FINAL PROJECT REPORT **YEAR**: 2 of 2

Project Title: ABA chemical fruit thinning of Bartlett Pears

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[1]Budget: **Year 1**: $10,483 **Year 2**: $10,871

Other funding sources: None presently.

Budget 1: Todd Einhorn
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Item	2013	2014
Salaries[1]	6,944	7,152
Benefits	745	745
Wages[2]	1,800	1,800
Benefits	198	198
Equipment	0	0
Supplies	0	0
Travel[3]	200	200
Miscellaneous[4]	776	776
Total	10,483	10,871

Footnotes: [1]Salaries are calculated as 4 months of a 0.49 FTE Graduate Student Research Assistantship at the monthly rate of $1,736. The increase in salary in year 2 reflects a 3% rate increase. Graduate student benefits for the period equate to $745. [2]Wages are for one part-time employee to work 150 hours ($12/hr) to aid in weekly plant measurements, bloom count, fruit set, harvest, and postharvest fruit quality assays. Part-time employee benefits are calculated at an 11% rate. [3]Travel includes trips to and from one regional PNW research site. [4]Miscellaneous costs account for MCAREC plot fees at a rate of $3,103/acre, prorated to ¼ acre (= $776) for field on-site field trials.

Objectives:

1. Identify the appropriate application timing of ABA for thinning Bartlett pears.
2. Identify the appropriate rates of ABA for thinning Bartlett pears.
3. Compare and contrast the response of Bartlett pears following ABA applications over multiple sites using meteorological data generated from individual test sites.

Significant Findings:

- A total of 5 trials were administered to evaluate the thinning efficacy of ABA (ProTone®, Valent Biosciences Corp.) on Bartlett pear trees. Thinning was rate-responsive but inconsistent from year-to-year: Strong thinning was observed in 60% of the trials and insignificant thinning in 40%. When ABA thinned, the most effective rate was ~100-150 ppm; higher concentrations removed too many fruits and lower concentrations not enough. Concentrations above 400 ppm were phytotoxic to leaves resulting in necrotic leaf spots and partial defoliation.
- We showed that ABA reduced photosynthesis, indirectly, by reducing stomatal conductance (partial closure of stomates). Photosynthesis responded to ABA rate. This action persisted in the plant for a relatively short period of time. Photosynthesis was inhibited 1 HR after application. Four days after application, 100 ppm ABA leaves were photosynthesizing at~ 80% of control levels and nearly 100% by day 12.
- ABA, on its own, did not appear to produce enough carbon stress to elicit sufficient thinning when sunny conditions prevailed the week after treatment. However, when combined with cloudy, overcast weather, thinning efficacy was high. Daily solar radiation (light) data supported this observation.
- In 2014, we conducted a shade x ABA trial to test the relationship between light level and ABA on thinning. Shade houses were constructed out of 30% or 60% shade cloth and placed over whole canopies for 15 d. ABA (125 ppm) was applied to trees in the presence or absence of shade. Under natural light (no shade), ABA-treated trees retained ~35% fewer fruits compared to unshaded controls. Fruit set of canopies under 30% shade had less fruit set than full-sun trees, but ABA + 30% shade did not improve the effect. Trees receiving 60% shade had ~ half the fruits of control trees; at this level of shade, ABA only slightly increased the thinning response.
- For the shade study, 125 ppm ABA reduced photosynthesis by 95% on day 1, but a recovery to 75-80% of control photosynthesis had occurred by day 3. Photosynthesis remained ~20% reduced until day 11. Leaf photosynthesis of trees shaded 30% were only slightly reduced from unshaded levels, indicating that sufficient light was available to saturate the response; however, leaf photosynthesis of 60% shaded trees was markedly reduced. Under this level of shade, interestingly, ABA did not augment the effect.
- In addition to solar radiation, tree age or, more importantly, carbon stores, may have played a key role in the inconsistent results. For the 3 trials where thinning was effective, trees were relatively small (between 7-10 years-old); however, the 2 trials in which relatively no thinning was observed were performed on 18 and 19-year-old trees with markedly larger canopies and, presumably, root systems.

Methods:

ABA (ProTone, Valent BioSciences Corp.) plus 0.1% surfactant was sprayed to drip using a pressurized handgun. Rates of ABA varied depending on the trial. In all 5 experiments, whole canopies of Bartlett pear trees were treated. At all sites, each treatment was replicated 4 times.

Timing of application occurred ~10mm fruit size, though one trial examined earlier applications at petal-fall. For each replicate tree, a minimum of 200 flower clusters were counted and tagged on scaffold limbs. Just prior to hand thinning timing (~35 days after bloom), fruit were counted on tagged sections of scaffolds and fruit set was determined. Scaffolds were roughly chest-height and adequately represented the condition of the lower and mid-canopy, but fruit set, in general, was greater in the top portion of the canopy (probably due to less spray coverage in the tops).

Photosynthesis measurements were intended to begin one day prior to each of the two treatment timings and continue every couple of days until the effect disappeared; however, our photosynthesis chamber malfunctioned, necessitating shipment to the (MA) for repairs in 2013. We received the instrument 5 days after the 10 mm treatments were applied and measurements began the next day, rendering only a partial data set. We did, however, repeat the trial in 2013 in Parkdale. Complete photosynthesis data sets were recorded for 2 of the 5 trials.

All fruits were counted at harvest and weighed. Fruit quality at harvest and PH was assessed in 2014.

Results and Discussion:

The 2013 Parkdale site was a good example of ABA's potential as a 'Bartlett' thinning compound (Figure 1). Fruit set, in general, at this site was high. Absolute fruit set and the number of fruit removed by hand-thinning, were significantly reduced by ABA; the effect was more pronounced with increasing rate. Rates of 200 and 400 ppm were excessive, resulting in too much drop for acceptable commercial harvest levels. In fact, rates of 400 ppm resulted in a high degree of leaf phyto-toxicity (necrotic spots and defoliation of ~25% of the canopy). Hence, the combined effects of 400 ppm ABA on photosynthesis and defoliation led to the drastic thinning response observed. The 100 ppm rate, however, reduced hand thinning by ~half.

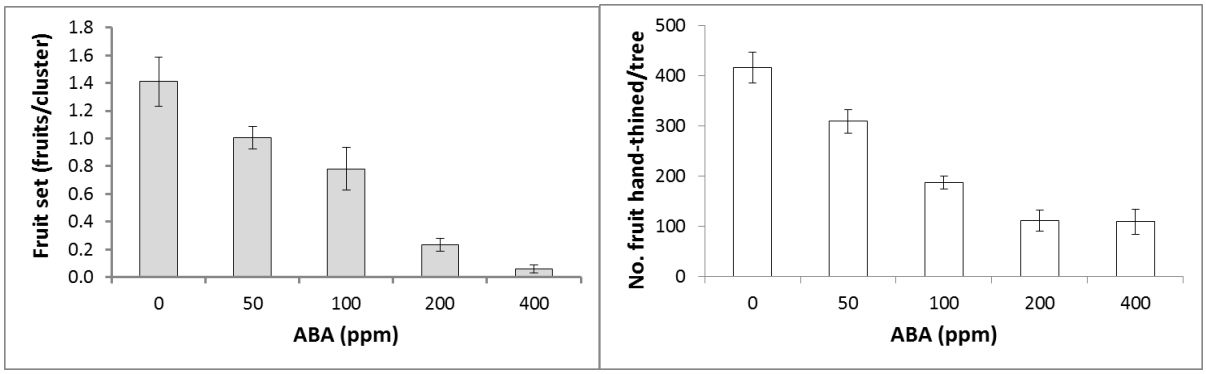


Figure 1. Fruit set (left) and hand thinned fruit (right) as affected by ABA rate. Both data sets are from the 2013 Parkdale trial.

In addition to reducing overall fruit set, ABA had a positive effect on the fruit density of individual spurs (Figure 2). An increase in the number of blank spurs (i.e., spurs failing to set any fruits) was observed with increasing ABA rate. For control trees, ~50% of the spurs were void of any fruit following natural 'June' drop, compared to 94% in trees sprayed with 400 ppm ABA. Prior to hand thinning, a higher percentage of the total fruit on ABA-treated trees resided singly on spurs. In general, fewer fruit were set on multiple-fruit spurs of ABA-treated trees compared to controls. However, for the concentration range best-suited for commercial use (~100 ppm), ABA still produced a small percentage of spurs possessing 3 or 4 fruits/spur.

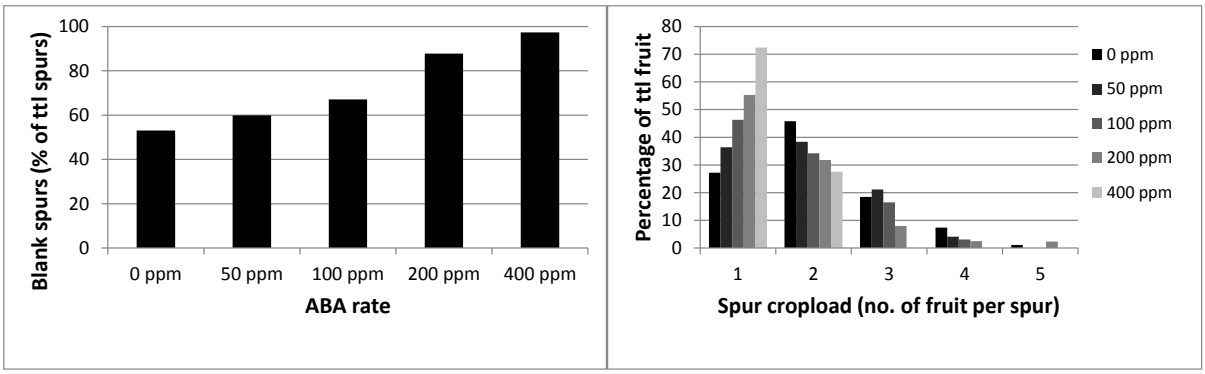


Figure 2. Percentage of total spurs on selected scaffold limbs that did not set fruit (i.e., blank) following applications of ABA at 10 mm timing (left). The right panel shows the distribution of spurs bearing 1 to 5 fruits as affected by ABA rate. Frequency distributions were done prior to hand-thinning, but after natural (and ABA induced) fruit drop occurred. Data were collected from pre-selected scaffolds and are means of 4 replicates.

After 3 years of trials, however, thinning was not consistent and good thinning efficacy of ABA was only observed in 3 of the 5 trials (Table 1). The lack of consistent thinning by ABA, as observed in the 2013 and 2014 (19-year-old trees) Hood River trials (Table 1), suggests that other factors contributed to the thinning efficacy of ABA. Recently, Middelberg et al. (2014) demonstrated that the uptake of ABA at the leaf cuticle was unresponsive to fluctuations in temperature or humidity. Hence, poor ABA uptake as a function of differing application temperatures from year-to-year or site-to-site can likely be ruled out as a limiting factor to thinning efficacy.

Summary of 5 ABA thinning trials (site and tree age provided). Data are percentage fruit set before hand thinning relative to controls (i.e., treatment fruit set as a percentage of control fruit set).

ABA (ppm)	2012 Hood River 8-year-old	2013 Hood River 18-year-old	2013 Parkdale 9-year-old	2014 Hood River 19-year-old	2014 Hood River 9-year-old
0	100	100	100	100	100
50		100	71	95	
100		100	57	86	
125	59				65
150		96			
200		78	18	79	
250	12				
400			7	58	
500	2				

ABA applied ~10 mm fruit diameter.

In fact, photosynthesis data support that ABA was taken up in the 2013 Hood River trial, despite our instrument issues described above. In that trial, photosynthesis of 200 ppm ABA leaves, 6 days after the 10 mm application timing, was 72% of control levels (Figure 3). In comparison, photosynthesis of leaves at Parkdale treated with 200 ppm ABA was 60% of control levels on day 4 and 73% of

control levels by day 8 from treatment application (Figure 3). The effects are harder to see for the lower rates of ABA (50 and 100 ppm) because they had largely disappeared by day 6 in Hood River (~10% reduced from control levels) and were undetectable by day 8 in Parkdale (Figure 3). In fact, at Parkdale photosynthesis of leaves treated with 100 ppm ABA recovered to ~82% of control levels by day 4 from application.

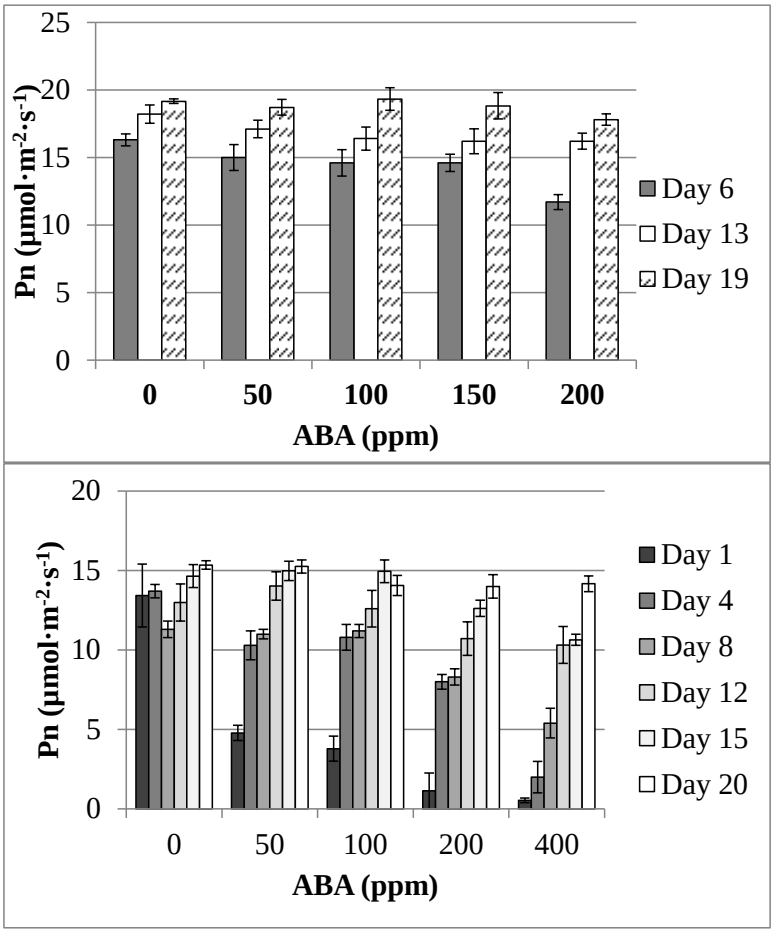


Figure 3. Photosynthesis of pear leaves 6, 13 and 19 days after applications of ABA at Hood River 2013 (above), and more frequently at Parkdale 2013 (below). Equipment malfunction precluded comprehensive measurements in Hood River. Data are means of 4 replicates (n=4).

The most efficacious rate of ABA for commercial thinning would likely fall between 100 and 125 ppm (as seen for trials whose columns are highlighted in grey in Table 1). Clearly, at these rates the effect of ABA on gas exchange (photosynthesis) is quite transient. Presumably, ABA thins by two possible modes of action. The first may be attributed to hormonal effects on fruits which likely involves ethylene (ABA stimulates ethylene synthesis) and possibly direct effects of ABA, though other hormonal interactions cannot be ignored. The second is an indirect effect, via carbon reduction associated with limited photosynthesis. Many chemical thinners work, in part, on this principle and thinning is the outcome of fruit competition for limited available carbohydrates (i.e., demand exceeds supply and weak fruit are at a competitive disadvantage). However, pear trees are quite large and likely have sufficient reserve carbohydrates (in wood and root tissues) to supplement such a short interruption in photosynthate production (i.e., only a day or two of severe reductions and up to 3 to 5 days at 70% to 80% of optimal levels). In fact, average tree age at trials where good thinning was

observed was 9-years, while those that appeared unresponsive to ABA were double in age (Table 1). This observation implies that the thinning activity of ABA may be better related to the compound's effect on carbon balance and gas exchange than hormone balance, per se.

In 2012 and 2013, we plotted daily solar radiation (light) values for each of the three trials (Figure 4). Interestingly, a general relationship was apparent between thinning and light intensity for the 10-day period after ABA application. Sites where thinning was strong (Hood River 2012 and Parkdale 2013) were also plagued by low light levels (typical cloudy spring conditions in OR); the poor thinning observed in 2013 at the Hood River site experienced full sun conditions for the entire 10-day period after ABA application (Figure 4). These data, though only correlative, provide additional support that ABA thinning is dependent upon the carbohydrate status in the plant. Incidentally, these data (carbohydrate status leading up to thinning in combination with forecasted conditions) largely form the basis for carbon driven (deficit : surplus) thinning models (i.e., Malusim).

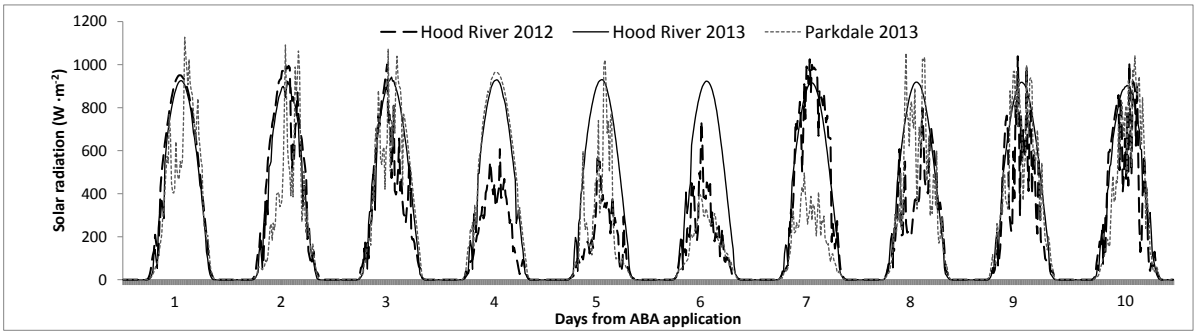
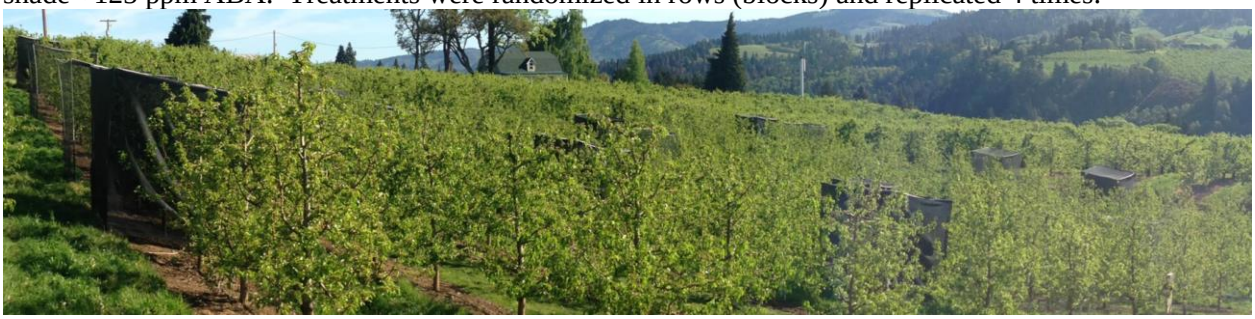


Figure 4. Daily solar radiation levels throughout the 10-day period immediately succeeding ABA applications at the 10 mm timing for 2012 and 2013 trials. Cloudy, low-light conditions in Hood River (2012) and Parkdale (2013) contrast the sunny period immediately following the Hood River 2013 experiment. Data were calculated from meteorological stations in Hood River and Parkdale.

Based on this assumption, we hypothesized that a lack of thinning (2013 Hood River and later observed in 2014) was attributed to the combination of a short-lived reduction in photosynthesis elicited by ABA and high light (sunny) conditions following treatment. In contrast, the high thinning achieved by ABA in 2012 and 2013 Parkdale were commensurate with several weeks of cloudy, overcast weather. In 2014 we designed a factorial trial to test this hypothesis.

Shade houses were constructed out of shade cloth (either 30% or 60% shade) and pvc tubing. Steel fence posts were inserted into the orchard and pre-assembled shade houses were fitted over entire trees and secured to fence posts immediately after ABA (125 ppm) application on 29-April. Control trees were left uncovered. There were a total of 6 treatments: 1) Uncovered control; 2) uncovered control + 125 ppm ABA; 3) 30% shade; 4) 30% shade +125 ppm ABA; 5) 60% Shade; and, 6) 60% shade +125 ppm ABA. Treatments were randomized in rows (blocks) and replicated 4 times.



Photos. Overview of shade experiment plot (top) and close-up of one replicate 60% shade house (left) and 30% shade house (right).

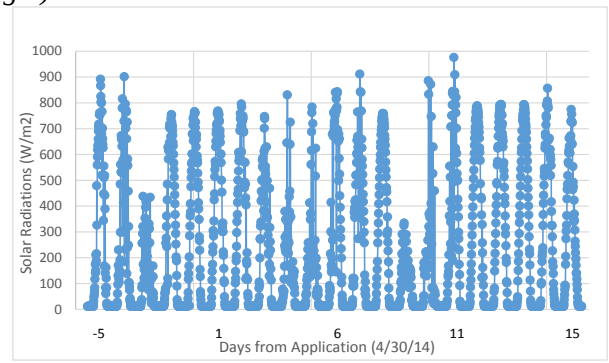


Figure 5. Daily solar radiation -5 days to +15 days after ABA and shade application.

Using forecasted weather, we targeted a cloud-free week between petal fall and 10 mm fruit size to conduct the experiment. Despite our best efforts, two partial-sun/cloud days occurred during the first week of the experimental period (Figure 5). Fruit set of ABA 125 ppm trees was ~35% reduced relative to untreated trees (Table 1 and Figure 6). Thirty percent shade alone reduced fruit set, compared to unshaded controls, but ABA 125 ppm applied to these trees did not improve the response. The highest level of shade resulted in more than 50% reduction in control fruit set. The addition of ABA did not, however, drastically alter thinning. These data indicate that both shade and ABA have the potential to thin, but when combined, the effect was not additive.

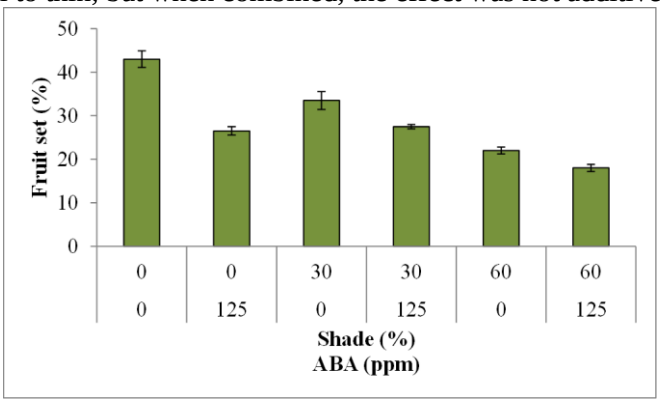
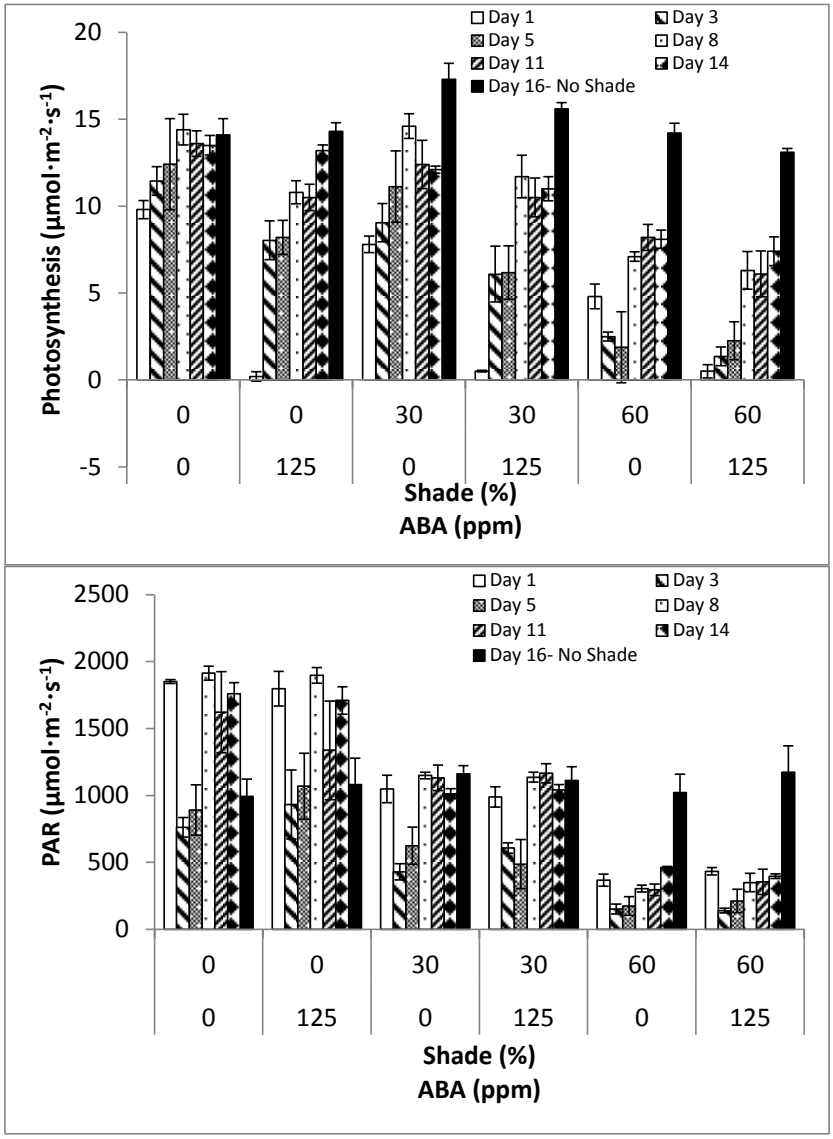


Figure 6. Fruit set as affected by ABA 125 ppm and/or shade (30% or 60%). Trees were 9-year-old Bartlett. Data are from 4 reps.

125 ppm ABA markedly reduced photosynthesis of control trees by 95% on day 1, but a recovery to 75-80% of control levels had occurred by day 3 (Figure 7 top panel). Photosynthesis remained ~20% reduced until day 11. Leaf photosynthesis of trees shaded 30% were reduced to levels similar to those induced by ABA on its own (i.e., without shade), with the exception of day 1 when photosynthesis was nearly 0 for ABA-treated trees. Only minor reductions in photosynthesis were observed for 30% shade leaves relative to unshaded trees, indicating that light was non-limiting for maximum photosynthesis. In fact, PAR (light levels) measured at the leaf surface of 30% shade trees was above saturating conditions (~750 to 1,000 units PAR) for leaf photosynthesis on most days (Figure 7 bottom panel). Despite the fact that these trees were small, this would not likely be the case for the whole canopy given that internal canopy leaves would experience more shade. The slightly lower photosynthetic rates of the 30% shade + ABA leaves during the first 5 days of the experiment had only minor effects on thinning relative to 30% shade alone (Figures 6 and 7 top panel). For trees shaded 60%, leaf photosynthesis was markedly reduced, as were light levels (Figure 7). Under this level of shade, interestingly, ABA did not augment the depression in photosynthesis. These data do not support the additive effects of ABA and shade on fruit set.



ABA did not reduce fruit size in the shade x ABA experiment (Table 2) as was the case for all other experiments (data not shown). In fact, when measured, fruit growth rates were unaffected by ABA rate (data not shown). Fruit quality (firmness, soluble solids and titratable acidity) was also unaffected at harvest and following ripening (data not shown).

Effect of ABA and Shade on Hand thinnning and harvest parameters.

Treatment		Fruits hand thinned	Harvest		
ABA	Shade	no./tree	Fruits/tree	lbs/tree	Fruit sz (g)
0 ppm	0%	18 a	225	21.9	193 d
ABA 125 ppm	0%	3 b	142	15	211 bc
0 ppm	30%	11 ab	165	15.1	197 cd
ABA 125 ppm	30%	3 b	144	16.8	215 b
0 ppm	60%	3 b	166	14.6	195 d
ABA 125 ppm	60%	0 bc	142	16.6	252 a
Pr (>F)		0.01	0.1	0.23	0.012

In summary, these results present somewhat of a challenge for moving forward with additional work on ABA. Ample carbon supplies of older trees might preclude the thinning activity of ABA for pear. When sunny, high-light conditions occurred during the first few days from application, ABA did not induce fruit drop. However, we demonstrated that ABA thinning was not drastically altered by shade when provided at a relatively high level (i.e., 60%). The effects of ABA and shade do not appear to be additive. Increasing ABA rate is not a solution due to phytotoxicity at high rates.

Executive Summary

Between 2012 and 2014, a total of 5 trials were administered to evaluate the thinning efficacy of ABA (ProTone®, Valent Biosciences Corp.) on Bartlett pear trees. Thinning was rate-responsive but inconsistent from year-to-year: Strong thinning was observed in 60% of the trials and insignificant thinning in 40%. When ABA thinned, the most effective rate was ~100-150 ppm; higher concentrations removed too many fruits and lower concentrations not enough. When effective, ABA significantly increased the proportion of blank spurs and spurs with a fruit density of 1. The frequency of multiple-fruited spurs was reduced as ABA rate increased.

ABA reduced photosynthesis, indirectly, by reducing stomatal conductance (partial closure of stomates). The rate of photosynthesis was inversely related to the rate of ABA. One hour after ABA application, photosynthesis was completely inhibited. Four days later, 100 ppm ABA leaves were photosynthesizing at~ 80% of control levels and nearly 100% by day 12. High rates of ABA (400 ppm) reduced control photosynthesis by roughly half for a period of 8 days beyond application. Photosynthesis only recovered to 75% of control levels by 15 days after application. Moreover, 400 ppm ABA was phytotoxic to leaves resulting in necrotic leaf spots and partial defoliation.

ABA, on its own, did not appear to produce enough carbon stress to elicit sufficient thinning when sunny conditions prevailed the week after treatment. However, when combined with cloudy, overcast weather, thinning efficacy was high. Daily solar radiation (light) data supported this observation.

Therefore, in 2014 we conducted a shade x ABA trial to test the relationship of these factors on thinning. Pre-assembled shade houses, constructed from PVC and 30% or 60% shade cloth, were placed over whole canopies immediately after ABA application and were maintained for 15 d. There were a total of 6 treatments: 1) Uncovered control [0% shade]; 2) uncovered control [0% shade] + 125 ppm ABA; 3) 30% shade; 4) 30% shade +125 ppm ABA; 5) 60% shade; and, 6) 60% shade +125 ppm ABA. Trees treated with ABA alone retained ~35% fewer fruits compared to uncovered controls. 30% shade produced fruit drop similar to ABA on its own, but the addition of 125 ppm ABA to 30% shaded trees did not improve the effect. Trees provided 60% shade had roughly half the fruit retention of control trees; at this level of shade, ABA only slightly increased the thinning response.

Application of 125 ppm ABA to unshaded trees reduced photosynthesis by 95% on day 1, but a recovery to 75-80% of control photosynthesis occurred by day 3 and remained ~20% reduced until day 11. Leaf photosynthesis of trees shaded 30% was only slightly reduced from unshaded levels for the first few days, indicating that light was nonlimiting for photosynthesis. PAR data at the leaf surface supported this conclusion. The addition of ABA to trees provided 30% shade only slightly lowered leaf photosynthesis relative to 30% shade alone. At 60% shade, photosynthesis was markedly reduced. Under this level of shade, interestingly, ABA did not augment the effect.

Tree age or, more importantly, carbon stores, may have played a key role in the inconsistent results. For the 3 trials where thinning was effective, trees were relatively small (between 7-9 years-old); however, the 2 trials in which no thinning was observed comprised 18 and 19-year-old trees, possessing markedly larger canopies and, presumably, root systems.

When sunny, high-light conditions occurred during the first few days from application, ABA did not induce fruit drop. However, we demonstrated that ABA thinning was not drastically altered by shade when provided at a relatively high level (i.e., 60%). The effects of ABA and shade do not appear to be additive. Improving thinning by increasing ABA rate is not a viable solution due to the phytotoxicity observed at high rates.