Progress Report

Development of a new method for breaking buds on ornamental flowering plants.

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Introduction

The focus of this research project is the development and testing of a new method for forcing bud break on plants. The method, developed by us, is effective at initiation of any particular dormant bud anywhere on a plant without breaking other dormant buds.

This method is based on our observation that localized axillary bud break on the flower stem of *Rosa hybrida* 'Kardinal' can be induced to break through mechanical manipulation of the stem by partially compressing the internode above a specific axillary bud. We call this treatment a "Partial Crush" (PC) treatment. It induces a bud break at the proximal node, which will grow to produce a flower stem for subsequent harvest without harming the current stem or successive growth. The effect on cut-flower rose was to generate a specific and timed bud break from 7 to 14 days earlier than stem pruning or flower harvesting. Applying this treatment can potentially increase production of cut flower roses. Furthermore, the method may be applicable in a variety of ornamental plants and it is part of this project to explore which other plants might respond to this treatment and how the treatment might need to be modified to maximize effectiveness.

The scientific basis for the process of bud breaking is that apical dominance inhibits axillary bud breaks and lateral shoot branching due to the inhibitory effects of auxin (IAA), which is biosynthesized in the shoot apex and polar transported in the plant (Sachs & Thimann 1967; Leyser 2003). Axillary buds lower on a stem have a higher degree of inhibition then apical buds (Le Bris et al 1998). Apical dominance inhibits axillary bud breaks because of the polar transport of auxin through the stem from the growing apex (Kitazawa et al. 2008). Cytokinin encourages cell division and is translocated in the plant from the root upwards (Sachs and Thimann 1967). We suspect that disruption in the translocation of the growth hormones due to partial compression of the rose stem reduces auxin's inhibitory effect on the axillary bud below the compression site; accumulation of cytokinin below the wound probably encourages cell division and release of the bud. The mode of action is basically unknown but that does not stop us from developing a protocol for use in horticulture. Our objective is to develop a practical protocol for application of the PC treatment and to screen plants to see which ones are

potential targets for this in horticulture. We are currently focused on studying how the depth of compression of the stem affects the PC's efficacy while continuing to try the PC method on other plants.

Development of a commercial protocol to induce forced axillary bud breaks using the Partial Crush treatment.

Previous research helped develop a method that induces specific axillary bud breaks by partially crushing the stem above a selected axillary bud (Lieth, Orsi, unpublished). This treatment utilizes mechanical manipulation of the growing stem to force specific and timed axillary bud breaks on the growing flower stem to break before harvest where apical dominance is removed by pruning. The potential application of this treatment include forcing bud break in hard to break plant species, and a reduction in time between harvest periods due to early bud break. A practical protocol of application of the partial crush (PC) treatment needs to be developed in order to make the treatment available to growers to use on their commercial flower crops. The depth of application of the compression to the stem, the plant's carrying capacity of the treatment and effects of the treatment to subsequent generations of stems needs to be analyzed in order to develop an effective protocol for use. The purpose of this trial is to test the depth of compression needed in order to induce uniform axillary bud break before stem harvest.

Hydrangea macrophylla

Earlier in the project we found that *Hydrangea macrophylla* is receptive to the PC treatment and buds broke on average 8 days after treatment. However, it was noted that 21 days after the application of PC some of the growing flower stems became necrotic at the crush site with the necrosis moving up the stem to the terminal flower bud. This caused wilting in the leaves and flower cyme, followed by cell necrosis on the stem and eventual stem death. Thus for Hydrangea we know that PC is effective, but we need to find ways to improve on the method to improve survival of both the crushed stem as well as the new flower.

Screening of Ornamental and Cut Flower Greenhouse Plants Receptiveness to the PC Treatment.

Our objective is to identify and evaluate the partial crush (PC) treatment's effectiveness on other horticultural plant species. A variety of plants from different plant families are being tested to determine whether the PC treatment is family specific or generally works on all species and to what extent. We are particularly interested in determining for which ornamental plants and cut flower species the PC treatment represents an effective tool. Once species that are receptive to the treatment are identified a commercial protocol can be established for the utilization of the PC to force axillary bud breaks.

Cut-flower commercial greenhouse testing

Testing the effectiveness of the PC treatment in a commercial greenhouse setting is important to get the method adopted by cut flower growers. We have begun to test the method at a commercial greenhouse in Petaluma, CA (Neve Brother's) on hybrid tea roses, spray roses, *Hydrangea* flowers and blind shoots for its efficiency on forced bud break. For the hybrid tea and spray roses varieties that will be tested are those identified by the grower as easy to time bud break or harvest and difficult to time bud break.

Materials and Methods

PC Protocol- Depth Compression for Forced Bud Break

In a block with two rows with 15 *Rosa hybrida* 'Kardinal' plants, for a total of 30 plants, different applications of the partial crush treatment were tested for the most efficient management practice. Plants were grown in 2 gallon containers of UC Mix (1 sand: 1 redwood sawdust: 1 peat) amended with Osmocote® slow release fertilizer and irrigated by drip irrigation (2 gal/hr emitters), with amended half-strength Hoagland's solution (ref.), 4 times a day for 2 minutes each cycle in order to maintain a leaching fraction of 30% to reduce build up of salts.

Plants were pruned hard, with all flower stems or new growth removed to create a canopy of flower stem growth. 3 weeks after pruning, when the new flower stems were at the visible bud stage, when the flower bud is first visible to the eye, this is also 10 to 14 days before the harvest date, the four different Partial Crush (PC) treatments were applied. The four treatments were used to determine the optimal compression depth for forced axillary bud break. All treatments were applied using needle nose pliers to crush the stem to the needed depth. Digital calipers were used to measure the stem width before the treatment was applied. The caliper of the stem was recorded, and the needed depth to compress the stem to for each treatment was calculated before the treatment was applied. The treatments were applied 0.5 to 1.0 cm above the most basipetal 5-leaflet leaf on the flower stem. The treatments include:

1.) Control (CTRL)- The stem was flagged where the compression zone would have been above the targeted axillary bud. The bud was observed for any changes.
2.) Partial Crush 20% (PC20)- The stem was compressed 20% of its caliper above the targeted bud.
3.) Partial Crush 40% (PC40)- The stem was compressed to a depth of 40% of its caliper above the targeted axillary bud.
4.) Partial Crush 60% (PC 60)- The stem was compressed to a depth of 60% of its caliper above the targeted axillary bud.

Trial 1- Winter 2010

Treatments were applied randomly within the planting block. 10 replicates of each treatment for a total of 40 stems treated were applied on December 4, 2009. Initial

measurements taken before any treatment were applied include: stem caliper (mm), stem length (cm), number of nodes along stem, and any general observations about the stem. The targeted axillary bud's date of bud break, and any general observation of the stem growth were recorded. Stems were harvested when all 5 sepals on the flower were fully extended. Before the stem was removed, its final stem length (cm) was recorded. All harvest dates occurred between 12/18/2009 to 12/28/2009. The average daily greenhouse temperature from the treatment date to the final harvest date was 18.6C with a mean PAR of 319.93 µmol m^{-2} s^{-1} at noon.

Trial 2- Spring 2010

The trial was replicated the same as trial 1 except for the time of year the treatments were applied. They were applied on April 26, 2010 with the daily average temperature of 22.5C and mean PAR of 1027.4 µmol m^{-2} s^{-1} at noon from April 26 to May 21, 2010. Stems were harvested on May 14 and May 21, 2010.

Trial 3- Increase in area crushed on stem

The trial was replicated the same as trial 1 and 2 with the exception of the time of year and the pliers used. For this trial standard pliers were used instead of needle-nose pliers. The zone of damage was 12mm with the standard pliers compared to 3mm of the stem receiving the crush with the needle-nose pliers. Due to overlapping of trail 2 and trial 3 in the same planting block the roses were not headed back entirely 3 weeks before the start of the trial, but because trial 2 flowers were harvested at about the same time the growing flower stems were almost completely removed 3 weeks before application of the treatments. Results are not yet available.

Ornamental and Cut Flower Crops Screening Trial

Several plant species were selected to be grown in the greenhouses to receive the PC treatment and to contrast the results with no treatment or other treatments that typically induce bud break. Plant species that were used in these trials consisted of ornamental plants: *Hydrangea macrophylla* 'Angel's Robe', *Rhododendron* sp. 'Florist Azalea', and Bachelor's buttons; and cut flower varieties tested include: *Antirrhuinum majus* (Snapdragon), *Helianthus annuus* (Sunflower), *Delphinium* elatum 'Guardian Blue', *Callistephus chinensis* (Aster), *Dianthus* sp. (Dianthus), *Matthiola incana* (Stock), *Tanacetum parthenium* (Matricaria), and *Calendula* sp..

Rhododendron sp. 'Florist Azalea', Bachelor's Button, Cut Flower Screening Trials

For each of these plant species only stems with an apical floral meristem were used. Stems chosen had flowers that were at the visible bud stage. The following were the measurements taken before any treatments were applied:

- Date of treatment, Plant ID, Replicate number
- Stem length (cm) - measured from the treated axillary bud to the stem apex.

- Node position – treated node position recorded measured from the bottom of the stem to the top
- Nodes per stem

After measurements were taken the stem was tagged above the targeted node with a tag containing the plant ID, and date; then the treatment was applied. The PC treatment was applied to the stem 0.5cm above the node that was targeted for bud break. The stem was crushed with needle nose pliers to a depth of 40% to 60% of the stem caliper. Control treated stems were flagged above the targeted node intended for bud break. After the stem was treated the date of axillary bud break was recorded, along with the following bud (or stem) length measurements at 5, 7, 14, 21 and 28 days of any resulting new stem. Any observations about the stem growth were noted.

Hydrangea macrophylla- trial 2

Hydrangea plants were grown in 1 gallon containers in UC Mix (1 sand: 1 redwood sawdust: 1 peat) amended with Osmocote® slow release fertilizer. Two treatments were tested: partial crush (PC) treatment and control (CTRL). The treatments were applied to growing flower stems above the targeted axillary bud when the flower cyme had approximately 20% of its bract color showing. This was estimated to be 3 weeks before the harvest date which was considered to be when the first flower opens. The PC and CTRL treatment were applied and observed the same as the ornamental and cut flower screening trials previously stated.

Results/Discussion

Partial Crush Protocol- Depth Compression for Forced Bud Break

Trial 1- Winter 2010

Preliminary data is shown in Table 1. After the stems were treated the targeted buds were observed for bud break before harvest. No stems reached bud break before harvest although several of the PC treated stems had swollen buds before harvest. After the treated stem was harvested, the targeted bud observed for bud break date and the bud's subsequent harvest date once it reaches maturity.

Table 1. Partial Crush (PC) protocol trial 1.

Treatment	Mean Actual % Crush	Mean Change in Stem Length (cm)	Days to bud break after treatment	n of stem to reach budbreak to date
PC60	58%	9.1 a	24.7 a	7
PC40	41%	10.1 a	27.5 a	6
PC20	22%	11.8 a	31.5 a	8
CTRL	0.0%	10.3 a	29.4 a	5

*Mean separations determined by standard t-test (0.05)

For the first time since the PC treatment trials have started there were no bud breaks prior to harvest on *Rosa hybrida* 'Kardinal'. This is found to be out of the norm for this variety of cut flower rose because in several previous trials, the growing flower stems and bottom canes have all shown to significantly be effected by forced bud break prior to harvest.

There are some differences in this trial which leads us to conclude that they are affecting the forced bud breaks. First, the time of year for application of the PC treatment has never been done to growing flower stems in the month of December; however, the bottom break trial was performed in the winter months in 2009 which showed the PC treatment significantly forced axillary bud break prior to removal of the upper portion of the stem. This suggests that carbohydrates might be a major player perhaps due to reduced photosynthesis in the winter months. To assuage this issue supplementary lighting will be added above the rose bench.

Secondly, when the treatment was applied, the crush zone was on the stem 0.5-1.0 cm above the targeted node, but the crush site was only about 3mm tall, the width of the needle nose pliers and was applied in one clean motion or compression. The PC treatment was applied in this way because of the need to measure the depth of crush with the digital calipers at the time of application. Previous studies with the PC treatment were applied by touch at an estimate of 40-60% of the stem caliper at 0.5-1.0cm above the targeted node, but the zone of the stem that was crushed was approximately 1.0cm in height on the stem and was crushed in a slow motion, first with the stem tissue being softened and then the crush was applied until the pliers could not be squeezed further. In the next trial the zone of crush will be a greater width on the stem and will not be just the width of needle nose pliers.

Trial 2- Spring 2010

Bud break was not significantly forced by any of the PC treatments as compared to the control treatment prior to harvest. However, two PC60 and two PC40 treated buds did break prior to harvest, but this was not found to be significant compared to the CTRL treatment (table 2). The PC treatment at the different depths did not reduce the time from treatment to bud break either. Data are still being collected for subsequent stem harvested that developed from the forced buds.

Table 2. Partial Crush (PC) treatment protocol trial 2.

Treatment	Targeted % Crush	Mean Actual % Crush	Mean Change in Stem Length (cm)	Days to bud break after treatment	n of stems to have BB prior to harvest	n of stem to reach BB to date
PC60	60.0%	60.2% ± 2.2%	10.3 ± 3.1 a	22.8 ± 8.1 a	2	10
PC40	40.0%	41.1% ± 0.9%	8.6 ± 3.3 a	21.5 ± 4.7 a	2	10
PC20	20.0%	20.9% ± 1.0%	13.1 ± 3.8 a	22.1 ± 1.1 a	0	8
CTRL	0.0%	0.0% ± 0.0%	4.5 ± 22.7 a	24.1 ± 3.3 a	0	9

*Mean separations determined by standard t-test (0.05)

PAR levels were higher for this trial compared to the previous winter trial, which had no bud break in any treatments prior to harvest. It is believed that light was not the only

factor that is inhibited early bud break and a new trial with a larger area compressed on the stem is performed during the higher light period of the summer.

Trial 3- Increase in area crushed on stem

Data are still being collected for this trial and only preliminary data are reported here. The larger area compressed on the stem appears to have increased the probability of forced bud break prior to stem harvest. Of 10 replicated stems 5 of the PC 60 stems had bud break compared to 0 of the CTRL stems (table 3). Buds and subsequent growing stems will need to be followed in order to see if the subsequent flower harvest is earlier for the PC treated buds then the CTRL buds.

Table 3. Partial Crush (PC) treatment protocol trial 3.

Treatment	Targeted % Crush	Mean Actual % Crush	Mean Change in Stem Length (cm)		n of stem to reach budbreak prior to harvest	n of stem to reach budbreak to date
PC60	60.0%	57.8% ± 2.1%	7.6 ± 4.6	a	5	9
PC40	40.0%	40.9% ± 1.4%	6.9 ± 3.1	a	1	9
PC20	20.0%	21.4% ± 2.0%	7.3 ± 5.3	a	2	6
CTRL	0.0%	0.0% ± 0.0%	8.9 ± 6.5	a	0	9

*Mean separations determined by standard t-test (0.05)

These preliminary data suggest that an increase in light along with area crushed on stem force early bud break. A follow up trial in lower PAR condition will further solidify our understanding to whether this is due to light levels or damaged done to the stem.

Ornamental and Cut Flower Crops Screening Trial

Out of the 9 species that have been analyzed, there was no significant difference between the PC and CTRL stem and the effect of forced bud break before harvest. All data indicates that the PC treatment did not affect the mean stem length (Table 4 and 5), or increase the number of bud breaks prior to harvest. Data are still being collected for one other cut flower species that is being screened.

Table 4. Ornamental and Cut flower species screened for receptiveness to PC treatment

Species	Treatment	n	number of plants to break before harvest	Mean Stem Length (cm)		Mean Days to Bud Break	
	PC	9	1	9.8	a	14.0	a
Rhododendron sp. (Azalea)	CTRL	9	1	10.0	a	7.0	a
	PC	9	6	59.3	a	5.0	a
Bachelor's Buttons	CTRL	9	2	60.4	a	5.0	a

*Mean separations determined by standard t-test (0.05)

More screening and research are needed to discover other cut flower or ornamental species that might also be receptive to the PC treatment. This will also help us determine if there is a way to determine if a plant might be receptive to this treatment either because that plant family is sensitive to the PC treatment or if it is due to the physiology of the plants (i.e. C3, C4 photosynthesis).

Table 5. Greenhouse partial crush trials with commercial cut flower plant varieties

Plant Name	n per trt	TRT	Change in Stem Length (cm)				n of plants per treatment with bud break prior to harvest
Callistephus chinensis 'Meteor Rose Pink'	10	CTRL	58.8	±	13.7	a	10
		PC	57.6	±	14.9	a	10
Delphinium sp. 'Guardian Blue'	10	CTRL	23.6	±	22.5	a	10
		PC	18.3	±	9.8	a	10
Dianthus sp. 'Amazon Neon Duo'	10	CTRL	52.3	±	10.6	a	9
		PC	45.2	±	10.3	a	9
Matthiola incana 'Kate Cherry Blossom'	10	CTRL	34.0	±	15.0	a	0
		PC	23.4	±	20.8	a	0
Helianthus annuus 'Prado Gold'	10	CTRL	34	±	15.0	a	0
		PC	23.4	±	20.8	a	0
Antirrhinum majus 'Plum Blossom'	10	CTRL	58.8	±	13.7	a	1
		PC	57.6	±	14.9	a	4
Tanacetum parthenium 'Magic Single'	10	CTRL	9.76	±	4.8	a	6
		PC	11.5	±	6.2	a	10

*Mean separations determined by standard t-test (0.05)

Hydrangea macrophylla- trial 2

Previous research showed that *H. macrophylla* 'Angel's Robe' is receptive to the PC treatment, however, the growing flower stems that received the treatment died on average 21 days after treatment. For this trial the PC treatment was applied at a later stage in the flower growth to induce forced bud break but the stem was removed for harvest before death could occur. Of the 7 stems that were treated with the PC treatment all of them had bud break before harvest (Table 6). Of the 8 Control treatment stems only 2 had bud break prior to harvest. The days to bud break show the time from treatment to bud break for those buds that broke prior to harvest.

Table 6. Hydrangea trial 2. Efficacy of later application of PC treatment on reducing stem death prior to harvest

Species	Treatment	n	number of plants to break before harvest	Mean Stem Length (cm)		Days to Bud Break	
Hydrangea macrophylla 'Angel's Robe'	PC	7	7	29.5	a	24.6	a
	CTRL	8	2	26.2	a	29.5	a

*Mean separations determined by standard t-test (0.05)

Applying the treatment at the first color stage instead of when the buds were the size of a quarter prevented any stem death prior to harvest in stage two.

Trial of PC treatment at a commercial cut-flower greenhouse on hybrid tea roses, spray roses, and Hydrangea.

Results from tests in commercial production are not yet at a point where we can report specific results. This will be the focus of the next progress report.

Conclusion

While it is not surprising that some species are not likely to benefit from this method, others, including *Hydrangea* and Rose, show great potential. It would be beneficial to continue the research in developing a PC method protocol for commercial application and screening of other high value cut flower species. Hydrangea needs further testing to better refine a commercial application protocol, as well as Rose. Further testing on cut flower Hydrangea varieties, could either prolong the growing season, increase harvestable yields per year by decreasing blind shoots in current or subsequent growing seasons.

The next step in the project is to identify other cut flower species that are receptive to the PC forcing method, further refine protocols for commercial application of the PC treatment on Roses and *Hydrangea*, and determine whether it is plant growth hormones that are causing the forced bud breaks due to the PC treatment or if the forced bud break is due to carbon partitioning and photosynthesis due to light quality or intensity. Seasonal variations in forced bud breaking and plant carrying capacity or PC treatment should also be investigated to tell growers the proper application of these methods to grow valuable cut flower ornamentals. Exploring the seasonal variation would be an important future line of research as that will have an impact on timing flowers for Valentine's Day.

Literature Citied

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