Progress Report

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

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Report Date: August 2009 (2008-09 Final Report)
Funded by the Joseph H. Hill Memorial Foundation, Inc.
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Introduction

Over the past five years we have carried out a considerable amount of research on induction of bud breaking in roses, gerbera and a variety of other plants. Much of this research has focused on using Plant Growth Regulators and cultural methods (e.g. photoperiodic control in gerbera) and was funded by the Hill Foundation as well as the national IR4 program. Unfortunately of these methods, the ones that were effective did not allow targeted initiation of one particular dormant bud. Separate from that research we also carried out research to find such a targeted method. The currently-funded work is aimed at exploiting a discovery by us of such a targeted bud-breaking method.

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 and better time 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 speculated 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. Research is needed to confirm this scientific basis or to discover what is actually going on so that we can tailor horticultural methods to it. At this time the most promising method is the Partial Crush method and the results of applying that method is described in this report.

Rose bottom break experiment

Cut flower rose growers have always sought methods to effectively force bottom breaks. These are axillary bud breaks on older stem tissue. Generally such tissue is very hard and woody and is the remnant of stems that may have been initiated months earlier. In traditional rose production bottom breaking was induced through severe pruning or harvesting very low on the plant; some growers used "arching" methods (which were later developed into "bending methods"). Even in hydroponic cut flower rose production with bending, there are many times where growers wish to initiate breaks on older stems near the substrate; such breaks can also be called "bottom breaks". Thus one of the important questions that we wanted to explore was whether the PC method could be used on older rose stem tissue as an aide in inducing bottom breaks.

Screening of Ornamental Greenhouse Plants Receptiveness to PC Treatment

Our objective is to identify and evaluate the partial crush (PC) treatment's effectiveness on other plant species that are commercially valuable. 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 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.

Materials and Methods

Rose Bottom Break Experiment

Trial 1:

Two treatments were used to test the efficacy of crushing the basipetal rose cane to induce specific axillary bud bottom breaks. *Rosa hybrida* 'Kardinal', grafted on 'Natal Briar' rootstock, grown in a 2 gallon pot with UC Mix amended with slow release Osmocote® encapsulated fertilizer, and irrigated with fertigation solution, using a Smith Injector with 2 gal/hr drip emitters, 5 times a day, for 8 minutes total per day. The nutrient solution was a half strength Hoagland's solution

The plants selected for the experiment from a set of 20 well-established plants had 3 to 4 major canes above the bud union where the scion was grafted onto the 'Natal Briar' rootstock. A flag was attached at each stem denoting the site of a possible treatment. 10 plants were select for the PC treatment where the PC treatment was applied at the location of the flag; the remaining 10 plants received no treatment at the marked internode.

Imposition of the PC treatment involved using needle-nosed pliers to crush the stem to about 30-40% of its diameter. About 0.5-1.0 cm above the target node.

Flower stems on each plant were harvested when the 5 sepals are fully extended downward.

The plants were inspected frequently and the date of bud break at the target node (or above) was recorded. The height and width of each plant was measured along with the number of leaves on each plant. Each flag was labeled with all pertinent information. Observations and measurements will carried out over a 6 week period.

Trial 2:

This trial was motivated by the results of the first bottom break trial which showed promising results. This time three treatments were imposed to test the efficacy of crushing the basipetal rose cane to induce specific axillary bud bottom breaks. The treated stems were selected as before randomly from among an experimental group. Flower stems on each plant were not harvested until 10 days after the treatment to reduce any additional hormonal changes in the plant that is not due to the treatments.

In addition to the PC and Control (as before) a new "Double Crush" (DC) treatment was imposed where the PC treatment is basically imposed twice. The second PC was imposed 24 hours later at the same location on the stem.

Before the treatments are applied all flower stems were removed from the plant canopy. Plants will be allowed to grow for 15 days after stem removal to reduce any hormonal fluctuations within the plant. At the end of the 15 days the height and width of each plant was measured. Every pot was labeled with the Plant ID and every stem emerging from the bud union was flagged just above the targeted node

During the first 10 days after the treatments no flower stems were harvested on the treated plants to reduce any changes in apical dominance that might influence bud breaking that are not do to the treatment. The stem above the crush site or flagged node were removed 10 days after the treatment is applied. Plants were evaluated at day 0, 5, 7, 14, 21, 28, 35, 42, and 48. The bud length was measured and the date of harvested of the secondary bud that was forced to break was recorded even if it extended past the 48^{th} day of measurement. The fresh weight and final length stem were also measured.

Ornamental Greenhouse 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: *Zinnia* sp., *Chrysanthemum* 'Nob Hill', and *Lillium longiflorum* 'Nellie White'. While the latter is generally used as a potted flowering plant, it was felt that it would serve well as a proxy for lily species in general. H*ydrangea. macrophylla* (Big leaf Hydrangea)*, P. x hortorum* (Zonal Geranium)*,* and *Nepeta* sp. (Catmint) were also included in the test to get data on potential other greenhouse ornamentals. Other plant species are currently being grown to test in the greenhouse but are not included in this progress report, *B. davidii* (Butterfly Bush)*, Moluccella laevis (*Bells of Ireland), *Ocimum basilicum* 'Genovese' (Genovese Basil), and *Rhododendron* sp. (Azalea).

Hydrangea macrophylla, Nepeta sp., Pelargonium x *hortorum,*

For each of these three plant species only stems with an apical floral meristem were used. Stems chosen had flowers that were starting to open. The following were the measurements taken before any treatments were applied:

- Date, Plant ID,
- 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 are taken the stem were tagged above the targeted node with a tag containing the plant ID, and date; then the treatment was applied. 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.

Chrysanthemum

Thirteen pots are planted with 5 rooted cuttings of *Chrysanthemum* 'Nob Hill' (a cut flower variety), obtained from Yoder Brothers, and were grown under photoperiodic condition to promote flowering. Each cutting was pinched to produce three axillary bud breaks, and subsequently three stems per cutting. Each cutting with 3 bud breaks received three treatments, one treatment on each of the three stems. In addition to the Control and PC treatment, one of the branches was pruned (PR) just above the targeted node. The PC treatment was imposed by crushing the stem 40-60% of the stem caliper. A total of 15 replicates of each treatment were used. After application of the treatments, axillary bud break dates were recorded.

Lillium longiflorum 'Nellie White'

The lilies bulbs were obtained from the Lily Research Institute, and went through vernalization to induce flowering. They were grown in 6" standard pots in UC mix, and the PC and CTRL treatments were applied when the flowers reached visible bud. The same measurements and cultural conditions were used for this trial as in the *Hydrangea macrophylla* trial. In addition, the flower number per stem, bulblets produced and any new stem growth were measured. 4 weeks after the PC and CTRL treatments were applied all the flower stems were removed. Since lilies' axillary buds are along the basal plate of the bulb we also counted the bulblets produced, if the PC treatment increases bulblet production this can be beneficial to lily bulb growers. Any new stem growth that emerges from the basal plate was also noted. Two weeks after stem harvest the bulbs were removed from the soil and the number of bulblets were counted.

Results and Discussion

Bottom Breaks on Roses

Trial 1:

Out of 37 replicate of CTRL and PC stems, 7 internodes in the PC treatment resulted in bud break within the experimental period while none of the stems in the Control treatment showed any bud breaking (Table 1). Of the 7 PC buds that broke 3 of them became blind shoots. This could be due to the fact that the stem above the broken bud was not removed. In past trials the PC treatment was on flowering rose stems that were harvested when the five sepals extended downward. Since the PC here was on older canes that emerged from the bud union there was most likely a stronger influence of apical dominance on the breaking axillary bud.

Table 1. Bottom break trial 1 on *Rosa hybrida* 'Kardinal'

TRT	n per trt	n per trt with BB	Mean days to bud break from treatment	
CTRL	37	0	0.0	a
PC	37	7	16.7	b

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

In a new trial, it would be noteworthy to prune the stems after bud break, instead of waiting 10 days after the treatment to remove the stem tissue. This could further speed up the release and growth of the PC treated bud by completely eliminating apical dominance after the forced bud break.

Picture 1. Blind shoot formed after bud break on PC treated stem.

Picture 2. Mature stem formed from forced bud break due to PC treatment.

Trial 2:

Out of ten replicates for each treatment, 5 of the PC and 5 of the DC treated stems broke prior to removal of the stem above the treated axillary bud compared to zero of the CTRL stems (Table 2, Picture 3).

Table 2. Bottom break experiment on roses, trial 2.

TRT	n per trt	n per trt with BB prior to Day 10	n per trt with BB	Mean days to bud break from treatment	n harvested	Mean days from bud break to harvest	Mean final fresh weight of BB stem (g)	Mean final stem length of BB stem (cm)
CTRL	10	0	10	24.3 a	7	29.7 a	43.1 a	58.0 a
PC	10	5	9	14.0 b	7	37.4 b	49.0 a	61.3 a
DC	10	5	8	13.4 b	8	40.0 b	48.6 a	62.9 a

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

Picture 3. PC stem bud break, day 7 after treatment

This indicates that the PC and DC treatments are producing a physiological response in the plant that is causing the forced bud break. Little is known about the mode of action causing the bud break, but it would be beneficial to the further investigate the cause of the forced axillary bud break so growers and production managers can better manipulate plants in novel ways. It is speculated that the PC and DC treatments might work either by blocking the transport of the hormone auxin which is basipetally transported in the phloem tissue (Sachs & Thimann 1967; Leyser 2003), and inhibits lateral bud breaks. In addition the cause of the forced bud break could be a wound reaction from the crush treatment. When callus tissue is produced it goes through many cell division caused by an increased production in cytokinins, which also promotes axillary buds breaks.

Table 3. Mean bud length of targeted axillary buds on days observed. Mean separations determined by standard t-test.

TRT	Mean Bud Length Day 5 (mm)	Mean Bud Length Day 7 (mm)	Mean Bud Length Day 10 (mm)*	Mean Bud Length Day 14 (mm)	Mean Bud Length Day 21 (mm)	Mean Bud Length Day 28 (mm)	Mean Bud Length Day 35 (mm)	Mean Bud Length Day 42 (mm)	Mean Bud Length Day 49 (mm)
CTRL	0.0 a	0.0 a	0.0 a	0.0 a	7.5 a	82.4 a	197.1 a	354.7 a	449.1 a
PC	0.0 a	0.4 a	0.8 ab	0.8 ab	15.8 a	76.0 a	206.2 a	381.9 a	461.4 a
DC	0.0 a	0.7 a	1.1 b	1.3 b	15.6 a	94.4 a	218.8 a	365.3 a	466.5 a

* On day 10 the all treated stems, CTRL, PC, and PR were pruned back above the targeted bud.

The mean fresh weight and final stem length of the treated stems that grew from the targeted axillary bud, known as the secondary stem, had no significant difference from the CTRL treated stems. They were slightly longer and had a greater fresh weight, which might not be statistically different, but every centimeter counts in cut flower production. Zero of the CTRL buds had broke until the 21st day of treatment, 11 days after stem removal, and 7 of the 10 axillary buds had been released. On that same day 9 of the PC treated, and 8 of the DC treated buds had broken. The bud lengths for DC were significantly longer than the CTRL buds on day 10 and 14, and they were not significantly different from the CTRL or DC for the PC treated buds (Table 3).

Picture 4. Growing stem from PC forced axillary bud. Day 21 after treatment.

While the DC treatment was effective at forcing axillary buds to break, 2 of the 10 replicate stems died after the above portion of the stem was removed on day 10. The bud and the remaining portion of the stem above the bud union turned necrotic and died. Even though it is effective it should be noted that the DC treatment increases the labor involved to perform the treatment, has the same effect on bud break as the PC treatment, and the DC treatment has the possibility of exposing the plant tissue to disease because it is crushed twice in a 24 hour period.

Further testing should be conducted to better refine and speed up the process of forced bottom break on the rose canopy. The end result would reduce the time growers spend trying to rejuvenate the rose canopy. The hard prune cuts could be made after the roses have already broke bud at the exact location on the plant that the grower intends.

Greenhouse Ornamentals screening trial

Hydrangea macrophylla

By day 7 eight of ten replicate PC stems and one CTRL stem had bud break (Picture 5). However, by day 21 after treatment eight of ten PC replicates had necrotic lesions starting at the crush site and moving up the stem towards the flower. The flower was starting to wilt and did not recover, eventually becoming necrotic and dying above

the crush site (Picture 6). During the time between day 7 and 21 there were a few days of very hot weather and the plants probably were stressed during this time as evidenced by some tissue necrosis. This was likely the cause of the stem death. This trial will be repeated under more-moderate conditions next spring.

Picture 5. Axillary bud break from PC Treatment on *H. macrophylla,* day 7.

Picture 6. Complete stem death above crush site 28 days after the treatment was applied.

Another possible cause of the stem death could be due to the fact that the stems received the PC treatment when the flower cyme was small, about a quarter in size, and was at least four weeks away from harvest. During the initial PC trials used on flowering rose stems, the treatment was applied about 10 days prior to harvest of the flower on the stem where the bus is being induced to break. In future hydrangea trials we will impose the treatment more similar to our earlier rose work. Since we observed that between day 7, when the buds first broke, and day 21, when the necrosis started, the PC treatment should be applied to the stems less than 21 days before harvest to induce bud break, but not cause necrosis or stem death. Applying the treatment when the flowers start to show color, approximately two weeks before harvest might be ideal.

Table 4. Greenhouse screening trial for plants receptive to partial crush treatment.

Plant Name	n per trt	TRT	Mean days to bud break from treatment	n of plants per treatment with bud break	Bud length day 1 (mm)	Bud length day 5 (mm)	Bud length day 7 (mm)	Bud length day 14 (mm)	Bud length day 21 (mm)	Bud length day 28 (mm)
Hydrangea macrophylla	10	CTRL	8.0 a	1	0.0 a	0.0 a	0.3 a	0.3 a	0.3 a	0.3 a
		PC	8.0 a	8	0.0 a	0.0 a	2.0 b	5.8 b	10.5 b	16.6 b
Zinnia sp.	10	CTRL	11.4 a	10	0.0 a	0.0 a	3.5 a	4.9 a	5.6 a	8.9 a
		PC	13.4 a	8	0.0 a	0.0 a	2.9 a	4.9 a	7.8 a	13.2 a
Pelargonium x hortorum	10	CTRL	14.8 a	7	0.0 a	0.0 a	0.0 a	2.9 a	12.4 a	17.8 a
		PC	8.7 a	4	0.0 a	0.0 a	4.8 b	14.5 b	27.1 a	41.5 a
Nepeta sp.	10	CTRL	8.0 a	10	0.0 a	0.0 a	3.0 a	5.2 a	8.5 a	12.1 a
		PC	8.0 a	10	0.0 a	0.0 a	4.0 a	8.4 a	14.1 a	24.2 a

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

The data showed that the bud length for the PC treatment was significantly longer than the CTRL treatment on days 7, 14, 21 and 28. On day 28 the buds had a mean length of 16.6 mm with some of the buds as long as 60.0 mm before the stem harvest (Table 4). This treatment could be beneficial for cut flower hydrangea production since they are a valuable cut flower crop. The treatment could potentially increase stems produced in the current growing season if it is shown the new bud breaks have flower bud primordial or for the subsequent growing season after the buds are vernalized. For 4 of the 8 PC stems that had bud break, they had multiple bud breaks, two or three BB, below the crush site.

Zinnia sp.

There was no significant difference between the CTRL and PC for the bud length or days to bud break (Table 4). The PC treatment cause severe callus formation on 4 of the 10 PC treated stems (Picture 7), but the callus did not affect the above portion of the flower stem. The flowers continued to grow and reach full bloom.

Picture 7. Callus formation and bud break due to PC treatment on *Zinnia sp.*

Pelargonium x *hortorum*

On day 7 and 14 the PC treated axillary buds were significantly longer than the CTRL buds (Table 4). The mean lengths of the PC buds were longer than the CTRL but not statistically significant on day 21 and 28. Two of the PC treated stem had large necrotic lesion at the crush site, but it did not kill the stem above the crush. The lesion became soft and the above portion did fall over, but never completely died.

Nepeta sp.

Because *Nepeta* sp. is not apically dominant all the buds broke by day 7 (Table 4). Because all the buds had broken naturally it was important to see if the PC treatment influences axillary bud length. However, the following weeks after the bud breaks there was no significant difference in the CTRL and PC bud lengths. The plants were all very healthy and in full flower during the study.

Lillium longiflorum 'Nellie White'

Since lilies have axillary buds along the basal plate on the bulb the PC treatment would not have forced bud break along the stem, but could possible cause an increase in bulblet formation on the stem and basal plate. The bulblets could be used for lily bulb

production. Two weeks after the stem was removed above the treated site the bulbs were removed from the pot and the bulblets counted. There was no significant difference found in the mean bulblets produced per bulb or the change in stem length from treatment day to harvest (Table 5). Monocots might not be the best plant type to use for the PC treatment because they do not have axillary buds to force to break.

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	Bulblets formed 2 weeks after harvest
Lillium longiflorum 'Nellie White'	20	CTRL	0.7 a	0	2 a
		PC	1.2 a	0	1.9 a
Chrysanthemum 'Nob Hill'	15	CTRL	14.8 a	0	--
		PC	14.5 a	1	--
		PR	--	14	--

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

Chrysanthemum 'Nob Hill'

Out of 15 replicates 14 of the PR treatment, 1 of the PC, and 0 of the CTRL broke before stem harvest (Table 5). There was also no significant difference in the change in stem length between the CTRL and PC treatments. There was significant callus tissue formation at the crush site on the PC stems. The callus did not affect the growing stem quality. It was speculated that the *Chrysanthemum* BB trial would have produced forced bud breaks, since mums are easy to grow and are resilient to different forms of stress. Since the mums were not receptive to the PC, but are resilient to stresses, this could point to that the PC treatment causes forced bud break by creating a stress reaction in plants and induction of hormone production in the form of cytokinin.

Conclusion

The project is progressing quite well. While it is not surprising that some species are not likely to benefit from this method (e.g. Lillium), 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 buds responded quite well to the PC treatment which could potentially increase floral production in cut flower operations. Further testing on cut flower Hydrangea varieties, could either prolong the growing season or harvestable yields per year. Furthermore, the ability for growers to force bottom breaks on rose plants prior to and in some cases, without hard pruning is extraordinary.

Literature Citied

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