Using Plant Growth Regulators to Improve the Quality of Containerized Herbaceous Peony (*Paeonia lactiflora* Pall.)

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ABSTACT

(Academic)

Herbaceous peonies (*Paeonia lactiflora* Pall.) are common perennials used both in gardens and the landscape as well as for cut flowers. Peonies require a chilling period to break dormancy but not for flower bud differentiation. For all studies discussed in this dissertation, two peony cultivars, Sarah Bernhardt and Inspecteur Lavergne, small (3–5 eye) crowns from Holland were potted in 3.8-L pots in mid-November of 2017 and 2018. Our overall objective was to determine if we could manipulate chilling time, along with application of gibberellic acid (GA_3) and growth retardants, to produce marketable containerized peonies from a small crown in a single season (November to May).

We evaluated chilling, GA_3 and a growth retardant (uniconazole; UNZ) under controlled chilling and greenhouse forcing conditions. All potted plants were held outdoors at Battlefield Farms (Rapidan, VA, 38˚ N) for 4 weeks [in 2017, 400 chilling units (CU) according to Fulton Chilling Model] or in a 10°C cooler for 5.5 weeks (in 2018, 400 CU) to root, then placed in a 5°C cooler for 3, 4 or 5 weeks (total 752, 869 or 986 CU). GA_3 was applied as a 0 or 100 $mg \cdot L^{-1}$ drench at 250 ml/pot after the plants were moved into the Virginia Tech greenhouse (Blacksburg, VA, 37˚ N) for forcing. Uniconazole drenches were applied to each cultivar under each chilling treatment at 355 ml/pot at 0, 15, or 20 $mg \cdot L^{-1}$ at 7 days after the GA_3 drench applications. Three weeks chilling at 5°C (752 CU total) provided sufficient chilling for ‘Sarah Bernhardt’ and ‘Inspecteur Lavergne’. Application of GA_3 reduced production time and resulted in a greater number of shoots, and, in three of the four studies, increased the number of flowering shoots in three of the four studies. Substrate drench application of 15 $mg \cdot L^{-1}$ UNZ prior to spring emergence reduced plant width moderately resulting in improved compactness of both cultivars.

We evaluated the effects of plant growth retardants applied with different methods at different stages of production on the growth and development of containerized peony under nursery conditions. All potted plants were placed in an unheated coldframe at the Virginia Tech Urban Horticulture Center (Blacksburg, VA, 37° N) for one month after potting to promote rooting and then were moved outdoors to a gravel pad to receive natural chilling from November to February. In 2017–18, substrate drenches of UNZ at 0, 15, 30 or 45 $mg \cdot L^{-1}$ or paclobutrazol (PBZ) at 0, 30, 60 or 90 $mg \cdot L^{-1}$ at 237 mL/pot were applied about 4 weeks after potting for both cultivars in mid-December 2017. In 2018–19, fall drenches of uniconazole at 0, 15, 30 or 45 $mg \cdot L^{-1}$ at 237 mL/pot were applied about 4 weeks after potting in mid-December 2018, or spring sprenches of uniconazole were applied at 0, 15, 30 or 45 $mg \cdot L^{-1}$ at 840 $mL \cdot m^{-2}$ in March 2019 after 50% shoot emergence for each cultivar. Plant growth retardant applications had little effect on plant growth of either cultivar, but treated plants were of a darker green color compared to the control plants. In addition, higher rates of uniconazole applied as a fall drench increased the number of flowering shoots of both cultivars and the percentage of plants flowering for 'Sarah Bernhardt' in the second season of the study where plants were more protected from spring freezes. Fall paclobutrazol drenches or spring uniconazole sprenches had little effect on flowering.

To determine the best timing for spring GA_3 applications under nursery conditions, we applied three models based on natural chilling accumulation. The models were a modified Fulton Chilling Model (FCM) for herbaceous peonies, Blackberry Chilling Model 5 (BCM5) for blackberry, or a visual development model (VDM) which was 10% of plants showing shoot emergence in the spring. We choose 1,000 CU for the first two chilling models as the chilling required to break dormancy and promote normal plant growth and flowering. All plants were

held in an unheated coldframe at the Virginia Tech Urban Horticulture Center for one month after potting to promote rooting, then were moved outdoors to a gravel pad to receive natural chilling over the winter months. Drenches of 0 or 100 $mg \cdot L^{-1}$ GA_3 were applied at 250 mL/pot to each cultivar under each chilling model when the specific conditions were met. Due to greater winter injury in the 2017–18 season, results varied by year. In the 2017–18 season, GA_3 applied according to BCM5 reduce days to emergence for both cultivars and reduce the plant width of 'Inspecteur Lavergne', and later application according to BCM5 and VDM reduced plant length and diameter of 'Sarah Bernhardt'. Reductions in plant size may have been due to greater winter injury due to the earlier emergence of GA_3 treated plants. In the 2018–19 season, earlier GA_3 drench applications tended to reduce days to emergence for both cultivars and the FCM application reduced days to bud for 'Inspecteur Lavergne', but GA_3 drench applications had no effect on plant size. GA_3 can be applied after chilling (1,000 CU) using a suitable chilling model such as FCM for peonies, or BCM5, or VDM, but GA_3 had little effect on plant development under nursery conditions.

We also evaluated GA_3 effects on peony bud differentiation and development during controlled chilling and early forcing, as well as effects on growth and flowering. All potted plants were held in a 10°C cooler for 5.5 weeks (400 CU) to root, then placed in a 5°C cooler for 4 weeks (total 869 CU). GA_3 was applied at 0 or 100 $mg \cdot L^{-1}$ pre-chilling or post-chilling as a 250 ml/pot drench. Bud differentiation and development of excised buds were evaluated using a stereomicroscope at potting, after rooting (before chilling), after 1, 2, 3 or 4 weeks of chilling, and at 5, 10 or 15 days after the beginning of forcing. All buds were removed from the sample plants, measured for bud length and diameter, and dissected under a stereomicroscope to assess differentiation stages. Root dry weights and crown dry weights were also determined after

rooting, after chilling, and at 15 days of forcing. Ten plants of each treatment were grown in the Virginia Tech greenhouse after chilling until flowering. GA_3 applications did not advance the bud development stage because most of buds were already in the reproductive stages before dormancy, but GA_3 enhanced bud elongation during chilling and the early forcing period. Our findings suggest that GA_3 applications can reduce the time to emergence and flowering, as well as increase the numbers of shoots and flowering shoots. GA_3 applied right after rooting in, prior to the chilling period, or before greenhouse forcing, resulted in earlier emergence and flowering with higher quality plants. However, earlier applications, pre-chilling, tended to produce plants with more shoots.

Overall, our experiments indicate that three weeks of chilling at 5°C (752 CU total) is a sufficient chilling regime for forcing 'Sarah Bernhardt' and 'Inspecteur Lavergne' peonies, and 1,000 CU of naturally accumulated chilling is sufficient for nursery production. GA_3 applications can reduce the time to emergence and flowering, as well as increase the numbers of total shoots and flowering shoots. Timing of GA_3 application is flexible; it can be applied right after rooting, before the chilling period, just before greenhouse forcing, or after shoots have begun to emerge. Plant growth retardant applications had a little effect on the growth of tested cultivars, but all plants treated with growth retardants are generally darker green in color. Additionally, growth retardant applications have some positive effects on flowering.

ABSTACT

(General Audience)

Herbaceous peonies (*Paeonia lactiflora* Pall.) are common perennials used both in gardens and the landscape as well as for cut flowers. Peonies require a chilling period to break dormancy but not for flower bud differentiation. For all studies, two peony cultivars, Sarah Bernhardt and Inspecteur Lavergne, 3 to 5 eye small crowns from Holland were potted in 3.8-L pots in mid November of 2017 and 2018. Our overall objective was to determine if we could manipulate chilling time, along with application of gibberellic acid (GA_3) and growth retardants, to produce marketable containerized peonies from a small crown in a single season (November to May). We evaluated chilling, GA_3 and a growth retardant (uniconazole) under controlled chilling and greenhouse forcing conditions. We evaluated the effects of plant growth retardants (uniconazole or paclobutrazol) applied with different methods (fall drenches or spring sprenches) at different stages of production on the growth and development of containerized peony under nursery conditions. To determine the best timing for spring GA_3 applications under nursery conditions, we applied three models based on natural chilling accumulation. We also evaluated GA_3 effects on peony bud differentiation and development during controlled chilling and early forcing, as well as growth and flowering. Overall, 3 weeks chilling at 5°C [752 chilling units (CU) total] is a sufficient chilling regime for forcing 'Sarah Bernhardt' and 'Inspecteur Lavergne' peonies, and 1000 CU naturally accumulated chilling is sufficient for nursery production. GA_3 applications can reduce the time to emergence and flowering, as well as increase the numbers of shoots and flowering shoots. Timing of GA_3 application is flexible, it can be applied right after rooting, after the chilling period, or after shoots have begun to emerge. Plant growth retardant applications had little effect on plant growth of either cultivar, but all plants treated with growth retardants were darker green in color. Additionally, growth retardant applications had some positive effects on flowering.

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Chapter 1

Literature Review of Using Plant Growth Regulators to Improve the Quality of Containerized Herbaceous Peony (*Paeonia lactiflora* Pall.)

Introduction

Herbaceous peony (*Paeonia lactiflora* Pall.) is a traditional flowering landscape and medicinal perennial in many countries of temperate regions with rising interest as cut flowers in recent years (Kamenetsky and Dole, 2012). *Paeonia* is the only genus in the Paeoniaceae. More than 30 woody and herbaceous *Paeonia* species have been identified to date. Most species are native to China, the remainder are from other parts of Asia, Europe and North America (Halda et al., 2004). Most herbaceous peony cultivars are derived from few species, mainly from *Paeonia lactiflora* Pall., also called Chinese peony which is native to China (Rogers, 1995). Peony is a beautiful plant with rich green foliage and huge masses of showy flowers. They are easy to grow and require little maintenance, making them suitable to any garden style. Peony flowers exhibit a wide range colors and shades. Some species and cultivars emit a sweet fragrance. Peony flowers also have six forms which include single, Japanese, anemone, bomb, semi-double, and double (Michener and Adelman, 2017). Each herbaceous peony plant consists of several herbaceous shoots; the actual number of shoots depends on cultivar, plant age and vigor. Each shoot has five to seven leaves. Flowers are at the terminal of shoots, with axillary flowers on some shoots.

Cultivation History

Cultivation of herbaceous peony plants started at least 3000 years ago in ancient China (Yu et al., 2011). Plants are used for both ornamental and medicinal purposes, and thus was named as

the “Prime Minister of Flowers”. Its Chinese name *Shaoyao* (or *Sho Yo*) translates as both “charming and beautiful” and “wealthy and honorable” (Rogers, 1995). Peony was in the “Classic of Poetry” (“*Shijing*” or “*Shih-ching*”) dating from the 11th to 7th century B.C. It has been regarded as the Chinese “love flower” since ancient times (Yu et al., 2014). Its gracious beauty made it a very popular flower in Chinese gardens with tree peonies (named as “King of the flowers”) in the Tang Dynasty (618–907 A.D.), with large scale peony gardens, especially royal gardens, existing in the cities of Xi’an and Luoyang, the two capitals of that time (Yuan and Yu, 2011). The peak of peony cultivation time was in the Song Dynasty (960–1279 A.D.), when peonies were widely planted all over the country. Herbaceous peonies remain extremely popular in China today. They can always be found in gardens along with tree peonies to extend the blooming time for their similar large showy flowers. In addition to its ornamental value, dried peony roots have anti-inflammatory and immunomodulatory effects and have been used in traditional Chinese medicine to treat muscle pain, fungal infections, spasmodic pain, fever, rheumatoid arthritis, and hepatitis for several thousand years (El Babili et al., 2013; He and Dai, 2011). Peony roots and flowers are also served as a traditional food and tea (Yu et al., 2011).

Chinese peony was introduced to Japan at the 10th century. It was quickly accepted and has been widely cultivated ever since then, becoming the symbol of wealth and status (Yu et al., 2011). The peak peony cultivation time in Japan was in 1700 A.D.; there were more than 100 cultivars with lots of bright colors at that time (Yu et al., 2011). Since then, a lot of new cultivars have been introduced.

In Europe, peonies (*Paeonia* spp.*)* were cultivated for medicine and spice from 8th century, then used as ornamental landscape plants from the 14th century (Yu et al., 2011). The genus *Paeonia* was first recorded as “the most ancient plant of all” in the book “Natural History”

written by Pliny the Elder around 79 A.D. (Harding, 1993). The genus was named after Paeon, the Greek mythical god of medicine and healing (Rogers, 1995). It was an important plant culturally in the western world with references in Homer's "Iliad" and Shakespeare's "Taming of the Shrew".

Chinese peony was widely introduced into European and American gardens in the 1800s from China and Japan (Rogers, 1995) with great interest and was widely used in gardens and as a cut flower. In 1903, peonies became popular in the United States to the extent that the American Peony Society (APS) formed to "promote cultivated peonies and foster studies to improve its worth as a garden plant" (APS, 2020). As the International Cultivar Registration Authority for peonies, APS has registered 6,854 peony cultivars to date, including 4,080 cultivars of *P. lactiflora* (APS, 2020). These numbers continue to increase as new cultivars are introduced and registered every year. A few hundred popular cultivars are available that may best fit gardener needs (Michener and Adelman, 2017). Containerized herbaceous peonies are popular in the potted herbaceous perennial plant sector, with more than half million plants, valued at almost $6 million USD, sold in 2018 (USDA, 2019).

Annual Life Cycle and Carbohydrate Storage

Most herbaceous peonies are grown from a perennial underground crown, which is an underground shoot that serves as an energy storage center for plant renewal (Din et al., 2015). A large number of buds develop on the surface of the crown and progress into monocarpic shoots with leaves and flowers after emergence in early spring. In northern hemisphere temperate regions, peonies flower once a year from May to July depending on location and cultivar, and flowers normally last for one to two weeks. The leaves remain green for a few months after

flowering until the leaves senescence, at which time the peony plant enters dormancy for three to four months (Barzilay et al., 2002). The renewal buds initiate after flower senescence and remain vegetative during the summer. After senescence of above ground shoots in fall, the meristem on the tip of the renewal buds begins its generative stage by initiating floral production and later differentiation until early winter. These growth stages of peony do not require a chilling period (Barzilay et al., 2002).

As a deciduous perennial and winter-dormant plant, the carbon supply for spring growth and development of the herbaceous peony is stored in the crowns. Walton et al. (2007) found starch, the main form of carbohydrate storage, is accumulated in developing flower buds. Its accumulation reaches the peak at the beginning of flower opening and declines during flower opening (Walton et al., 2007). Starch concentration in the crown declines in spring right after shoot emergence due to plant development, and begins to increase again at 45 days after the emergence of shoots, and increases throughout the flowering season, reaching its highest level in mid-summer (150 days after shoot emergence). Starch concentration begins to decline in the late summer/autumn period to a stable level which is maintained during dormancy (Walton et al., 2007). Starch accumulation enables peonies to survive cold winter extremes, enhancing their cold hardiness. Carbohydrate storage was previously reported in other herbaceous perennials like rhizomes of *Veratrum*, crowns of *Dicentra* and roots of *Panax* (Kleijn et al., 2005; Risser and Cottam, 1968; Follet et al., 2004). Plant species with similar life cycles and environmental conditions often employ similar carbohydrate storage and mobilization strategies to survive harsh growing conditions (Walton et al., 2007). These plants produce a rapidly growing shoot in the spring. If it is damaged by frost or herbivory, plants can still survive on the stored carbohydrates throughout the rest of the year (Walton et al., 2007).

Flower Bud Differentiation and Development

Peony is a day neutral plant, which means that the flower initiation is independent of photoperiod (Fearnley-Whittingstall, 1999). The development of flower buds of peony 'Sarah Bernhardt' was described by Barzilay et al. (2002) in Israel (lat. 33° N, long. 35° E). Renewal buds grow on the surface of the crown. Four to eight leaf scales tightly cover and protect the inside of each renewal bud, which starts to develop as a monocarpic shoot beginning with leaf primordia formation just after flower senescence in late June. After bracts are produced from the apical meristem, the buds transition from the vegetative growth stage to the generative stage in September. Flower differentiation begins in October with sepals, petals and petaloids produced from apical meristems, and ceases in early December. Each renewal bud contains a central flowering shoot, and three to six axillary shoots. Large axillary shoots can also differentiate into flowering shoots at the same time with central shoots, and small buds normally remain vegetative (Barzilay et al., 2002).

Floral development studies of five Chinese peony cultivars, including early-flowering (Da Fu Gui and Dongfang Hong), mid-flowering (Zi Fengyu), and late-flowering (Qingwen and Taohua Feixue), in Beijing, China (lat. 40° N, long. 116° E) showed that all cultivars grown in the field began the formation of leaf primordia in renewal buds in early June, bract primordia formation in late August, sepal primordia formation in late September, petals in early October, and stamen primordia in late October (Ai, 2016; Zhou, 2012). In these studies, most renewal buds were already in the reproductive stage when plants entered dormancy. The bud studies of a Japanese wild type peony also reported renewal buds started entering the reproductive stage in October, and all buds were in the reproductive stage at the end of October and in November (Aoki, 1991).

Bud differentiation was also studied for 12 field grown Chinese cultivars including single, Japanese and double type flowers in Beijing, China (Ai, 2016). For single type (such as Fen Yunu), the bud differentiation order was: starting with bract primordium, then sepal primordium, after that petal primordium, flowed with stamen primordium and pistil primordium. For Japanese type (such as Lian Tai), the bud differentiation order was: starting with bract primordium, then sepal primordium, after that petal primordium, flowed with stamen primordium, pistil primordium, and petaloid stage. For double type (such as Da Fu Gui), the bud differentiation order was: starting with bract primordium, then sepal primordium, flowed with petal primordium, stamen primordium, secondary petaloid formation period, the upper floral structure formation stage, and petaloid stage. More than 50% of the flower buds for most cultivars (11 of 12) were in pistil primordium stage when the soil started freezing (late November) (Ai, 2016). Over 90% of the flower buds for early-flowering cultivars and over 50% of the flower buds for late-flowering cultivars were in the pistil primordium differentiation stage when the soil started to defrost in spring (early to mid-March). The differentiation of peony floral buds did not have a specific physiological dormancy period; the dormancy start time was mainly affected by the environmental conditions. Flower buds kept differentiating while the soil was frozen.

As the most popular double-flowered *P. lactiflora* cultivar used for research in China, Da Fu Gui (also written as Dafugui) is very suitable for containerized forcing culture. Floral bud differentiation of field-grown Da Fu Gui from autumn (late September) to early spring (mid-April) was studied using different sampling methods including paraffin sections or hand-made sections, followed by stereomicroscopy in Beijing, China (Zhang et al., 2019b). The differentiation of the double flower bud has two parts including differentiation of lower parts (differentiation periods for the bracts, sepals, petals, stamens and pistillodes which happens

before winter dormancy) and upper parts (separate differentiation periods for the petals, stamens and pistil which happens early the following spring, which is the 'double' formation), and finally develops to a double-flower. The floral bud differentiation closely depends on the seasonal temperature changes.

Bud Dormancy

Bud dormancy is a special state for perennial plants characterized by a temporary suspension of bud meristem growth that allows the buds to adapt and survive over a cold winter period. There are two types of dormancy, endodomancy (true dormancy) which is signaled by plant physiological factors, and ecodormancy (climatic dormancy) which is controlled by environmental factors (Considine and Considine, 2016; Lloret et al., 2018). Endodormancy release requires sufficient cold accumulation, and ecodormacy release requires heat accumulation. Cold accumulation affects flower quality and fruit production directly for perennial fruit species (Beauvieux et al., 2018). Although growth is suspended during dormancy, overwintering buds continue active development with extensive transcriptomic and hormonal changes (Lloret et al., 2018). Dormancy, cold accumulation and flowering are the three major processes for a perennial reproductive bud, and have important functions in bud dynamics, plant survival, growth resumption and quality. Specific mechanisms and gene expressions related to dormancy and dormancy release in perennial buds are mainly regulated by environmental signaling, hormonal signaling, carbohydrate metabolism, oxidative stress, and others such as the role of plasma membrane and mitochondrial respiration (Lloret et al., 2018).

Environmental factors including photoperiod and temperature lead to bud dormancy and growth cessation (Lloret et al., 2018). Short daylength induces some photoperiodic control genes

such as *CONSTANS (CO)/ FLOWERING LOCUS T (FT)* module, *FT* protein interactor gene *FLOWERING LOCUS D*, and *APETALA1 (AP1),* that control growth cessation in some tree species. Low temperatures induce *DORMANCY-ASSOCIATED MAD-BOX (DAM)* genes which regulate dormancy induction and growth cessation in many perennial plants (Lloret et al., 2018).

Plant hormones are endogenous molecules regulating plant growth and development, classically grouped in auxins, gibberellins (GAs), cytokinins (CK), abscisic acid (ABA), and ethylene (Rademacher, 2015). Hormones including GAs, ABA, auxin and CK have paramount functions in regulating transcript levels of dormancy-related genes in several temperate tree species (Beauvieux et al., 2018). Higher ABA levels induce dormancy in early winter and higher GA_3 (gibberellic acid, one of GAs) and CK levels promote dormancy release for many perennial plants, including herbaceous peony (Beauvieux et al., 2018; Yu et al., 2012). Directly applied CK can inhibit apical dominance and induce bud break of axillary buds of garlic (*Allium sativum*). However, indole-3-acetic acid (IAA) does not regulate dormancy, but promotes bud differentiation and development after dormancy release (Yu et al., 2012). Differentially expressed genes (DEGs) of *P. lactiflora* 'Da Fu Gui' also showed that the most frequent DEGs involved in plant hormone signal transduction during dormancy and dormancy release are related to auxin, CK, GAs and ABA biosynthetic and signaling pathways (Guo et al., 2017).

Carbohydrate metabolism is essential during the bud dormancy periods; starch is degraded to soluble sugars during dormancy and starch increases again right before dormancy release for fruit tree species such as sweet cherry (*Prunus avium*) (Kaufmann and Blanke, 2017). In walnut trees (*Juglans regia* and *J. regia* × *nigra*), soluble sugars increase during dormancy and significantly decrease after dormancy release (Charrier et al., 2017). Bud dormancy of axillary buds of grapevine (*Vitis vinifera*) and apical buds of poplar (*Populus tremula* × *P. alba*) was

related to carbon supply starvation and sugar deficit (Tarancón et al., 2017). After dormancy release, due to the increasing expression and activity of cell membrane transporters, the carbohydrate uptake capacity increases in the buds (Tarancón et al., 2017). Bud dormancy is induced by carbon supply starvation syndrome linked to sugar deficit in dormant axillary buds of all eukaryotes (Tarancón et al., 2017). The carbohydrates are in the crown and bud tissue of herbaceous peonies during the dormancy (Walton et al., 2007). Carbohydrates are stored in the crowns of herbaceous peonies mainly as starch; starch accumulation is related to developing hardiness so the peony plant can survive in the cold winter extremes (Walton et al., 2007). Starch concentration in peony crowns reaches its maximum level in the fall just before dormancy, then decreases during dormancy as sugar (glucose, fructose and sucrose) content increases; starch is degraded to sugars to overcome cold stress and helps peony buds survive during dormancy (Zhang et al., 2017). After dormancy, sugars are transported towards the buds through the sap.

Oxidative stresses and reactive oxygen species (ROS) are also involved in bud dormancy release (Beauvieux et al., 2018). Oxidative stresses trigger ROS production including H_2O_2, and research showed exogenous H_2O_2 can partially replace chilling and induce bud dormancy release in small fruits such as grapevines (Beauvieux et al., 2018; Pérez et al., 2008). In herbaceous peony during dormancy, H_2O_2 concentrations in buds fluctuate with temperature changes, being lower at low temperatures, which may help herbaceous peonies survive at low temperatures (Zhang et al., 2017).

Herbaceous peonies can experience endodormancy (Barzilay et al., 2002; Byrne and Halevy, 1986; Cheng et al., 2009; Evans et al., 1990; Fulton et al., 2001; Kamenetsky et al., 2003; Rhie et al., 2012; Zhang et al., 2019a) and ecodormancy (Yeo et al., 2012). For herbaceous peony ‘Da Fu Gui’, Guo et al. (2017) characterized differentially expressed genes (DEGs) during

bud dormancy release; three bud types were examined: dormant buds (S1), endodormancy-released buds under controlled chilling (S2), and ecodormancy-released buds under natural chilling (S3). In this study, 1772 DEGs were detected from the S1/S2 comparison transition set and 3119 DEGs from the S2/S3 set (Guo et al., 2017). Gene ontology (GO) classification of DEGs from both transitions showed that the majority of DEGs were from the biological process (biological metabolic processes), cellular component (cell parts) and molecular function (catalytic activity) categories. Kyoto Encyclopedia of Genes and Genomes analysis showed that the plant hormone signal transduction (environmental information processing), DNA replication (genetic information processing) and flavonoid biosynthesis (metabolism) were critical to both transitions, while the photosynthesis pathway was significant for S2/S3 transition exclusively (Guo et al., 2017). About 45% of the annotated DEGs among the two transitions of 'Da Fu Gui' were homologous to DEGs from grapevine, which indicates that the two temperate perennial plants have similar bud dormancy patterns; their large array of DEGs were homologous to those of cacao tree (*Theobroma cacao,* 15%), peach (*P. persica*, 8%) and California poplar (*P. trichocarpa*, 7%) (Guo et al., 2017).

Gibberellins (GAs) Biosynthesis and Commercial Use in Horticulture Crops

Gibberellins (GAs) are a large group of tetracyclic diterpenoid carboxylic acids with tetracyclic *ent*-gibberellane (C_{20}) or 20-nor-*ent*-gibberellane (C_{19}) skeletons. Currently 136 fully characterized GAs have been identified in fungi, bacteria and higher plants (Hedden and Thomas, 2012). Among the 136 GAs, only six have intrinsic biological activity, namely GA_1, GA_3, GA_4, GA_5, GA_6 and GA_7. GAs are known for regulating the development of many higher plants, by promoting cell elongation and plant growth, inducing hydrolytic enzymes in seed germination, inducing bolting in long-day plants, promoting flowering, and fruit set and

development (Rademacher, 2015, 2016).

The GA biosynthetic pathway for GA_1, GA_3 and GA_4 has three stages (Hedden and Thomas, 2012). In stage I, which occurs in plastids, terpene cyclases catalyze the synthesis of *ent*-kaurene from trans-geranylgeranyl diphosphate (GGPP). In stage II, which occurs in the endoplasmic reticulum, involving the cytochrome P450 monooxygenases, *ent*-kaurene is sequentially oxidized to yield GA_{12} and GA_{53}. In stage III, which occurs in the cytosol, involving dioxygenase, GA_{12} and GA_{53} are further oxidized to other C_{20}-GAs, and C_{19}-GAs (Hedden and Thomas, 2012; Sponsel and Hedden, 2010).

Commercial GAs are produced from fermentation of the fungus *Gibberella fujikuroi,* which was found by Japanese scientists in early 1950s (Rademacher, 2016). GA_3 is the most widely used GA in horticulture, viticulture and agriculture (Rademacher, 2015). Other commonly used GAs are GA_4 and GA_7 as a mixture due to their close chemical similarity and the difficulty in separating them in the fermentation extract of *G. fujikuroi*. $GA_{4/7}$ is commercially used to reduce apple fruit russetting (Rademacher, 2015).

GA_3 can be used to enhance flowering for ornamental crops (Latimer and Whipker, 2019). It can be used as an alternative of chilling for some woody and herbaceous ornamentals in the greenhouse, such as containerized florist azalea (*Rhododendron* sp. cv.) (Ballantyne, 1960; Boodley and Mastalerz, 1959) or Korean chamchwi (*Aster scaber*) for cut flowers (Seong, et al., 1996), as well as geophytes like tulip (*Tulipa* sp.) and blazing star (*Liatris* sp.) (Metzger, 1995). Additionally, GA_3 improves flowering and flower size of camellia (*Camellia* sp.) and baby's breath (*Gypsophila* sp.), encourages early flowering and increases overall production of statice (*Limonium* spp.) and peace lily (*Spathiphyllum* spp.) (Latimer and Whipker, 2019).

Furthermore, it increases stem length of cut flowers such as stock (*Matthiola* sp.), larkspur (*Delphinium* sp.), and carnation (*Dianthus* spp.). The time of GA_3 application is critical to its efficacy, as late applications can result in excessive plant stretching and weak and spindly stems (Latimer and Whipker, 2019). GA_3 applied as foliar spray at 4 and 6 weeks after transplanting four-week-old seedlings not only increased vegetative height, but also produced a larger numbers of flowers of chrysanthemum (*Chrysanthemum indicum*) (Moond and Gehlot, 2006, 2007). Dahlia (*Dahlia variabilis*) plants treated with a 200 $mg \cdot L^{-1}$ GA_3 spray had larger numbers of flowers than the untreated control; this treatment also resulted in significant improvement in the plant growth and quality, such as number of leaves, branches, and flowers, size of flowers, early flower emergence, and improved shelf life (Singh et al., 2017). For bleeding heart (*Dicentra spectabilis*), GA_3 application did not replace the cold requirement (Song et al., 2002), but promoted elongation under a short-day photoperiod (Weiler and Lopes, 1977).

GA's Effects on Cold Acclimation and Dormancy Release

GAs play a central role in bud dormancy either directly or by cross-talking with other biochemical and hormonal pathways that regulate dormancy maintenance and release (Beauvieux at el., 2018; Lloret et al., 2018; Turnbull, 2011). GAs can affect important bud dormancy release mechanisms in woody or herbaceous plants, such as hormonal signaling pathways and florigen *FLOWERING LOCUS T (FT)* gene expression, carbohydrate metabolism and oxidative stress (Beauvieux at el., 2018).

GAs can also act as mobile florigen signals themselves and/or control other mobile floral signals (Turnbull, 2011). Endogenous GA levels are regulated by photoperiod and increase under long-days following the expression of biosynthetic and catabolic genes (Turnbull, 2011). The

protein *FT* is a common florigen also mobile in the phloem, and a main factor of bud dormancy release in trees (Turnbull, 2011). Low temperatures during dormancy induce GA biosynthesis gene up-regulation and GA catabolic genes down-regulation in hybrid aspen (*P. tremula* × *tremuloides*) (Karlberg et al. 2010), and *FT1* gene up-regulation in poplar (*P.* spp.) (Hsu et al., 2011). GAs can also induce expression of *FT* genes, and the relative level of *FT* genes could determine dorancy release (Turnbull, 2011). GAs can also affect carbohydrate metabolism during dormancy and dormancy release; soluble sugars such as glucose and sucrose are hydrolyzed from starch during dormancy to overcome carbon supply starvation (Beauvieux et al., 2018; Walton et al., 2007; Zhang et al., 2017). Exogenous GA_4 can induce sucrose synthesis and increase the soluble sugar content leading to dormancy release in Japanese apricot (*P. mume*) (Zhuang et al., 2015). GAs can also affect oxidative stress; GA_4 application up-regulated oxidation-induction proteins and led to the dormancy release in apricot flower buds due to the induction of oxidative stress (Zhuang et al., 2013). Also, reactive oxygen species (ROS) interact with GA signaling in barley (*Hordeum vulgare*) seed dormancy; exogenous H_2O_2 treatment increased endogenous GA content in barley seed by up-regulating the GA-induced gene expression, inhibiting the GA catabolism gene expression and strongly inducing GA synthesis gene expression (Bahin et al., 2011).

Dormancy, cold acclimation/accumulation and flowering are the three major processes for a perennial reproductive bud, which are important to bud dynamics, plant survival, growth resumption and flower quality. Overwintering buds of perennials grown in temperate regions must deal with cold and dehydration stress during dormancy. Cold acclimation processes enhance plant tolerance to cold weather and desiccation related to gene expression (Wisniewski et al., 2003). Cold acclimation and dormancy are related. Both are induced by coldness and

short-day photoperiod, and both are associated with cessation of growth (Welling et al., 2002). Cold-dependent C-Repeat Binding Factor (*CBF*) genes regulate both dormancy and cold acclimation (Lloret et al., 2018). *CBF* genes induce synthesis of protective proteins, modify the lipid composition of membranes, affect carbohydrate metabolism, and produce storage and antioxidant compounds to overcome cold, drought and oxidative stresses during the cold acclimation process (Welling and Palva, 2006).

Although floral initiation and differentiation do not require a chilling period, peony flower buds require cold accumulation to break dormancy and promote plant growth and flowering (Barzilay et al., 2002). Byrne and Halevy (1986) reported a duration up to 28 days at 5.6°C can break dormancy. In fact, increasing the chilling time to 6 weeks or reducing the temperature to 1°C increased the number of shoots for 'Sarah Bernhardt' and 'Festiva Maxima'. Evans et al. (1990) reported that peony required more than 28 days of cooling at 5.5°C for sprouting. Aoki (1991) also reported that chilling at 4°C for 30 days resulted in peony plants with the highest flowering percentage. Kamenetsky et al. (2003) reported the best dormancy-release treatment for 'Sarah Bernhardt' was under chilling regimes of 2°C or 6°C for 60 or 70 days, respectively. Cheng et al. (2009) found that chilling at 0 to 4°C for 3 weeks was sufficient for dormancy release and flowering, but 4 weeks was optimal for several Chinese cultivars. Zhou (2012) reported that at least 7 weeks at 2±1°C was sufficient to break dormancy as well as to retain the flower quality for five Chinese cultivars; she also suggested that the peony plant can be sensitive to low temperatures when the flower buds are in the petal primordia differentiation stage. Single-flowered cultivar 'Hang Baishao' which is the cultivar best adapted to low latitude regions (N 30°) in southern China, grows and flowers optimally under chilling at 0 to 4°C for 28 days (Zhang et al., 2019a).

Fulton et al. (2001) developed a chilling model to calculate the chilling units (CU) accumulated by peony in order to quantify the amount of chilling required to break dormancy using three cultivars and three temperature regimes. CU were calculated by the linear equation ($y = -0.0605x + 1$, $R^2 = 0.9943$), where y is the number of CU and x is the temperature in °C. The optimal accumulated CU varies among cultivars (Fulton et al., 2001; Rhie et al., 2012; Yeo et al., 2012). The chilling requirements of *P. lactiflora* cultivars from previous studies are listed in **Table 1-1**, in which we calculated the CUs by applying Fulton's Model to the chilling regime described by the authors of the individual studies.

The chilling requirement of herbaceous peony has some similarity with fruit crops such as apple and crabapple; both are *Malus* spp. (Michener and Adelman, 2017). Other chilling models were used and listed to calculate natural accumulation CUs received to break dormancy of fruit trees such as sweet cherry (*P. avium*), almond (*P. dulcis*), peach (*P. persica*) or apricot (*P. armeniaca*) (Alburquerque et al., 2008, Egea et al., 2003; Erez and Fishman, 1998; Ruiz et al., 2007). Among those models, an easy to access model is the Blackberry Chilling Model (BCM) developed by North Carolina State University (Warmund and Krumme, 2005). It is a commercial model of chilling accumulation for the fruit crop blackberry (*Rubus* spp.). In this model, the accumulated CUs are calculated using instantaneous temperatures of local weather stations, or hourly average temperatures are used where available. The Blackberry Chilling Models (2020) have four different formulas to calculate accumulated CU which are Model 1, Model 2, Model 5 and Model 6. The Blackberry Chilling Model 5 (BCM5) considers temperatures between 0 to 12.4°C as effective temperatures for dormancy release, and attribute negative effects for dormancy release when temperatures are above 15.9°C, which is similar to the peony chilling requirements reported from known studies (Byrne and Halevy, 1986; Evans et al., 1990; Fulton

et al., 2001; Halevy et al., 2002; Kamenetsky et al., 2003).

Overall, herbaceous peony needs a chilling period to break dormancy and enhance growth and development. The chilling requirement is satisfied by a range of low temperatures accumulated over time. Increasing chilling time and decreasing temperature can advance the time of emergence and flowering (Byrne and Halevy, 1986; Evans et al., 1990; Kamenetsky et al., 2003). But excessive chilling can decrease flowering percentage and even prevent flowering (Aoki, 1991; Byrne and Halevy, 1986; Halevy et al., 2002). The optimal accumulated chilling units varies among cultivars (Fulton et al., 2001; Rhie et al., 2012; Yeo et al., 2012). The chilling requirement of peony cultivars from previously published studies are listed in **Table 1-1** (CU were calculated according to the Fulton Model).

GA_3 can also be used for herbaceous peonies to partially replace the chilling requirement when plants receive insufficient chilling (Evans et al., 1990). Non-chilled 'Scarlet O'Hara' peony crowns subjected to a GA_3 substrate drench emerged in 8 days, while untreated control plants failed to emerge, but all flower buds on plants treated with GA_3 aborted (Evans et al., 1990). Halevy et al. (2002) reported that very early flowering and high quality 'Sarah Bernhardt' flowers were obtained with a combination of chilling at 2°C for 13 weeks and GA_3 treatments; the optimal GA_3 treatment for these 4-year-old plants was a soil drench of 250 mL of 100 $mg \cdot L^{-1}$ GA_3 to 4-year-old peony. In addition, flower production was doubled with this GA_3 treatment of two other field-grown peony cultivars (Karl Rosenfeld and Duchesse de Nemours). One GA_3 drench was sufficient for 'Sarah Bernhardt' and 'Karl Rosenfeld', but repeated GA_3 applications (drench or spray) reduced 'Sarah Bernhardt' flower production by causing more flower abortion (Halevy et al., 2002). Cheng et al. (2009) also reported that GA_3 application enhanced plant growth and development, as well as shortened the time to emergence and flowering of peony;

chilling at 0 to 4°C for 4 weeks before a drench application of 250 mL of 200 $mg \cdot L^{-1}$ GA_3 was optimal for forcing 'Da Fu Gui'. In other research, GA_3 treatment replaced the chilling requirement for 'Taebaek' peony when plants received insufficient chilling, and GA_3 treatment effectively reduced the flowering time and increased flowering percentage (Yeo et al., 2012).

Forcing herbaceous peonies often results in flower bud abortion or blasting. Evans et al. (1990) reported that the abortion of all flower buds on non-chilled 'Scarlet O'Hara' peonies subjected to GA_3 application might have been due to the high rate of GA_3 (118 mg a.i./pot). Kamenetsky et al. (2003) reported that high night temperatures (22°C) during the early forcing period caused high numbers of young flower buds to abort at an early growth stage on 'Sarah Bernhardt' plants. Park et al. (2015) reported that chilling at 0°C for 6 weeks after a pre-chilling treatment at 10°C for 2 weeks promoted flowering and reduced flower bud abortion of peony 'Taebaek'. The optimal chilling time for peony 'Taebaek' was after November, where more than 80% of plants flowered, and the optimal pre-chilling treatment was 2 weeks at 10°C in October, which induced 89.6% of plants into flower with about three flowers per plant.

The flowering percentages of herbaceous peonies under forcing conditions are highly cultivar-dependent. Cheng (et al. 2009) studied 20 Chinese herbaceous peony cultivars for forcing for both landscape use and cut flower production and founded that less than five cultivars were suitable for forcing with a flowering percentage greater than 75% while the remaining cultivars had less than 50% of plants flowering. Less than 50% of 2 to 4-year-old 'Sarah Bernhardt' plants grown under container production flowered after sufficient chilling (Halevy et al., 2002; Kamenetsky et al., 2003), and less than 70% of 3-year-old plants flowering with GA_3 application (Halevy et al., 2002). Hall et al. (2007) reported less than 16% of 3-year-old 'Sarah Bernhardt' plants flowered even under optimal forcing conditions after sufficient chilling.

PGRs and Growth Retardants

PGRs

Plant growth regulators (PGRs) are natural or synthetic organic chemicals used to regulate growth and development of higher plants (Rademacher, 2015). PGRs are used to not only control cell elongation, but also affect other plant growth processes such as flowering, fruit formation, ripening and drop, defoliation, and other quality traits. PGRs are widely used in agriculture, horticulture, and viticulture production systems to increase resistance to biotic and abiotic stresses, improve morphological structure, increase quality and yield, and facilitate harvesting (Rademacher, 2015). PGRs have several uses in greenhouse and nursery production such as regulating shoot growth, enhancing lateral branching, enhancing plant flowering or flower removal, all intended to improve the quality and marketability of containerized crops (Latimer and Whipker, 2019).

PGRs can inhibit the biosynthesis of natural hormones or their translocation by blocking hormone receptors (Rademacher, 2015). PGRs are divided into six different categories including compounds related to auxins, GAs, growth retardants (inhibitors of GA biosynthesis), CK, ABA, and compounds affecting the ethylene status (Rademacher, 2015). The factors affecting efficacy of PGRs are application methods and timing, concentration, plant species and the plant growing conditions (Latimer and Whipker, 2019).

Application methods of PGRs for containerized ornamental plants are mainly foliar sprays, substrate drenches, plant, root or bulb dips, and pre-plant soaking of liners, bulbs or crowns. The application method varies by the absorption organs (roots, leaves, or stems etc.) of PGRs by the plant. Some PGRs are absorbed by only one organ type such as roots, leaves, or stems, whereas

some PGRs can be absorbed by several plant organs. For example, daminozide is absorbed exclusively through foliar sprays, while paclobutrazol and uniconazole are absorbed through the stems, petioles, and roots (Latimer and Whipker, 2019), and ancymidol is absorbed by the roots, stems, and leaves (Whipker et al., 2003). For foliar sprays, using the same compressed air sprayer with the same nozzle for all the plants is recommended for uniformity (Latimer and Whipker, 2019). PGRs can be more effective in controlling specific plant characteristics if applied at the correct plant growth stage. Also foliar sprays can be applied several times. Substrate drenching can be applied uniformly to each plant and used at relatively lower doses of PGRs compared with foliar sprays (Latimer and Whipker, 2019).

Growth retardants

Plant growth retardants (inhibitors of GA biosynthesis) are the most important PGR group to reduce shoot growth (Rademacher, 2015). Growth retardants are either inhibitors of the GA-biosynthetic pathway, or inhibitors that specifically block the active site of GA-metabolizing enzymes (Sponsel and Hedden, 2010; Rademacher, 2015; Rademacher, 2016). Depending on how they affect the different stages of GA biosynthesis, growth retardants are divided into three groups (Rademacher, 2016). The first group, chlormequat chloride and mepiquat chloride, inhibit the early part of GA-biosynthetic pathway in the plastid. They are quaternary ammonium compounds that inhibit cyclases in early GA biosynthesis which blocks ent-kaurene formation. The second group, ancymidol, flurprimidol and triazoles (paclobutrazol and uniconazole), inhibit the second part of the GA-biosynthetic pathway. They are compounds with a nitrogen-containing heterocycle that inhibits cytochrome P450-dependent monooxygenases which catalyze the oxidation of *ent*-kaurene to *ent*-kaurenoic acid, and other monooxygenases in the endoplasmic reticulum. The third group, daminozide and other structural mimics of 2-oxoglutaric acid inhibit

the GA biosynthesis enzyme dioxygenases in the late stage of the pathway.

Growth retardants are widely used in greenhouse and nursery production to regulate shoot growth of containerized plants, especially plant height (Hartmann et al., 2011; Megersa et al., 2018). Common growth retardants used in the industry include paclobutrazol (Bonzi, Downsize, Pac O, Piccolo, or Piccolo 10 XC), chlormequat chloride (Citadel or Cycocel), ancymidol (Abide or A-Rest), uniconazole (Concise or Sumagic), daminozide (B-Nine or Dazide), and flurprimidol (Topflor) (Latimer and Whipker, 2019; Megersa et al., 2018). They primarily affect elongation in stems, petioles and flower stalks, and improve plant market value by maintaining plant size and shape in proportion with the containers. Growth retardants can maintain the desired plant size, by limiting the plant growth rate. They must be applied prior to rapid shoot growth.

Chlormequat chloride can suppress stem elongation of kalanchoe (*Kalanchoe streptantha*) (Currey and Erwin, 2012). Chlormequat chloride also can delay chrysanthemum (*Chrysanthemum indicum*) flowering as well as increase the number of flowers (Moond and Gehlot, 2007). Ancymidol can suppress stem elongation of kalanchoe, but paclobutrazol and uniconazole are able to provide broad efficacy on the inhibition of stem elongation of 11 kalanchoe species (Currey and Erwin, 2012). Application of daminozide reduced final vegetative plant height of anise hyssop (*Agastache foeniculum*) relative to untreated plants (Latimer and Freeborn, 2011a).

Uniconazole and paclobutrazol (triazoles) are two popular growth retardants used in floriculture for controlling plant length and diameter in recent years. They are both persistent on plastic surfaces and in media substrate, and uniconazole is more potent than paclobutrazol (Latimer and Scoggins, 2018). Uniconazole dips reduced tiger lily (*Lilium lancifolium*) plant

height by 52% at 6 weeks after potting, while drench applications reduced final plant height by 37% at 9 weeks after potting (Latimer and Freeborn, 2011b). Paclobutrazol drenches as well as the dips reduced height of tiger lily plants by 23% at 6 weeks after potting. The uniconazole dip or drench at higher rates also reduced the height of Aurelian lily (*Lilium* × *aurelianense*). These PGR applications had no effect on the time of flowering, flower number or width of first open flower in either crop (Latimer and Freeborn, 2011b). Uniconazole applied by either spraying onto the foliage or substrate drenching reduced plant height of anise hyssop, and uniconazole or paclobutrazol application reduced the plant width of St. John's wort (*Hypericum calycinum*) (Latimer and Freeborn, 2011a).

Uniconazole sprays at 25 to 50 $mg \cdot L^{-1}$ upon sprouting caused a reduction of shoot growth for tree peony (*P. suffruticosa*) 'Taiyoh' and 'Hanakisoi' without affecting flowering, but paclobutrazol spray applications at 500 $mg \cdot L^{-1}$ or 1000 $mg \cdot L^{-1}$ were less effective in reducing shoot growth of 'Hanakisoi' (Hamada et al., 1990). Few growth retardants are used in growing herbaceous peonies, and most of the research has been done in China. Zhu et al. (2002) reported that 0.015% paclobutrazol or mepiquat chloride sprays in spring can control plant size, keeping plants compact without reducing flowering percentage of a few herbaceous peony cultivars such as 'Da Fu Gui'. Wang et al. (2014) studied the effect of paclobutrazol foliar sprays applied at the emergence stage on growth of five cultivars; paclobutrazol reduced plant length and diameter as well as increased plant stem diameters, with 100 $mg \cdot L^{-1}$ concentration resulting in the best integrated effect; however, no flowering data were presented. Also 100 $mg \cdot L^{-1}$ paclobutrazol spray decreased lateral bud number by 97% as well as decreased the length of lateral branches by 78% and the diameter of the plant by 42% of 'Zi Fengyu' peonies (Zhao et al., 2015). Four weekly 100 $mg \cdot L^{-1}$ paclobutrazol foliar spray applications increased photosynthetic

characteristics of herbaceous peony ‘Zi Fengyu’, resulting in darker green leaves and plants that were 15% shorter than untreated plants (Xia et al., 2018).

Plant growth retardants also enhance plant color and tolerance to stress and disease. With plant size reductions in response to plant growth retardants, plant respiration losses are reduced, resulting in increased tolerance to the adverse environmental conditions during shipping, handling, and retail marketing, elongating shelf life and making plants more marketable (Latimer and Whipker, 2019). Trees such as sugar maples (*Acer saccharum*) treated with paclobutrazol have a higher chlorophyll content and darker green leaves, along with reduced drought stress due to increased concentrations of ABA which caused stomates to close, reducing transpirational water loss (Channey, 2005). Paclobutrazol application not only reduced plant height of herbaceous peony significantly, but also significantly increased leaf greenness and photosynthetic characteristics such as photosynthetic rate and transpiration rate, as well as water use efficiency (Xia at el., 2018). Paclobutrazol can also induce water deficit tolerance in tomato (*Solanum lycopersicum*) by reducing plant height, increasing the diameter of stem and number of leaves, improving root architecture, and enhancing overall metabolism (Pal at el., 2016). Daminozide can affect flower colors of herbaceous peony by inhibiting the flavonoid biosynthetic genes, reducing the accumulation of anthocyanin, thus the plants produce less red colored flowers (Tang at el., 2018).

Our lab performed preliminary studies on the responses of different PGR application methods of containerized peonies during the 2016-17 season (Appendix 1). Uniconazole was applied by fall sprenches in November 2016 on two peony cultivars, Sarah Bernhardt and Karl Rosenfeld, under natural chilling. Neither cultivar had a significant growth response in spring 2017. Paclobutrazol, uniconazole or benzyladenine were applied as 2-minute crown soaks before

potting on two peony cultivars, Sarah Bernhardt and Inspecteur Lavergne, under natural chilling. None of the PGR soaks had a significant impact on growth or development of the plants. PGR soaks did not cause plant death, a side effect previously observed with longer soak times. GA_3 drenches and uniconazole (UNZ) drenches were applied to two peony cultivars, Sarah Bernhardt and Inspecteur Lavergne, under controlled chilling (chilled at 5°C for 6 weeks following with one month of natural rooting) and greenhouse forcing conditions. GA_3 increased the shoot number of both cultivars and the number of flowering shoots in 'Inspecteur Lavergne'. UNZ drenches resulted in moderate growth regulation. However, the percentage of plants flowering was low (less than 50%). Our preliminary studies concluded that a spring applied drench was the most effective PGR application method for containerized herbaceous peony. A 100 $mg \cdot L^{-1}$ GA_3 drench prior to emergence increased numbers of shoots effectively. UNZ provided some control of vegetative growth for both cultivars and no significant interactions with GA_3 applications. But the percentage of plants flowering was low (20% to 50%).

Overall, research on chilling effects and PGRs use on forcing herbaceous peony are still limited, especially on the interaction of chilling, GA_3 and growth retardant applications. For the nursery industry, knowing the CU requirements for sufficient chilling, PGR effects and the effects of the timing of those applications could be very beneficial for enhancing the production and flowering of containerized herbaceous peony to meet the increasing market demand. Also, from our preliminary studies, we determined which PGR application method is the best for containerized peonies. Objectives of this project are to identify the effects of PGRs, chilling, and application timing on the plant growth and flowering of containerized herbaceous peony.

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Table 1-1. Chilling regimes and calculated chilling units (CU) of *Paeonia lactiflora* cultivars.

Cultivars	Rooting	Starting date	Temp (°C)	Duration	CU*	Plant age	Results	Place	Reference
Sarah Bernhardt Festiva Maxima An unnamed red double		Oct 31	5.6	4 weeks	444	1	Break dormancy	Davis, CA	Byrne & Halevy, 1986
Sarah Bernhardt Festiva Supreme Krinkled White	16°C /0,2 or 4 weeks	Oct-Nov	5.5	4 weeks or 6 weeks	448 or 673	1	Break dormancy for all Cultivars (CVs)	St. Paul, MN	Evans et al., 1990
Scarlet O'Hara	16°C/4 weeks	Nov	5.5	6 weeks	673	1	Break dormancy	St. Paul, MN	Evans et al., 1990
Sarah Bernhardt		Oct 3	4	30 days	546	2	High flowering percentage 67% (2 plants/pot)	Kamihonjocho, Japan	Aoki, 1991
Sarah Bernhardt Karl Rosenfeld		Aug 25	2	10 weeks	1477	3	High flowering percentage (70%) with GA of both CVs	Golan Heights, Israel	Halevy et al., 2002
Sarah Bernhardt		Aug 4	2	13 weeks	1920	4	Higher flowering percentage (90%) with GA	Golan Heights, Israel	Halevy et al., 2002
Sarah Bernhardt Sarah Bernhardt		Nov Oct	2 6	60 days 70 days	1266 1070	2 2	Maximum number of shoots/plant Fewer shoots than 2°C/60 days	Bet Dagan, Israel	Kamenetsky et al., 2003
Karl Rosenfeld Monsieur Jules Elie Sarah Bernhardt Coral Sunset	27-12°C /10 days	Early April (autumn)	1	10 weeks	1578	3	Sufficient for growth and flowering for all CVs	Manawatu, New Zealand	Hall et al., 2007
Da Fu Gui	6-8°C/2 weeks	Oct	0-4	4 weeks	≈784	3	Sufficient for plant growth and flowering with GA	Beijing, China	Cheng et al., 2009
Taebaek		Sep	Natural	2 months	1222	2	Subsequent growth and normal flowering	Suwon, Korea	Yeo et al., 2012
Taebaek, Mlsurae Taebaek, Mlsurae		Sep Sep	0 5	6 weeks 9 weeks	1008 1058	1 1	Break dormancy and induce flowering for both CVs Mlsurae require more chilling than Taebaek	Suwon, Korea	Rhie et al., 2012
Da Fu Gui Dong Fang Hong QingWen		Oct 30	2±1	7 weeks	1034	4	Sufficient chilling for all CVs	Beijing, China	Zhou, 2012
Taebaek	10°C/2 weeks	Nov 12	0	6 weeks	1141	2	More than 80% plants flowering	Suwon, Korea	Park at el., 2015
Hang Baishao		Nov 29	0-4	4 weeks	590	1	Earlier and more flowering with humic acid	Hangzhou, China	Zhang et al., 2019a

*CU (Chilling units) as calculated by applying Fulton's Model (Fulton et al., 2001) to the chilling regime described by the references cited.

Chapter 2

The Manipulation of Chilling Duration and Growth Regulators to Produce Single Season Containerized Herbaceous Peonies

Abstract

Herbaceous peonies (*Paeonia lactiflora* Pall.) are common perennials used both in gardens and the landscape as well as for cut flowers. Peonies require a chilling period to break dormancy but not for flower bud differentiation. Using two peony cultivars, Sarah Bernhardt and Inspecteur Lavergne, small (3–5 eye) crowns from Holland were potted in 3.8-L pots in mid-November of 2017 and 2018. Our objective was to determine if we could manipulate chilling time, along with application of gibberellic acid (GA_3) and growth retardants, to produce marketable containerized peonies from a small crown in a single season (November to May).

We evaluated chilling requirements, GA_3 and a growth retardant (uniconazole; UNZ) under controlled chilling and greenhouse forcing conditions. All potted plants were held outdoors at Battlefield Farms (Rapidan, VA, 38˚ N) for 4 weeks [in 2017, 400 chilling units (CU)] or in a 10°C cooler for 5.5 weeks (in 2018, 400 CU) to root, then placed in a 5°C cooler for 3, 4 or 5 weeks (total 752, 869 or 986 CU). GA_3 was applied as 0 or 100 $mg \cdot L^{-1}$ drench at 250 ml/pot after the plants were moved into the Virginia Tech greenhouse (Blacksburg, VA, 37˚ N) for forcing. Uniconazole drenches were applied to each cultivar under each chilling treatment at 355 ml/pot at 0, 15, or 20 $mg \cdot L^{-1}$ at 7 days after the GA_3 drench applications. Three weeks chilling at 5°C (752 CU total) provided sufficient chilling for ‘Sarah Bernhardt’ and ‘Inspecteur Lavergne’. Application of GA_3 reduced production time and resulted in a greater number of shoots, and increased the number of flowering shoots. Application of 15 $mg \cdot L^{-1}$ UNZ as a substrate drench

prior to spring emergence reduced plant width moderately resulting in improved compactness of both cultivars.

Introduction

Herbaceous peony (*Paeonia lactiflora* Pall.) is a traditional flowering landscape and medicinal perennial plant in many countries of temperate regions that also is of significant value as a commercial cut flower (Kamenetsky and Dole, 2012; Rogers, 1995). There are 6,854 registered peony cultivars according to the American Peony Society (APS), which serves as the International Cultivar Registration Authority for peonies, and 4,080 of these cultivars are *P. lactiflora* (APS, 2020). Containerized herbaceous peonies are popular in the potted herbaceous perennial plant sector, with more than half million plants, valued at almost $6 million USD, sold in 2018 (USDA, 2019).

Most herbaceous peonies are grown from a perennial underground crown, which is an underground shoot that serves as an energy storage center for plant renewal (Din et al., 2015). A large number of buds develop on the surface of the crown and grow into monocarpic shoots with leaves and flowers after emergence in early spring. In northern hemisphere temperate regions, peonies flower once a year from May to July depending on location and cultivar, and flowers normally last for one to two weeks. The leaves remain green for a few months after flowering until the leaves senesce, at which time the peony plant enters dormancy for 3 to 4 months (Barzilay et al., 2002). The renewal buds initiate after flower senescence and remain vegetative during the summer. After senescence of the above ground shoots in the fall, the apical meristem of renewal buds reaches the generative stage, and begins floral initiation and differentiation until early winter. Floral initiation and differentiation of peony do not require a chilling period

(Barzilay et al., 2002). However, peonies require a period of cold accumulation to break dormancy and promote plant growth and flowering (Barzilay et al., 2002). In the United States, herbaceous peonies can be grown across USDA hardiness zones 3 to 8 (Michener and Adelman, 2017; Rogers, 1995). Several studies have determined chilling regimes for commercial *P. lactiflora* cultivars all over the world (Aoki, 1991; Byrne and Halevy, 1986; Cheng et al. 2009; Evans et al., 1990; Kamenetsky et al., 2003; Zhang et al., 2019; Zhou, 2012). Overall, the chilling requirement of herbaceous peony is satisfied by a range of low temperatures accumulated over time. Increasing chilling time and decreasing temperature can advance the time of emergence and flowering (Byrne and Halevy, 1986; Evans et al., 1990; Kamenetsky et al., 2003). But excessive chilling can decrease flowering percentage and even prevent flowering (Aoki, 1991; Byrne and Halevy, 1986; Halevy et al., 2002).

Fulton et al. (2001) developed a chilling model to calculate the chilling units (CU) accumulated by peony in order to quantify the amount of chilling required to break dormancy using three cultivars and three temperature regimes. Chilling units were calculated by the linear equation ($y = -0.0605x + 1$, $R^2 = 0.9943$), where y is the number of CU and x is the temperature in °C. The optimal accumulated CU varies among cultivars (Fulton et al., 2001; Rhie et al., 2012; Yeo et al., 2012). The chilling requirements of *P. lactiflora* cultivars from previous studies are listed in **Table 1-1**, in which we calculated the chilling units by applying Fulton's Model to the chilling regime described by the authors of the individual studies.

Gibberellins (GAs) are known as regulators of many developmental phases of higher plants, promoting cell elongation and plant growth, inducing hydrolytic enzymes during seed germination, inducing bolting in long-day plants, promoting flowering and fruit set and development (Rademacher, 2015; 2016). GA_3 is the most widely used GA in horticulture,

viticulture and agriculture (Rademacher, 2015). GA_3 can be used to enhance flowering of perennial ornamental crops (Moond and Gehlot, 2006, 2007; Singh et al., 2017). GA_3 was also used on herbaceous peonies to partially replace the chilling requirement when plants received insufficient chilling, and to enhance plant growth, development and flowering (Cheng et al., 2009; Evans et al., 1990; Halevy et al., 2002; Yeo et al., 2012).

Plant growth retardants (inhibitors of GA biosynthesis), which reduce shoot growth, are the most widely used plant growth regulator (PGR) group in commercial plant production (Rademacher, 2015). Growth retardants are widely used in greenhouse or nursery production to regulate shoot growth of containerized plants, especially to manage plant height (Hartmann et al., 2011; Latimer and Freeborn, 2011a, b; Megersa et al., 2018). Uniconazole was used to control shoot growth of tree peony (*P. suffruticosa*) (Hamada et al., 1990).

Overall, herbaceous peonies have been one of the more difficult crops for plant growth control in containers in the nursery production (I. Brantingham, personal communication, Riverbend Nursery, Riner, VA). The standard for marketable peonies varies by country. In China and Japan, the standard for commercial production is 80% of plants flowering (Aoki, 1991; Zhou, 2012). In the United States, some retailers require at least three flowering shoots per pot for commercial growers (P. Wierstra, personal communication, Oregon Perennial Company, Woodburn, OR). Some commercial growers are using larger crowns (6–8 buds) and larger containers (2 gallon) in order to produce first year market-ready plants (I. Brantingham, personal communication, Riverbend Nursery, Riner, VA). Other growers like Battlefield Farms (Rapidan, VA) are using small crowns (3–5 buds) and small containers (1 gallon), but they grow plants for 18 to 24 months in order to reach the same required standard (J. Zeijlmaker, personal communication). Most of the herbaceous peony studies described above were using 3 or 4 year-

old plants. In this project, our objective was to determine if we could manipulate chilling time, along with the application of GA_3 and a growth retardant (UNZ), to produce marketable containerized peonies with three or more flowering shoots per pot from a small (3-5 buds) crown in a single season (November to May).

Materials and Methods

Plant materials

Three to five bud peony crowns of 'Sarah Bernhardt' and 'Inspecteur Lavergne' were imported to Battlefield Farms, Rapidan, VA (lat. 38°N, long. 78°W) from a Dutch commercial source in fall of 2017 and 2018. 'Sarah Bernhardt' is a double, late flowering type with very large dark rose pink flowers, medium height, floriferous, strong stems, and good foliage (**Fig. 2-1**). 'Inspecteur Lavergne' is a double, early flowering type, with globular crimson flower with frilled petals in the center, and long straight stems (**Fig. 2-1**). All crowns were potted in 3.8-L pots using Battlefield Farms' peony substrate (60% hydro fiber, 40% peat) with 5 cm of media over the crown in mid-November 2017 and 2018. Controlled release fertilizer (17N–2.2P–9.1K, Osmocote Pro 12–14 month, ICL Specialty Fertilizers, Summerville, SC) was incorporated at 5.3 kg/m^3 at time of potting. All crowns were sorted for uniformity of number of eyes and size of crown and roots prior to potting. A fungicide drench (Subdue Maxx, Syngenta, Greensboro, NC) was applied at 0.08 $mL \cdot L^{-1}$ at 237 mL/pot two weeks after potting to protect plants from fungal infection. Insecticide and fungicide drenches were applied in spring as needed.

Rooting and chilling treatments

All potted plants were held under natural condition at Battlefield Farms for 4 weeks (in

2017, 400 CU received according to Fulton Model) or in a 10°C cooler for 5.5 weeks (in 2018, in order to match the 400 CU with the previous season) without light to allow rooting, irrigating as necessary. Then all plants were placed on plant racks in the Battlefield Farms cooler at 5°C for 3, 4, or 5 weeks (total 752, 869, 986 CU, respectively) without light.

Greenhouse forcing

After chilling, plants were transported to the Virginia Tech double layer polyethylene greenhouse (Blacksburg, VA, lat. 37° N, long. 80° W) in January and grown under natural day length (9 to 13 hours' daylight). In 2018, mean greenhouse temperatures were 18.4°C day / 10.9°C night (light data unavailable). In 2019, mean greenhouse temperatures were 18.9°C day / 15.9°C night with an average daily light integral (DLI) of 10.6 $mol \cdot m^{-2} \cdot d^{-1}$. The air temperature and DLI were measured hourly and using a thermo data logger (WatchDog Model 1000/2000, Spectrum Technologies, Inc. Plainfield, IL). Irrigation was applied as needed when media was dry on top of the pot.

PGR drenches

GA_3 (Florgib, Fine Americas, Walnut Creek, CA) drenches were applied to each cultivar under each chilling treatment at 250 mL/pot at 0 or 100 $mg \cdot L^{-1}$ (0 or 25 mg a.i./pot) the day after the plants were moved into the greenhouse (day 0). We used this regime for both cultivars based on the study by Halevy et al. (2002), which reported a drench with 250 mL of 100 $mg \cdot L^{-1}$. GA_3 was optimal for 'Sarah Bernhardt' and two other peony cultivars. No GA_3 research results have been reported on 'Inspecteur Lavergne'. Uniconazole (UNZ; Concise, Fine Americas) drenches were applied to each cultivar under each chilling and GA_3 treatment at 355 mL/pot at 0, 15, or 20 $mg \cdot L^{-1}$ (0, 5.3, or 7.1 mg a.i./pot) at 7 days after the GA_3 drench applications.

Measurements and data analysis

Days to emergence, days to bud (cracked color) and days to first open flower were recorded by checking plants twice weekly in the 2017–18 season and daily in the 2018–19 season. Plant height, flower height and plant width, as well as number of shoots, number of vegetative shoots, number of shoots with blasted buds, and number of flowering shoots were recorded at finish (when >50% of plants in a treatment group had flowered). According to Kamenetsky et al. (2003), a shoot with a blasted bud is a shoot with a flower bud that aborted/blasted in the later stages of bud development and a vegetative shoot is a shoot without a flower bud or with flower bud that aborted in the early stages of development, i.e. bud smaller than 2 mm (**Fig. 2-2**). Flowering percentage was recorded as the percentage of plants in a treatment group flowering at the end of the study when all plants have finished flowering (for each individual plant of a treatment group, 1 = flowering and 0 = no flowering; the average flowering rate was converted to a percentage).

The experiments were three-way randomized plot designs (3 chilling regimes × 2 GA_3 applications × 3 UNZ applications) with each cultivar set up as a separate experiment in each growing season. Two plots were set up, with each whole plot consisting of five single plant replicates for a total of 10 single plant replicates in both plots with treatments completely randomized, 180 plants for each cultivar. For all experimental datasets, data were analyzed by analysis of variance with mean separation by Student's *t* test at $P \leq 0.05$ using JMP® Pro 15, © SAS Institute Inc. (Cary, NC).

Results

'Sarah Bernhardt'

We did not have sufficient data to analyze treatment effects on days to bud, days to flower and flower height because of lack of flowering (40–55% in the 2017–18 season, 20–57% in the 2018–19 season).

2017–18 Season. Although chilling time showed significant effects on days to emergence, the difference was within our 3 to 4-day sampling frequency (**Table 2-1**). Application of 100 $mg \cdot L^{-1}$ GA_3 significantly reduced the days to emergence by 5 days, however UNZ had no effect. There were no treatment effects on plant height, but GA_3 increased plant width while chilling time had no effect. GA_3 and UNZ applications had significant interactions on plant width; 15 or 20 $mg \cdot L^{-1}$ UNZ application reduced plant width by 15 to 19% with 0 $mg \cdot L^{-1}$ GA_3 plants, but only 20 $mg \cdot L^{-1}$ UNZ application reduced plant width by 7% of plants treated with 100 $mg \cdot L^{-1}$ GA_3. Chilling time and UNZ had no effect on number of shoots but GA_3 significantly increased the number of shoots from 3.4 shoots/pot in plants treated with 0 $mg \cdot L^{-1}$ to 5.0 shoots/pot in plants treated with 100 $mg \cdot L^{-1}$ GA_3. Chilling time and UNZ applications had no effect on the number of vegetative shoots, while GA_3 application increased the number of vegetative shoots by one shoot/plant. Increased chilling time reduced flower bud blasting while GA_3 application increased the number of shoots with blasted buds by 0.5 shoot/plant. UNZ application had no effect. There were no treatment effects on the number of flowering shoots or the percentage of plants flowering.

2018–19 Season. Chilling time and GA_3 application had significant effects on days to emergence; plants chilled for 3 weeks emerged most quickly, and plants treated with 100 $mg \cdot L^{-1}$

GA_3 emerged 4.5 days earlier than untreated plants; UNZ had no effect on days to emergence (**Table 2-2**). There were interactions between chilling time and GA_3 application on plant height, where application of 100 $mg \cdot L^{-1}$ GA_3 increased plant height by 20% on plants chilled for 3 weeks (**Fig. 2-3**), by 15% on plants chilled for 5 weeks but had no effect on plants chilled for 4 weeks (**Table 2-2**). UNZ had no effect on plant height. There was a similar interaction between chilling time and GA_3 application on plant width where application of 100 $mg \cdot L^{-1}$ GA_3 increased plant width by 13% but only on plants chilled for 3 weeks. Application of 15 or 20 $mg \cdot L^{-1}$ UNZ reduced plant width by 10%. While chilling duration had no effect, GA_3 application increased the number of shoots from 3.5 shoots/plant with untreated plants to 4.7 shoots/plant in plants treated with 100 $mg \cdot L^{-1}$ GA_3. UNZ had no effect on number of shoots. There were no treatment effects on the number of vegetative shoots. Longer chilling times increased the number of shoots with blasted buds. There were interactions between chilling time and GA_3 application on the number of shoots with blasted buds, where application of 100 $mg \cdot L^{-1}$ GA_3 increased the number of shoots with blasted buds (by 0.8 shoots/pot) on plants chilled for 4 weeks, while plants chilled for 3 or 5 weeks were not affected. UNZ had no effect on bud blasting. While chilling duration had no effect, GA_3 application increased the number of flowering shoots from 0.2 shoots/plant with untreated plants to 0.9 shoots/plant in plants treated with 100 $mg \cdot L^{-1}$ GA_3, and UNZ had no effect. While chilling duration had no effect, GA_3 application increased the percentage of plants flowering from 20% with untreated plants to 57% in plants treated with 100 $mg \cdot L^{-1}$ GA_3. UNZ had no effect on flowering.

'Inspecteur Lavergne'

2017–18 Season. Although statistically significant, chilling and GA_3 application effects on days to emergence and days to bud were within our 3 to 4-day sampling frequency, and UNZ

applications had no effect (**Table 2-3**). The 5 week chilling time reduced days to flower. There was a significant interaction between chilling time and GA_3 application on days to flower where applications of 100 $mg \cdot L^{-1}$ GA_3 reduced days to flower only for plants chilled for 3 weeks. UNZ had no effect on days to flower. Plant height at time of flowering was not affected by chilling time or GA_3 application, but there was an interaction between the GA_3 and UNZ applications where 15 $mg \cdot L^{-1}$ UNZ treated plants were 13% shorter than control plants only in the absence of GA_3. Flower height increased 10% with increased chilling, but plants treated with 100 $mg \cdot L^{-1}$ GA_3 had flower stalks 7% shorter than those of the control plants; UNZ had no effect. Plant width was not affected by chilling time or GA_3 application, but there was an interaction between the GA_3 and UNZ applications where 15 or 20 $mg \cdot L^{-1}$ UNZ treated plants were 15 to 18% more compact than control plants only in the absence of GA_3. Chilling for 5 weeks increased the number of shoots over plants chilled for 4 weeks. There was a significant interaction between effects of chilling time and GA_3 application on number of shoots, where GA_3 at 100 $mg \cdot L^{-1}$ increased the number of shoots from 3.3 shoots/pot with untreated plants to 6.2 shoots/pot only on plants with 5 weeks chilling. UNZ had no effect on number of shoots. The number of vegetative shoots was greater for plants chilled for 5 weeks than for those chilled 3 or 4 weeks. There were interactions between the effects of chilling and GA_3 application on the number of vegetative shoots, where 100 $mg \cdot L^{-1}$ GA_3 significantly increased the number vegetative shoots only of plants chilled for 5 weeks (by 2.2 shoots/plant). UNZ had no effect on the number of vegetative shoots. There were also interactions between the effects of chilling and GA_3 application, and between chilling and UNZ applications on the number of shoots with blasted buds. GA_3 application significantly increased the number shoots with blasted buds of plants chilled for 4 weeks (by 0.4 shoots/plant) or 5 weeks (by 0.3 shoots /plant) but not for plants

chilled for 3 weeks. UNZ applications significantly reduced the number of shoots with blasted buds (by 0.7 shoots/plant) on plants chilled for 3 weeks, but had no effect on plants subjected to longer chilling times. The number of flowering shoots declined from 2.1 shoots/pot to 1.6 shoots/pot with increasing chilling time, and plants treated with 100 $mg \cdot L^{-1}$ GA_3 had more flowering shoots per pot than untreated plants, but UNZ application had no effect. The percentage of plants flowering declined from 98% to 80% with increasing chilling time, but GA_3 and UNZ applications had no effect on the percentage of plants flowering.

2018–19 Season. Chilling time and UNZ application had no effect on days to emergence but plants treated with 100 $mg \cdot L^{-1}$ GA_3 emerged 3.7 days earlier than untreated plants (**Table 2-4**). There were interactions between chilling time and GA_3 application on days to bud (cracked color), where application of 100 $mg \cdot L^{-1}$ GA_3 reduced days to bud by 6.7 days on plants chilled for 3 weeks, but not for those chilled for 4 or 5 weeks. UNZ had no effect on days to bud. Chilling time and UNZ application had no effect on days to flower while plants treated with 100 $mg \cdot L^{-1}$ GA_3 flowered 3.2 days earlier than untreated plants. There was a significant three-way interaction between chilling time, GA_3 and UNZ application on plant height (**Table 2-4**, **2-5**), wherein the application of 15 $mg \cdot L^{-1}$ UNZ reduced plant height by 10.8 cm only on plants chilled for 3 weeks with no GA_3 treatment (**Table 2-5**, **Fig. 2-3**). Longer chilling times resulted in taller flowers, while GA_3 and UNZ had no effect (**Table 2-4**). Chilling time and GA_3 application had significant effects on plant width; plants chilled for 5 weeks were widest, and plants treated with 100 $mg \cdot L^{-1}$ GA_3 were 1.7 cm wider than untreated plants. There were interactions between the effects of chilling time and UNZ on plant width wherein the application of 15 or 20 $mg \cdot L^{-1}$ UNZ reduced plant width significantly in plants from each chilling duration. However, the magnitude of the difference on plant width varied from 3.8 cm with 4 weeks of

chilling to 10.4 cm with 5 weeks of chilling. There were also interactions between the effects of GA_3 and UNZ applications on plant width wherein the application of 15 or 20 $mg \cdot L^{-1}$ UNZ reduced plant width significantly, but again, the magnitude of the difference in plant width varied from 3.1 cm with 100 $mg \cdot L^{-1}$ GA_3 treatment to 10.1 cm with no GA_3. There were interactions between chilling time and GA_3 applications on the number of shoots. The application of 100 $mg \cdot L^{-1}$ GA_3 significantly increased the number of shoots on plants from all chilling durations but the increases varied: 1.8 shoots on plants chilled for 3 weeks, 0.9 shoots for 4 weeks and 2.5 shoots for 5 weeks. UNZ had no effect on the number of shoots. There were interactions between chilling time and GA_3 application on the number of vegetative shoots, where application of 100 $mg \cdot L^{-1}$ GA_3 increased 1.7 vegetative shoots/plant on plants chilled for 5 weeks, but not those chilled for 3 or 4 weeks. UNZ had no effect on the number of vegetative shoots. Although chilling time had no effect, application of 100 $mg \cdot L^{-1}$ GA_3 significantly increased the number of shoots with blasted buds, and 15 $mg \cdot L^{-1}$ UNZ significantly reduced the number of shoots with blasted buds. Chilling time and UNZ application had no effect on number of flowering shoots but plants treated with 100 $mg \cdot L^{-1}$ GA_3 had 0.5 more flowering shoots per pot than untreated plants. There was a significant three-way interaction between chilling time, GA_3 and UNZ application on the percentage of plants flowering, wherein the application of 20 $mg \cdot L^{-1}$ UNZ increased flowering percentage by 100% only on plants chilled for 4 weeks with no GA_3 treatment (**Table 2-5**). The percentage of plants flowering varied from 50% to 100% across treatment combinations.

All plants treated with UNZ appeared to have greener leaves than the non-treated ones (**Fig. 2-3**).

Discussion

Our research objective was to determine if we could manipulate chilling time, along with application of GA_3 and a growth retardant (UNZ), to produce marketable containerized peonies from a small (3–5 buds) crown in a single season (November to May). Not unexpectedly, the results varied with the cultivars, but we did not produce marketable plants, plants having three or more shoots with flower buds, with any of our treatment regimes.

Chilling duration affected both cultivars. According to Fulton's chilling units (CU) model for herbaceous peonies (Fulton et al., 2001), plants received around 400 CU during the rooting period. After rooting, 3, 4 or 5 weeks of controlled chilling at 5°C (total 752, 869 or 986 CU) was sufficient to satisfy the CU requirement of both cultivars, Sarah Bernhardt and Inspecteur Lavergne. Our results were within the chilling requirements range reported by other researchers for herbaceous peonies (**Table 1-1**). Byrne and Halevy (1986) reported that a minimum of 4 weeks at 5.6°C (444 CU) can break dormancy of one-year-old plants of 'Sarah Bernhardt' and 'Festiva Maxima', but increasing the chilling time to 6 weeks (666 CU) or reducing the temperature to 1°C (631 CU) increased the number of shoots. Increasing the chilling time did not significantly affect the number of shoots in our studies. Evans et al. (1990) used a production regime very similar to ours, three to five bud crowns were planted and immediately subjected to various chilling durations but the authors reported no increase in the number of shoots of three peony cultivars with 4 to 6 weeks chilling time, and only 'Krinkled White' had more than three shoots/plant. Plants in our studies had a greater number of shoots (3 to 6 per pot) with both cultivars. Aoki (1991) reported chilling at 4°C for 30 days (546 CU) resulted in the highest flowering percentage (67%) for one-year-old plants of 'Sarah Bernhardt' grown for cut flowers, however the number of shoots was not reported. Kamenetsky et al. (2003) reported dormancy

release of two-year-old plants of 'Sarah Bernhardt' under chilling regimes of 2°C for 60 days (1266 CU) or 6°C for 70 days (1070 CU), with the 1070 CU regime resulting in plants with greater numbers of shoots per plant (7–8 shoots/plant) and flowers under forcing conditions. In our two-year study, plants of both 'Sarah Bernhardt' and 'Inspecteur Lavergne', after rooting (400 CU) with 3 weeks controlled chilling at 5°C (total 752 CU), had a sufficient number of shoots and a high enough percentage of plants flowering to indicate that 752 CU is sufficient to break dormancy of the two cultivars trialed.

In our studies, GA_3 application had positive effects on production timing and flowering of 'Sarah Bernhardt' and 'Inspecteur Lavergne' peonies in our studies. GA_3 consistently reduced days to emergence of both cultivars and tended to reduce days to bud (cracked color) and days to flower, which tended to hasten bud and flower development of 'Inspecteur Lavergne'. Cheng et al. (2009) reported a drench with 250 mL of 200 $mg \cdot L^{-1}$ GA_3 (50 mg a.i./pot) as optimal for forcing 'Da Fu Gui' peony chilled at 0 to 4°C for 4 weeks after 2 weeks rooting at 6 to 8°C (approximately 784 CU), with early emergence and a shorter flowering time and an increased flowering percentage. Yeo et al. (2012) reported a soil drench of 300 mL of 100 $mg \cdot L^{-1}$ GA_3 (30 mg a.i./pot) reduced the days to emergence and flower while increasing the percentage of plants flowering with 'Taebaek' peony subjected to insufficient natural chilling accumulation (429–876 CU). GA_3 application at 25 mg a.i./pot in our studies, increased the number of shoots by 1.0 to 2.5 shoots/pot and in some cases the number of flowering shoots as well as the percentage of plants flowering in both cultivars. A substrate drench of GA_3 at 118 mg a.i./pot released freshly potted 'Scarlet O'Hara' peony plants from dormancy without chilling, producing 4.1±1.7 shoots per pot (control plants did not grow), but all flower buds aborted (Evans et al., 1990). In our studies, GA_3 application also tended to increase the number of vegetative shoots or shoots with

blasted buds for both cultivars, which may indicate insufficient development of the additional renewal buds stimulated to grow by the GA_3 applications. However, the number of flowering shoots was also higher with GA_3 application for both cultivars of our studies, except for 'Sarah Bernhardt' in the 2017–18 season. Halevy et al. (2002) reported that a drench with 250 mL of 100 mg·L^{-1} GA_3 (25 mg a.i./pot) was optimal for 4-year-old peony 'Sarah Bernhardt' chilled at 2°C for 13 weeks (1920 CU), in terms of increasing the percentage of plants flowering (70–90%) and the number of flowers per plant (4.6 ± 0.9 flowers). The marketing standard for herbaceous peonies sold by Battlefield Farms is three or more flowering shoots per plant, which normally are two-year-old plants (J. Zeijlmaker, personal communication). Our GA_3 treated first year plants had 4.4 to 6.2 shoots per plant which would be marketable if we could reduce flower bud abortion and/or blasting, subsequently reducing the production time by one year.

Flower bud abortion/blasting is a major issue in forcing herbaceous peonies, and may be attributed to various abiotic or endogenous/exogenous factors (Evans et al., 1990; Kamenetsky et al., 2003). Evans et al. (1990) reported all flower buds aborting on non-chilled 'Scarlet O'Hara' peonies treated with GA_3 may be attributed to the high rate of GA_3 (118 mg a.i./pot) applied. Kamenetsky et al. (2003) reported high night temperatures (22°C) during the early forcing period caused high numbers of young flower buds abort during early development in 'Sarah Bernhardt' plants. In contrast to these reports, our GA_3 rate was much lower (25 mg a.i./pot and our night temperatures never exceeded 18°C (data not reported). Furthermore, Park et al. (2015) reported that delaying the start of chilling until November, following a pre-chilling treatment of 2 weeks at 10°C, promoted flowering of 'Taebaek' peony and reduced flower bud abortion. This pre-chilling treatment is comparable to our rooting period, which in our 2018-2019 studies pre-chilling 5.5 weeks at 10°C, resulted in the flowering of 57% of the 'Sarah Bernhardt' plants

treated with GA_3, a very high percentage for first year plants. In contrast, plants of 'Inspecteur Lavergne' exhibited 50% to 100% flowering depending on the treatment in 2018-2019 season. Therefore, it is speculated that a pre-chilling or acclimation period prior to chilling or GA_3 application is required to alleviate flower bud blasting.

The flowering percentages of herbaceous peonies under forcing conditions are highly cultivar dependent. Cheng et al. (2009) studied 20 Chinese herbaceous peony cultivars for forcing for both landscape and cut flower production and found that only five cultivars were suitable for forcing with a high flowering percentage (>75%), while the remaining cultivars had less than 50% of plants flowering. Overall, 'Inspecteur Lavergne' had a higher percentage of plants flowering (50% to100%) than 'Sarah Bernhardt' (20% to 57%) in our studies. 'Inspecteur Lavergne' frequently reached 80% of plants flowering, especially with GA_3 application, which is the standard for commercial production in China and Japan (Aoki, 1991; Zhou, 2012), but 'Sarah Bernhardt' did not. We expected our first year 'Sarah Bernhardt' plants to be a less floriferous cultivar in container production, with reports of less than 50% of 2 to 4-year-old plants flowering after sufficient chilling (Halevy et al., 2002; Kamenetsky et al., 2003), and less than 70% plants of 3-year-old plants flowering with GA_3 application (Halevy et al., 2002). Hall et al. (2007) reported less than 16% of 3-year-old 'Sarah Bernhardt' plants flowering even under optimal forcing conditions after sufficient chilling. All of our studies used one-year-old plants for 'Sarah Bernhardt', and percentage of plants flowering was determined to be normal or even higher than expected based on previous reports.

UNZ applied as drenches at 15 or 20 $mg \cdot L^{-1}$ (5.3 or 7.1 mg a.i./pot) resulted in statistically significant reductions in plant width of both cultivars in our studies. However, these small differences were not commercially significant in production and UNZ had less effect in

controlling plant height. UNZ did not reduce the number of shoots or the percentage of plants flowering, but in some cases reduced the number of shoots with blasted buds in 'Inspecteur Lavergne' plants. Foliar sprays of 25 or 50 $mg \cdot L^{-1}$ UNZ applied twice at a 5-day interval upon bud break significantly reduced shoot length of tree peonies while foliar sprays of 500 or 1000 $mg \cdot L^{-1}$ paclobutrazol three times at a 5-day interval upon bud break had less effect without affecting flowering (100% flowering with all treatments) (Hamada et al., 1990). Foliar sprays of 100 $mg \cdot L^{-1}$ paclobutrazol applied at the time of emergence in spring controlled the plant size of five Chinese herbaceous peony cultivars, resulting in plants that were shorter and less wide with greater stem diameters than untreated plants; however, no flowering data were presented (Wang et al., 2014). We have no explanation for the lack of plant growth response to our UNZ applications.

Both 'Sarah Bernhardt' and 'Inspecteur Lavergne' plants with UNZ drenches visually appeared to have greener leaves than the non-treated plants, similar to the increase of leaf greenness of 'Zi Fengyu' peony plants treated with 100 $mg \cdot L^{-1}$ paclobutrazol sprays applied once a week for 4 weeks from April to May (Xia at el., 2018).

Conclusion

According to our data in two growing seasons, after one-month outdoor natural rooting (or the equivalent condition with 400 CU), 3 weeks chilling at 5°C (752 CU total) is a sufficient chilling regime for 'Sarah Bernhardt' and 'Inspecteur Lavergne' peony plants. Application of GA_3 hastens production time by a few days and results in a greater number of shoots, and may increase the number of flowering shoots and the percentage of plants flowering. Application of 15 $mg \cdot L^{-1}$ UNZ as a substrate drench prior to spring emergence is only slightly effective in

improving compactness of both cultivars. It is speculated that if we can mitigate flower bud abortion/blasting, and turn more vegetative shoots into flowering shoots, then marketable containerized peonies could be produced from a small (3 to 5 buds) crown in a single season (November to May).

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Table 2-1. Effects of chilling duration (pre-chilled at 5°C for 3, 4 or 5 weeks), GA_3 and/or uniconazole (UNZ) drench application on growth and flowering of *Paeonia lactiflora* 'Sarah Bernhardt' (SB) in the 2017–18 season.

SB (2017–18)		Days to emergence[z]	Plant height (cm)[y]	Plant width (cm)		Number of shoots	Number of vegetative shoots	Number of shoots with blasted buds	Number of flowering shoots	Flowering %
Chilling time	3 weeks	15.0 a[x]	44.4	44.2		4.4	2.6	1.1 a	0.8	55
	4 weeks	13.5 ab	45.2	42.7		4.0	2.5	0.8 ab	0.7	52
	5 weeks	11.3 b	45.4	43.2		4.2	3.1	0.6 b	0.6	40
	P-value	0.0205	0.7796	0.1493		0.1997	0.1538	0.0075	0.3833	0.2312
GA ($mg \cdot L^{-1}$)	0	15.9	45.7	42.3		3.4	2.2	0.6	0.6	49
	100	10.6	44.2	44.4		5	3.2	1.1	0.7	49
	P-value	<0.0001	0.2229	0.0013		<0.0001	0.0003	<0.0001	0.3089	1.0000
				GA ($mg \cdot L^{-1}$)						
				0	100					
UNZ ($mg \cdot L^{-1}$)	0	11.9	46.0	47.7 a	45.8 a	4.3	2.9	1.9	0.5	42
	15	14.4	44.2	40.7 b	44.6 ab	4.1	2.5	1.7	0.8	55
	20	13.5	44.8	38.4 b	42.8 b	4.2	2.8	2.0	0.7	50
	P-value	0.1602	0.4544	<0.0001	0.0397	0.5878	0.3911	0.1778	0.1576	0.3417
Chilling time		0.0205	0.7796	0.1493		0.1997	0.1538	0.0075	0.3833	0.2312
GA		<0.0001	0.2229	0.0013		<0.0001	0.0003	<0.0001	0.3089	1.0000
Chilling Time x GA		0.5871	0.2546	0.4978		0.7363	0.3122	0.7103	0.521	0.8201
UNZ		0.1602	0.4544	<0.0001		0.5878	0.3911	0.1778	0.1576	0.3417
Chilling Time x UNZ		0.8578	0.1295	0.0845		0.7630	0.2402	0.1701	0.3431	0.1828
GA x UNZ		0.8562	0.0550	<0.0001		0.2984	0.8459	0.0649	0.9914	0.9360
Chilling x GA x UNZ		0.2194	0.6773	0.5839		0.6685	0.7548	0.3012	0.2223	0.3314

[z] Days to emergence, measured from the beginning of greenhouse forcing.

[y] Plant height, tallest height of vegetative growth.

[x] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 10)

Table 2-2. Effects of chilling duration (pre-chilled at 5°C for 3, 4 or 5 weeks), GA_3 and/or uniconazole (UNZ) drench application on growth and flowering of *Paeonia lactiflora* 'Sarah Bernhardt' (SB) in the 2018–19 season.

SB (2018–19)		Days to emergence[z]	Plant height (cm)[y]			Plant width (cm)			Number of shoots	Number of vegetative shoots	Number of shoots with blasted buds			Number of flowering shoots	Flowering %
Chilling Time	3 weeks	5.0 c[x]	37.2 a			40.3			3.9	2.6	0.5 b			0.7	45
	4 weeks	12.9 a	29.8 b			37.9			4.2	2.6	1.1 a			0.4	29
	5 weeks	8.1 b	35.8 a			40.3			4.2	2.3	1.3 a			0.6	42
	P-value	<0.0001	<0.0001			0.0503			0.3288	0.6235	<0.0001			0.1379	0.1246
			Chilling Time			Chilling Time					Chilling Time				
			3 weeks	4 weeks	5 weeks	3 weeks	4 weeks	5 weeks			3 weeks	4 weeks	5 weeks		
GA ($mg \cdot L^{-1}$)	0	10.9	33.6	29.9	33.1	37.8	38.1	40.6	3.5	2.4	0.4	0.7	1.4	0.2	20
	100	6.4	40.7	29.7	38.4	42.9	37.7	39.9	4.7	2.6	0.6	1.5	1.1	0.9	57
	P-value	<0.0001	0.0011	0.9088	0.0130	0.0012	0.8041	0.6679	<0.0001	0.3229	0.3616	0.0009	0.2973	<0.0001	<0.0001
UNZ ($mg \cdot L^{-1}$)	0	7.8	35.6			42.4 a			4.2	2.6	1.0			0.5	35
	15	8.9	33.7			37.8 b			4.2	2.6	0.9			0.6	39
	20	9.2	33.4			38.3 b			3.9	2.3	0.9			0.7	42
	P-value	0.492	0.3036			<0.0001			0.5098	0.6235	0.7112			0.7311	0.7111
Chilling time		<0.0001	<0.0001			0.0503			0.3288	0.6235	<0.0001			0.1379	0.1246
GA		<0.0001	0.0012			0.0456			<0.0001	0.3229	0.0595			<0.0001	<0.0001
Chilling Time x GA		0.4666	0.0424			0.0307			0.3639	0.7748	0.0080			0.3367	0.3067
UNZ		0.4920	0.3036			<0.0001			0.5098	0.6235	0.7112			0.7311	0.7111
Chilling Time x UNZ		0.9971	0.6140			0.2645			0.4869	0.7865	0.5805			0.0718	0.1646
GA x UNZ		0.1164	0.2944			0.2292			0.0971	0.0624	0.3979			0.0796	0.0510
Chilling x GA x UNZ		0.9634	0.9257			0.0383			0.1876	0.2826	0.3531			0.1906	0.8251

[z] Days to emergence, measured from the beginning of greenhouse forcing.

[y] Plant height, tallest height of vegetative growth.

[x] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 10)

Table 2-3. Effects of chilling duration (pre-chilled at 5°C for 3, 4 or 5 weeks), GA_3 and/or uniconazole (UNZ) drench application on growth and flowering of *Paeonia lactiflora* 'Inspecteur Lavergne' (IL) in the 2017–2018 season.

IL (2017–18)		Days to emergence[z]	Days to bud	Days to flower			Plant height (cm)[y]		Flower height (cm)	Plant width (cm)		Number of shoots			Number of vegetative shoots			Number of shoots with blasted buds			Number of flowering shoots	Flowering %		
Chilling time	3 weeks	16.1 ab[x]	46.2 a	79.8 a			43.8		50.2 b	40.8		4.2 ab			1.8 b			0.3			2.1 a	98 a		
	4 weeks	18.3 a	45.0 a	78.3 a			45.5		52.9 ab	39.1		4.0 b			1.8 b			0.4			1.8 ab	90 ab		
	5 weeks	14.5 b	42.0 b	72.8 b			46.3		55.3 a	41.0		4.8 a			2.8 a			0.4			1.6 b	80 b		
	P-value	0.0008	<0.0001	<0.0001			0.1737		0.0101	0.1793		0.0386			0.0004			0.6987			0.0124	0.0049		
				Chilling Time								Chilling Time			Chilling Time			Chilling Time				Chilling Time		
				3 weeks	4 weeks	5 weeks						3 weeks	4 weeks	5 weeks	3 weeks	4 weeks	5 weeks	3 weeks	4 weeks	5 weeks		3 weeks	4 weeks	5 weeks
GA ($mg \cdot L^{-1}$)	0	19	45.8	83.0	80.0	73.0	46.2		54.8	39.6		3.8	3.6	3.3	1.4	1.7	1.7	0.4	0.2	0.2	1.7	100	93	70
	100	13.6	43.1	76.5	76.6	72.7	44.2		50.8	41.0		4.7	4.4	6.2	2.0	1.8	3.9	0.6	0.6	0.5	2	97	87	90
	P-value	<0.0001	0.0005	0.0001	0.2151	0.9999	0.0653		0.0034	0.1127		0.2181	0.3384	<0.0001	0.1449	0.7582	<0.0001	0.3661	0.0137	0.0431	0.0246	0.9982	0.9572	0.1149
							GA ($mg \cdot L^{-1}$)			GA ($mg \cdot L^{-1}$)								Chilling Time						
							0	100		0	100							3 weeks	4 weeks	5 weeks				
UNZ ($mg \cdot L^{-1}$)	0	16.5	45.0	77.0			48.8 a	42.1	51.4	44.5 a	42.4	4.5			2.2			0.8 a	0.4	0.3	1.9	90		
	15	16.6	44.5	76.2			42.3 b	45.5	52.6	36.3 b	40.5	3.9			1.9			0.2 b	0.3	0.4	1.7	87		
	20	15.7	43.8	77.7			47.5 a	44.9	54.4	37.9 b	40.1	4.5			2.2			0.1 b	0.6	0.4	2.0	92		
	P-value	0.616	0.3274	0.5531			0.0074	0.0921	0.1898	<0.0001	0.1391	0.0634			0.5833			0.0001	0.3231	0.8647	0.3155	0.6559		
Chiiling time		0.0008	<0.0001	<0.0001			0.1737		0.0101	0.1793		0.0386			0.0004			0.6987			0.0124	0.0049		
GA		<0.0001	0.0005	0.0003			0.0653		0.0034	0.1127		<0.0001			0.0001			0.0379			0.0246	0.462		
Chilling Time x GA		0.3116	0.9731	0.0061			0.7284		0.5844	0.6119		0.0003			0.0025			0.0356			0.9663	0.0343		
UNZ		0.616	0.3274	0.5531			0.2213		0.1898	<0.0001		0.0634			0.5833			0.2069			0.3155	0.6559		
Chilling Time x UNZ		0.1748	0.6259	0.5923			0.9989		0.0869	0.3092		0.1833			0.2605			0.0034			0.3862	0.7920		
GA x UNZ		0.8230	0.5482	0.3807			0.0017		0.9913	0.0188		0.7849			0.6513			0.3203			0.6997	0.8344		
Chilling x GA x UNZ		0.2641	0.5117	0.7705			0.5698		0.2757	0.4971		0.4202			0.6616			0.1500			0.5857	0.8351		

[z] Days to emergence, measured from the beginning of greenhouse forcing.

[y] Vegetative height, tallest height of vegetative growth.

[x] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 10)

Table 2-4. Effects of chilling duration (pre-chilled at 5°C for 3, 4 or 5 weeks), GA_3 and/or uniconazole (UNZ) drench application on growth and flowering of *Paeonia lactiflora* 'Inspecteur Lavergne' (IL) in the 2018–19 season.

IL (2018–19)		Days to emergence[z]	Days to bud	Days to flower	Plant height (cm)[y]	Flower height (cm)	Plant width (cm)	Number of shoots	Number of vegetative shoots	Number of shoots with blasted buds	Number of flowering shoots	Flowering %
Chilling Time	3 weeks	9.5	37.2	59.6		41.0 b[x]	37.8 b	4.3	2.3 b	0.5	1.5	
	4 weeks	11.7	36.8	58.1		42.1 b	36.2 b	4.5	2.3 b	0.5	1.9	
	5 weeks	10.6	35.9	58.0		47.3 a	41.9 a	4.9	3.2 a	0.4	1.6	
	P-value	0.0536	0.4822	0.2211	Table 2-5	0.0004	<0.0001	0.1452	0.0067	0.5322	0.1663	Table 2-5

			Chilling Time						Chilling Time			Chilling Time					
			3 weeks	4 weeks	5 weeks				3 weeks	4 weeks	5 weeks	3 weeks	4 weeks	5 weeks			
GA ($mg \cdot L^{-1}$)	0	12.4	40.6	37.4	37.3	60.2		44.4	37.8			3.2	4.0	3.6	2.0	2.3	2.3	0.1	1.4	
	100	8.7	33.9	36.3	34.6	57.0		42.5	39.5			5.4	4.9	6.1	2.6	2.3	4.0	0.7	1.9	
	P-value	<0.0001	<0.0001	0.4651	0.0801	0.0002	Table 2-5	0.1730	0.0155			<0.0001	0.0311	<0.0001	0.1915	0.9386	0.0002	<0.0001	0.0064	Table 2-5

								Chilling Time			GA ($mg \cdot L^{-1}$)					
								3 weeks	4 weeks	5 weeks	0	100				
UNZ ($mg \cdot L^{-1}$)	0	11.1	36.5			57.9		45.4	42.0 a	39.1 a	47.7 a	44.8 a	41.7 a	4.5	2.5	0.6 a	1.5	
	15	11.3	37.6			59.3		42.5	34.6 b	34.4 b	40.7 b	34.9 b	38.2 b	4.6	2.8	0.3 b	1.6	
	20	9.3	36.0			58.5		42.5	35.8 b	35.3 b	37.3 c	33.7 b	38.6 b	4.6	2.4	0.4 ab	1.9	
	P-value	0.0625	0.3253			0.4461	Table 2-5	0.1381	<0.0001	0.0045	<0.0001	<0.0001	0.0073	0.8394	0.4548	0.0394	0.1997	Table 2-5

Chiiling time	0.0537	0.4822	0.2211	0.0006	0.0004	<0.0001	0.1452	0.0067	0.5322	0.1663	0.2489
GA	<0.0001	0.0002	0.0002	0.4265	0.1730	0.0155	<0.0001	0.0043	<0.0001	0.0064	0.0596
Chilling Time x GA	0.4622	0.0434	0.2541	0.7621	0.4731	0.2528	0.0161	0.0240	0.0951	0.7307	0.6271
UNZ	0.0625	0.3253	0.4461	0.4675	0.1381	<0.0001	0.8394	0.4548	0.0394	0.1997	0.0514
Chilling Time x UNZ	0.2089	0.8275	0.4538	0.4985	0.6000	0.0140	0.4758	0.9075	0.2829	0.9649	0.9292
GA x UNZ	0.3550	0.2684	0.1022	0.0834	0.1450	<0.0001	0.5871	0.1432	0.0742	0.4840	0.8660
Chilling x GA x UNZ	0.3996	0.8272	0.5292	0.0173	0.5222	0.1143	0.1215	0.8269	0.1861	0.1565	0.0432

[z] Days to emergence, measured from the beginning of greenhouse forcing.

[y] Plant height, tallest height of vegetative growth.

[x] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 10)

Table 2-5. Breakout of plant height and flowering percentage for *Paeonia lactiflora* 'Inspecteur Lavergne' (IL) due to the significant three-way interactions of chilling duration (pre-chilled at 5°C for 3, 4 or 5 weeks), GA_3 (0 or 100 mg·L^{-1}) and uniconazole (UNZ, 0.15, or 20 mg·L^{-1}) applications in the 2018–19 season.

IL (2018–19) Plant Height (cm)		Chilling Time					
		3 weeks		4 weeks		5 weeks	
		GA 0	GA 100	GA 0	GA 100	GA 0	GA 100
UNZ (mg·L^{-1})	0	36.6 a[z]	30.8	31.8	35.2	40.1	36.0
	15	25.8 b	33.6	31.1	34.9	37.9	37.5
	20	31.4 ab	35.0	35.9	31.0	33.6	37.9
	P-value	0.0185	0.2670	0.3850	0.2060	0.2142	0.7457

IL (2018–19) Flowering %		Chilling Time					
		3 weeks		4 weeks		5 weeks	
		GA 0	GA 100	GA 0	GA 100	GA 0	GA 100
UNZ (mg·L^{-1})	0	70	60	50 b	90	80	70
	15	60	90	80 ab	100	90	80
	20	70	90	100 a	90	70	100
	P-value	0.8469	0.2323	0.0052	0.7383	0.5258	0.2283

[z] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 10)

Fig. 2-1. Typical flowers of *Paeonia lactiflora*: 'Sarah Bernhardt' and 'Inspecteur Lavergne'.

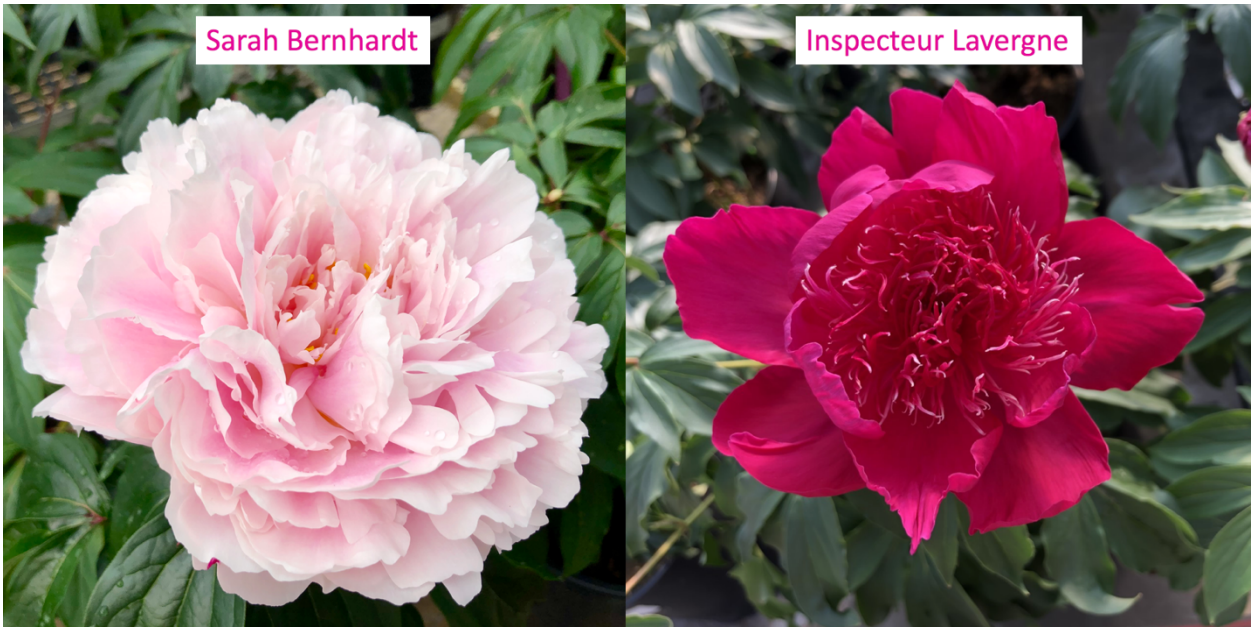

Fig. 2-2. Flower buds of the vegetative shoot (A, flower abortion in earlier stage), shoot of blasted bud (B, flower abortion/blasting in later stage) and normal flower bud (C) of *Paeonia lactiflora* 'Sarah Bernhardt'.

Fig. 2-3. Effect of GA_3 and/or uniconazole (UNZ, Concise) on plant size and development of *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne' under 3 weeks chilling at 5°C. Photo at finish (when >50% of plants in a treatment group had flowered) in March 2019.

Chapter 3

Uniconazole and Paclobutrazol Have Little Effect on Growth and Flowering of Containerized Peonies under Nursery Production

Abstract

Herbaceous peonies (*Paeonia lactiflora* Pall.) are widely used perennials for gardens, landscape and cut flower industry. Using two peony cultivars, Sarah Bernhardt and Inspecteur Lavergne, small (3–5 eye) crowns from Holland were potted in 3.8-L pots in mid-November of 2017 and 2018. We evaluated the effects of plant growth retardants applied with different methods at different stages of production on the growth and development of containerized peony under nursery conditions. All potted plants were placed in an unheated coldframe at the Virginia Tech Urban Horticulture Center (Blacksburg, VA, 37° N) for one month after potting to promote rooting and then were moved outdoors to a gravel pad to receive natural chilling over the winter. In 2017-18, substrate drenches of uniconazole (UNZ) at 0, 15, 30 or 45 $mg \cdot L^{-1}$ or paclobutrazol (PBZ) at 0, 30, 60 or 90 $mg \cdot L^{-1}$ at 237 mL/pot were applied about 4 weeks after potting for both cultivars in mid-December 2017. In 2017-18, fall drenches of UNZ at 0, 15, 30 or 45 $mg \cdot L^{-1}$ at 237 mL/pot were applied about 4 weeks after potting in mid-December 2018, or spring sprenches of UNZ were applied at 0, 15, 30 or 45 $mg \cdot L^{-1}$ at 840 $mL \cdot m^{-2}$ in March 2019 after 50% shoot emergence for each cultivar. Plant growth retardant applications had little effect on plant growth of either cultivar, but foliage of treated plants were darker green compared with the untreated plants. In addition, higher rates of UNZ applied as a fall drench increased the number of flowering shoots of both cultivars and the percentage of plants flowering for 'Sarah Bernhardt' in the second season of the study where plants were more protected from spring freezes. Fall PBZ drenches or spring UNZ sprenches had little effect on flowering.

Introduction

Herbaceous peony (*Paeonia lactiflora* Pall.) is a traditional flowering landscape and medicinal perennial plant in many countries of temperate regions that also is of significant value as a commercial cut flower (Kamenetsky and Dole, 2012; Rogers, 1995). There are 6,854 registered peony cultivars according to the American Peony Society, which serves as the International Cultivar Registration Authority for peonies, and 4,080 of these cultivars are *P. lactiflora* (APS, 2020). Containerized herbaceous peonies are popular in the potted perennial plant sector, with more than half million plants, valued almost $6 million USD, sold in 2018 (USDA, 2019).

Plant growth retardants (inhibitors of gibberellin biosynthesis) are widely used in the greenhouse/nursery industry to regulate shoot growth of containerized plants, especially plant height (Hartmann et al., 2011; Megersa et al., 2018; Rademacher, 2016). They primarily affect elongation in stems, petioles and flower stalks, and improve plant appearance by maintaining plant size and shape in proportion with the containers as well as increase the shipping capacity with more compact plants (Hartmann et al., 2011; Latimer and Freeborn, 2011a, b). Plant growth retardants also increase the plant tolerance to the shipping and handling stresses, improve the shelf life and result more marketable plants (Latimer and Whipker, 2019).

Growth retardants are also used for growing peonies. Most of the research has been done in China. Uniconazole (UNZ) or paclobutrazol (PBZ) foliar sprays upon bud break (when the sprouts were 3 cm long) controlled shoot growth for ‘Taiyoh’ and ‘Hanakisoi’ tree peony (*P. suffruticosa*) (Hamada et al., 1990). PBZ or mepiquat chloride foliar sprays of different rates applied in the spring controlled plant size of a few herbaceous peony cultivars such as ‘Da Fu

Gui' (Zhu et al., 2002; Wang et al., 2014). However, PBZ application can reduce the number of lateral buds per branch as well as reduce the length and diameter of lateral branches of 'Zi Fengyu' plants, a Chinese herbaceous peony cultivar that tends to have multiple lateral branches (Zhao et al., 2015). PBZ foliar spray applications also increase photosynthetic characteristics of herbaceous peony 'Zi Fengyu', resulting in darker green leaves and shorter plants (Xia et al., 2018).

In our preliminary studies on the responses of containerized peonies to different PGR application methods in 2016–17, we found no response to UNZ applied as fall sprenches or pre-plant crown soaks (2 minutes), but moderate growth regulation with 10 or 20 $mg \cdot L^{-1}$ drenches (355 mL/3.8-L pot) in the spring (Appendix 1).

Herbaceous peonies have been one of the more difficult crops for plant growth control in containers in the nursery production (I. Brantingham, personal communication, Riverbend Nursery, Riner, VA). Most of the herbaceous peony studies described above were using 3 or 4 year-old plants. In this research, our objective was to evaluate effects of growth retardants applied with different methods at different stages of production on the growth and development of containerized herbaceous peony grown from small (3–5 buds) crowns under nursery conditions.

Materials and Methods

Plant materials

Three to five eye peony crowns of 'Sarah Bernhardt' and 'Inspecteur Lavergne' were imported to Battlefield Farms (Rapidan, VA) from a Dutch commercial source in fall of 2017

and 2018. ‘Sarah Bernhardt’ is a double, late flowering type with very large dark rose pink flowers, medium height, floriferous, strong stems, and good foliage. And ‘Inspecteur Lavergne’ is a double, early flowering type, with globular crimson flower with frilled petals in the center, and long straight stems (**Fig. 2-1**). All crowns were potted in 3.8-L pots using a peat and pine bark substrate (Fafard 52 Mix, Sun Gro Horticulture, Agawam, MA) amended with 50% composted pine bark, with 5 cm of substrate over the crown in mid-November of 2017 and 2018 at Virginia Tech’s Urban Horticulture Center (Blacksburg, VA, lat. 37° N, long. 80° W). Substrate was also amended with pulverized dolomitic limestone to adjust pH, and 17N–2.2P–9.1K Osmocote Pro 12–14 month CRF (ICL Specialty Fertilizers, Summerville, SC) was incorporated at a rate of 5.3 kg/m^3 at time of potting. All crowns were sorted for uniformity of number of eyes and size of crown and roots prior to potting.

Growth retardants

In 2017–18, substrate drenches of UNZ (Concise, Fine Americas, Walnut Creek, CA) at 0, 15, 30 or 45 mg·L^{-1} (0, 3.56, 7.11 or 10.7 mg a.i./pot) or PBZ (Piccolo 10 XC, Fine Americas) at 0, 30, 60 or 90 mg·L^{-1} (0, 7.11, 14.2 or 21.3 mg a.i./pot) were applied to both cultivars at 237 mL/pot about 4 weeks after potting peony crowns, in mid-December 2017, while the air temperature was 10°C.

In 2018–19, we applied UNZ at 0, 15, 30 or 45 mg·L^{-1} to both cultivars as fall drenches in 2018, or spring sprenches in 2019. As in 2017, UNZ at 0, 15, 30 or 45 mg·L^{-1} was applied as a fall drench at 237 mL/pot (0, 3.56, 7.11 or 10.7 mg a.i./pot) about 4 weeks after potting peony crowns in mid-December 2018, while the air temperature was 10°C. UNZ at 0, 15, 30 or 45 mg·L^{-1} was applied as a spring sprench at 840 mL·m^{-2} (4 times label recommended volume, 23.9 mg a.i. per plant) in March 2019 after 50% shoot emergence for each cultivar.

Plant maintenance, measurements and data analysis

All plants were held in an unheated coldframe in Virginia Tech's Urban Horticulture Center for one month after potting to promote rooting, then moved outdoors to a gravel pad to receive natural chilling over the winter months (December through February/March). Buffer pots filled with media were used around the experimental plot. All pots were covered with a double layer of 2.5 oz. frost blanket (DeWitt's Ultimate, Strong, ME) and a single layer of 5-mil opaque poly for winter protection when the temperature was below -6.7°C and uncovered when the temperature was above 5°C outdoors. All plants were moved back to the coldframe in spring (light data unavailable) after emergence began, when they could no longer be safely covered for freeze protection. A fungicide drench (Subdue Maxx, Syngenta, Greensboro, NC) at 0.08 $mL \cdot L^{-1}$ was applied at 237 mL/pot two weeks after potting to protect plants from fungal infection. Additional insecticide and fungicide applications were applied in the spring as needed. Rodenticide bait stations were placed among the plants before covering and filled periodically during the winter. Irrigation was applied as needed when media was dry on top of the pot by hand-watering.

Evaluation began with the first plant emergence in the spring. Days to emergence, days to bud (cracked color) and days to first open flower were recorded twice weekly in the season of 2017–18 and daily in the season of 2018–19. Plant height, flower height and plant width, as well as number of shoots, number of vegetative shoots, number of shoots with blasted buds, and number of flowering shoots were recorded at finish (when >50% of plants in a treatment group had flowered). According to Kamenetsky et al. (2003), a shoot with a blasted bud is a shoot with a flower bud that aborted/blasted in the later stages of bud development and a vegetative shoot is a shoot without a flower bud or with flower bud that aborted in the early stages of development (bud smaller than 2 mm) (**Fig. 2-2**). Flowering percentage was recorded as the percentage of

plants in a treatment group flowering at the end of the study when all plants have finished flowering (for each individual plant of a treatment group, 1 = flowering and 0 = no flowering, the average flowering rate was converted to a percentage).

The experiments were simple completely randomized designs with type of growth retardant (2017–18) or application time (2018–19). Each cultivar was set up as a separate experiment. Each treatment consisted of ten replications completely randomized. For all experimental datasets, data were analyzed by analysis of variance with mean separation by Student's *t* test at $P \leq 0.05$ using JMP® Pro 15, © SAS Institute Inc. (Cary, NC).

Results

2017–18 Season fall paclobutrazol (PBZ) drenches

For 'Sarah Bernhardt', fall PBZ drenches had no significant effect on days to emergence or height of plants evaluated the following spring, but PBZ treated plants were 22% less wide (**Table 3-1**). PBZ treated plants had fewer shoots, as low as 3.3 shoots/pot in 60 $mg \cdot L^{-1}$ PBZ treated plants compared to 6.1 shoots/pot for untreated plants. Fall PBZ drenches reduced the number of vegetative shoots, but increased the number of shoots with blasted buds. PBZ treatments did not affect the number of flowering shoots or the percentage of plants flowering. However, the percentage of plants flowering was very low (0–30%).

For 'Inspecteur Lavergne', fall PBZ drenches had no significant effects on growth or flowering (**Table 3-1**). Plants treated with PBZ visually appeared greener than the untreated plants. The percentage of plants flowering was not affected by PBZ but was generally high, reaching 90% with the 60 $mg \cdot L^{-1}$ PBZ treatment.

2017–18 Season fall uniconazole (UNZ) drenches

For ‘Sarah Bernhardt’, fall UNZ drenches had no significant effects on growth or flowering of plants evaluated the following spring (**Table 3-2**).

For ‘Inspecteur Lavergne’, fall UNZ drenches did not affect days to emergence or plant height of plants evaluated the following spring (**Table 3-2**). However, plants drenched with 30 or 45 mg·L^{-1} UNZ were more narrow, resulting in slightly more compact plants. Although the numbers of total shoots and vegetative shoots were not affected, fall UNZ drenches increased the numbers of shoots with blasted buds when plants were treated with 45 mg·L^{-1} UNZ. Fall UNZ drenches affected the number of flowering shoots, but the number of flowering shoots on treated plants did not differ from that of untreated plants. Although the percentage of plants flowering was not significantly affected by fall UNZ drenches, plants treated with higher concentrations (30 or 45 mg·L^{-1} UNZ) had 90% or 80% of plants flowering.

2018–19 Season fall uniconazole (UNZ) drenches

For ‘Sarah Bernhardt’, fall UNZ drenches had no significant effect on days to emergence or plant growth evaluated the following spring (**Table 3-3**). However higher concentrations (30 or 45 mg·L^{-1}) significantly reduced the number of shoots with 2.7 shoots/plant on 30 mg·L^{-1} UNZ treated plants compared to 4.6 shoots/plant for untreated plants. Plants treated with 30 or 45 mg·L^{-1} UNZ had fewer vegetative shoots, and with 30 mg·L^{-1} UNZ had fewer shoots with blasted buds. Application of 30 or 45 mg·L^{-1} UNZ increased the number of flowering shoots by 0.5 shoot/plant and the percentages of plants flowering up to 50% compared to untreated plants (**Fig. 3-1**).

For ‘Inspecteur Lavergne’, fall UNZ drenches had no significant effects on days to emergence or plant growth, however different concentrations of UNZ treatment had various effects on total number of shoots (Table 3-3). Application of 45 $mg \cdot L^{-1}$ UNZ reduced the number of vegetative shoots by 2.0 shoots/plant compared to untreated plants, but there was no effect on the number of shoots with blasted buds. Application of 45 $mg \cdot L^{-1}$ UNZ significantly increased the number of flowering shoots by 0.7 shoot/plant compared to untreated plants.

2018–19 Season spring uniconazole (UNZ) sprenches

For ‘Sarah Bernhardt’, spring UNZ sprenches had very little effect on plant growth or flowering (**Table 3-4**, **Fig. 3-2**). UNZ spring sprenches did not affect days to emergence or plant height; only 45 $mg \cdot L^{-1}$ UNZ significantly reduced plant width by 12% compared to untreated plants, with no effect on the number of shoots or flowering.

For ‘Inspecteur Lavergne’, spring UNZ sprenches did not affect plant growth or flowering (**Table 3-4**, **Fig. 3-2**).

Discussion

Our research goal was to find the best combination of growth retardant, concentration, application method and application timing to control the growth of herbaceous peony, without negatively affecting flowering. Although Hamada et al. (1990), Zhu et al. (2002) and Wang et al. (2014) showed that peonies were responsive to growth retardants, our results showed no commercial value. This lack of response might be due to the timing of application, the plant uptake of the products, the application rate of the growth retardants, or frequency of the application.

UNZ and PBZ are two popular plant growth retardants (PGRs) used in floriculture to control plant length and diameter in recent years. UNZ and PBZ are both persistent on plastic and media particles and work well as a media/substrate drench, while UNZ is more potent than PBZ (Latimer and Scoggins, 2018).

Fall PBZ drenches did not reduce plant height for either of our peony cultivars, only the higher concentration (90 $mg \cdot L^{-1}$) reduced 'Sarah Bernhardt' plants width by 22%. The negative effect was that the higher concentrations of paclobutrazol (60 or 90 $mg \cdot L^{-1}$) reduced the total number of shoots of 'Sarah Bernhardt' (3.3 shoots/pot on PBZ treated plants than 6.1 shoots with untreated plants). In China, foliar sprays of 500 or 1000 $mg \cdot L^{-1}$ paclobutrazol applied three times at a 5-day interval upon bud break significantly reduced shoot length of tree peonies, but no flowering data were reported (Hamada et al., 1990). Foliar sprays of 100 $mg \cdot L^{-1}$ paclobutrazol applied at emergence in spring controlled the plant size of five Chinese herbaceous peony cultivars, resulting in plants that were shorter and less wide with greater stem diameters than untreated plants; however, no flowering data were presented (Wang et al., 2014). Foliar sprays of 150 $mg \cdot L^{-1}$ paclobutrazol applied once a day for 10 days beginning 20 days prior to flowering reduced plant height of 'Da Fu Gui' herbaceous peony by 24% (Zhu et al., 2003). Perhaps our studies did not show significant growth control of herbaceous peonies because our paclobutrazol drenches were applied in fall when the plants were dormant, not in active growth stages, although paclobutrazol is soil active and can stay in the substrate for a period of time (Latimer and Whipker, 2019; Rademacher, 2016). Also the cold air temperature at the time of the growth retardants application (10°C in fall 2017 and 5°C in fall 2018) might have an effect on their uptake by plants. Previous research showed there were temperature effects on plant response to UNZ application where UNZ promoted of larkspur (*Delphinium elatum)* seedlings leaf

differentiation when growing under greenhouse conditions (>15°C) but not under chilling conditions (5°C) (Ogasawara et al., 2001).

In our studies, plants treated with PBZ appeared greener than the untreated plants, perhaps due to increased photosynthetic capacity as described by Xia et al. (2018), where 100 $mg \cdot L^{-1}$ PBZ foliar spray applications to herbaceous peony 'Zi Fengyu', resulted in darker green leaves and plants that were 15% shorter than untreated plants.

Although during the two seasons' studies, fall UNZ drenches provided little growth control, higher concentrations (30 or 45 $mg \cdot L^{-1}$ on 'Sarah Bernhardt', and 45 $mg \cdot L^{-1}$ on 'Inspecteur Lavergne') had positive but inconsistent effects on flowering of both peony cultivars. In the 2018–19 season, UNZ drenches increased the number of flowering shoots by 0.5 to 0.7 per plant in both cultivars and the percentage of plants flowering, 50% in 'Sarah Bernhardt'. The increase in the number of flowering shoots may be due to the reduction in the number vegetative shoots, i.e., a reduction in early bud abortion. This might be related to the ability of growth retardants to enhance tolerance to stress and disease (Latimer and Whipker, 2019). The total number of shoots was reduced, but UNZ application protected the ones that developed. Perhaps some buds on the crown had not transitioned to reproductive stages at the time of UNZ application (mid-December), and perhaps the UNZ inhibited the reproductive bud transition or growth. UNZ is very persistent both in plants and in the soilless media, lasting for approximately six months (Latimer and Whipker, 2019; Rademacher, 2016). Unlike PBZ, UNZ has been tested in few studies on peonies. Foliar sprays of 25 or 50 $mg \cdot L^{-1}$ UNZ applied to tree peony twice at a 5-day interval upon bud break reduced shoot growth more effectively than foliar sprays of 500 or 1000 $mg \cdot L^{-1}$ PBZ applied three times at a 5-day interval upon bud break without affecting flowering (100% flowering with all treatments) (Hamada et al., 1990).

Spring UNZ sprenches provided little control of plant height or width of herbaceous peony plants, although they were applied when plants were in active growth (50% emergence). Perhaps the application volume which was 840 $mL \cdot m^{-2}$, only about 23.9 mg a.i. per plant, was too low.

Our greenhouse studies of UNZ applied as drenches at 15 or 20 $mg \cdot L^{-1}$ in spring after plants had received sufficient chilling and had begun to emerge resulted in statistically significant reductions in plant width on both cultivars (Chapter 2). However, these small differences were not commercially significant in production and UNZ had even less effect in controlling plant height. UNZ did not reduce the number of shoots or the percentage of plants flowering, but in some cases reduced the number of shoots with blasted buds in 'Inspecteur Lavergne' plants in our greenhouse studies (Chapter 2).

The poor flowering of the peonies, especially with 'Sarah Bernhardt' (0–30%) in 2017–18 was most likely due to the spring cold damage (**Fig. 3-3**). The cold damage happened during a freezing event in late March 2018 when most of the plants had already emerged and we could not apply protective covering, causing the loss of many of the primary shoots. In 2019, plants were moved to coldframe after emergence and less cold damage was observed. Flowering of 'Sarah Bernhardt' improved (0–50%), but the percentage of plants flowering was still very low. Plants still experienced periods of cold temperature and damage although we applied cold protection (covered with a double layer of 2.5 oz. frost blanket and a single layer of 5-mil opaque poly) even inside the coldframe. Cold damage also increased the susceptibility of plants to diseases such as gray mold (*Botrytis spp*.) and tobacco rattle virus, which reduced the flowering and plant quality.

All plants had relatively desirable plant size, and plants treated with growth retardant were

darker green in color compared with the control plants.

Conclusion

Overall, all our plant growth retardant applications had little effect on plant growth of either cultivar, but foliage of treated plants were darker green color compared with the control plants. In addition, higher rates of UNZ applied as a fall drench increased the number of flowering shoots of both cultivars and the percentage of plants flowering for ‘Sarah Bernhardt’ in the second season of the study where plants were more protected from spring freezes. Fall PBZ drenches or spring UNZ sprenches had little effect on flowering.

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Table 3-1. Effect of paclobutrazol (PBZ, Piccolo 10 XC) fall drenches on nursery grown containerized *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne' in 2017–18.

Fall PBZ Drench (2017–18)	mg·L^{-1}	Days to emergence[z]	Plant height (cm)[y]	Plant width (cm)	Number of shoots	Number of vegetative shoots	Number of shoots with blasted buds	Number of flowering shoots	Flowering %
Sarah Bernhardt	0	52.3	35.5	38.2 a[x]	6.1 a	5.5 a	0.3 b	0.3	30
	30	54.3	33.3	35.1 ab	4.1 ab	3.1 b	0.8 ab	0.2	20
	60	53.3	31.6	32.2 ab	3.3 b	2.0 b	1.3 ab	0.0	0
	90	60.6	29.3	29.9 b	3.9 b	2.1 b	1.7 a	0.1	10
	P-value	0.7006	0.4144	0.0180	0.0041	0.0004	0.0067	0.2880	0.2880
Inspecteur Lavergne	0	40.1	39.9	35.1	4.5	2.4	0.9	1.2	60
	30	53.6	36.4	30.3	4.4	3.2	0.6	0.6	40
	60	54.1	34.1	24.8	4.3	2.3	0.4	1.6	90
	90	41.9	36.8	40.0	4.3	2.2	0.9	1.2	70
	P-value	0.1222	0.3149	0.2745	0.9815	0.4509	0.3829	0.2234	0.1314

[z] Days to emergence, measured from the day the first plant emerged (day 0).

[y] Plant height, tallest height of vegetative growth.

[x] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 10)

Table 3-2. Effect of uniconazole (UNZ, Concise) fall drenches on nursery grown containerized *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne' in 2017–18.

Fall UNZ Drench (2017–18)	mg·L^{-1}	Days to emergence[z]	Plant height (cm)[y]	Plant width (cm)	Number of shoots	Number of vegetative shoots	Number of shoots with blasted buds	Number of flowering shoots	Flowering %
Sarah Bernhardt	0	49.4	34.9	34.8	5.0	3.9	1.1	0	0
	15	50.9	35.1	32.2	4.1	2.9	1.0	0.2	10
	30	46.4	31.2	29.5	5.0	4.2	0.7	0.1	10
	45	55.6	35.4	32.1	4.5	2.9	1.3	0.3	20
	P-value	0.5731	0.6984	0.3762	0.6761	0.2896	0.5936	0.5596	0.5548
Inspecteur Lavergne	0	36.1	37.5	34.8a	4.6	3.2	0.4b	1.0ab	50
	15	52.0	37.6	32.5ab	4.3	2.6	0.9ab	0.7b	50
	30	42.1	42.9	30.2bc	4.5	2.0	0.6ab	1.9a	90
	45	47.2	38.2	27.3c	3.9	1.8	1.2a	0.9ab	80
	P-value	0.2940	0.4768	0.0005	0.7157	0.3773	0.0491	0.0215	0.1256

[z] Days to emergence, measured from the day the first plant emerged (day 0).

[y] Plant height, tallest height of vegetative growth.

[x] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 10)

Table 3-3. Effect of uniconazole (UNZ, Concise) fall drenches on nursery grown containerized *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne' in 2018–19.

Fall UNZ Drench (2018–19)	mg·L^{-1}	Days to emergence[z]	Plant height (cm)[y]	Plant width (cm)	Number of shoots	Number of vegetative shoots	Number of shoots with blasted buds	Number of flowering shoots	Flowering %
Sarah Bernhardt	0	33.8	38.7	43.7	4.6 a[x]	3.4 a	1.2 a	0.0 b	0 b
	15	34.5	43.2	41.6	4.7 a	2.8 ab	1.9 a	0.0 b	0 b
	30	26.6	45.0	41.0	2.7 b	1.9 bc	0.2 b	0.5 a	50 a
	45	29.4	47.8	42.2	2.8 b	1.1 c	1.2 a	0.5 a	40 a
	P-value	0.5876	0.1209	0.6894	<0.0001	0.0004	0.0057	0.011	0.0049
Inspecteur Lavergne	0	39.9	37.3	39.2	5.1 ab	4.2 ab	0.7	0.2 b	20
	15	40.8	37.0	37.0	6.0 a	5.5 a	0.3	0.2 b	20
	30	41.3	34.7	33.9	4.4 ab	3.6 bc	0.3	0.5 ab	40
	45	38.2	38.6	33.5	3.5 b	2.2 c	0.3	0.9 a	70
	P-value	0.7042	0.8138	0.1056	0.0237	0.0037	0.3379	0.0369	0.0663

[z] Days to emergence, measured from the day the first plant emerged (day 0).

[y] Plant height, tallest height of vegetative growth.

[x] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 10)

Table 3-4. Effect of uniconazole (UNZ, Concise) spring sprenches on nursery grown containerized *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne' in 2018–19.

Spring UNZ Sprench (2018–19)	mg·L^{-1}	Days to emergence[z]	Plant height (cm)[y]	Plant width (cm)	Number of shoots	Number of vegetative shoots	Number of shoots with blasted buds	Number of flowering shoots	Flowering %
Sarah Bernhardt	0	24.1	42.4	47.9 a[x]	4.4	2.9	1.3	0.2	20
	15	30.8	43.0	43.1 ab	3.6	1.8	1.8	0.0	0
	30	32.7	42.6	46.4 ab	4.6	2.5	2.0	0.1	10
	45	26.0	37.7	42.3 b	4.3	2.8	1.4	0.0	0
	P-value	0.5632	0.3379	0.0105	0.4601	0.5994	0.5429	0.2829	0.2829
Inspecteur Lavergne	0	38.8	35.9	38.5	5.6	4.9	0.4	0.3	20
	15	39.9	34.7	35.3	5.3	4.5	0.6	0.2	20
	30	38.6	35.8	34.8	5.5	4.7	0.6	0.1	10
	45	38.1	35.4	36.2	5.9	5.2	0.6	0.1	10
	P-value	0.9498	0.982	0.0676	0.9138	0.9149	0.9350	0.7258	0.8679

[z] Days to emergence, measured from the day the first plant emerged (day 0).

[y] Plant height, tallest height of vegetative growth.

[x] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 10)

Fig. 3-1. Effect of fall uniconazole (UNZ, Concise) drenches on plant size and development of nursery grown containerized *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne' in 2018–19. (Pictures taken in May 2019)

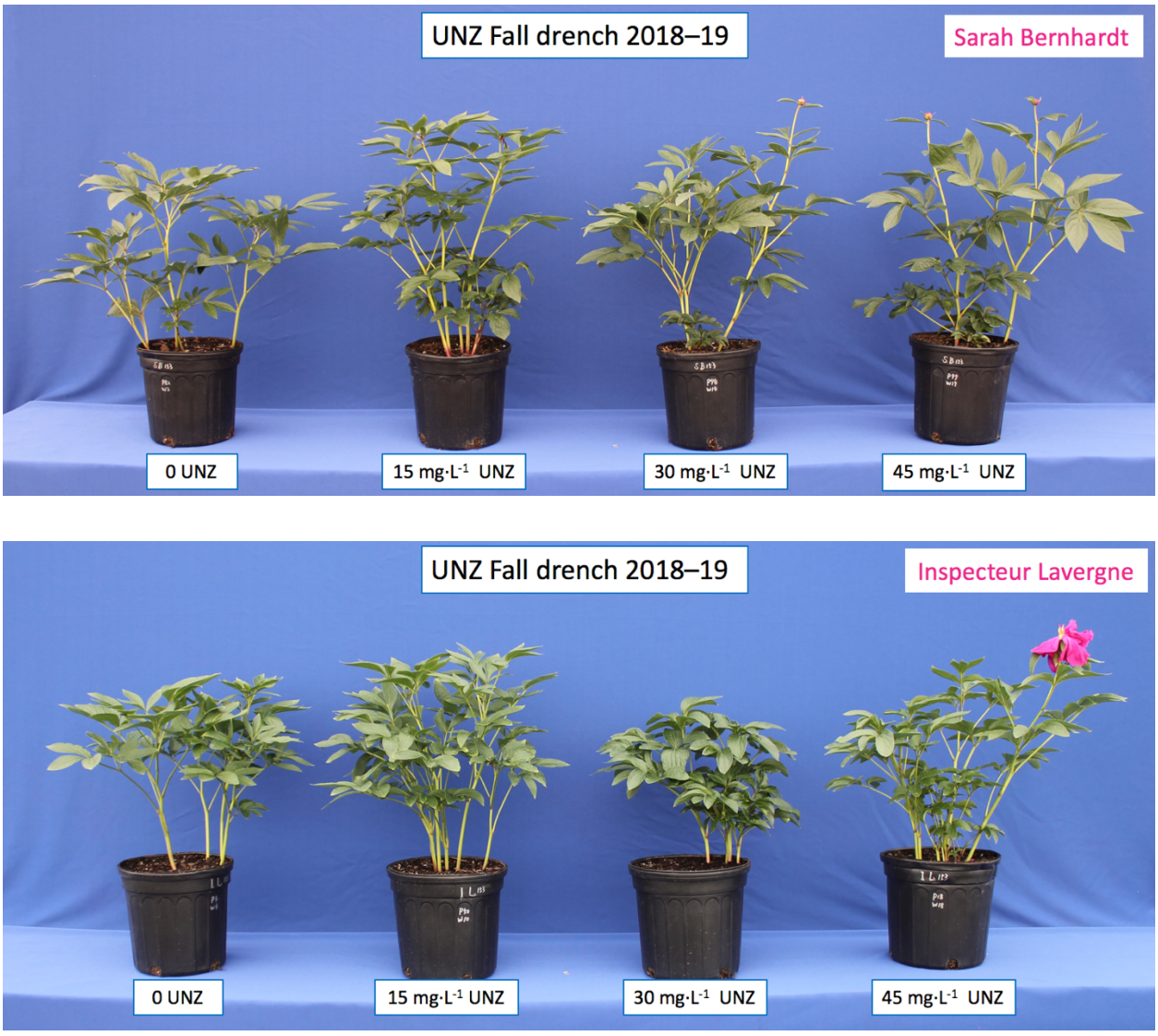

Fig. 3-2. Effect of spring uniconazole (UNZ, Concise) sprenches on plant size and development of nursery grown containerized *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne' in 2018–19. (Pictures taken in May 2019)

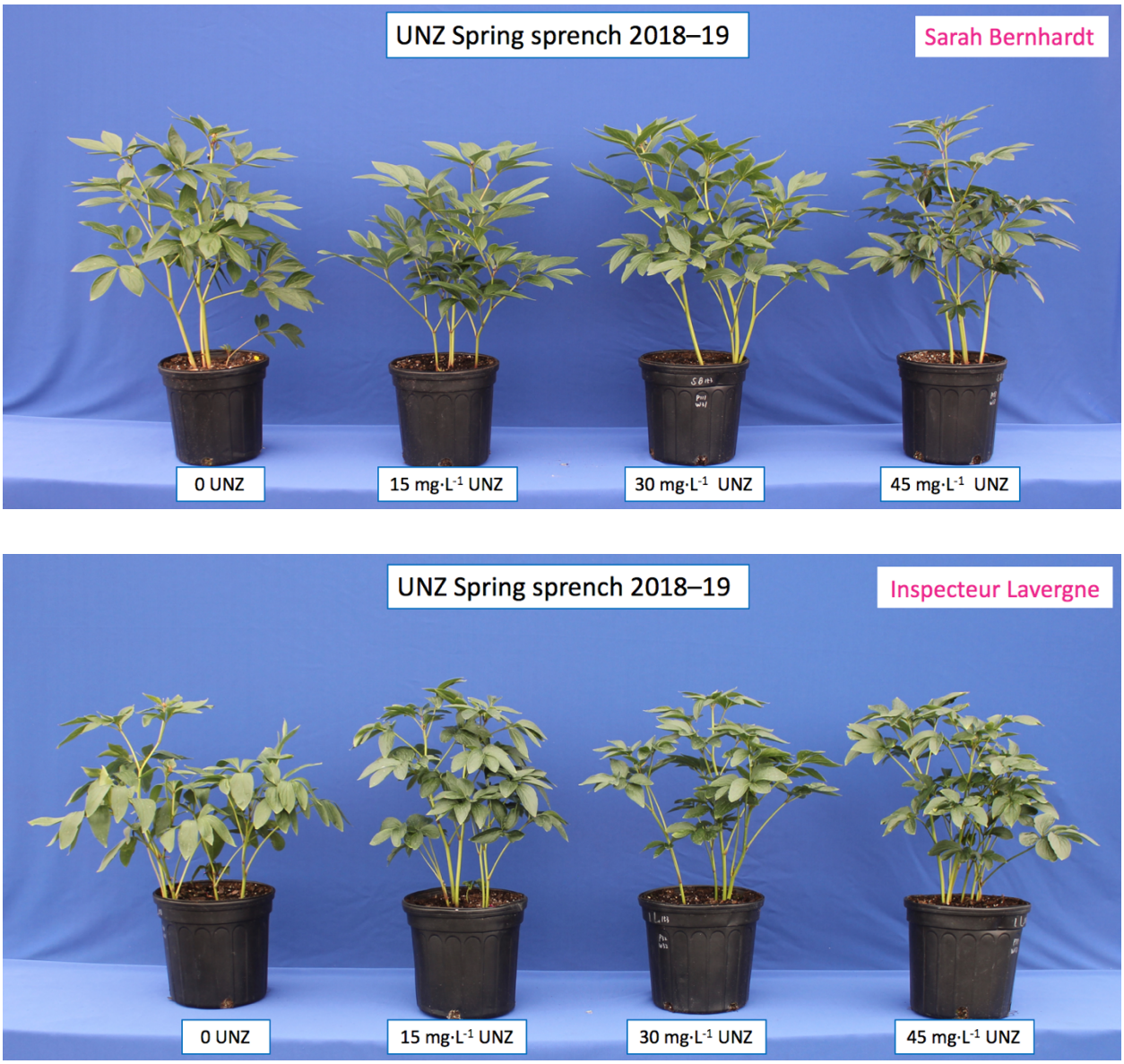

Fig. 3-3. Spring freezing event in late March 2018 when most of the nursery grown containerized *Paeonia lactiflora* had already emerged (picture taken on March 22, 2018).

Chapter 4

Chilling Accumulation Models as Guidelines for Timing of Spring GA_3 Drench Application to Containerized Peonies under Nursery Conditions

Abstract

Herbaceous peonies (*Paeonia lactiflora* Pall.) are common perennials used both in gardens and the landscape as well as for cut flowers. Peonies require a chilling period to break dormancy but not for flower bud differentiation. Using two peony cultivars, Sarah Bernhardt and Inspecteur Lavergne, small (3–5 eye) crowns from Holland were potted in 3.8-L pots in mid-November of 2017 and 2018. To determine the best timing for spring GA_3 applications under nursery conditions, we applied three models based on natural chilling accumulation. The models were a modified Fulton Chilling Model (FCM) for herbaceous peonies, Blackberry Chilling Model 5 (BCM5) for blackberry, or a visual development model (VDM) which was 10% of plants showing shoot emergence in the spring. We selected 1,000 chilling units (CU) based on Fulton et al. (2001) for the first two chilling models as the chilling required to break dormancy and promote normal plant growth and flowering. All plants were held in an unheated coldframe at the Virginia Tech Urban Horticulture Center (Blacksburg, VA, 37° N) for one month after potting to promote rooting, then were moved outdoors to a gravel pad to receive natural chilling over December through February/March. Drenches of 0 or 100 $mg \cdot L^{-1}$ GA_3 were applied at 250 mL/pot to each cultivar under each chilling model when the specific conditions were met. Due to greater winter injury in the 2017–18 season, results varied by year. In the 2017–18 season, GA_3 applied according to BCM5 reduce days to emergence for both cultivars and reduce the plant width of 'Inspecteur Lavergne', and later application according to BCM5 and VDM reduced plant length and diameter of 'Sarah Bernhardt'. Reductions in plant size may have been due to

greater winter injury due to the earlier emergence of GA_3 treated plants. In the 2018–19 season, earlier GA_3 drench applications tended to reduce days to emergence for both cultivars and the FCM application reduced days to bud for 'Inspecteur Lavergne', but GA_3 drench applications had no effect on plant size. GA_3 can be applied after chilling (1,000 CU) using a suitable chilling model such as FCM for peonies, or BCM5, or VDM, but GA_3 had little effect on plant development under nursery conditions.

Introduction

Because of its various types of large showy flowers and medicinal values, herbaceous peony (*Paeonia lactiflora* Pall.) has been a traditional landscape and medicinal perennial plant in many countries for many decades, and recently also used in commercial cut flower industry (Kamenetsky and Dole, 2012; Rogers, 1995). Peonies are popular in the United States and there are more than 4,000 registered herbaceous peony cultivars according to the American Peony Society, which serves as the International Cultivar Registration Authority for peonies (APS, 2020). Containerized herbaceous peonies are popular in the potted perennial plant market, with more than half million plants sold in 2018, valued almost $6 million USD (USDA, 2019).

Peonies require cold accumulation to break dormancy and promote plant growth and flowering (Barzilay et al., 2002). The chilling requirement is satisfied by a range of low temperatures accumulated over time. Increasing chilling time and decreasing temperature can advance the time of emergence and flowering (Byrne and Halevy, 1986; Evans et al., 1990; Kamenetsky et al., 2003). But excessive chilling can decrease flowering percentage and even prevent flowering (Aoki, 1991; Byrne and Halevy, 1986; Halevy et al., 2002). The optimal accumulated chilling units varies among cultivars (Fulton et al., 2001; Rhie et al., 2012; Yeo et

al., 2012). For the most popular cultivar ‘Sarah Bernhardt’, 4 weeks at 5.5 or 5.6°C can break the dormancy (Byrne and Halevy, 1986; Evans et al., 1990), and 60 days at 2°C or 70 days at 6°C can result in optimal growth and flowering (Kamenetsky et al., 2003).

Using natural low temperatures is the traditional way to satisfy the dormancy requirements of perennial plants grown in temperate zones. In the United States, herbaceous peonies can be grown across USDA hardiness zone 3 to 8 (Michener and Adelman, 2017; Rogers, 1995). But natural low temperatures fluctuate, even in the same area where winter climate and temperatures vary from year to year.

Fulton et al. (2001) developed a chilling model to calculate the chilling unit (CU; 1 CU is the sufficient chilling in an hour) received by peony in order to calculate the amount of chilling required to break dormancy. CU can be calculated by the linear equation $y = -0.0605x + 1$ ($R^2 = 0.9943$), where y is the number of chilling units and x is the mean hourly temperature in °C. The sufficient chilling required for 95% of ‘Sarah Bernhardt’ plant to emerge and to produce shoots and flowers is 1,002 CU. Using this chilling model, researchers studied the CU required to break dormancy and to induce flowering of Korean peony cultivars ‘Taebaek’ and ‘Mulsurae (Rhie et al., 2012), and to verify that natural chilling accumulation is practical for dormancy release, normal growth and flowering of ‘Taebaek’ (Yeo et al., 2012).

Other chilling models were used and listed to calculate natural accumulation chilling units received to break dormancy of fruit trees such as cherry, almond, peach or apricot (Alburquerque et al., 2008, Egea et al., 2003; Erez and Fishman, 1998; Ruiz et al., 2007). Among those models, an easy to access model is Blackberry Chilling Model developed at North Carolina State University (Warmund and Krumme, 2005). It is a commercial model of chilling accumulation

for the fruit crop blackberry (*Rubus* spp.). The accumulated CU of the model are calculated using instantaneous hourly average temperatures of local weather station where available. The Blackberry Chilling Model has four different formulas to calculate accumulated chilling units (CU) which are Model 1, Model 2, Model 5 and Model 6. The Blackberry Chilling Model 5 considers temperatures between 0 to 12.4°C as effective temperatures for dormancy release, and attributes negative effects for dormancy release when temperatures are above 15.9°C, which is similar to the peony chilling requirements reported from known studies (Byrne and Halevy, 1986; Evans et al., 1990; Fulton et al., 2001; Halevy et al., 2002; Kamenetsky et al., 2003).

According to the literature and our own peony experiments, 1,000 CU (using Fulton Chilling Model) are required to break dormancy and to induce flowering of many peony cultivars (Fulton et al., 2001; Halevy et al., 2002; Kamenetsky et al., 2003; Rhie et al., 2012; Yeo et al., 2012; Zhou, 2012).

Gibberellins (GAs) are known as regulators of many developmental phases of higher plants, promoting cell elongation and plant growth, inducing hydrolytic enzymes in seed germination, inducing bolting in long-day plants, promoting flowering, and fruit setting and development (Rademacher, 2015; 2016). GA_3 is the most widely used GA in horticulture, viticulture and agriculture (Rademacher, 2015). GA_3 can be used to enhance flowering of perennial ornamental crops (Moond and Gehlot, 2006, 2007; Singh et al., 2017). GA_3 can also be used to completely or partially replace the chilling requirement of some woody and herbaceous ornamentals typically forced in the greenhouse, such as containerized florist azalea (*Rhododendron* sp. cv.) (Ballantyne, 1960) or Korean chamchwi (*Aster scaber*) for cut flowers (Seong, et al., 1996), as well as geophytes like tulip (*Tulipa* sp.) and blazing star (*Liatris* sp.) (Metzger, 1995). GA_3 was also used on herbaceous peonies to partially replace the chilling requirement when plant received

insufficient chilling, and to enhance plant growth, development and flowering (Cheng et al., 2009; Evans et al., 1990; Halevy et al., 2002; Yeo et al., 2012).

Overall, most research on chilling effects and GA_3 use on herbaceous peony have been conducted under greenhouse forcing conditions. No literature reporting on the interaction of GA_3 and chilling as it relates to application timing exists. In this project, we developed three models based on natural chilling accumulation to determine the best timing for GA_3 application to affect plant growth and flowering of containerized herbaceous peonies under nursery conditions.

Materials and Methods

Plant materials

Three to five eye peony crowns of 'Sarah Bernhardt' and 'Inspecteur Lavergne' were imported to Battlefield Farms (Rapidan, VA) from a Dutch commercial source in fall of 2017 and 2018. 'Sarah Bernhardt' is a double, late flowering type with very large dark rose pink flower, medium height, floriferous, strong stems, and good foliage. And 'Inspecteur Lavergne' is a double, early flowering type, with globular crimson flower with frilled petals in the center, and long straight stems (Fig. 2-1). All crowns were potted in 3.8-L pots (1-gallon) using a peat and pine bark substrate (Fafard 52 Mix, Sun Gro Horticulture, Agawam, MA) amended with 50% composted pine bark with 5 cm of substrate over the crown in mid-November of 2017 and 2018 at Virginia Tech's Urban Horticulture Center (Blacksburg, VA, lat. 37° N, long. 80° W). Substrate was also amended with pulverized dolomitic limestone to adjust pH, and 17N–2.2P–9.1K Osmocote Pro 12–14 month CRF (ICL Specialty Fertilizers, Summerville, SC) was incorporated at a rate of 5.3 kg/m^3 at time of potting. All crowns were sorted for uniformity of number of eyes and size of crown and roots prior to potting.

Natural chilling accumulation models

Three chilling models were used to help interpret when natural accumulation chilling was sufficient for herbaceous peonies.

1) A modified Fulton Chilling Model (FCM) for herbaceous peonies, the amount of CU can be calculated by the linear equation $y = -0.0605x + 1$, where y is the number of CU and x is the temperature from 0°C to 10°C. One hour below 0°C is set equal to 1 CU, and one hour over 10°C is set equal to 0 CU, because 0°C is effective for dormancy breaking and temperatures over 10°C do not affect the chilling requirement for perennial plants (Yeo et al., 2012). The sufficient chilling required of 'Sarah Bernhardt' is 1,002 CU (Fulton et al., 2001).

2) Blackberry Chilling Model 5 (BCM5), is one of the four formulas of commercial model of chilling accumulation for blackberry (Warmund and Krumme, 2005). The BCM5 accumulates 1 CU when air temperature is between 0 and 9.1°C; 0.5 CU between 9.2 and 12.4°C; 0 CU between 12.5 and 15.9°C; -0.5 CU between 16 and 18°C; and -1 CU above 18°C. Chilling inception occurs at the first incidence of -2.2°C. A weather station on the Blackberry Chilling Model station is close to our research center and allowed us to have accurate cumulative chilling units from their website. As with the FCM, we targeted 1,000 CU as the completion of the required chilling.

3) A visual development model (VDM), is 10% of plants showing shoot emergence in the spring. Shoot emergence is an indicator of dormancy breaking for perennial plants. And normally the rest of the peony plant will emerge and grow rapidly after shoot emergence begins.

Based on published reports for 'Sarah Bernhardt', we choose 1,000 CU for the first two

chilling models as the chilling required to break dormancy and promote regular plant growth and flowering, and to use as our indicator of the time for GA_3 applications. Since no chilling requirement recommendations have been reported for 'Inspecteur Lavergne', we used the same 1,000 CU for the first two models as for 'Sarah Bernhardt'. The air temperature was measured every hour using a thermo data logger (WatchDog Model 1000/2000, Spectrum Technologies, Inc. Plainfield, IL). In the 2018–19 season, the media temperature in the peony root-zone (at a depth of 5 cm) was measured from Dec. 7, 2018 to the end of the growing season on May 22, 2019.

GA_3

GA_3 (Florgib, Fine Americas, Inc., Walnut Creek, CA) drenches at 250 mL/pot were applied for each cultivar under each chilling model at 100 $mg \cdot L^{-1}$ (25 mg a.i./pot) or no GA_3 control. Applications were made right after the sufficient natural chilling accumulation (1,000 CU, FCM and BCM5) or shoot emergence reached 10% (VDM) unless the pots were frozen. If pots were frozen at that time, GA_3 drenches were applied as soon after that date as pots were thawed. According to these guidelines, GA_3 drenches were applied for FCM on 20 Jan. 2018, BCM5 on 8 Feb. 2018, VDM on 19 Feb. 2018 for the 2017–18 season (**Fig. 4-1**), and for FCM on 18 Jan. 2019, BCM5 on 6 Feb. 2019, VDM on 21 Feb. 2019 for 'Sarah Bernhardt' and on 26 Feb. 2019 for 'Inspecteur Lavergne' (visual 10% of plants showing shoot emergence were observed on different dates for each cultivar) for the 2018–19 season (**Fig. 4-2**).

Plant maintenance, measurements and data analysis

All plants were held in an unheated coldframe at Virginia Tech's Urban Horticulture Center (Blacksburg, VA) for one month after potting to promote rooting, then moved outdoors to a

gravel pad to receive natural chilling over the winter months (December through February/March). Buffer pots filled with media were used around the experimental plot. All pots were covered with a double layer of 2.5 oz. frost blanket (DeWitt's Ultimate, Strong, ME) and a single layer of 5-mil opaque poly for winter protection when the temperature was below -6.7°C and uncovered when the temperature was above 5°C outdoors. All plants were moved back to the coldframe in spring (light data unavailable) after emergence began, when they could no longer be safely covered for freeze protection. A fungicide drench (Subdue Maxx, Syngenta, Greensboro, NC) at 0.08 $mL \cdot L^{-1}$ was applied at 237 mL/pot two weeks after potting to protect plants from fungal infection. Additional insecticide and fungicide applications were applied in the spring as needed. Rodenticide bait stations were placed among the plants before covering and filled periodically during the winter. Irrigation was applied as needed when media was dry on top of the pot.

Evaluation began with the first plant emergence in the spring. Days to emergence, days to bud (cracked color) and days to first open flower were recorded twice weekly in the season of 2017–18 and daily in the season of 2018–19. Plant height, flower height and plant width, as well as number of shoots, number of vegetative shoots, number of shoots with blasted buds, and number of flowering shoots were recorded at finish (when >50% of plants in a treatment group had flowered). According to Kamenetsky et al. (2003), a shoot with a blasted bud is a shoot with a flower bud that aborted/blasted in the later stages of bud development and a vegetative shoot is a shoot without a flower bud or with flower bud that aborted in the early stages of development (bud smaller than 2 mm) (**Fig. 2-2**). Flowering percentage was recorded as the percentage of plants in a treatment group flowering at the end of the study when all plants have finished

flowering (for each individual plant of a treatment group, 1 = flowering and 0 = no flowering, the average flowering rate was converted to a percentage).

The experiment was a completely randomized design with three different GA_3 applications (applications timing according to chilling models) plus a no GA_3 control. Each cultivar set up as a separate experiment in each growing season. Each treatment consisted of 10 single plant replicates with treatments completely randomized. For all experimental datasets, data were analyzed by analysis of variance with mean separation by Student's *t* test at $P \leq 0.05$ using JMP® Pro 15, © SAS Institute Inc. (Cary, NC).

Results

2017–18 season

'Sarah Bernhardt'. We did not have sufficient data to analyze treatment effects on days to bud, days to flower and flower height because of lack of flowering (no flowers this season). GA_3 applied earlier according to the FCM and BCM5 reduced the time to emergence by more than one week compared to the control plant with no GA_3, while the later application using the VDM had no effect (**Table 4-1**). GA_3 applied later using the BCM5 and VDM reduced the plant height by 31% and 18% and reduced plant width by 14% and 3% compared to the control plants with no GA_3, while the earlier application using the FCM had no effect. GA_3 had no effected on the numbers of shoots and flowering regardless of application time. No flowering was observed this year.

'Inspecteur Lavergne'. GA_3 applied using the BCM5 reduced the time to emergence by more than one week compared to the control plants with no GA_3, while the earlier application

using the FCM and later application using VDM had no effect (**Table 4-1**). The GA_3 application had no effect on the days to bud (cracked color) regardless of timing. However, GA_3 applied later using the VDM increased the days to flower by more than one week compared to the control plants with no GA_3, or those plants with GA_3 applied earlier according to the FCM and BCM5 models. GA_3 application tended to reduce the plant height and plant width, especially GA_3 applied using the BCM5 reduced the plant width by 21% compared to the control plant with no GA_3. GA_3 applications had no significant effect on the numbers of shoots or percent of plants flowering. Low flowering was observed this year (10–20% with GA_3 treated plants and 40% with untreated plants).

2018–19 Season

In addition to air temperature, we also measured the substrate temperature during the 2018–19 season (7 Dec. 2018 through 22 May 2019), which covered the chilling period after the plants were moved to the nursery pad. Mean substrate temperature fluctuated less than air temperature and seldom dropped below 0°C compared to the air temperatures (**Fig. 4-3**).

'Sarah Bernhardt'. We did not have sufficient data to analyze treatment effects on days to bud, days to flower and flower height because of lack of flowering (0–10% this season). GA_3 applied earlier according to the FCM and BCM5 reduced the days to emergence by more than 10 days compared to the control plants with no GA_3, while the later application using the VDM had no effect (**Table 4-2**). GA_3 applications had no effects on plant height, plant width, or plant development (**Fig. 4-4**). The flowering on 'Sarah Bernhardt' was still very low in this season (0–10%).

'Inspecteur Lavergne'. GA_3 applications had no effect on days to emergence compared to

the control plants with no GA_3 (**Table 4-2**). GA_3 applied earlier according to the FCM reduced the days to bud (cracked color) by 7 days compared to the control plants with no GA_3, while the later application using the BCM5 and VDM had no significant effect. GA_3 applications had no effect on days to flower, plant height, plant width, the number of shoots, or percent plants flowering compared to the control plants with no GA_3 (**Fig. 4-3**).

Discussions

For herbaceous peonies grown under nursery conditions, the effect of the timing of GA_3 application has not been reported before. We developed these three models according to natural chilling accumulation to determine the best timing for GA_3 application and their effects on plant growth and flowering of peonies. According to the literature and our greenhouse (Chapter 2) peony experiments, 1,000 CU are required to break dormancy and induce flowering of many peony cultivars (Fulton et al., 2001; Halevy et al., 2002; Kamenetsky et al., 2003; Rhie et al., 2012; Yeo et al., 2012; Zhou, 2012). We chose 1,000 CU for the FCM and BCM5 as the timing guideline for GA_3 applications. Over our two seasons' study, the timing of GA_3 applications had less effect than expected. Also GA_3 cannot be applied when media/substrate is frozen. In the 2017–18 season, the time to reach the 1,000 natural CU according to the FCM was 25 days earlier than the time according to BCM5, but the continuous cold weather and frozen pots delayed our FCM application for 6 days. In the 2018–19 season, the time to reach the 1,000 natural CU according to the FCM was 14 days earlier than the time according to BCM5, but the continuous cold weather and frozen pots delayed our BCM5 application for 5 days.

We used air temperature for the FCM and BCM5 models just as most of other peony chilling studies have reported. Yeo at el. (2012) used the field soil temperature (at a depth of 5

cm) obtained from their weather station to calculate the CU using the FCM. Using media temperatures with the FCM, the peonies accumulated 680 CU as compared to 737 CU using air temperature over the chilling period on the nursery pad, 7 Dec. 2018 to the end of 1,000 CU on 18 Jan. 2019, a difference of only 57 CU.

Overall, the timing of the GA_3 applications according to the FCM or BCM5 was about a two to four weeks earlier than that of the VDM. The earlier GA_3 application according to the FCM or BCM5 reduced days to emergence of 'Sarah Bernhardt' peony plants in both seasons, while only GA_3 application according to the BCM5 reduced days to emergence of 'Inspecteur Lavergne' plants in 2017–18. Earlier application according to the FCM reduced days to bud for 'Inspecteur Lavergne' plants in 2018–19, and later GA_3 application according to the VDM increased days to flower for 'Inspecteur Lavergne' plants in 2017–18.

In our studies on herbaceous peonies under controlled chilling and greenhouse forcing conditions, GA_3 applications also reduced days to emergence of both cultivars in both seasons, and increased the number of shoots of 'Sarah Bernhardt' plants in both seasons, and of 'Inspecteur Lavergne' plants in 2018–19 (Chapter 2). GA_3 was used for herbaceous peonies to partially replace the chilling requirement when plants received insufficient chilling (Evans et al., 1990). 'Sarah Bernhardt' peony exhibited early flowering and high quality flowers with GA_3 treatment under forcing conditions, with an optimal GA_3 treatment of one 250 mL substrate drench of 100 $mg \cdot L^{-1}$ GA_3 (Halevy et al., 2002). GA_3 application enhanced plant growth and development, as well as shortened the time to emergence and flowering of Chinese forcing peony cultivar 'Da Fu Gui', where 250 mL of 200 $mg \cdot L^{-1}$ GA_3 was applied as a substrate drench (Cheng et al., 2009). GA_3 treated Korean peony cultivar Taebaek grown under natural chilling accumulation then forced under greenhouse conditions also had shorter days to emergence and

greater flowering than those not treated with GA_3 (Yeo et al., 2012). In these studies, GA_3 applications were all made after chilling, at the start of forcing.

In the 2017–18 season, all nursery grown peonies suffered spring cold damage which contributed to the low percentage of plant flowering ('Inspecteur Lavergne' only 20–30% flowering, and no flowers on 'Sarah Bernhardt'). The cold damage happened in late March 2018 when most plants had already emerged and were in active growth and still on the gravel pad where we could not apply the covering (**Fig. 3-3**). Both the untreated and GA_3 treated plants suffered cold damage to many shoots that did not recover later in the season. Damaged shoots were more susceptible to diseases such as gray mold (*Botrytis spp*.) and tobacco rattle virus, which reduced flowering and plant quality. Due to the earlier emergence, GA_3 treated plants may have suffered more damage than untreated plants which resulting in plants that were smaller than the control plants.

Plant growth in 2018–19 season was more protected than the previous season because we moved the plants to the coldframe when emergence began. The standard marketing quality of herbaceous peonies sold by Battlefield Farms is three and more flowering shoots per plant and these are normally two-year-old plants (J. Zeijlmaker, personal communication). In 2018–19 season, both cultivars of our GA_3 treated first year plants had around six shoots per plant, which would be marketable if we can reduce flower bud abortion and/or blasting, subsequently reducing the production time by one year.

Because of limitations to root growth, most containerized peonies have a lower flowering percentage compared with the field grown ones (Cheng, at el. 2009). Tree peony (*Paeonia suffruticosa* Andr.) showed similar results with 58% flowering of field grown plants as compared

to 21% in containers (Mornya and Cheng, 2018). All of our research plants were first year plants. Our two-year-old naturally chilled peonies of the two cultivars, Sarah Bernhardt and Inspecteur Lavergne, also proved that the flowering percentage of the peonies grown in containers (25%) was much lower than that of plants growing in the landscape (>90%) (data not shown). 'Sarah Bernhardt' peony especially has low flowering percentages in containers even when using two or three year-old plants under optimal growing conditions (Halevy et al., 2002; Kamenetsky et al., 2003).

Conclusion

Overall, GA_3 drench applications had positive effects on reducing the time to emergence of containerized peonies under nursery conditions. GA_3 can be applied after plants have acquired sufficient chilling (1,000 CU) using a suitable chilling model such as Fulton Chilling Model for peonies or Blackberry Chilling Model 5, or when 10% of plants showing shoot emergence. Timing of the GA_3 application was not critical to the effectiveness, but earlier applications tended to reduce time to emergence. In southwest Virginia (lat. 37° N, long. 80° W), our GA_3 application time was over a one-month period, from mid-late January to 10% of plants showing shoot emergence which happened in late February. Either a Fulton Chilling Model or Blackberry Chilling Model 5 can be used to determine the timing of spring GA_3 drench applications to containerized peonies under nursery conditions.

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Table 4-1. Chilling models as guidelines for spring GA_3 drenches under cold frame/nursery conditions of containerized *Paeonia lactiflora* 'Sarah Bernhardt' (SB) and 'Inspecteur Lavergne' (IL) in 2017–18 (FCM. Fulton Chilling Model using the WatchDog temperature sensors; BCM5. Blackberry Chilling Model 5; VDM. Visual shoot emergence in the spring after 10% shoot emergence).

Cultivar	Chilling Model	Days to emergence[z]	Days to bud	Days to flower	Plant Height (cm)[y]	Plant Width (cm)	Number of shoots	Number of vegetative shoots	Number of shoots with blasted buds	Number of flowering shoots	Flowering %
SB	Control	46.2 a[x]	*	*	36.9 a	36.4 a	4.6	3.3	1.3	0.0	0
	FCM	32.8 b	*	*	35.2 ab	36.6 a	5.6	5.0	0.6	0.0	0
	BCM5	36.9 b	*	*	25.6 c	31.3 b	6.6	6.2	0.4	0.0	0
	VDM	47.1 a	*	*	30.4 bc	35.2 b	5.3	4.4	0.9	0.0	0
	P-value	0.0064	*	*	0.0002	0.0191	0.2859	0.0971	0.0724	1.0000	1.0000
IL	Control	41.0 a	107.5	123.0 b	35.0	36.9 a	4.8	2.6	1.9	0.4	40
	FCM	48.0 a	110.0	128.0 ab	28.0	31.5 ab	4.8	3.2	1.2	0.4	20
	BCM5	30.4 b	113.0	126.0 ab	28.5	29.0 b	5.0	4.0	0.8	0.2	10
	VDM	42.9 a	117.7	134 a	31.1	32.3 ab	4.9	3.0	1.8	0.1	10
	P-value	0.0038	0.0773	0.0433	0.0536	0.0386	0.9937	0.3939	0.1029	0.6135	0.3090

[z] Days to emergence, measured from the day the first plant emerged (day 0).

[y] Plant height, tallest height of vegetative growth.

[x] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 10).

* Insufficient data to analyze.

Chilling Model:
FCM. Fulton Chilling Model using the WatchDog temperature sensors (GA applied on 20 Jan. 2018)
BCM5. Blackberry Chilling Model 5, North Crolina State University (GA applied on 8 Feb. 2018)
VDM. Visual shoot emergence in the spring (after 10% emergence) (GA applied on 19 Feb. 2018)

Table 4-2. Chilling models as guidelines for spring GA_3 drenches under cold frame/nursery conditions of containerized *Paeonia lactiflora* 'Sarah Bernhardt' (SB) and 'Inspecteur Lavergne' (IL) in 2018–19 (FCM. Fulton Chilling Model using the WatchDog temperature sensors; BCM5. Blackberry Chilling Model 5; VDM. Visual shoot emergence in the spring after 10% shoot emergence).

Cultivar	Chilling Model	Days to emergence[z]	Days to bud	Days to flower	Plant Height (cm)[y]	Plant Width (cm)	Number of shoots	Number of vegetative shoots	Number of shoots with blasted buds	Number of flowering shoots	Flowering %
SB	Control	35.4 a[x]	*	*	45.8	45.7	4.5	3.2	1.2	0.1	10
	FCM	20.4 c	*	*	39.7	43.7	6.5	5.1	1.4	0.0	0
	BCM5	25.0 bc	*	*	46.3	43.3	5.7	3.8	1.8	0.1	10
	VDM	33.1 ab	*	*	41.3	41.5	6.9	5.7	1.1	0.1	10
	P-value	0.0031	*	*	0.3429	0.4100	0.0838	0.1264	0.6125	0.8013	0.8013
IL	Control	35	81.6 ab	100.2	44.7	40.5	5.3	3.5	1.1	0.7	50
	FCM	27	74.8 c	96.3	42.7	40.5	6.5	5.2	0.8	0.5	40
	BCM5	27	75.8 bc	95.3	44.2	40.1	6.5	4.7	1.0	0.8	40
	VDM	29	83.8 a	98.0	43.3	41.3	6.6	4.0	1.6	1.0	70
	P-value	0.3376	0.0090	0.2323	0.9000	0.9218	0.1690	0.2511	0.3001	0.6952	0.5206

[z] Days to emergence, measured from the day the first plant emerged (day 0).

[y] Plant height, tallest height of vegetative growth.

[x] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 10).

* Insufficient data to analyze.

Chilling Model:
FCM. Fulton Chilling Model using the WatchDog temperature sensors (GA applied on 18 Jan. 2019)
BCM5. Blackberry Chilling Model 5, North Crolina State University (GA applied on 6 Feb. 2019)
VDM. Visual shoot emergence in the spring (after 10% emergence) (GA applied on 21 Feb. 2019 for SB or 26 Feb. 2019 for IL)

Fig. 4-1. Changes in the daily air temperatures and accumulated chilling units (CU) according to Fulton Chilling Model (FCM) and Blackberry Chilling Model 5 (BCM5) from November 2017 to February 2018. Arrows indicate the spring GA_3 drench applications according to the different chilling models for *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne'.

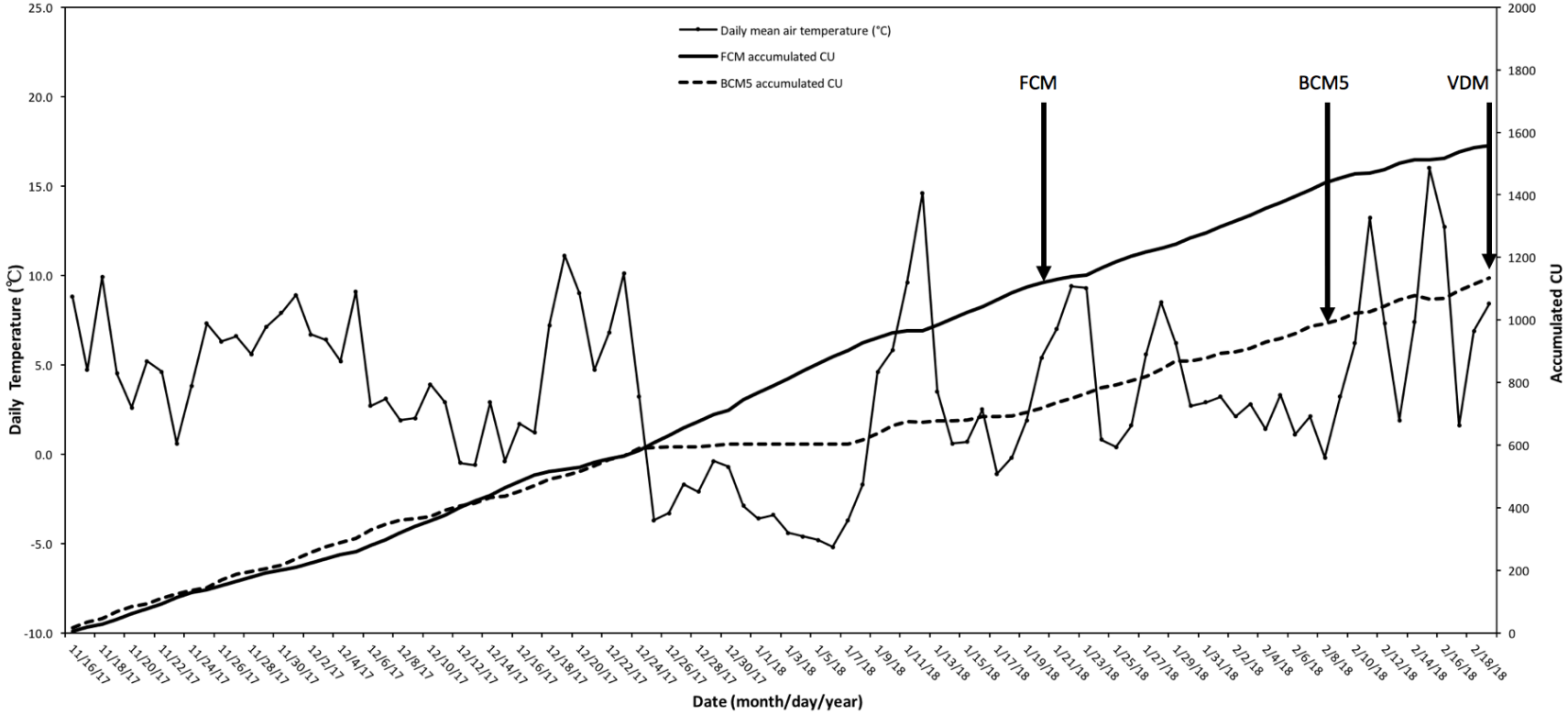

Fig. 4-2. Changes in the daily air temperatures and accumulated chilling units (CU) according to Fulton Chilling Model (FCM) and Blackberry Chilling Model 5 (BCM5) from November 2018 to February 2019. Arrows indicate the spring GA_3 drench applications according to the different chilling models for *Paeonia lactiflora* 'Sarah Bernhardt' (SB) and/or 'Inspecteur Lavergne' (IL).

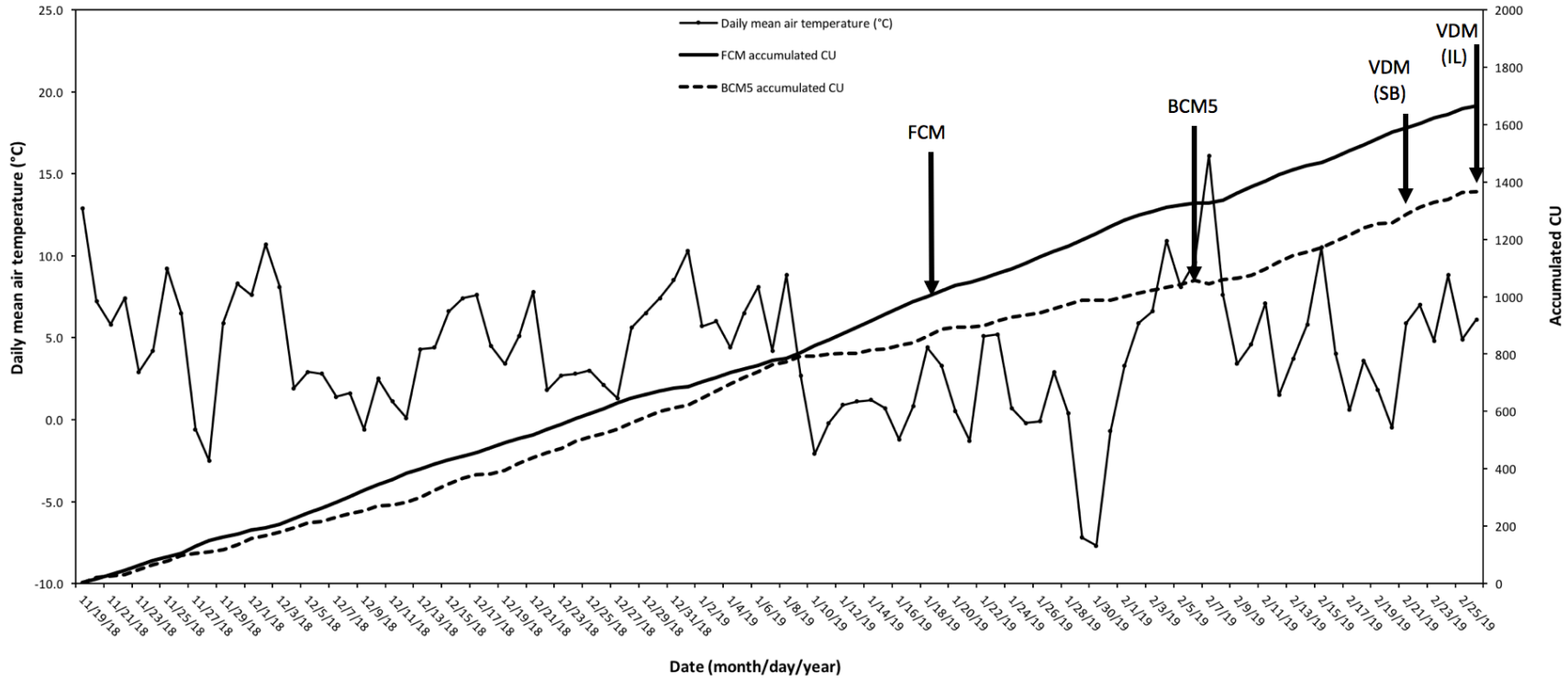

Fig. 4-3. Changes in the daily mean air temperatures and daily mean media/substrate temperatures and related accumulated chilling units (CU) according to Fulton Chilling Model from 7 Dec. 2018 to 18 Jan. 2019.

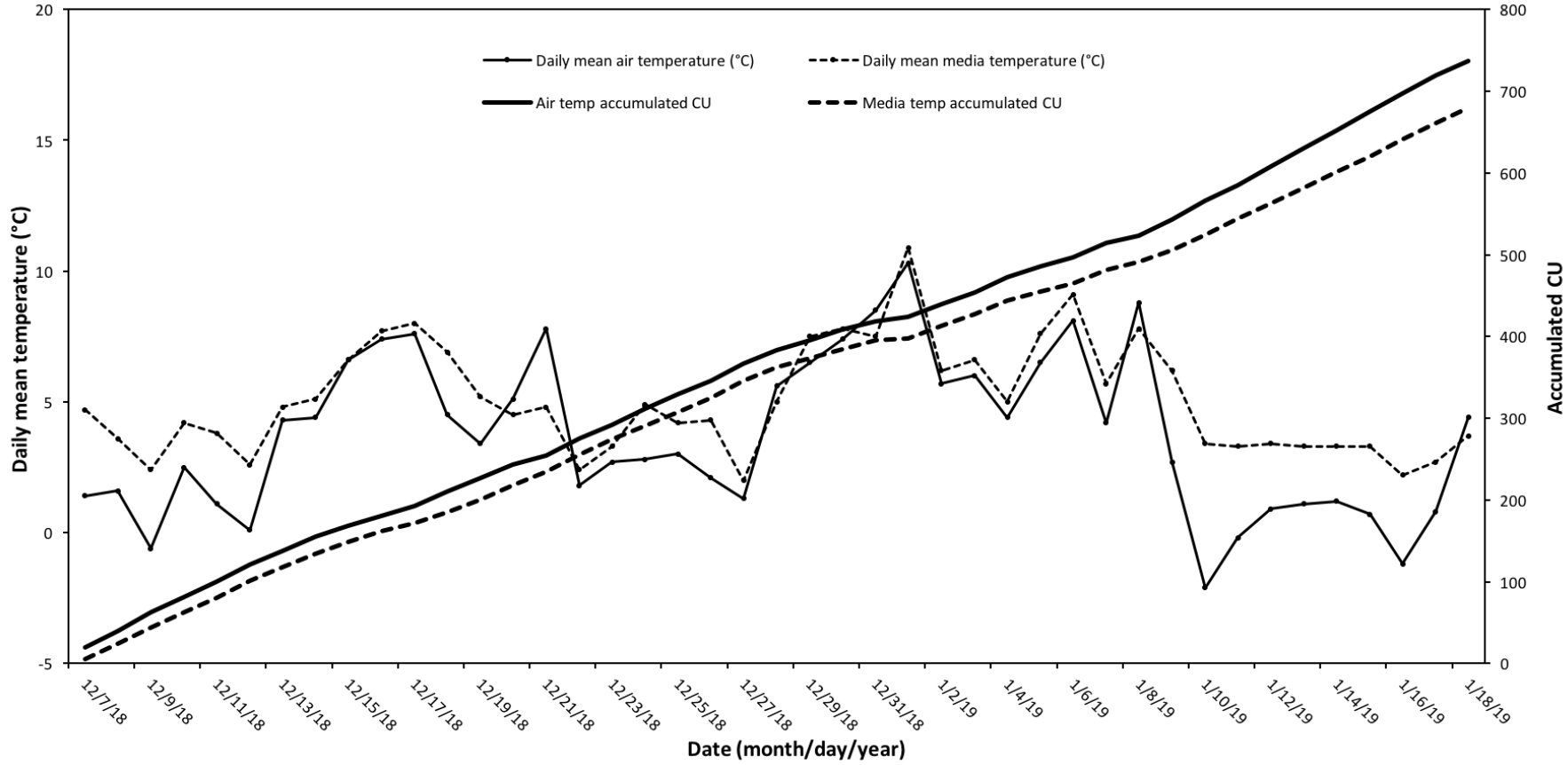

Fig. 4-4. Effects of chilling models as guidelines for spring GA_3 drenches under coldframe/field conditions of containerized *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne' in 2018–19. (FCM. Fulton Model using the WatchDog temperature sensors; BCM5. Blackberry Chill Model 5; VDM. visual 10% shoot emergence)

Chapter 5

Effect of GA_3 and Application Time on Bud Differentiation and Development of Containerized Herbaceous Peonies

Abstract

Herbaceous peonies (*Paeonia lactiflora* Pall.) are popular perennials used both in the landscape and commercial cut flower industry. Peonies require a chilling period to break dormancy but not for flower bud differentiation. We evaluated GA_3 effects on peony bud differentiation and development during controlled chilling and early forcing, as well as growth and flowering. Using two *P. lactiflora* cultivars, Sarah Bernhardt and Inspecteur Lavergne, small (3–5 eye) crowns from Holland were potted in 3.8-L pots in mid-November 2018. All potted plants were held in a 10°C cooler for 5.5 weeks (400 CU) to root, then placed in a 5°C cooler for 4 weeks (total 869 CU). GA_3 was applied at 0 or 100 $mg \cdot L^{-1}$ pre-chilling or post-chilling as a 250 ml/pot drench. Bud differentiation and development of excised buds were evaluated using a stereomicroscope at potting, after rooting (before chilling), after 1, 2, 3 or 4 weeks of chilling, and at 5, 10 or 15 days after the beginning of forcing. All buds were removed from the sample plants, measured for length and diameter, and dissected under a stereomicroscope to assess differentiation stages. Root dry weights and crown dry weights were also determined after rooting, after chilling, and at 15 days of forcing. Ten plants of each treatment were grown in the Virginia Tech greenhouse (Blacksburg, VA, 37° N) after chilling until flowering. GA_3 applications did not advance the bud development stage because most of buds were already in the reproductive stages before dormancy, but GA_3 enhanced bud elongation during chilling and the early forcing period. GA_3 applications can reduce the time to emergence and flowering, as well as increase the numbers of shoots and flowering shoots. GA_3 can be applied right after

rooting in, prior to the chilling period, or before greenhouse forcing, resulting earlier emergence and flowering with higher quality plants. However, earlier applications, pre-chilling, tended to produce plants with more shoots.

Introduction

As a traditional ornamental and medicinal perennial plant, herbaceous peony (*Paeonia lactiflora* Pall.) has more than 3,000 years' cultivation history in China and widely planted in many countries of temperate regions (Kamenetsky and Dole, 2012; Rogers, 1995; Yu et al., 2011)). There are more than 4,000 herbaceous peony cultivars registered by the American Peony Society, and hundreds of cultivars are still widely grown today across North America (APS, 2020). Recent years containerized herbaceous peonies are one of the main perennials in the potted perennial plant sector, with more than half million plants sold in 2018, valued at $6 million USD (USDA, 2019).

Most herbaceous peonies are grown from a perennial underground crown, which is an underground shoot that serves as an energy storage center for plant renewal (Din et al., 2015). A large number of buds develop on the surface of the crown and grow into monocarpic shoots with leaves and flowers after emergence in early spring. In northern hemisphere temperate regions, peonies flower once a year from May to July depending on location and cultivar, and blooms normally last for one to two weeks. The leaves remain green for a few months after flowering until the leaves senescence, at which time the peony plant enters dormancy for 3 to 4 months (Barzilay et al., 2002). The renewal buds initiate after flower senescence and remain vegetative during the summer. After senescence of the above ground shoots in the fall, the apical meristem of renewal buds reaches the generative stage, and begins floral initiation and differentiation until

early winter. Floral initiation and differentiation of peony do not require a chilling period (Barzilay et al., 2002).

Floral development of 'Sarah Bernhardt' was described in Israel (lat. 33° N, long. 35° E; Barzilay et al., 2002), and floral development of a few Chinese peony cultivars (early-, mid- or late flowering types) grown in the field were studied in Beijing, China (lat. 40° N, long. 116° E; Ai, 2016). Leaf primordia of renewal buds begin to form just after above ground flower senescence in June, and the buds turn from the vegetative stage to the generative stage after bracts appear in September, then flower differentiation begins in October with sepals, petals produced from the apical meristems, and slows down when enters dormancy in late November to early December. More than 50% of the flower buds for most of the three Chinese cultivars were in pistil primordium stage when the soil started freezing (Ai, 2016). The differentiation of peony floral buds did not have a specific physiological dormancy period; the dormancy start time was mainly affected by the environmental conditions and flower buds kept differentiating while the soil was frozen (Ai, 2016).

However, peonies require a period of cold accumulation to break dormancy and promote plant growth and flowering (Barzilay et al., 2002). In the North America, herbaceous peonies can be grown across USDA hardiness zone 3 to 8 (Michener and Adelman, 2017; Rogers, 1995). Several studies have determined chilling regimes for several commercial *P. lactiflora* cultivars all over the world (Aoki, 1991; Byrne and Halevy, 1986; Cheng et al. 2009; Evans et al., 1990; Kamenetsky et al., 2003; Zhang et al., 2019; Zhou, 2012). Overall, the chilling requirement of herbaceous peony is satisfied by a range of low temperatures with chilling hours accumulated over time. Increasing chilling time and decreasing temperature can reduce the time of emergence and increase flowering (Byrne and Halevy, 1986; Evans et al., 1990; Kamenetsky et al., 2003).

But excessive chilling can decrease flowering percentage and even prevent flowering (Aoki, 1991; Byrne and Halevy, 1986; Halevy et al., 2002). Fulton et al. (2001) developed a chilling model to calculate the chilling units (CU) accumulated by peony, in order to quantify the amount of chilling required to break dormancy. In this model, chilling units were calculated by the linear equation ($y = -0.0605x + 1$, $R^2 = 0.9943$), where y is the number of CU and x is the temperature from 0°C to 10°C. The optimal accumulated CU varies among cultivars (Fulton et al., 2001; Rhie et al., 2012; Yeo et al., 2012). The chilling requirement of *Paeonia lactiflora* cultivars from previous studies are listed in **Table 2-1**, where we calculated the chilling units by applying Fulton's Model to the chilling regime described by the authors of peony studies.

Gibberellins (GAs) are known as regulators of many developmental phases of higher plants, promoting cell elongation and plant growth, inducing hydrolytic enzymes in seed germination, inducing bolting in long-day plants, promoting flowering, and fruit setting and development (Rademacher, 2015; 2016). GA_3 is the most widely used GA in horticulture, viticulture and agriculture (Rademacher, 2015). GA_3 can be used to enhance flowering of perennial ornamental crops (Moond and Gehlot, 2006, 2007; Singh et al., 2017). GA_3 was also used on herbaceous peonies to partially replace the chilling requirement when plant received insufficient chilling, and to enhance plant growth, development and flowering (Cheng et al., 2009; Evans et al., 1990; Halevy et al., 2002; Yeo et al., 2012).

Our preliminary GA_3 and chilling studies showed that GA_3 applied after chilling and before shoot emergence of herbaceous peonies, significantly reduced days to emergence, days to bud (cracked color) and days to flower and increased the number of shoots and in some cases the number of flowering shoots (Chapter 2 and 4, Appendix 1). However, how GA_3 is applied pre- or post-chilling affects bud differentiation and development has not been reported. In this study,

our objective was to evaluate the effects of pre-chilling or post-chilling GA_3 application on bud differentiation and development of peony plants during dormancy and early forcing to better understand the effects of GA_3 on growth and flowering of the finished plants.

Materials and Methods

Preliminary study of bud differentiation stages

Bud differentiation stages were studied in October 2018 before the experiment using 2-year-old peony plants grown in the Urban Horticulture Center of Virginia Tech (Blacksburg, VA, lat. 37° N, long. 80° W). Each plant of the two cultivars, Sarah Bernhardt and Inspecteur Lavergne, were removed from the pot and the crowns were carefully washed clean of the substrate. All buds were excised from the surface of the crown, measured with a caliper for length and diameter, then cut into half using a razor blade, stained with 0.1% Toluidine Blue and observed under a dissecting microscope (Carl Zeiss SterREO Discovery V12, Göttingen, Germany) for the differentiation and development stages. More than 100 buds of each cultivar were examined. Bud differentiation was categorized into five stages (**Fig. 5-1** and **5-2**).

Stage 1: Vegetative, no reproductive parts;

Stage 2: Vegetative, hollow center forming;

Stage 3: Transition between vegetative and reproductive, leaf primordia forming;

Stage 4: Reproductive, leaf primordia and bracts present;

Stage 5: Reproductive, bracts and sepal presents, flower differentiation begins with sepal primordia, petal primordia and other reproductive parts produced from apical meristems, flower forming.

Data showed that the length and diameter of the bud had no correlation with the bud differentiation stage (data not shown), so we determined that each bud from all treatment plants will be cut and observed under the dissecting microscope to assess the development stage.

Plant materials

Three to five eye peony crowns of 'Sarah Bernhardt' and 'Inspecteur Lavergne' were imported to Battlefield Farms (Rapidan, VA) from a Dutch commercial source in the fall of 2018. 'Sarah Bernhardt' is a double, late flowering type with very large dark rose pink flower, medium height, floriferous, strong stems, and good foliage (**Fig. 5-1f**). 'Inspecteur Lavergne' is a double, early flowering type, with globular crimson flower with frilled petals in the center, and long straight stems (**Fig. 5-2F**). All crowns were potted in 3.8-L pots using Battlefield Farm's peony substrate (60% hydro fiber, 40% peat) with 5 cm of media over the crown in mid-November 2018. Controlled release fertilizer (17N–2.2P–9.1K, Osmocote Pro, 12-14 month, ICL Specialty Fertilizers, Summerville, SC) was incorporated at 5.3 kg/m^3 at time of potting. All crowns were sorted for uniformity of number of eyes and size of crown and roots prior to potting.

Rooting, chilling and greenhouse forcing conditions

All potted plants were placed on plant racks and in 10°C cooler for 5.5 weeks (400 CU, respectively) to allow rooting in, irrigating on the rack as necessary at Battlefield Farms

(Rapidan, VA, lat. 38°N, long. 78°W). After 400 CU, all plants were placed in a cooler at 5°C for 4 weeks (total 869 CU received) without light. After chilling, plants were transported to the Virginia Tech double layer polyethylene greenhouse (Blacksburg, VA, lat. 37° N, long. 80° W) which maintained an average 18.7°C day / 15.7°C night temperature with an average daily light integral (DLI) of 11.0 $mol \cdot m^{-2} \cdot d^{-1}$. The air temperature and DLI were measured hourly using a thermo data logger (WatchDog Model 1000/2000, Spectrum Technologies, Inc. Plainfield, IL).

GA_3 drenches

Based on previous studies (Chapter 2 and Chapter 4), GA_3 (Florgib, Fine Americas, Walnut Creek, CA) drenches were applied to each cultivar at 0 or 100 $mg \cdot L^{-1}$ at 250 ml/pot at one of two times: 1) pre-chilling: after the rooting period (400 CU) and before chilling commenced; or 2) post-chilling: after the plants were chilled for 4 weeks (total 869 CU received), the day after plants were moved into the Virginia Tech greenhouse for forcing.

Bud differentiation and development data collecting

Buds were collected from plants of each cultivar for evaluation at multiple times: 1) before potting; 2) from untreated plants and plants treated of GA_3 pre-chilling at 1, 2, 3 or 4 weeks of chilling; and 3) from untreated, pre-chilling GA_3 treated, and post-chilling GA_3 treated plants at 5, 10 and 15 days of greenhouse forcing. Each plant was removed from the pot and the crown was carefully washed clean of the potting substrate. All buds were excised from the surface of each crown, measured with a caliper for their length and diameter, then cut into half using a razor blade, stained with 0.1% Toluidine Blue and observed under dissecting microscope for the differentiation and development stages.

Root and crown measurements

After the buds were measured, the roots and crown of each plant were carefully separated to determine dry weights. Samples were harvested after rooting in (untreated plants); after 4 weeks chilling (untreated and pre-chilling GA_3 treated plants); and after 15 days of greenhouse forcing (untreated, pre-chilling GA_3 treated and post-chilling GA_3 treated plants). The root and crown tissues of each plant were put into paper bags separately and placed in a dryer at 60°C for 72 hours until their weight became constant. Root dry weight and crown dry weight of each plant were recorded and analyzed.

The bud development study was arranged in a completely randomized design with six single plant replicate. Each cultivar was set up as a separate experiment.

Spring measurements and data analysis

To evaluate the effect of timing GA_3 application on peony growth and development, ten plants of each treatment (0 GA_3 control, 100 $mg \cdot L^{-1}$ pre-chilling and 100 $mg \cdot L^{-1}$ post-chilling) of each cultivar were grown in the Virginia Tech Greenhouse through flowering. Irrigation was applied as needed when media was dry on top of the pot, generally daily by hand-watering. Plant height, flower height and plant width, as well as number of shoots, number of vegetative shoots, number of shoots with blasted buds, number of flowering shoots, and number of flowers were recorded at finish (when >50% of plants in a treatment group had flowered). According to Kamenetsky et al. (2003), a shoot with a blasted bud is a shoot with a flower bud that aborted/blasted in the later stages of bud development and a vegetative shoot is a shoot without a flower bud or with flower bud that aborted in the early stages of development (bud smaller than 2 mm) (**Fig. 2-2**). Flowering percentage was recorded as the percentage of plants in a treatment

group flowering at the end of the study when all plants have finished flowering (for each individual plant of a treatment group, 1 = flowering and 0 = no flowering, the average flowering rate was converted to a percentage).

The experiment to evaluate plant growth and flowering was arranged in a completely randomized design with each cultivar set up as a separate experiment. Each treatment consisted of 10 single plant replicates with treatments completely randomized within cultivar. For all experimental datasets, data were analyzed by analysis of variance with mean separation by Student's *t* test at $P \leq 0.05$ using JMP® Pro 15, © SAS Institute Inc. (Cary, NC).

Results

Bud differentiation and development

The number of buds was relatively constant over the chilling period (**Table 5-1**). Pre-chilling GA_3 application did not affect the number of buds per crown during each chilling period as compared to plants not treated with GA_3. However, the pre-chilling GA_3 treatment increased the number of buds significantly at 15 days of greenhouse forcing, from 5.2 (untreated) to 10.7 buds (pre-chilling treated) for 'Sarah Bernhardt' plants. Post-chilling GA_3 application also increased the number of buds significantly at 15 days of greenhouse forcing from 5.2 (untreated) to 11.8 buds (treated post-chilling) for 'Sarah Bernhardt' plants. GA_3 application did not affect the number of buds per crown for 'Inspecteur Lavergne' plants.

With respect to the bud development stage over time, buds of 'Sarah Bernhardt' were fully reproductive (Stage 4.3) at the time of potting (**Table 5-2**) and were not affected by pre- or post-chilling GA_3 application. Buds of 'Inspecteur Lavergne' were also fully reproductive (Stage 4.4)

at the time of potting. Pre-chilling GA_3 application only significantly affected the bud stage of 'Inspecteur Lavergne' by enhancing the bud development during the 1st week of chilling and 10 days of greenhouse forcing. Post-chilling GA_3 application only affected the bud stage of 'Inspecteur Lavergne' by hastening the bud development during the 10 days of greenhouse forcing.

Pre-chilling GA_3 application significantly increased the bud length (elongation) of 'Sarah Bernhardt' plants during the 2nd and 4th week of chilling and the first 10 days of initiation of greenhouse forcing, but post-chilling GA_3 application had no effect (**Table 5-3**). Pre-chilling GA_3 application increased the bud length of 'Inspecteur Lavergne' plants during the 1st, 3rd and 4th week of chilling and the first 15 days of greenhouse forcing. Post-chilling GA_3 application only significantly increased bud length of 'Inspecteur Lavergne' plants at 15 days of greenhouse forcing, relative to buds from untreated plants.

Pre-chilling and post-chilling GA_3 application reduced bud diameter of 'Sarah Bernhardt' plants only at 15 days of greenhouse forcing (**Table 5-4**). Only post-chilling GA_3 application reduced bud diameter of 'Inspecteur Lavergne' plants at 5 and 15 days of greenhouse forcing.

Neither pre-chilling nor post-chilling GA_3 application affected root dry weight of either cultivar (**Table 5-5**, **Fig. 5-3**). Pre-chilling GA_3 application reduced the crown dry weight of 'Inspecteur Lavergne' plants after 4 weeks of chilling but there were no differences at 15 days of forcing (**Table 5-6**).

Plant growth and flowering

'Sarah Bernhardt'. The pre-chilling GA_3 drench application reduced days to emergence by 7

days, days to bud (cracked color) by 15 days, and days to flower by 13 days, while the post-chilling GA_3 drench application had no effect on days to emergence or days to bud, but reduced the time to flower by 6 days compared to untreated plants (**Table 5-7**). Neither pre-chilling nor post-chilling GA_3 application affected plant height or plant width. Pre-chilling GA_3 application increased the number of shoots by 1.4 shoots/plant but post-chilling GA_3 application had no significant effect (**Table 5-7**, **Fig. 5-4**). Neither pre-chilling nor post-chilling GA_3 application had a significant effect on the number of vegetative shoots, shoots with blasted buds, or flowering.

'Inspecteur Lavergne'. Both pre-chilling or post-chilling GA_3 drench applications reduced days to emergence by about 14 days, days to bud (cracked color) by 15 or 16 days, and days to flower by 13 or 12 days (**Table 5-7**). Neither pre-chilling nor post-chilling GA_3 application affected plant height or width. Pre-chilling GA_3 application significantly increased the number of shoots by 2 shoots/plant but post-chilling GA_3 application had no effect (**Table 5-7**, **Fig. 5-4**). Neither pre-chilling nor post-chilling GA_3 application affected the number of vegetative shoots or shoots with blasted buds. Post-chilling GA_3 application increased the number of flowering shoots by 1.4 shoots/plant and the number of flowers by 1.6 flowers/plant, while pre-chilling GA_3 application had a non-significant effect.

Discussion

Neither pre-chilling nor post-chilling GA_3 application affected the number of buds developing during the chilling period while plants were in dormancy. GA_3 application also had little effect on the bud development stage because most of the buds on the crown were already in the reproductive stages (Stages 4–5) at the time of potting. We received our peony crowns in

November from commercial growers in the Netherlands, just like many nursery operators. At that time, the renewal buds on the peony crown were already developed to the reproductive stages. Research of floral development with several herbaceous peony cultivars in different locations also found early development of renewal buds. Renewal buds of 'Sarah Bernhardt' in Israel (lat. 33° N, long. 35° E) transitioned from the vegetative stage to the generative stage in September, and flower differentiation began in October with sepals, petals and petaloids produced from apical meristems, and ceased at early December (Barzilay et al., 2002). Floral development studies of five Chinese peony cultivars, including early-flowering ('Da Fu Gui' and 'Dongfang Hong'), mid-flowering ('Zi Fengyu'), and late-flowering ('Qingwen' and 'Taohua Feixue'), in Beijing, China (lat. 40° N, long. 116°E) showed all the cultivars grown in the field began the formation of leaf primordia in renewal buds in early June, bract primordia formation in late August, sepal primordia formation in late September, petals in early October, and stamen primordia in late October (Ai, 2016; Zhou, 2012). As in our studies, most renewal buds were already in the reproductive stage when plants entered dormancy from these studies. The bud studies of a Japanese wild type peony also reported renewal buds started entering reproductive stage in October, and all buds were in reproductive stage in early November (Aoki, 1991).

Over the one month rooting period, the renewal buds continued to develop, with 38% increase in length on 'Sarah Bernhardt' buds and 43% increase in length on 'Inspecteur Lavergne' buds compared to buds on crown at the time of potting. That is similar with Park et al. (2015) studies, where 'Taebaek' peony buds elongated during a two-week pre-chilling treatment at 10°C. Pre-chilling GA_3 application had significant effects on bud development of both cultivars. GA_3 application increased bud length which is an indication of bud growth during chilling and reduced the time to emergence which promoted more uniform shoot emergence. Bud

diameter was reduced at early greenhouse forcing due to the rapid shoot elongation. All these indicate GA_3 application can enhance bud development.

GA_3 application did not affect root growth of herbaceous peonies. Roots started growing after potting and grew slowly during chilling for both cultivars. After initiation of the greenhouse forcing (0 to 15 days), roots kept growing slowly on 'Sarah Bernhardt', but grew rapidly on 'Inspecteur Lavergne' plants. Crown dry weights are related with carbohydrate supply for bud growth and development because the carbohydrate supply for spring growth and development of the herbaceous peony is stored in the crowns (Walton et al., 2007). Pre-chilling GA_3 application reduced crown dry weight of 'Inspecteur Lavergne', perhaps due to the hydrolysis of non-structural starch in the crowns to sugars to supply the rapid bud development as well as the rapid root growth. Starch would be restored after shoots emerge and growth leads to starch accumulation.

Neither pre-chilling nor post-chilling GA_3 application increased the number of buds on crowns at early forcing of 'Sarah Bernhardt' plants. But overall, there were more renewal buds (7 to 8) on the crowns before dormancy release than the number of shoots that emerged and developed (2 to 4 shoots per plant on 'Sarah Bernhardt' or 3 to 6 shoots per plant on 'Inspecteur Lavergne'). Apparently only the stronger buds emerged and developed into the shoots.

In addition, our study showed GA_3 applications had significant effects on plant growth and flowering of both cultivars especially with the pre-chilling application, which consistently reduced days to emergence, days to bud (cracked color) and days to flower of plants of both cultivars. Post-chilling GA_3 treatment had similar effects only on 'Inspecteur Lavergne' plants. Pre-chilling GA_3 application also significantly increased the numbers of shoots for both cultivars

while post-chilling application did not. GA_3 application right after chilling is the recommended method for herbaceous peonies in several studies. Our other GA_3 studies on greenhouse forcing herbaceous peonies also found a reduction in days to emergence on both cultivars in two seasons with GA_3 applications (Chapter 2). In addition, GA_3 applications increased the number of shoots of 'Sarah Bernhardt' plants in two seasons, and of 'Inspecteur Lavergne' plants in 2018-2019 (Chapter 2). Also in our nursery studies, GA_3 applied earlier, after sufficient natural chilling, reduced days to emergence of 'Sarah Bernhardt' peony plants in two seasons, and of 'Inspecteur Lavergne' plants in 2017-2018 (Chapter 4). GA_3 applications also increased the number of shoots on both cultivars grown in the nursery in the 2018-2019 season (Chapter 4). Researchers from Israel found very early flowering and high quality 'Sarah Bernhardt' flowers were obtained with a soil drench of 250 mL of 100 $mg \cdot L^{-1}$ GA_3 applied after chilling (Halevy et al., 2002). In addition, flower production was doubled with GA_3 treatment for two other field-grown cultivars (Karl Rosenfeld and Duchesse de Nemours). Chinese researchers found 250 mL of 200 $mg \cdot L^{-1}$ GA_3 enhanced plant growth and development of 'Da Fu Gui' peony, as well as reduced the time to emergence and increased flowering (Cheng et al., 2009). Yeo et al. (2012) reported a soil drench of 300 mL of 100 $mg \cdot L^{-1}$ GA_3 reduced the days to emergence and to flower while increasing the percentage of plants flowering with Korea cultivar Taebaek subjected to insufficient natural chilling accumulation (429–876 CU).

GA_3 application did not affect bud blasting for either cultivars in our study. Park et al. (2015) reported that a pre-chilling treatment of two weeks at 10°C prior to an appropriate chilling period after November, promoted flowering of 'Taebaek' peony and reduced flower bud abortion. Our studies using 5.5 weeks pre-chilling at 10°C in November, along with a GA_3 application, our 'Inspecteur Lavergne' had 90% of plants flowering with about two flowers per

plant.

Depending on the cultivar, pre-chilling GA_3 application can have plants ready earlier with more shoots than post-chilling application. Plants of both cultivars treated with GA_3 prior to chilling flowered 13 days earlier than untreated plants. Plants treated with GA_3 prior to chilling also had 1.6 to 2 more shoots per plant. 'Inspecteur Lavergne' plants treated with GA_3 after chilling had three times the number of flowering shoots. The standard marketing quality of herbaceous peonies sold by Battlefield Farms is three and more flowering shoots per plant, and normally are two-year-old plants (J. Zeijlmaker, personal communication). Although our pre-chilling and post-chilling GA_3 treated first year plants exhibited some differences, but both had 3.7 to 5.8 shoots per plant which would make them marketable if we could reduce flower bud abortion/blasting and promote more flowering, subsequently reducing the production time by one year.

Conclusion

Overall, GA_3 applications have positive effects on greenhouse forcing peony production. GA_3 applications did not advance the bud development stage because most of buds were already in the reproductive stages before dormancy, but GA_3 enhances bud growth during chilling and the early forcing period. GA_3 applications can reduce the time to emergence and flowering, as well as increase the numbers of shoots and flowering shoots. GA_3 can be applied right after rooting, during the chilling period, or before greenhouse forcing, resulting in earlier production (about 10 to 15 days) with higher quality plants. However, earlier applications pre-chilling tended to produce plants with more shoots.

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Table 5-1. Effect of GA_3 application on number of buds of *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne' at each production stage.

Number of buds	GA (100 mg·L^{-1})	Potting	After rooting	1-week chilling	2-week chilling	3-week chilling	4-week chilling	5-day forcing	10-day forcing	15-day forcing
Sarah Bernhardt	0	5.3	9.8	5.8	7.5	5.7	7.0	8.0	7.8	5.2 b[z]
	Pre-chilling			6.2	5.3	7.8	8.5	7.5	8.0	10.7 a
	Post-chilling							6.8	8.1	11.8 a
	P-value			0.8458	0.2001	0.1045	0.3476	0.6531	0.9719	0.0010
Inspecteur Lavergne	0	7.7	8.8	8.8	9.2	9.0	9.0	7.3	7.0	7.0
	Pre-chilling			10.0	8.0	7.7	6.7	9.0	7.8	9.2
	Post-chilling							9.5	8.0	9.5
	P-value			0.4935	0.1504	0.5189	0.0749	0.3696	0.7949	0.2767

[z] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 6)

Table 5-2. Effect of GA_3 application on development stage of buds of *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne' at each production stage.

Bud stage	GA (100 mg·L^{-1})	Potting	After rooting	1-week chilling	2-week chilling	3-week chilling	4-week chilling	5-day forcing	10-day forcing	15-day forcing
Sarah Bernhardt	0	4.3	4.0	4.3	4.3	4.5	4.5	4.5	4.5	4.8
	Pre-chilling			4.3	4.7	4.6	4.7	4.6	4.7	4.7
	Post-chilling							4.7	4.6	4.7
	P-value			0.8970	0.1395	0.8802	0.2622	0.3761	0.3906	0.2513
Inspecteur Lavergne	0	4.4	4.5	4.3	4.7	4.4	4.8	4.6	4.2 b[z]	5
	Pre-chilling			4.6	4.6	4.6	4.7	4.6	4.7 a	5
	Post-chilling							4.5	4.8 a	5
	P-value			0.0173	0.4537	0.1678	0.7985	0.8114	<0.0001	0.9340

[z] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 6)

Table 5-3. Effect of GA_3 application on bud length of *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne' at each production stage.

Bud length (mm)	GA (100 mg·L^{-1})	Potting	After rooting	1-week chilling	2-week chilling	3-week chilling	4-week chilling	5-day forcing	10-day forcing	15-day forcing
Sarah Bernhardt	0	10.1	13.9	18.9	20.6	27.6	24.9	20.5 b[z]	27.6 b	41.4
	Pre-chilling			18.3	42.8	23.8	46.9	37.3 a	83.8 a	58.6
	Post-chilling							26.3 ab	54.0 ab	38.1
	P-value			0.8273	<0.0001	0.4124	0.0034	0.0411	0.0001	0.0663
Inspecteur Lavergne	0	9.6	13.7	12.0	13.7	12.3	28.1	16.8 b	17.8 b	25.6 b
	Pre-chilling			16.2	15.9	20.9	56.0	34.8 a	37.1 a	44.5 a
	Post-chilling							19.3 ab	26.8 b	46.4 a
	P-value			0.0053	0.0975	0.0004	0.0251	0.0005	<0.0001	0.0022

[z] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 6)

Table 5-4. Effect of GA_3 application on bud diameter of *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne' at each production stage.

Bud diameter (mm)	GA (100 mg·L^{-1})	Potting	After rooting	1-week chilling	2-week chilling	3-week chilling	4-week chilling	5-day forcing	10-day forcing	15-day forcing
Sarah Bernhardt	0	5.2	4.4	5.3	4.4	5.3	4.6	5.0	4.4	5.8 a[z]
	Pre-chilling			4.5	5.1	4.9	3.8	3.9	4.3	4.0 b
	Post-chilling							4.8	4.1	3.9 b
	P-value			0.0932	0.1832	0.2689	0.0674	0.0707	0.8155	0.0004
Inspecteur Lavergne	0	5.0	5.1	5.5	5.8	5.2	4.6	5.4 a	5.4	6.3 a
	Pre-chilling			5.2	5.7	4.7	5.1	5.3 a	5.5	6.3 a
	Post-chilling							4.4 b	5.5	5.3 b
	P-value			0.4882	0.7863	0.1227	0.2607	0.0090	0.8850	0.0271

[z] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 6)

Table 5-5. Effect of GA_3 application on root dry weight of *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne' at each production stage.

Roots dry weight (mg)	GA (100 mg·L^{-1})	After rooting	After 4-week chilling	After 15-day forcing
Sarah Bernhardt	0	0.25	0.36	0.41
	Pre-chilling		0.35	0.37
	Post-chilling			0.42
	P-value		0.9356	0.8870
Inspecteur Lavergne	0	0.22	0.26	0.95
	Pre-chilling		0.16	1.06
	Post-chilling			0.73
	P-value		0.1422	0.5755

Student's *t* Test, $P \leq 0.05$, n = 6.

Table 5-6. Effect of GA_3 application on crown dry weight of *Paeonia lactiflora* 'Sarah Bernhardt' and 'Inspecteur Lavergne' at each production stage.

Crown dry weight (mg)	GA (100 mg·L^{-1})	After Rooting	After 4-week chilling	After 15-day forcing
Sarah Bernhardt	0	42.26	42.72	50.81
	Pre-chilling		43.63	39.96
	Post-chilling			47.40
	P-value		0.8500	0.2914
Inspecteur Lavergne	0	42.83	49.40	40.51
	Pre-chilling		28.40	38.50
	Post-chilling			52.46
	P-value		0.0074	0.3264

Student's *t* Test, $P \leq 0.05$, n = 6.

Table 5-7. Effect of GA_3 application on growth and flowering of *Paeonia lactiflora* 'Sarah Bernhardt' (SB) and 'Inspecteur Lavergne' (IL) at each production stage.

Cultivar	GA (100 mg·L^{-1})	Days to emergence[z]	Days to bud	Days to flower	Plant height (cm)[y]	Plant width (cm)	Number of shoots	Number of vegetative shoots	Number of shoots with blasted buds	Number of flowering shoots	Number of flowers	Flowering %
SB	0	8.8 a[x]	47.0 a	67.5 a	37.9	45.7	2.7 b	1.6	0.8	0.2	0.4	20
	Pre-chilling	1.9 b	31.6 b	54.6 c	36.5	40.8	4.1 a	2.2	0.9	0.9	0.9	50
	Post-chilling	11.1a	45.1 a	61.8 b	37.6	45.1	3.7 ab	1.4	1.7	0.6	0.6	40
	P-value	0.0013	<0.0001	0.0011	0.8962	0.1183	0.0125	0.2928	0.0571	0.1264	0.4312	0.3922
IL	0	21.8 a	49.0 a	67.4 a	34.7	43.3	3.8 b	2.8	0.3	0.7 b	0.7 b	50
	Pre-chilling	7.8 b	34.2 b	54.1 b	40.1	40.4	5.8 a	3.8	0.5	1.4 ab	1.7 ab	90
	Post-chilling	8.0 b	33.4 b	55.6 b	39.2	41.4	4.9 ab	2.2	0.3	2.1 a	2.3 a	80
	P-value	<0.0001	<0.0001	0.0005	0.0748	0.4433	0.0182	0.0722	0.6653	0.0369	0.0347	0.1156

[z] Days to emergence, measured from the beginning of greenhouse forcing.

[y] Plant height, tallest height of vegetative growth.

[x] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 10).

Fig. 5-1. Development Stages of *Paeonia lactiflora* 'Sarah Bernhardt' buds: a. Stage 1, vegetative, no reproductive parts; b. Stage 2, vegetative, hollow center forming; c. Stage 3, transition between vegetative and reproductive, leaf primordia forming; d. Stage 4, reproductive, leaf primordia and bracts present; e. Stage 5, reproductive, bracts and sepal presents, flower forming; f. Flower. (LP=Leaf primordia; Br=Bract; Sep=Sepal)

Fig. 5-2. Development Stages of *Paeonia lactiflora* 'Inspecteur Lavergne' buds: A. Stage 1, vegetative, no reproductive parts; B. Stage 2, vegetative, hollow center forming; C. Stage 3, transition between vegetative and reproductive, leaf primordia forming; D. Stage 4, reproductive, leaf primordia and bracts present; E. Stage 5, reproductive, bracts and flower primordia presents, flower forming; F. Flower. (LP=Leaf primordia; Br=Bract; Sep=Sepal)

Fig. 5-3. Comparison of the effect of GA_3 applied pre-chilling and post-chilling on buds and roots of *Paeonia lactiflora* 'Sarah Bernhardt' (SB) and 'Inspecteur Lavergne' (IL) at 15 days after greenhouse forcing.

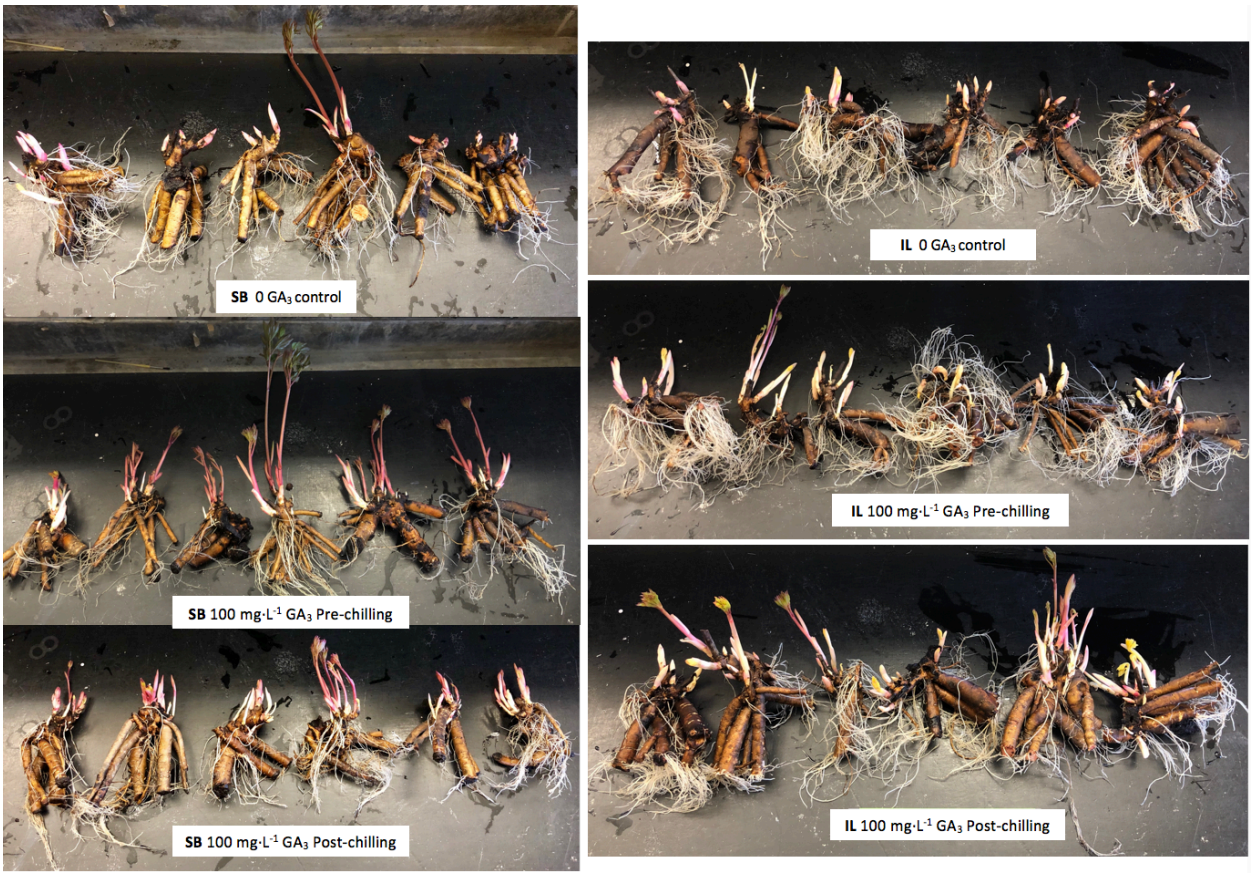


Fig. 5-4. Effect of GA_3 applied pre-chilling and post-chilling on plant growth and flowering of *Paeonia lactiflora* 'Sarah Bernhardt' (SB) and 'Inspecteur Lavergne' (IL).

APPENDIX 1

2016–17 Preliminary Studies on Containerized Herbaceous Peonies: Responses to Different PGR Application Methods

(Performed by Rachel Mack and Joyce Latimer, data not published)

Uniconazole was applied by three different methods in the preliminary studies in fall 2016: sprenches, dips (2-minutes soaks) and drenches.

1) PGR (Uniconazole) sprenches (natural chilling)

Material and methods: Two peony cultivars, Sarah Bernhardt and Karl Rosenfeld, were treated with uniconazole (Concise, Fine Americas, Walnut Creek, CA) sprenches at 0, 10 or 20 $mg \cdot L^{-1}$ at 840 $mL \cdot m^{-2}$ (4 times of spray volume) in November 2016.

Results: Neither cultivar had a significant growth response in spring 2017 following the fall sprench applications (data not presented).

2) PGR dips (natural chilling)

Material and methods: Two peony cultivars, Sarah Bernhardt and Inspecteur Lavergne, of two crown sizes (3-5 eyes in 3.8-L pot, or 3-5 eyes in 7.6 L pot) were used. Three PGRs were applied as 2-minute soaks: paclobutrazol (Piccolo 10 XC, Fine Americas, Walnut Creek, CA, 10 or 20 $mg \cdot L^{-1}$), uniconazole (Concise, Fine Americas, 3 or 6 $mg \cdot L^{-1}$), or benzyladenine (Configure, Fine Americas, Walnut Creek, CA, 250 or 500 $mg \cdot L^{-1}$).

Results: Plants from large crowns were slightly wider and had a greater number of shoots than those from small crowns in both cultivars (data not presented). In ‘Sarah Bernhardt’, plants

from large crowns also had a greater number of flowering shoots and a higher flowering percentage than plants from small crowns (data not presented). None of the PGR soaks had a significant impact on growth or development of the plants (data not presented). PGR soaks did not cause plant death, a side effect previously observed with longer soak times.

3) PGR (GA_3 and uniconazole) drenches (controlled chilling and greenhouse forcing)

Material and methods: Two peony cultivars, Sarah Bernhardt and Inspecteur Lavergne, 3 to 5 eyes in 3.8-L pot, were pre-chilled at 5°C for 6 weeks followed by one month of natural rooting. GA_3 (Florgib, Fine Americas, Walnut Creek, CA) at 0 or 100 $mg \cdot L^{-1}$ drench (250 ml/pot) was applied soon after moving plants from the cooler to the greenhouse. Uniconazole (UNZ; Concise, Fine Americas) at 0, 10 or 20 $mg \cdot L^{-1}$ drench (355 mL/ pot) was also applied at 7 days after the GA_3 application.

Results: GA_3 increased the shoot number of both cultivars and the number of flowering shoots in 'Inspecteur Lavergne' (**Table A1-1**, **A1-2**). UNZ drenches resulted in moderate growth regulation. However, the percentage of plants flowering was low (less than 50%) (**Table A1-1**, **A1-2**; **Fig. A1-1**).

Conclusion: The spring applied drench was the most effective PGR application method for containerized herbaceous peony. A 100 $mg \cdot L^{-1}$ GA_3 drench prior to emergence increased numbers of shoots effectively. UNZ provided some control of vegetative growth for both cultivars and no significant interactions with GA_3 applications. The percentage of plants flowering was low (20% to 50%).

Table A1-1. Effect of GA_3 and/or uniconazole (UNZ, Concise) drenches on growth and flowering of *Paeonia lactiflora* 'Sarah Bernhardt' (SB) forced in the greenhouse after 6 weeks of pre-chilling at 5°C in 2016–17.

SB (2016–17)		Days to emergence[z]	Plant height (cm)[y]	Plant width (cm)	Number of shoots	Number of flowering shoots	Flowering %
GA ($mg \cdot L^{-1}$)	0	12.8	25.2	37.5	3.3	0.5	30
	100	12.6	23.9	36.8	4.7	0.4	17
	P-value	0.9166	0.2769	0.5634	<0.0001	0.7024	0.2260
UNZ ($mg \cdot L^{-1}$)	0	11.2 b [x]	27.2 a	42.3 a	4.3	0.6	25
	10	11.8 ab	24.6 ab	34.5 b	4.3	0.2	15
	20	15.1 a	21.3 b	34.6 b	3.5	0.6	30
	P-value	0.0323	0.0004	<0.0001	0.1269	0.1739	0.5229
GA		0.9166	0.2769	0.5634	<0.0001	0.7024	0.2260
UNZ		0.0323	0.0004	<0.0001	0.1269	0.1739	0.5229
GA x UNZ		0.2223	0.8821	0.9448	0.1981	0.5006	0.1782

[z] Days to emergence, measured from the beginning of greenhouse forcing.

[y] Plant height, tallest height of vegetative growth.

[x] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 10)

Table A1-2. Effect of GA_3 and/or uniconazole (UNZ, Concise) drenches on growth and flowering of *Paeonia lactiflora* 'Inspecteur Lavergne' (IL) forced in the greenhouse after 6 weeks of pre-chilling at 5°C in 2016–17.

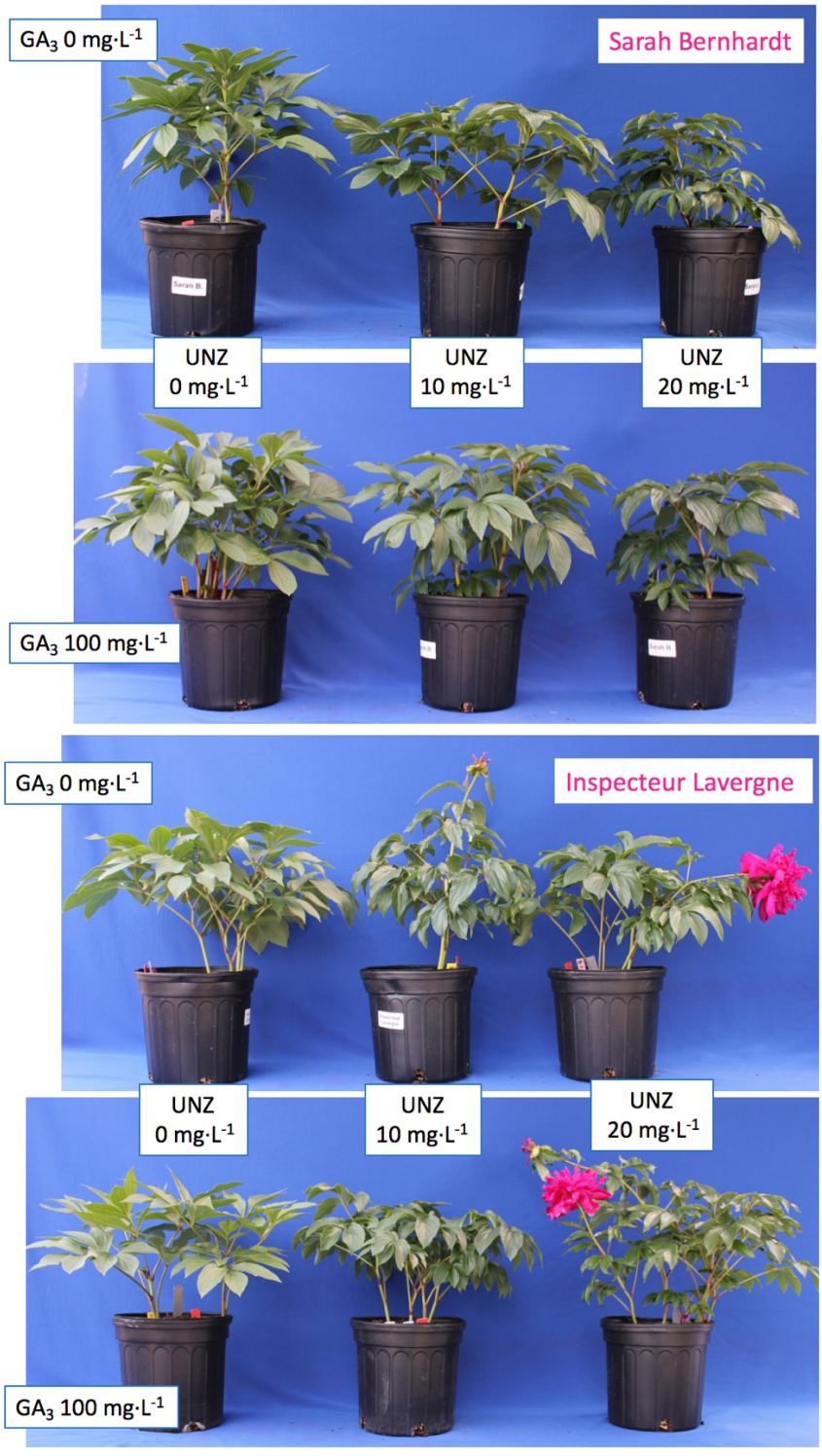
IL (2016–17)		Days to emergence[z]	Plant height (cm)[y]	Plant width (cm)	Number of shoots	Number of flowering shoots	Flowering %
GA ($mg \cdot L^{-1}$)	0	13.7	25.9	39.1	2.8	0.6	50
	100	10.5	26.5	41.5	4.2	0.8	40
	P-value	0.0014	0.5913	0.0351	0.0002	0.4407	0.4453
UNZ ($mg \cdot L^{-1}$)	0	11.9	28.1	45.0 a [x]	3.7	0.4	30
	10	11.9	24.6	38.3 b	3.2	0.8	55
	20	12.5	26.0	37.5 b	3.7	0.9	50
	P-value	0.8117	0.0661	<0.0001	0.3930	0.1585	0.2604
GA		0.0014	0.5913	0.0351	0.0002	0.4407	0.4453
UNZ		0.8117	0.0661	<0.0001	0.3930	0.1585	0.2604
GA x UNZ		0.2876	0.9705	0.0863	0.7674	0.6347	0.5572

[z] Days to emergence, measured from the beginning of greenhouse forcing.

[y] Plant height, tallest height of vegetative growth.

[x] Means within a column followed by the same letter are not significantly different (Student's *t* Test, $P \leq 0.05$, n = 10).

Fig. A1-1. Effect of GA_3 and/or uniconazole (UNZ, Concise) on plant size and development of *Paeonia lactiflora* 'Inspecteur Lavergne' and 'Sarah Bernhardt'. Photo at finish (when >50% of plants in a treatment group had flowered) in May 2017.



Appendix 2 (VCE Publication)

Container Production of Herbaceous Peonies

Information for Greenhouse and Nursery Operators

Dongfang Zhou, Graduate Assistant; Holly Scoggins, Associate Professor; Joyce Latimer, Professor
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Introduction

Herbaceous peonies (*Paeonia lactiflora* Pall.) are popular low maintenance landscape perennials in temperate regions. They produce large showy flowers with many forms and colors from early May to July (Figure A2-1). There are more than 4000 herbaceous peony cultivars registered by the American Peony Society, and hundreds of cultivars are still widely grown today across North America (APS, 2020). Peonies need cold winter climates to grow well. They can grow successful in USDA hardiness zone 3 to 8, and can survive down to zone 9 but are unlikely to flower. Rule of thumb, if you can grow apples, flowering crabapples (both *Malus*) and lilacs (*Syringa*) you can also grow herbaceous peonies (Michener and Adelman, 2017). USDA statistics show more than half million potted herbaceous peonies, valued at closely to $6 million USD, were sold in 2018 (USDA, 2019).

Fig. A2-1. Herbaceous peonies in the landscape

Herbaceous peony is also a popular cut flower worldwide, grown traditionally for the Mother's Day holiday market and wedding markets. The Netherlands is the world's largest

supplier of cut peonies, with approximately 55 million stems sold annually (Kamenetsky and Dole, 2012). The most popular cultivar for cut peonies is Sarah Bernhardt which accounts for more than 50% of the cut peony production. Other popular cut peony cultivars in the United States are Karl Rosenfeld, Dr. Alexander Fleming, Duchesse De Nemours, Kansas, Festiva Maxima and Red Charm.

As a grower of containerized herbaceous peony production in Virginia, the main market is for the home landscape. The popular landscape cultivars in Virginia are Sarah Bernhardt, Karl Rosenfeld, Festiva Maxima, Dr. Alexander Fleming, Inspecteur Lavergne, Felix Crousse, Mother's Choice, Raspberry Sundae, Coral Sunset, Do Tell and Bowl of Beauty. The best time to market peonies in Virginia is from March through May when the plants are in bud or flower.

Challenges with Container Production

Production challenges of containerized peonies include plant size control and flowering. Container-grown peony plants typically have fewer shoots and flowers compared to plants grown in open fields. Applying proper plant growth regulators (PGRs) can improve these production challenges.

Young Plants/Propagules

Peony propagules are crowns, which are perennial underground stems that serve as an energy storage center with a number of renewal buds growing on the surface.

Peony crowns with buds are also described as "crowns with eyes". They are commonly sold as crowns with 3 to 5 or more eyes. Most of the peony crowns sold in the U.S. are grown and harvested in the Netherlands. They are also available from some U.S. growers located mainly in Oregon and Michigan.

A 1-gallon pot is suitable for potting up smaller peony crowns while 2- or 3-gallon pots should be used for larger crowns. Crowns should be planted with 2" of soilless media covering the crowns. The best time for potting peony crowns is late-October to November when most plants are entering dormancy.

Fig. A2-2. Peony crown with renewal buds

Production Inputs and Root Zone Management

The growing substrate must be able to hold moisture but have enough drainage and porosity for good root development. Nursery mixes such as 60% hydro fiber and 40% peat, or commercially formulated potting mixes amended with composted pine bark (by volume) at a 1:1 ratio, worked well in our research studies.

Controlled released fertilizers (CRF, such as 17–5–11 Osmocote Pro 12–14 month) may be incorporated into the substrate at a rate of 9 lbs/yd^3 prior to potting.

Irrigate the plants immediately after potting to ensure that crowns are in good contact with the substrate. Overhead irrigate peonies during the morning and if possible, avoid late-afternoon or evening irrigations. The amount of water applied depends on temperature and evaporation. The potting substrate should not dry out during production.

Avoid wet roots, especially in the winter because peonies are dormant and more prone to root rots. Ensure plants are grown in a location that is well-drained such as a container pad, greenhouse, or coldframe. Avoid placing peonies in area where irrigation or rainfall may pool.

The recommended range for pH is 5.5 to 7.0, and for electrical conductivity (EC) is 0.8 to 3.0 ds/m as tested by the Pour Thru method. Monitor the substrate pH and EC. If the substrate pH drifts below the optimal pH range, apply liquid lime according to label directions. When EC levels begin to rise or soluble fertilizer salts accumulate above the optimal EC level, clear water applications can be used to flush salts thereby reducing EC.

Chilling/Vernalization

As with many perennial species, herbaceous peonies need a cold winter period (vernalization) for spring shoot emergence and flowering.

After potting up in late-October to mid-November, plants can be left outdoors for about a month or placed in a 50°F cooler for about 5 weeks for rooting. For controlled chilling, potted plantsshould be moved to a 50°F cooler for about 5 weeks for rooting and then moved to a cooler at 40°F for a minimum of 3 weeks. If you skip the rooting period, potted plants should be kept in a cooler at 40°F for a minimum of 6 weeks. Containerized peonies also can be held outdoors from potting until late-January, two to three months, to ensure sufficient natural chilling (Zone 3–6, longer for Zone 7–8). During the winter, pots should be covered with frost blankets when temperatures are below 20°F for winter protection, and uncovered to ventilate when temperatures are above 40°F. Rodenticide bait stations should be placed among the plants before covering and filled periodically during the winter.

If forcing plants in a heated greenhouse after sufficient chilling, plants can be market ready in 6 to 8 weeks, i.e., buds showing color. Maximum day / minimum night temperatures of the greenhouse should be maintained at 73/50°F (Hall et al., 2007). High night temperatures late in the season cause peony flower bud blasting/abortion, leading to less marketable plants.

If growing plants in an unheated coldframe after sufficient chilling, plants will generally flower earlier than the plants in landscape. Keep the coldframe well ventilated late in the season to avoid high temperatures.

Fig. A2-3. Herbaceous peonies under greenhouse forcing, average day/night temperatures at 66/60°F (Virginia Tech, Blacksburg, VA)

Fig. A2-4. Flower buds of the vegetative shoot (A, flower abortion in early stage), shoot with blasted bud (B, flower abortion/blasting in late stage) and normal flower bud (C) of *Paeonia lactiflora* 'Sarah Bernhardt'.

Plant Growth Regulators

Gibberellic acid (GA_3) can reduce the production time by enhancing earlier and more uniform shoot emergence and earlier flowering. GA_3 may also increase the numbers of shoots and flowering shoots, and the percentage of plants flowering, resulting higher quality plants. GA_3

can be applied to herbaceous peonies to partially replace the chilling (vernalization) requirement, especially in southern areas (USDA Hardiness Zone 7-8).

A 100 ppm GA_3 drench at 8.5 oz/pot for 1-gallon plants is recommended for herbaceous peony, and can be applied any time after rooting in the fall until shoot emergence begins in the spring. Earlier applications result in shorter production time and a greater number of shoots and flowers in some cultivars. For peonies grown under controlled chilling and greenhouse forcing, GA_3 applied just before chilling reduced production time of 'Sarah Bernhardt' and 'Inspecteur Lavergne' plants by 15 days. Florgib (Fine Americas) is a commercial GA_3 that can be used for peonies. A 1-gallon jug of Florgib can treat 6,000 peony plants at this rate resulting in a cost of less than $0.02 per plant.

Under greenhouse forcing conditions, plant growth retardants (such as uniconazole) can be used early at shoot emergence to make plant more compact and greener, to enhance marketability of plants. Drenches of 15 ppm uniconazole with 12 oz./pot are recommended for 1-gallon potted peonies, and can be applied one week after moving plants into the greenhouse. Growth retardants have been less effective under outdoor or coldframe conditions.

Pests and Pathogens

Botrytis blight and tobacco rattle virus are the most common diseases of containerized peony. Botrytis blight can cause shoots and flower buds to blacken and die, reducing the flowering rate and resulting unattractive and unmarketable plants. Tobacco rattle virus can cause discoloration or distortion of plant foliage, resulting in damaged and unmarketable plants. Especially for plants grown under nursery conditions, spring cold damage after shoot emergence can increase susceptibility to Botrytis blight and tobacco rattle virus. Thrips can also be found in greenhouse grown plants.

Prevention is the best practice for pests and pathogens. Make sure the production site is clean. Always use clean, disease free plant materials, substrate and pots. Peony crowns should be treated with a fungicide before potting. Fungicide drenches should be applied 2 weeks after potting to protect plants from fungal infection. Keep proper spacing and ventilation between plants for good air flow during the growing season, and avoid temperature extremes. For nursery grown peonies, moving plants to a coldframe after emergence in spring may avoid spring cold damage.

Monitor plants for pathogens and pests during production. Remove infested plant parts or plants and apply fungicide spray or drench when Borytis blight is observed on shoots, foliage or flower buds in the spring. Also, an appropriate insecticide should be applied as soon as thrips are observed. Consult Virginia Cooperative Extension Pest Management Guide (Hong and Day, 2020) for appropriate pesticide recommendations for commercial production.

Peony pests and pathogens can be controlled by a combination of cultural management practices and chemicals.

Carryover plant care – plants to be held over for future sales

Unsold peony plants should be kept in a cool sunny place for sale the following year. Do not remove the leaves before natural leaf senescence in October. Early removal may interrupt bud development on the underground crowns, resulting in poor growth and flowering in the next growing season.

Sample Production Schedule

Potting: Late October–November

Rooting: One-month outdoors

Outdoor winter chilling (vernalization): November–January

Cooler based chilling (vernalization): Minimum 3 weeks at 32–41°F

Greenhouse forcing: February through April

Spring nursery growing to flowering: May

Carryover plants: June–October

Citations and Resources

American Peony Society (APS) 2020. Available online: http://americanpeonysociety.org, download on February 2020.

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Hong C. and E. Day. 2020. Pest Management Guide: Horticulture and Forest Crops. VA Coop. Ext. Publ. 456–017. Available online: <https://www.pubs.ext.vt.edu/content/dam/pubs_ext_vt_edu/456/456-017/ENTO-337.pdf>

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