PETAL DROP IN SUNFLOWERS:

VARIETAL DIFFERENCES AND POSSIBLE REMEDIES

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A complaint among sunflower growers is that the petals are easily knocked off the flower, which ruins its appearance and destroys its market value. Varietal susceptibility to petal drop among different sunflower genotypes, grouped into the red, bicolor, orange and yellow colored varieties, was measured using a novel technique. Mean break strength of varieties in the yellow group were higher and significantly different ($p<0.05$) from the orange and bicolor groups, which were in turn higher and significantly different from varieties in the red group. Mean vase life (12 days) of sunflower varieties in the yellow group was longer than the orange group (10 days), which in turn were longer and significantly different ($p<0.05$) from the bicolor group (9 days), and significantly different from the red group (8 days). There was no relationship between head diameter, petal length and abscission tendency in sunflowers. It was also observed that petal drop in all the sunflower varieties was ethylene insensitive.

Three types of phytohormones (cytokinins, ABA and auxins) were present in abscission zone tissues of 5 sunflower genotypes, Procut Yellow Lite (PYL: yellow group), Procut Lemon (PL: yellow group), Strawberry Blonde (SB: red group). Procut Bicolor (PBC: bicolor group), and Moulin Rouge (MR: red group). Two groups of cytokinins were detected: the zeatin + zeatin riboside (Z + ZR) group and the iso-

pentenyl adenosine (iPA) group. The levels of 'Z+ZR' in PYL and PBC were significantly higher ($p<0.05$) than in PL, SB, and MR, while levels of 'iPA' in PYL, PL and PBC were significantly higher than in SB and MR. It was also observed that PYL and PBC were significantly high in cytokinins but low in ABA. There were significantly low ($p<0.05$) levels of auxin in sunflower abscission zone tissues within 1 hour of flower opening in all genotypes. The genotypes with the shorter vase life (PBC, SB and MR) had the highest levels of ABA in their petals while those with the longer vase life (PYL and PL) had the smallest amounts of ABA in their petals.

Petal detachment forces were significantly higher ($p<0.05$) when dipped in cytokinins (BAP-10 and Fascination- BA+GA4+7) than in the controls. At higher concentrations of up to 300 ppm, detachment forces decreased in Fascination treated plants more than in BAP-10 treated plants. When sunflower heads were dipped for 10 minutes in different concentrations of BAP-10 and BA+GA4+7 and kept for 14 days, abscission was delayed and vase life was extended by 4 and 6 days, respectively.

Anatomical analysis revealed a differentiated region (the abscission zone) at the junction of the petal and achene consisting of cells with a different morphology from those above and below it. Cell division at the abscission zone of the short-lived variety occurred earlier and faster than that in the long-lived variety. These differences indicate that whereas the anatomical and cellular nature of the abscission zone is similar in the two lines (PYL and PBC), the tempo of development differs. Specifically, the abscission layer reaches full differentiation, or maturity, sooner in line PBC, hence its earlier petal drop, than in line PYL.

BIOGRAPHICAL SKETCH

Joyous was born in Kumba, in the South West Region of the Republic of Cameroon. She completed her primary, secondary and undergraduate education in this part of Cameroon. Her father was an officer in the Cameroonian Army and her mother a secondary school teacher. Joyous' interest in horticulture was nurtured by her father during her childhood. He loved gardening and would allocate flower plots around the house for Joyous and her sister to nurture. This peculiar upbringing helped Joyous develop skills in floriculture, planning and time management from a very young age.

Her interest in plants inspired her to study Environmental Science at the University of Buea, Cameroon in 1998. During this period, she joined the Environmental Science Students' Association and through this body undertook visits to the Limbe Botanic Garden (LBG), a leading horticultural institution in Central Africa. This strengthened Joyous' interest in specializing in plant sciences. On graduating from Buea, Joyous gained admission into the masters' program in forestry at the renowned University of Ibadan, Nigeria in 2002; she graduated in 2004 with distinction.

In 2005, Joyous was employed to head the production unit of the LBG's Floriculture Project funded by the governments of Cameroon and the United States. The objective was to build the production and export marketing capacity of the floriculture industry in Cameroon. In 2006, she was offered an assistantship in the Department of Horticulture, Cornell University to pursue a Ph.D. with Professor Hans Christian Wien. Joyous is married and has a two-year old daughter Ezra Mormandem Nuesiri.

To my husband Dr. Emmanuel Nuesiri and our daughter Ezra

For all your encouragement, support, and patience through this journey.

To my parents, Simon and Lydia Tata, and my siblings

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Chapter 1

Introduction to Dissertation

Ornamental sunflowers have become increasingly popular as cut flowers the world over (Blacquiere et al., 2002; Devecchi, 2005; Hayata and Imaizumi, 2000). The development of new cultivars provides a wide range of colors to meet the needs of the flower industry. In 2001 there were six new cultivars that were evaluated in the field trials of the Association of Specialty Cut Flower Growers (Dole, 2002). The 17 cultivars included in the on-farm field trials in 2002 had a range of bloom colors such as green, peach, bronze, as well as traditional, dark-disc with yellow petals (Dole, 2003). The wholesale value of cut sunflowers sold in the Unites States in 1998 was $4,423,000 (U.S. Dept. of Agriculture, 1998).

Despite its popularity as a cut flower, a growing complaint heard among sunflower growers is that the petals of some varieties are easily knocked off the flower, which ruins its appearance and destroys its market value. This can happen within a day of the flowers' opening and the petals flattening out. There has been no systematic study of this problem in literature, although sunflower growers have been actively selecting for lines less prone to this disorder.

Abscission of petals, buds and flowers is a common problem in cut flowers and potted plants (van Doorn and Stead, 1997). Loss of quality in leaves, stems and flowers of cut flowers poses a financial constraint to growers and marketers everywhere, and may result in rejection in the market place. There are several reasons why some ornamentals lose quality. These include wilting of petals or leaves, yellowing of leaves, and phototropic bending of scapes and stems. Some authors suggest that the fall of turgid petals in several species is solely due to the forces generated by the growing fruit and not to cell wall dissolution (Reiche, 1885; van Doorn and Stead, 1997). Petal abscission is known to be a developmental process regulated by the plant, but

can also be induced to occur when the plant is under stress (Mao et al., 1989). Thus increasing the shelf life of cut flowers is an important area of research which has engaged horticulturists for many decades. However, there is little published work comparing the postharvest life of various cultivars of cut ornamentals (van Der Meulen-Muisers et al., 1999). While several experiments have been carried out to study the process of petal drop in cut flower species and to look for ways of prolonging the shelf life, little research has been done with sunflowers.

The sunflower (*Helianthus annuuss.* L) is native to southwestern United States and Mexico (Heiser, 1978). Historically, sunflowers were used for various purposes such as food crops (Putt, 1978), garden plants (Hedrick, 1950), for medicine (Heiser, 1951a), as flowering plants and more recently as cut flowers (Armitage and Laushman, 2003; Starman et al., 1995). Sunflowers are considered easy to grow by cut flower growers. The time from seeding to harvest evaluated for 29 cut sunflower cultivars ranged from 51.6 to 69.22 days (Sloan and Harkness, 2006). Vase life of sunflowers varies depending on the species. It was reported to range from 4 to 13 days (Gast, 1995). Yanez et al. (2005) found a vase life of 6.8 to 11.2 days for some 28 ornamental sunflower cultivars. Gast (1995) stated that a postharvest life of at least 10 days is desirable in the wholesale of fresh cut flowers. It is worth noting that many commercial cut flower cultivars and cut greens (including sunflowers) have been patented, and are characterized by specific attributes such as color, form, disease resistance and size.

Breeders of these new cultivars fail to take into consideration important commercial attributes when developing these varieties. For example, some of the modern alstroemeria cultivars have wonderfully attractive flowers, but their display life is short because of rapid leaf yellowing under commercial conditions. This is similar to the beautiful dark red and bicolored cultivars of sunflowers that lose their petals easily.

Abscission of inflorescence parts (petals, sepals, styles and stamens) is widespread. Anatomical findings suggest that prior to the abscission of a plant organ, cell division occurs at the base of the organ (Sexton and Roberts, 1982). Anatomical and physiological literature indicates that flower abscission is the result of active middle lamella solubilization and in some species also of breakdown of the primary wall. Osborne (1989) stated that levels of insoluble wall pectins decreased prior to abscission.

It has been well established that plant hormones participate in the endogenous regulation of abscission. The level of endogenous cytokinins in petals of a short-lived rose variety (Golden Wave) was lower than in a long-lived variety (Lovita). Further, application of exogenous cytokinins increased longevity of the short-lived variety (Mayak and Halevy, 1970). Abscisic acid content in petals of cut roses increased prior to abscission. This rise also occurred earlier in a cultivar showing early abscission than in one with late abscission (Mayak et al., 1972). Flower abscission has also been shown to be advanced by ethylene. Exposure of plants to exogenous ethylene hastened inflorescence abscission in several species (Woltering, 1987). Exposure of plants to exogenous ethylene hastened abscission in olives (Weis et al., 1988). The rate of ethylene production always increases prior to flower abscission in many species (Jackson et al., 1972; Mor et al., 1984b; Roberts et al., 1984).

Major research objectives

The research 'Petal drop in sunflowers: varietal differences and possible remedies' had the following overarching objectives:

- To characterize the age at which petals become susceptible to abscission
- To confirm varietal susceptibility to brushing

- To study the anatomy and morphology of flowers susceptible and those resistant to petal loss
- To study the relationship between endogenous levels of plant hormones and petal drop tendencies
- To check the effect of commercial cytokinin products on the vase life of cut sunflowers

The outline of the thesis

This thesis investigates the factors regulating early loss of petals in some sunflower varieties which are used as cut flowers. Chapter 1 provides the motivation behind the study, a literature review on abscission in cut flowers and the major research objectives. Chapter 2 is a study of the physical techniques used to measure varietal susceptibility in sunflowers to drop their petals. The study characterized the age at which petals become susceptible to abscission and the relationship between petal detachment forces, color and vase life of flowers.

In Chapter 3, we analyzed phytohormone levels in abscission zone tissues of sunflower varieties from the susceptible and resistant lines, to determine if differences in hormone levels correlated with abscission tendency in the genotypes tested. We also present results of the effect of exogenously applied plant hormones on the vase life of sunflowers. Chapter 4 is a study of the anatomy of petal drop in two lines of sunflowers; the short-lived line Procut Bicolor (PBC) and a long-lived line Procut Yellow Lite (PYL). The study determined if differences in the nature and/or development of an abscission zone between lines correlates with differences in timing with respect to the conclusion of petal drop and end of vase life. Finally, Chapter 5 concludes the thesis, and presents lessons learnt and recommendations for future study.

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Chapter 2: Physical Techniques in Characterizing Varietal Susceptibility to Petal Drop in Sunflowers

Introduction

It has been observed that some varieties of sunflower grown as cut flowers are easily subject to loss of flower petals if the flower is brushed against a hard object. This can happen as early as when the flowers first opens, thus it is not linked with flower death (the usual senescence process). This loss of petals from newly opened flowers is very detrimental to flower appearance, causing a reduction in their market value. We noticed this problem for the first time when carrying a bunch of freshly-harvested 'Procut Bicolor' flowers through a narrow doorframe, and observed that petals were knocked off when they brushed against the door. Growers have known for years that there are varietal differences in this characteristic, but lacked a reliable method of measuring petal loss objectively. Breeders of sunflowers are also aware of the varietal differences which exist in this characteristic and have been actively selecting for lines which are less prone to the disorder. Unfortunately, the varieties with dark or bicolored petals which are considered by some to be the most beautiful in bouquets tend to be more susceptible to this disorder than those with yellow or orange petals. A proper understanding of the varietal differences in petal drop of sunflowers and reliable testing methods are beneficial to breeding programs directed towards the improvement of sunflower longevity. This knowledge will also enable growers to increase overall sales of the dark colored varieties. There has been no systematic study of the problem so far in literature.

The overall goal of this study was to measure and characterize varietal susceptibility in the tendency of sunflowers to drop their petals.

Morphology of sunflower

The sunflower belongs to the family *Asteraceae*, a plant family with one of the largest inflorescences. The inflorescence consists of an outer whorl of showy ray flowers which varies in color from yellow to orange, to red, dark red and white and from about 300 to 8000 disk flowers (Knowles, 1978; Pustovoit, 1975). Variation in petal color has been discussed by Cockerell (1912, 1915, 1918) and Fick (1976). Rudorf (1961) also reported the rare occurrence of white ray flowers. Ray flowers are normally sterile, with a rudimentary pistil and vestigial style and stigma, but no anther. The disk flowers are arranged in arcs radiating from the center of the head. Disk flowers are epigynous (petals and stamens attached at the summit of an ovary). Involucral bracts or phyllaries, which vary in form, surround the head. The inflorescence (head) of sunflowers is of great interest to breeders because the head size influences procedures and crossing techniques (Fick, 1989). When a sunflower is in full bloom it is a very photogenic crop because of its large inflorescence with showy yellow-orange or dark-red-bicolored ray flowers. Head diameter is an important factor in the determination of yield in sunflowers. It is measured by including the area of the disk flowers and may vary from 6 to 75cm (Heiser, 1976). Head shape is another morphological characteristic used to differentiate sunflower cultivars. The head shape can be flat, concave, convex, irregular or trumpet-shaped. Some authors suggest that head shape also influences the thickness of the receptacle, with the concave head types having larger, thicker receptacles often associated with a fluted or horn-shaped appearance (Fick and Miller, 1997). A description of various head shapes can be found in the International Sunflower Database Collection (IBPGR, 1985). The purpose of the morphology experiments was to find out if there was any meaningful relationship between head size and abscission tendency among sunflower cultivars and to investigate if petal length influenced abscission.

Physical techniques in petal abscission study

Many researchers (Evensen et al., 1993; Fernandez et al., 2000; Lease et al., 2006; Mckenzie and Lovell, 1992; Patterson and Bleecker, 2004) have used physical methods such as measurement of breakstrength to study petal abscission. Breakstrength is a quantitative measure of the force required to detach an organ from the body of a plant. Evensen et al (1993) used an electronic force gauge (Shimpo, Graham and White Instruments, St. Albans, Herts, UK) to measure the speed of separation and the time taken for separation to be completed in ethylene treated plants of *Pelargonium x hortorum*.

To gain a better understanding of varietal differences in susceptibility to this abnormal loss of petals in sunflowers, we first devised a simple brushing test which was used to compare the varieties. After this, another technique was devised called the petal breakstrength method. Both methods are discussed in this chapter.

I. The Brushing Test

The brushing test consists of bending back the petals of the newly-opened flowers with the edge of the index finger, brushing the flower at right angles. One stroke was used for each of two sides of each flower, and six flowers were brushed in this manner on any one day. The brushing score is the number of petals dislodged by each stroke. For most of the varieties examined, the test was applied on at least two occasions.

Figure 2.1a: The brushing test – bending back the petals with the index

Figure 2.1b: The brushing test – brushing the flower at right angles

II. The Petal Detachment Force Technique

The petal detachment force technique was adapted from a method described by Moebius-Clune et al. (2008) for intact soil core penetration resistance measurement in the laboratory. This device consists of a scale that is connected to a computer. The flower was held face up on a scale by a weight of 2kg draped across the upper pedicel. An alligator clip inserted into the drill press chuck was attached to a random petal. The scale was tared to zero. The drill press lever was slowly manually raised (at about 0.5cm/sec) and the force recorded on the computer (onto an Excel spreadsheet) each second for data capture.

The lowest recorded value before breakage was used to represent the "breakstrength." This value represents the force needed to detach that petal from the receptacle of the flower. The force required to remove petals from sunflower heads of various sunflower genotypes was measured at different stages of flower life. A minimum of 4 petals was pulled on each flower head and the readings averaged. Data was analyzed using analysis of variance (ANOVA) on

JMP 10 statistical software (http://www.jmp.com/industries/manufacturing/). Petal detachment force was the dependent variable while sunflower variety and day of harvest were the independent variables.

Figure 2.2: The petal break strength meter (purpose built for this study): pulling on the petal while the flower is pinned to an electronic weighing scale measures the weight which is recorded directly into an Excel spread sheet in a computer

Figure 2.3: A close up of the petal detachment process showing the flower being held down face up by a 2kg weight and the alligator clip attached to a random petal The flower is resting on a scale, connected to a computer which measures the force reduction on the load cell as the petal is pulled slowly upward.

Figure 2.4: The Petal Pulling Machine v.2 (purpose built for this study)

Research objectives

1.) To measure varietal susceptibility to petal drop in different sunflower cultivars

2.) To confirm varietal susceptibility to petal loss using detachment force technique

3.) To characterize the age at which petals become susceptible to abscission

4.) To study the morphology of flowers susceptible and those resistant to petal loss

Materials and Methods

The plants were started in greenhouses from seed in seedling trays in redi-earth artificial soil mix, at recommended temperatures (19-25^{o}C). Seedlings were transferred to the field (with an Arkport Sandy Loam soil type) in 4 rows, with a spacing of about 23cm between plants and rows. Different sunflower varieties were harvested within 1 hour of the flower opening. The stems were immediately placed in water and taken to the laboratory where detachment force measurements were made using the petal breakstrength meter. In another experiment to evaluate the age at which sunflower genotypes became susceptible to petal loss, cut flowers were harvested within one hour of the flower opening. They were left in distilled water in glass vases in a post-harvest room at a temperature of 20^{o}C and 60% RH. Detachment forces were measured on days 1, 3, 6, 9, and 12.

For the brushing test, six flowers were brushed on any one day. The brushing score is the number of petals dislodged by each stroke. For most of the varieties examined, the test was applied on at least two occasions.

The force required to remove petals from the heads of various sunflower genotypes was measured at different stages of flower life. A minimum of 4 petals was pulled from each head and the readings (which were recorded directly into a computer) averaged. Seven to ten replicates per variety were used in all experiments.

To develop a regression equation that could predict vase life, breakstrength measurements were obtained for 13 sunflower varieties on Day 1, 3, 6, 9 and 12. Cut sunflowers were harvested at a commercial stage (within one hour of the flower opening and the petals flattening out) and placed in a post-harvest room (at 20 ^{o}C and 60%RH). Peduncles were cut to a length of 40cm and placed in clean vases with distilled water. Vases were washed and water changed every other day or as soon as it became dirty / cloudy. A minimum of four petals were pulled on each side of the flower head and the readings averaged. At least seven replicates were used per cultivar. A total of 2056 measurements were recorded. The variables analyzed were petal detachments force, sunflower variety, time (in days), vase life (in days) and color. Means were separated using Tukey's HSD adjustment test on JMP 10 statistics (http://www.jmp.com/industries/manufacturing/).

Relationship between head diameter, petal length and drop rates

To find out if there were relationships between head diameter, petal length and drop rates, an average of 17 flowers each of three varieties: Sunrich Orange, Strawberry Blonde and Moulin Rouge were used. Four petals were pulled on each flower head. Head diameter of the flower, petal length and detachment force of the petal was recorded. Head diameter and petal length as they relate to drop rates are of major interest to agronomists and breeders because they influence procedures and crossing techniques (Fick, 1989). We observed that the varieties that were more prone to drop their petals early had smaller heads, slimmer and longer petals. So we were trying to determine if these factors (head diameter, petal length) had any effect on drop rates (detachment force).

Results and discussion

The brushing test revealed that some cultivars have petals that are held tightly, and none are dislodged, but others consistently lose 3 or more petals when the edge of the finger is brushed against the flower (Table 2.1). Two of the most susceptible to petal loss were Chianti and Procut Bicolor, confirming the general impression that varieties with darker petals tend to be more abscission-prone. However, there are exceptions to that rule, as seen with varieties like Sunrich Gold, Sunbright and Sunrich Orange Summer. Double Quick Orange also appears to be susceptible, but this is the only cultivar tested that has many thin ray flowers that can inflate the total of abscised petals when brushed. Although this test gave a general indication of petal loss susceptibility, we decided to refine the test by using a less subjective technique, the break strength meter (Figure 2.2).

Table 2.1: The effect of brushing against the petals of open sunflower flowers on petal abscission, 2006. The brushing score is the number of petals dislodged with each brush stroke. Six flowers were brushed on any one day. For most of the varieties examined, the test was applied on at least two occasions.

Cultivar	Brushing score	Standard dev.
Procut Yellow Lite	0.29	0.42
Valentine	0.4	0.47
Sunny	0.42	--
Ring of Fire	0.51	0.38
Full Sun Improved	0.53	0.3
The Joker	0.63	0.47
Sonya	0.69	0.55
Procut Peach	0.76	0.58
Sunrich Orange	1.05	0.29
Sunbright Supreme	1.11	0.5
Florenza	1.28	1.08
TH 742	1.42	1.09
Premier Yellow	1.82	0.8
Premier Light Yellow	1.83	1.2
Moonbright	1.92	--

Cultivar	Brushing score	Standard dev.
Procut Lemon	1.94	0.54
Sunrich Gold	2.12	1.95
Sunbright	2.12	1.94
Strawberry Blonde	2.62	1.22
Sunrich Orange Summer	2.62	2.01
Chianti	3.33	--
Double Quick Orange	3.47	3.16
Procut Bicolor	3.54	0.37

Varietal susceptibility to petal loss was tested using the breakstrength meter in 2007, 2008 and 2009. Twenty-three cultivars were tested in 2006 using the brushing test while seventeen cultivars were tested in 2007, 2008 and 2009 using the detachment force technique. The results from both techniques in all 4 years show that there were highly significant differences in varietal susceptibility to petal abscission among the cultivars tested using student's t-test (Table 2.2). The results from the detachment force technique were similar to those of the brushing test carried out in 2006 (Table 2.1). The results show repeatedly that the dark red and bicolored varieties (Moulin Rouge, Strawberry Blonde, Cherry Rose and Procut Bicolor), were most prone to lose petals while the yellow to orange flowers (Procut Lemon, Procut Yellow Lite and Sunrich Orange) were less likely to lose their petals at an early age. In 2006 to 2008, we measured varietal susceptibility to petal drop at one stage of flower development (within 1 hour of the flower opening and the petals flattening out). However, our experiments in 2009 were more focused on trying to determine if there was a correlation between varietal differences in petal drop tendencies and vase life. At what age did abscission begin in these different cultivars? To answer this question, in 2009, we measured petal drop at different stages of flower development.

Table 2.2: Pair-wise comparison of varietal susceptibility of sunflower varieties based on petal detachment force (in grams), arranged in a decreasing order. The higher the number, the harder it is to pull out the petals. Four petals were pulled from each flower. Detachment forces were measured within one hour of the flower opening. The test was applied on at least three occasions for each variety.

2007		2008	
Varieties	**Mean Force**	**Varieties**	**Mean Force**
Sunrich Orange	263.09a[z]	Procut Lemon	249.83a
Procut Lemon	260.03a	Procut Yellow	246.77a
Tosca	229.44b	Procut Yellow Lite	241.67a
Procut Yellow Lite	218.22b	Premier Lemon	237.59b
Procut Apricot Lite	214.14b	Procut Early Orange	225.36b
Procut Orange	202.92b	Tosca	208.02b
Procut Early Orange	192.73b	Sunrich Orange	194.77b
Apricot Lite	186.61b	Procut Apricot Lite	187.63b
Procut Red Lemon Bicolor	184.57b	Orange King	182.53b
Orange King	184.57b	Procut Red Lemon Bicolor	167.23b
Orange Glory	182.53b	Sun for You Bicolor	151.94b
Premiere Lemon	179.47b	Procut White Lite	147.86b
Procut Peach	159.08b	Procut Peach Blush	139.70c
Procut Peach Blush	138.68c	Procut Bicolor	129.50c
Procut Bicolor	118.29c	Cherry Rose	100.95c
Strawberry Blonde	117.27c	Moulin Rouge	98.91c
Moulin Rouge	91.77c	Strawberry Blonde	91.77c

[z]Means followed by the same letter are not significantly different at $p < 0.05$.

The petal break strength meter measures the force required to detach a petal from the receptacle of the flower. The results from the detachment forces revealed that while some sunflower varieties are tightly attached to the receptacle of the flower when opened, and are difficult to pull out thus producing higher detachment force readings, others are loosely attached and can be removed with a lower force (Table 2.2). The results from the separation of means shown in Table 2.2 above arranges the varieties in order of susceptibility with the less susceptible varieties appearing at the top of the table while the more susceptible cultivars are at the bottom. The results also revealed that the varieties with dark red and bicolored petals (Cherry Rose, Moulin Rouge, Procut Bicolor, Strawberry Blonde, Procut Peach Blush), were more susceptible to petal drop than the yellow or orange colored ones (Table 2.2). The more

susceptible varieties based on the pulling force measurements that ranged from 91.77 to 138.68g in 2007 were Moulin Rouge, Strawberry Blonde, Procut Bicolor and Procut Peach Blush. The more susceptible varieties based on pulling force measurements that ranged from 91.77 to 129.50g in 2008 were Strawberry Blonde, Moulin Rouge, Cherry Rose and Procut Bicolor.

The least susceptible (resistant) varieties with pulling forces ranging from 208.02 to 249.83 grams in 2008 include Procut Early Orange, Premier Lemon, Procut Yellow Lite, Procut Yellow and Procut Lemon. In 2007, the resistant varieties were Sunrich Orange, Procut Lemon and Tosca. The probability of finding dark red and bicolored varieties with the lowest force of detachment was quite high, but this was not always the case. As can be seen from the table, Procut White Lite, Sun For You Bicolor and Procut Red Lemon Bicolor also produced lower detachment forces although not as low as the most susceptible cultivars. The moderately susceptible varieties with pulling force ranging from 147.86 to 194.77 grams in 2008 included Procut White Lite, Sun For You Bicolor, Procut Red Lemon Bicolor, Orange King, Procut Apricot Lite and Sunrich Orange. In 2007, the moderately susceptible varieties had detachment forces ranging from 229.44 grams (Tosca) down to 159.08 grams (Procut Peach).

We preferred the petal detachment force technique for our research because it was quantitative and an easily reproducible way of measuring petal abscission.

After repeated measurements of the detachment forces of different sunflower cultivars, we were able to group sunflower cultivars into different categories based on their susceptibility to petal abscission by using their detachment forces (Table 2.3).

Table 2.3: Varietal susceptibility to petal drop in sunflowers

Least Susceptible	Moderately Susceptible	Susceptible	Most susceptible
Procut Lemon	Tosca	Apricot Lite	Procut Bicolor
Procut Yellow Lite	Premiere Lemon	Procut Red Lemon Bicolor	Strawberry Blonde
Sunrich Orange	Procut Apricot Lite	Orange King	Moulin Rouge
Procut Yellow	Procut Orange	Orange Glory	Cherry Rose
	Procut Early Orange	Premier Lemon	Chianti
		Procut Peach	
		Procut Peach Blush	
		Sun For You Bicolor	
		Procut White Lite	

Regression analysis on petal breakstrength data

The results of the data analyses presented below were carried out using JMP 10 statistical software. The data were obtained from breakstrength experiments carried out on 13 sunflower varieties. The aim was to develop a regression equation that could predict vase life of a sunflower variety using physical characteristics of color and petal break strength. In the analysis, the innate physical characteristic of color was used to distinguish the different sunflower varieties rather than their varietal names. It was felt that the varietal name was an extrinsic characteristic and therefore not a suitable independent proxy for the physical characteristics of the sunflower species. Color being an independent physical characteristic of sunflowers permits an aggregation of the 13 sunflower varieties used in this study into major color groups shown in Table 2.4 below. The experimental data analyzed to obtain the results shown below is provided in the appendix. The regression analysis of breakstrength data shown below is grouped by sunflower variety, color and vase life.

Table 2.4: Color category and coding of 13 sunflower varieties

Variety	Code	Color Category
Chianti	CH	Red Group
Cherry Rose	CR	Red Group
Moulin Rouge	MR	Red Group
Procut Bicolor	PBC	Bicolor Group
Procut Red Lemon Bicolor	PRLB	Bicolor Group
Strawberry Blonde	SB	Bicolor Group
Orange Glory	OG	Orange Group
Procut Early Orange	PEO	Orange Group
Procut Orange	PO	Orange Group
Procut Peach	PP	Orange Group
Sunrich Orange	SO	Orange Group
Procut Lemon	PL	Yellow Group
Procut Yellow Lite	PYL	Yellow Group

1. Results of regression analysis on breakstrength (force) data by variety

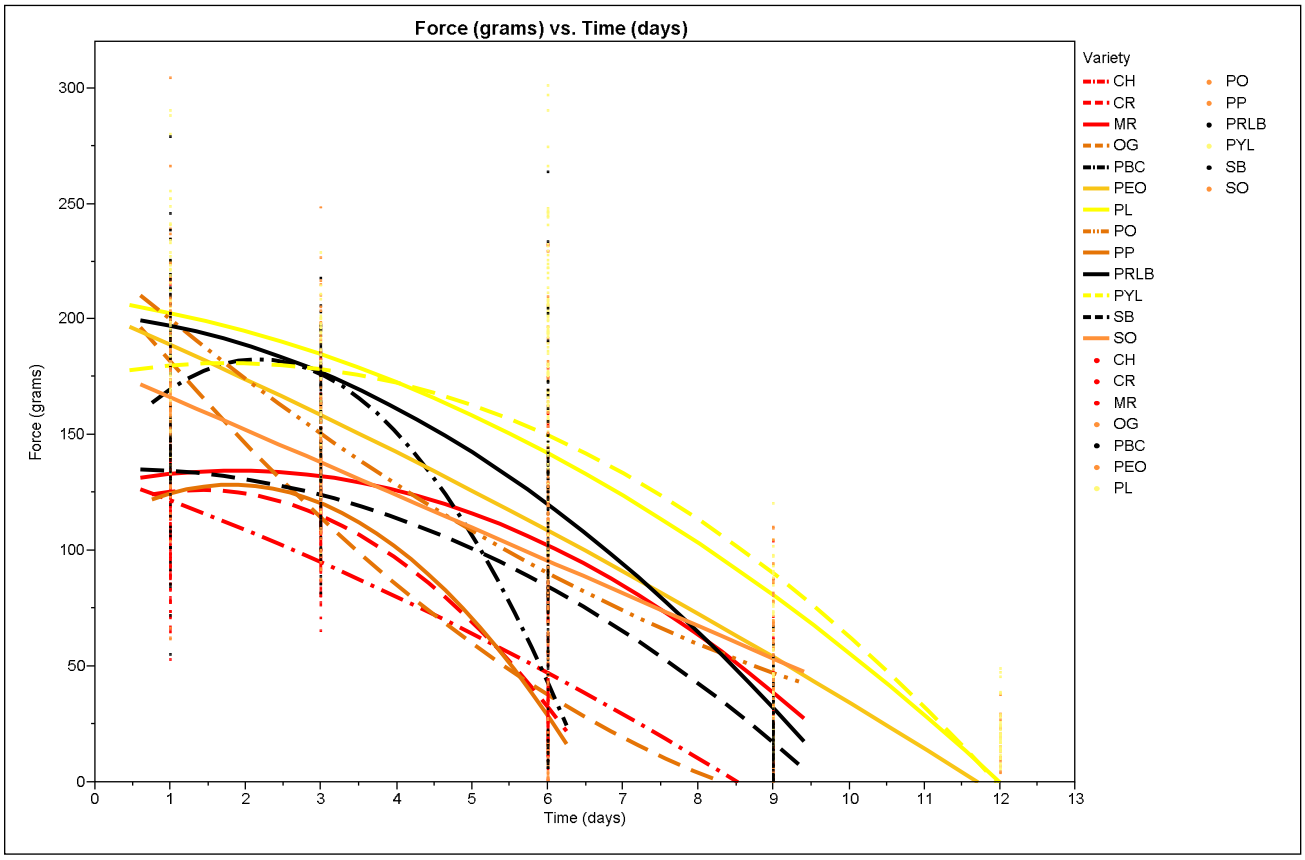

Figure 2.5: Regression plot of the change in breakstrength with time of the 13 sunflower varieties in this study

2. Regression analysis of breakstrength data grouped by color

Table 2.5: Relationship between flower color and petal breakstrength for 13 sunflower varieties. Mean separation was by Tukey's HSD adjustment test (n=2056).

	Color category			
Least square means	Yellow	Orange	Bicolor	Red
	122.46a[z]	111.93b	108.37b	91.85c

[z]Means followed by the same letter are not significantly different at p<0.05

Table 2.5 shows that the mean break strength of varieties in the yellow group are higher and significantly different (at p<0.05) from varieties in the orange and bicolor groups, which are in turn higher and significantly different from varieties in the red group.

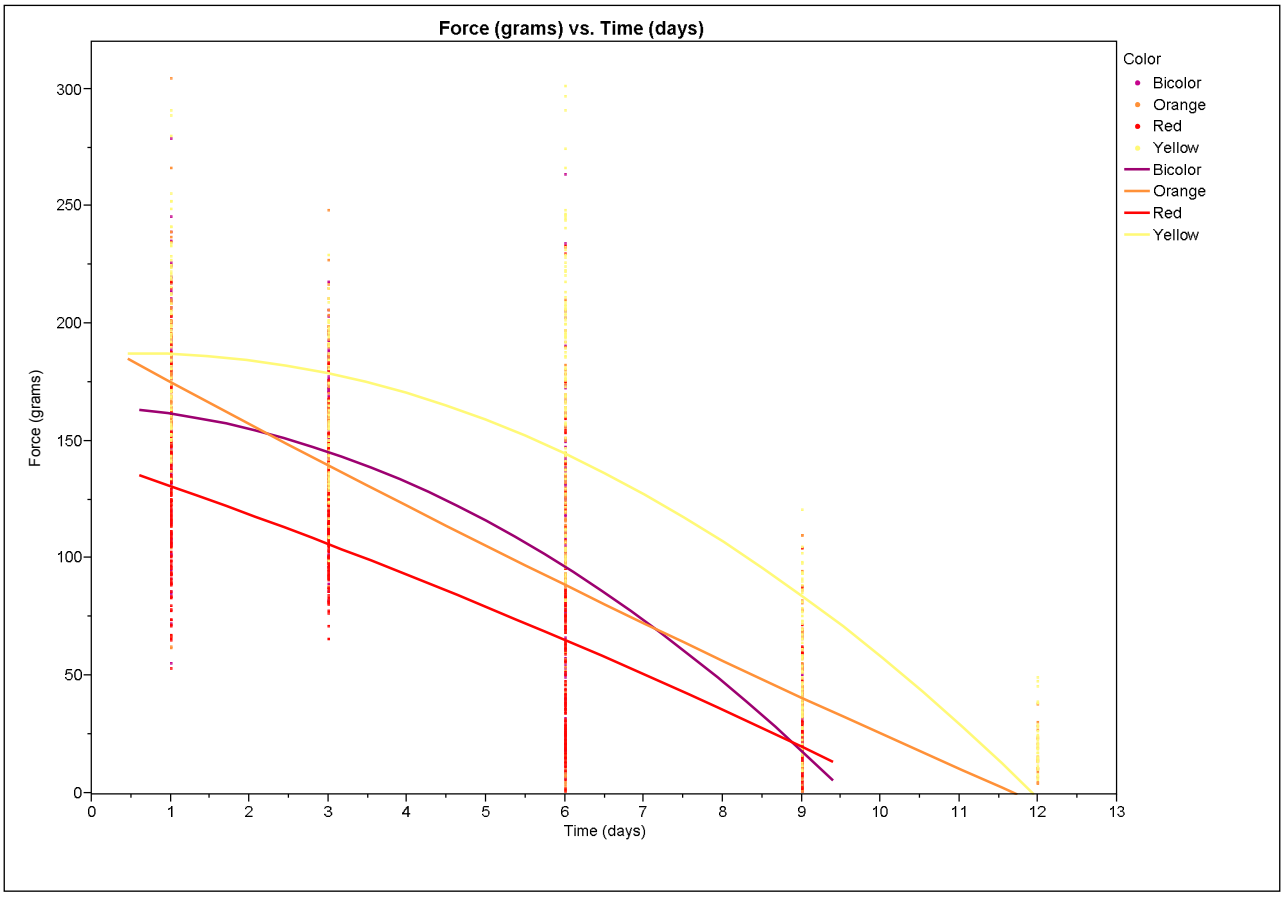

Figure 2.6: The change in breakstrength with time of the 13 sunflower varieties grouped by color

3. Regression of breakstrength by vase life data grouped by time after harvest

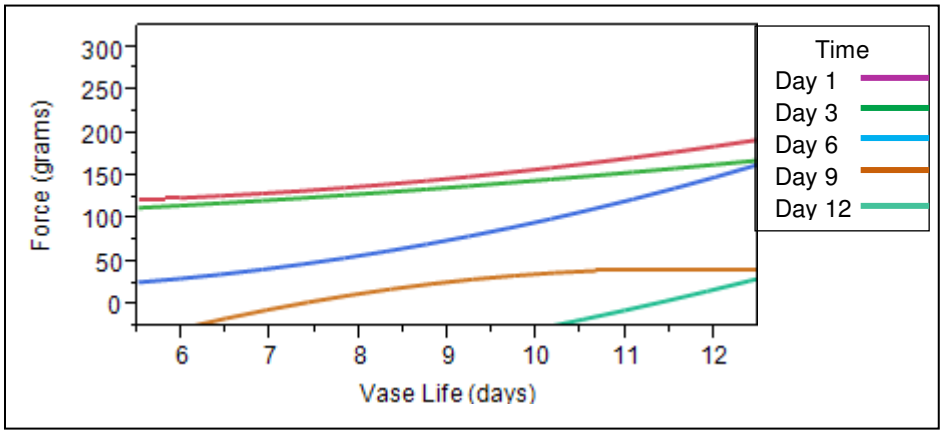

Figure 2.7: Breakstrength by vase life grouped by time after harvest

Based on the parameter estimates and the quadratic prediction model, the regression equation for predicting vase life of the sunflower varieties in this study, from breakstrength values obtained 1 and 6 days after harvest are:

$$Force_{(day1)} = 126.83 - 5.38(vase\ life) + 0.85(vase\ life)^2 \qquad \text{(Equation 1)}$$

$$Force_{(day6)} = 29.03 - 9.17(vase\ life) + 1.6(vase\ life)^2 \qquad \text{(Equation 2)}$$

Table 2.6: Observed and predicted vase life computed using regression equation

Variety	Average Force (day 1)	Average Force (day6)	Observed Vase life	Predicted vase life (force @ day1)	Predicted Vase life (force @ day6)
CH[z]	108.09	29.57	9	7	6
CR	125.43	32.63	6	6	6
MR	130.52	95.85	9	7	10
OG	168.25	12.24	9	11	4
PBC	170.29	42.83	6	11	7
PEO	178.45	120.33	12	12	11
PL	215.16	169.27	12	14	13
PO	202.92	92.79	9	13	10
PP	124.41	28.55	6	6	6
PRLB	204.96	134.60	9	13	12
PYL	181.51	170.29	12	12	13
SB	134.60	95.85	9	8	10
SO	168.25	99.93	9	11	10

[z]CH: Chianti, CR: Cherry Rose, MR: Moulin Rouge, OG: Orange Glory, PBC: Procut Bicolor, PEO: Procut Early Orange, PL: Procut Lemon, PO: Procut Orange, PP: Procut Peach, PRLB: Procut Red Lemon Bicolor, PYL: Procut Yellow Lite, SB: Strawberry Blonde, SO: Sunrich Orange

Table 2.7: Correlation between observed and predicted vase life based on values in Table 6

		Observed V[z]	Predicted V1[y]	Predicted V2[w]
Observed Vaselife	Pearson Correlation	1	.616*	.717**
	Sig. (2-tailed)		.025	.006
	N	13	13	13
Predicted Vaselife 1	Pearson Correlation	.616*	1	.608*
	Sig. (2-tailed)	.025		.028
	N	13	13	13
Predicted Vaselife 2	Pearson Correlation	.717**	.608*	1
	Sig. (2-tailed)	.006	.028	
	N	13	13	13
*. Correlation is significant at the 0.05 level (2-tailed).				
**. Correlation is significant at the 0.01 level (2-tailed).				

[z]Observed V: Time after harvest at which all the petals have completely fallen off
[y]Predicted V1: Values for vase life based on vase life regression equation 1 above. The values for predicted V1 are shown in Table 2.6
[w]Predicted V2: Values for vase life based on vase life regression equation 2 above. The values for predicted V2 are shown in Table 2.6

Table 2.7 above shows that breakstrength data obtained 6 days after harvesting is a better predictor of vase life compared with breakstrength data obtained 1 day after harvest.

Table 2.8: Relationship between flower color and vase life (in days) for 13 sunflower varieties. Mean separation was by Tukey's HSD adjustment test (n=2056).

	Color category			
Least square means	Yellow	Orange	Bicolor	Red
	12.0a[z]	9.93b	9.0b	8.13c

[z]Means followed by the same letter are not significantly different at $p<0.05$

Table 2.8 shows that the mean vase life (12 days) of the sunflower varieties in the yellow group is longer than varieties in the orange group (10 days), which in turn are higher and significantly different from the bicolor group (9 days), which are in turn higher and significantly different

from varieties in the red group (8 days). The orange and bicolor groups do not differ. Thus vase life has a strong relationship with flower color, with the darker varieties in this study having a shorter vase life compared with the lighter varieties.

Relationship between head diameter, petal length and drop rates

Our results show that there were no significant differences between head diameter, petal length and drop rates at $p<0.05$ (Table 2.9). This means that there was no meaningful relationship between head diameter, petal length and drop rates. Head diameter and/or petal length do not affect the tendency of sunflowers to drop petals.

Table 2.9: Relationship between head diameter, petal length and drop rates

	Fixed Effects Tests			
Source	DF	DFDen	F Ratio	Prob>F
Variety	2	48.97	3.42	0.0409*
Head Diameter(cm)	1	51.82	1.39	0.2437
Petal Length(cm)	1	191	1.86	0.1743

The breakstrength meter technique used in this study has been used by other authors to study vase life in other flowers species (McKenzie and Lovell, 1992). However, it has never been tried on sunflowers. The vase life for sunflower varieties can be determined by using the prediction equation we derived in this study. When the prediction equation was used to determine the vase life of sunflower varieties, the results were similar to vase life results found by other authors (Gast, 1995; Yaňez et al., 2005)

Conclusion and future studies

A proper understanding of the varietal differences in susceptibility to petal drop among sunflower cultivars and reliable testing methods are crucial and beneficial to breeding programs directed towards the improvement of sunflower longevity. This knowledge will also help growers to increase overall sales of sunflowers. The petal breakstrength meter provides a quantitative and easily reproducible way of measuring varietal susceptibility to petal drop in sunflowers. A faster way is just to brush against the flower head near the base of the petal, but this method is easily biased. The petal break strength meter was also used to determine the start of abscission and the end of vase life of 17 sunflower varieties. We found a relationship between varietal susceptibility to petal drop and vase life in sunflowers. More experiments on post-harvest conditions as they relate to drop tendency amongst sunflower cultivars are recommended. We found no relationship between head diameter, petal length and abscission tendency in sunflowers.

For future studies, detachment force experiments should be repeated with more sunflower cultivars. In our experiments to obtain an equation for vase life, we measured detachment force on Days 1, 3, 6, 9 and 12, in future experiments, detachment forces should be measured every day for 12-14 days. Other morphological characteristics such as head shape, sepal/bract style as it affects drop rates should be carried out. Also, research to understand the breeding lines used in the development of the red, dark colored and bicolor varieties should be done. What is the cause of the susceptibility to petal drop which seems to be inherent in the red and dark colored lines? Is it possible for breeders to use parents which are not susceptible to abscission in their crossings?

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Chapter 3: Endogenous Phytohormones in Sunflowers and Their Relation to Petal Drop

Introduction

This chapter deals with the quantification of endogenous levels of cytokinins, abscisic acid and indole-3-acetic acid in abscission zone tissues of sunflower petals in varieties that differ in their tendency to drop petals. Cytokinins [zeatin + zeatin riboside (Z +ZR) and iso-pentenyl adenosine (iPA)] and abscisic acid (ABA) were quantified using enzyme-linked immunosorbent assay (ELISA) while indole-3-acetic acid (IAA) was quantified using liquid chromatography-mass spectrometry (LC-MS) technique. All phytohormones were measured within 1 hour of the flower opening. The chapter begins with a brief review of literature, followed by the test for ethylene sensitivity in sunflowers and the quantification of phytohormones in abscission zone tissues of sunflower petals. Finally, an evaluation of the effect of growth hormones (cytokinins) on petal drop rates in sunflowers was carried out and the results presented here.

Davies (2004) defined plant hormones as a group of naturally occurring, organic substances that influence physiological processes at low concentrations. It has now been well established that natural abscission of flowers is regulated by endogenous plant hormones, although little attention has been given to their quantification in petal abscission zones. There is a limited amount of literature on the quantification of phytohormones in sunflower petal abscission zone tissues.

Ethylene plays an important role in the senescence of many cut flowers (Kim et al. 2007; van Doorn, 2001; van Doorn, 2002; van Doorn and Stead, 1997 Woltering and van Doorn, 1988). Woltering and van Doorn (1988) studied petal senescence in 93 species from 22 families and the sensitivity of these species to exogenous ethylene. Their result showed that petal abscission in all tested species from the Asteraceae family was ethylene insensitive. These results

have not been verified for sunflowers. In our study of petal drop in sunflowers, we decided to start by checking the effect of exogenous ethylene on petal drop rates and to determine if petal drop in sunflowers was ethylene dependent.

As far back as 1972, Mayak and Halevy reported an increase in ABA levels during flower senescence in roses. In another study, endogenous ABA was analyzed during the vase life of cut rose flowers, and the results showed a decrease in ABA levels in rose petals during the first 3 days followed by a steady low level and then an increase in late senescence. The same authors reported an inverse relationship between ABA levels in the petal and flower longevity. They showed that the higher the ABA levels at harvest, the shorter the subsequent vase life.

Cytokinins are adenine derivatives with a unique characteristic of inducing cell division in tissue culture. The most common cytokinin in plants is zeatin. Cytokinins can also occur as ribosides and ribotides (Davies. 2004). Cytokinins occur in roots and in developing seeds. They are known to be synthesized in the roots and then transported via the xylem to the shoots and other developing plant parts (Davies, 2004). The action of cytokinin in plants depends on the type of cytokinin, its internal translocation of and regulation by other growth regulators such as auxin and ethylene (Mor et al., 1983; Staden et al., 1987).

Mayak and Halevy (1970) reported that cytokinin activity was higher in young rose petals than in old ones and that the content of endogenous cytokinin was lower in petals of a short-lived rose variety than in a long-lived variety. Application of a cytokinin directly to the flower buds of the short-lived rose variety retarded senescence (Mayak and Halevy, 1970). Cytokinins also delayed senescence of carnation flowers (Staden and Joughin, 1988), inflorescences of *Grevillea* ‘Sylvia’ (Setyadjit et al., 2004), but this has not been tested on cut sunflowers. A further study on the correlative changes in phytohormones in relation to

senescence processes in rose petals by Mayak and Halevy (1972) showed a decrease in the activity of cytokinins and a simultaneous increase in abscisic acid and ethylene in senescing petals of two rose cultivars. Staden et al. (1987) detected the presence of cytokinins in the petals of carnation flowers. Their study further revealed that the basal part of the petals which had a higher cytokinin activity senesced more slowly than the upper parts of the petals. Lukazewska et al. (1994) detected the presence of two groups of cytokinins in rose petals and indicated that these cytokinins delayed flower senescence when they were applied as holding solutions.

Literature shows that the mechanism of cytokinin-induced delay of senescence involves a reduction in the production of ethylene and a decrease in the sensitivity of petals to ethylene (Staden et al., 1987; Setyadjit et al., 2004). In a study by Chang et al. (2003), petunia flowers (*Petunia x hybrid* cv V26) transformed with a cytokinin biosynthetic gene for extended flower longevity, were used to study the effects of elevated cytokinin content on ethylene synthesis and sensitivity and ABA accumulation in petunia corollas. Their results showed a delay in floral senescence in the transformed lines by 6 to 10 days compared to the wild type flowers. The researchers induced endogenous ethylene production by pollination in both transformed and wild type corollas and they found a delay in endogenous ethylene production in the transformed flowers compared to wild type. They also found a significantly higher accumulation of ABA in the wild type flowers compared to transformed flowers. Finally, the transformed flowers were less sensitive to exogenous ethylene compared to the wild type. These results indicate the different hormone interactions that regulate flower senescence.

Mor et al. (1983) reported that peak ethylene production was reduced from detached outer whorl of carnation 'White Aim' petals that had been pulsed for 24 hrs with 0.1 mM Benzyladenine (BA). A range of studies using natural and synthetic cytokinins show that these

chemicals have the potential of delaying senescence of cut flowers, but the results have been variable. Stadena and Joughin (1988) found that a low concentration of BA accelerated senescence in cut carnation flowers while higher concentrations retarded senescence.

Very little is known about auxin effects in petal abscission of sunflowers. However, it has been established that a basipetal flux of IAA through the abscission zone prevents abscission by causing the abscission zone to become insensitive to ethylene (Woodward and Bartel, 2005). Abscission can be prevented by the continuous polar supply of IAA to the abscission zone (Taylor and Whitelaw, 2001), and the removal of the IAA source results in the sensitivity of the abscission zone to ethylene and abscission commences (Addicott, 1982; Meir et al., 2003; Sexton and Robert, 1982). Guinn and Brummett (1987) reported that IAA content of abscission zones was positively correlated with boll retention in cotton. They showed that the concentration of IAA in cotton abscission zones was highest when boll retention was highest and lowest when boll retention was lowest. Rodgers (1981) used a bioassay to determine IAA levels in cotton bolls and his results revealed that abscising bolls had lower levels of IAA compared to retained bolls. The hypothesis that the abortion of unfertilized flowers may be due to low auxin content and production in the ovary has been investigated. Yager and Muir (1985) reported a lower quantity of ovary auxin in unpollinated tobacco flowers compared to pollinated ones. Muir (1942) reported that the ripening of tobacco ovaries produced an increase in diffusible auxin.

Exogenous application of auxins has been shown to prevent or delay flower abscission in Lupinus sp. (Aarts, 1957; Warne, 1947), Geraldton wax flowers (Joyce, 1989), and apple (van Overbeek, 1952). Auxin also delayed the onset of petal abscission in *Linum lewisii* by about 5 h (Addicott, 1977). Nevertheless, at certain concentrations, auxins may increase rather than decrease abscission of flowers (Abeles et al., 1992). Armitage et al. (1980) reported that auxin

failed to delay petal abscission in *Pelargonium x hortorum*. McKenzie and Lovell (1992b) found that auxin accelerated petal abscission in *Crocosmia*. Overall, there is very little in literature on phytohormones and sunflower abscission.

Objectives of phytohormone study:

1. To verify if petal drop in sunflowers is ethylene insensitive
2. To quantify the levels of trans-zeatin (Z) + trans-zeatin riboside (ZR), iso-pentenyl adenosine (iPA), abscisic acid (ABA) and indole-3-acetic acid (IAA) in abscission zone tissues in a range of sunflower genotypes that differ in their abscission tendencies
3. To determine if differences in the levels of phytohormones measured within one hour of the flower opening are correlated with differences in abscission tendencies in the genotypes tested
4. To check the effect of commercial cytokinin products on petal drop rates in sunflowers

Research hypotheses:

1. Petal drop some sunflower varieties is independent of ethylene
2. Endogenous cytokinin levels will differ between genotypes in a way that correlates with differences in tendency to drop petals
3. Petal drop in sunflowers can be delayed by external application of cytokinins

Materials and Methods

I. Ethylene sensitivity experiments

Cut sunflower of Strawberry Blonde (SB) a variety which has a tendency to drop its petals early were used. The plants were started in greenhouses from seed in seedling trays at recommended temperatures for the species. They were then transplanted to the field in 4 rows at 9 inch spacing between plants and rows. Fully opened flowers were harvested and immediately placed in water. They were transported to the postharvest room with a temperature of 20 ^{o}C and 60% RH with 12 hrs fluorescent lighting for 2 hrs before treating with ethylene. The stems were cut to a length of about 30 cm and immediately placed in clean vases with distilled water. The flowers were placed in 20 liter air tight buckets at 20^{o}C in darkness, and then treated with ethylene. Flowers were left in water during the ethylene treatments. Control flowers were placed under identical conditions in an ethylene-free environment.

Ethylene Treatment

Ten parts per million (ppm) ethylene at a flow rate of 250µl/min, was applied to plants in the air tight buckets for 24 hrs. The temperature of the growth chamber was kept at 20 ^{o}C. All treatments were left in darkness for 24 hrs. Six plants (three in each vase) were used in each treatment. Plants were taken out of the treatment after 24 hrs. Petal detachment forces were measured 48 hrs after treating with ethylene (see Fig 1 and Table 1 below). Data were analyzed using one-way analysis of variance (ANOVA) with the JMP software, where petal detachment force was the dependent variable while sunflower variety and ethylene treatment were the independent variables. (http://www.jmp.com/industries/manufacturing/). These experiments were repeated at least three times using different concentrations of ethylene (3 ppm and 6 ppm),

and twice using 10 ppm ethylene, and the results were the same. We have presented here results from the experiment using 10 ppm ethylene for Strawberry Blonde.

II. Measuring petal abscission

Petal abscission was studied by a determination of the force required to detach the petal from the receptacle of the flower using separation force measurements. Many researchers (Fernandez et al., 2000; McKenzie and Lovell, 1992; Patterson and Bleecker, 2004) have used detachment force methods to study abscission in plants. Separation forces were measured in these experiments using a modification of a soil core micro-penetrometer apparatus. This device consists of a scale which is connected to a computer. The flower was held down face up on a scale by a weight of 2 kg draped across the upper stem. An alligator clip inserted into the drill press chuck was attached to a random petal. The scale was tared to zero. The drill press lever was slowly raised (at about 0.5 cm/sec) and the force recorded on the computer each second. The highest negative recorded value before breakage was used to represent the "break strength." This value represents the force needed to detach that petal from the receptacle of the flower. The force required to remove petals from sunflower heads of Strawberry Blonde (SB) and their controls were measured 24 hrs after treating with ethylene. A minimum of 4 petals were pulled on each flower head and the readings averaged. Results were analyzed using analysis of variance (ANOVA) on JMP, where petal detachment force was the dependent variable while sunflower variety, day (time after harvest) and vase life were the independent variables.

III. Enzyme-linked Immunosorbent Assay (ELISA)

For ELISA analysis, abscission zone tissues of ray florets from 5 sunflower genotypes were collected. All abscission zone tissues were taken from flowers within 1 hr of the flower opening. We randomly selected 3 flower heads from each genotype, and harvested abscission zones of ray florets from these flowers. Abscission zone tissues were collected in small aluminum foil boxes, immediately frozen in liquid nitrogen in a styrofoam box and then transferred from liquid nitrogen to -80^{o}C where they were stored until extraction. All plants were grown in one batch. Harvesting of abscission zone tissues of all genotypes and freezing in liquid nitrogen took place within one week in August 2009.

For extraction, abscission zone tissues were first homogenized in liquid nitrogen by grinding using a mortar and pestle. The grinding consisted of first adding liquid nitrogen into a mortar, tissue was then taken out of -80^{o}C refrigerator where they had been stored and immediately placed in the liquid nitrogen in the mortar and ground with a pestle. One hour before extraction, 80% methanol was placed in ice in a styrofoam box; 10 ml of this 80% methanol was put into 50 ml pre-labeled tubes and placed in ice; then 0.5 g of ground tissue was added into the 80% methanol in 50 ml tubes. The tubes were left in ice for 60 min; all samples were removed after 60 min and put on a rotary shaker at 150 rpm speed at room temperature for 3-4 hrs. The samples were centrifuged (centrifugation speed up to about 20,000rpm), and the resulting supernatant was transferred into 12 ml tubes. Two milliliters of 80% methanol were added to the plant tissue and again placed on the shaker for 15-30 min. Supernatant was decanted into the first tube as before; samples were then kept in -4 ^{o}C until the following day. One milliliter aliquot each of extract was transferred to a 96-well plate and dried with turbulent air-flow at 35-40^{o}C.

Cation-exchange chromatographic separation of zeatin-related compounds used columns made from phenyl sulfate-coupled to a 40-µm-diameter surface-modified styrene divinyl benzene solid support (model DSC-18 Strata-X-C, Phenomenex, Torrance, CA). Columns contained 25 mg packing material and were formatted in a 96-well arrangement. Samples were redissolved in 1200 µL of Solvent A (30% methanol + 0.2 M formic acid) and 100-µL aliquots were loaded into 12 columns; columns were washed with 200 µL of Solvent A and then with 400 µL of Solvent B (65% methanol + 0.2 M formic acid); cytokinins were eluted with 400 µL of Solvent C (65% methanol + 0.35 M NH_4OH). The resulting fractions for ABA and IAA were collected and stored under -80 degrees, while the fractions for cytokinin compounds were further purified using HPLC. ELISA was then performed on both fractions. Data were analyzed using analysis of variance (ANOVA) on JMP software.

ELISA method (same as described above) was used in our preliminary experiments in 2008 to analyze zeatin. Here zeatin was measured in three sunflower cultivars: Procut Bicolor, Strawberry Blonde and Procut Yellow Lite at two stages of flower growth: 24 hours after flower opening and 8 days after harvest for PBC and SB and 14 days after harvest for PYL.

IV. Exogenous application of cytokinins on petal drop rates

Cut sunflowers (30 cm stems) harvested at a commercial stage were used throughout these investigations. The plants were started in greenhouses from seed in seedling trays at recommended temperatures for the species. They were then transplanted to the field in 4 rows at 22 cm spacing between plants and rows. Some preliminary experiments were first carried out. In this first experiment, we dipped sunflower heads in Benzyladenine (a cytokinin) solutions at different concentrations for 5 min and immediately measured petal detachment forces. Seven

flowers of Strawberry Blonde (SB) variety were used per treatment and 4 petals were pulled from each flower head. The results we obtained from using BA as dips in this first experiment were positive. Our results showed that petals treated with BA had significantly higher detachment forces than untreated ones. These results encouraged us to do a more thorough search on commercial cytokinin products that could be potential agents in extending the vase life of sunflowers. We came across two of such commercial cytokinin products (BAP-10 and Fascination). BAP-10 is an N-6-Benzyladenine solution (cytokinin) manufactured by Plant-Wise Biostimulant Co. (www.plant-wise.com). Fascination is registered and manufactured by Valent Biosciences Corp, Libertyville, IL. Their website is http://www.valentbiosciences.com. The active ingredients in Fascination are N-(phenylmethyl)-1H-purine 6-amino (1.8% w/w) and Gibberellins A_4A_7 (1.8% w/w). Chemical names used for Fascination are: N-(phenylmethyl)-1H -purine-6-amine [benzyladenine (BA)]; gibberellin (GA4+7): [BA+GA4+7]. These products have been used on other cut flowers but not sunflowers.

Our experiments with commercial cytokinin products were conducted in a postharvest room set at a temperature of 20 ^{o}C and 60% RH and 12h fluorescent lighting. 'Procut Bicolor' flowers were used for all experiments. In the first experiment, flowers were dipped in different concentrations of BAP-10 and Fascination. The treatments used were as follows: control (DI water), 100 ppm and 300 ppm of each chemical. Five flowers per treatment were dipped for 10 mins, and 10 petals per flower were pulled on the same day. For the pulsing experiments, two (50 ppm and 100ppm) concentrations of Fascination were compared with a control of deionized water. Procut Bicolor stems were left in the treatment for 8 days. Chrysal CVBN was used to control bacterial contamination. Chrysal CVBN is a chlorine product with 2465 dichloroisocyanuric acid salt as the active ingredient. It is manufactured and distributed by

Chrysal International, P.O.Box 5300, 1411 DD NAARDEN, The Netherlands (Email: info@chrysal.nl; http://www.chrysal.com/chrysal_cvbn.aspx?id=367&tg=2348&lan=557. Vase solution was changed every other day. Four flowers were used in each treatment. Ten petals were pulled per flower head on day 1, 4 and 8. Petal detachment force measurements were used to determine petal abscission in these experiments.

In order to confirm the results of the dipping experiments, sunflower heads of Procut Bicolor were dipped in different concentrations of BAP-10 and BA+GA4+7 for 10 mins and left in a vase with distilled water for 14 days to see which treatment was most effective in prolonging vase life. Two concentrations (100 ppm and 300 ppm) of each chemical was used and compared with a control of deionized water. Control plants were dipped in deionized water, and left in a vase with distilled water. Four stems were used in each treatment; they were cut to an initial length of 30 cm and left in distilled water for 14 days undisturbed. Distilled water was changed every other day or as often as the vases became contaminated. The experiment was started on August 26th and ended on September 10th. Pictures were taken on days 5, 8, 10, 12 and 14.

Results:

I. Ethylene sensitivity test

Preliminary experiments were carried out using concentrations of 3 ppm and 6 ppm ethylene to check sunflower sensitivity to this hormone, but there was no effect. Therefore a concentration of 10 ppm was used in large-scale investigations. The results from ethylene sensitivity of sunflowers showed that there were no significant differences in the detachment forces between ethylene treated flowers and the controls at $p < 0.05$ (Table 3.1). Figure 3.1

shows pictures taken 48 hours after treating with ethylene for control and ethylene-treated plants.

This indicates that petal abscission in sunflowers is ethylene insensitive.

Table 3.1: Mean force in grams to detach petals of sunflower (Strawberry Blonde) 48 hours after harvest, in the presence and absence of 10 ppm ethylene (n = 16 for each treatment). Flowers were exposed to ethylene for 24 hours. Detachment forces were measured 24 hours after the conclusion of ethylene treatment.

Time after harvest	**Average detachment force (grams)**	
	Control (n=16)	**10ppm (n=16)**
48 hours	130.09a[z]	123.47a

[z]Means followed by the same letter are not significantly different at $p < 0.05$.

Figure 3.1: Cut sunflowers (Strawberry Blonde) 48 hrs after ethylene treatment; the flowers on the right were treated with 10 ppm ethylene at a flow rate of 250µl/min for 24 hrs, while those on the left were the controls. Note that the differences in color between the two flowers is not due to ethylene exposure: cultivar varies in color among individuals

II. ELISA results for phytohormone quantification

Five sunflower genotypes were tested; three [Procut Bicolor (PBC), Strawberry Blonde (SB) and Moulin Rouge (MR)] were susceptible to petal drop while two [Procut Lemon (PL) and Procut Yellow Lite (PYL)] were less susceptible to petal drop. Results from the test for zeatin + zeatin riboside, iPA and ABA show that there were highly significant differences in the levels of these phytohormones among the five sunflower genotypes tested at $p < 0.05$ (Table 3.2). Results from the test for IAA revealed that there were no significant differences in levels of IAA among the five sunflower genotypes tested at $p < 0.05$ (Table 3.2). Auxin levels were generally very low in abscission zone tissues compared to those of the other hormones measured. There was no association between hormone level and abscission tendency in the genotypes tested.

Table 3.2: Phytohormone quantity in petal abscission zone tissues of five sunflower genotypes. Phytohormone levels were measured within 1 hour of the flower opening in all genotypes. Levels were measured in picomoles per gram fresh mass (pmol/gFM). Separation of means was done using Student's t-test with a pair wise comparison of the level of each phytohormone measured (n=3 for each sunflower genotype). There were three replications in each genotype making a total of 15 distinct flowers. Mean separation was performed on log transformed data.

Phytohormone measured	Sunflower genotypes				
	Procut Yellow Lite	Procut Lemon	Procut Bicolor	Strawberry Blonde	Moulin Rouge
Z+ZR (pmol/g FM)	6.25a[z]	1.07b	7.02a	1.11b	1.01b
iPA (pmol/g FM)	8.74a	3.84ab	7.05a	1.37b	1.36b
ABA (pmol/gFM)	0.95b	2.56a	3.47a	2.89a	2.27a
IAA (ng/gFM)	1.59c	1.74c	1.86c	1.87c	1.37c

[z]Mean separation within rows was by least square means with a Student's t-test adjustment. Means within rows followed by the same letter are not significantly different at $p < 0.05$.

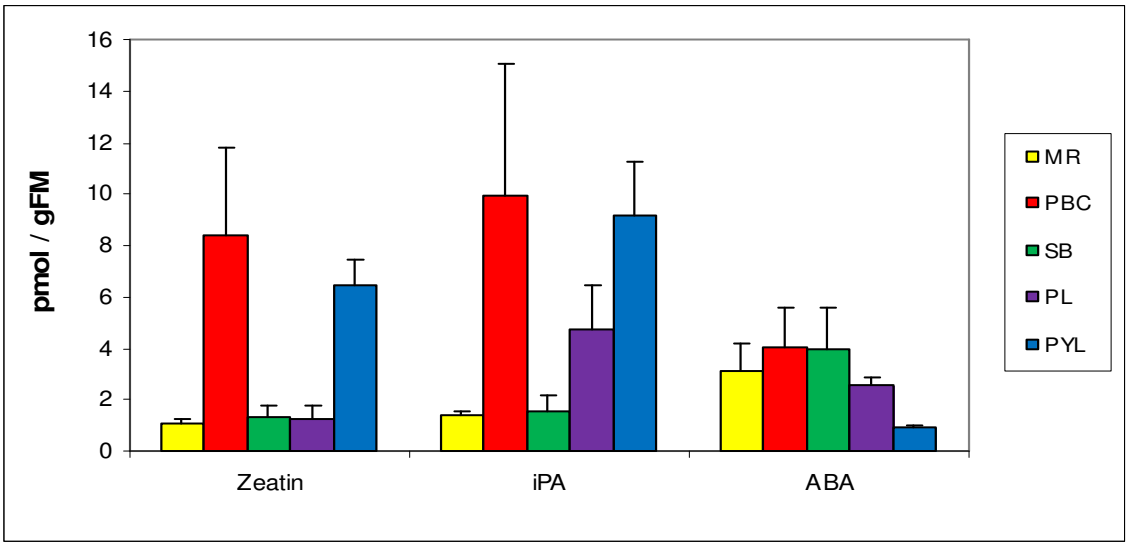


Figure 3.2: A graphical representation of phytohormone levels in abscission zone tissues of five sunflower genotypes. Cultivars arranged in order of abscission tendency from the most susceptible (MR, PBC and SB) to the least susceptible (PL and PYL).

Figure 3.2 above shows the levels (pmol/g FM) of Z +ZR, iPA and ABA in the five genotypes tested. Procut Bicolor (PBC) and Procut Yellow Lite (PYL) were significantly high in cytokinins: Zeatin (Z+ZR) and iPA, but low in ABA. Procut Lemon (PL) was high in iPA cytokinin group but low in Zeatin (Z +ZR) and ABA. Moulin Rouge (MR) and Strawberry Blonde (SB) were extremely low in Zeatin (Z+ZR) and iPA but high in ABA.

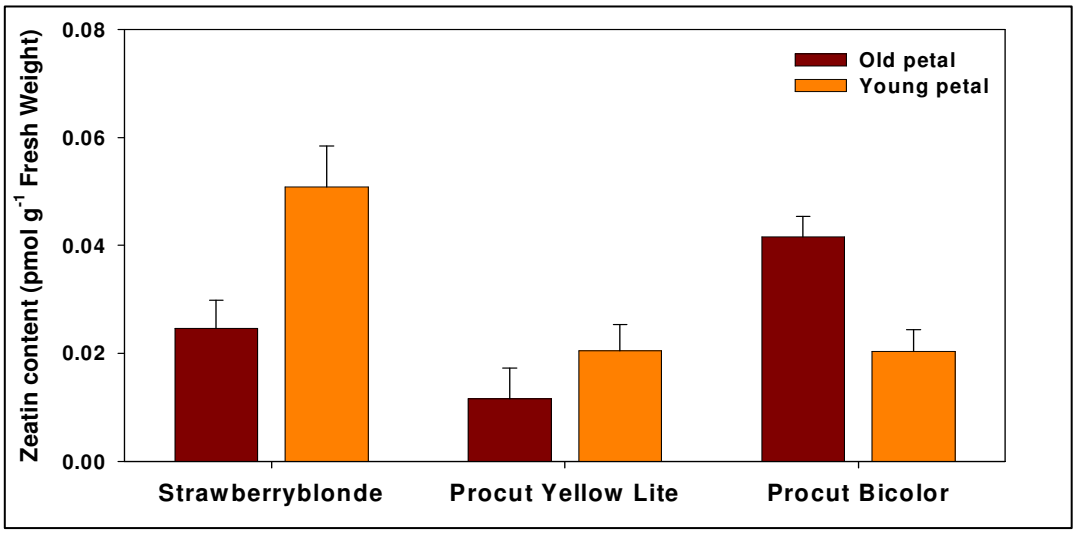


Figure 3.3: Cytokinin content in young (24 hours after flower opening) and old petals (8 days after opening for PBC and SB and 14 days for PYL) of three sunflower varieties

Figure 3.3 shows the levels (pmol/g FM) of zeatin in petals of 3 sunflower genotypes measured at 2 stages of flower development; a young stage (when the flower is fully open and the petals have flattened out, 24 hours after flower opening), and an old stage (during flower senescence, 8 days after flower opening for Procut Bicolor and Strawberry Blonde and 14 days for Procut Yellow Lite). Procut Yellow Lite and Strawberry Blonde had high levels of zeatin at the young stage than the old one, while Procut Bicolor had high levels of zeatin at the old stage than the young stage.

III. Exogenous application of cytokinins on petal drop rates in sunflowers

Tables 3.3 and 3.4 below show the results of exogenous application of BA+GA4+7 and BAP-10 on petal drop rates in sunflowers using vase solution and dipping methods, respectively.

Table 3.3: The effect of different concentrations of BA+GA4+7 supplied as vase solution on petal drop rates in sunflower (Procut Bicolor). The mean separation force in grams required to detach petals from the abscission zone was recorded on days 1, 4 and 8 after treatment.

Time in vase solution (days)	Average detachment force (grams)		
	Control	50 ppm	100 ppm
1 (n=39 for each treatment)	89.03b[z]	129.57a	140.80a
4 (n=40 for each treatment)	135.59b	165.04a	155.54a
8 (n=40 for each treatment)	47.87a	26.71b	22.63b

[z]Mean separation within rows was by least square means using Student's t-test and a pair wise comparison of the average detachment force recorded. Means within rows followed by the same letter are not significantly different at $p < 0.05$.

Table 3.3 above shows that using BA+GA4+7 either at 50 ppm or 100 ppm in vase solution had a higher resistance to petal abscission in Procut Bicolor (PBC) on day 1 and 4, but not on day 8. Petal detachment forces were significantly higher in BA+GA4+7 treated plants. Results from our study of the anatomy of petals of 'Procut Bicolor' at different developmental stages revealed that by day 8 the petal separation layer was fully formed and mature, such that a gentle touch of the

petals led to immediate abscission. Thus, the cytokinin treatment when supplied in vase solution did not delay petal abscission on day 8.

Table 3.4: The effect of dipping the heads of sunflower (Procut Bicolor) for 10 minutes in different concentrations of BAP-10 and Fascination (BA+GA4+7). The mean force required to detach petals from the abscission zone was recorded immediately after treatment.

Cytokinin product	**Average detachment force (grams)**		
	Control (n=50)	**100 ppm (n=50)**	**300 ppm (n=50)**
BAP-10	75.32b[z]	105.54a	115.82a
Fascination (BA+GA4+7)	80.85c	114.31a	98.32b

[z]Mean separation within rows was by least square means using Student's t-test and a pair wise comparison of the average detachment force recorded. Means within rows followed by the same letter are not significantly different at $p < 0.05$.

In order to confirm the results of the dipping experiments from Table 3.4 above, fresh cut sunflower heads of Procut Bicolor were dipped in different concentrations of BAP-10 and BA+GA4+7 for 10 minutes and left in a vase with deionized water for 14 days to see which treatment was most effective in prolonging vase life. Control plants were dipped in deionized water. Pictures were taken on day 5, 8, 10, 12 and 14.

On day 5 (plate 3.1), all plants in all treatments were healthy, petals were all fully open, healthy, succulent and the colors in the petals were rich. Note that stems of the control plants were a little shorter because they were recut as they were wilting faster. I had to wash the vase with Clorox bleach and recut the stems to avoid bacterial contamination. Petals usually fell off the flower heads during deionized water change in all treatments. However, I tried to be very gentle with the plants to prevent this from happening.

On day 8 (plate 3.2), petals of BAP-10 and BA+GA4+7 treated plants are still very healthy, succulent, full of color and fully open while the stems and leaves are also healthy. While

petals of BAP-10 and BA+GA4+7 treated plants were still firmly attached to the flower head, the control plants had started losing petals quickly. Our results from a study of the anatomy of sunflowers revealed that by day 8, the abscission layer of PBC was already fully mature and abscission of petals happened at this time. **[See plates 3.1 and 3.2 on pages 46 and 47]**

Plate 3.1: The effect of BAP-10 and Fascination (BA+GA4+7) on Procut Bicolor 5 days after dipping

Plate 3.2: The effect of BAP-10 and Fascination (BA+GA4+7) on Procut Bicolor 8 days after dipping

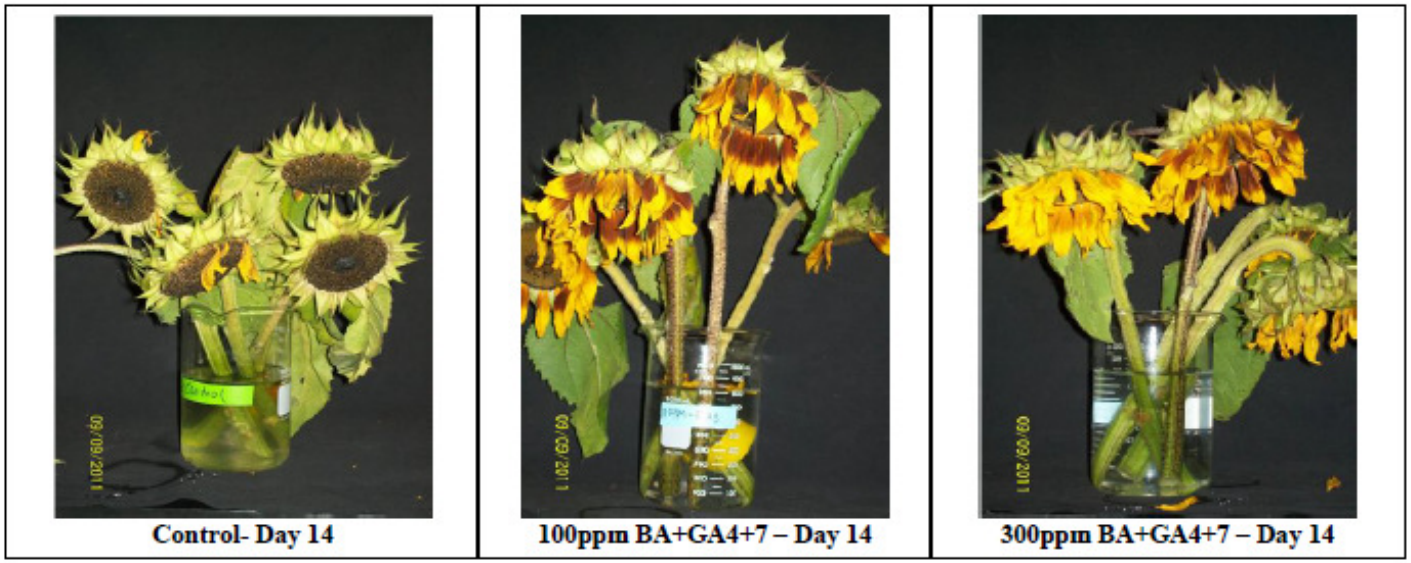
On day 10 (plate 3.3), most of the plants in the control treatment had lost most of their petals. It is interesting to note that these petals fell off even though they were healthy, succulent and rich in color. This explains the importance of the presence of a mature separation layer. Once the separation layer was fully formed, by day 10, a gentle breeze or touch of the petals or even a touch of the stems of the plant as water was being changed caused the petals to abscise even if they were still succulent and rich in color. The BAP-10 and BA+GA4+7 treated petals, were succulent, rich in color and firmly attached to the flower head. Their stems and leaves were also healthy. There was head bending of one of the stems in the 300 ppm BAP-10 treated plants as can be seen in the pictures taken on day 10. Note that distilled water change was done before pictures were taken on all the days. While the petals of the two BA+GA4+7 treated plants and that of 300 ppm BAP-10 treatment were healthy, succulent, full of color and their stems holding well in their vases, those of the 100 ppm BAP-10 treated plants had lost a few petals on day 10 as can be seen on the photos. The 300 ppm BAP-10 treated plants had one bent head. The two BA+GA4+7 treatments and the 300 ppm BAP-10 treated plants had not lost any petals by day 10. The petals were firmly attached to the flower head. The readily available cytokinins provided to their petals seemed to cause the petals to remain attached to the flower head.

On day 12 (plate 3.4), the petals of all BA+GA4+7 treated plants were very healthy, succulent, full of color and firmly attached to the flower head. Their stems and leaves were also very healthy and holding very well in the vase. There was no head bending in all BA+GA4+7 treated plants and they will still look good in bouquets. The 100 ppm BAP-10 treated plants had lost some petals. The petals of the 100 ppm BAP-10 treated plants were twisted and drying up. **[See plates 3.3, 3.4, and 3.5 on pages 49 to 51]**

Plate 3.3: The effect of BAP-10 and Fascination (BA+GA4+7) on Procut Bicolor 10 days after dipping

Plate 3.4: The effect of BAP-10 and Fascination (BA+GA4+7) on Procut Bicolor 12 days after dipping

Plate 3.5: The effect of BAP-10 and Fascination (BA+GA4+7) on Procut Bicolor 14 days after dipping



The petals of the 300 ppm BAP-10 treated plants were healthy, but were not as succulent and full of color as those of the BA+GA4+7 treated plants. This was because they had begun to wilt, lose color and had one bent head. While the plants of both BA+GA4+7 treatments would still look great in bouquets on day 12, those of the 300 ppm BAP-10 treatment was manageable, but the plants in the 100 ppm BAP-10 treatment were almost gone. Petals of the control plants had all fallen off.

On day 14 (plate 3.5), the petals of all BAP-10 treated plants had wilted, lost color, dried up and were ready to be discarded. However, petals in both of the BA+GA4+7 treatments still had quite some color. The petals were still full and many were still attached to the flower head. It is important to note that the petals in both of the BA+GA4+7 treatments hardly fell off, even though water change (which caused slight shaking of the stems) was done every 2 days. The petals were firmly attached to the receptacle and instead of falling off, the petals could instead be seen losing color though still attached to the flower head. At the time I discarded the plants of both BA+GA4+7 treatments (on day 17), the petals had not fallen off, they had just wilted while attached to the flower head (numerical evidence not shown).

In conclusion, exogenous application of cytokinins (by dipping sunflower heads for 10 minutes in either 100 ppm or 300 ppm BA+GA4+7) delayed petal abscission and prolonged vase life by 6 days. Using 100 ppm or 300 ppm of BAP-10 also delayed petal abscission and prolonged vase life of sunflower petals by 4 days.

Discussion and Conclusion

Although it is assumed that abscission of petals is generally sensitive to ethylene (van Doorn, 2001), ethylene insensitive abscission of petals has been reported in some species in the Orchidaceae, Liliaceae and some Saxifragaceae species (van Doorn, 2001). This is also the case in some members of the Asteraceae family (Woltering and van Doorn, 1988). In our study, we exposed sunflower petals to 10 ppm ethylene for 24 h, compared to the 3 ppm exposure for 24 h used by van Doorn (2001). Van Doorn's (2001) study showed that species which were sensitive to ethylene immediately showed rapid wilting (turgor loss and desiccation) or abscission (falling of petals without wilting). Evensen et al. (1993) also report abscission of petals of geranium after just 2 h of ethylene treatment. Our results did not show any rapid wilting, desiccation, turgor loss, or immediate petal drop of sunflower petals which were treated with ethylene (Figure 3.1). There were no significant differences in petal detachment forces of ethylene treated flowers and the controls (Table 3.1). These results show that petal abscission in sunflowers is ethylene insensitive and may not be regulated by endogenous ethylene, confirming the results of Woltering and van Doorn (1988) with other members of the Asteraceae.

The initial goal of the study on phytohormone quantification was to measure cytokinins in petals of sunflowers which were resistant and those susceptible to petal loss at different stages of flower development. Our hypothesis was that endogenous cytokinins had a regulatory role in abscission of sunflower petals. However, results from preliminary quantification of cytokinins at different stages of flower life revealed that there were extremely low levels of cytokinins at the later stages (when petals begin to abscise) of flower life (figure 3.4). In fact, some of the levels at the later stages were extremely difficult to measure because they were very low. Quantifying cytokinins is quite challenging and timing is very crucial. Plant samples should be taken at a time when the

hormone is most abundant. After our preliminary experiments, and a careful study of the literature on cytokinins in flowers, we found that cytokinins were usually more abundant in petals which were at a younger stage of flower development, and sometimes just before bloom (Mayak and Halevy, 1970; Saha et al., 1985). After these preliminary experiments, we decided to adjust our experimental design to measure cytokinins at the stage when the flower just opens (within 1 hour of the flower opening) in all five genotypes. Table 2.2 in Chapter 2 shows detachment forces of petals taken within an hour of the flower opening and the petals flattening out. We also decided to measure ABA alongside cytokinins because the procedures were basically the same.

Abscission is a physiological process which takes place in a restricted region (the abscission zone) located at the base of a plant organ. Abscission is largely controlled by five plant hormones: auxin, cytokinins, abscisic acid, ethylene and gibberellic acid (Addicott, 1970). This study is the first to measure phytohormone levels in petals of sunflowers and their relation to petal drop. Our results show that 3 types of phytohormones; cytokinins, ABA and auxins were present in abscission zone tissues of 5 sunflower genotypes. Two groups of cytokinins were detected: the zeatin + zeatin riboside (Z + ZR) group and the iso-pentenyl adenosine (iPA) group. The levels of Z+ZR cytokinins in Procut Bicolor and Procut Yellow Lite were significantly higher than in Procut Lemon, Moulin Rouge and Strawberry Blonde (Figure 3.2, Table 3.2), while the levels of iPA cytokinins in Procut Yellow Lite, Procut Bicolor and Procut Lemon were significantly higher than in Moulin Rouge and Strawberry Blonde (Table 3.2). These results were similar to those of Lukaszewska et al. (1994), who measured endogenous cytokinins in rose petals. Procut Bicolor (PBC) and Procut Yellow Lite (PYL) were significantly high in cytokinins: Zeatin (Z+ZR) and iPA, but low in ABA. The results obtained for PYL (a variety resistant to petal drop) was expected. The high levels of cytokinins (Z +ZR and iPA) and low levels of ABA in PYL explains why this genotype last longer (12 days) in vases than the

other genotypes. Procut Lemon (PL) (another resistant variety to petal drop) was high in iPA cytokinin group but low in Zeatin (Z +ZR) and ABA. The high levels of cytokinins iPA and low levels of ABA in PL also explains the reason this variety lasts longer (12 days) in vases than other sunflower varieties. The result we got for PL was also expected. Moulin Rouge (MR) and Strawberry Blonde (SB) were extremely low in both cytokinin groups: Zeatin (Z+ZR) and iPA but high in ABA. The significantly high levels of ABA and low levels of cytokinins in SB and MR (varieties which are susceptible to petal loss) explains the reason these varieties lose their petals faster (8 days after harvest). PBC (a variety which loses its petals early) had significantly high levels of cytokinins (Z+ZR and iPA) but low levels of ABA. This result was not predictable. However, some authors have suggested that PBC has been undergoing improvements over the years, although the seed companies didn't release the information of the version of PBC released into the market at the time we carried out these experiments. Of all the hormones analyzed, cytokinins (Z +ZR and iPA) were the most abundant in all five genotypes tested (Table 3.2). Of the two types of cytokinin detected, iPA was more abundant that Z+ZR cytokinin. Lukaszewska et al. (1994) reported that the cytokinin group of iso-pentenyl adenosine (2iP) was more abundant in young rose petals than that of Zeatin (Z).

Cytokinins and auxin function to maintain biochemical processes, and in nutrient partitioning within the plant (Addicott, 1970). Their function at times may retard abscission and at other times may promote abscission. Auxin promotes abscission by directing nutrients from weak organs to actively growing organs thereby promoting the abscission of the weak organs. Our results showed that there were significantly low levels of auxin in sunflower abscission zone tissues within 1 hour of flower opening in all genotypes. The following reasons may account for the low auxin levels:

1. The type of tissue tested. A recent study comparing the distribution of auxin in Arabidopsis during flower development confirmed that petals are areas of low auxin (Aloni, 2006). A careful

study of the growth and development physiology of sunflowers is imperative in understanding the distribution and role that auxin plays in petal abscission.

2. Site of quantification: In this study we quantified auxin levels in petal abscission zone tissues (located at the base of the petal, where the petal meets the achene), and not in the ovaries. Aloni (2006) reported that auxin was lower in petals but higher in ovaries of Arabidopsis flowers. Abscission zone tissues were collected at a young stage within one hour of the flower opening.

It has been well established that cytokinins are synthesized in the roots and transported via the xylem to the shoots where they regulate growth, development and senescence. Our results revealed the presence of two groups of cytokinins: zeatin and its riboside (zeatin riboside) [Z + ZR] and that of iso-pentenyl adenosine [iPA] in petal abscission zone tissues of 5 sunflower genotypes. The presence of both groups of cytokinins was detected in carnations (Van Staden et al., 1987) and roses (Lukaszewska et al., 1994). The iso-pentenyl adenosine cytokinin group was more abundant than zeatin and its riboside (zeatin riboside) (Table 3.2). The low levels of Z + ZR groups of cytokinin measured compared to that of iso-pentenyl adenosine group could be due to their utilization in flower development or their conversion to glucosides.

The levels of all cytokinin groups combined were much higher than that of ABA and IAA (Table 3.2). The increased cytokinins levels in varieties which are resistant to petal drop at a young stage indicate the possible involvement of cytokinins in flower development of sunflowers and the eventual increased vase life in these varieties. On the other hand, the low levels of cytokinins and high levels of ABA in varieties which are susceptible to petal drop further explains the possible role cytokinins play in abscission of sunflower petals. Furthermore, the genotypes with the longest vase life had the high levels of cytokinins, which indicates the role cytokinins play in petal drop of sunflowers. Our present results are similar to those of Saha et al. (1985) and we conclude that cytokinin levels are higher in

abscission zones tissues of sunflower petals at a younger stage than an old stage, during senescence of petals. (see Figure 3.3).

How do the cytokinin levels measured relate to petal drop in these genotypes? The results we obtained here reveal that each genotype behaves differently. It will be incorrect to conclude that petal drop in the genotypes which drop their petals easily is due to a low level of cytokinins in their petals. If this were the case then the genotypes which are resistant to petal drop would have consistently produced the highest amounts of cytokinins. Instead, Procut Bicolor which loses its petals early had the highest amount zeatin + zeatin riboside in petal abscission zone tissues followed by Procut Yellow Lite as seen in Table 3.2. However, our results reveal generally with one exception that cytokinin levels were much higher at a younger stage (within 1 hour of the flower opening, Figure 3.2) and lower at an older stage (time of senescence, Figure 3.3) in sunflowers. Procut Lemon, a genotype which is on the resistant side, produced low levels of Z + ZR compared to PBC and SB which are more susceptible to petal drop.

The hypothesis suggested by Mayak and Halevy (1972) that the higher the endogenous ABA levels at harvest, the shorter the subsequent vase life in roses was similar to our findings in cut sunflowers. The results from ABA analysis revealed that the genotypes with the shortest vase life (PBC, SB and MR) had the highest levels of ABA in their petals while those with the longest vase life (PYL and PL) had the smallest amounts of ABA in their petals (see Table 3.2, Figure 3.2 above). This result indicates that ABA is an important regulator of petal drop in sunflowers. These increased levels of endogenous ABA at a young stage in petals of the susceptible varieties might be responsible for the increased abscission of petals observed in these lines. We did not measure ABA levels at other stages of flower life.

The results from exogenous application of cytokinins (BAP-10 and Fascination- BA+GA4+7) showed that these chemicals have the potential of extending vase life of cut sunflowers. When Fascination was supplied continuously as vase solution and petals pulled on Day 1, 4 and 8, the results showed that petal detachment forces were higher for the treated petals than the controls (Table 3.3). Fascination had an effect on Day 1 and 4 but not on Day 8. The lack of an effect of Fascination on Day 8 could be due to the method of application and concentration. Higher concentrations of cytokinins applied as vase solution for up to 8 days may be toxic to the plant and instead result in a decrease in detachment force. Some authors found that cytokinins delayed abscission when applied as a dipping solution rather than a vase solution. Results from the dipping experiments with BA+GA4+7 and BAP-10 showed that when petals were dipped in these chemicals their detachment forces were significantly higher than in the controls (Table 3.4). However, at higher concentrations of up to 300 ppm, detachment forces decreased in Fascination treated plants more than in BAP-10 treated plants. Finally, when sunflower heads were dipped for 10 minutes in different concentrations of BAP-10 and BA+GA4+7 and kept for 14 days, these chemicals delayed petal abscission and extended vase life by 4 and 6 days, respectively. These chemicals appeared to arrest and delay cell division at the abscission zone.

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Chapter 4: Anatomy of Petal Drop in Sunflowers

Introduction

This chapter is devoted to investigations related to petal drop via the formation and maturation of an abscission zone and separation layer at the juncture of petal and achene. Two approaches to this problem were applied. The first of these was based on a measurement of the force required to pull petals from the flower head, the second investigated changes in the anatomy of the petal-achene juncture. Both approaches were used over a meaningful time course that relates to the course of petal drop as seen in the trade.

Many researchers (Fernandez et al., 2000; McKenzie and Lovell, 1992; Patterson and Bleecker, 2004) have used physical methods (e.g. breakstrength) to study abscission in plants. The breakstrength provides a quantitative measure of the force required to detach an organ from the main body of the plant. Evensen et al. (1993) used an electronic force gauge (Shimpo, Graham and White Instruments, St. Albans, Herts, UK) to measure the speed of separation and the duration of the abscission process in ethylene treated petals of *Pelargonium x hortorum*.

Abscission is the shedding away of plant parts such as leaves, fruits, flowers and petals (Taylor and Whitelaw, 2001). It is a regulated developmental process by the plant, but can also be induced to occur due to environmental stresses such as water stress (Jones et al., 1980; McCree, 1986), light quality and quantity (Mao et al., 1989), wounding and pathogen attack (Ryan, 1987). Although abscission enables plants to cope with the challenge of diseases and pathogens, and temperate plants to overwinter, it can lead to significant losses in the commercial cut flower industry where premature loss of petals reduces marketability of cut flowers.

Studies of flower and leaf abscission have shown that the shedding of leaves and other organs is often preceded by localized cell division at the base of the leaf or the organ being shed (Sexton and Roberts, 1982). This cell division results in the formation of a thin transverse layer which is referred to as the abscission layer. Further studies revealed that abscission occurs at predictable regions, and that these regions have cells which are morphologically distinct before the abscission event (Brown and Addicott, 1950; Gawadi and Avery, 1950). The cells that comprise the separation layer have been shown to be discreet, small, isodiametric, closely packed, with a dense cytoplasm and appear to have lost the capacity to enlarge although they do enlarge during abscission (Sexton and Roberts, 1982; Sexton et al., 1985).

A breakdown of the middle lamella, followed by swelling of the cell walls causes the weakening of the primary cell walls and results in the separation of abscission zone cells (Polito and Lavee, 1980; Webster, 1973). This process happens in association with the secretion of cell wall-degrading hydrolases (Roberts et al., 2002). Sexton and Redshaw (1981) reported that cells at the separation layer expand and become rounded during abscission.

The number of cells that make up the abscission zone can vary widely from species to species. The separation process of pedicel abscission zone in tomato flowers takes place between two discrete layers of cells. On either side of these layers are three to four layers of much smaller closely packed cells which lie across the diameter of the pedicel (Tabuchi and Arai, 2000). In contrast, the abscission zone in *Sambucus nigra* has up to 50 cell layers which separate over many layers during the process of abscission.

It is well known that abscission can be induced to occur experimentally, and this has been used to study the time course of abscission in different plant species. In most of these studies, the plant tissues were treated with ethylene to synchronize and accelerate such experimentally induced

abscission (Evensen et al., 1993). The time it takes between induction and the completion of separation (when the part is ready to fall off) varies depending on the tissue and the levels of factors promoting or retarding abscission of the tissue. Previous research shows that leaf and fruit abscission is completed in 10-48 h while floral organs are shed very rapidly; in tomatoes, the flower pedicels abscise in 4 h after the start of ethylene exposure (Roberts et al., 1984).

The abscission of explanted abscission zones of fruits and leaves takes at least 12-24 h (Jackson et al., 1972). A decrease in the strength required to detach the corollas of *Digitalis purpurea* was noticed within 8 h of ethylene exposure (Stead and Moore, 1983) and that of *Pelargonium x hortorium* was noticed within only 30 mins of the start of ethylene treatment and separation was completed 60-90 min later (Evensen et al., 1993). There is an interesting report (Fitting, 1911) that revealed that petals could fall off within minutes of induction of abscission. However, this is being debated (see Sexton and Roberts, 1982; Taylor and Whitelaw, 2001). Evensen et al. (1993) showed that the force required to remove petals decreased within 30 mins of the commencement of ethylene treatment, and in some cases separation was complete after just 60 mins. The sunflower is a member of the Asteraceae family of flowering plants. It consists of an outer whorl of showy flowers known as the ray flowers, and the disk flowers which occupy the remainder of head. This study focuses mainly on the ray petals of sunflowers. Woltering and van Doorn (1988) studied petal senescence in 93 species from 22 families and the sensitivity of these species to exogenous ethylene. Their result showed that petal abscission in all tested species from the Asteraceae family was ethylene insensitive.

Objectives of anatomical studies

1.) To characterize an abscission zone, if present, at the base of petals of sunflower florets in varieties that differ with respect to petal drop, and

2.) To determine if differences in the nature and/or development of the abscission zone between lines are correlated with differences in timing with respect to petal drop.

Research hypothesis

1.) A specific set of anatomical and micro-chemical details that might enhance the tendency of sunflowers to drop their petals can be used to characterize an abscission zone, and

2.) The anatomical details that characterize the abscission zone will differ between lines in a way that correlates with differences in tendency to drop petals.

Materials and methods

Two sunflower lines: Procut Bicolor (PBC), a variety which loses its petals easily (short-lived), and Procut Yellow Lite (PYL), a variety which holds unto its petals much longer (long-lived), were selected for this study. The two lines were chosen to represent differences observed in the tendency of sunflowers to drop their petals.

I. In the first approach, separation force measurements were taken for the two sunflower varieties using a modification of a soil cone micro-penetrometer apparatus which measures separation forces in the opposite direction. This device consists of a scale which is connected to a computer. The flower was held down on the scale by a weight of 2 kg. An alligator clip inserted into the drill press chuck was attached to a random petal. The scale was tared to zero. The drill press lever was slowly raised (at about 0.5 cm/sec) and the force by which the weight on the scale diminishes was recorded

on the computer each second. The highest negative recorded value before breakage was used to represent the "breakstrength." This value represents the force needed to detach that petal from the receptacle of the flower. The force that was required to remove the petals from sunflower heads of PBC and PYL was sampled over a 12 day period after anthesis. Flowers were first harvested, put in glass vases with tap water and stored at a temperature of 20^{o}C. Force readings for both varieties were taken on day 1,3,6,9 and 12. A minimum of 4 petals were pulled on each flower head and the readings averaged. Results were analyzed using ANOVA and GLM regression procedures on JMP. Petal detachment force was the dependent variable while sunflower variety, day (time after harvest) and vase life were the independent variables.

II. In the second approach, we studied the changes in the anatomy of the petal-achene juncture of the two lines. Three stages from line 1 and 4 stages from line 2 were studied. These stages represent a time course with physiological relevance; when the flower just opens, 4 days after harvest, the end of flower life for PBC (8 days) and the end of flower life for PYL (12 days). The end of flower life was defined as the time (in days) when detachment force equals zero; a gentle touch of petals with the index finger causes them to fall off easily. Flowers were harvested, put in vases with distilled water and stored at a temperature of 20^{o}C. The vases were washed and sterilized, and the deionized water was changed daily to avoid bacterial contamination. Florets were fixed in FAA for at least 72 hours and then dehydrated through an ethanol and butanol series. Paraffin sections (approx. 10µm) at the stages described above for both varieties were stained with Toluidine Blue O and examined by light microscopy (LM). Tests for suberin, lignin and pectic substances were done using thin hand sections. Three florets each from two inflorescences in each stage were studied. Three sections from each floret were studied. These experiments were repeated 3 times.

A list of light micrographs of abscission zone sections studied

Two sunflower cultivars from two different lines were chosen for this study as follows:

Line 1: Procut Bicolor (PBC) is the variety which drops its petals early, and

Line 2: Procut Yellow Lite (PYL) is the variety which holds its petals longer

The two lines were chosen to represent differences observed in the tendency of sunflowers to drop their petals. Three stages from line 1 and 4 stages from line 2 were studied. These stages represent a time course with physiological importance; when the flower just opens, four days after harvest, and the end of flower life for both varieties. Three petals each from two flowers in each stage were studied. Abscission zone sections from each petal were collected. 3 sections from each petal were studied and photographed. A total of 9 images were studied and photographed from each flower head and a total of 18 images were taken at each stage. In general, I studied a total of 18x7=126 sections and photographed them. Higher Magnification photos were also studied and photos taken for each section making a total of 252 sections studied and photographed for the anatomical analysis.

Staining protocols used for micro-chemical tests

A.) Suberin: A saturated solution of Sudan IV in 70% alcohol imparts a red color to fatty substances. Fresh sections were left in the stain for 20 min, washed with 50% alcohol to remove excess stain and transferred to a drop of glycerin for observation. Fats may be distinguished from other colored substances by the fact that they exist as drops within the cells. Fuelgen's reaction also distinguishes cutin, suberin, and lignified elements (Johansen, 1940).

B.) Lignin: Sections were placed on a slide in a large drop of solution of 0.1g phloroglucin in 10 cc. of 95% alcohol and covered with a cover slip. The solution was allowed to partially evaporate, and

then a little 25% hydrochloric acid was diffused in at the edge of the coverslip. The appearance of a red- violet color indicates the presence of lignin (Johansen, 1940).

C.) Pectic Substances: Ruthenium Red is the classical dye indicator for pectic substances (Johansen, 1940). It is a very expensive dye, and its solutions are quite unstable. Consequently, a small quantity of the solution was prepared. Two or three tiny crystals of the dye were dissolved in a drop of deionized water until the solution was clear reddish-pink in color. Sections were left in the Ruthenium Red solution for 30 min, then washed thoroughly, and mounted on a slide in glycerin. All pectic substances acquire a red color (Johansen, 1940).

D.) General Cell Morphology: Citrate buffer (0.05% in 50mM), pH<5 was used. Sections were stained for 2 min and then were rinsed in distilled water. Cells containing pectic substances stain pink to reddish purple, while cells containing lignin (e.g. tracheary elements/sclerenchyma) stain blue or blue green. Nuclei stain blue to greenish blue and various phenolic substances stain green to blue green. Thin walled parenchyma stains reddish purple (Ruzin, 1999).

Results and Discussion:

The results from the two approaches used in this study point in the same direction:

I. Detachment force analysis: Results from the physical study (Figure 4.1) show that there was a difference in timing in the formation and maturation of the separation layer between the two lines. Detachment forces were initially high for both varieties (PBC, short-lived and PYL, long-lived) on Day1 and Day 3. However, on Day 6, PYL produced a high reading for strength of attachment, while PBC had a low reading. The strength of attachment switched from an initial high to low in both lines because of the maturation of the separation layer. This maturation occurred earlier in the line that is first to lose its petals (line PBC). Figure 4.1 shows that the variety PYL had an initial high

detachment force on Day 1 (180.58 grams), Day 3 (158.74 grams) and Day 6 (181.76.6 grams). After Day 6, the strength of attachment of petals in variety PYL then switched from high to low on Day 9 (34.52 grams), and Day 12 (15.39 grams).

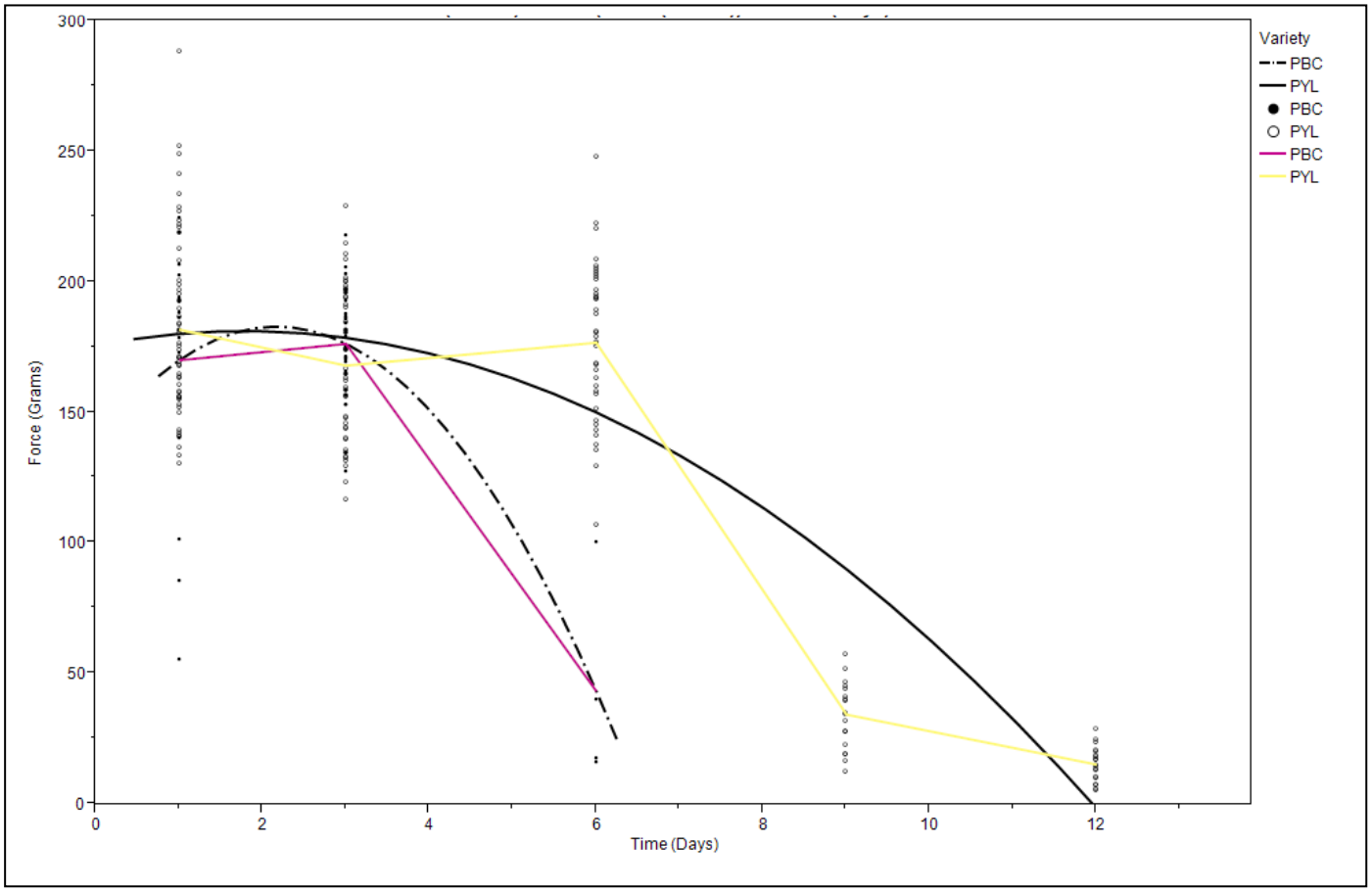

Figure 4.1: The break-strength or force required to remove petals the receptacle of variety PBC vs. PYL measured on day 1, 3, 6, 9 and 12 after harvest. The colored lines are the line plots while the black lines are the regression plots

Variety PBC also had high detachment force readings on Day 1 (170.29 grams) and Day 3 (176.41 grams). However on Day 6 the forces declined significantly to 42.83 grams, compared to that of the long-lived line (181.76 grams) on the same day. While the strength of attachment switched from high to low after Day 6 in the long-lived line, that in the short- lived line was faster (only after Day

3). Attachment forces were significantly lower on Day 6 (42.83 grams), compared to that of the long-lived line on Day 6 (181.76 grams). There were no force readings for the short-lived line on Day 9 and Day 12 because the flower had wilted by Day 9.

II. Anatomical analysis: To understand the anatomical changes taking place at the petal-achene juncture, we examined sections from both lines stained with Toluidine Blue O. Three stages from the short-lived and 4 stages from the long-lived line were studied as described above. Results showed a differentiated region at the junction of the petal and achene consisting of cells with a different morphology from those above and below. In the final stages of development in both lines (Fig. 2 below), the cells at this region were oval to elliptical in shape as seen in sections parallel to the axis of the achene and petal, with their long dimensions transverse to that axis. The more distal cells (technically in the petal) are elongated in the direction of the axis of the petal. The proximal cells (those in the achene) are polygonal, and more or less isodiametric. Those proximal to the region are more closely packed compared to the distal cells. Because the petals separate from the achenes by the destruction of cells within the region of transversely oriented cells (the separation layer), it is permissible to refer to the region as an abscission zone.

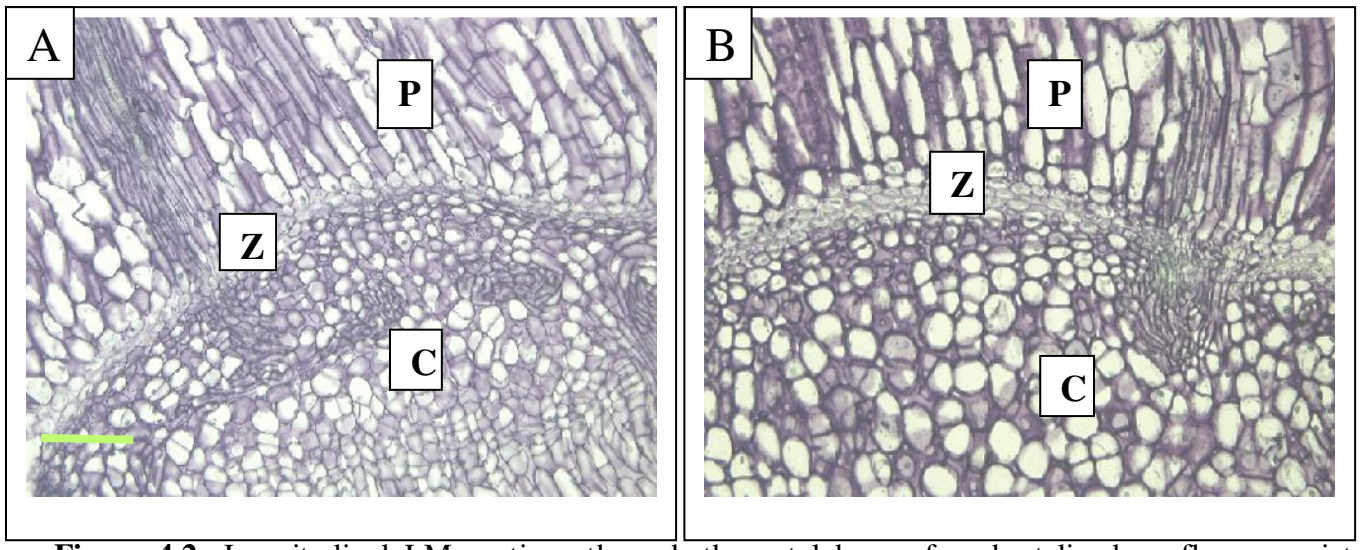

Figure 4.2: Longitudinal LM sections through the petal base of a short-lived sunflower variety PBC (A) on Day 8 and a long lived variety PYL (B) on Day 12. P is the petal, C is the achene consisting of isodiametric cells, and Z is the location of the abscission zone in both varieties. X 120. Bar = 100µm

The abscission zone as seen in the final stages of development in both lines (Figure 4.2) had between 4-5 layers of smaller transversely oriented cells which laid horizontally across the diameter at the junction between the petal and the achene, where the separation takes place. The separation layer was uninterrupted, and traversed the entire plane of the abscission zone. Apart from the separation layer cells (those found in the newly differentiated region where separation eventually occurs), the separation layer also consisted of cells that were destined to be part of the surface of the achene after it is free of the petal. These cells were different from the separation zone cells in that they were polygonal, and more or less isodiametric (Figure 4.2). The cells at the separation layer in both lines were relatively cytoplasmic with intact organelles, plastids containing small starch grains, and no visible intercellular spaces (Figure 4.2). Results from tests for the presence of lignin, suberin and pectic substances revealed that there was no accumulation of these substances in the abscission zones of sunflowers (Figure 4.3 below).

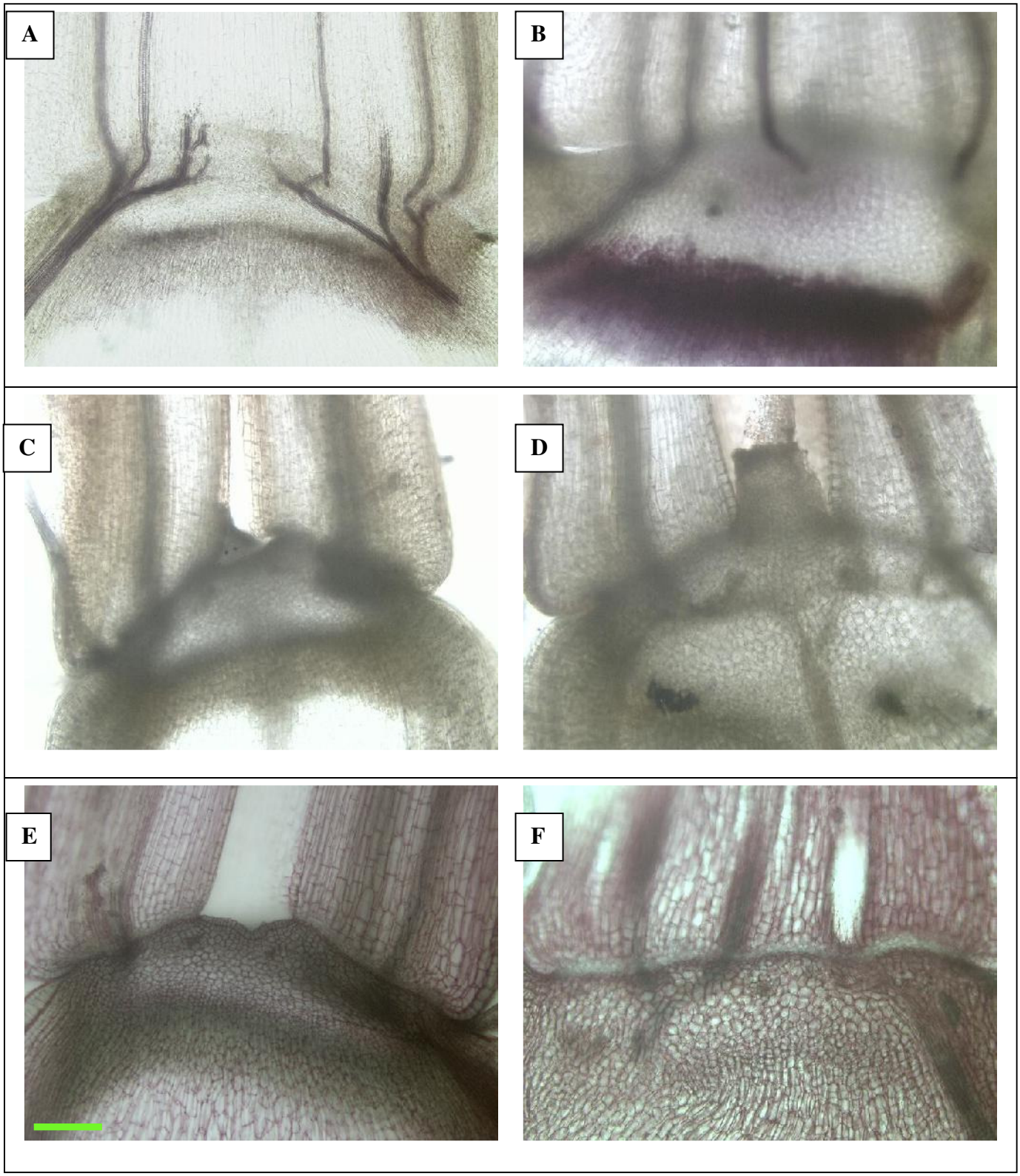

Figure 4.3: Sections from the tests for lignin (A, B), suberin (C, D), and pectic substances (E, F) in PBC and PYL lines, respectively; the sections show no accumulation of lignin, suberin and pectic substances in the separation layer. Sections taken at the most advanced stages of development in both lines; Day 8 for variety PBC and Day 12 for variety PYL. X 60. Bar = 200µm

The separation layer was seen to change over time from stage 1 to stage 3 in line PBC and from stages 1 through 4 in line PYL. In stage 1 (Figure 4.4A and 4.4B), the cells at the separation layer were tightly packed and more or less undifferentiated across both lines. At stage 3 for line PBC (Figure 4.4E), the cells in the separation layer were seen to be more defined, expanded, and rounded. It is assumed that PBC (the short-lived variety) attains maximum cell differentiation at this stage. Results from petal detachment forces confirm that by stage 3 (Day 8) the force of attachment was zero (Figure 4.1) due to the formation and maturation of the separation layer which causes the petals to drop by a gentle touch at this time. The long-lived variety continued to hold its petals until day 12 (Figure 4.1). At stage 4 (Day 12), the cells at the separation layer of the long-lived variety (Figure 4.7) in turn expanded and became rounded. Petal detachment force measurements confirmed that by stage 4 (day 12), the detachment forces decreased significantly (15.39 grams-Figure 4.1 above) and the flower died.

While the separation layer of PYL was only 2 layers thick on Day 8 that of PBC was 4 layers on Day 8 when it drops its petals. PYL dropped its petals four days later (Day 12) with a 5 layers thick separation layer. This result reveals that cell division at the abscission zone of the short-lived variety occurred earlier and faster than that in the long-lived variety. These differences indicate that whereas the anatomical and cellular nature of the abscission zone is similar in the two lines, the tempo of development differs in a way that seems to account for earlier petal drop in line PBC. Specifically, the abscission layer reaches full differentiation, or maturity, sooner in line PBC than in line PYL. This result further reveals the horticultural importance of abscission in petal drop of sunflowers in general, and that the differences in timing of the formation and maturation of an abscission zone accounts for the differences in timing to petal drop in sunflower varieties that differ with respect to petal drop.

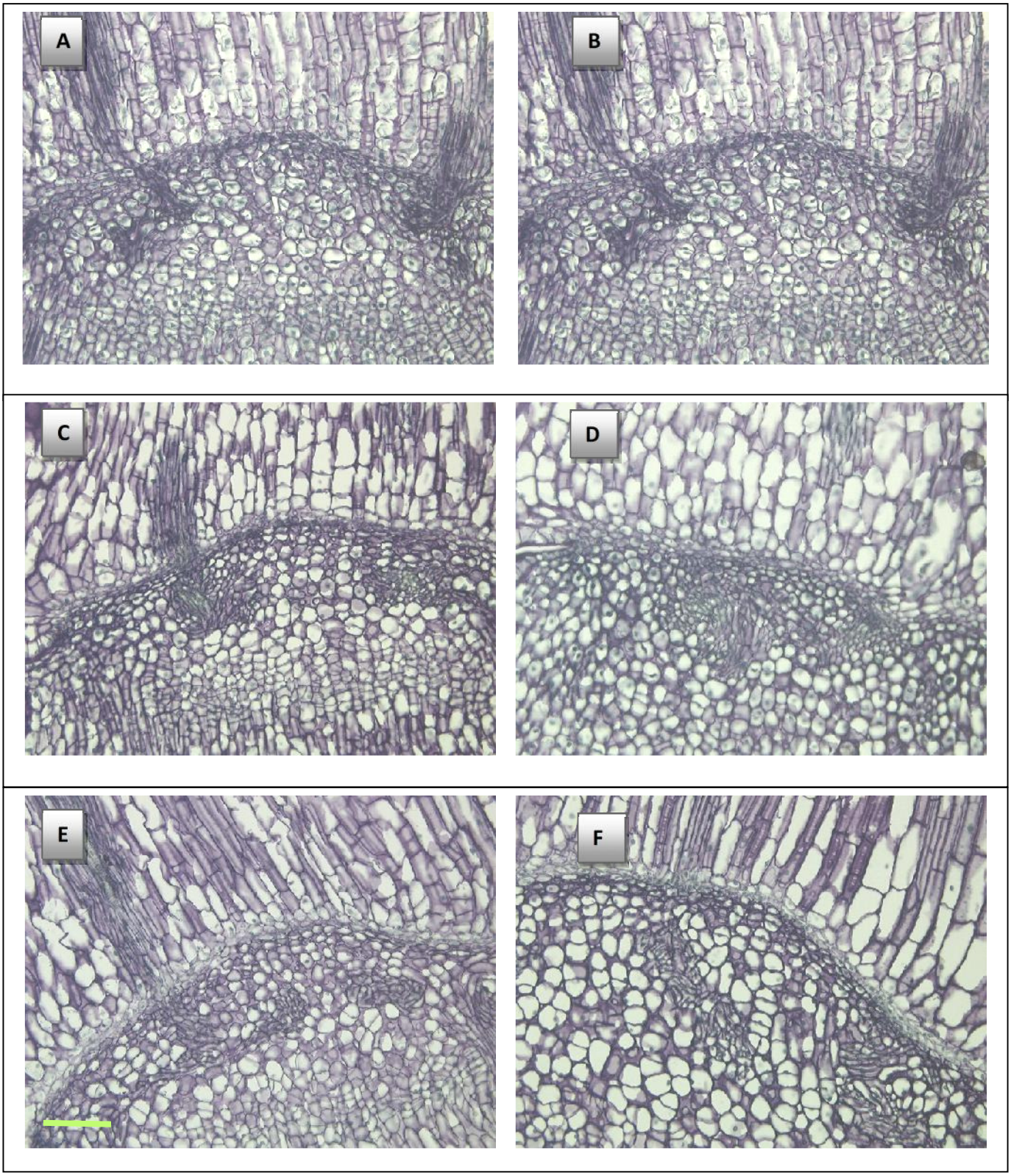

Figure 4.4: The change in the appearance of the separation layer over time, for line PBC (A) and line PYL (B) at stage 1(Day 1); for PBC (C) and PYL (D) at stage 2(Day 4); and for PBC (E) and PYL (F) at stage 3(Day 8). X 120. Bar = 100µm

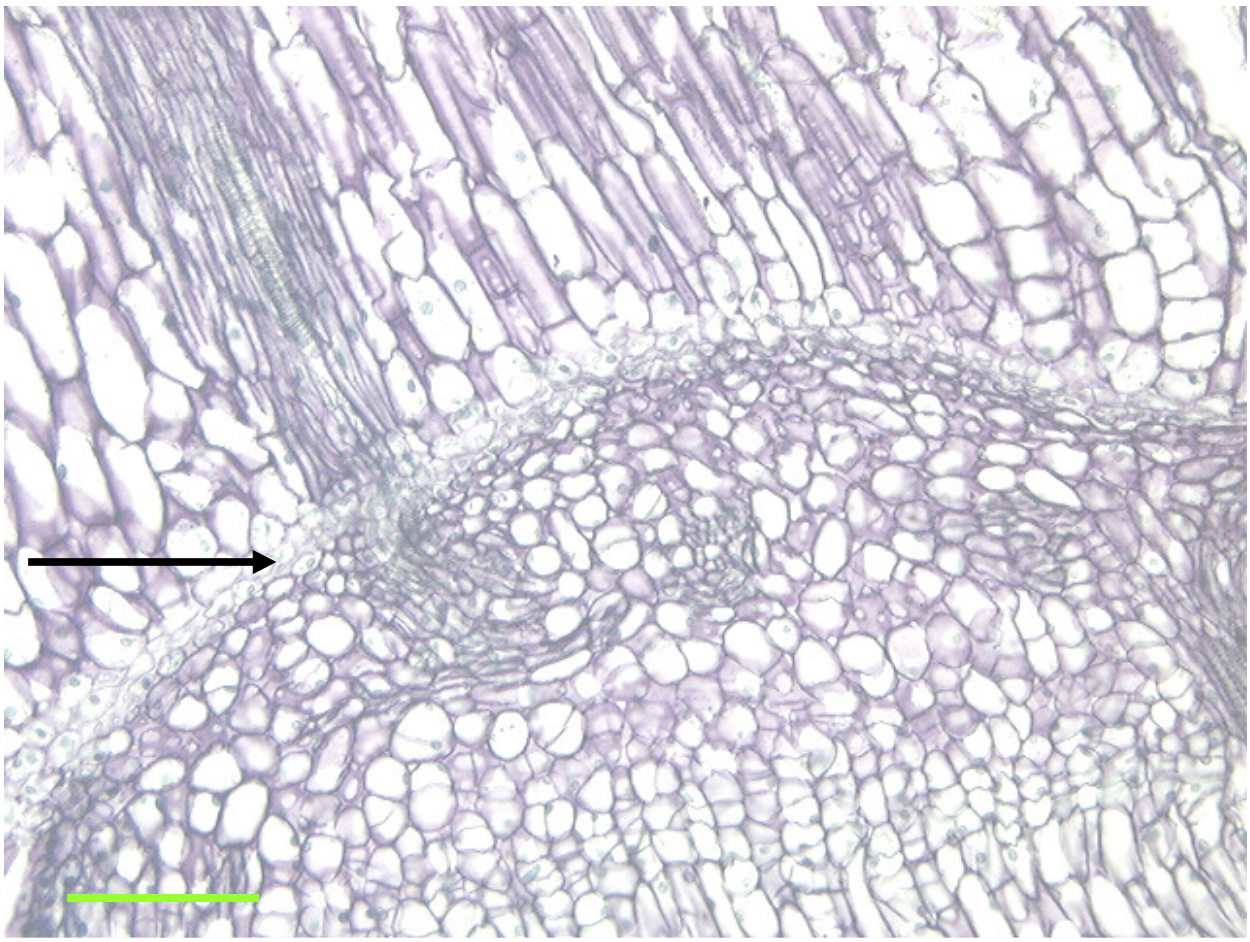

Figure 4.5: A high magnification picture of the short-lived variety PBC (picture E stage 3 in figure 4 above) taken on Day 8 showing a fully differentiated and mature separation layer (see arrow). 220X Bar=100µm

Figure 4.5 and 4.6 are higher magnification pictures taken on Day 8 for variety PBC and PYL respectively. Figure 4.5 shows that the speed of development and maturation of the separation layer was faster in the short-lived variety PBC than in the long-lived variety (Figure 4.6 below) on the same day. This can be seen by the number of cell layers at the separation zone in both varieties (indicated by the arrows). The short-lived variety had 4 cell layers (Figure 4.5 above) at the separation zone on Day 8 whereas the long-lived variety had only 2 (Figure 4.6 below). These results reveal that the separation layer differentiates and matures faster in variety PBC than in variety PYL, which causes PBC to drop its petals earlier than PYL.

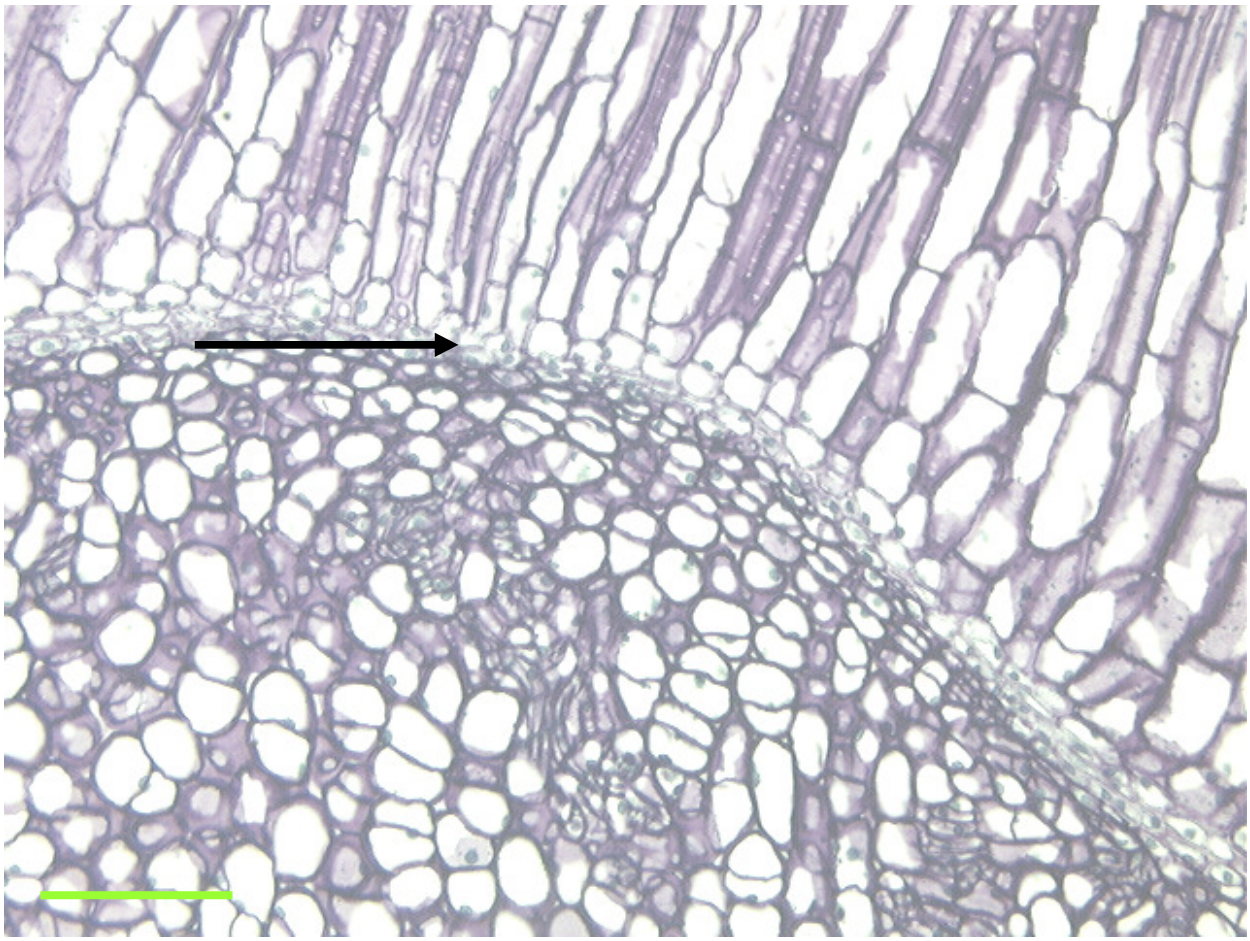

Figure 4.6: A high magnification picture of the long-lived variety PYL (Picture F stage 3 in figure 4.4 above) taken on Day 8 showing the progression of cell differentiation at the separation layer (see arrow). The separation layer is less developed in PYL on Day 8 compared to PBC. Note that variety PYL attains a fully differentiated and mature separation layer on Day 12 as seen in Fig 7 below. 220X Bar=100µm

In Figure 4.4, note that at stage 1, the cells are tightly glued together and no separation layer is apparent. By stage 3 (plates E and F in Figure 4.4), the weakening process appears faster in line PBC than PYL and petal drop happened for this variety at this time, while line PYL continues to hold its petals until stage 4.

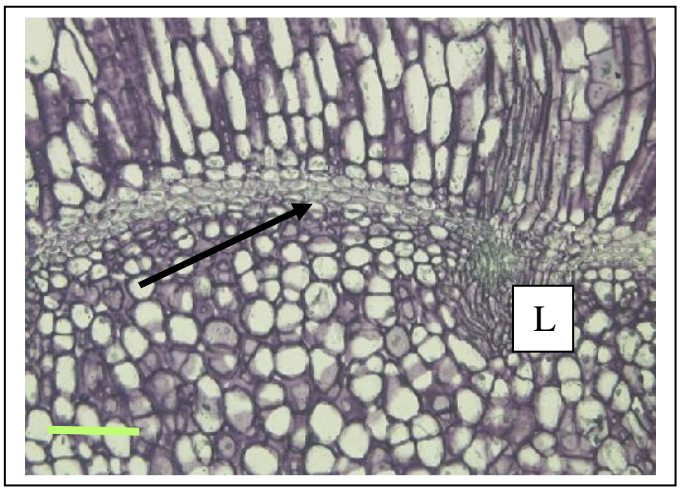

Figure 4.7: Light Micrograph showing a mature separation layer (see arrow) for line PYL at stage 4 (Day 12) when the variety drops its petals; by this time, flowers for line PBC have lost their petals. L shows tracheary elements. Tracheary elements appear less well developed during the separation process. X 120. Bar = 100µm

There were recognizable tracheary elements from the achene up through the separation layer into the petal in all treatments in both lines on Day 1. However, the tracheary elements seemed to stop when they reached the abscission zone by Day 8 and Day 12 when the separation layer was fully differentiated and mature in PBC and PYL, respectively (Figure 4.7). The separation layer in both lines was continuous and uninterrupted.

Anatomy of sunflower petal drop

The anatomical features of petal drop in sunflower appear identical to the majority of established descriptions of abscission (Sexton and Roberts, 1982; Sexton et al., 1985, Sexton, 1994; Sexton et al., 2000). The process involved the separation of 4-5 rows of smaller transversely oriented cells which laid horizontally across the diameter at the juncture between the petal and the achene, the separation layer. While separation in tulip tepal abscission happens along a fracture line (Sexton et al., 2000), in sunflower it happens in a separation layer.

The separation layer in sunflower was produced by cell division before the separation process happens. The separation layer was uninterrupted and traversed the entire plane of the abscission zone. The cells at the separation layer were smaller and oval to elliptical in shape and morphologically distinct from those distal and proximal to that region. The petal separates from the achene by the destruction of cells at the separation layer. The process of cellular separation has been proposed to be restricted to 1-5 layers of cells, the 'separation layer' which is found in a flat uninterrupted plane that cuts across the whole structure (Gawadi and Avery, 1950; Hodgson, 1918; Tison, 1900). The separation zone cells in sunflower appear to remain intact, expanded and rounded during the separation process. Sexton and Redshaw (1981) reported that cells at the separation layer expand and become rounded during abscission. The separation layer cells in sunflower were also relatively cytoplasmic with intact organelles and plastids. These results are similar to the separation process in tulip tepal abscission (Sexton et al., 2000).

Although cell separation in tulip tepal abscission and many other species results from the breakdown of the middle lamella and adjacent areas of the primary wall (Polito and Lavee, 1980; Sexton et al., 2000; Webster, 1973), in sunflower, there is no visible breakdown of the middle lamella and the cells at the separation layer appear to have contact with other surrounding cells during separation. In *P. X hortorum* (Evensen et al., 1993b), the middle lamella was extensively degraded with some cells in the abscission zone appearing to have no contact with most of the surrounding cells. This was not the case in sunflower. In tulip, the separation process appeared to start at the periphery of the tepal base and spread centripetally, but in sunflowers there appeared to be no specific location where the separation began.

There was a difference in timing in the formation and maturation of the separation layer between the two lines, which accounts for the difference in timing to petal drop in sunflower varieties

that differ with respect to petal drop. Although the anatomical and cellular nature of the abscission zone is similar in the two lines, the tempo of development differs in a way that seems to account for earlier petal drop in PBC compared to PYL. The development and maturation of the separation layer was faster in PBC compared to PYL.

There was no accumulation of lignin, suberin or pectic substances in the separation layer. This has been confirmed in abscission processes of other species. Failure to lignify is a characteristic of the separation layer (Facey, 1950). Osborne (1989) suggested that pectins are degraded and their bonds with other molecules cleaved by hydrolytic enzymes.

Recommendation for future studies

The results show clearly that the separation process in sunflower petal abscission happens at a separation layer at the juncture between petal and achene. A separation layer is a layer of cells within an abscission zone which becomes physiologically active and secretes the hydrolytic enzymes that weaken the cell walls and permit separation (Addicott, 1982). It is possible to modify the position of the separation layer by treatments which modify the length of time before abscission during which the cell division can occur (Addicott, 1982). Future anatomical studies to confirm the effect of chemicals like BAP-10 and BA+GA4+7 which delayed abscission in sunflower should be a reasonable follow up to this study. Also, the anatomical study carried out here should be repeated for more sunflower varieties.

In Chapter one of the thesis, we identified a relationship between color of variety and petal break strength. The red and dark colored varieties dropped their petals earlier than the orange or yellow varieties. Anatomical studies to check the relationship between the timing of the formation and maturation of the separation layer with color of varieties should be carried out. Detail studies on the

cell shape, cell size, histological differentiation, cell content, chemical changes at the cell level for distinct color groups comparing within and between color groups should be done. Finally, hormone analysis should be done at different stages of flower development, at the start and end of the abscission process for more varieties to understand the physiological reason for changes in hormone levels between lines and its effects on abscission.

Conclusion

The concept that the timing of the maturation of the separation layer in the abscission zone helps determine the timing of petal drop is strongly supported by the finding that both physical (detachment force experiments) and anatomical investigations point in this direction. However, anatomical appearances do not tell us when the changes in enzymes have occurred that render the separation layer ready for destruction.

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Chapter 5: Conclusions, Recommendations and Future Studies

The loss of petals from newly opened flowers in some of the most attractive sunflower cultivars used as cut flowers is detrimental to the flower appearance and commonly leads to rejection in the market, causing a reduction in overall sales. Growers of sunflowers have been aware of this characteristic, but have lacked a reliable method of measuring petal loss, and consequently vase life, objectively. Breeders of sunflowers have also been aware of this disorder, and have been actively selecting for lines which are less prone to this disorder and have longer vase life.

The research 'petal drop in sunflowers: varietal differences and possible remedies' has investigated the morphological, anatomical and physiological factors responsible for the loss of petals in some sunflower genotypes used as cut flowers and proposes remedies. A detailed description of the petal breakstrength methodology and procedure is presented in the Materials and Methods section in Chapter two of this thesis. The petal breakstrength meter proved to be a reliable method of measuring susceptibility to petal drop objectively. A faster method is to brush against the flower head near the base of the petal (the brushing test technique), but this method is easily biased. The petal breakstrength meter provides an accurate, reproducible way of measuring varietal susceptibility to petal drop. Using the breakstrength meter, we were able to confirm varietal susceptibility to this characteristic and group sunflower varieties into different categories based on the detachment force readings. These were the susceptible, moderately susceptible and less susceptible categories.

In Chapter two, the physical studies carried out point to the fact that there is a meaningful relationship between petal color and petal abscission in sunflowers. We found that the red colored varieties had a shorter vase life followed by the bicolor and the orange groups. The yellow colored varieties were the most resistant to petal drop, and recorded the highest vase life. These findings reveal that there is a relationship between color and abscission in sunflowers. Future studies to

investigate this relationship at a genetic level should be carried out. Could it be that the plant breeders of these new varieties are using susceptible varieties as the parent plants to make crosses for the red and dark red cultivars? Are there dark parent varieties that are less susceptible to petal loss that could be used? Future research into less susceptible parent lines should be done.

We used the physical characteristic of flower color and detachment force to develop a regression equation that would enable growers to estimate the vase life of sunflowers. With this equation it is easy to estimate the vase life of sunflowers if they have information on the color, breakstrength or detachment force, and the number of days after harvesting that the breakstrength was measured. For future studies, the same experiments should be repeated with an increased number of sunflower cultivars from color groups not included in this study. The regression equation for vaselife and other results from this study will be beneficial to breeding programs which are directed towards the improvement of sunflower longevity. Furthermore, this knowledge will also lead to overall increase in sale of sunflowers by growers.

Growers of cut sunflowers have informed us that most of the new commercial cut flower cultivars are hybrids and are being produced by specific breeders. These breeders and companies do not release information on the identity of parental lines of these new cultivars. Some of these modern cut flower cultivars have very attractive petals, but have shorter vaselife. Therefore, it is likely that the parent plants from which the red/dark red cultivars are developed from are susceptible to petal loss and thus this trait is passed on to progeny. However, future studies into the relationship between parent plants and vase life should be carried out. Experiments on postharvest life and longevity of the parent plants and the modern cultivars should be done before releasing them into the market.

Future studies should be carried out to use the petal breakstrength meter on additional sunflower cultivars. In our study to predict the vaselife of sunflowers, we measured detachment forces on Day 1, 3, 6, 9 and 12. Future studies should measure detachment forces every day from Day 1 to 14 in order to fully capture the varietal differences in this characteristic. There are more sunflower color groups that have recently been released in the market. Additional experiments to test the effect of color on vaselife using the petal breakstrength meter should be carried out.

In our morphology experiments, we investigated the relationship between head diameter and petal length on petal drop tendency. Future studies to determine the relationship between head shape and petal drop tendency should be done. In these experiments we had no information of which version of Procut Bicolor we used because the breeders don't release that information to seed companies. In future experiments, different versions of the cultivars undergoing improvements should be used where possible. In addition, future work should be done jointly with breeders or with companies which have patented these new cultivars.

Chapter three presents results from phytohormone analyses in abscission zone tissues in five sunflower genotypes. It shows that there were genotypic differences in the levels of phytohormones in sunflower abscission zone tissues. Every genotype behaved differently. Future studies should analyze phytohormone at different stages of flower development: when the flower just opens, mid-way and at the end of the flower life. Other phytohormones such as GA should also be analyzed. From these extended physiological experiments, it could be possible to establish a hormonal basis for the difference in petal drop characteristic between lines. Our results from experiments using commercial cytokinin products to extend vase life of sunflowers revealed that Fascination was effective in significantly extending the vase life of cut sunflowers which are prone to petal loss. We therefore recommend the use of Fascination to extend postharvest life of sunflowers for growers.

Future research on new commercial cytokinin products to extend vase life in cut sunflowers should be done. Additional experiments on methods of application of Fascination such as spraying the buds before full bloom should also be done.

Chapter four presents results from the study of sunflower anatomy. It reveals that the separation process in sunflower petal abscission happens within a separation layer which is located at the juncture between petal and achene. Future anatomical studies to show the effect of chemicals like BAP-10 and BA+GA4+7 which delayed abscission in sunflower should be carried out to show anatomical effects of these chemicals on the separation layer. Also, the anatomical study carried out in this thesis should be repeated for more sunflower cultivars. Anatomical studies to check the relationship between the timing of the formation and maturation of the separation layer with color of varieties should also be done. Detail studies on the cell shape, cell size, histological differentiation, cell content, chemical changes at the cell level for various distinct color groups comparing within and between color groups should be done.

APPENDIX A

Raw data pertaining to Chapter 2

Detachment force, time (days), vase life, and color category of 13 sunflower cultivars. CH: Chianti, CR:Cherry Rose, MR:Moulin Rouge, PBC:Procut Bicolor, PRLB:Procut Red Lemon Bicolor, SB:Strawberry Blonde, OG:Orange Glory, PEO:Procut Early Orange, PO:Procut Orange, PP: Procut Peach, SO:Sunrich Orange, PL:Procut Lemon, PYL:Procut Yellow Lite.

Table A1: Data table for Chapter 2, Tables 2.5, 2.6, 2.7, 2.8; Figures 2.5, 2.6 and 2.7

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
CH	1	93.9	9	Red
CH	1	83.5	9	Red
CH	1	118.8	9	Red
CH	1	96.2	9	Red
CH	1	129	9	Red
CH	1	142.7	9	Red
CH	1	123.5	9	Red
CH	1	101	9	Red
CH	1	168.3	9	Red
CH	1	140.4	9	Red
CH	1	140.6	9	Red
CH	1	124.4	9	Red
CH	1	114.3	9	Red
CH	1	120.8	9	Red
CH	1	124	9	Red
CH	1	135	9	Red
CH	1	134.3	9	Red
CH	1	116.1	9	Red
CH	1	108.7	9	Red
CH	1	110.6	9	Red
CH	1	132.4	9	Red
CH	1	102.5	9	Red
CH	1	144	9	Red
CH	1	144.4	9	Red
CH	1	107.1	9	Red
CH	1	97.3	9	Red
CH	1	92.5	9	Red
CH	1	105	9	Red
CH	1	71.4	9	Red
CH	1	88.7	9	Red
CH	1	79.4	9	Red
CH	1	73.7	9	Red
CH	1	89.7	9	Red
CH	1	92.8	9	Red
CH	1	108.3	9	Red

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
CH	1	133.9	9	Red
CH	1	82.7	9	Red
CH	1	88.9	9	Red
CH	1	93.4	9	Red
CH	1	107	9	Red
CH	1	89.4	9	Red
CH	1	95.3	9	Red
CH	1	105.9	9	Red
CH	1	113.5	9	Red
CH	1	104.9	9	Red
CH	1	98.5	9	Red
CH	1	104.4	9	Red
CH	1	95.5	9	Red
CH	1	77.9	9	Red
CH	1	85.3	9	Red
CH	1	77.2	9	Red
CH	1	92.4	9	Red
CH	3	126.5	9	Red
CH	3	152.7	9	Red
CH	3	137.5	9	Red
CH	3	121.9	9	Red
CH	3	137.6	9	Red
CH	3	140	9	Red
CH	3	118.8	9	Red
CH	3	137.6	9	Red
CH	3	100.2	9	Red
CH	3	113.1	9	Red
CH	3	128.7	9	Red
CH	3	102.5	9	Red
CH	3	146.7	9	Red
CH	3	139.2	9	Red
CH	3	116.2	9	Red
CH	3	126.1	9	Red
CH	3	146.6	9	Red
CH	3	132.7	9	Red
CH	3	155.7	9	Red
CH	3	151.3	9	Red
CH	3	108.3	9	Red
CH	3	114.9	9	Red
CH	3	117.2	9	Red
CH	3	144.2	9	Red
CH	3	117.4	9	Red
CH	3	125.3	9	Red
CH	3	125.8	9	Red
CH	3	130.9	9	Red
CH	3	105.6	9	Red
CH	3	121.7	9	Red
CH	3	115.8	9	Red
CH	3	100.8	9	Red
CH	3	162.6	9	Red

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
CH	3	107.7	9	Red
CH	3	137.4	9	Red
CH	3	114.5	9	Red
CH	3	97.1	9	Red
CH	3	128.2	9	Red
CH	3	130.3	9	Red
CH	3	139.1	9	Red
CH	3	87.1	9	Red
CH	3	136.4	9	Red
CH	3	133.3	9	Red
CH	3	135.5	9	Red
CH	3	92.9	9	Red
CH	3	106.6	9	Red
CH	3	91.6	9	Red
CH	3	134.1	9	Red
CH	3	116.9	9	Red
CH	3	125.6	9	Red
CH	3	134.5	9	Red
CH	3	108.5	9	Red
CH	3	90.4	9	Red
CH	3	99.9	9	Red
CH	3	113.6	9	Red
CH	3	122.7	9	Red
CH	3	113.1	9	Red
CH	3	112.8	9	Red
CH	3	84	9	Red
CH	3	98.1	9	Red
CH	3	115.2	9	Red
CH	3	114.2	9	Red
CH	3	97.5	9	Red
CH	3	90.9	9	Red
CH	3	70.6	9	Red
CH	3	95.6	9	Red
CH	3	93.5	9	Red
CH	3	95.9	9	Red
CH	3	103.6	9	Red
CH	3	81.7	9	Red
CH	3	85.7	9	Red
CH	3	87.3	9	Red
CH	3	104.5	9	Red
CH	3	76	9	Red
CH	3	99.5	9	Red
CH	3	111.5	9	Red
CH	6	20.9	9	Red
CH	6	34.7	9	Red
CH	6	33.6	9	Red
CH	6	37.1	9	Red
CH	6	24	9	Red
CH	6	18.3	9	Red
CH	6	10.6	9	Red

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
CH	6	29	9	Red
CH	6	24.8	9	Red
CH	6	25.2	9	Red
CH	6	30.6	9	Red
CH	6	14.4	9	Red
CH	6	18.7	9	Red
CH	6	20.4	9	Red
CH	6	19.8	9	Red
CH	6	27.2	9	Red
CH	6	25.1	9	Red
CH	6	18.7	9	Red
CH	6	13.9	9	Red
CH	6	30.7	9	Red
CH	6	20.7	9	Red
CH	6	25.4	9	Red
CH	6	23.7	9	Red
CH	6	9.2	9	Red
CH	6	51.1	9	Red
CH	6	35.7	9	Red
CH	6	37.6	9	Red
CH	6	35.1	9	Red
CH	6	16.1	9	Red
CH	6	47.3	9	Red
CH	6	55.3	9	Red
CH	6	38.8	9	Red
CH	6	23	9	Red
CH	6	25.9	9	Red
CH	6	25.2	9	Red
CH	6	24.7	9	Red
CH	6	12.6	9	Red
CH	6	25.7	9	Red
CH	6	21.5	9	Red
CH	6	21.5	9	Red
CH	6	20.4	9	Red
CH	6	12.3	9	Red
CH	6	22	9	Red
CH	6	12.8	9	Red
CH	6	17.6	9	Red
CH	6	80.9	9	Red
CH	6	92.5	9	Red
CH	6	83.1	9	Red
CH	6	81.8	9	Red
CH	6	26.4	9	Red
CH	6	35.1	9	Red
CH	6	26.1	9	Red
CH	6	25.4	9	Red
CH	6	37.5	9	Red
CH	6	41.3	9	Red
CH	6	29	9	Red
CH	6	23.4	9	Red

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
CH	6	25	9	Red
CH	6	20.8	9	Red
CH	6	28	9	Red
CH	6	19	9	Red
CH	6	17.9	9	Red
CH	6	23.2	9	Red
CH	6	44.1	9	Red
CH	6	18.4	9	Red
CH	6	38.6	9	Red
CH	6	39.7	9	Red
CH	6	21.7	9	Red
CH	6	25.2	9	Red
CH	6	24.5	9	Red
CH	6	30.9	9	Red
CH	6	27	9	Red
CH	6	25.4	9	Red
CH	9	1.6	9	Red
CH	9	1.9	9	Red
CH	9	8.8	9	Red
CH	9	4.5	9	Red
CH	9	12.6	9	Red
CH	9	15.6	9	Red
CH	9	7.9	9	Red
CH	9	13.2	9	Red
CH	9	9.1	9	Red
CH	9	12.3	9	Red
CH	9	14	9	Red
CH	9	12	9	Red
CH	9	13.2	9	Red
CH	9	15.7	9	Red
CH	9	15	9	Red
CH	9	9.4	9	Red
CH	9	12.6	9	Red
CH	9	12	9	Red
CH	9	9.5	9	Red
CH	9	11.6	9	Red
CR	1	183.1	6	Red
CR	1	182.4	6	Red
CR	1	161.9	6	Red
CR	1	173.5	6	Red
CR	1	158	6	Red
CR	1	156.6	6	Red
CR	1	170.8	6	Red
CR	1	124	6	Red
CR	1	146.9	6	Red
CR	1	151.4	6	Red
CR	1	143.7	6	Red
CR	1	126.8	6	Red
CR	1	122.6	6	Red
CR	1	113.9	6	Red

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
CR	1	128.2	6	Red
CR	1	114	6	Red
CR	1	134.3	6	Red
CR	1	122.3	6	Red
CR	1	126.2	6	Red
CR	1	117.2	6	Red
CR	1	119.4	6	Red
CR	1	109.5	6	Red
CR	1	118.4	6	Red
CR	1	117.5	6	Red
CR	1	93.1	6	Red
CR	1	100	6	Red
CR	1	98.8	6	Red
CR	1	98.4	6	Red
CR	1	120.8	6	Red
CR	1	131.6	6	Red
CR	1	108.2	6	Red
CR	1	137.8	6	Red
CR	1	143	6	Red
CR	1	122.3	6	Red
CR	1	145.2	6	Red
CR	1	139.5	6	Red
CR	1	92.7	6	Red
CR	1	117	6	Red
CR	1	105.1	6	Red
CR	1	105.2	6	Red
CR	1	87.8	6	Red
CR	1	124.8	6	Red
CR	1	117.6	6	Red
CR	1	133.4	6	Red
CR	1	119.3	6	Red
CR	1	106.8	6	Red
CR	1	114.8	6	Red
CR	1	95.7	6	Red
CR	1	104.8	6	Red
CR	1	131.6	6	Red
CR	1	109.7	6	Red
CR	1	97.1	6	Red
CR	3	120	6	Red
CR	3	175.3	6	Red
CR	3	131	6	Red
CR	3	94.6	6	Red
CR	3	110.9	6	Red
CR	3	130.4	6	Red
CR	3	98.5	6	Red
CR	3	107.1	6	Red
CR	3	112.1	6	Red
CR	3	123.6	6	Red
CR	3	128.5	6	Red
CR	3	113.2	6	Red

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
CR	3	159.5	6	Red
CR	3	140.8	6	Red
CR	3	140.1	6	Red
CR	3	139.3	6	Red
CR	3	139.2	6	Red
CR	3	139.3	6	Red
CR	3	102.7	6	Red
CR	3	112.4	6	Red
CR	3	123.4	6	Red
CR	3	157.8	6	Red
CR	3	109	6	Red
CR	3	134.7	6	Red
CR	3	109.8	6	Red
CR	3	111.8	6	Red
CR	3	108.2	6	Red
CR	3	125.1	6	Red
CR	3	95.5	6	Red
CR	3	110.3	6	Red
CR	3	108.9	6	Red
CR	3	110.6	6	Red
CR	3	97.6	6	Red
CR	3	94	6	Red
CR	3	110.1	6	Red
CR	3	126.4	6	Red
CR	3	118	6	Red
CR	3	138.2	6	Red
CR	3	114	6	Red
CR	3	124.4	6	Red
CR	3	108.9	6	Red
CR	3	80.6	6	Red
CR	3	115.8	6	Red
CR	3	125	6	Red
CR	3	100.3	6	Red
CR	3	103.6	6	Red
CR	3	81.3	6	Red
CR	3	107.1	6	Red
CR	3	65.1	6	Red
CR	3	82.8	6	Red
CR	3	77.5	6	Red
CR	3	79.9	6	Red
CR	3	117.3	6	Red
CR	3	92	6	Red
CR	3	118.6	6	Red
CR	3	126.3	6	Red
CR	3	122.4	6	Red
CR	3	107.1	6	Red
CR	3	114.2	6	Red
CR	3	107.9	6	Red
CR	3	117.9	6	Red
CR	3	117.2	6	Red

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
CR	3	109.9	6	Red
CR	3	127.2	6	Red
CR	3	152.7	6	Red
CR	3	129.3	6	Red
CR	3	136.1	6	Red
CR	3	126.6	6	Red
CR	3	92.6	6	Red
CR	3	86.5	6	Red
CR	3	96.9	6	Red
CR	3	109.5	6	Red
CR	6	43.9	6	Red
CR	6	39.2	6	Red
CR	6	37.9	6	Red
CR	6	69.1	6	Red
CR	6	36.7	6	Red
CR	6	30.4	6	Red
CR	6	20	6	Red
CR	6	19.5	6	Red
CR	6	35.5	6	Red
CR	6	43.3	6	Red
CR	6	35.9	6	Red
CR	6	45.8	6	Red
CR	6	42.1	6	Red
CR	6	27.4	6	Red
CR	6	20	6	Red
CR	6	39	6	Red
CR	6	77.3	6	Red
CR	6	69.8	6	Red
CR	6	61.5	6	Red
CR	6	63	6	Red
CR	6	68.3	6	Red
CR	6	63.1	6	Red
CR	6	77.7	6	Red
CR	6	61.5	6	Red
CR	6	50.3	6	Red
CR	6	34.6	6	Red
CR	6	56.2	6	Red
CR	6	34.8	6	Red
CR	6	28.7	6	Red
CR	6	18.2	6	Red
CR	6	34.9	6	Red
CR	6	0.5	6	Red
CR	6	18.8	6	Red
CR	6	15	6	Red
CR	6	25.3	6	Red
CR	6	12.5	6	Red
CR	6	27.7	6	Red
CR	6	26.9	6	Red
CR	6	10.8	6	Red
CR	6	15	6	Red

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
CR	6	11.3	6	Red
CR	6	0.6	6	Red
CR	6	5.5	6	Red
CR	6	15.2	6	Red
CR	6	5.4	6	Red
CR	6	20.8	6	Red
CR	6	1.3	6	Red
CR	6	17.7	6	Red
CR	6	22	6	Red
CR	6	29.7	6	Red
CR	6	17	6	Red
CR	6	19.5	6	Red
CR	6	18.9	6	Red
CR	6	40.1	6	Red
CR	6	36.6	6	Red
CR	6	28	6	Red
MR	1	115.7	9	Red
MR	1	179.8	9	Red
MR	1	132.8	9	Red
MR	1	138.8	9	Red
MR	1	111.3	9	Red
MR	1	110.9	9	Red
MR	1	113.3	9	Red
MR	1	116.8	9	Red
MR	1	193.7	9	Red
MR	1	194.1	9	Red
MR	1	214.7	9	Red
MR	1	217.3	9	Red
MR	1	156	9	Red
MR	1	195.1	9	Red
MR	1	190.8	9	Red
MR	1	187.5	9	Red
MR	1	149.2	9	Red
MR	1	144	9	Red
MR	1	140.9	9	Red
MR	1	152.9	9	Red
MR	1	157.6	9	Red
MR	1	153.9	9	Red
MR	1	147.9	9	Red
MR	1	202.5	9	Red
MR	1	161	9	Red
MR	1	160.6	9	Red
MR	1	194.6	9	Red
MR	1	200.5	9	Red
MR	1	172.1	9	Red
MR	1	163.9	9	Red
MR	1	162.1	9	Red
MR	1	163.7	9	Red
MR	1	106.2	9	Red
MR	1	144	9	Red

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
MR	1	136.5	9	Red
MR	1	157.9	9	Red
MR	1	104.9	9	Red
MR	1	65.6	9	Red
MR	1	90.8	9	Red
MR	1	106.3	9	Red
MR	1	85.2	9	Red
MR	1	88	9	Red
MR	1	102.6	9	Red
MR	1	127	9	Red
MR	1	98.4	9	Red
MR	1	104.4	9	Red
MR	1	110.1	9	Red
MR	1	120.4	9	Red
MR	1	65.4	9	Red
MR	1	70.5	9	Red
MR	1	65	9	Red
MR	1	66.7	9	Red
MR	1	78.1	9	Red
MR	1	77.3	9	Red
MR	1	97.2	9	Red
MR	1	88.5	9	Red
MR	1	53	9	Red
MR	1	83.2	9	Red
MR	1	64.9	9	Red
MR	1	73.4	9	Red
MR	3	167.4	9	Red
MR	3	151.6	9	Red
MR	3	161.8	9	Red
MR	3	180.9	9	Red
MR	3	164.2	9	Red
MR	3	153.1	9	Red
MR	3	140.3	9	Red
MR	3	173.6	9	Red
MR	3	132.4	9	Red
MR	3	151.7	9	Red
MR	3	124	9	Red
MR	3	179.2	9	Red
MR	3	110.5	9	Red
MR	3	108.5	9	Red
MR	3	146.6	9	Red
MR	3	145.8	9	Red
MR	3	127.8	9	Red
MR	3	144.5	9	Red
MR	3	198.2	9	Red
MR	3	196.7	9	Red
MR	3	182.6	9	Red
MR	3	182.9	9	Red
MR	3	140.2	9	Red
MR	3	163	9	Red

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
MR	3	160.2	9	Red
MR	3	123.5	9	Red
MR	3	116.7	9	Red
MR	3	138.1	9	Red
MR	3	134.8	9	Red
MR	3	123.2	9	Red
MR	3	146.8	9	Red
MR	3	136.7	9	Red
MR	3	132	9	Red
MR	3	119.6	9	Red
MR	3	116	9	Red
MR	3	129.1	9	Red
MR	3	107.8	9	Red
MR	3	130.7	9	Red
MR	3	117.2	9	Red
MR	3	112.4	9	Red
MR	3	126.5	9	Red
MR	3	117.9	9	Red
MR	3	104.7	9	Red
MR	3	118.8	9	Red
MR	3	129.8	9	Red
MR	3	107.1	9	Red
MR	3	124	9	Red
MR	3	129.3	9	Red
MR	3	108.6	9	Red
MR	3	105	9	Red
MR	3	100.3	9	Red
MR	3	102.4	9	Red
MR	3	166.1	9	Red
MR	3	167.7	9	Red
MR	3	146.1	9	Red
MR	3	148.5	9	Red
MR	6	232.1	9	Red
MR	6	159	9	Red
MR	6	229.3	9	Red
MR	6	232.6	9	Red
MR	6	154.4	9	Red
MR	6	64.1	9	Red
MR	6	88.1	9	Red
MR	6	106.7	9	Red
MR	6	21.1	9	Red
MR	6	40.8	9	Red
MR	6	40.2	9	Red
MR	6	33.9	9	Red
MR	6	43.2	9	Red
MR	6	70.8	9	Red
MR	6	55.1	9	Red
MR	6	72.5	9	Red
MR	6	178.3	9	Red
MR	6	58.9	9	Red

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
MR	6	153.3	9	Red
MR	6	174.8	9	Red
MR	6	128.7	9	Red
MR	6	122.4	9	Red
MR	6	98	9	Red
MR	6	144.4	9	Red
MR	6	120.8	9	Red
MR	6	162.1	9	Red
MR	6	139.8	9	Red
MR	6	112.9	9	Red
MR	6	61.1	9	Red
MR	6	85.3	9	Red
MR	6	79.3	9	Red
MR	6	90.8	9	Red
MR	6	119.3	9	Red
MR	6	11.1	9	Red
MR	6	112.9	9	Red
MR	6	85.8	9	Red
MR	6	16.1	9	Red
MR	6	70.4	9	Red
MR	6	60.3	9	Red
MR	6	69.9	9	Red
MR	6	75.8	9	Red
MR	6	77.4	9	Red
MR	6	74.5	9	Red
MR	6	84.3	9	Red
MR	6	73.6	9	Red
MR	6	81.6	9	Red
MR	6	91.5	9	Red
MR	6	112.9	9	Red
MR	6	62.8	9	Red
MR	6	95.1	9	Red
MR	6	107.1	9	Red
MR	6	79.9	9	Red
MR	6	83.5	9	Red
MR	6	75.3	9	Red
MR	6	74.3	9	Red
MR	6	89.9	9	Red
MR	9	57.1	9	Red
MR	9	87.5	9	Red
MR	9	66	9	Red
MR	9	69	9	Red
MR	9	61.3	9	Red
MR	9	38.7	9	Red
MR	9	53.1	9	Red
MR	9	104	9	Red
MR	9	71.3	9	Red
MR	9	77.2	9	Red
MR	9	59.1	9	Red
MR	9	58	9	Red

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
MR	9	14.8	9	Red
MR	9	31.9	9	Red
MR	9	39.6	9	Red
MR	9	33.3	9	Red
MR	9	23.8	9	Red
MR	9	25.8	9	Red
MR	9	18	9	Red
MR	9	19.6	9	Red
MR	9	7.1	9	Red
MR	9	7	9	Red
MR	9	7.9	9	Red
MR	9	7.1	9	Red
MR	9	67.7	9	Red
MR	9	59.5	9	Red
MR	9	62.1	9	Red
MR	9	52.6	9	Red
MR	9	25.5	9	Red
MR	9	30.3	9	Red
MR	9	28.2	9	Red
MR	9	29.2	9	Red
MR	9	37.6	9	Red
MR	9	22	9	Red
MR	9	21.5	9	Red
MR	9	23.7	9	Red
MR	9	47.3	9	Red
MR	9	37.2	9	Red
MR	9	31.9	9	Red
MR	9	24.8	9	Red
MR	9	30.4	9	Red
MR	9	37.7	9	Red
MR	9	42.8	9	Red
MR	9	30.2	9	Red
MR	9	21.5	9	Red
MR	9	42.2	9	Red
MR	9	45.1	9	Red
MR	9	52.1	9	Red
OG	1	186	9	Orange
OG	1	186.8	9	Orange
OG	1	206.8	9	Orange
OG	1	180.5	9	Orange
OG	1	168.7	9	Orange
OG	1	180.6	9	Orange
OG	1	157.4	9	Orange
OG	1	145.3	9	Orange
OG	1	150.1	9	Orange
OG	1	165.9	9	Orange
OG	1	175.9	9	Orange
OG	1	165.7	9	Orange
OG	1	162	9	Orange
OG	1	115.8	9	Orange

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
OG	1	152.5	9	Orange
OG	1	166.8	9	Orange
OG	1	165.6	9	Orange
OG	1	178	9	Orange
OG	1	179.4	9	Orange
OG	1	176.7	9	Orange
OG	3	149.4	9	Orange
OG	3	136	9	Orange
OG	3	136.6	9	Orange
OG	3	139.6	9	Orange
OG	3	147	9	Orange
OG	3	138	9	Orange
OG	3	139.9	9	Orange
OG	3	151.4	9	Orange
OG	3	129.6	9	Orange
OG	3	152.4	9	Orange
OG	3	147	9	Orange
OG	3	142.4	9	Orange
OG	3	161	9	Orange
OG	3	147	9	Orange
OG	3	144.8	9	Orange
OG	3	141.2	9	Orange
OG	3	152.7	9	Orange
OG	3	149.9	9	Orange
OG	3	125	9	Orange
OG	3	135.6	9	Orange
OG	6	19.9	9	Orange
OG	6	27.1	9	Orange
OG	6	15.5	9	Orange
OG	6	56.1	9	Orange
OG	6	8.7	9	Orange
OG	6	20.2	9	Orange
OG	6	10.3	9	Orange
OG	6	7.2	9	Orange
OG	6	15.9	9	Orange
OG	6	16.3	9	Orange
OG	6	16.9	9	Orange
OG	6	6.4	9	Orange
OG	6	12.4	9	Orange
OG	6	2.4	9	Orange
OG	6	0.3	9	Orange
OG	6	8.9	9	Orange
OG	6	1.4	9	Orange
OG	6	3.9	9	Orange
OG	6	0.5	9	Orange
OG	9	1.5	9	Orange
OG	9	1.4	9	Orange
OG	9	0.1	9	Orange
OG	9	1.9	9	Orange
OG	9	3.8	9	Orange

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
OG	9	1.1	9	Orange
OG	9	0.04	9	Orange
OG	9	0.6	9	Orange
OG	9	1.5	9	Orange
OG	9	1.4	9	Orange
OG	9	0.1	9	Orange
OG	9	1.9	9	Orange
OG	9	1.5	9	Orange
OG	9	1.4	9	Orange
OG	9	0.1	9	Orange
OG	9	1.9	9	Orange
PBC	1	186.1	9	Bicolor
PBC	1	187.9	9	Bicolor
PBC	1	224.5	9	Bicolor
PBC	1	193.7	9	Bicolor
PBC	1	206.4	9	Bicolor
PBC	1	218.9	9	Bicolor
PBC	1	202.5	9	Bicolor
PBC	1	192.6	9	Bicolor
PBC	1	192.3	9	Bicolor
PBC	1	181.5	9	Bicolor
PBC	1	178.2	9	Bicolor
PBC	1	173	9	Bicolor
PBC	1	85.4	9	Bicolor
PBC	1	140	9	Bicolor
PBC	1	101.3	9	Bicolor
PBC	1	55.1	9	Bicolor
PBC	3	153	9	Bicolor
PBC	3	174.4	9	Bicolor
PBC	3	186.4	9	Bicolor
PBC	3	152.7	9	Bicolor
PBC	3	127.1	9	Bicolor
PBC	3	179.1	9	Bicolor
PBC	3	134.6	9	Bicolor
PBC	3	169	9	Bicolor
PBC	3	175.5	9	Bicolor
PBC	3	170.1	9	Bicolor
PBC	3	181.2	9	Bicolor
PBC	3	175.5	9	Bicolor
PBC	3	217.7	9	Bicolor
PBC	3	185.5	9	Bicolor
PBC	3	184.1	9	Bicolor
PBC	3	170.3	9	Bicolor
PBC	3	170.8	9	Bicolor
PBC	3	187.6	9	Bicolor
PBC	3	158.4	9	Bicolor
PBC	3	171.3	9	Bicolor
PBC	3	156.3	9	Bicolor
PBC	3	174.9	9	Bicolor
PBC	3	195.6	9	Bicolor

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PBC	3	175.8	9	Bicolor
PBC	3	165.5	9	Bicolor
PBC	3	168.9	9	Bicolor
PBC	3	185.8	9	Bicolor
PBC	3	196.8	9	Bicolor
PBC	3	164.5	9	Bicolor
PBC	3	173.6	9	Bicolor
PBC	3	192.5	9	Bicolor
PBC	3	180.4	9	Bicolor
PBC	3	196.7	9	Bicolor
PBC	3	205.6	9	Bicolor
PBC	3	202.9	9	Bicolor
PBC	3	185.3	9	Bicolor
PBC	6	100.1	9	Bicolor
PBC	6	15.7	9	Bicolor
PBC	6	39.9	9	Bicolor
PBC	6	17.2	9	Bicolor
PEO	1	160.7	12	Orange
PEO	1	129.8	12	Orange
PEO	1	141.8	12	Orange
PEO	1	223.8	12	Orange
PEO	1	124.3	12	Orange
PEO	1	159.9	12	Orange
PEO	1	127.9	12	Orange
PEO	1	161.6	12	Orange
PEO	1	238.9	12	Orange
PEO	1	159.7	12	Orange
PEO	1	234.6	12	Orange
PEO	1	266.2	12	Orange
PEO	1	190.7	12	Orange
PEO	1	165.7	12	Orange
PEO	1	133.2	12	Orange
PEO	1	130	12	Orange
PEO	1	149.9	12	Orange
PEO	1	236.5	12	Orange
PEO	1	177.6	12	Orange
PEO	1	181.8	12	Orange
PEO	1	208.2	12	Orange
PEO	1	217.6	12	Orange
PEO	1	304.4	12	Orange
PEO	1	195.6	12	Orange
PEO	1	157.2	12	Orange
PEO	1	182.7	12	Orange
PEO	1	176.5	12	Orange
PEO	1	147.5	12	Orange
PEO	1	199.9	12	Orange
PEO	1	173.7	12	Orange
PEO	1	177.4	12	Orange
PEO	1	199.9	12	Orange
PEO	1	173.7	12	Orange

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PEO	1	177.4	12	Orange
PEO	1	170.9	12	Orange
PEO	1	155.3	12	Orange
PEO	1	162.8	12	Orange
PEO	1	148.4	12	Orange
PEO	1	152.6	12	Orange
PEO	1	170.8	12	Orange
PEO	1	173.1	12	Orange
PEO	1	184.6	12	Orange
PEO	1	185.6	12	Orange
PEO	1	177	12	Orange
PEO	1	162.7	12	Orange
PEO	1	147.5	12	Orange
PEO	1	173.6	12	Orange
PEO	1	155.6	12	Orange
PEO	3	180.1	12	Orange
PEO	3	155.7	12	Orange
PEO	3	214.8	12	Orange
PEO	3	165.1	12	Orange
PEO	3	178.9	12	Orange
PEO	3	148.9	12	Orange
PEO	3	210.2	12	Orange
PEO	3	247.9	12	Orange
PEO	3	92.1	12	Orange
PEO	3	143.6	12	Orange
PEO	3	114.7	12	Orange
PEO	3	165.1	12	Orange
PEO	3	165.8	12	Orange
PEO	3	190.8	12	Orange
PEO	3	185	12	Orange
PEO	3	170.2	12	Orange
PEO	3	158.7	12	Orange
PEO	3	161.4	12	Orange
PEO	3	151.2	12	Orange
PEO	3	156	12	Orange
PEO	3	190.5	12	Orange
PEO	3	158.8	12	Orange
PEO	3	226.6	12	Orange
PEO	3	166.9	12	Orange
PEO	3	205.3	12	Orange
PEO	3	186.1	12	Orange
PEO	3	216.5	12	Orange
PEO	3	189.4	12	Orange
PEO	3	184.4	12	Orange
PEO	3	186.5	12	Orange
PEO	3	196.4	12	Orange
PEO	3	141.9	12	Orange
PEO	3	159.8	12	Orange
PEO	3	165.7	12	Orange
PEO	3	148.7	12	Orange

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PEO	3	166.4	12	Orange
PEO	3	182.4	12	Orange
PEO	3	185	12	Orange
PEO	3	189.4	12	Orange
PEO	3	203.5	12	Orange
PEO	3	180.2	12	Orange
PEO	3	182.1	12	Orange
PEO	3	192.8	12	Orange
PEO	3	162.7	12	Orange
PEO	3	159.6	12	Orange
PEO	3	158.4	12	Orange
PEO	3	155	12	Orange
PEO	3	174.9	12	Orange
PEO	3	142.5	12	Orange
PEO	3	152.9	12	Orange
PEO	3	145.3	12	Orange
PEO	6	153.7	12	Orange
PEO	6	169.3	12	Orange
PEO	6	180.1	12	Orange
PEO	6	115.3	12	Orange
PEO	6	115.2	12	Orange
PEO	6	87.6	12	Orange
PEO	6	89.6	12	Orange
PEO	6	41.8	12	Orange
PEO	6	90.7	12	Orange
PEO	6	149.7	12	Orange
PEO	6	143.6	12	Orange
PEO	6	189.8	12	Orange
PEO	6	92	12	Orange
PEO	6	82.4	12	Orange
PEO	6	101.2	12	Orange
PEO	6	137.6	12	Orange
PEO	6	15.1	12	Orange
PEO	6	15.6	12	Orange
PEO	6	13	12	Orange
PEO	6	14	12	Orange
PEO	6	21.4	12	Orange
PEO	6	45.7	12	Orange
PEO	6	56.6	12	Orange
PEO	6	46.9	12	Orange
PEO	6	167.7	12	Orange
PEO	6	139.7	12	Orange
PEO	6	166	12	Orange
PEO	6	95.3	12	Orange
PEO	6	103.9	12	Orange
PEO	6	73.1	12	Orange
PEO	6	103.6	12	Orange
PEO	6	99.2	12	Orange
PEO	6	119.1	12	Orange
PEO	6	174.7	12	Orange

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PEO	6	159.2	12	Orange
PEO	6	160.4	12	Orange
PEO	6	165.8	12	Orange
PEO	6	173.8	12	Orange
PEO	6	191.7	12	Orange
PEO	6	156.3	12	Orange
PEO	6	99	12	Orange
PEO	6	60.1	12	Orange
PEO	6	53.1	12	Orange
PEO	6	69.2	12	Orange
PEO	6	209.6	12	Orange
PEO	6	205.5	12	Orange
PEO	6	164.3	12	Orange
PEO	6	196.5	12	Orange
PEO	6	113.3	12	Orange
PEO	6	143.2	12	Orange
PEO	6	125.8	12	Orange
PEO	6	116.1	12	Orange
PEO	6	88	12	Orange
PEO	6	115.2	12	Orange
PEO	6	155.3	12	Orange
PEO	6	134.2	12	Orange
PEO	6	128.8	12	Orange
PEO	6	134.6	12	Orange
PEO	6	126.2	12	Orange
PEO	6	122.2	12	Orange
PEO	6	120.6	12	Orange
PEO	6	125.7	12	Orange
PEO	6	123.4	12	Orange
PEO	6	113.7	12	Orange
PEO	6	128.3	12	Orange
PEO	6	132.6	12	Orange
PEO	6	124.6	12	Orange
PEO	6	123.3	12	Orange
PEO	6	122.6	12	Orange
PEO	6	112.8	12	Orange
PEO	6	133.6	12	Orange
PEO	6	135.2	12	Orange
PEO	6	112.1	12	Orange
PEO	6	140.5	12	Orange
PEO	6	140.1	12	Orange
PEO	6	137.1	12	Orange
PEO	6	104.1	12	Orange
PEO	6	82.6	12	Orange
PEO	6	99.7	12	Orange
PEO	6	99.1	12	Orange
PEO	6	73.8	12	Orange
PEO	6	82.2	12	Orange
PEO	6	79	12	Orange
PEO	6	71.4	12	Orange

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PEO	9	9.4	12	Orange
PEO	9	44.5	12	Orange
PEO	9	44.2	12	Orange
PEO	9	13.1	12	Orange
PEO	9	38.4	12	Orange
PEO	9	25.7	12	Orange
PEO	9	15.9	12	Orange
PEO	9	16.8	12	Orange
PEO	9	33.7	12	Orange
PEO	9	44.5	12	Orange
PEO	9	17.7	12	Orange
PEO	9	10.9	12	Orange
PEO	9	29.4	12	Orange
PEO	9	35.2	12	Orange
PEO	9	23.4	12	Orange
PEO	9	19.1	12	Orange
PEO	9	4.8	12	Orange
PEO	9	24.3	12	Orange
PEO	9	16.6	12	Orange
PEO	9	20.3	12	Orange
PEO	9	39.9	12	Orange
PEO	9	40.8	12	Orange
PEO	9	37.4	12	Orange
PEO	9	45	12	Orange
PEO	9	5.7	12	Orange
PEO	9	32.4	12	Orange
PEO	9	11.8	12	Orange
PEO	9	19.2	12	Orange
PEO	9	6.2	12	Orange
PEO	9	10.4	12	Orange
PEO	9	14.8	12	Orange
PEO	9	9.4	12	Orange
PEO	9	1.7	12	Orange
PEO	9	12.5	12	Orange
PEO	9	3.9	12	Orange
PEO	9	9.8	12	Orange
PEO	9	7	12	Orange
PEO	9	8.6	12	Orange
PEO	12	4.3	12	Orange
PEO	12	13.8	12	Orange
PEO	12	13.3	12	Orange
PEO	12	12.8	12	Orange
PEO	12	8.5	12	Orange
PEO	12	6.5	12	Orange
PEO	12	13.2	12	Orange
PEO	12	4.5	12	Orange
PEO	12	9.6	12	Orange
PEO	12	12.6	12	Orange
PEO	12	15.1	12	Orange
PEO	12	9.6	12	Orange

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PEO	12	37.3	12	Orange
PEO	12	13.1	12	Orange
PEO	12	23.3	12	Orange
PEO	12	20.6	12	Orange
PEO	12	28.4	12	Orange
PEO	12	29.6	12	Orange
PEO	12	26.7	12	Orange
PEO	12	19.2	12	Orange
PEO	12	10.5	12	Orange
PEO	12	22.8	12	Orange
PEO	12	10.1	12	Orange
PEO	12	3.9	12	Orange
PL	1	279.6	12	Yellow
PL	1	219.4	12	Yellow
PL	1	203.8	12	Yellow
PL	1	232.7	12	Yellow
PL	1	150.5	12	Yellow
PL	1	191.9	12	Yellow
PL	1	158.3	12	Yellow
PL	1	180.6	12	Yellow
PL	1	215.4	12	Yellow
PL	1	194.9	12	Yellow
PL	1	199	12	Yellow
PL	1	212.1	12	Yellow
PL	1	255.3	12	Yellow
PL	1	290.3	12	Yellow
PL	1	251.7	12	Yellow
PL	1	204.9	12	Yellow
PL	1	199	12	Yellow
PL	1	240.7	12	Yellow
PL	1	224.7	12	Yellow
PL	1	194.1	12	Yellow
PL	3	133.9	12	Yellow
PL	3	144.9	12	Yellow
PL	3	139.7	12	Yellow
PL	3	151.6	12	Yellow
PL	3	108.6	12	Yellow
PL	3	123.4	12	Yellow
PL	3	109.1	12	Yellow
PL	3	155.4	12	Yellow
PL	3	128.4	12	Yellow
PL	3	131.1	12	Yellow
PL	3	138.3	12	Yellow
PL	3	113.2	12	Yellow
PL	3	130.9	12	Yellow
PL	3	149.6	12	Yellow
PL	3	126	12	Yellow
PL	3	156.4	12	Yellow
PL	3	157.7	12	Yellow
PL	3	161	12	Yellow

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PL	3	186.8	12	Yellow
PL	3	155	12	Yellow
PL	3	136.2	12	Yellow
PL	3	123.4	12	Yellow
PL	3	98.5	12	Yellow
PL	3	130	12	Yellow
PL	6	176.4	12	Yellow
PL	6	229	12	Yellow
PL	6	192.1	12	Yellow
PL	6	163.7	12	Yellow
PL	6	225.4	12	Yellow
PL	6	169.4	12	Yellow
PL	6	172.9	12	Yellow
PL	6	246.4	12	Yellow
PL	6	175.9	12	Yellow
PL	6	206.2	12	Yellow
PL	6	166.6	12	Yellow
PL	6	180.4	12	Yellow
PL	6	245.5	12	Yellow
PL	6	296.8	12	Yellow
PL	6	231.1	12	Yellow
PL	6	300.8	12	Yellow
PL	6	143.3	12	Yellow
PL	6	177.5	12	Yellow
PL	6	143.9	12	Yellow
PL	6	201.1	12	Yellow
PL	6	143.1	12	Yellow
PL	6	213.2	12	Yellow
PL	6	199.6	12	Yellow
PL	6	164.1	12	Yellow
PL	6	232.4	12	Yellow
PL	6	201.2	12	Yellow
PL	6	185.9	12	Yellow
PL	6	204.8	12	Yellow
PL	6	210.9	12	Yellow
PL	6	195	12	Yellow
PL	6	207.8	12	Yellow
PL	6	225.2	12	Yellow
PL	6	160.9	12	Yellow
PL	6	223.9	12	Yellow
PL	6	207.2	12	Yellow
PL	6	244.1	12	Yellow
PL	6	121.5	12	Yellow
PL	6	133.3	12	Yellow
PL	6	156.2	12	Yellow
PL	6	126.7	12	Yellow
PL	6	217.6	12	Yellow
PL	6	185.2	12	Yellow
PL	6	195.7	12	Yellow
PL	6	199.3	12	Yellow

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PL	6	240.3	12	Yellow
PL	6	245.8	12	Yellow
PL	6	290.3	12	Yellow
PL	6	221.8	12	Yellow
PL	6	274.2	12	Yellow
PL	6	227.7	12	Yellow
PL	6	162	12	Yellow
PL	6	266.1	12	Yellow
PL	6	243.6	12	Yellow
PL	6	243.8	12	Yellow
PL	6	223.8	12	Yellow
PL	6	193.5	12	Yellow
PL	6	81.8	12	Yellow
PL	6	100.5	12	Yellow
PL	6	111.5	12	Yellow
PL	6	123.2	12	Yellow
PL	6	98.2	12	Yellow
PL	6	109.1	12	Yellow
PL	6	98.9	12	Yellow
PL	6	112.2	12	Yellow
PL	6	99.6	12	Yellow
PL	6	119.3	12	Yellow
PL	6	106.6	12	Yellow
PL	6	91.6	12	Yellow
PL	6	89.9	12	Yellow
PL	6	88.4	12	Yellow
PL	6	97.2	12	Yellow
PL	6	90.6	12	Yellow
PL	6	109.5	12	Yellow
PL	6	103.1	12	Yellow
PL	6	92.2	12	Yellow
PL	6	95.6	12	Yellow
PL	6	102.7	12	Yellow
PL	6	88.3	12	Yellow
PL	6	89.2	12	Yellow
PL	6	92.9	12	Yellow
PL	6	103.9	12	Yellow
PL	6	81.6	12	Yellow
PL	6	103.3	12	Yellow
PL	6	81.5	12	Yellow
PL	9	71.6	12	Yellow
PL	9	101.6	12	Yellow
PL	9	93.1	12	Yellow
PL	9	76.7	12	Yellow
PL	9	57.7	12	Yellow
PL	9	90.7	12	Yellow
PL	9	76.6	12	Yellow
PL	9	63.8	12	Yellow
PL	9	60.9	12	Yellow
PL	9	66.9	12	Yellow

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PL	9	78.4	12	Yellow
PL	9	93.4	12	Yellow
PL	9	52.6	12	Yellow
PL	9	45.1	12	Yellow
PL	9	77.4	12	Yellow
PL	9	52.4	12	Yellow
PL	9	56.5	12	Yellow
PL	9	51.1	12	Yellow
PL	9	85.9	12	Yellow
PL	9	97.9	12	Yellow
PL	9	120.3	12	Yellow
PL	9	85.1	12	Yellow
PL	9	97.5	12	Yellow
PL	9	69.7	12	Yellow
PL	9	53.8	12	Yellow
PL	9	58.5	12	Yellow
PL	9	65.2	12	Yellow
PL	9	67.5	12	Yellow
PL	9	9.1	12	Yellow
PL	9	11.6	12	Yellow
PL	9	16.7	12	Yellow
PL	9	9.9	12	Yellow
PL	9	15.5	12	Yellow
PL	9	15.4	12	Yellow
PL	9	9.4	12	Yellow
PL	9	11.3	12	Yellow
PL	9	36.9	12	Yellow
PL	9	46.5	12	Yellow
PL	9	89.9	12	Yellow
PL	9	72.8	12	Yellow
PL	9	35.5	12	Yellow
PL	9	40.5	12	Yellow
PL	9	38.4	12	Yellow
PL	9	42.9	12	Yellow
PL	9	33.4	12	Yellow
PL	9	33.8	12	Yellow
PL	9	27.6	12	Yellow
PL	9	32.8	12	Yellow
PL	9	38.1	12	Yellow
PL	9	35.9	12	Yellow
PL	9	20.8	12	Yellow
PL	9	24.6	12	Yellow
PL	9	15.3	12	Yellow
PL	9	18.2	12	Yellow
PL	9	15.5	12	Yellow
PL	9	16.2	12	Yellow
PL	9	42.5	12	Yellow
PL	9	65.7	12	Yellow
PL	9	69.2	12	Yellow
PL	9	80.9	12	Yellow

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PL	9	71.4	12	Yellow
PL	9	104.5	12	Yellow
PL	9	93.8	12	Yellow
PL	9	88.3	12	Yellow
PL	9	75.4	12	Yellow
PL	9	46.8	12	Yellow
PL	9	69.2	12	Yellow
PL	9	73.2	12	Yellow
PL	9	48.8	12	Yellow
PL	9	36.2	12	Yellow
PL	9	45	12	Yellow
PL	9	35.6	12	Yellow
PL	9	56.3	12	Yellow
PL	9	77.3	12	Yellow
PL	9	58	12	Yellow
PL	9	54	12	Yellow
PL	12	48.8	12	Yellow
PL	12	45	12	Yellow
PL	12	47.5	12	Yellow
PL	12	38.5	12	Yellow
PL	12	25.8	12	Yellow
PL	12	38.2	12	Yellow
PL	12	23.2	12	Yellow
PL	12	24.4	12	Yellow
PL	12	27.7	12	Yellow
PL	12	18.6	12	Yellow
PL	12	20.9	12	Yellow
PL	12	23.1	12	Yellow
PL	12	20.1	12	Yellow
PL	12	24.2	12	Yellow
PL	12	18.5	12	Yellow
PL	12	13.2	12	Yellow
PL	12	18.1	12	Yellow
PL	12	24.4	12	Yellow
PL	12	22.2	12	Yellow
PL	12	14.7	12	Yellow
PL	12	19.4	12	Yellow
PL	12	18.3	12	Yellow
PL	12	16.8	12	Yellow
PL	12	11.5	12	Yellow
PO	1	198.9	9	Orange
PO	1	177.4	9	Orange
PO	1	203.5	9	Orange
PO	1	197.3	9	Orange
PO	1	205.6	9	Orange
PO	1	203.2	9	Orange
PO	1	206.8	9	Orange
PO	1	224.4	9	Orange
PO	1	203	9	Orange
PO	1	217.8	9	Orange

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PO	1	216.8	9	Orange
PO	1	209.8	9	Orange
PO	1	182.8	9	Orange
PO	1	200.4	9	Orange
PO	1	201	9	Orange
PO	1	192	9	Orange
PO	3	122.4	9	Orange
PO	3	122.5	9	Orange
PO	3	141.9	9	Orange
PO	3	163.2	9	Orange
PO	3	136.7	9	Orange
PO	3	136.6	9	Orange
PO	3	164.9	9	Orange
PO	3	129.4	9	Orange
PO	3	129.5	9	Orange
PO	3	198.6	9	Orange
PO	3	163.5	9	Orange
PO	3	194.9	9	Orange
PO	3	173	9	Orange
PO	3	158.1	9	Orange
PO	3	130.8	9	Orange
PO	3	158.7	9	Orange
PO	3	116.4	9	Orange
PO	3	146.4	9	Orange
PO	3	126.3	9	Orange
PO	3	137.5	9	Orange
PO	3	107.5	9	Orange
PO	6	119.4	9	Orange
PO	6	106.2	9	Orange
PO	6	95.6	9	Orange
PO	6	95.3	9	Orange
PO	6	102.4	9	Orange
PO	6	103.6	9	Orange
PO	6	126.1	9	Orange
PO	6	110.1	9	Orange
PO	6	86.6	9	Orange
PO	6	90.5	9	Orange
PO	6	96.3	9	Orange
PO	6	82.3	9	Orange
PO	6	107.3	9	Orange
PO	6	60.7	9	Orange
PO	6	119.9	9	Orange
PO	6	110.9	9	Orange
PO	6	103.8	9	Orange
PO	6	97.4	9	Orange
PO	6	62.3	9	Orange
PO	6	62.9	9	Orange
PO	6	92.8	9	Orange
PO	6	84.2	9	Orange
PO	6	81.2	9	Orange

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PO	6	92.9	9	Orange
PO	6	84.7	9	Orange
PO	6	81.4	9	Orange
PO	6	73.6	9	Orange
PO	6	80.4	9	Orange
PO	9	2.8	9	Orange
PO	9	0.6	9	Orange
PO	9	0.2	9	Orange
PO	9	2.1	9	Orange
PO	9	8.7	9	Orange
PO	9	2	9	Orange
PO	9	0.1	9	Orange
PO	9	2.7	9	Orange
PO	9	18.6	9	Orange
PO	9	29.1	9	Orange
PO	9	33.3	9	Orange
PO	9	12.3	9	Orange
PO	9	80.5	9	Orange
PO	9	86.9	9	Orange
PO	9	85.8	9	Orange
PO	9	81.6	9	Orange
PO	9	57.5	9	Orange
PO	9	53.7	9	Orange
PO	9	69.5	9	Orange
PO	9	60.9	9	Orange
PO	9	109.4	9	Orange
PO	9	109.7	9	Orange
PO	9	94.2	9	Orange
PO	9	104.3	9	Orange
PP	1	122.8	6	Orange
PP	1	134.2	6	Orange
PP	1	148.6	6	Orange
PP	1	137.3	6	Orange
PP	1	126.7	6	Orange
PP	1	162.3	6	Orange
PP	1	130.3	6	Orange
PP	1	137.2	6	Orange
PP	1	137.1	6	Orange
PP	1	113.5	6	Orange
PP	1	151.9	6	Orange
PP	1	143.7	6	Orange
PP	1	158.3	6	Orange
PP	1	147.1	6	Orange
PP	1	127.4	6	Orange
PP	1	124.6	6	Orange
PP	1	103.8	6	Orange
PP	1	61.3	6	Orange
PP	1	66.4	6	Orange
PP	1	61.8	6	Orange
PP	3	109.9	6	Orange

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PP	3	108.6	6	Orange
PP	3	107.3	6	Orange
PP	3	129	6	Orange
PP	3	87.5	6	Orange
PP	3	137.9	6	Orange
PP	3	118.9	6	Orange
PP	3	94.5	6	Orange
PP	3	112.6	6	Orange
PP	3	131.3	6	Orange
PP	3	146	6	Orange
PP	3	144	6	Orange
PP	3	123.7	6	Orange
PP	3	131.6	6	Orange
PP	3	125.2	6	Orange
PP	3	136.6	6	Orange
PP	3	91.3	6	Orange
PP	3	126.9	6	Orange
PP	3	118.2	6	Orange
PP	3	97.3	6	Orange
PP	3	120.6	6	Orange
PP	3	125.8	6	Orange
PP	3	121.4	6	Orange
PP	3	126.8	6	Orange
PP	3	130.5	6	Orange
PP	3	123.3	6	Orange
PP	3	125.3	6	Orange
PP	6	3.9	6	Orange
PP	6	12.7	6	Orange
PP	6	4.1	6	Orange
PP	6	26.9	6	Orange
PP	6	14.1	6	Orange
PP	6	21.9	6	Orange
PP	6	12	6	Orange
PP	6	1.5	6	Orange
PP	6	0.3	6	Orange
PP	6	3.5	6	Orange
PP	6	7.7	6	Orange
PP	6	8.1	6	Orange
PP	6	55.9	6	Orange
PP	6	59.1	6	Orange
PP	6	42.8	6	Orange
PP	6	67.8	6	Orange
PP	6	56.3	6	Orange
PP	6	51.5	6	Orange
PP	6	55.4	6	Orange
PP	6	69	6	Orange
PRLB	1	213.3	9	Bicolor
PRLB	1	202.7	9	Bicolor
PRLB	1	191.8	9	Bicolor
PRLB	1	217.2	9	Bicolor

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PRLB	1	178.3	9	Bicolor
PRLB	1	245.2	9	Bicolor
PRLB	1	184.5	9	Bicolor
PRLB	1	194.7	9	Bicolor
PRLB	1	278.7	9	Bicolor
PRLB	1	212.8	9	Bicolor
PRLB	1	210.4	9	Bicolor
PRLB	1	188.8	9	Bicolor
PRLB	1	189	9	Bicolor
PRLB	1	183.9	9	Bicolor
PRLB	1	176.1	9	Bicolor
PRLB	1	179.3	9	Bicolor
PRLB	1	213.5	9	Bicolor
PRLB	1	238.6	9	Bicolor
PRLB	1	190.2	9	Bicolor
PRLB	1	202.7	9	Bicolor
PRLB	1	225.4	9	Bicolor
PRLB	1	234.8	9	Bicolor
PRLB	1	219.5	9	Bicolor
PRLB	1	197.2	9	Bicolor
PRLB	1	209.4	9	Bicolor
PRLB	1	199.8	9	Bicolor
PRLB	1	182.7	9	Bicolor
PRLB	1	183.6	9	Bicolor
PRLB	3	156.3	9	Bicolor
PRLB	3	114.7	9	Bicolor
PRLB	3	152.6	9	Bicolor
PRLB	3	136.9	9	Bicolor
PRLB	3	153	9	Bicolor
PRLB	3	141.3	9	Bicolor
PRLB	3	125.7	9	Bicolor
PRLB	3	155	9	Bicolor
PRLB	3	179.8	9	Bicolor
PRLB	3	133.6	9	Bicolor
PRLB	3	160.2	9	Bicolor
PRLB	3	143	9	Bicolor
PRLB	3	125.4	9	Bicolor
PRLB	3	171.6	9	Bicolor
PRLB	3	155.6	9	Bicolor
PRLB	3	164.3	9	Bicolor
PRLB	3	195.4	9	Bicolor
PRLB	3	157.4	9	Bicolor
PRLB	3	152.8	9	Bicolor
PRLB	3	150.9	9	Bicolor
PRLB	3	197	9	Bicolor
PRLB	3	163.8	9	Bicolor
PRLB	3	195	9	Bicolor
PRLB	3	162.8	9	Bicolor
PRLB	3	195.3	9	Bicolor
PRLB	3	179.1	9	Bicolor

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PRLB	3	172.1	9	Bicolor
PRLB	3	175.1	9	Bicolor
PRLB	6	150.3	9	Bicolor
PRLB	6	138	9	Bicolor
PRLB	6	144.2	9	Bicolor
PRLB	6	166.9	9	Bicolor
PRLB	6	134.9	9	Bicolor
PRLB	6	127.5	9	Bicolor
PRLB	6	132.7	9	Bicolor
PRLB	6	133.3	9	Bicolor
PRLB	6	133.4	9	Bicolor
PRLB	6	125.3	9	Bicolor
PRLB	6	133.6	9	Bicolor
PRLB	6	127	9	Bicolor
PRLB	6	108.4	9	Bicolor
PRLB	6	105	9	Bicolor
PRLB	6	117.6	9	Bicolor
PRLB	6	121.9	9	Bicolor
PRLB	6	141.2	9	Bicolor
PRLB	6	130.5	9	Bicolor
PRLB	6	136.5	9	Bicolor
PRLB	6	138.5	9	Bicolor
PRLB	6	137.1	9	Bicolor
PRLB	6	149.4	9	Bicolor
PRLB	6	142.8	9	Bicolor
PRLB	6	155.8	9	Bicolor
PRLB	6	141.9	9	Bicolor
PRLB	6	115.8	9	Bicolor
PRLB	6	140.1	9	Bicolor
PRLB	6	124.9	9	Bicolor
PRLB	9	44.2	9	Bicolor
PRLB	9	50.1	9	Bicolor
PRLB	9	54.6	9	Bicolor
PRLB	9	53.9	9	Bicolor
PRLB	9	23.4	9	Bicolor
PRLB	9	20.7	9	Bicolor
PRLB	9	22.9	9	Bicolor
PRLB	9	15.9	9	Bicolor
PRLB	9	44.1	9	Bicolor
PRLB	9	21	9	Bicolor
PRLB	9	24.4	9	Bicolor
PRLB	9	13.4	9	Bicolor
PRLB	9	29.9	9	Bicolor
PRLB	9	21.2	9	Bicolor
PRLB	9	25.2	9	Bicolor
PRLB	9	15.3	9	Bicolor
PRLB	9	4.7	9	Bicolor
PRLB	9	12	9	Bicolor
PRLB	9	10	9	Bicolor
PRLB	9	6.4	9	Bicolor

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PYL	1	155.7	12	Yellow
PYL	1	186.3	12	Yellow
PYL	1	168.4	12	Yellow
PYL	1	152.8	12	Yellow
PYL	1	198.6	12	Yellow
PYL	1	226.9	12	Yellow
PYL	1	167.6	12	Yellow
PYL	1	252.1	12	Yellow
PYL	1	160.3	12	Yellow
PYL	1	183.7	12	Yellow
PYL	1	180.1	12	Yellow
PYL	1	158.1	12	Yellow
PYL	1	175.1	12	Yellow
PYL	1	248.7	12	Yellow
PYL	1	241.1	12	Yellow
PYL	1	200.5	12	Yellow
PYL	1	189.5	12	Yellow
PYL	1	184.2	12	Yellow
PYL	1	221.7	12	Yellow
PYL	1	218.8	12	Yellow
PYL	1	228.4	12	Yellow
PYL	1	220.7	12	Yellow
PYL	1	233.8	12	Yellow
PYL	1	171.2	12	Yellow
PYL	1	192.7	12	Yellow
PYL	1	195.2	12	Yellow
PYL	1	196.8	12	Yellow
PYL	1	208	12	Yellow
PYL	1	288.1	12	Yellow
PYL	1	212.5	12	Yellow
PYL	1	198.8	12	Yellow
PYL	1	223.2	12	Yellow
PYL	1	133.5	12	Yellow
PYL	1	170.6	12	Yellow
PYL	1	142.3	12	Yellow
PYL	1	163.5	12	Yellow
PYL	1	130.3	12	Yellow
PYL	1	141.2	12	Yellow
PYL	1	149.9	12	Yellow
PYL	1	176.3	12	Yellow
PYL	1	140.5	12	Yellow
PYL	1	151.8	12	Yellow
PYL	1	169.2	12	Yellow
PYL	1	163.1	12	Yellow
PYL	1	152	12	Yellow
PYL	1	157.2	12	Yellow
PYL	1	155.4	12	Yellow
PYL	1	170.9	12	Yellow
PYL	1	156	12	Yellow
PYL	1	155	12	Yellow

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PYL	1	180.3	12	Yellow
PYL	1	181.4	12	Yellow
PYL	1	155.2	12	Yellow
PYL	1	164.8	12	Yellow
PYL	1	143.2	12	Yellow
PYL	1	173.7	12	Yellow
PYL	1	186.5	12	Yellow
PYL	1	136.6	12	Yellow
PYL	3	200.4	12	Yellow
PYL	3	214.6	12	Yellow
PYL	3	158.2	12	Yellow
PYL	3	166.8	12	Yellow
PYL	3	181.6	12	Yellow
PYL	3	143.9	12	Yellow
PYL	3	191.4	12	Yellow
PYL	3	190	12	Yellow
PYL	3	210.6	12	Yellow
PYL	3	159.7	12	Yellow
PYL	3	208.6	12	Yellow
PYL	3	174.3	12	Yellow
PYL	3	201.2	12	Yellow
PYL	3	197.3	12	Yellow
PYL	3	174	12	Yellow
PYL	3	228.8	12	Yellow
PYL	3	196.6	12	Yellow
PYL	3	184.3	12	Yellow
PYL	3	180.3	12	Yellow
PYL	3	193.8	12	Yellow
PYL	3	198.3	12	Yellow
PYL	3	193.6	12	Yellow
PYL	3	200.1	12	Yellow
PYL	3	210.6	12	Yellow
PYL	3	194.4	12	Yellow
PYL	3	180.5	12	Yellow
PYL	3	184.3	12	Yellow
PYL	3	195.9	12	Yellow
PYL	3	123	12	Yellow
PYL	3	144	12	Yellow
PYL	3	134.4	12	Yellow
PYL	3	116.7	12	Yellow
PYL	3	131.4	12	Yellow
PYL	3	134.4	12	Yellow
PYL	3	139.5	12	Yellow
PYL	3	145.7	12	Yellow
PYL	3	148.1	12	Yellow
PYL	3	135.6	12	Yellow
PYL	3	143.5	12	Yellow
PYL	3	156.6	12	Yellow
PYL	3	133.1	12	Yellow
PYL	3	161.8	12	Yellow

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PYL	3	140.2	12	Yellow
PYL	3	134.2	12	Yellow
PYL	3	164.6	12	Yellow
PYL	3	155.7	12	Yellow
PYL	3	134.3	12	Yellow
PYL	3	129.5	12	Yellow
PYL	3	147.6	12	Yellow
PYL	3	132.4	12	Yellow
PYL	3	163	12	Yellow
PYL	6	176.2	12	Yellow
PYL	6	157.7	12	Yellow
PYL	6	168.4	12	Yellow
PYL	6	135.7	12	Yellow
PYL	6	208.5	12	Yellow
PYL	6	146.7	12	Yellow
PYL	6	194.4	12	Yellow
PYL	6	176.6	12	Yellow
PYL	6	222.2	12	Yellow
PYL	6	180.2	12	Yellow
PYL	6	151.1	12	Yellow
PYL	6	195	12	Yellow
PYL	6	201	12	Yellow
PYL	6	187.6	12	Yellow
PYL	6	157	12	Yellow
PYL	6	196.6	12	Yellow
PYL	6	157.5	12	Yellow
PYL	6	165.9	12	Yellow
PYL	6	178.8	12	Yellow
PYL	6	106.7	12	Yellow
PYL	6	129.1	12	Yellow
PYL	6	160.2	12	Yellow
PYL	6	143.3	12	Yellow
PYL	6	141	12	Yellow
PYL	6	145.1	12	Yellow
PYL	6	162.8	12	Yellow
PYL	6	137.2	12	Yellow
PYL	6	137.3	12	Yellow
PYL	6	151.2	12	Yellow
PYL	6	193.3	12	Yellow
PYL	6	206	12	Yellow
PYL	6	203.7	12	Yellow
PYL	6	202.1	12	Yellow
PYL	6	203.1	12	Yellow
PYL	6	193.8	12	Yellow
PYL	6	205.8	12	Yellow
PYL	6	175.5	12	Yellow
PYL	6	220.1	12	Yellow
PYL	6	247.8	12	Yellow
PYL	6	208.5	12	Yellow
PYL	6	204.9	12	Yellow

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
PYL	6	181	12	Yellow
PYL	6	168.9	12	Yellow
PYL	6	189.3	12	Yellow
PYL	9	43.9	12	Yellow
PYL	9	18.8	12	Yellow
PYL	9	35	12	Yellow
PYL	9	57.3	12	Yellow
PYL	9	27.7	12	Yellow
PYL	9	46.7	12	Yellow
PYL	9	39.7	12	Yellow
PYL	9	41.1	12	Yellow
PYL	9	34.5	12	Yellow
PYL	9	22.3	12	Yellow
PYL	9	51.7	12	Yellow
PYL	9	39.6	12	Yellow
PYL	9	16.3	12	Yellow
PYL	9	45.1	12	Yellow
PYL	9	12.5	12	Yellow
PYL	9	27.4	12	Yellow
PYL	9	39.9	12	Yellow
PYL	9	31.8	12	Yellow
PYL	9	19.2	12	Yellow
PYL	12	28.7	12	Yellow
PYL	12	18.5	12	Yellow
PYL	12	18.1	12	Yellow
PYL	12	5.3	12	Yellow
PYL	12	10.3	12	Yellow
PYL	12	12.8	12	Yellow
PYL	12	13.4	12	Yellow
PYL	12	20.1	12	Yellow
PYL	12	14.2	12	Yellow
PYL	12	24.4	12	Yellow
PYL	12	15.1	12	Yellow
PYL	12	20.7	12	Yellow
PYL	12	16.7	12	Yellow
PYL	12	9.6	12	Yellow
PYL	12	23.7	12	Yellow
PYL	12	17	12	Yellow
PYL	12	5.7	12	Yellow
PYL	12	7.1	12	Yellow
PYL	12	7.4	12	Yellow
PYL	12	12.8	12	Yellow
SB	1	136	9	Bicolor
SB	1	144.9	9	Bicolor
SB	1	137.4	9	Bicolor
SB	1	140.8	9	Bicolor
SB	1	133.6	9	Bicolor
SB	1	124	9	Bicolor
SB	1	154.3	9	Bicolor
SB	1	149.9	9	Bicolor

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
SB	1	133.6	9	Bicolor
SB	1	106.2	9	Bicolor
SB	1	157.1	9	Bicolor
SB	1	101.1	9	Bicolor
SB	1	109.8	9	Bicolor
SB	1	84.6	9	Bicolor
SB	1	114.5	9	Bicolor
SB	1	71.8	9	Bicolor
SB	1	146.8	9	Bicolor
SB	1	131.7	9	Bicolor
SB	1	157.3	9	Bicolor
SB	1	101.4	9	Bicolor
SB	1	141.2	9	Bicolor
SB	1	152.9	9	Bicolor
SB	1	200.7	9	Bicolor
SB	1	150	9	Bicolor
SB	1	153.7	9	Bicolor
SB	1	119.8	9	Bicolor
SB	1	128.8	9	Bicolor
SB	1	128.9	9	Bicolor
SB	1	130.4	9	Bicolor
SB	1	145.5	9	Bicolor
SB	1	109.6	9	Bicolor
SB	1	143.3	9	Bicolor
SB	1	162.3	9	Bicolor
SB	1	143.8	9	Bicolor
SB	1	147.7	9	Bicolor
SB	1	155.8	9	Bicolor
SB	1	140.6	9	Bicolor
SB	1	153.1	9	Bicolor
SB	1	131.4	9	Bicolor
SB	1	129.8	9	Bicolor
SB	1	134.9	9	Bicolor
SB	1	138.6	9	Bicolor
SB	1	142.3	9	Bicolor
SB	1	148.3	9	Bicolor
SB	1	153.3	9	Bicolor
SB	1	149.8	9	Bicolor
SB	1	149.7	9	Bicolor
SB	1	142.1	9	Bicolor
SB	1	91.2	9	Bicolor
SB	1	94.7	9	Bicolor
SB	1	108.1	9	Bicolor
SB	1	114.7	9	Bicolor
SB	1	144.4	9	Bicolor
SB	1	148	9	Bicolor
SB	1	141.6	9	Bicolor
SB	1	107.8	9	Bicolor
SB	3	177.3	9	Bicolor
SB	3	188.7	9	Bicolor

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
SB	3	172.4	9	Bicolor
SB	3	167.4	9	Bicolor
SB	3	130.1	9	Bicolor
SB	3	81.6	9	Bicolor
SB	3	112.1	9	Bicolor
SB	3	111	9	Bicolor
SB	3	105.5	9	Bicolor
SB	3	119.4	9	Bicolor
SB	3	100.6	9	Bicolor
SB	3	98.2	9	Bicolor
SB	3	135.1	9	Bicolor
SB	3	118.4	9	Bicolor
SB	3	129.9	9	Bicolor
SB	3	134.4	9	Bicolor
SB	3	129.8	9	Bicolor
SB	3	123.7	9	Bicolor
SB	3	141.1	9	Bicolor
SB	3	101.4	9	Bicolor
SB	3	147.3	9	Bicolor
SB	3	127	9	Bicolor
SB	3	95.9	9	Bicolor
SB	3	100.2	9	Bicolor
SB	3	120.2	9	Bicolor
SB	3	140.6	9	Bicolor
SB	3	132.8	9	Bicolor
SB	3	110.6	9	Bicolor
SB	3	132.6	9	Bicolor
SB	3	111.4	9	Bicolor
SB	3	85.3	9	Bicolor
SB	3	121.8	9	Bicolor
SB	3	109.5	9	Bicolor
SB	3	88.8	9	Bicolor
SB	3	100.6	9	Bicolor
SB	3	146.2	9	Bicolor
SB	3	129.8	9	Bicolor
SB	3	112.7	9	Bicolor
SB	3	129.4	9	Bicolor
SB	3	133.5	9	Bicolor
SB	3	113.6	9	Bicolor
SB	3	112.9	9	Bicolor
SB	3	124.7	9	Bicolor
SB	3	144.2	9	Bicolor
SB	3	124.8	9	Bicolor
SB	3	131.7	9	Bicolor
SB	3	143.7	9	Bicolor
SB	3	135.3	9	Bicolor
SB	3	146.9	9	Bicolor
SB	3	118.3	9	Bicolor
SB	3	146	9	Bicolor
SB	3	133	9	Bicolor

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
SB	3	109.8	9	Bicolor
SB	3	114.9	9	Bicolor
SB	3	118.2	9	Bicolor
SB	6	17.9	9	Bicolor
SB	6	11.9	9	Bicolor
SB	6	7.8	9	Bicolor
SB	6	8.5	9	Bicolor
SB	6	73.5	9	Bicolor
SB	6	48.9	9	Bicolor
SB	6	31.7	9	Bicolor
SB	6	56.8	9	Bicolor
SB	6	14.5	9	Bicolor
SB	6	16	9	Bicolor
SB	6	8.8	9	Bicolor
SB	6	19.9	9	Bicolor
SB	6	46.5	9	Bicolor
SB	6	89.3	9	Bicolor
SB	6	81.3	9	Bicolor
SB	6	86.1	9	Bicolor
SB	6	97.8	9	Bicolor
SB	6	76.1	9	Bicolor
SB	6	101	9	Bicolor
SB	6	88.3	9	Bicolor
SB	6	12.3	9	Bicolor
SB	6	9.4	9	Bicolor
SB	6	5.9	9	Bicolor
SB	6	6.2	9	Bicolor
SB	6	64	9	Bicolor
SB	6	127.2	9	Bicolor
SB	6	147	9	Bicolor
SB	6	84.5	9	Bicolor
SB	6	134.1	9	Bicolor
SB	6	111.3	9	Bicolor
SB	6	109.9	9	Bicolor
SB	6	131.1	9	Bicolor
SB	6	161.3	9	Bicolor
SB	6	190.3	9	Bicolor
SB	6	233.7	9	Bicolor
SB	6	172.4	9	Bicolor
SB	6	202.2	9	Bicolor
SB	6	263.3	9	Bicolor
SB	6	173.1	9	Bicolor
SB	6	204.8	9	Bicolor
SB	6	83.6	9	Bicolor
SB	6	74.7	9	Bicolor
SB	6	79.3	9	Bicolor
SB	6	100.3	9	Bicolor
SB	6	169.2	9	Bicolor
SB	6	169.1	9	Bicolor
SB	6	118	9	Bicolor

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
SB	6	131	9	Bicolor
SB	6	123.2	9	Bicolor
SB	6	98.1	9	Bicolor
SB	6	103.3	9	Bicolor
SB	6	57.4	9	Bicolor
SB	6	79	9	Bicolor
SB	6	54.2	9	Bicolor
SB	6	22.2	9	Bicolor
SB	6	57.2	9	Bicolor
SB	6	90.1	9	Bicolor
SB	6	90.8	9	Bicolor
SB	6	92.9	9	Bicolor
SB	6	93	9	Bicolor
SB	6	80.9	9	Bicolor
SB	6	65.6	9	Bicolor
SB	6	50	9	Bicolor
SB	6	61.5	9	Bicolor
SB	6	62.4	9	Bicolor
SB	6	50.5	9	Bicolor
SB	6	53	9	Bicolor
SB	6	48.8	9	Bicolor
SB	6	88.4	9	Bicolor
SB	6	102.2	9	Bicolor
SB	6	76.5	9	Bicolor
SB	6	78.4	9	Bicolor
SB	6	80.4	9	Bicolor
SB	6	80	9	Bicolor
SB	6	83.9	9	Bicolor
SB	6	95.4	9	Bicolor
SB	6	62.2	9	Bicolor
SB	6	75.5	9	Bicolor
SB	6	70.3	9	Bicolor
SB	6	70.5	9	Bicolor
SB	6	92	9	Bicolor
SB	6	81.9	9	Bicolor
SB	6	75	9	Bicolor
SB	6	74.1	9	Bicolor
SB	6	76.5	9	Bicolor
SB	6	60.3	9	Bicolor
SB	6	67.9	9	Bicolor
SB	6	55.9	9	Bicolor
SB	6	73.1	9	Bicolor
SB	6	86.4	9	Bicolor
SB	6	81.6	9	Bicolor
SB	6	86.3	9	Bicolor
SB	6	86.5	9	Bicolor
SB	6	83.3	9	Bicolor
SB	6	76.3	9	Bicolor
SB	6	76	9	Bicolor
SB	9	2.1	9	Bicolor

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
SB	9	0.4	9	Bicolor
SB	9	1.6	9	Bicolor
SB	9	0.4	9	Bicolor
SB	9	6.3	9	Bicolor
SB	9	16	9	Bicolor
SB	9	8.7	9	Bicolor
SB	9	9.7	9	Bicolor
SB	9	41.2	9	Bicolor
SB	9	42.7	9	Bicolor
SB	9	42	9	Bicolor
SB	9	36.2	9	Bicolor
SB	9	25.9	9	Bicolor
SB	9	34.4	9	Bicolor
SB	9	7.4	9	Bicolor
SB	9	29.4	9	Bicolor
SB	9	14.2	9	Bicolor
SB	9	19.6	9	Bicolor
SB	9	13.5	9	Bicolor
SB	9	10.6	9	Bicolor
SB	9	10.6	9	Bicolor
SB	9	17.1	9	Bicolor
SB	9	16.1	9	Bicolor
SB	9	17.3	9	Bicolor
SB	9	11.5	9	Bicolor
SB	9	20.5	9	Bicolor
SB	9	11.4	9	Bicolor
SB	9	18.1	9	Bicolor
SB	9	16.2	9	Bicolor
SB	9	49.9	9	Bicolor
SB	9	34.6	9	Bicolor
SB	9	31.6	9	Bicolor
SB	9	18.7	9	Bicolor
SB	9	21.6	9	Bicolor
SB	9	24.2	9	Bicolor
SB	9	59.4	9	Bicolor
SB	9	17.3	9	Bicolor
SB	9	35	9	Bicolor
SB	9	24.4	9	Bicolor
SB	9	29.9	9	Bicolor
SB	9	23.2	9	Bicolor
SB	9	18.1	9	Bicolor
SB	9	26.1	9	Bicolor
SB	9	66.9	9	Bicolor
SB	9	16.1	9	Bicolor
SB	9	29.6	9	Bicolor
SB	9	34.9	9	Bicolor
SB	9	34.2	9	Bicolor
SB	9	12.2	9	Bicolor
SB	9	25.2	9	Bicolor
SB	9	22	9	Bicolor

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
SB	9	29.2	9	Bicolor
SB	9	1.2	9	Bicolor
SB	9	2	9	Bicolor
SB	9	0.6	9	Bicolor
SB	9	10.2	9	Bicolor
SB	9	0.1	9	Bicolor
SB	9	0.8	9	Bicolor
SB	9	0.3	9	Bicolor
SB	9	0.3	9	Bicolor
SB	9	0.9	9	Bicolor
SB	9	2.5	9	Bicolor
SB	9	0.8	9	Bicolor
SB	9	0.2	9	Bicolor
SB	9	2.9	9	Bicolor
SB	9	6.8	9	Bicolor
SB	9	0.5	9	Bicolor
SB	9	0.7	9	Bicolor
SB	9	0.7	9	Bicolor
SB	9	3.4	9	Bicolor
SB	9	2.1	9	Bicolor
SB	9	2.3	9	Bicolor
SO	1	208.8	9	Orange
SO	1	178.1	9	Orange
SO	1	193.9	9	Orange
SO	1	199.7	9	Orange
SO	1	153	9	Orange
SO	1	152.9	9	Orange
SO	1	160.7	9	Orange
SO	1	185.8	9	Orange
SO	1	170.7	9	Orange
SO	1	166	9	Orange
SO	1	157.6	9	Orange
SO	1	162.9	9	Orange
SO	1	172.2	9	Orange
SO	1	178.6	9	Orange
SO	1	186.5	9	Orange
SO	1	181.9	9	Orange
SO	1	150.4	9	Orange
SO	1	150.3	9	Orange
SO	1	160.1	9	Orange
SO	1	160	9	Orange
SO	1	167.7	9	Orange
SO	1	170.6	9	Orange
SO	1	161.3	9	Orange
SO	1	153.1	9	Orange
SO	1	160.7	9	Orange
SO	1	155.7	9	Orange
SO	1	158.9	9	Orange
SO	1	163.3	9	Orange
SO	3	87.2	9	Orange

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
SO	3	100.1	9	Orange
SO	3	126.7	9	Orange
SO	3	133.8	9	Orange
SO	3	139.4	9	Orange
SO	3	161.5	9	Orange
SO	3	122	9	Orange
SO	3	132.9	9	Orange
SO	3	99.8	9	Orange
SO	3	105	9	Orange
SO	3	125.2	9	Orange
SO	3	144.3	9	Orange
SO	3	109.2	9	Orange
SO	3	148.1	9	Orange
SO	3	117.5	9	Orange
SO	3	146.7	9	Orange
SO	3	163	9	Orange
SO	3	124	9	Orange
SO	3	147.4	9	Orange
SO	3	134.6	9	Orange
SO	3	156.7	9	Orange
SO	3	154.9	9	Orange
SO	3	146.8	9	Orange
SO	3	139.1	9	Orange
SO	6	85.7	9	Orange
SO	6	83.5	9	Orange
SO	6	81.8	9	Orange
SO	6	75.7	9	Orange
SO	6	92.6	9	Orange
SO	6	182	9	Orange
SO	6	151	9	Orange
SO	6	159.4	9	Orange
SO	6	80.2	9	Orange
SO	6	53.1	9	Orange
SO	6	63.5	9	Orange
SO	6	75.1	9	Orange
SO	6	77.1	9	Orange
SO	6	65	9	Orange
SO	6	75.3	9	Orange
SO	6	86.7	9	Orange
SO	6	74.8	9	Orange
SO	6	100.8	9	Orange
SO	6	85.8	9	Orange
SO	6	83.7	9	Orange
SO	6	127.1	9	Orange
SO	6	104.3	9	Orange
SO	6	127.6	9	Orange
SO	6	116.7	9	Orange
SO	6	113.5	9	Orange
SO	6	131.9	9	Orange
SO	6	129.5	9	Orange

Variety	Time (Days)	Force (Grams)	Vase Life (Days)	Color
SO	6	115.8	9	Orange
SO	9	43.5	9	Orange
SO	9	59.6	9	Orange
SO	9	68.4	9	Orange
SO	9	52.4	9	Orange
SO	9	35.7	9	Orange
SO	9	54.4	9	Orange
SO	9	48.3	9	Orange
SO	9	68.8	9	Orange
SO	9	43.9	9	Orange
SO	9	27.1	9	Orange
SO	9	41.8	9	Orange
SO	9	39.5	9	Orange
SO	9	55.1	9	Orange
SO	9	48.5	9	Orange
SO	9	40.3	9	Orange
SO	9	40	9	Orange
SO	9	51.3	9	Orange
SO	9	77.9	9	Orange
SO	9	67.2	9	Orange
SO	9	65.4	9	Orange

Table A2: Data table for Chapter 2, Figure 2.5

Prediction model of regression plot grouped by variety

Prediction Model

$a+b*\text{Time (days)}+c*\text{Time (days)}^2$

a=Intercept

b=Slope

c=Quadratic

Table A3: Data table for Chapter 2, Figure 2.5

Summary of fit for regression model grouped by variety

Summary of Fit

AICc	20374.923
BIC	20598.436
SSE	2328931.8
MSE	1154.6514
RMSE	33.980162
R-Square	0.7252426

Table A4: Data table for Chapter 2, Figure 2.5

Parameter estimates for regression equation for each variety

Parameter Estimates

Parameter	Group	Estimate	Std Error	Lower 95%	Upper 95%
Intercept	CH	133.61091	7.0437208	119.80547	147.41635
Slope	CH	-11.36662	3.5629229	-18.34982	-4.383419
Quadratic	CH	-0.505395	0.3763859	-1.243097	0.2323083
Intercept	CR	117.34917	9.4236532	98.879146	135.81919
Slope	CR	12.574722	6.4249545	-0.017957	25.167402
Quadratic	CR	-4.448889	0.8712948	-6.156595	-2.741182
Intercept	MR	128.03568	6.6329931	115.03526	141.03611
Slope	MR	7.0816067	3.4078586	0.4023265	13.760887
Quadratic	MR	-1.89195	0.3356361	-2.549784	-1.234115
Intercept	OG	220.35844	11.471199	197.8753	242.84157
Slope	OG	-40.50959	5.8611151	-51.99716	-29.02202
Quadratic	OG	1.6829811	0.5765345	0.5529941	2.812968
Intercept	PBC	138.31639	16.656442	105.67036	170.96242
Slope	PBC	41.145046	11.074166	19.440079	62.850014
Quadratic	PBC	-9.498935	1.701628	-12.83406	-6.163806
Intercept	PEO	203.19826	6.1602486	191.1244	215.27213
Slope	PEO	-14.02376	2.305855	-18.54315	-9.504363
Quadratic	PEO	-0.285431	0.1822856	-0.642704	0.0718425
Intercept	PL	208.49777	8.8076618	191.23507	225.76047
Slope	PL	-4.712925	2.7854355	-10.17228	0.746428
Quadratic	PL	-1.055588	0.2063871	-1.460099	-0.651077
Intercept	PO	227.19602	12.037559	203.60283	250.7892
Slope	PO	-28.39054	5.5475838	-39.2636	-17.51747
Quadratic	PO	0.9316357	0.525239	-0.097814	1.9610852
Intercept	PP	109.96756	15.235737	80.106059	139.82905
Slope	PP	20.525019	10.443972	0.0552103	40.994827
Quadratic	PP	-5.677574	1.4219099	-8.464466	-2.890682
Intercept	PRLB	202.21229	9.6734198	183.25273	221.17184
Slope	PRLB	-3.166806	4.9381106	-12.84533	6.5117125
Quadratic	PRLB	-1.75232	0.4905824	-2.713844	-0.790796
Intercept	PYL	175.60307	6.0029653	163.83747	187.36866
Slope	PYL	6.2505721	2.5822808	1.1893946	11.311749
Quadratic	PYL	-1.75207	0.2067826	-2.157356	-1.346783
Intercept	SB	135.16211	6.47562	122.47013	147.85409
Slope	SB	1.0172629	3.0185945	-4.899074	6.9335994
Quadratic	SB	-1.573826	0.2904643	-2.143126	-1.004526
Intercept	SO	180.30843	9.6735603	161.3486	199.26826
Slope	SO	-14.05938	4.9754218	-23.81103	-4.307733
Quadratic	SO	-0.005794	0.4961099	-0.978151	0.9665636

Table A5: Data table for Chapter 2, Table 2.5 and Figure 2.6

Prediction model of regression plot grouped by color

Prediction Model

$a + b*\text{Time (days)} + c*\text{Time (days)}^2$

a=Intercept

b=Slope

c=Quadratic

Table A6: Data table for Chapter 2, Table 2.5 and Figure 2.6

Summary of fit for regression model grouped by flower color

Summary of Fit	
AICc	20924.572
BIC	20997.564
SSE	3125888
MSE	1529.2994
RMSE	39.106258
R-Square	0.6312212

Table A7: Data table for Chapter 2, Table 2.5 and Figure 2.6

Parameter estimates for regression equation for each color group

Parameter Estimates

Parameter	Group	Estimate	Std Error	Lower 95%	Upper 95%
Intercept	Bicolor	164.79247	5.7405961	153.5411	176.04383
Slope	Bicolor	-1.588434	2.8032931	-7.082787	3.9059197
Quadratic	Bicolor	-1.637989	0.2738202	-2.174667	-1.101312
Intercept	Orange	193.20512	4.4687404	184.44655	201.96369
Slope	Orange	-18.40422	1.8623542	-22.05437	-14.75407
Quadratic	Orange	0.1602237	0.1619109	-0.157116	0.4775633
Intercept	Red	142.24845	4.6228849	133.18776	151.30914
Slope	Red	-11.33003	2.3776399	-15.99012	-6.669938
Quadratic	Red	-0.255024	0.2477734	-0.740651	0.230603
Intercept	Yellow	186.88773	5.5360431	176.03729	197.73818
Slope	Yellow	1.6577553	2.0543092	-2.368617	5.6841273
Quadratic	Yellow	-1.452691	0.1620288	-1.770261	-1.13512

Table A8: Data table for Chapter 2, Figure 2.7

Prediction model for regression model grouped by time after harvest

Prediction Model

$a + b*\text{Vase Life (days)} + c*\text{Vase Life (days)}^2$

a=Intercept

b=Slope

c=Quadratic

Table A9: Data table for Chapter 2, Figure 2.7

Summary of fit for regression model grouped by time after harvest

Summary of Fit	
AICc	20783.871
BIC	20873.661
SSE	2910494.3
MSE	1426.0139
RMSE	37.762599
R-Square	0.6566324

Table A10: Data table for Chapter 2, Figure 2.7

Parameter estimates for regression model grouped by time after harvest

Parameter Estimates

Parameter	Group	Estimate	Std Error	Lower 95%	Upper 95%
Intercept	1	126.82899	33.845752	60.49253	193.16544
Slope	1	-5.383635	7.448766	-19.98295	9.2156778
Quadratic	1	0.8545692	0.3998078	0.0709604	1.6381781
Intercept	3	92.682966	30.153301	33.583582	151.78235
Slope	3	1.9980956	6.7465489	-11.2249	15.221088
Quadratic	3	0.3288209	0.3675602	-0.391584	1.0492256
Intercept	6	29.030967	31.794855	-33.2858	91.347738
Slope	6	-9.168711	6.8841156	-22.66133	4.3239079
Quadratic	6	1.5996998	0.3629046	0.8884199	2.3109797
Intercept	9	-251.9219	14.149462	-279.6543	-224.1894
Slope	9	50.620681	1.3825817	47.910871	53.330491
Quadratic	9	-2.175	0	-2.175	-2.175
Intercept	12	-137.2647	4.5793877	-146.2401	-128.2893
Slope	12	1	0	1	1
Quadratic	12	1	0	1	1