Bitter Pit Mitigation and the 'Honeycrisp' Apple: Prohexadione-calcium and Bourse Pinching Effects on Bitter Pit, Shoot Extension, and Fruit Size

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The profitability of the 'Honeycrisp' variety is critical to the continued growth of the New York State apple industry. As more young orchards reach full bearing potential, we can expect market supply will increase, likely resulting in downward pressure on grower returns. Substantial losses of fruit to storage issues such as bitter pit (BP) will be an ever-growing challenge to the grower's bottom line. Our group is now in the third season of a study focused on the development of practical recommendations growers can implement to reduce bitter pit losses in their 'Honeycrisp' orchards. Research objectives of the entire project include the identification of factors that contribute to BP development, the use of early season calcium applications to reduce BP, the use of plant growth regulators to reduce BP, and the influence of thinning timing on BP expression.

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According to our survey data from 34 orchard blocks collected in 2016 and 35 blocks in 2017, BP incidence in Eastern New York orchards averaged 26.7% and 13.8%, respectively (Donahue and Wallis, unpublished). Historically, more than 50% of fruit is unmarketable due to BP (Rosenberger 2001, 2004) and pack-out percentages can be even less after long-term storage. 2016 was an especially difficult BP year in the Hudson Valley, and 'Honeycrisp' has developed a reputation for being a challenging apple to grow. Despite this, an increasing number of acres continue to be planted each year, and many blocks have yet to reach full bearing. A better understanding of factors contributing to BP and management solutions is necessary to realize the potential and maintain the viability of HC production in NY.

BP is a physiological disorder resulting from a complex interaction between certain fruit physiological factors, as well as certain environmental and horticultural conditions. Specifically, it is associated with low Ca in fruit tissue, which is influenced by soil and weather conditions, nutrient availability, tree age and vigor, crop load, and fruit size (Ferguson and Watkins 1989; Rosenberger 2004; De Freitas 2010). Enormous variation in BP incidence can be observed among commercial plantings, likely related to variations in local environmental and horticultural conditions, rootstock selection, soil fertility management programs, and foliar calcium programs being implemented.

Foliar calcium (Ca) sprays have been demonstrated as an effective treatment for BP (Ferguson and Watkins 1989; Rosenberger 2004; Biggs 2015). Summer Ca sprays are currently the industry standard for mitigation of BP, with some growers applying Ca weekly throughout the growing season. This is an expensive practice and can be very corrosive to equipment, and not always effective in commercial orchards. The direct consequences of a mineral deficiency(ies) may not be the only factor contributing to the BP problem. An alternative hypothesis considers calcium status as an indirect factor, with other factor(s) triggering the expression of the disorder, such as environmental conditions or plant hormones (Saure 1996). The unpredictability of BP could be related to the interaction of plant hormones and mineral nutrient transport in the tree, and those relationships may vary by tree phenology. There may be other parameters that contribute to the expression of BP symptoms. Competition with vigorously growing shoot tissue may be a significant contributor to deficiency of Ca in fruit tissue (De Freitas 2010). Apple fruit, in general, and BP-susceptible cultivars such as 'Braeburn' (Drazeta et al. 2004) in particular lack extensive xylem development, especially in the calyx end. This condition forces an even heavier reliance on the phloem sap stream to deliver calcium to rapidly dividing fruit cells (Kirkby 1984), and it has been shown that Ca transport via the phloem is minimal in apples. Therefore, grower practices that limit shoot growth may help tip the competitive balance for Ca away from the shoot and toward the fruit.

Prohexadione-calcium (prohex) (Kudos^R, Fine Americas Inc; Apogee^R, BASF Corp.) acts as an inhibitor of gibberellins, reducing levels of the highly active GA_1, resulting in the accumulation of the non-active precursor GA_{20} (Evans et al. 1999), and whose use is widespread in commercial orchards to reduce shoot growth. Researchers at Pennsylvania State University recently implemented a BP prediction protocol (Baugher et al. 2017), which includes measurements of terminal shoot extension as a factor influencing BP incidence in Pennsylvania orchards.

The objective of this two-year study was to evaluate the efficacy of two specific practices for the mitigation of bitter pit in apple *Malus domestica* Borkh cv. 'Honeycrisp': 1) pre-bloom applications of prohex (Apogee^{R}) and 2) bourse shoot pinching.

Materials and Methods

Two experiments were conducted in two different commercial orchards in two consecutive growing seasons, with the trees managed by the grower according to generally accepted best management practices common to the region.

2016 Experiment*:* A field trial with six single-tree replications, in a randomized complete block design, was conducted in a commercial orchard in Clintondale (Ulster Co., NY). The orchard planting was in its eighth leaf, planted at 698 trees/A (1,730 trees/ha) using the M.9-T337 rootstock in a tall spindle system. Experimental treatments and application/task timings are presented in Table 1. Prohex was applied at a rate of 9 oz/A in four timing strategies. Two bourse shoot pinching treatments, 9 days and 24 days post-full bloom were implemented by the hand removal of apical meristem tissue from all bourse shoots found on each treatment tree in the study. All experimental trees, including the untreated control, were hand thinned 51 days post-full bloom. Treatments were applied at 100 GPA rate using a Solo model 451 backpack mist blower calibrated for a 1.5X concentration, with buffer trees separating treatments. Mature fruit were harvested by whole tree strip-pick, and then 30 apples were randomly subsampled. Apples were numbered individually, preconditioned at 50°F for six days, and held in refrigerated storage at 38°F for a total of 90 days. Individual apples were rated at 45 days post-harvest for BP incidence, counts of BP lesions, visually assessed for percent surface area afflicted with BP in 10% increments, diameter (mm), length (mm), and weight (grams). A second and final evaluation of BP incidence and BP surface area was made at 90 days post-harvest.

Ten random terminal shoots without fruit present were selected from each tree, tagged, and the season's shoot extension (TSE) recorded at 33 and 100 days post-full bloom, by which time terminal buds had set. Ten random fruits growing on spurs in the interior of each tree were tagged and the extension of bourse shoot(s) associated with the fruit at that location (BSE) was recorded at 33 and 100 days post-full bloom. At harvest, the available fruits from tagged spurs were harvested, labeled as to tree and spur location, and stored for later BP evaluation according to the previously described preconditioning and storage regime. At 60 days post-harvest, these individually labeled apples were evaluated for BP incidence, counts of BP lesions, percent surface area afflicted with BP in 10% increments, and weight (grams). Measurements of bourse shoot extension at each spur were then associated with the relevant labeled apple.

2017 Experiment*:* A field trial with eight single-tree replications, in a randomized complete block design, was conducted in a commercial orchard in Milton (Ulster Co., NY). The orchard was in its eighth leaf, planted 1,784 trees/A (4,425 trees/ha) using the M.9-T337 rootstock in a tall spindle system. Experimental treatments and application/task timings are presented in Table 2. Prohex applied at a rate of 6 oz/A in four timing strategies, and at 3 oz/A in back-to-back applications at tight cluster (TC) and pink stages pre-bloom. All experimental trees, including the untreated control, were hand thinned at 33 days post-full bloom to the largest fruit in each cluster, and again at 59 days post-full bloom to the target fruit load of 4.6 fruits/cm^2 trunk cross-sectional area (TCSA). Treatments were applied at 100 GPA rate using a Solo model 451 backpack mist blower calibrated for a 1.5X concentration, with buffer trees separating treatments. Mature fruit were harvested as a strip-pick from the top down until a sample size of 40 apples per tree was achieved. Apples were numbered individually at harvest, preconditioned at 50°F for seven days,

Table 1. 2016 Prohexadione-calcium and Bourse Shoot Pinching Treatments and Timings

		Days (Pre) or Post Full Bloom			
Treatment	**Rate/Task**	**Final No. Fruits/cm^2 TCSA****	**Timing 1**	**Timing 2**	**Timing 3**
Control	Hand Thinning	4.5	51*		
Prohex at Pink	9 oz./A	2.7	-15		
Prohex: 3 Tri-Weekly Starting Pink	9 oz./A	2.2	-15	7	27
Prohex at Petal Fall	9 oz./A	2.1	3		
Prohex: 3 Tri-Weekly Starting PF	9 oz./A	1.6	3	25	45
Bourse Shoot Pinching at 9 Days Post FB	All bourse shoots	3.4	9		
Bourse Shoot Pinching at 24 Days Post FB	All bourse shoots	2.7	24		

* Hand-thinned to the target fruit load/tree

** Treatment crop loads were not significantly different, JMP ANOVA Tukey HSD $p \leq 0.05$

Note: All treatments were hand-thinned to the same protocol as "Control". Full bloom date was 30-Apr.

Table 2. 2017 Prohexadione-alcium Treatments and Timings.

		Days (Pre) or Post Full Bloom			
Treatment	**Rate/Task**	**Final No. Fruits/cm^2 TCSA**	**Timing 1**	**Timing 2**	**Timing 3**
Control	Hand Thinning	4.6	33*	59**	
Prohex at Tight Cluster	6 oz./A	4.6	-12		
Prohex at Tight Cluster & Pink	3 oz./A each appl.	4.6	-12	-7	
Prohex at Pink	6 oz./A	4.6	-7		
Prohex at Petal Fall	6 oz./A	4.6	9		
Prohexadione-Ca: 3 Tri-Weekly Starting PF	6 oz./A	4.6	9	31	51

* Hand-thinned to the largest fruit/cluster

** Hand-thinned to the target fruit load/tree

Note: All treatments were thinned to the same protocol as "Control". Full bloom date was 7-May then held in refrigerated storage at 38°F for a total of 105 days. Individual apples were rated at 53 days post-harvest for BP incidence, counts of BP lesions, visually assessed for percent surface area afflicted with BP in 10% increments, and weight (grams). A second and final evaluation of BP incidence, BP surface area, diameter (mm), and length (mm), was made at 105 days post-harvest.

Ten random terminal shoots without fruit present were selected from each tree after terminal buds had set, and the season's extension (TSE) was recorded. Ten random fruits set from terminal buds in the exterior canopy of each tree were selected at harvest, tagged, and the TSE associated with each fruit location was recorded. The ten fruits from tagged terminal buds were harvested at maturity, labeled as to tree and terminal bud location, and stored for later BP evaluation according to the previously described preconditioning and storage regime. At 60 days post-harvest, individually labeled apples were evaluated for BP incidence, counts of BP lesions, percent surface area afflicted with BP in 10% increments, and weight (grams). Measurements of TSE at each fruiting location were then associated with the relevant labeled apple.

Data Analysis: Whenever possible, data was recorded and analyzed at the level of individual fruits using JMP statistical analysis software (JMP Pro v.14.0.0, SAS Institute Inc. 2018). All data sets were tested to determine the type of distribution, and whether a data transformation to approximate normality was required prior to analysis. Data sets that did not fit a normal distribution, and whose normality could not be approximated following a log transformation, were analyzed using non-parametric statistical procedures with numerical data transformed into ranks. Modern *Analysis of Means* (AOM) procedures (Nelson et al. 2005) as implemented in JMP software were utilized for the analysis of binomial bitter pit incidence data at the level of individual fruits. For continuity, appropriate AOM procedures were utilized for the analysis of all data sets. To maximize clarity for the reader, the graphical output of these analyses are not presented, and statistical differences between means are represented by more traditional letters. Differing letters indicate statistical significance at a 95% confidence interval.

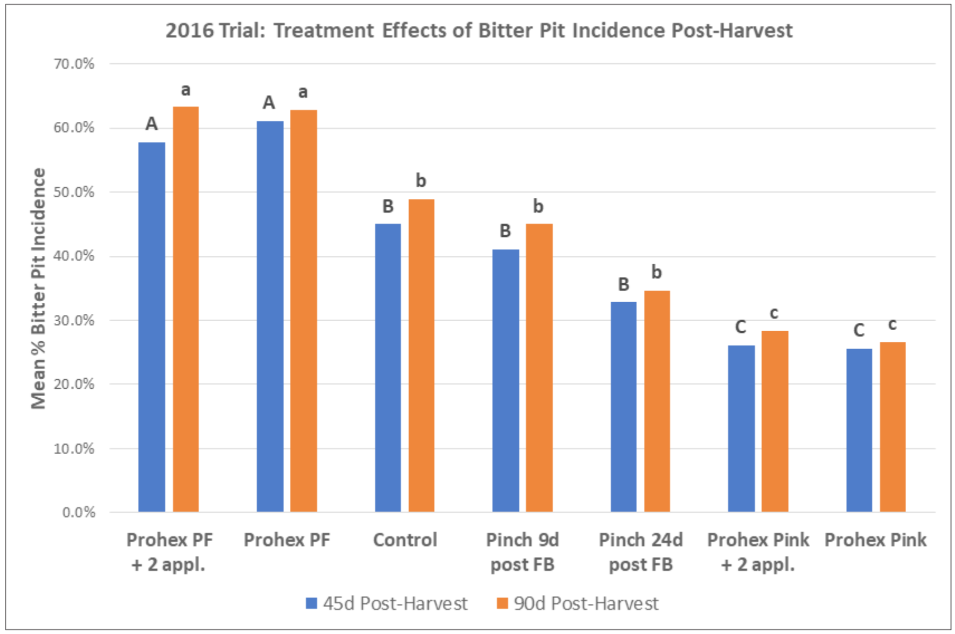

Figure 1. Bitter pit incidence after 45 and 90 days of refrigerated storage. Differing letters indicate statistically significant treatment results within each storage rating timing. The upper-case letters reference the 45-day rating, the lower case letters reference the 90-day rating.

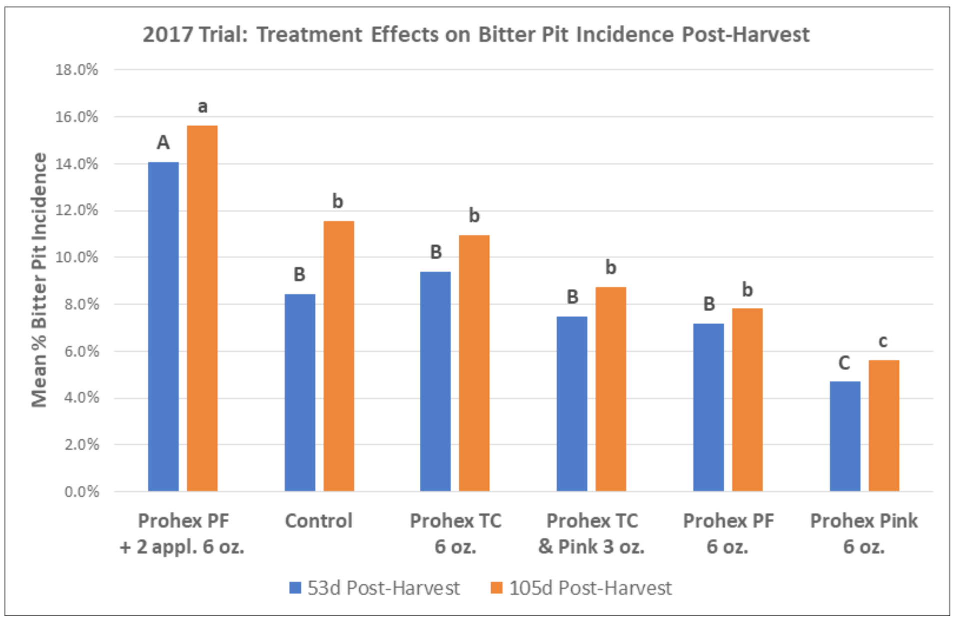

Figure 2. Bitter pit incidence after 53 and 105 days of refrigerated storage. Differing letters indicate statistically significant treatment results within each storage rating timing. The upper-case letters reference the 53-day rating, the lower case letters reference the 105-day rating.

Results

Effects on Bitter Pit: Spring of 2016 was extraordinarily challenging for Hudson Valley apple producers. Temperatures as low as the single digits (°F) were experienced around the early tight cluster stage of pre-bloom flower development during the first week of March. The damage to flower clusters was significant, and is documented in an earlier article (Reig and Donahue 2016). As a result, the distribution of fruit set was highly clustered, and even hand thinning to an equivalent crop load within the experiment was an additional challenge. In the end, crop load ranged from 1.6–4.5 fruits per cm^2 TCSA, with no statistical significance found between treatments. Spring of 2017 offered favorable conditions, with a heavy bloom and uniformly distributed fruit set allowing for very precise hand thinning to 4.6 fruits per cm^2 TCSA across all treatments.

Prohex was found in 2016 (Figure 1) and 2017 (Figure 2) to have both positive and negative effects on bitter pit incidence, depending on application timing. In 2016, prohex applied at a rate of 9 oz/A at the pink stage of flower development was found to significantly reduce bitter pit incidence, 26.7% versus 48.9% (control) after 90 days in refrigerated storage at 38°F. A conventional three-spray program on a tri-weekly interval, but started earlier than commonly practiced, at pink, also resulted in significantly reduced bitter pit incidence (28.3%). In contrast, prohex applied as a single petal fall application, or as in a conventional three-spray tri-weekly program starting at petal fall, significantly increased the incidence of BP when compared with the control (48.9%), 62.8% and 53.6% respectively. Removing the apical meristem (pinching) of bourse shoots at 9 days post-full bloom did not significantly affect BP incidence, while pinching at 24 days post full-bloom did significantly reduce BP incidence (34.4%). Overall, BP incidence was found to increase slightly (approximately 7%) in all treatments as storage extended from 45 to 90 days.

Table 3. 2016 Prohexadione Calcium and Pinching Treatment Effects on Terminal and Bourse Shoot Extension (TSE and BSE, respectively), Contrasted with BP Incidence.

Treatment	TSE 33d PFB (mm)	*1	TSE 100d PFB (mm)	*1	BSE 33d PFB (mm)	*1	BSE 100d PFB (mm)	*1	90d BP % Inc.	*2
Control	181	a	212	a	65.4	a	74.6	a	48.9	b
Prohex at Pink	125	b	142	b	66.1	a	76.8	a	26.7	c
Prohex: 3 Tri-Weekly Starting Pink	96	c	104	c	36.2	a	44.6	a	28.3	c
Prohex at Petal Fall	126	b	134	b	45.4	a	58.2	a	62.8	a
Prohex: 3 Tri-Weekly Starting PF	123	b	133	c	56.7	a	65.4	a	53.6	a
Bourse Pinching at 9 Days Post-FB	208	a	239	a	43.5	a	71.7	a	45	b
Bourse Pinching at 24 Days Post-FB	172	a	187	b	68.3	a	78.3	a	34.4	c

*1 Non-normal (Weibull) data distribution, JMP Fit Y by X Platform, Analysis of Means Methods on Transformed Ranks, alpha = 0.05

*2 Binomial data distribution, JMP Fit Y by X Platform, Analysis of Means of Proportions, alpha = 0.05

In 2017, prohex applied at a rate of 6 oz/A at the pink stage of flower development was found to significantly reduce bitter pit incidence, 5.3% versus 11.6% (control) after 105 days in refrigerated storage at 38°F. Prohex applied at tight cluster, five days earlier than pink, produced no significant effect (10.9%). Prohex applied at a rate of 3 oz/A, but in back-to-back applications at TC and pink, appeared to do slightly better than TC alone at 8.8%, significantly worse that pink alone, and not significantly different from the control. In contrast to our results from 2016, prohex applied as a single petal fall application did not significantly worsen BP incidence (7.8%) compared with the control. As in 2016, the conventional three-spray tri-weekly program starting at petal fall significantly increased the incidence of BP (15.6%) when compared with the control at 11.6%. As was found in 2016, BP incidence increased as storage progressed from 53 to 105 days, but this time at a rate of approximately 16%.

Effects on Shoot Extension: In 2016, all prohex treatments were found to significantly reduce TSE when measured at 33 days, and again at 100 days post-full bloom (Table 3). Not surprisingly, three applications resulted in the most growth suppression by the end of the vegetative growth period, with the treatment starting at pink offering significantly more growth suppression (96 mm) than starting at petal fall (123 mm), compared with the control at 181 mm. The rate of growth slowed during the period between 33 and 100 days, with the shoots in the pink three-spray treatment trees adding only eight additional mm of extension, while the PF-start treatment trees added 10 mm. At the end of the vegetative growth period, neither of the three-spray treatment timings were statistically different, but both remained significantly less than the control. A single application of prohex at pink fell in the middle range (125 mm), almost identical to a single application of prohex at PF (126 mm) when measured 33 days post bloom, with a similar result observed at 100 days post-full bloom. Pinching bourse shoots at either nine or 24 days post-full bloom did not have a significant effect on TSE measured at 33 days, but pinching bourse shoots at 24 days post-full bloom did significantly reduce TSE compared with the control at 100 days (187 vs. 212 mm). Finally, none of the prohex or pinching treatments resulted in a significant reduction of bourse shoot growth when compared with the control.

In 2017, prohex application timing appeared to play a role in the TSE results, as did the presence or absence of fruit at the terminal (Table 4). A single application of prohex at PF, and the three-spray program starting at PF resulted in the greatest suppression of TSE (170 mm, 172 mm) compared with the control at 286 mm, but only if fruit was absent. If a fruit was present, prohex did not significantly reduce TSE. Prohex applied at pink, or in two consecutive applications, TC and pink, significantly suppressed TSE compared with the control, but not as effectively as later timings. Once again, if an apple was present on the terminal, prohex at pink did not significantly reduce TSE compared with the control. Finally, prohex applied at TC had no significant effect on TSE.

Effects on Fruit Size: In 2016, fruit size (weight) of apples treated with a single application of prohex at pink (170 g) or the

Table 4. 2017 Prohexadione-calcium TSE Effects Contrasted with BP Incidence.

Treatment	TSE wo/ fruit (mm)	*1	TSE w/ fruit (mm)	*1	105d BP % Inc.	*2
Control	286	a	113	a	11.6	b
Prohex at Tight Cluster	297	a	n/a		10.9	b
Prohex at Tight Cluster & Pink	252	b	n/a		8.8	b
Prohex at Pink	225	b	82	a	5.3	c
Prohex at Petal Fall	170	c	84	a	7.8	b
Prohex: 3 Tri-Weekly Starting PF	172	c	81	a	15.6	a

*1 Non-normal data distribution, JMP Fit X by Y Platform, Analysis of Means Methods on Transformed Ranks.

*2 Binomial data distribution, JMP Fit X by Y Platform, Analysis of Means of Proportions.

For all tests, alpha = 0.05

three-spray program starting at pink (168 g) was significantly lower compared with the control (193 g), a reduction of 12.6%. Fruit size (weight) of apples treated with a single application of prohex at PF (198 g) or the three-spray program starting at PF (203 g) was significantly greater compared with the control, an increase of 3.7%. Bourse shoot pinching at nine days significantly increased fruit size (200 g) compared with the control, a 3.7% increase, while bourse shoot pinching at 24 days post-full bloom had no significant effect on fruit size.

For 2017, the prohex application rate was reduced from the previous year's rate of 9 oz./A, to 6 oz./A. We found no significant reduction of fruit size in 2017 when applied at pink. In a repeat of our 2016 findings, prohex applied in a conventional three-spray program increased fruit size, this time by 10.9%. In 2016, bourse shoot pinching effects on fruit size was dependent on timing. Pinching at 9 days post-full bloom resulted in a slight, but significant increase in fruit size, 200 g vs. 193 g; pinching at 24 days post-full bloom was not found to significantly affect fruit size (size data not shown).

Discussion

From tests over two seasons with very different weather conditions, under high and low-moderate levels of BP pressure, in two different orchard locations, 1,539 apples rated in 2016 and 2,240 in 2017, we've learned the following:

- Prohexadione-calcium applied in a single application prebloom at the pink stage has the potential to reduce bitter pit incidence in 'Honeycrisp' by approximately 50%.
- Prohexadione-calcium applied in a traditional three-spray program starting at PF can significantly increase bitter pit incidence in Honeycrisp.
- Our data suggests there is a prohexadione-calcium rate and timing response:
  - The 9 oz/A rate reduced fruit size, the 6 oz/A rate did not.
  - Tight cluster application at the 6 oz/A rate is too early to suppress BP.
  - The 3 oz/A rate appears to be too low for effective BP suppression.

Based on the efficacy data produced by this study, BASF Corp. obtained a New York State 2(ee) label in 2018 for the use of Apogee[R] applied at pink for the suppression of bitter pit in the 'Honeycrisp' variety when applied at a rate of 6 oz/A and not adjusted for tree-row-volume (TRV). The economic value to the grower of a 50% reduction in bitter pit incidence is potentially thousands of dollars per acre and is straightforward enough to calculate; however, understanding why prohex at this early timing has a positive effect, and "conventional" application timing has a negative effect, is not clear, and likely not very straightforward. Our original hypothesis was based on the following assumptions:

- Actively growing shoots and young fruitlets aggressively compete for calcium during the cell division (mitosis) period of fruit development. Research conducted by Cheng and Sazo (2018) has shown that the varieties 'Honeycrisp' and 'Gala' partition calcium between leaves and fruits differently, and that when measured at fruit maturity, 'Honeycrisp' was found to have relatively more calcium in the leaves and less in the fruits than 'Gala'.
- Due to the relatively higher transpiration rate of shoots compared with fruits, understanding that calcium moves throughout the tree via the xylem, and the transpiration stream is the "engine", fruitlets would be inherently a poor competitor for calcium.
- By reducing TSE, especially in the immediate vicinity of the fruit, one might tip the competitive balance away from the shoot and towards the fruit. Lakso and Goffinet (2017), in their recent review, described the significance of shoot position, shading and timing in the partitioning of carbohydrates between shoots and fruits.

The data presented here does not appear to support our hypothesis. The right-hand columns in Table 3 (2016) and Table 4 (2017) summarize BP incidence for each treatment, allowing the reader to compare BP% incidence with various shoot extension measurements. Regression analysis conducted on the 2016 and 2017 data (analysis not shown) did not find any significant relationship between shoot extension, terminal or bourse, and bitter pit incidence. In both 2016 and 2017, we found that the presence of a fruit on the spur or terminal appeared to negate the shoot extension reduction effects of prohex that we observed when fruit was not present. Since prohex is known to inhibit the synthesis of GA_1, and basal translocation is not a characteristic of prohex, perhaps the transformation of GA_{20} to GA_1 by the seeds of the associated fruit are not affected by prohex application to the fruit surface and adjacent leaves, so there remain localized levels of gibberellins adequate to stimulate shoot extension. If competition for calcium between fruits and shoots was a significant factor in bitter pit development, it would be reasonable to surmise that fruits and shoots that are near each other would be the competitive epicenter of the relationship. The data from our study indicates this is not the case. However, let's make a distinction between fruit calcium content and bitter pit incidence. We have preliminary data (Donahue et al. unpublished) from the 2017 experiment that suggests the reduction of TSE following prohex treatments does result in a significantly higher concentration of calcium in apple peels at harvest, but with no corresponding reduction in observed bitter pit incidence. Additional research exploring this finding is being conducted in 2018.

Our data on fruit size suggest that in some cases, BP incidence appears to increase with a modest increase in fruit size and decrease when there is a modest reduction in fruit size. However, we do not consider this relationship to be causal, as has been the conventional wisdom. This was demonstrated by our results in 2017, when prohex at pink reduced BP but did not reduce fruit size, and in 2016 where pinching at 24 days post-full bloom reduced BP but did not reduce fruit size. Work continues on this project in 2018, additional findings will be reported in future articles.

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