory), treatments failed to produce significant differences (Reese and Erwin 1997). Therefore, the authors subsequently analyzed the data in terms of ADT. They found that plants did not flower when exposed to an ADT below 22˚ C and that total flower bud number per plant increased from 0 to 6.3 as ADT increased from 22 to 30˚ C (Reese and Erwin 1997). However, this response is not without limits in that production of a given crop at a temperature above its critical level can either fail to promote faster development or inhibit floral initiation. For example, analysis of constant air temperature treatments ranging from 15 to 27˚ C indicated a maximal rate of development occurred at ≈25˚ C for *Celosia argentea* L. var. *plumosa* L. (celosia) "Gloria Mix" and ≈26˚ C for *Impatiens walleriana* Hook. f. (impatiens) 'Accent Red', above which a further increase in temperature did not accelerate flowering (Pramuk and Runkle 2005). Results from research on *Viola* x *wittrockiana* Gams. (pansy) 'Delta Yellow Blotch' and 'Delta Primrose Blotch' flowering showed that time to flower decreased as plant ADT increased from 16.3 to 25.7˚ C regardless of differences between day and night temperatures (DIF) (Niu et al. 2000). This indicates DIF does not influence time to flower for pansy. However flower morphology was affected as flower peduncle length increased with increasing DIF (Niu et al. 2000). In addition, a study on *Phalaenopsis* Blume. (moth orchid) Brother Goldsmith '720' and Miva Smartissimo x Canberra '450', showed that inflorescence initiation was inhibited by treatments with a high day temperature set point (e.g., 29˚ C), even when the night temperature set point was cool (e.g., 17˚ C) (Blanchard and Runkle 2006). This indicates that DIF does not override day temperature effects for orchid flowering. Overall, these findings indicate that the

effect of temperature on flowering is highly variable and species specific and, as such, should be examined with a range of temperature treatments. Furthermore, these studies indicate that ADT or constant temperature may be more relevant to flower induction than DIF.

1.3.3 Plant Hormones and Growth Regulators

Plant growth regulators (PGRs) are commonly used to alter plant conformation and need to be evaluated for potential impacts on flowering before widespread use on a new crop. Many commercial PGRs are used to counter the effects of gibberellins (GA), as high GA content is positively associated with internode elongation. However, there is evidence that GAs can also act as a promoter/inhibitor of flowering (Erwin 2006). Applications of commonly used PGRs (e.g., ancydimol, chlormequat, daminozide, paclobutrazol, and uniconazole) are designed to reduce internode length. Unfortunately, the modes of action for these commonly used commercial PGRs are not fully understood. For example, daminozide has been categorized by some as a gibberellin (GA) biosynthesis inhibitor (Heins et al. 2000) while others claim that daminozide is not a GA biosynthesis inhibitor but instead works by either inhibition of GA translocation or promotion of GA degradation (Gent and McAvoy 2000). Gent and McAvoy reference that daminozide acts as an inhibitor of the "late stages of gibberellin metabolism" whereas the conclusion of the article states that daminozide works in part by inhibiting the "later stages of GA biosynthesis" and thereby reduces endogenous biologically active GA levels (Brown et al. 1997). The conflicting theories about the mode of action of daminozide may be an issue of semantics as the end results are very similar. Nonetheless, there is certainly a connection between daminozide and GA. Therefore, PGRs, especially anti-GA compounds, have the potential to affect flowering and need to be evaluated before widespread use.

The effect of PGRs and GA on flowering has been documented for some species. A decline in endogenous GA levels prior to floral initiation was observed in *Boronia megastigma*

Nees. (boronia) (Roberts et al. 1991), with the investigators concluding that boronia may require a reduction in the concentration of GA (i.e., GAs 1, 3 and 20) before plants will initiate flowers. Applications of anti-GA compounds have been shown to result in a similar reduction in endogenous GA levels (Brown et al. 1997) and therefore could affect flower development. For example, flower development of *Oenothera fruticosa* L. (sundrops) `Youngii-lapsley' was affected by uniconazole at 15 $mg \cdot L^{-1}$ (15 ppm). Following application, the flower diameter of treated plants was 36% smaller than that of controls, resulting in a 58% reduction in total floral area (Clough et al. 2001). Research examining the effects of anti-GA treatments should also include GA treatments as a positive control. Therefore, a good option for simulating commercial conditions is Fascination®, a commercial formulation comprised of 1.8 % $GA_{4,7}$ and 1.8% benzyladenine (6BA; a synthetic cytokinin) and labelled for use on potted ornamentals to promote internode elongation at concentrations of 1 to 25 ppm $GA_{4,7}$ and 6BA. At this concentration, the BA content is considered negligible, based on published reports (Blanchard and Runkle 2008, Carey 2008), so any effect would be attributed to GA alone. Given the variable effects of PGRs on different species, research is needed to evaluate the effect of these compounds on both time to flower/flower development and plant architecture for any new crop including *E.* Styer Group.

The plant hormone ethylene has varied affects on plants including both floral promotion and inhibition. It is an important regulator and promoter of flower bud formation for Bromeliads, such as *Ananas comosus* (L.) Merr. (pineapple) (Burg and Burg 1966; Erwin 2006) and ethephon (an ethylene gas generator) treatments stimulated early flowering in *Mangifera indica* L. (mango) (Chacko et al. 1976). However, ethylene also causes floral senescence and abscission in many floriculture crops (Jones et al. 2001, Reid and Wu 1992 and Serek and Sisler 2001), a phenomenon which is sometimes useful for stock plant management where vegetative shoots are required for propagation. For example, exposure to ethylene gas causes flower

abscission in *Impatiens balsamina* L. (rose balsam) `Tempo Pink' and *Impatiens* × *hybrida* (sym. *I. hawkeri*) (New Guinea impatiens) `Aruba', without negative secondary effects, justifying use of ethephon for stock plant management (Tamari et al., 1998). Recently, guidelines for stock plant management of many crops include the application of ethephon to abort flower buds and promote vegetative growth (Runkle 2006). However, plant responses to ethylene are sometimes graded based on concentration. For example, low concentration treatments of ethephon (200 ppm) on *I. balsamina* significantly reduced the number of flowers per plant (<10%) compared to the control plants while plants treated with higher concentrations (400 or 800 ppm) produced no flowers (Tamari et al. 1998). Therefore, the observed reduction in flowers resulted from bud abscission rather than from inhibition of flower initiation or development (Tamari et al. 1998). An experiment examining the combined effect of 400 ppm ethephon and 25 ppm gibberellin (approximately 92% GA_3) on *I. balsamina* flowering also resulted in bud abscission, with a complete absence of flowers observed for treated plants (Tamari et al. 1998). This indicates that the bud abscission caused by ethylene is not overridden by GA applications, despite the fact that GA application has been shown to result in more flower buds and earlier flowering for this species (Sharma et. al.1978). These findings are relevant because the ability to maintain a plant in a vegetative state can reveal information about the factors/stimuli required for reproductive growth; if you can prevent floral induction, you may gain a better understanding of what causes floral induction. Accordingly, the effects of GA and ethylene on flowering of a new crop should be examined.

1.3.4 Flowering of Gentianaceae

Exacum Styer Group's closest relative in the horticulture industry, *Exacum affine* (Persian violet), offers an ideal candidate from which to base new production protocols. *E. affine* has been identified as a day neutral plant meaning that flower induction is not affected by night

length (Erwin 2006). However, flower induction and development in *E. affine* is promoted by increased irradiance provided by supplemental lighting of 183 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a 16 hour photoperiod (Holcomb and Craig, 1983). Results from experiments using various radiant energy levels (irradiance) indicated the most rapid progression to flowering in *E. affine* was positively correlated to radiant energy level, with lower radiant energy levels (85 $\mu mol \cdot m^{-2} \cdot s^{-1}$) increasing time to flower (first flower open) and higher radiant energy levels (345 $\mu mol \cdot m^{-2} \cdot s^{-1}$) reducing time to flower (first flower open) (Williams et al. 1983). A study on *E. affine* plant quality when grown under supplementary light conditions (70 and 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) showed increased irradiance reduced the production time (to "commercial maturity" with approximately 20 open flowers) and resulted in increased number of flowers per plant during a simulated post-production period (Serek and Trolle 2000). Similarly, a low-irradiance environment was shown to result in both reduced flowers per plant and flower colour intensity (Rubino 1991). Of the aforementioned studies, only Williams et al. (1983) considered the potential affects of temperature increases with increased irradiance. For their highest radiant energy level, the temperature increase was only 1˚ C above the set air temperature for all light treatments (21˚ C), leading them to conclude that the predominant factor affecting flowering was radiant energy level and not temperature (Williams et al. 1983). Later research on various genotypes of *E. affine* which examined the effect of season in Beltsville, Maryland (39 °N) on time to flower, resulted in earlier flowering (first flower open and full bloom) for the summer season (February to October, 22° C ADT for two greenhouses) for some genotypes while other genotypes flowered earlier during the winter season (October to June, 17 and 19° C ADT for two greenhouses) (Rubino 1993). Rubino suggests the winter season delayed flowering for some genotypes because of low irradiance (i.e., short days at higher latitudes) with no mention of the possible effects of temperature (i.e., lower ADT). While it appears radiant energy may be the

primary factor affecting flowering of *E. affine*, the effects of air and/or plant temperature may also be significant and require further evaluation.

Investigations on the effects of PGRs on flowering of *E. affine* may be useful for *E.* Styer Group. Research examining the affect of gibberellin application to *E. affine* has shown faster flowering time (days to five open flowers) with increased concentrations of GA_3 (Neumaier et al. 1987). This finding raises questions about the possible effect of anti-GA compounds on flowering of *E.* Styer Group. Besides possible effects on flowering, the use of PGRs may be beneficial for commercial production of *E.* Styer Group as some genotypes have shown the tendency to 'bolt' (i.e., internode elongation associated with flower induction) resulting in a less desirable conformation. For height control of *E. affine*, daminozide (an anti-gibberellin) has been recommended (Sweet 1982) and has been shown to effectively reduce plant height (Barrett and Nell 1989). In addition, flurprimidol, a gibberellin biosynthesis inhibitor, has been shown to reduce plant height of *E. affine* "Blue Champion" (Whipker et al. 2006). The study described above examining *E. affine* in post-production also addressed the impact of ethylene on flower number after anthesis (Serek and Trolle 2000). *E. affine* plants at "commercial maturity" (with approximately 20 open flowers) were placed in gas tight chambers and ventilated with 3.5 ppm ethylene or ethylene-free air (control) for seven days. Ethylene treated plants had 50% fewer open flowers than control plants. Unfortunately, studies of PGR use on *E. affine* have not addressed their impact on flowering time.

Another relative of *E.* Styer Group from Gentianaceae, *Eustoma grandiflorum* (Raf.) Shinn. (lisianthus), may also serve as a useful model for investigating flowering. A study examining the effect of photoperiod and temperature on flowering of *E. grandiflorum* cultivars ('Yodel White', 'Heidi Pink', 'Blue Lisa' and 'GCREC-Blue') indicates both photoperiod and temperature influence the number of days to anthesis (Harbaugh 1995). For all cultivars, the fewest 'days to flowering' was achieved when plants were grown under LD at 28° C while SD at

12° C resulted in the greatest 'days to flowering'. This was confirmed by work on *E. grandiflorum* 'Heidi Deep Blue' which demonstrated that growth and flowering can be easily manipulated through temperature and photoperiod treatments (Zaccai and Edri 2002). This experiment included a two-by-two factorial design of two planting times with different temperature regimes (26.5° C/14.1° C, winter planting and 27.0° C/17.5° C, summer planting) under two photoperiod regimes: long day (LD) and short day (SD), 16 h/8 h and 8 h/16 h (light/dark), respectively. Summer planting combined with LD treatment resulted in the fastest floral transition. Furthermore, Zaccai and Edri state that floral transition apparently requires a certain minimal number of nodes and a minimal stem length. Later research indicated that *E. grandiflorum* is a quantitative long-day plant (Islam et al. 2005). Results from this study showed that increased irradiance reduced the time to flower initiation (Islam et al. 2005). However, most recently, the ratio between red (R: 660 ± 30 nm) and far red (FR: 730 ± 30 nm) wavelengths during long-day treatments have been implicated in *E. grandiflorum* flowering where either a promotion or delay of flower bud initiation and development was observed (Yamada et al. 2009). Specifically, the threshold R:FR ratio demarcating the promotion or delay of visible bud was approximately 5.3. Therefore, Yamada et al. concluded that long-day treatments using light sources with a R:FR ratio above 5.3 would delay flowering and those with a R:FR ratio below 5.3 would promote it. Together, these data suggest that photoperiod, temperature, light intensity, light quality, and plant size and/or maturity may all impact flowering of *E. grandiflorum* and point to potential areas of inquiry for *E.* Styer Group.

For potted plant production of *E. grandiflorum*, stem length reduction is desired and is normally achieved via PGR application. A few studies have evaluated the most effective PGRs for this purpose as well as measured their affect on flowering. For example, daminozide (foliar spray of 2500 ppm applied twice) delayed flowering (defined as half the flower buds on the plant had opened) of one cultivar when grown at 13° C while daminozide did not delay flowering of

any cultivars when grown at 18° C (Halevy and Kofranek 1984). The same study included ancymidol treatments (foliar spray of 100 ppm applied twice or soil drench of 0.5 mg per pot) which resulted in earlier flowering, by about 10 days, for both application methods (Halevy and Kofranek 1984). These results were confirmed by later research for which 'days to flower' (DTF; number of days from seed sowing until the first flower within a pot opened, i.e., the corolla flared, exposing pistil and stamens) for *E. grandiflorum* was delayed by daminozide (foliar spray of 2500 ppm applied twice or 7500 ppm applied once) compared to ancymidol (foliar spray of 66 ppm or soil drench of 0.25 mg per pot) (Starman 1991). Furthermore, Starman found that uniconazole foliar sprays at high concentrations (10 or 20 ppm) delayed DTF of *E. grandiflorum* while drenching with the same chemical did not delay DFT; the high drench concentration (1.60 mg per pot) actually hastened flowering by approximately four days (Starman 1991). Overall, flowering of *E. grandiflorum* is impacted by PGRs differently depending on chemical and application method.

1.3.5 Flowering of *Exacum* Styer Group

Little is currently known about factors controlling the flowering of *E*. Styer Group. Previous studies have demonstrated that flower induction of *E*. Styer Group is not regulated by photoperiod (Anon 1994). Later work on floral induction indicated irradiance as a primary factor for some *E.* Styer Group genotypes, but with high levels of genotypic variation observed (Krishnasamy 2007). Based upon her observations, Krishnasamy postulated that additional primary factors besides irradiance may influence floral initiation, and suggested investigating node number and/or temperature as future areas of research. Results from an experiment on the effect of anti-GA compounds, (i.e., chlorocholinchloride and ancymidol) and a GA antagonist compound (i.e., abscisic acid) on floral initiation showed a delayed flowering response across all treatments. However no treatment completely prevented flowering (Krishnasamy 2007). While

this PGR experiment adds to our understanding of the flowering response for *E.* Styer Group, the application method (injection to apical meristem) is not practical for commercial production. These studies clearly demonstrate the need for further research on factors regulating *E.* Styer Group flowering, with temperature, node count and PGR application all likely to have significant roles in this process.

1.4 Summary

Before *E*. Styer Group can thrive in the horticulture industry, reliable production protocols must be established. Most importantly, floral induction and development must be characterized and understood. Thorough evaluation of plant responses to individual factors is necessary before interactions among factors can be evaluated, leading to a better understanding of floral requirements and an effective production scheme. Accordingly, this research focuses on the evaluation of plant response to individual factors which are manipulated with commercially relevant methods.

Chapter 2 Materials and Methods

2.1 Plant Material

Eleven genotypes of *Exacum* Styer Group (Table 2.1.1) were micropropagated in the Centre for Plant Research plant tissue culture laboratory at the University of British Columbia following the protocols of Riseman and Chennareddy (Riseman and Chennareddy, 2004). Harvested propagules were treated with 10 mM NAA solution (i.e., dip in solution for 60 seconds) and placed in Oasis® plug trays (Smithers-Oasis North America, Kent, OH) and acclimated in a laboratory fog chamber for approximately two weeks. Plug trays were then moved to the UBC Horticulture Greenhouse to continue rooting and acclimation under mist (10 seconds of mist every 15 minutes) and ambient light. The mist bench was covered in shade cloth (40% shade) to maintain high relative humidity and provide partial shade. Rooting time varied by genotype but once under mist, adequate rooting occurred for most plants after two weeks. Once rooted (i.e., when oasis plugs were lifted from the trays, roots were visible on the outside of the oasis foam), plants were removed from mist and acclimated to greenhouse conditions by placement under shade cloth (20% shade) for approximately one week with sub-irrigation as needed with fertilizer water described below for the UBC Greenhouse.

2.2 Plant Growth Regulator Experiments

2.2.1 Growth conditions

Once rooted and acclimated (approximately five weeks from tissue culture), plants were moved to one of two locations: 1) University of British Columbia Horticulture Greenhouse, Vancouver, BC (June to December 2006) and 2) Westcan Greenhouses, Langley, BC (April to October 2006). Rooted cuttings were potted into 4" pots with a soil-less potting mix composed of 75% peat and 25% perlite including a fertilizer starter charge (approximately 1.8 EC) and a wetting agent with the pH adjusted to between 5.5 and 6.5 with dolomite limestone (West Creek

Farms, Fort Langley, BC). Constant fertilization was delivered via the irrigation water as needed. At the UBC Greenhouse, fertilizer solution was made with Scotts® Peters® Excel® 15-5-15 Cal-Mag fertilizer at a rate of 100 ppm total N (0.66 EC). At Westcan Greenhouse, fertigation solution was maintained at 1.6 EC with injection from two stock solutions; Plant Prod® 20-8-20 fertilizer (Plant Products Co. Ltd., Brampton, ON) and EPSO Top® magnesium sulphate epsom salts (K+S KALI GmbH, Postfach, Kassel, Germany). At both locations, supplemental zinc was applied in clean water biweekly in the form of Zn-EDTA (14% zinc, Plant Products Co. Ltd., Brampton, On.) at 300 ppm. At the UBC Greenhouse, plants received a combination of ambient light plus supplemental light for the duration of the experiment (June to December 2006). Ambient light was supplemented with 48 $\mu mol \cdot m^{-2} \cdot s^{-1}$ by high pressure sodium (HPS) lamps (400 watts each) placed two meters above the bench between 6.00 and 22.00 hours when solar radiation was below 1828 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Therefore supplemental lights were turned off between 6.00 and 22.00 hours when solar radiation was at or above 1828 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At Westcan Greenhouse, plants also received ambient light plus supplemental light for the duration of the experiment (April to October 2006). Ambient light was supplemented with 99 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at table height by HPS lamps (600 watts each) between 4.00 and 17.30 hours when solar radiation was below 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Therefore supplemental lights were turned off between 4.00 and 17.30 hours when solar radiation was at or above 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Constant temperature for the UBC Greenhouse was set between 20 and 24° C. The average daily temperature for the duration of the experiment at UBC was 23.3° C. Constant temperature for Westcan Greenhouse was set at 22° C with ventilation starting when the temperature reached 25° C. The average daily temperature for the duration of the experiment at Westcan was 23.5° C. Pests were managed as needed, only requiring release of biocontrol insects for aphids, use of Safer® soap for spider mites, and biweekly fungicide applications (only at UBC). Fungicide applications alternated between Rovral® (Bayer CropScience, Inc., Calgary, AB, active

ingredient: iprodione) at a rate 1000 ppm and Sovran® (BASF Canada Inc., Toronto, ON, active ingredient: kresoxim methyl) at 240 ppm.

2.2.2 Experimental design

Ten genotypes of *Exacum* Styer Group were used for the plant growth regulator experiments, with two genotypes common to both locations. Genotypes used at the UBC Greenhouse were 01-9-1, 01-37-8, 01-42-3, 01-47-49, 01-48-10 and 02-174-9. Genotypes used at Westcan Greenhouse were 01-37-37, 01-37-50, 01-37-61, 01-42-3, 01-47-21 and 01-47-49. Experiments used a completely randomized design with chemical and genotype analyzed as main effects. Treatments were randomly assigned to plants with plants repositioned biweekly to eliminate possible effects of bench location. Each genotype x treatment unit included at least four plants. Treatments included two concentrations each of three PGRs: daminozide (B-Nine®, Chemtura Corporation., Middlebury, CT, 2500 and 3500 ppm), ethephon (Ethrel®, Bayer Crop Science, Calgary, AB, 500 and 1000 ppm), gibberellin (Fascination® [1.8 % $GA_{4,7}$ and 1.8% benzyladenine], Valent BioSciences Corporation, Libertyville, IL,10 and 15 ppm), each mixed with tap water. Ethrel® and Fascination® were supplemented with two drops (approximately 0.1 ml) Tween 20® (Fisher Scientific Co., Ottawa, ON.) per litre of water. Tween 20® was not used for B-Nine® treatments as the formulation already contained a surfactant. The control treatment consisted of tap water plus the surfactant (two drops per litre). Starting one week after potting, plants were treated five times, once every seven to ten days. Treatment solutions were applied by spray to the foliage until run-off. At Westcan Greenhouse, reproductive stage (e.g., vegetative, visible bud, macro/coloured bud and anthesis) of all plants was recorded biweekly. Data collected biweekly at the UBC Greenhouse included developmental/reproductive stage as with Westcan Greenhouse plus plant height (cm) and node count. Reproductive stages were defined as follows: vegetative - non-reproductive to the naked eye (only having vegetative

growth visible); visible bud - a reproductive bud visible to the naked eye; macrobud - at least three buds that were beginning to colour with sepal separation; and anthesis - at least three flower buds open with petals perpendicular to pistil. Data were collected until either all plants reached anthesis or six months had passed. Descriptive statistics and analysis of variance (ANOVA) were performed with Statistica 7.0 (Statsoft, Inc.).

2.3 Temperature Experiments

2.3.1 Growth conditions

Once rooted and acclimated as described above, plants were potted into 2.5" cone-tainers (with comparable volume to 4" square pots) with the same medium used for the PGR experiment. For the first temperature experiment (winter; October 2007 to March 2008), the general health and vigour of propagules for each genotype was noted during potting based on root growth (i.e., number of visible roots on the outside of oasis foam plug) and stem and leaf vigour (i.e., leaf size and number). For the second temperature experiment (summer; June to October 2008), all potted propagules were individually rated based on root health / vigour and shoot health / vigour (1-5 scale with 5 being most vigorous). Once potted, cone racks were placed on a greenhouse bench to harden off for one week before placement in growth chambers and commencement of treatments. Temperature treatments were conducted in three growth chambers (Conviron E-15) located in the Land & Food Systems building (MacMillan) and the Forest Sciences Centre. Plants were watered as needed. Fertilizer was given as solution in irrigation water made with Scotts® Peters® Excel® 15-5-15 Cal-Mag fertilizer at a rate of 100 ppm total N. Supplemental zinc was applied in fertilizer water biweekly in the form of Zn-EDTA (14% zinc Plant Products Co. Ltd., Brampton, On.) at 300 ppm. Pests were managed as needed, only requiring use of Safer® soap for spider mites, mealybugs and white flies, and biweekly fungicide applications (for winter experiment only). Fungicide included alternations

between Rovral® (Bayer CropScience, Inc., Calgary, AB, active ingredient: iprodione) at a rate 1000 ppm and Nova 40W® (Dow AgroSciences Canada Inc., Calgary, AB, active ingredient: myclobutanil [triazole]) at 113 ppm. Photoperiod and thermoperiods followed 16 hours of light / daytime temperature and 8 hours of dark / night time temperature. Light was provided by a combination incandescent bulbs (60 watts each) and cool white fluorescent bulbs (160 watts each) resulting in light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at potting medium level. For the first experiment (winter), temperature treatments were 16° C DT/12° C NT, 23° C DT/19° C NT and 30° C DT/26° C NT. For the second experiment (summer), temperature treatments were 23° C DT/19° C NT, 30° C DT/26° C NT and 35° C DT/31° C NT.

2.3.2 Experimental design

Six genotypes of *Exacum* Styer Group used for temperature experiments in growth chambers were 01-37-37, 01-37-50, 01-42-3, 01-47-21, 01-50-46 and 02-174-9. Each growth chamber was set with a different diurnal temperature scheme to represent typical commercial growing conditions (i.e., day time temperature higher than night time temperature). Experiments utilized a completely randomized design with sub-samples. Plants were randomly repositioned in chambers every week to eliminate possible effects of location within chamber. Each chamber contained 33 plants per genotype (sub-samples) to allow for destructive harvests over time. Weekly data collection included plant height (cm), node count, developmental/reproductive stage (vegetative, visible bud, macro/coloured bud and anthesis), and destructive root and shoot biomass measurements (for the first experiment only). Reproductive stages were defined as for the PGR experiments. Data collection continued until either all plants had reached anthesis or 18 or 23 weeks had passed (for the summer and winter experiments, respectively). Reproductive data for all plants was characterized with descriptive statistics. Descriptive statistics and ANOVA were performed with Statistica 7.0 (Statsoft, Inc.).

Table 2.1.1. List of *Exacum* Styer Group genotypes used in plant growth regulator (PGR) experiments in two locations (Westcan Greenhouse and UBC Greenhouse) and temperature experiments in two seasons (winter and summer).

	PGR Experiments		Temperature Experiments	
Genotype	Westcan	UBC	Winter	Summer
01-9-1		x		
01-37-8		x		
01-37-37	x		x	x
01-37-50	x		x	x
01-37-61	x			
01-42-3	x	x	x	x
01-47-21	x		x	x
01-47-49	x	x		
01-48-10		x		
01-50-46			x	x
02-174-9		x	x	x

Chapter 3 Results

3.1 Plant Growth Regulator Experiments

3.1.1 Overview of effects

Two plant growth regulator (PGR) experiments were conducted in two locations (Westcan Greenhouse and UBC Greenhouse) and with mostly different genotypes. Therefore, these two experiments were not pooled (i.e., each analyzed separately). However, two genotypes (01-42-3 and 01-47-49) were included in both locations. For these two genotypes, analyses included location as a main effect to account for this possible source of variation. Analysis of variance (ANOVA) of the PGR experiment conducted at Westcan Greenhouse revealed significant main effects for genotype ($P \leq 0.001$), treatment ($P \leq 0.001$), as well as significant interactions between them ($P \leq 0.01$), for weeks to visible bud (VB), weeks to macrobud (MB), weeks to anthesis (AN) and weeks between VB and AN (with the exception of treatment which was not significant for weeks between VB and AN) (Table 3.1.1). ANOVA of the PGR experiment conducted at the UBC Greenhouse revealed significant main effects for genotype ($P \leq 0.001$), treatment ($P \leq 0.001$), as well as a significant interaction between them ($P \leq 0.01$), for weeks to visible bud (VB), weeks to macrobud (MB), weeks to anthesis (AN), weeks between VB and AN, plant height (cm) and node number at VB and number of reproductive branches at anthesis (Table 3.1.2). These significant interactions between genotype and treatment indicate that genotypes did not behave uniformly in response to the various treatments. Mean weeks to anthesis for individual effects are presented for Westcan Greenhouse (Figure 3.1.1) and UBC Greenhouse (Fig. 3.1.2) with data summaries (Tables 3.1.3 and 3.1.4). Full ANOVA results including Type III Sums of Squares decomposition (sums of squares, degrees of freedom, mean squares, and F and *P* values) are detailed in Appendix A. A brief description of additional observations of treatment effects (i.e., treatment induced symptoms) are presented in Appendix

B. Photographs are presented of representative plants (i.e., closest to average reproductive stage, node, height and general appearance) for the experiment conducted at the UBC Greenhouse (week 20) (Fig. 3.1.3). Another separate ANOVA was conducted for the two common genotypes (01-42-3 and 01-47-49) to examine the effect of location on weeks to anthesis (Table 3.1.5). All interactions were significant ($P \leq 0.01$) indicating that data from the two locations cannot be pooled.

3.1.2 PGR treatment effects

When compared to the control and summarised across genotypes, several treatments promoted earlier flowering (mean weeks to anthesis) while others delayed flowering (Tables 3.1.1 and 3.1.2). However, treatments effects were not consistent across locations. At Westcan, the treatment effects were very similar across genotypes; plants treated with Daminozide 3500 ppm flowered earliest (approximately 15 weeks), plants treated with Ethephon 1000 ppm flowered latest (approximately 16.5 weeks) and all other treatments (including the control) flowered in between 15 and 16.5 weeks (Table 3.1.1). At the UBC Greenhouse, treatment effects were more variable (i.e., greater range of flowering times among treatments) than at Westcan Greenhouse. The control plants flowered earliest (approximately 14 weeks) while plants treated with Ethephon 1000 ppm flowered latest (approximately 17.5 weeks). Plants from all other treatments flowered either during week 15 or week 16 (Table 3.1.2).

Treatment of either GA (e.g., Fascination at 10 or 15 ppm $GA_{4,7}$ and 6BA) or anti-GA (e.g., B-Nine at 2500 or 3500 ppm daminozide) compounds had similar effects on flowering times (i.e., statistically equivalent) for most genotypes. Specifically, at Westcan, all plants within a genotype were statistically equivalent for both GA and anti-GA treatments (in relation to the same genotype from different treatments) for mean weeks to anthesis (Fig. 3.1.1). At

UBC, four of six genotypes were statistically equivalent for GA and anti-GA treatments for mean weeks to anthesis (Fig. 3.1.2).

3.1.3 Genotype effects

Summarised across treatments, several genotypes consistently flowered significantly earlier (mean weeks to anthesis) than other genotypes. When pooled across treatments conducted at Westcan Greenhouse, genotype 01-37-61 flowered the earliest (10 weeks) whereas genotype 01-37-37 flowered the latest (18 weeks) while all other genotypes flowered between weeks 12 and 17. At the UBC Greenhouse, genotype 01-37-8 flowered the earliest (11 weeks) whereas genotype 01-9-1 flowered the latest (18 weeks) while all other genotypes flowered between weeks 14 and 17.

3.1.4 Genotype x treatment interaction

Due to the significant interaction between genotype and treatment, the main effects described above do not give a full understanding of the impacts of PGR treatments on flowering of various *Exacum* Styer Group genotypes. For example, the effect of PGR treatments on the time to anthesis for different genotypes for both locations are presented (Figs. 3.1.1 and 3.1.2). It appears that later flowering genotypes (i.e., 01-37-37 and 01-42-3) were less impacted by PGR treatments while earlier flowering genotypes (i.e., 01-37-8, 01-37-50 and 01-37-61) were more impacted; typically by a delay in flowering. The greatest impact was from the Ethephon 1000 ppm treatment on genotype 01-37-8 (Table 3.1.4 and Fig. 3.1.2) where flowering was delayed by seven weeks as compared to the control.

It appears that the basis for the significant interactions arise from the change in rank order of genotypes and treatments across locations. The ranking of genotypes at Westcan Greenhouse was relatively constant across treatments (though still dependent on genotype confirming the

interaction) while ranking of genotypes at UBC Greenhouse was variable depending on treatment. At Westcan Greenhouse, genotype 01-37-61 flowered the earliest for all treatments, though genotype 01-37-50 was statistically equal in response, while genotype 01-37-37 flowered latest for all treatments. At the UBC Greenhouse, genotype 01-37-8 flowered the earliest for all treatments while the latest flowering genotype was dependent on treatment; 01-9-1 flowered the latest for the Ethephon (500 ppm) and GA (10 and 15 ppm) treatments; 01-42-3 flowered the latest for the control and Daminozide (2500 ppm) treatments; 01-48-10 flowered the latest for the Ethephon (1000 ppm) and Daminozide (3500 ppm) treatments. However, most of the late flowering genotypes, regardless of treatment, were statistically equivalent for weeks to anthesis. Overall, whether genotype ranking was consistent across treatments or not, genotype appeared to be more influential on time to flower than treatment.

The PGR experiment conducted at Westcan Greenhouse revealed several trends for genetic families (i.e., siblings from a common parental combination). Specifically, the siblings from family 01-47 (01-47-21 and 01-47-49) behaved similarly (statistically equivalent) to PGR treatments, while siblings from family 01-37 (01-37-37, 01-37-50 and 01-37-61) exhibited significant variation. Two of the three 01-37 genotypes (01-37-50 and 01-37-61) were ranked with the first and second shortest time to flower across all treatments (excluding 01-37-50 for daminozide 2500 ppm which was third fastest) while the third sibling (01-37-37) had the longest time to flower across all treatments.

3.1.5 Location x genotype x treatment interaction

Rank of genotypes across treatments, based on mean weeks to anthesis, revealed differences both between locations and among genotypes used at each location (Table 3.1.6). Overall, genotypes (with the exception of 01-47-21) treated at Westcan Greenhouse remained in approximately the same rank order for flowering time in response to the different PGR

treatments. However, genotypes treated at UBC Greenhouse exhibited significantly more variation when ranked for flowering time in response to the different PGR treatments with genotypes 01-42-3 and 01-48-10 displaying the most variation across treatments. While these differences may reflect inherent variation in the locations or seasons when the experiments were conducted, it may also indicate that some genotypes (i.e., those used at UBC Greenhouse versus those used at Westcan Greenhouse) were simply more variable in their flowering response following the various PGR treatments in comparison to the control.

Since only two genotypes (01-42-3 and 01-47-49) were included in both locations, only those genotypes were used to examine the interaction among location, genotype and treatment for mean weeks to anthesis (Table 3.1.7). When location x treatment groups were ranked by genotype, it appears that location had greater influence on time to flower than treatment for these genotypes. If location was not significant, any given treatment pair (i.e., from each location) should appear together (or in close proximity). However, this was not the case. For example, location was clearly significant for genotype 01-47-49 as the five earliest flowering treatments were from Westcan Greenhouse and the six latest flowering treatments were from the UBC Greenhouse. Furthermore, the time to flower rank orders for treatments for each location were not consistent for either of these genotypes. Therefore, the locations were not comparable, likely due to environmental and/or seasonal differences.

3.2 Temperature Experiments

3.2.1 Deviations from experimental design

The two temperature experiments conducted were intended to be replicates. Since season was not known to be a factor, the only difference between the experiments that was intentional was the elimination of the coldest treatment (16° C DT/12° C NT) and the addition of a warmer treatment (35° C DT/31° C NT). However, more differences arose between the

experiments as the summer experiment progressed. First, many plants from three genotypes (01-37-50, 01-42-3 and 02-174-9) had visible buds before plants were moved to growth chambers and the commencement of treatments. In addition, within the first three weeks of treatment, more plants from these genotypes set bud along with some plants from genotype 01-47-21. For these plants, initiation occurred before treatments commenced. This may indicate that these plants have an internal clock as they were propagated in an artificial environment with no known link to season (i.e., tissue culture laboratory). With the objective to investigate the effect of temperature on flower induction and development, it would be naïve to consider plants which budded before the commencement of treatments to be replicates for plants which had not set bud before treatment. For these reasons, the two experiments do not fulfill the requirements of replicates and will not be referred to as such.

Besides the differences described above, the results presented for the temperature experiments deviate from the experimental design outlined in Chapter 2 in three ways. First, the growth chamber in which the repeated 30° C DT/26° C NT treatment was conducted malfunctioned during week five and all plants within died. It was unclear exactly why the plants died but thermometers in the chamber indicated a brief temperature increase to 40° C. Therefore, this treatment (30° C DT/26° C NT – summer season) was excluded from the results. Second, across all temperature treatments, one genotype (01-50-46) of the six responded poorly. Specifically, very few plants reached anthesis in the 23° C DT/19° C NT treatment and only four plants (12% of total) flowered during the winter and only five plants (15% of total) flowered during the summer. Furthermore, all 01-50-46 plants in the 35° C DT/31° C NT treatment died before producing visible buds. This is the only genotype that experienced a mortality rate greater than 20% from any treatment. For these reasons, genotype 01-50-46 was excluded from all analyses. However, summary data for 01-50-46 are presented in Appendix C. Third, biomass measurements, which were collected for the winter season only, are not presented here as the

results did not indicate significant differences. However, these data are presented in Appendix D. In addition, root and shoot ratings for the summer temperature experiment are presented in Appendix E.

3.2.2 Overview of effects

As with the PGR experiments, the genotypes included in the temperature experiments did not behave uniformly. ANOVA revealed a significant interaction between genotype and treatment for all metrics for the winter temperature experiment (Table 3.2.1). Furthermore, the highest temperature treatment (35° C DT/31° C NT) prevented flowering for three (01-37-37, 01-42-3 and 01-47-21) of five genotypes. For this reason, a factorial ANOVA could not be performed as planned. Instead, subsequent ANOVA only included the successfully repeated temperature treatment (23° C DT/19° C NT) (Table 3.2.2) and only plants from the two genotypes (01-37-50 and 02-174-9) that did flower (Table 3.2.3). Individual effects of temperature on the time to anthesis across genotypes are presented (Figs. 3.2.1 and 3.2.2). Analysis Type III Sums of Squares decomposition (sums of squares, degrees of freedom, mean squares, and F and *P* values) is presented in Appendix A. A brief description of additional observations of treatment effects (i.e., treatment induced symptoms) are presented in Appendix B. Photographs of representative plants (closest to average reproductive stage, node, height and general appearance) for individual effects (week 19 of the winter experiment) are also presented (Fig. 3.2.3). The observed variation within a genotype x treatment combination is presented as photographs (Fig. 3.2.4) for individual effects (week 18 of the summer experiment).

3.2.3 Temperature treatment effects

Two temperature treatments prevented flowering for some or all genotypes**.** The lowest temperature treatment, 16° C DT/12° C NT, prevented flowering for all plants and therefore is

not included in analysis of days to anthesis. As stated above, the highest temperature treatment, 35° C DT/31° C NT, prevented flowering for three of five genotypes. In contrast to the PGR experiments, many genotype x treatment groups did not reach anthesis or included plants at various reproductive stages (i.e., pre-anthesis) at the conclusion of the experiments (week 18 or week 23, for winter and summer, respectively). For treatments that did include flowering plants for all genotypes for the winter season, on average the warmer treatment (30° C DT/26° C NT; 93 days to anthesis) promoted earlier flowering than the colder treatment (23° C DT/19° C NT; 120 days to anthesis) when pooled across genotypes.

3.2.4 Genotype effects

Similar to the genotype effects from the PGR experiments, plants of some genotypes flowered earlier than others. When analyzed across treatments for the winter season, genotypes 01-42-3 and 02-174-9 flowered earliest (mean 90 days or approximately 13 weeks to anthesis) while plants from genotype 01-37-37 flowered the latest (mean 122 days or approximately 17 weeks to anthesis). Mean days to anthesis for the remaining genotypes were 98 (01-37-50) and 118 (01-47-21) or approximately 14 or 17 weeks, respectively.

3.2.5 Genotype x treatment effects

Due to the significant interaction between genotype and treatment for the winter season, the description above does not reveal the complete effect of temperature on flowering of *Exacum* Styer Group genotypes. When genotypes are ranked by time to flower, the order is not consistent across temperature treatments. For the winter season, one genotype (01-47-21) flowered later in the warmer treatment (30° C DT/26° C NT) whereas all other genotypes flowered later in the colder treatment (23° C DT/19° C NT). Also, genotype 01-47-21 had the weakest propagules (compared to all other genotypes) for the winter season and therefore may

not have been able to respond to the warmer temperature (i.e., faster growth) as quickly as the other genotypes. Unfortunately, due to chamber malfunction, this cannot be confirmed by the summer temperature experiment as no data were collected from the 30° C DT/26° C NT treatment.

Since plants from only two genotypes flowered in both temperature treatments in the summer season, only those genotypes were analyzed for temperature effects. Summaries of these effects are presented (Table 3.2.3). There was a significant interaction ($P \leq 0.05$) between genotype and temperature for weeks to anthesis but treatment alone was not significant. Genotypes flowered at statistically equivalent times for both treatments, though 01-37-50 flowered earlier at 35° C DT/31° C NT than at 23° C DT/19° C NT (72 versus 89 days, respectively) while 02-174-9 flowered earlier at 23° C DT/19° C NT than at 35° C DT/31° C NT (52 versus 61 days, respectively). Since the warmest treatment prevent flowering of three genotypes (01-37-37, 01-42-3 and 01-47-21) and did not result in faster flowering for 02-174-9, this treatment (35° C DT/31° C NT) promoted faster flowering for only one of five genotypes.

3.2.6 Season x genotype effects

Data from both seasons were compared for the 23° C DT/19° C NT treatment and indicate a seasonal effect. ANOVA revealed a significant interaction between temperature and season (Table 3.2.2) and therefore prevents pooling the repeated treatments. Nonetheless, it is interesting to note that the genotype with weak propagules from the winter season (01-47-21) flowered dramatically earlier during the summer season (69 days versus 112 days). However, all other genotypes also flowered significantly earlier in the summer season compared to the winter season, thus confirming that season did have a significant effect.

3.2.7 Reproductive stage and node number

As stated above, the coldest treatment (16° C DT/12 ° C NT) prevented flowering for all genotypes, the warmest treatment (35° C DT/31° C NT) prevented flowering for three of five genotypes, and many genotype x treatment groups included plants at various reproductive stages at the conclusion of the experiments. To more clearly report these effects, summaries of these data (i.e., percent plants at each reproductive stage, percent mortality, and average node number) are presented (Table 3.2.4). For the lowest temperature treatment, lack of flowering was linked to reduced growth rate (i.e., low node count relative to flowering plants from other treatments). In contrast, the highest temperature treatment promoted plant growth (i.e., high node count relative to flowering plants from other treatments) but prevented the transition to reproduction. Overall, the coldest treatment prevented flowering and growth for all genotypes while the warmest treatment prevented flowering for some genotypes while promoting vegetative growth.

Table 3.1.1. Main and significant effects of plant growth regulator treatments applied to six *Exacum* Styer Group genotypes on weeks to visible bud (VB), macrobud (MB), anthesis (AN), and weeks from visible bud to anthesis at Westcan Greenhouse.

	Weeks to VB	Weeks to MB	Weeks to AN	Weeks from VB to AN
		Main effects		
Genotype				
01-37-37	14.10	17.12	18.89	4.80
01-37-50	7.35	10.35	12.13	4.77
01-37-61	5.73	8.43	10.33	4.60
01-42-3	12.07	15.00	16.97	4.79
01-47-21	9.54	13.12	14.69	5.15
01-47-49	8.45	13.15	14.83	6.38
Treatment				
Control	10.02	13.74	15.46	5.37
Ethephon 500 ppm	10.20	13.85	15.59	5.39
Ethephon 1000 ppm	11.18	14.76	16.63	5.45
Daminozide 2500 ppm	10.74	13.91	15.59	4.85
Daminozide 3500 ppm	10.13	13.30	15.05	4.93
$GA_{4,7}$ 10 ppm	9.88	13.53	15.28	5.40
$GA_{4,7}$ 15 ppm	9.90	13.60	15.45	5.55
		Significant effects		
Source of Variation				
Genotype (G)	***	***	***	***
Treatment (T)	***	***	***	NS
G x T	**	***	**	**

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

Table 3.1.2. Main and significant effects of plant growth regulator treatments applied to *Exacum* Styer Group genotypes on weeks to visible bud (VB), macrobud (MB), anthesis (AN), weeks from visible bud to anthesis, plant height and number of nodes to first bud, height/node ratio, and number of reproductive branches at anthesis at UBC Greenhouse.

	Weeks to VB	Weeks to MB	Weeks to AN	Weeks from VB to AN	Height (cm)	Node (#)	Height/Node Ratio	Number of Reproductive Branches
				Main effects				
Genotype								
01-9-1	12.10	15.10	18.06	5.96	21.26	16.31	1.30	15.43
01-37-8	5.00	8.70	11.17	6.17	13.29	11.31	1.18	8.07
01-42-3	11.19	14.56	17.41	6.22	23.71	16.74	1.42	14.37
01-47-49	11.67	14.37	17.04	5.37	22.20	16.19	1.38	15.33
01-48-10	10.88	13.52	16.60	5.71	17.14	14.23	1.22	13.67
02-174-9	10.07	12.50	14.84	4.77	18.09	14.11	1.29	13.34
Treatment								
Control	9.00	11.58	14.27	5.27	20.66	14.79	1.40	11.23
Ethephon 500 ppm	10.13	13.41	16.33	6.20	16.60	14.30	1.15	11.46
Ethephon 1000 ppm	11.23	14.95	17.50	6.27	13.63	12.84	1.07	14.39
Daminozide 2500 ppm	10.91	13.53	16.14	5.23	19.94	15.93	1.24	14.14
Daminozide 3500 ppm	11.04	13.96	16.65	5.61	18.18	15.70	1.15	14.52
$GA_{4,7}$ 10 ppm	9.38	12.28	15.04	5.66	23.01	15.15	1.52	14.15
$GA_{4,7}$ 15 ppm	9.38	12.19	15.00	5.62	22.47	14.87	1.52	13.72
				Significant effects				
Source of Variation								
Genotype (G)	***	***	***	***	***	***	***	***
Treatment (T)	***	***	***	***	***	***	***	***
G x T	***	***	***	***	***	***	***	***

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

Table 3.1.3. Individual effects of plant growth regulator treatments applied to six *Exacum* Styer Group genotypes on weeks to visible bud (VB), macrobud (MB), anthesis (AN), and weeks from visible bud to anthesis at Westcan Greenhouse.

		Weeks to VB	Weeks to MB	Weeks to AN	Weeks from VB to AN
			Individual Effects		
Genotype	Treatment				
01-37-37	Control	13.67	16.73	18.47	4.80
	Ethephon 500 ppm	14.46	17.46	19.38	4.92
	Ethephon 1000 ppm	14.42	17.00	19.17	4.75
	Daminozide 2500 ppm	14.07	16.93	18.50	4.43
	Daminozide 3500 ppm	14.31	17.38	19.08	4.77
	$GA_{4,7}$ 10 ppm	13.46	17.23	18.92	5.46
	$GA_{4,7}$ 15 ppm	14.38	17.15	18.85	4.46
01-37-50	Control	8.00	10.00	11.80	3.80
	Ethephon 500 ppm	7.20	10.00	12.20	5.00
	Ethephon 1000 ppm	8.50	13.25	15.00	6.50
	Daminozide 2500 ppm	9.20	12.20	13.80	4.60
	Daminozide 3500 ppm	7.00	9.50	10.75	3.75
	$GA_{4,7}$ 10 ppm	5.00	8.50	10.25	5.25
	$GA_{4,7}$ 15 ppm	6.00	8.75	10.75	4.75
01-37-61	Control	5.00	8.20	9.60	4.60
	Ethephon 500 ppm	5.50	9.25	11.25	5.75
	Ethephon 1000 ppm	8.50	11.25	12.50	4.00
	Daminozide 2500 ppm	5.00	7.40	10.00	5.00
	Daminozide 3500 ppm	5.00	7.50	9.75	4.75
	$GA_{4,7}$ 10 ppm	6.00	8.25	10.25	4.25
	$GA_{4,7}$ 15 ppm	5.50	7.50	9.25	3.75
01-42-3	Control	13.33	16.00	18.33	4.00
	Ethephon 500 ppm	12.00	14.50	17.00	5.00
	Ethephon 1000 ppm	12.00	14.50	17.00	5.00
	Daminozide 2500 ppm	11.60	14.80	16.60	5.00
	Daminozide 3500 ppm	12.67	15.67	17.67	5.00
	$GA_{4,7}$ 10 ppm	11.60	15.00	16.20	4.60
	$GA_{4,7}$ 15 ppm	12.00	15.00	16.80	4.80
01-47-21	Control	10.50	14.00	15.25	4.75
	Ethephon 500 ppm	10.25	13.75	15.00	4.75
	Ethephon 1000 ppm	13.50	15.50	18.50	5.00
	Daminozide 2500 ppm	8.75	12.50	13.75	5.00
	Daminozide 3500 ppm	7.00	10.25	12.00	5.00
	$GA_{4,7}$ 10 ppm	10.00	13.75	15.50	5.50
	$GA_{4,7}$ 15 ppm	8.75	13.25	14.75	6.00
01-47-49	Control	7.79	13.29	15.07	7.29
	Ethephon 500 ppm	7.55	12.82	13.91	6.36
	Ethephon 1000 ppm	9.08	14.17	15.58	6.50
	Daminozide 2500 ppm	10.23	13.92	15.46	5.23
	Daminozide 3500 ppm	8.75	12.50	14.25	5.50
	$GA_{4,7}$ 10 ppm	8.31	12.38	14.31	6.00
	$GA_{4,7}$ 15 ppm	7.33	12.92	15.08	7.75

Table 3.1.4. Individual effects of plant growth regulator treatments applied to *Exacum* Styer Group genotypes on weeks to visible bud (VB), macrobud (MB), anthesis (AN), weeks from visible bud to anthesis, plant height and number of nodes to first bud, height/node ratio, and number of reproductive branches at anthesis at UBC Greenhouse.

		Weeks to VB	Weeks to MB	Weeks to AN	Weeks from VB to AN	Height (cm)	Node (#)	Height/Node Ratio	Number of Reproductive Branches
					Individual effects				
Genotype	Treatment								
01-9-1	Control	10.25	12.25	15.25	5.00	22.25	16.63	1.35	12.63
	Ethephon 500 ppm	11.57	14.71	17.71	6.14	16.43	13.71	1.20	10.57
	Ethephon 1000 ppm	13.00	16.00	18.57	5.57	14.71	14.14	1.04	14.57
	Daminozide 2500 ppm	12.71	15.57	18.57	5.86	24.43	17.14	1.43	16.71
	Daminozide 3500 ppm	11.00	15.29	17.57	6.57	19.07	16.29	1.18	14.71
	$GA_{4,7}$ 10 ppm	13.57	16.57	20.29	6.71	27.00	19.43	1.40	20.57
	$GA_{4,7}$ 15 ppm	12.75	15.63	18.75	6.00	24.38	16.75	1.47	18.25
01-37-8	Control	5.00	7.00	9.00	4.00	14.13	10.50	1.34	7.38
	Ethephon 500 ppm	5.00	11.00	13.86	8.86	12.71	11.29	1.13	6.43
	Ethephon 1000 ppm	5.00	13.00	16.00	11.00	10.63	11.13	0.96	8.63
	Daminozide 2500 ppm	5.00	7.00	9.00	4.00	11.81	12.00	0.99	9.25
	Daminozide 3500 ppm	5.00	7.50	9.50	4.50	11.56	11.38	1.02	8.13
	$GA_{4,7}$ 10 ppm	5.00	7.75	10.00	5.00	15.38	11.50	1.33	8.63
	$GA_{4,7}$ 15 ppm	5.00	7.86	11.14	6.14	17.21	11.43	1.51	7.86
01-42-3	Control	10.50	15.25	17.50	7.00	24.44	16.25	1.50	12.25
	Ethephon 500 ppm	11.00	13.00	16.00	5.00	21.00	15.75	1.34	11.50
	Ethephon 1000 ppm	11.00	13.00	16.00	5.00	14.07	14.29	0.98	15.14
	Daminozide 2500 ppm	12.75	16.00	19.00	6.25	23.94	18.63	1.29	16.00
	Daminozide 3500 ppm	12.14	15.14	18.57	6.43	23.57	18.00	1.31	16.43
	$GA_{4,7}$ 10 ppm	10.75	14.88	17.50	6.75	31.00	17.00	1.83	15.25
	$GA_{4,7}$ 15 ppm	10.25	14.50	17.25	7.00	26.75	17.13	1.57	14.38

Table 3.1.4 continued. Individual effects of plant growth regulator treatments applied to *Exacum* Styer Group genotypes on weeks to visible bud (VB), macrobud (MB), anthesis (AN), weeks from visible bud to anthesis, plant height and number of nodes to first bud, height/node ratio, and number of reproductive branches at anthesis at UBC Greenhouse.

		Weeks to VB	Weeks to MB	Weeks to AN	Weeks from VB to AN	Height (cm)	Node (#)	Height/Node Ratio	Number of Reproductive Branches
					Individual effects				
Genotype	Treatment								
01-47-49	Control	11.00	13.00	16.00	5.00	25.88	17.13	1.52	13.75
	Ethephon 500 ppm	12.50	15.25	17.50	5.00	22.25	16.88	1.32	15.13
	Ethephon 1000 ppm	13.00	16.00	18.33	5.33	14.75	14.33	1.06	13.83
	Daminozide 2500 ppm	12.75	16.00	18.75	6.00	22.69	16.75	1.37	15.63
	Daminozide 3500 ppm	12.75	15.63	17.75	5.00	19.69	16.00	1.22	18.50
	$GA_{4,7}$ 10 ppm	10.25	13.00	16.00	5.75	24.81	16.63	1.50	15.38
	$GA_{4,7}$ 15 ppm	9.75	12.13	15.25	5.50	23.50	15.13	1.55	14.75
01-48-10	Control	8.50	11.00	14.88	6.38	17.38	14.25	1.23	10.38
	Ethephon 500 ppm	11.25	13.38	16.75	5.50	13.94	13.63	1.03	13.50
	Ethephon 1000 ppm	13.00	16.25	19.00	6.00	13.19	11.75	1.13	17.38
	Daminozide 2500 ppm	12.50	15.25	18.50	6.00	16.13	15.75	1.02	13.25
	Daminozide 3500 ppm	14.50	17.13	20.13	5.63	17.81	16.88	1.06	15.13
	$GA_{4,7}$ 10 ppm	8.25	11.00	14.13	5.88	19.94	13.38	1.52	12.63
	$GA_{4,7}$ 15 ppm	9.00	11.50	13.75	4.75	21.13	14.75	1.46	13.25
02-174-9	Control	8.75	11.00	13.00	4.25	19.88	14.00	1.42	11.00
	Ethephon 500 ppm	9.00	13.00	16.00	7.00	12.75	14.13	0.90	10.88
	Ethephon 1000 ppm	13.00	15.63	17.25	4.25	14.88	12.13	1.23	16.75
	Daminozide 2500 ppm	10.75	12.50	14.50	3.75	19.31	15.38	1.25	13.88
	Daminozide 3500 ppm	11.00	13.38	16.75	5.75	18.19	16.00	1.14	14.50
	$GA_{4,7}$ 10 ppm	9.00	11.00	13.00	4.00	20.44	13.50	1.51	13.25
	$GA_{4,7}$ 15 ppm	9.00	11.00	13.38	4.38	21.19	13.63	1.55	13.13

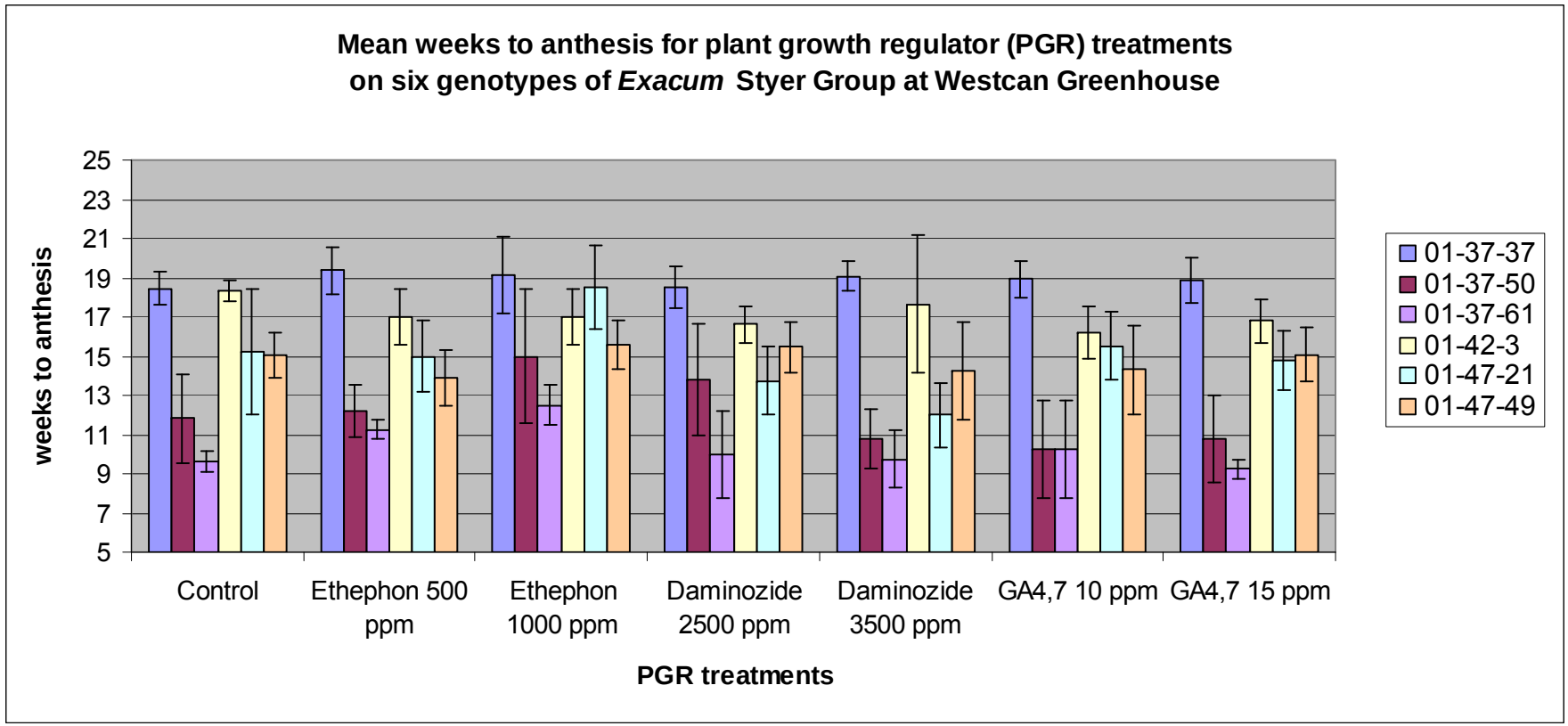

Figure 3.1.1. Mean weeks to anthesis for plant growth regulator treatments on six genotypes of Exacum Styer Group at Westcan Greenhouse.
Error bars = standard deviation

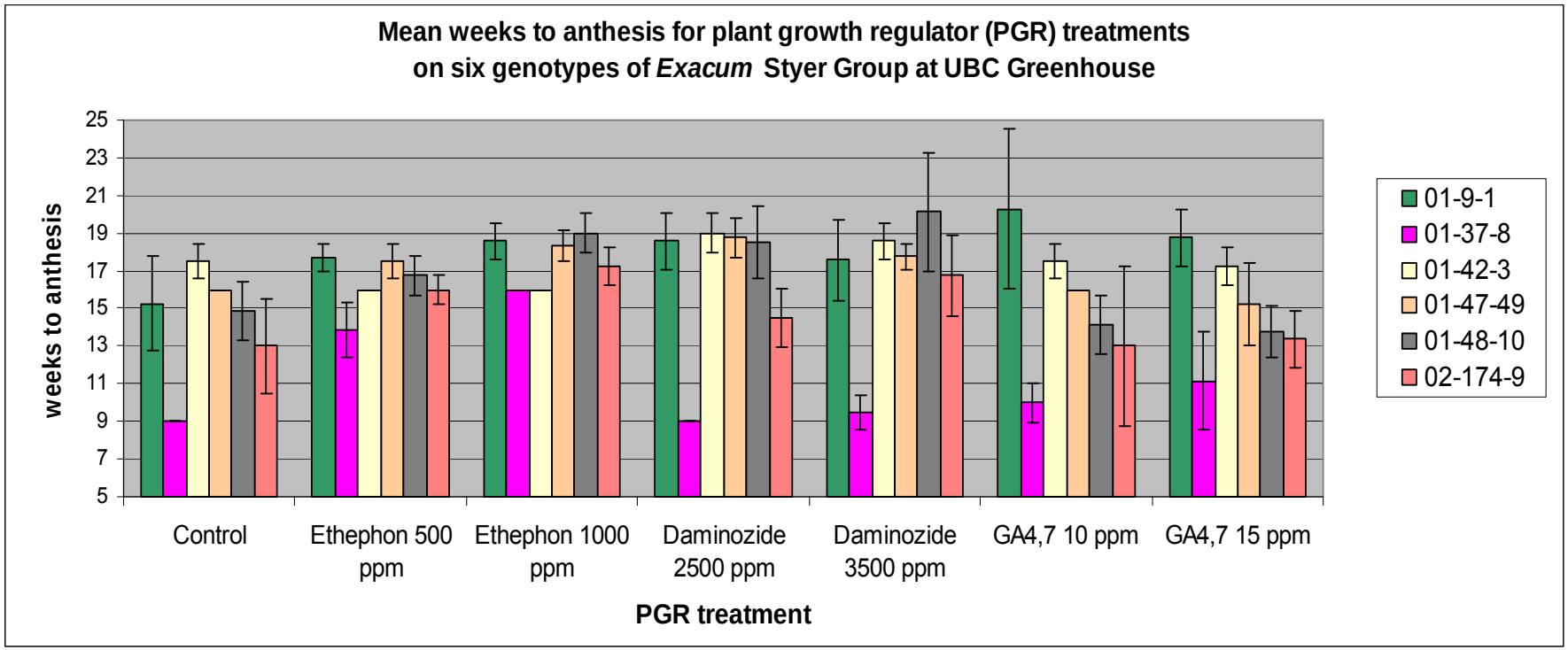

Figure 3.1.2. Mean weeks to anthesis for plant growth regulator treatments on six genotypes of Exacum Styer Group at the UBC Greenhouse.
Error bars = standard deviation

Fig. 3.1.3. Photographs of representative plants (i.e., closest to average reproductive stage, number of nodes, height and general appearance) from each genotype x treatment group for the PGR experiment conducted at the UBC Greenhouse taken on October 24, 2006 (week 20). Ruler = 40cm

Fig. 3.1.3 continued. Photographs of representative plants (i.e., closest to average reproductive stage, number of nodes, height and general appearance) from each genotype x treatment group for the PGR experiment conducted at the UBC Greenhouse taken on October 24, 2006 (week 20). Ruler = 40cm.

Table 3.1.5. Type III Sums of Squares decomposition and significant effects of plant growth regulator treatments applied to two *Exacum* Styer Group genotypes (01-42-3 and 01-47-49) in two locations (Westcan Greenhouse and UBC Greenhouse) on weeks to anthesis (AN)

Effect	Sums of Squares	Degrees of Freedom	Mean Squares	F value	*P* value	Significance
Intercept	51900.79	1	51900.79	27710.44	0.000000	***
Genotype (G)	79.37	1	79.37	42.38	0.000000	***
Location (L)	79.08	1	79.08	42.22	0.000000	***
Treatment (T)	52.95	6	8.82	4.71	0.000167	***
G x L	45.09	1	45.09	24.07	0.000002	***
G x T	38.33	6	6.39	3.41	0.003180	**
L x T	41.22	6	6.87	3.67	0.001783	**
G x L x T	33.47	6	5.58	2.98	0.008321	**
Error	367.10	196	1.87			

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

Table 3.1.6. Rank of mean weeks to anthesis (AN) by treatment for all genotypes for two locations (Westcan Greenhouse and UBC Greenhouse) of plant growth regulator experiments.

Control		Ethephon 500 ppm		Ethephon 1000 ppm		Daminozide 2500 ppm		Daminozide 3500 ppm		$GA_{4,7}$ 10 ppm		$GA_{4,7}$ 15 ppm	
Genotype	Weeks to AN	Genotype	Weeks to AN	Genotype	Weeks to AN	Genotype	Weeks to AN	Genotype	Weeks to AN	Genotype	Weeks to AN	Genotype	Weeks to AN
Westcan													
01-37-61	9.60	01-37-61	11.25	01-37-61	12.50	01-37-61	10.00	01-37-61	9.75	01-37-61	10.25	01-37-61	9.25
01-37-50	11.80	01-37-50	12.20	01-37-50	15.00	01-47-21	13.75	01-37-50	10.75	01-37-50	10.25	01-37-50	10.75
01-47-49	15.07	01-47-49	13.91	01-47-49	15.58	01-37-50	13.80	01-47-21	12.00	01-47-49	14.31	01-47-21	14.75
01-47-21*	15.25	01-47-21	15.00	01-42-3	17.00	01-47-49	15.46	01-47-49	14.25	01-47-21	15.50	01-47-49	15.08
01-42-3	18.33	01-42-3	17.00	01-47-21	18.50	01-42-3	16.60	01-42-3	17.67	01-42-3	16.20	01-42-3	16.80
01-37-37	18.47	01-37-37	19.38	01-37-37	19.17	01-37-37	18.50	01-37-37	19.08	01-37-37	18.92	01-37-37	18.85
UBC													
01-37-8	9.00	01-37-8	13.86	01-37-8	16.00	01-37-8	9.00	01-37-8	9.50	01-37-8	10.00	01-37-8	11.14
02-174-9	13.00	01-42-3	16.00	01-42-3	16.00	02-174-9	14.50	02-174-9	16.75	02-174-9	13.00	02-174-9	13.38
01-48-10*	14.88	02-174-9	16.00	02-174-9	17.25	01-48-10	18.50	01-9-1	17.57	01-48-10	14.13	01-48-10	13.75
01-9-1	15.25	01-48-10	16.75	01-47-49	18.33	01-9-1	18.57	01-47-49	17.75	01-47-49	16.00	01-47-49	15.25
01-47-49	16.00	01-47-49	17.50	01-9-1	18.57	01-47-49	18.75	01-42-3	18.57	01-42-3	17.50	01-42-3	17.25
01-42-3*	17.50	01-9-1	17.71	01-48-10	19.00	01-42-3	19.00	01-48-10	20.13	01-9-1	20.29	01-9-1	18.75

*Highlighted genotypes show the most variation in rank across treatments

Table 3.1.7. Rank of mean weeks to anthesis (AN) by genotype for plant growth regulator treatments at two locations (Westcan Greenhouse and UBC Greenhouse).

Genotype	Location	Treatment	Weeks to AN	Genotype	Location	Treatment	Weeks to AN
01-42-3	UBC	Ethephon 500 PPM	16.00	01-47-49	Westcan	Ethephon 500 PPM	13.91
	UBC	Ethephon 1000 PPM	16.00		Westcan	Daminozide 3500 PPM	14.25
	Westcan	$GA_{4,7}$ 10 ppm	16.20		Westcan	$GA_{4,7}$ 10 ppm	14.31
	Westcan	Daminozide 2500 PPM	16.60		Westcan	Control	15.07
	Westcan	$GA_{4,7}$ 15 ppm	16.80		Westcan	$GA_{4,7}$ 15 ppm	15.08
	Westcan	Ethephon 500 PPM	17.00		UBC	$GA_{4,7}$ 15 ppm	15.25
	Westcan	Ethephon 1000 PPM	17.00		Westcan	Daminozide 2500 PPM	15.46
	UBC	$GA_{4,7}$ 15 ppm	17.25		Westcan	Ethephon 1000 PPM	15.58
	UBC	Control	17.50		UBC	Control	16.00
	UBC	$GA_{4,7}$ 10 ppm	17.50		UBC	$GA_{4,7}$ 10 ppm	16.00
	Westcan	Daminozide 3500 PPM	17.67		UBC	Ethephon 500 PPM	17.50
	Westcan	Control	18.33		UBC	Daminozide 3500 PPM	17.75
	UBC	Daminozide 3500 PPM	18.57		UBC	Ethephon 1000 PPM	18.33
	UBC	Daminozide 2500 PPM	19.00		UBC	Daminozide 2500 PPM	18.75

Table 3.2.1. Main, individual and significant effects of temperature treatments applied to five *Exacum* Styer Group genotypes on days to visible bud (VB), macrobud (MB), anthesis (AN), days from visible bud to anthesis, plant height and number of nodes to first bud, height/node ratio, number of reproductive branches and number of flowers and MBs at anthesis during the winter.

		Days to VB	Days to MB	Days to AN	Days from VB to AN	Height (cm)	Node (#)	Height/ Node Ratio	Number of Reproductive Branches	Number of Flowers and MBs
					Main effects					
Genotype										
01-37-37		90.83	108.15	121.73	30.90	28.05	19.39	1.45	14.73	64.24
01-37-50		56.88	82.63	98.00	41.13	11.42	12.59	0.84	10.44	50.66
01-42-3		50.73	75.30	89.82	39.09	9.73	12.85	0.74	8.73	45.58
01-47-21		75.63	100.83	117.58	41.96	13.81	15.46	0.89	11.21	46.29
02-174-9		50.86	74.43	90.20	39.34	10.07	12.97	0.75	10.23	40.60
Treatment										
23° C/19° C		76.27	101.35	119.86	43.59	15.11	14.37	0.96	15.19	61.83
30° C/26° C		58.90	80.42	93.32	34.42	15.38	15.12	0.96	8.78	43.10
					Individual effects					
Genotype	Treatment									
01-37-37	23° C/19° C	106.50	126.56	142.17	35.67	28.75	19.28	1.49	20.94	86.89
	30° C/26° C	78.57	93.74	105.74	27.17	27.50	19.48	1.41	9.87	46.52
01-37-50	23° C/19° C	54.22	93.67	113.78	59.56	6.17	10.33	0.58	14.33	61.89
	30° C/26° C	57.91	78.30	91.83	33.91	13.48	13.48	0.94	8.91	46.26
01-42-3	23° C/19° C	50.70	75.30	92.20	41.50	6.50	11.20	0.58	7.60	31.60
	30° C/26° C	50.74	75.30	88.78	38.04	11.13	13.57	0.81	9.22	51.65
01-47-21	23° C/19° C	72.00	91.83	112.33	40.33	11.00	12.92	0.86	13.92	53.67
	30° C/26° C	79.25	109.83	122.83	43.58	16.63	18.00	0.93	8.50	38.92
02-174-9	23° C/19° C	73.50	100.64	121.29	47.79	13.00	14.14	0.89	14.86	58.14
	30° C/26° C	35.76	56.95	69.48	33.71	8.12	12.19	0.66	7.14	28.90
					Significant effects					
Source of Variation										
	Genotype (G)	***	***	***	***	***	***	***	***	***
	Treatment (T)	***	***	***	***	***	***	*	***	***
	G x T	***	***	***	***	***	***	***	***	***

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

Table 3.2.2. Main, individual and significant effects of season for 23° C DT/19° C NT temperature treatments on five *Exacum* Styer Group genotypes on days to visible bud (VB), macrobud (MB), anthesis (AN), days from visible bud to anthesis, plant height and number of nodes to first bud, height/node ratio, number of reproductive branches and number of flowers and MBs at anthesis.

		Days to VB	Days to MB	Days to AN	Days from VB to AN	Height (cm)	Node (#)	Height/ Node Ratio	Number of Reproductive Branches	Number of Flowers and MBs
					Main effects					
Genotype										
01-37-37		100.71	120.21	135.83	35.13	28.40	18.83	1.51	6.34	78.83
01-37-50		33.43	78.96	98.96	65.52	7.09	10.35	0.65	7.59	42.09
01-42-3		33.89	61.22	77.06	43.17	7.19	10.44	0.70	2.18	27.78
01-47-21		49.39	69.16	85.77	36.39	12.52	13.29	0.94	4.47	44.35
02-174-9		36.61	59.97	77.71	41.11	8.53	11.11	0.72	5.47	35.00
Season										
Winter		76.27	101.35	119.86	43.59	15.11	14.37	0.96	8.19	61.83
Summer		26.94	54.10	70.20	43.25	10.34	11.37	0.84	4.44	30.56
					Individual effects					
Genotype	Season									
01-37-37	Winter	106.50	126.56	142.17	35.67	28.75	19.28	1.49	7.25	86.89
	Summer	83.33	101.17	116.83	33.50	27.33	17.50	1.56	1.47	54.67
01-37-50	Winter	54.22	93.67	113.78	59.56	6.17	10.33	0.58	10.07	61.89
	Summer	20.07	69.50	89.43	69.36	7.68	10.36	0.70	4.92	29.36
01-42-3	Winter	50.70	75.30	92.20	41.50	6.50	11.20	0.58	1.90	31.60
	Summer	12.88	43.63	58.13	45.25	8.06	9.50	0.84	2.60	23.00
01-47-21	Winter	72.00	91.83	112.33	40.33	11.00	12.92	0.86	6.72	53.67
	Summer	35.11	54.84	69.00	33.89	13.47	13.53	0.99	2.38	38.47
02-174-9	Winter	73.50	100.64	121.29	47.79	13.00	14.14	0.89	7.45	58.14
	Summer	15.08	36.25	52.29	37.21	5.92	9.33	0.62	1.52	21.50
					Significant effects					
Source of Variation										
	Genotype (G)	***	***	***	***	***	***	***	***	***
	Season (S)	***	***	***	NS	NS	***	NS	**	***
	G x S	***	***	***	**	***	***	***	NS	*

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

Table 3.2.3. Main, individual and significant effects of temperature treatments on two *Exacum* Styer Group genotypes on days to visible bud (VB), macrobud (MB), anthesis (AN), days from visible bud to anthesis, plant height and number of nodes to first bud, height/node ratio, number of reproductive branches and number of flowers and MBs at anthesis during the summer.

		Days to VB	Days to MB	Days to AN	Days from VB to AN	Height (cm)	Node (#)	Height/ Node Ratio	Number of Reproductive Branches	Number of Flowers and MBs
					Main effects					
Genotype										
01-37-50		15.61	62.57	80.75	65.14	6.95	9.89	0.67	7.89	24.39
02-174-9		14.65	37.65	56.23	41.58	5.85	9.05	0.64	7.65	18.67
Treatment										
23° C/19° C		16.92	48.50	65.97	49.05	6.57	9.71	0.65	8.92	24.39
35° C/31° C		12.85	46.30	65.82	52.97	5.95	9.00	0.65	6.39	16.94
					Individual effects					
Genotype	Treatment									
01-37-50	23° C/19° C	20.07	69.50	89.43	69.36	7.68	10.36	0.70	9.29	29.36
	35° C/31° C	11.14	55.64	72.07	60.93	6.21	9.43	0.65	6.50	19.43
02-174-9	23° C/19° C	15.08	36.25	52.29	37.21	5.92	9.33	0.62	8.71	21.50
	35° C/31° C	14.11	39.42	61.21	47.11	5.76	8.68	0.66	6.32	15.11
					Significant effects					
Source of Variation										
	Genotype (G)	NS	***	***	***	NS	*	NS	NS	**
	Treatment (T)	NS	NS	NS	NS	NS	NS	NS	***	***
	G x T	NS	NS	*	*	NS	NS	NS	NS	NS

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

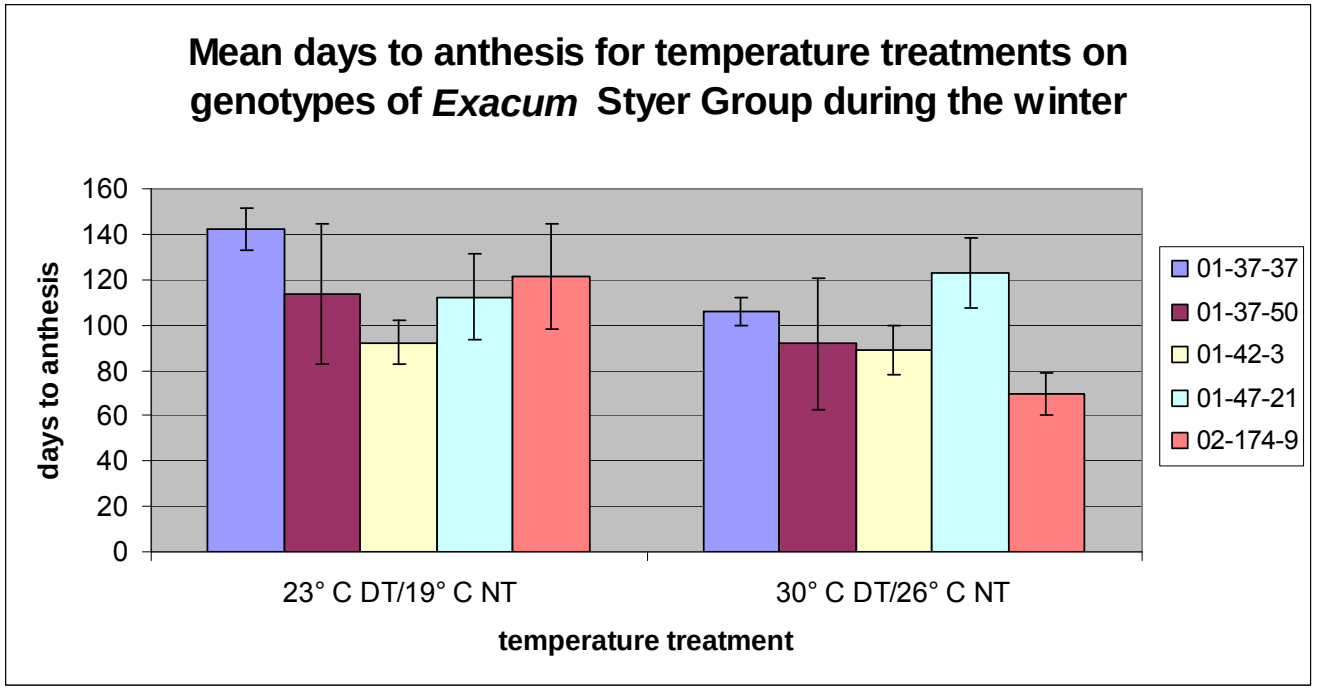

Fig. 3.2.1. Mean days to anthesis for temperature treatments on genotypes of five *Exacum* Styer Group during the winter. Error bars = standard deviation.

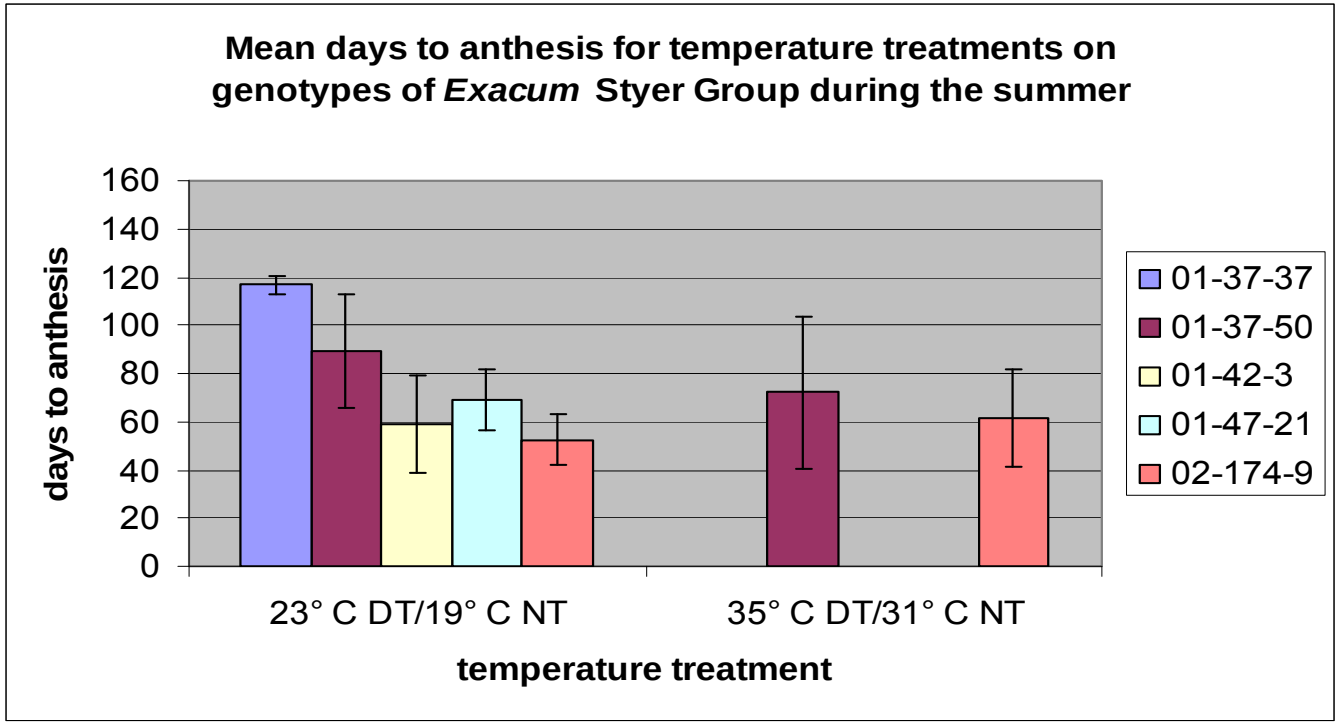

Fig. 3.2.2. Mean days to anthesis for temperature treatments on five genotypes of *Exacum* Styer Group during the summer. Error bars = standard deviation.

Fig. 3.2.3. Photographs of representative plants (i.e., closest to average reproductive stage, number of nodes, height and general appearance) from each genotype x treatment group for the temperature experiment conducted during the winter taken on February 26, 2008 (week 19). Ruler = 40cm. Low temperature is 16° C DT/12 ° C NT, medium temperature is 23° C DT/19° C NT and high temperature is 30° C DT/26° C NT.

Fig. 3.2.4. Photographs of representative plants (i.e., closest to average reproductive stage, number of nodes, height and general appearance) in the middle with extremes on either side from each genotype x treatment group for the temperature experiment conducted during the summer taken on October 2, 2008 (week 18). Ruler = 40cm.

Fig. 3.2.4 continued. Photographs of representative plants (i.e., closest to average reproductive stage, number of nodes, height and general appearance) in the middle with extremes on either side from each genotype x treatment group for the temperature experiment conducted during the summer taken on October 2, 2008 (week 18). Ruler = 40cm.

Table 3.2.4. Effect of temperature treatments during two seasons (winter and summer) on five *Exacum* Styer Group genotypes on percent plants dead or at four reproductive stages; vegetative (VEG); visible bud (VB); macrobud (MB); and anthesis (AN), with corresponding mean number of nodes to first bud at week 18 and standard deviation.

	Dead	VEG		VB		MB		AN		Total Plants
Genotype by Treatment	% plants	% plants	Node	% plants	Node	% plants	Node	% plants	Node	
01-37-37										
16/12 C (W)	9%	91%	11 (0.94)	0%		0%		0%		33
23/19 C (W)	0%	24%	18 (1.83)	48%	20 (0.88)	28%	18 (0.92)	0%		29
23/19 C (S)	0%	12%	20 (0.58)	12%	20 (0.58)	52%	19 (0.93)	24%	18 (1.05)	25
30/26 C (W)	0%	0%		0%		0%		100%	19 (1.04)	23
35/31 C (S)	0%	100%	27 (1.07)	0%		0%		0%		33
01-37-50										
16/12 C (W)	6%	94%	9 (1.54)	0%		0%		0%		33
23/19 C (W)	0%	26%	13 (2.50)	44%	14 (3.92)	7%	13 (3.54)	22%	10 (1.67)	27
23/19 C (S)	0%	0%		25%	11 (5.40)	17%	10 (2.87)	58%	10 (2.34)	24
30/26 C (W)	0%	0%		17%	19 (1.26)	4%	17 (0.00)	79%	12 (2.38)	24
35/31 C (S)	8%	0%		21%	11 (2.92)	13%	8 (0.58)	58%	9 (2.10)	24
01-42-3										
16/12 C (W)	3%	97%	10 (1.37)	0%		0%		0%		33
23/19 C (W)	0%	58%	16 (3.54)	4%	10 (0.00)	0%		38%	11 (0.63)	26
23/19 C (S)	0%	35%	13 (6.06)	27%	13 (4.86)	4%	9 (0.00)	35%	10 (2.54)	26
30/26 C (W)	0%	0%		0%		0%		100%	14 (1.50)	23
35/31 C (S)	4%	14%	30 (1.29)	82%	13 (4.93)	0%		0%		28
01-47-21										
16/12 C (W)	9%	91%	9 (1.30)	0%		0%		0%		33
23/19 C (W)	19%	23%	15 (3.22)	15%	14 (2.08)	8%	13 (2.83)	35%	13 (1.42)	26
23/19 C (S)	0%	9%	24 (0.70)	9%	20 (7.78)	0%		83%	14 (1.92)	23
30/26 C (W)	18%	32%	19 (3.77)	14%	20 (4.20)	14%	18 (1.71)	21%	17 (1.75)	28
35/31 C (S)	15%	79%	30 (4.26)	0%		0%		6%	12 (0.70)	33
02-174-9										
16/12 C (W)	0%	100%	11 (1.04)	0%		0%		0%		33
23/19 C (W)	0%	33%	19 (1.27)	19%	14 (2.34)	22%	16 (1.47)	26%	13 (2.15)	27
23/19 C (S)	0%	4%	10 (0.00)	0%		0%		96%	9 (1.27)	25
30/26 C (W)	0%	9%	24 (0.00)	0%		0%		91%	12 (2.36)	23
35/31 C (S)	13%	0%		0%		4%	11 (0.00)	83%	9 (1.11)	23

(W) = winter season, (S) = summer season

Node = mean number of nodes (standard deviation)

Chapter 4 Discussion

The goal of this research was to generate new information about *Exacum* Styer Group for use in developing commercial production guidelines. Specifically, the control of flowering, both initiation and development, was evaluated in terms of the impacts of various plant growth regulators (PGRs) and temperature. For the experiments, PGR treatments were designed to evaluate only commonly used commercial formulations while the temperature treatments evaluated only commercially viable temperature regimes. Therefore, the scope of this research did not include the full range of PGRs or temperatures commonly used in basic plant physiological research but rather, used a narrower scope concentrating on only commercially viable options.

Throughout the research, significant levels of variation were observed and presented challenges to data interpretation. Sources of variation included genotypic variation where individual genotypes responded differently based on treatments but also individuals within a genotype were variable in response to a given treatment. In addition, variation was present among treatments, locations (and/or season), as well as many significant interactions between main effects. Significant variation within the *E*. Styer Group has been observed before on seed populations indicating that this germplasm has an inherently high level of variation attributable to genetics (Krishnasamy 2007). While plants used for my experiments were asexually propagated (i.e., clones), it is not entirely surprising that variation would arise among genotypes since they are the product of interspecific hybrids from both heterogeneous populations and heterozygous individuals. Furthermore, the taxa from which *E*. Styer Group was developed are native to different environmental (i.e., dry vs. wet) and topographical (i.e., high altitude vs. low elevation) areas (Riseman 1997). Such differences in these native locations would include differences in light (i.e., under story vs. open), temperature, and moisture and may have led to the evolution of distinct flowering control mechanisms. Therefore, the hybrids of which *E*. Styer

Group is compromised may have recombined flowering control mechanisms and may respond differently to external stimuli.

4.1 Plant Growth Regulators and Flowering

The primary objectives of plant growth regulator (PGR) experiments were to examine the impact of these chemicals on flower initiation and development and subsequent effects on plant growth under typical commercial conditions. Therefore, I only used commercial formulations of plant hormones and growth regulators. Gibberellin (GA) has been shown to be involved in the transition from vegetative to reproductive growth. To determine the effect of exogenously applied GA on *E*. Styer Group flowering and development, a commercially available formulation, Fascination® was used. Fascination® is comprised of 1.8 % $GA_{4,7}$ and 1.8% benzyladenine and labelled for use on potted ornamentals to promote internode elongation at concentrations of 1 to 25 ppm $GA_{4,7}$ and 6BA. As stated in Chapter 1, at the concentrations used in this research (10 and 15 ppm), the BA content is considered negligible, based on published reports (Blanchard and Runkle 2008, Carey 2008), so any effect is attributed to the GA. While there is on-going discussion about the exact mode of action of the most commonly used PGRs, daminozide (2,2-dimethyl-hydrazide succinic acid) has been linked to GA (as an anti-GA) so there is a potential impact of daminozide application on flowering. This potential impact was examined by using B-Nine®. Ethylene has also been shown to affect flowering, both promotion and inhibition depending on the species in question. Therefore, applications of ethephon (2-chloroethyl phosphoric acid) were used to examine potential affects of ethylene on flowering. Within plant tissue, ethephon decomposes into ethylene plus chloride and phosphate ions (Gent and McAvoy 2000). The commercial formulation, Ethrel®, was used for these experiments. Using these commercial formulations allowed me to generate data most relevant for commercial growers interested in producing *E.* Styer Group.

It might be expected that GAs and anti-GAs would have different and possibly opposite effects on flowering, however that is not necessarily the case. In my experiments, both GA and anti-GA compounds resulted in similar flowering times with plants either flowering before or after the control plants, depending on concentration and location of experiments (Westcan Greenhouse or UBC Greenhouse). It should be noted that there are over 130 known GAs but experiments reported here used a combination of only two (GA_4 and GA_7). As the mode of action of anti-GA compounds is not exactly known, these compounds may have different interactions with other forms of GA which may help explain the observed similarity in flowering times between GA and anti-GA compounds. Several other anti-GA compounds (i.e., abscisic acid, chlorocholinchloride, and ancymidol) have been tested on *E*. Styer Group genotypes and were found to significantly delay flowering but were applied with a dramatically different application method (injection to apical meristem vs. foliar spray) (Krishnasamy 2007). From research on the related species, *Exacum affine*, application of GA_3 reduced time to flowering (days to five open flowers) (Neumaier 1987). If GA promotes flowering in *E. affine*, I would expect treatment with daminozide to delay or inhibit flowering. Unfortunately, subsequent research using daminozide on *E. affine*, while reporting a reduction in plant height indicating sensitivity of this species to this compound, made no comment concerning secondary effects on flowering (Barrett and Nell 1989). I would speculate that if a significant delay or prohibition of flowering had occurred, they would have reported the observation. Applications of daminozide on *Eustoma grandiflorum,* a species from a closely related genus, delayed flowering at low night temperature (13˚ C) but not at warmer night temperature (18˚ C) (Halevy and Kofranek 1984) indicating that daminozide and temperature significantly impact flowering of a close relative of *E*. Styer Group. Overall, based on my results and previous work, the effect of GA and anti-GA compounds on flowering is dependent on chemical, mode of application and species or genotype.

Examination of genotypic variation and interactions between GA-related PGRs (i.e., GA and anti-GA) and genotype reveal significant effects on flowering. In my research, genotypes did not behave uniformly within treatments resulting in a significant interaction. Working with *E*. Styer Group, Krishnasamy (2007) identified a significant treatment x population interaction from PGR treatments. She concluded that high levels of variation were likely due to the level of heterozygosity present in populations due to their interspecific pedigrees. Unfortunately, many studies examining the effect of GA and anti-GA compounds on flowering of other Gentians (*E. affine* and *E. grandiflorum*) only included one cultivar so genotypic variation was not addressed. However, treatment of three cultivars of *E. grandiflorum* with daminozide did reveal cultivar differences (i.e., different numbers of applications needed to reduce stem height for different cultivars) but an interaction between cultivar and treatment was not reported (Halevy and Kofranek 1984). Nonetheless, evidence remains for significant differences among genotypes of Gentians. Interactions between treatments and genotypes are discussed further in the following sections.

Ethephon treatments, though promoting flowering in some crops, interfered with flower development for Gentians. Ethephon applications in my experiments delayed flowering but only significantly for early flowering genotypes. In addition, the delay observed following ethephon treatments was not due to flower bud abscission, at least not visibly. However, treatments of 1000 ppm ethephon did cause unusual symptoms including severely reduced stem and leaf growth and apparent loss of apical dominance. Therefore, the delay to flowering caused by ethephon may be related to other internal physiological pathways indirectly associated with flowering. To date, no other studies have been conducted to determine the effect of ethephon on flowering of *E*. Styer Group. Research on the effect of ethylene and ethephon on flowering of other Gentians does confirm a negative impact on flower development. Ethylene treatment on *E. affine* plants after anthesis reduced flower number per plant in a post production environment

(Serek and Trolle 2000). This impact of ethylene on flower number was observed for two cultivars for which both experienced a similar reduction in flowers compared to the control indicating no significant genotypic effects (Serek and Trolle 2000). I was unable to identify literature on the effects of ethylene (or ethephon) on flowering of *E. grandiflorum* nor literature which addressed interactions between ethylene and genotype for Gentians. In contrast to GA and anti-GA treatments, ethephon had a significant impact on flowering of *E.* Styer Group as well as *E. affine*. However, ethephon was not shown to prevent flowering.

Overall, results presented here for PGR experiments on ten genotypes of *E.* Styer Group were variable depending on genotype. Genotypes that normally flower earlier were more affected by PGR applications than later flowering genotypes. Earlier flowering genotypes could have been in the process of floral induction or development during the PGR applications. Therefore, the active ingredients may have been more available during this crucial period of transition resulting in a greater treatment effect. For the later flowering genotypes, the treatment timing may have been such that the plants were not at a critical developmental stage when exposed to the compounds, thereby minimizing the effect. These observations could be confirmed by repeating the experiments with PGR applications linked to the time of flowering of the control plants (i.e., application commencement approximately five weeks before visible bud for control plants).

I also observed significant variation among members of genetic families. Siblings from family 01-47 (01-47-21 and 01-47-49) behaved similarly (statistically equivalent) in response to various PGR treatments at Westcan Greenhouse. The only sibling treated at UBC Greenhouse, 01-47-49, flowered later in general (for each treatment) than the same genotype at Westcan Greenhouse. This was likely due to differences in environment (i.e., total irradiance). However, siblings from family 01-37 (01-37-8, 01-37-37, 01-37-50 and 01-37-61) were much more variable in their response to PGR treatments with both the earliest flowering genotypes (01-37-8,

01-37-50 and 01-37-61) and the latest flowering genotype (01-37-37) across all treatments included in this family. This trend cannot be associated with location differences exclusively as only one of the earlier genotypes was treated at the second location. Instead, I conclude this dramatic difference is attributable to the inherent genetic variation among siblings. Put in the context of commercial production, this indicates that a cultivar series based on siblings may not perform with sufficient uniformity for commercial production. However, siblings with desirable traits for a cultivar series could be tested for such variation and selected based on common responses.

The effect of location was analyzed using the two genotypes common to both locations resulting in a significant interaction among location, genotype and treatment. Therefore, the two locations were not comparable. This is likely to due to environmental and/or seasonal effects. The average daily temperature of each location was virtually identical (23.5° C vs. 23.3° C for Westcan Greenhouse and UBC Greenhouse, respectively). However, the total irradiance was potentially different for each location since supplemental lighting was controlled by different minimum ambient levels before lamps turned on. Furthermore, total ambient irradiance based on daylength would also be different for each location as the experiments were conducted during different times of the year. Finally, this observed difference could also be affected by internal plant physiology if the plants have an internal clock regulating flowering. The issue of season/internal clock is examined further in the discussion of the temperature experiments.

4.2 Temperature and Flowering

The primary objectives of the temperature experiments were to examine the effect of average daily temperature (ADT) on flower initiation and development, and on plant development and growth in the context of commercial production. Other environmental factors (i.e., photoperiod and total irradiance) were already studied as outlined in Chapter 1. This

literature indicates that flowering of *E.* Styer Group is not regulated by photoperiod (Anon 1994) and that floral induction is influenced by total irradiance (Krishnasamy 2007). Based upon observations during her research, Krishnasamy suggested that additional primary factors besides irradiance may influence floral initiation and proposed investigating node number and temperature as the focus of future research. The temperature experiments reported here were designed with these previous findings in mind along with typical commercial greenhouse conditions. With limited resources (i.e., number of growth chambers available) and literature indicating differences between day and night temperatures (DIF) do not influence flowering, ADT treatments were selected with sufficient range to gather information about the base-line responses of this germplasm. While ADT was the primary consideration for the treatments, the night temperature for each treatment was set 4° C below the day temperature to mimic typical commercial greenhouse conditions.

Understanding the effect of temperature on flowering of ornamental crops is most relevant to commercial producers for temperatures that prevented flowering or when an optimal temperature for development is identified. Effects of temperature treatments of *E*. Styer Group genotypes reported here were most telling for the most extreme temperatures treatments. Two temperature treatments, the coolest and warmest, prevented flowering for some or all genotypes with the coolest preventing flowering for all genotypes and the warmest preventing flowering for three of five genotypes. Unfortunately, few previous studies have addressed the effects of temperature on flowering of Gentian relatives (*E. affine* and *E. grandiflorum*). Previous research on flowering of *E. affine* did not address the direct effect of temperature. However, one study did identify earlier flowering during the summer season for some genotypes while other genotypes flowered earlier during the winter season (Rubino 1993). Rubino suggested that the winter season may have delayed flowering because of low irradiance but made no comment on the possible effects of temperature. However, research which examined the effect of temperature

and photoperiod on flowering of two *E. grandiflorum* cultivars reported that the fastest flowering was achieved with long days and warm temperatures (Harbaugh 1995) for which it appears that temperature had a greater effect than photoperiod; the lowest constant temperature (12° C), regardless of photoperiod, prevented flowering of one cultivar but not the other. Another study on the flowering of *E. grandiflorum* did not address temperature directly but concluded that planting time/season had a direct effect on floral transition, probably acting through temperature (Zaccai and Edri 2002). Unfortunately, neither Harbaugh nor Zaccai and Edri identified an optimal temperature for development (i.e., above which development was delayed or prevented). The aforementioned results indicate temperature can impact flowering of Gentians but interactions must be considered before making conclusions.

Examination of genotypic variation and interactions between temperature and genotype can reveal significant individual effects. In my research, a significant interaction between temperature and genotype was identified for the intermediate temperature treatments (23° C DT/19° C NT and 30° C DT/26° C NT) with neither identified as an optimal general temperature for *E.* Styer Group. A significant treatment x population interaction from environmental treatments (i.e., irradiance) of *E.* Styer Group was already seen in previous work (Krishnasamy 2007). Previous work with *E. affine* also identified significant genotypic variation. Variation among genotypes was observed for flower count and colour under low light treatments but time to flower was not addressed (Rubino 1991). Later work by Rubino (1993) that evaluated genotype x season interactions identified significant variation among genotypes for days to first flower and full bloom. In addition, Rubino reported genotype rank for days to first flower changed dramatically for some genotypes with growing season. Rubino concluded that the effect of various environmental factors may be genotype-dependent. As discussed above, the effect of PGRs and temperature on *E. grandiflorum* flowering resulted in cultivars differences with a delay in flowering observed for one cultivar but not the others; however an interaction was not

reported (Halevy and Kofranek 1984). The study described above on the effect of photoperiod and temperature on flowering of different *E. grandiflorum* cultivars was designed to ignore cultivar differences; data for each cultivar were analyzed separately because distinct cultivars were initially chosen (Harbaugh 1995). Therefore, only results for two of four cultivars which varied the most were presented. Overall, genotypic variation can interact with temperature and therefore, results must be carefully evaluated before inclusion in commercial recommendations. This challenge can be addressed by reporting recommendations separately for individual cultivars, or selecting germplasm based on uniform response.

While my experiments were intended to determine the effect of external conditions (i.e., temperature) on flowering, the observation of a potential seasonal effect allows for speculation about the presence of a yet unidentified internal mechanism controlling flowering. For example, plants transplanted from their native provenance can behave as if they remained in their original environment despite being exposed to a different set of environmental cues directing development (Cronk 2005). For the results presented here, the conditions for propagation were artificial (i.e., controlled growth room in tissue culture laboratory) and constant throughout the year, therefore, it is surprising to observe a strong seasonal effect, especially for plants that transitioned to reproduction before commencement of the treatments. Many plants of four genotypes either had visible buds before commencement of treatments or budded within the first three weeks of the experiment. It should be noted that once moved to the greenhouse for rooting, propagules were exposed to ambient conditions for approximately three weeks. The daylength (and potentially total irradiance) during this greenhouse period for the summer experiment (May) would have been longer than for the winter experiment (September). However, since many plants had visible buds before being moved to the growth chambers (i.e., less than three weeks since the move from the artificial environment to the greenhouse), it is likely that floral induction occurred while in the tissue culture laboratory environment (i.e., constant conditions year round).

However, no flowering has been observed on the stock cultures while in tissue culture. While further work is needed to confirm the source of the seasonal effect, plants of *E.* Styer Group may have an internal clock and somehow sense season without external cues.

One internal cue that may influence the transition from vegetative to reproductive growth is plant size and age, which can be quantified by node number at flower initiation. As stated above, the coldest treatment (16° C DT/12° C NT) prevented flowering for all genotypes, the warmest treatment (35° C DT/31° C NT) prevented flowering for three of five genotypes and many genotype x treatment groups included plants at various reproductive stages at the conclusion of the experiments. For the lowest temperature treatment, lack of flowering was coupled with an overall lack of growth. In contrast, the highest temperature treatment did not inhibit plant growth but did prevent flowering. Previous research on flowering of *E. grandiflorum* indicates that floral transition will not occur without a certain minimal number of nodes (Zaccai and Edri 2002). While my results cannot confirm that *E.* Styer Group genotypes will not flower until a certain number of nodes is reached, results do indicate that plants are not pre-conditioned to flower after a set number of nodes are produced if external conditions are not favourable for flowering. Clearly, the optimal temperature for plant development is between the two extreme temperatures, but significant genotypic variation is present and needs to be evaluated.

4.3 Summary and Conclusions

Genotypic variation is significant among the ten genotypes evaluated and may represent inherent variation in this germplasm. As stated previously, a commercially viable cultivar series may not be easily developed. However, commercial introductions could be produced, either as siblings or unrelated genotypes that were selected for common response. Overall, some genotypes appear more promising for commercial introduction. Specifically, genotype 02-174-9

could be an ideal candidate for use as a potted flowering or bedding plant. Genotype 01-37-50 may be most suitable for commercial production of cut flowers. In addition, I would recommend continued research on genotypes that had early or intermediate time to flower (i.e, 01-37-8, 01-37-50, 01-37-61, 01-42-3, and 02-174-9).

Overall, the effect of PGR treatments on flowering is impacted more by genotype than by chemical. Individual effects of PGR and GA were not found to be essential factors for flower initiation. For genotypes prone to stem elongation, daminozide was effective at reducing stem length without a dramatic impact on flowering time for most genotypes. Depending on the desired phenotype (i.e., tall vs. compact plants), either concentration tested (i.e., 2500 or 3500 ppm) would be effective.

The effect of temperature on flowering was dependent on genotype with the exception of the coldest temperature treatment which prevented flowering for all genotypes. For some of the genotypes evaluated (01-37-37, 01-37-50, 01-42-3 and 02-174-9), the optimal temperature for fast flowering and attractive plant conformation was 30° C DT/26° C NT (or ADT of 28° C) However, for one genotype (01-47-21), the colder temperature treatment, 23° C DT/19° C NT (or ADT of 22 ° C), was optimal. Therefore, empirical studies on temperature effects on individual genotypes should concentrate on temperatures within this range.

Finally, plants of *E*. Styer Group may have an internal clock controlling flowering as seasonal differences were apparent despite the artificial environments used. Further work is needed to identify the source of this seasonal variation.

4.4. Future Work

To refine information for commercial growers, more temperature experiments should be conducted with treatments between 21 and 33° C ADT and if resources allow, they should also include DIF treatments. These experiments should be designed to determine whether DIF can be

used for height control as well as any secondary impacts on flowering. Furthermore, daminozide should be tested in different temperature treatments to examine potential interactions.

Research should be conducted to identify the cause of seasonal variation as this information would be useful for commercial growers and the scientific community. In order to determine the potential effect of internal controls (i.e., an internal clock), ambient seasonal conditions (i.e., total irradiance during propagation) must be carefully monitored and controlled. One way to address this issue would be to propagate plants at weekly intervals throughout the year and grow under common conditions until flowering. The time to flower could then be clearly associated with time of propagation.

Finally, to evaluate other factors that may influence flowering, experiments that examine the effect of moisture/water availability on flowering may be informative since plants flower in their native habit (Sri Lanka) in relation to monsoon seasons.

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Appendix A: Type III Sums of Squares Decomposition for ANOVAs in Chapter 3

Table A.1. Type III Sums of Squares decomposition from analysis of variance of effects of plant growth regulator treatments applied to six *Exacum* Styer Group genotypes on weeks to visible bud (VB), macrobud (MB), anthesis (AN), and weeks from visible bud to anthesis at Westcan Greenhouse.

	Effect	SS	Degrees of Freedom	MS	F	*P*
Weeks to VB	Intercept	20036.45	1	20036.45	6098.103	0.000000
	Genotype (G)	2634.48	5	526.90	160.361	0.000000
	Treatment (T)	79.88	6	13.31	4.052	0.000673
	G x T	196.66	30	6.56	1.995	0.002313
	Error	834.56	254	3.29		
Weeks to MB	Intercept	36200.85	1	36200.85	14440.24	0.000000
	Genotype (G)	2326.59	5	465.32	185.61	0.000000
	Treatment (T)	77.85	6	12.98	5.18	0.000048
	G x T	169.53	30	5.65	2.25	0.000375
	Error	636.76	254	2.51		
Weeks to AN	Intercept	47010.02	1	47010.02	18005.26	0.000000
	Genotype (G)	2330.91	5	466.18	178.55	0.000000
	Treatment (T)	95.88	6	15.98	6.12	0.000005
	G x T	163.85	30	5.46	2.09	0.001186
	Error	663.17	254	2.61		
Weeks from VB to AN	Intercept	5612.507	1	5612.507	3302.384	0.000000
	Genotype (G)	154.100	5	30.820	18.134	0.000000
	Treatment (T)	9.152	6	1.525	0.897	0.497271
	G x T	93.836	30	3.128	1.840	0.006489
	Error	431.681	254	1.700		

Table A.2. Type III Sums of Squares decomposition from analysis of variance of effects of plant growth regulator treatments applied to six *Exacum* Styer Group genotypes on weeks to visible bud (VB), macrobud (MB), anthesis (AN), weeks from visible bud to anthesis, plant height and number of nodes to first bud, height/node ratio, and number of reproductive branches at anthesis at UBC Greenhouse.

	Effect	SS	Degrees of Freedom	MS	F	*P*
Weeks to VB	Intercept	32805.73	1	32805.73	47617.68	0.000000
	Genotype (G)	1850.47	5	370.09	537.19	0.000000
	Treatment (T)	254.11	6	42.35	61.47	0.000000
	G x T	324.83	30	10.83	15.72	0.000000
	Error	192.21	279	0.69		
Weeks to MB	Intercept	54808.97	1	54808.97	54564.51	0.000000
	Genotype (G)	1480.20	5	296.04	294.72	0.000000
	Treatment (T)	393.12	6	65.52	65.23	0.000000
	G x T	567.51	30	18.92	18.83	0.000000
	Error	280.25	279	1.00		
Weeks to AN	Intercept	79914.78	1	79914.78	40154.43	0.000000
	Genotype (G)	1720.37	5	344.07	172.89	0.000000
	Treatment (T)	360.35	6	60.06	30.18	0.000000
	G x T	670.30	30	22.34	11.23	0.000000
	Error	555.26	279	1.99		
Weeks from VB to AN	Intercept	10316.16	1	10316.16	6603.921	0.000000
	Genotype (G)	86.44	5	17.29	11.066	0.000000
	Treatment (T)	39.75	6	6.63	4.241	0.000418
	G x T	436.95	30	14.56	9.324	0.000000
	Error	435.83	279	1.56		
Height (cm)	Intercept	116520.8	1	116520.8	13234.93	0.000000
	Genotype (G)	3747.0	5	749.4	85.12	0.000000
	Treatment (T)	2971.0	6	495.2	56.24	0.000000
	G x T	900.1	30	30.0	3.41	0.000000
	Error	2456.3	279	8.8		
Node	Intercept	69433.01	1	69433.01	25111.22	0.000000
	Genotype (G)	1095.56	5	219.11	79.24	0.000000
	Treatment (T)	265.46	6	44.24	16.00	0.000000
	G x T	243.61	30	8.12	2.94	0.000002
	Error	771.44	279	2.77		
Height/Node Ratio	Intercept	526.5580	1	526.5580	14258.10	0.000000
	Genotype (G)	1.9764	5	0.3953	10.70	0.000000
	Treatment (T)	9.4351	6	1.5725	42.58	0.000000
	G x T	2.7921	30	0.0931	2.52	0.000046
	Error	10.3036	279	0.0369		
Number of Reproductive Branches	Intercept	56444.86	1	56444.86	7441.604	0.000000
	Genotype (G)	2001.56	5	400.31	52.777	0.000000
	Treatment (T)	583.79	6	97.30	12.828	0.000000
	G x T	644.88	30	21.50	2.834	0.000004
	Error	2116.23	279	7.59		

Table A.3. Type III Sums of Squares decomposition from analysis of variance of effects of temperature treatments applied to five *Exacum* Styer Group genotypes on days to visible bud (VB), macrobud (MB), anthesis (AN), days from visible bud to anthesis, plant height and number of nodes to first bud, height/node ratio, number of reproductive branches and number of flowers and MBs at anthesis during the winter.

	Effect	SS	Degrees of Freedom	MS	F	*P*
Days to VB	Intercept	636307.4	1	636307.4	3234.794	0.000000
	Genotype (G)	43690.1	4	10922.5	55.527	0.000000
	Treatment (T)	4380.9	1	4380.9	22.271	0.000005
	G x T	12801.7	4	3200.4	16.270	0.000000
	Error	30489.6	155	196.7		
Days to MB	Intercept	1191891	1	1191891	4318.222	0.000000
	Genotype (G)	29645	4	7411	26.851	0.000000
	Treatment (T)	7990	1	7990	28.949	0.000000
	G x T	17840	4	4460	16.158	0.000000
	Error	42782	155	276		
Days to AN	Intercept	1646846	1	1646846	5428.116	0.000000
	Genotype (G)	26884	4	6721	22.153	0.000000
	Treatment (T)	15569	1	15569	51.317	0.000000
	G x T	18177	4	4544	14.978	0.000000
	Error	47026	155	303		
Days from	Intercept	235813.4	1	235813.4	3382.781	0.000000
VB to AN	Genotype (G)	4184.4	4	1046.1	15.006	0.000000
	Treatment (T)	3432.6	1	3432.6	49.241	0.000000
	G x T	3096.2	4	774.0	11.104	0.000000
	Error	10805.0	155	69.7		
Height (cm)	Intercept	29642.82	1	29642.82	1974.924	0.000000
	Genotype (G)	9400.29	4	2350.07	156.571	0.000000
	Treatment (T)	191.54	1	191.54	12.761	0.000472
	G x T	807.05	4	201.76	13.442	0.000000
	Error	2326.49	155	15.01		
Node	Intercept	30614.70	1	30614.70	6625.536	0.000000
	Genotype (G)	1326.12	4	331.53	71.749	0.000000
	Treatment (T)	114.49	1	114.49	24.777	0.000002
	G x T	216.52	4	54.13	11.715	0.000000
	Error	716.21	155	4.62		
Height/Node	Intercept	122.5613	1	122.5613	4116.450	0.000000
Ratio	Genotype (G)	14.0580	4	3.5145	118.041	0.000000
	Treatment (T)	0.1868	1	0.1868	6.273	0.013292
	G x T	1.6748	4	0.4187	14.063	0.000000
	Error	4.6149	155	0.0298		
Number of	Intercept	19467.64	1	19467.64	720.5252	0.000000
Reproductive	Genotype (G)	876.65	4	219.16	8.1115	0.000006
Branches	Treatment (T)	1148.90	1	1148.90	42.5225	0.000000
	G x T	692.63	4	173.16	6.4088	0.000085
	Error	4187.89	155	27.02		
Number of	Intercept	372667.5	1	372667.5	768.0744	0.000000
Flowers	Genotype (G)	15015.5	4	3753.9	7.7368	0.000010
and MBs	Treatment (T)	9356.8	1	9356.8	19.2846	0.000021
	G x T	16293.5	4	4073.4	8.3953	0.000004
	Error	75205.6	155	485.2		

Table A.4. Type III Sums of Squares decomposition from analysis of variance of effects of season for 23° C DT/19° C NT temperature treatments on five *Exacum* Styer Group genotypes on days to visible bud (VB), macrobud (MB), anthesis (AN), days from visible bud to anthesis, plant height and number of nodes to first bud, height/node ratio, number of reproductive branches and number of flowers and MBs at anthesis.

	Effect	SS	Degrees of Freedom	MS	F	*P*
Days to VB	Intercept	319022.5	1	319022.5	1535.351	0.000000
	Genotype (G)	47019.5	4	11754.9	56.572	0.000000
	Season (S)	41103.2	1	41103.2	197.816	0.000000
	G x S	4605.9	4	1151.5	5.542	0.000386
	Error	25973.1	125	207.8		
Days to MB	Intercept	729254.3	1	729254.3	2703.424	0.000000
	Genotype (G)	33451.5	4	8362.9	31.002	0.000000
	Season (S)	38210.5	1	38210.5	141.650	0.000000
	G x S	8057.7	4	2014.4	7.468	0.000020
	Error	33719.0	125	269.8		
Days to AN	Intercept	1083312	1	1083312	3698.987	0.000000
	Genotype (G)	32567	4	8142	27.800	0.000000
	Season (S)	44208	1	44208	150.950	0.000000
	G x S	9709	4	2427	8.288	0.000006
	Error	36608	125	293		
Days from	Intercept	226577.9	1	226577.9	2081.738	0.000000
VB to AN	Genotype (G)	12273.0	4	3068.3	28.190	0.000000
	Season (S)	56.5	1	56.5	0.519	0.472470
	G x S	1631.5	4	407.9	3.747	0.006486
	Error	13605.1	125	108.8		
Height (cm)	Intercept	18608.61	1	18608.61	1476.731	0.000000
	Genotype (G)	5822.00	4	1455.50	115.505	0.000000
	Season (S)	9.92	1	9.92	0.787	0.376771
	G x S	479.69	4	119.92	9.517	0.000001
	Error	1562.55	124	12.60		
Node	Intercept	19050.75	1	19050.75	5777.407	0.000000
	Genotype (G)	836.63	4	209.16	63.430	0.000000
	Season (S)	62.90	1	62.90	19.076	0.000026
	G x S	141.40	4	35.35	10.720	0.000000
	Error	412.18	125	3.30		
Height/Node	Intercept	94.08891	1	94.08891	1909.438	0.000000
Ratio	Genotype (G)	10.28226	4	2.57057	52.167	0.000000
	Season (S)	0.06126	1	0.06126	1.243	0.266984
	G x S	0.98962	4	0.24740	5.021	0.000872
	Error	6.15946	125	0.04928		
Number of	Intercept	19595.21	1	19595.21	702.5324	0.000000
Reproductive	Genotype (G)	1538.87	4	384.72	13.7930	0.000000
Branches	Season (S)	198.91	1	198.91	7.1314	0.008582
	G x S	234.59	4	58.65	2.1026	0.084378
	Error	3486.53	125	27.89		
Number of	Intercept	244367.1	1	244367.1	790.0556	0.000000
Flowers	Genotype (G)	18702.6	4	4675.7	15.1167	0.000000
and MBs	Season (S)	17959.4	1	17959.4	58.0640	0.000000
	G x S	3794.2	4	948.6	3.0667	0.018949
	Error	38663.0	125	309.3		

Table A.5. Type III Sums of Squares decomposition from analysis of variance of effects of temperature treatments on two *Exacum* Styer Group genotypes on days to visible bud (VB), macrobud (MB), anthesis (AN), days from visible bud to anthesis, plant height and number of nodes to first bud, height/node ratio, number of reproductive branches and number of flowers and MBs at anthesis during the summer.

	Effect	SS	Degrees of Freedom	MS	F	*P*
Days to VB	Intercept	15384.46	1	15384.46	72.62822	0.000000
	Genotype (G)	17.30	1	17.30	0.08168	0.775910
	Treatment (T)	413.83	1	413.83	1.95363	0.166807
	G x T	266.54	1	266.54	1.25829	0.265980
	Error	14192.27	67	211.82		
Days to MB	Intercept	170041.4	1	170041.4	487.6657	0.000000
	Genotype (G)	10320.1	1	10320.1	29.5972	0.000001
	Treatment (T)	481.5	1	481.5	1.3809	0.244102
	G x T	1222.7	1	1222.7	3.5065	0.065493
	Error	23361.8	67	348.7		
Days to AN	Intercept	318888.8	1	318888.8	714.4123	0.000000
	Genotype (G)	9714.3	1	9714.3	21.7630	0.000015
	Treatment (T)	300.2	1	300.2	0.6726	0.415040
	G x T	2911.3	1	2911.3	6.5222	0.012934
	Error	29906.5	67	446.4		
Days from	Intercept	194188.6	1	194188.6	630.5468	0.000000
VB to AN	Genotype (G)	8911.6	1	8911.6	28.9367	0.000001
	Treatment (T)	9.1	1	9.1	0.0295	0.864100
	G x T	1416.1	1	1416.1	4.5980	0.035640
	Error	20633.9	67	308.0		
Height (cm)	Intercept	2757.526	1	2757.526	333.6852	0.000000
	Genotype (G)	20.651	1	20.651	2.4990	0.118631
	Treatment (T)	11.036	1	11.036	1.3355	0.251941
	G x T	7.245	1	7.245	0.8767	0.352475
	Error	553.678	67	8.264		
Node	Intercept	6025.949	1	6025.949	2146.616	0.000000
	Genotype (G)	13.183	1	13.183	4.696	0.033789
	Treatment (T)	10.496	1	10.496	3.739	0.057386
	G x T	0.329	1	0.329	0.117	0.733053
	Error	188.081	67	2.807		
Height/Node	Intercept	29.08463	1	29.08463	965.9387	0.000000
Ratio	Genotype (G)	0.01714	1	0.01714	0.5694	0.453149
	Treatment (T)	0.00123	1	0.00123	0.0408	0.840533
	G x T	0.02859	1	0.02859	0.9495	0.333340
	Error	2.01739	67	0.03011		
Number of	Intercept	4002.633	1	4002.633	479.3824	0.000000
Reproductive	Genotype (G)	2.446	1	2.446	0.2929	0.590151
Branches	Treatment (T)	113.067	1	113.067	13.5416	0.000467
	G x T	0.652	1	0.652	0.0781	0.780796
	Error	559.421	67	8.350		
Number of	Intercept	30746.17	1	30746.17	365.7378	0.000000
Flowers	Genotype (G)	625.60	1	625.60	7.4417	0.008130
and MBs	Treatment (T)	1123.53	1	1123.53	13.3648	0.000505
	G x T	52.66	1	52.66	0.6264	0.431479
	Error	5632.43	67	84.07		

Appendix B: Summary of Treatment Induced Symptoms

B.1 Plant Growth Regulator Experiments

In general, plants subjected to plant growth regulator treatments (and control) remained healthy and stress-free with the exception of plants treated with Ethephon. In response to treatments of Ethephon at 500 ppm, plants showed signs of rosetting (loss of apical dominance and lack of leaf and stem growth). In response to treatments of Ethephon at 1000 ppm, plants exhibited severe rosetting to the point where it was very difficult to distinguish nodes and internodes because of restricted of stem elongation.

B.2 Temperature Experiments

In general, plants subjected to temperature treatments remained healthy and stress-free with the exception of plants in chambers with the coldest (16° C DT/12° C NT) and warmest (35° C DT/31° C NT) treatments. Plants subjected to 16° C DT/12° C NT exhibited very slow growth which, on average, resulted in no more than 2cm of stem growth over 18 weeks. In addition, leaves were chlorotic and curled under (i.e., leaves did not expand). Also, stem lesions were discovered on many plants which usually led to stem and/or root rot and death. Plants subjected to 35° C DT/31° C NT had many foliar symptoms including curling, distortion (i.e., dimpling and uneven leaf surface), full leaf and tip necrosis, and wilted/soft leaf tissue. In addition, plants had elongated, weak (i.e., broken easily), and floppy (i.e., soft) stems compared to plants subjected to lower temperatures.

Appendix C: Data for Excluded Genotype 01-50-46

Table C.1. Combined and partitioned effects of temperature treatments applied to one *Exacum* Styer Group genotype (01-50-46) on days to visible bud (VB), macrobud (MB), anthesis (AN), days from visible bud to anthesis, plant height and number of nodes to first bud, height/node ratio, number of reproductive branches and number of flowers and MBs at anthesis during two seasons.

Season	Treatment	Days to VB	Days to MB	Days to AN	Days from VB to AN	Height (cm)	Node (#)	Height/ Node Ratio	Number of Reproductive Branches	Number of Flowers and MBs
						Combined effects				
Winter	(Combined)	73.93	115.40	136.67	62.73	13.70	15.33	0.89	7.33	30.13
						Partitioned effects				
Winter	23/19 C	73.00	99.00	123.00	50.00	10.50	12.25	0.83	9.50	53.75
Winter	30/26 C	74.27	121.36	141.64	67.36	14.86	16.45	0.91	6.55	21.55
Summer	23/19 C	29.80	54.60	76.40	46.60	7.90	10.60	0.74	8.20	25.00

Table C.2. Effect of temperature treatments on one *Exacum* Styer Group genotype (01-50-46) on percent plants dead or at four reproductive stages; vegetative (VEG); visible bud (VB); macrobud (MB); and anthesis (AN), with corresponding mean nodes to first bud at week 18 and standard deviation.

	Dead	VEG		VB		MB		AN		Total Plants
Treatment	% plants	% plants	Node	% plants	Node	% plants	Node	% plants	Node	
16/12 C (W)	0%	100%	9 (2.87)	0%		0%		0%		33
23/19 C (W)	0%	70%	17 (2.08)	20%	14 (4.23)	0%		10%	11 (0.00)	30
23/19 C (S)	7%	77%	18 (2.87)	0%		0%		17%	11 (0.55)	30
30/26 C (W)	0%	24%	22 (2.15)	48%	18 (2.71)	28%	16 (2.17)	0%		29
35/31 C (S)	100%	0%		0%		0%		0%		33

(W) = winter season, (S) = summer season

Node = mean number of nodes (standard deviation)

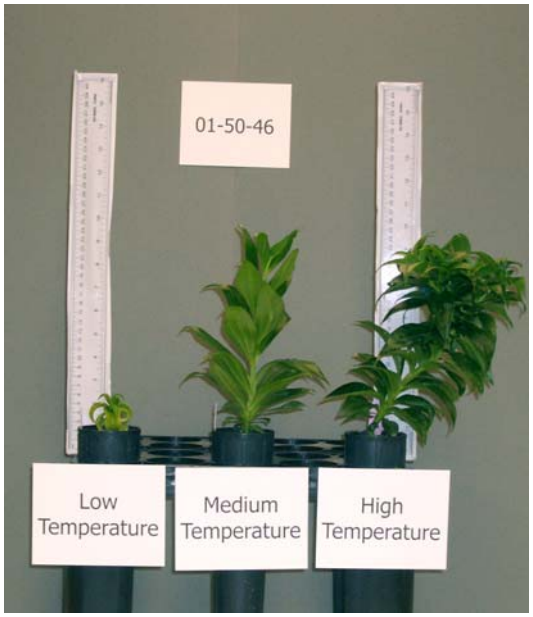

Fig. C.1. Photographs of representative plants (i.e., closest to average reproductive stage, number of nodes, height and general appearance) from excluded genotype (01-50-46) x treatment groups for the temperature experiment conducted during the winter taken on February 26, 2008 (week 19). Ruler = 40cm. Low temperature is 16° C DT/12° C NT, medium temperature is 23° C DT/19° C NT and high temperature is 30° C DT/26° C NT.

Fig. C.2. Photographs of representative plants (i.e., closest to average reproductive stage, number of nodes, height and general appearance) in the middle with extremes on either side from excluded genotype (01-50-46) x treatment groups for the temperature experiment conducted during the summer taken on October 2, 2008 (week 18). No plants are shown for the 35° C DT/31° C NT treatment since all plants had died by week 18. Ruler = 40cm.

Appendix D: Biomass Data for the Winter Temperature Experiment

Selected plants from the temperature experiment conducted in the winter were measured for root and shoot dry biomass. The goal of collecting these data was to determine if plants transitioned between reproductive stages depending on plant biomass (i.e., is plant biomass an internal cue for flower development).

All plants were grown and treated as described in Chapter 2 for the winter temperature experiment. Biomass data were collected on five plants per reproductive stage (visible bud, macrobud and anthesis) for each genotype x treatment group. In order to get representative data, a plant sampling system was determined prior to harvesting for destructive biomass measurement. For each genotype x treatment group, the following plants were harvested:

- Visible bud (33 plants): the 4th, 10th, 16th, 22nd and 28th plant to reach visible bud
- Macrobud (28 plants remain): the 4th, 9th, 14th, 19th and 24th plant to reach macrobud
- Anthesis (23 plants remain): the 3rd, 7th, 11th, 15th and 19th plant to reach anthesis

Once harvested, plants were immediately separated into shoot (plant parts above potting medium level) and root (plant parts below potting medium level). Potting medium was carefully removed from roots with repeated cycles of soaking and rinsing in clean water. Roots and shoots for each plant were placed in separate paper bags and put in an oven set at 40° C until completely dried (approximately one to four days). Dried samples were weighed to the nearest 0.0001 gram. Data are presented in the following tables and figures.

Since we now understand that genotypes of *E*. Styer Group do not behave uniformly, we were most interested in the interaction between genotype and reproductive stage. Since the interaction of genotype x reproductive stage was significant for only two of four metrics (root biomass and root/shoot biomass ratio), those means were graphed for visual assessment. While

some standard deviation bars do not overlap (indicating statistical difference), most genotypes did not have meaningful differences for these metrics. Furthermore, a clear trend was not visible and standard deviations indicate dramatic variability for biomass of some genotypes. Therefore, I concluded that plant biomass does not associate with reproductive stage.

Table D.1. Main and significant effects of temperature treatments and reproductive stage of six *Exacum* Styer Group genotypes on root biomass (g), shoot biomass (g), root/shoot biomass ratio and total plant biomass (g) during the winter.

	Root Biomass (g)		Shoot Biomass (g)		Root/Shoot Ratio		Total Biomass (g)	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
				Main effects				
Genotype (G)								
01-37-37	2.79	0.89	11.99	4.65	0.24	0.04	14.79	5.46
01-37-50	1.24	1.04	5.27	4.96	0.27	0.11	6.51	5.95
01-42-3	0.82	0.27	3.29	1.90	0.34	0.24	4.11	2.07
01-47-21	1.06	0.60	4.21	3.02	0.29	0.12	5.27	3.54
01-50-46	1.41	1.00	5.29	3.92	0.29	0.08	6.70	4.85
02-174-9	1.29	0.89	5.53	4.17	0.26	0.11	6.82	4.93
Temperature (T)								
23/19 C	1.86	1.12	7.50	5.82	0.33	0.17	9.36	6.87
30/26 C	1.14	0.88	4.96	3.59	0.24	0.07	6.11	4.42
Reproductive Stage (R)								
VB	1.03	0.80	3.23	2.80	0.37	0.16	4.27	3.55
MB	1.68	1.11	6.71	4.50	0.26	0.08	8.38	5.57
AN	1.82	1.11	9.13	5.42	0.20	0.05	10.95	6.47
				Signifcant effects				
Source of Variation								
Genotype (G)	***		***		*		***	
Temperature (T)	***		***		***		***	
Reproductive Stage (R)	***		***		***		***	
G x T	***		***		**		***	
G x R	**		NS		*		NS	
T x R	NS		NS		***		NS	
G x T x R	NS		NS		NS		NS	

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

Table D.2. Individual effects of genotype (G) by reproductive stage (R) interaction for temperature treatments and reproductive stage of six *Exacum* Styer Group genotypes on root biomass (g), shoot biomass (g), root/shoot biomass ratio and total plant biomass (g) during the winter.

		Root Biomass (g)		Shoot Biomass (g)		Root/Shoot Ratio		Total Biomass (g)	
		Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
		Individual effects of G x R Interaction							
Genotype (G)	Reproductive Stage (R)								
01-37-37	VB	2.21	0.54	7.75	1.72	0.28	0.03	9.96	2.23
	MB	3.05	1.13	12.42	4.23	0.24	0.02	15.46	5.34
	AN	3.13	0.62	15.81	3.54	0.20	0.02	18.94	4.11
01-37-50	VB	0.51	0.40	1.58	1.35	0.36	0.12	2.10	1.74
	MB	1.71	1.10	6.29	4.30	0.27	0.08	8.00	5.33
	AN	1.53	1.10	8.24	5.93	0.18	0.02	9.77	7.03
01-42-3	VB	0.73	0.26	1.79	1.33	0.51	0.28	2.52	1.49
	MB	0.76	0.25	3.21	0.90	0.25	0.10	3.97	0.99
	AN	1.02	0.20	5.49	0.99	0.19	0.04	6.51	1.09
01-47-21	VB	0.75	0.46	2.16	1.27	0.36	0.13	2.90	1.66
	MB	1.23	0.47	4.53	1.44	0.28	0.10	5.77	1.73
	AN	1.28	0.76	6.52	4.07	0.21	0.04	7.80	4.80
01-50-46	VB	0.75	0.37	2.47	1.13	0.32	0.09	3.22	1.47
	MB	1.44	0.55	5.87	2.66	0.26	0.05	7.31	3.17
	AN	2.58	1.17	9.64	4.41	0.28	0.06	12.22	5.37
02-174-9	VB	1.21	1.05	3.46	3.29	0.36	0.10	4.66	4.21
	MB	1.34	0.90	5.78	4.08	0.25	0.09	7.12	4.93
	AN	1.33	0.81	7.38	4.45	0.18	0.05	8.71	5.21

Table D.3. Type III Sums of Squares decomposition from analysis of variance of effects of temperature treatments and reproductive stage of six *Exacum* Styer Group genotypes on root biomass (g), shoot biomass (g), root/shoot biomass ratio and total plant biomass (g) during the winter.

	Effect	SS	Degrees of Freedom	MS	F	*P*
Root Biomass (g)	Intercept	311.1640	1	311.1640	925.2257	0.000000
	Genotype (G)	64.8617	5	12.9723	38.5724	0.000000
	Treatment (T)	11.8760	1	11.8760	35.3125	0.000000
	Reproductive Stage (R)	16.5492	2	8.2746	24.6040	0.000000
	G x T	14.4389	5	2.8878	8.5866	0.000001
	G x R	8.4365	10	0.8436	2.5085	0.009015
	T x R	1.4294	2	0.7147	2.1250	0.123937
	G x T x R	5.0393	10	0.5039	1.4984	0.148190
	Error	40.0211	119	0.3363		
Shoot Biomass (g)	Intercept	5369.405	1	5369.405	743.9738	0.000000
	Genotype (G)	1263.131	5	252.626	35.0034	0.000000
	Treatment (T)	159.676	1	159.676	22.1243	0.000007
	Reproductive Stage (R)	793.441	2	396.720	54.9688	0.000000
	G x T	246.198	5	49.240	6.8225	0.000013
	G x R	97.103	10	9.710	1.3454	0.214442
	T x R	23.821	2	11.910	1.6503	0.196362
	G x T x R	69.888	10	6.989	0.9684	0.474618
	Error	858.846	119	7.217		
Root/Shoot	Intercept	11.09819	1	11.09819	1521.731	0.000000
Biomass Ratio	Genotype (G)	0.11258	5	0.02252	3.087	0.011750
	Treatment (T)	0.22758	1	0.22758	31.204	0.000000
	Reproductive Stage (R)	0.64911	2	0.32455	44.501	0.000000
	G x T	0.16102	5	0.03220	4.416	0.001005
	G x R	0.17560	10	0.01756	2.408	0.012128
	T x R	0.11089	2	0.05544	7.602	0.000782
	G x T x R	0.05942	10	0.00594	0.815	0.615045
	Error	0.86788	119	0.00729		
Total Plant	Intercept	8265.731	1	8265.731	805.7179	0.000000
Biomass (g)	Genotype (G)	1898.716	5	379.743	37.0162	0.000000
	Treatment (T)	258.645	1	258.645	25.2119	0.000002
	Reproductive Stage (R)	1035.163	2	517.581	50.4522	0.000000
	G x T	372.523	5	74.505	7.2625	0.000006
	G x R	151.699	10	15.170	1.4787	0.155593
	T x R	28.337	2	14.169	1.3811	0.255295
	G x T x R	106.402	10	10.640	1.0372	0.416824
	Error	1220.802	119	10.259		

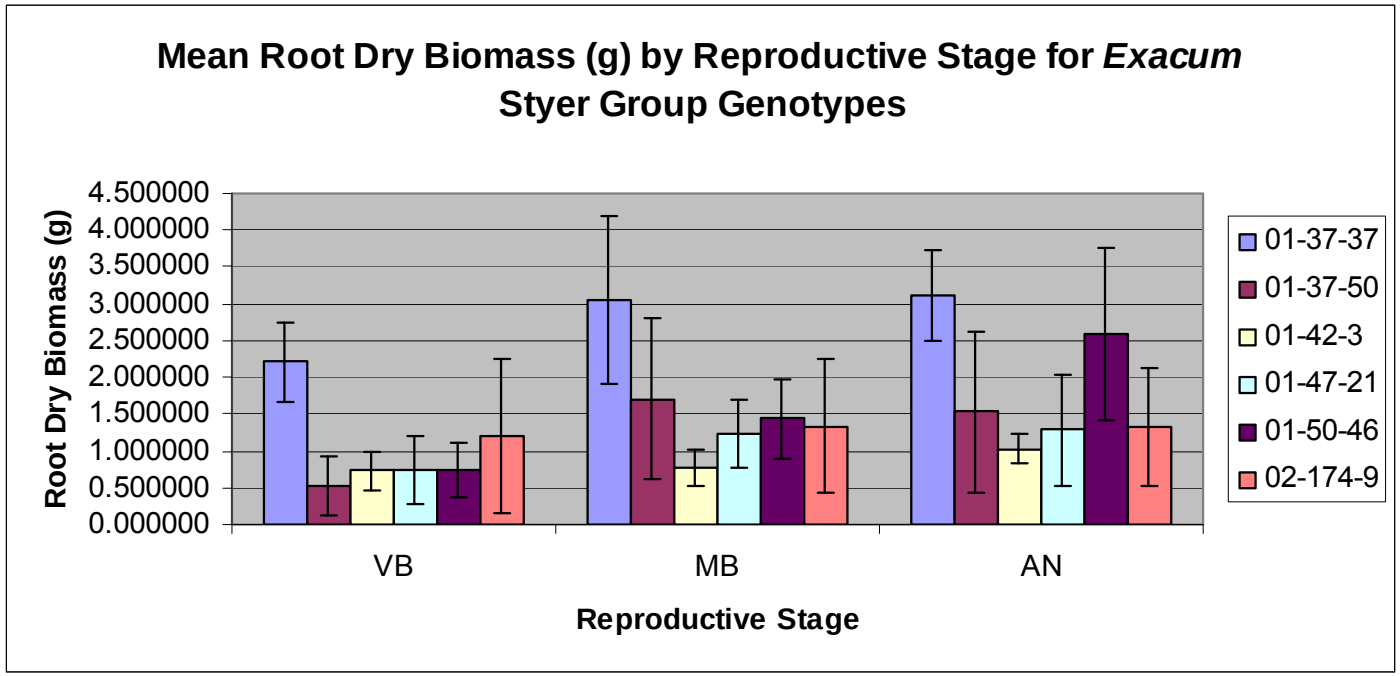

Fig. D.1. Mean root dry biomass by reproductive stage of six genotypes of *Exacum* Styer Group during the winter. Error bars = standard deviation

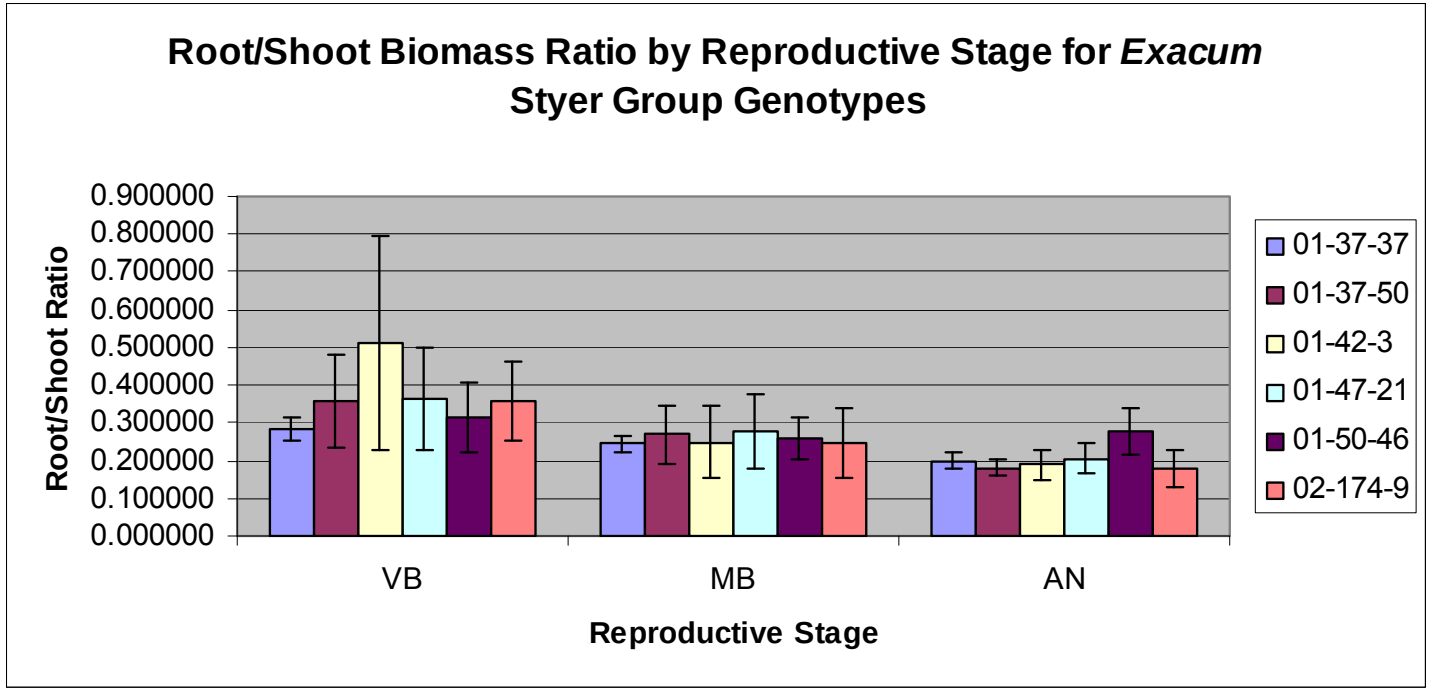

Fig. D.2. Mean root/shoot dry biomass ratio by reproductive stage of six genotypes of *Exacum* Styer Group during the winter. Error bars = standard deviation

Appendix E: Average Propagule Rating for the Summer Temperature Experiment

Table E.1. Mean root and shoot ratings (1 to 5 from least to most vigourous) with standard deviation for *Exacum* Styer Group propagules by genotype for the summer temperature experiment.

	Root rating		Shoot Rating	
Genotype	Mean	Standard deviation	Mean	Standard deviations
01-37-37	4.71	0.52	4.70	0.50
01-37-50	4.66	0.56	4.75	0.48
01-42-3	5.00	0.00	4.78	0.56
01-47-21	4.71	0.46	4.48	0.54
01-50-46	4.73	0.51	4.47	0.52
02-174-9	4.94	0.24	4.37	0.65