AN ABSTRACT OF THE THESIS OF

Matthew A. Arrington for the degree of Master of Science in Horticulture presented on March 20, 2015

Title: Novel Methods for Crop Load Management of Pear Cultivars in the Pacific Northwest

Abstract approved:

________________________________________
Todd C. Einhorn

Novel approaches to crop load management of pear orchards in the Pacific Northwest (PNW) are necessary, but will need to vary according to the unique vegetative and reproductive growth habits of the cultivar. ‘D’Anjou’ is vigorous and non-precocious; thus, strategies to limit vigor and induce early fruiting are required. In contrast, ‘Bartlett’ possesses a high fruit-setting efficiency and, consequently, is prone to over-setting fruit. Thus, crop reduction is necessary to achieve marketable fruit size, but hand thinning is time-consuming and costly. Two methods were investigated to resolve these issues: Root pruning of ‘d’Anjou’ pear trees and, chemical thinning of ‘Bartlett’ pears using abscisic acid (ABA).

Root pruning was imposed on one or both sides of the tree row and compared to an untreated control plot at two sites: Moderate-density, 6th leaf ‘d’Anjou’/OH x F 87; and, high-density, 4th leaf ‘d’Anjou’/OH x F 87. Root pruning two-sides of the tree row consistently reduced shoot growth and the effects were partially dependent on tree age at the time of root pruning. Return bloom was positively affected by root pruning, but fruit weight was often reduced. Reduced fruit weight was not associated with mid-season
water or nutrient deficits. Yield and yield efficiency the year following double-sided root pruning were improved with the greatest response occurring in the younger orchard (i.e., ~70% yield increase over control plots). Root pruning is a viable strategy to reduce vigor and improve precocity in high-density ‘d’Anjou’ plantings when performed in the 3rd or 4th year after planting.

Thinning efficacy of abscisic acid (ABA) applied to ‘Bartlett’ pear trees, between petal fall and 12mm fruit size, was inconsistent. Among four trials, ABA produced a rate-responsive, transient reduction in stomatal conductance and net photosynthesis (Pn) of ~ 80% to 95% within hours of application, the effect lasted one to two days. By day three, Pn returned to ~80% of control plots and was fully recovered by 7-10 days after application. Thinning was best achieved at 100-125 ppm ABA. Higher rates caused greater fruitlet abscission but rates exceeding 400 ppm resulted in phytotoxicity and leaf abscission. In field trials, good thinning was observed in years when low natural light (i.e., cloudy conditions) occurred during the week immediately succeeding ABA applications. Only a few days of low light appeared necessary to elicit ABA-induced fruit abscission. To test the additive effect of shade on ABA-induced thinning, an experiment was designed to expose whole canopies to one of three levels of shade (0%, 44%, or 77%) and two levels of ABA (0 or 125 ppm). Shade houses were erected within hours of ABA application (~petal fall) and were left in place for 15 days. Both ABA and shade affected fruit abscission, but 44% shade did not significantly reduce fruit set compared to control plots. Photosynthetic active radiation (PAR) was reduced relative to the intensity of shade, but Pn was not. Moderate shade (i.e., 44%) led to only minor reductions of Pn, while 77% shade reduced Pn by 50% to 75% for the duration of the
treatment period. ABA-induced thinning was not significantly improved by the addition of shade; however, two days of non-forecasted, cloudy conditions (i.e., low light) within the first week of the experiment invalidated comparisons to a true control (i.e., 0% shade). Given the short-term $P_n$ limitation induced by ABA, in combination with the high carbon reserves of pear trees, ABA application may not be consistent enough to warrant commercial application.
Novel Methods for Crop Load Management of Pear Cultivars in the Pacific Northwest

by
Matthew A. Arrington

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Major Professor, representing Horticulture

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Dr. Mateus de Silvero Pasa and Mr. Aritz Kerman were international graduate students fulfilling internships at the Mid-Columbia Agriculture Research and Extension Center. They contributed to data collection in both root pruning and ABA thinning projects. Dr. Todd Einhorn contributed to the experimental design, methodology, interpretation of data, and editing of all manuscripts.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Chapter 1: Root pruning reduces vegetative growth, improves return bloom and fruit set, and increases yield efficiency of young ‘d’Anjou’ pear trees</td>
<td>3</td>
</tr>
<tr>
<td>Abstract</td>
<td>3</td>
</tr>
<tr>
<td>1.1 Introduction</td>
<td>5</td>
</tr>
<tr>
<td>1.2 Materials and Methods</td>
<td>9</td>
</tr>
<tr>
<td>Planting material and experimental design</td>
<td>9</td>
</tr>
<tr>
<td>Measurements and Procedures</td>
<td>10</td>
</tr>
<tr>
<td>Statistical Analyses</td>
<td>13</td>
</tr>
<tr>
<td>1.3 Results</td>
<td>13</td>
</tr>
<tr>
<td>1.4 Discussion</td>
<td>17</td>
</tr>
<tr>
<td>1.5 Cited Work</td>
<td>23</td>
</tr>
<tr>
<td>Chapter 2: Abcisic Acid (ABA) inconsistently thins ‘Bartlett’ pear</td>
<td>39</td>
</tr>
<tr>
<td>Abstract</td>
<td>39</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>40</td>
</tr>
<tr>
<td>2.2 Materials and Methods</td>
<td>44</td>
</tr>
<tr>
<td>Planting material and experimental design</td>
<td>44</td>
</tr>
<tr>
<td>Measurements and procedures</td>
<td>45</td>
</tr>
<tr>
<td>Statistical analyses</td>
<td>47</td>
</tr>
<tr>
<td>2.3 Results</td>
<td>47</td>
</tr>
<tr>
<td>2.4 Discussion</td>
<td>50</td>
</tr>
<tr>
<td>2.5 Works Cited</td>
<td>53</td>
</tr>
<tr>
<td>Chapter 3: Shade and ABA effectively thin fruits of ‘Bartlett’ pear</td>
<td>68</td>
</tr>
<tr>
<td>Abstract</td>
<td>68</td>
</tr>
<tr>
<td>3.1 Introduction</td>
<td>69</td>
</tr>
<tr>
<td>3.2 Materials and Methods</td>
<td>72</td>
</tr>
<tr>
<td>Planting material and experimental design</td>
<td>72</td>
</tr>
<tr>
<td>Measurements and procedures</td>
<td>73</td>
</tr>
<tr>
<td>Topic</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>75</td>
</tr>
<tr>
<td>3.3 Results</td>
<td>75</td>
</tr>
<tr>
<td>3.4 Discussion</td>
<td>77</td>
</tr>
<tr>
<td>3.5 Works Cited</td>
<td>80</td>
</tr>
<tr>
<td>General Conclusion</td>
<td>90</td>
</tr>
<tr>
<td>Common Bibliography</td>
<td>92</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGURE 1.1 – Effects of root pruning on shoot growth of 6th leaf ‘d’Anjou’ of Exp. 1 (A), 4th leaf (B) and 5th leaf ‘d’Anjou’ of Exp. 2 (C) .................................................................36</td>
<td></td>
</tr>
<tr>
<td>FIGURE 1.2 – Effect of year one and year 2 root pruning treatments on ‘d’Anjou’ fruit growth. Data were taken on 5th leaf trees of Exp. 2. Means are from 4 replicates (n=20)..................37</td>
<td></td>
</tr>
<tr>
<td>FIGURE 1.3 – Effect of root pruning on stem water potential of 5th leaf ‘d’Anjou’ trees (A) and 4th leaf ‘d’Anjou’ trees (B) of Exp. 2 .................................................................38</td>
<td></td>
</tr>
<tr>
<td>FIGURE 2.1 – Effect of abscisic acid (ABA) rate on 8-year-old ‘Bartlett’ pear shoot growth. In-set is the percent increase in trunk cross-sectional area between 20 and 171 days after full bloom .........................................................64</td>
<td></td>
</tr>
<tr>
<td>FIGURE 2.2 – Effect of abscisic acid (ABA) rate on the percentage of spurs with varying fruit density for 9th leaf ‘Bartlett’ pear. Data are means of 4 replicates ......................65</td>
<td></td>
</tr>
<tr>
<td>FIGURE 2.3 – Effect of abscisic acid (ABA) rate on single-leaf photosynthesis of 18-year-old ‘Bartlett’/OH x F 87 pear trees of Exp. 2 (A) and 19-year-old Bartlett / OH x F 87 trees of Exp. 3 (B Data are means of 4 replicates (n=6). .........................................................66</td>
<td></td>
</tr>
<tr>
<td>FIGURE 2.4 – Diurnal solar radiation from -5 to 15 days from ABA application ..................................67</td>
<td></td>
</tr>
<tr>
<td>FIGURE 3.1 – Randomized complete block design with 4 single-tree replicates selected for uniformity .................................................................86</td>
<td></td>
</tr>
<tr>
<td>FIGURE 3.2 – Single replicates of 77% (left) or 44% shade cloth (right). PVC shade enclosures measured 3 x 3 x 4 m (L X W X H). .................................................................86</td>
<td></td>
</tr>
<tr>
<td>FIGURE 3.3 – Diurnal solar radiation from -5 to 15 days from abscisic acid (ABA) application. Data were collected from a weather station ~100 m from trial site ........................................87</td>
<td></td>
</tr>
<tr>
<td>FIGURE 3.4– Average photosynthetic active radiation (PAR) intercepted throughout 10-year-old ‘Bartlett’/OH x F 87 canopies at 10:00, 13:00 and 16:00 HR under 0% (i.e., exposed canopies), 44% and 77% shade .........................................................88</td>
<td></td>
</tr>
<tr>
<td>FIGURE 3.5– Photosynthetic active radiation (PAR) (A) and single-leaf photosynthesis (B) of 10-year-old ‘Bartlett’/OH x F 87 pear trees during a 15-day shade treatment with and without 125 ppm abscisic acid (ABA) .........................................................89</td>
<td></td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.1 - Treatment summary of Exp. 2 applied to 4th (2013) and 5th (2014) leaf ’d’Anjou’ trees. Year one (2013) replicates were divided in half to accommodate year 2 (2014) treatments.</td>
<td>29</td>
</tr>
<tr>
<td>Table 1.2 – Effect of root pruning on trunk cross-sectional area (TCA), average annual shoot length and photosynthetic active radiation (PAR) interception of 6th leaf (2012), 7th leaf (2013) and 8th leaf (2014) ‘d’Anjou’ trees of Exp. 1.</td>
<td>30</td>
</tr>
<tr>
<td>Table 1.3 – Effect of root pruning on trunk cross-sectional area (TCA), average shoot length, photosynthetic active radiation (PAR) interception, average node number per shoot and average leaf area.</td>
<td>31</td>
</tr>
<tr>
<td>Table 1.4 – Effects of root pruning on return bloom and yield characteristics of 6th leaf (2012), 7th leaf (2013) and 8th leaf (2014) ‘d’Anjou’ trees of Exp. 1.</td>
<td>32</td>
</tr>
<tr>
<td>Table 1.5 – Effects of root pruning on return bloom and yield characteristics of 4th and 5th leaf ‘d’Anjou’ pear trees at high density.</td>
<td>33</td>
</tr>
<tr>
<td>Table 1.6 – Effect of root pruning on fruit firmness (FF), soluble solids content (SSC) and titratable acidity (TA) at harvest and after 60 days of RA storage with or without ripening of 8th leaf ‘d’Anjou’ (2014, Exp. 1) and 5th leaf ‘d’Anjou’ (2014, Exp. 2) fruits.</td>
<td>34</td>
</tr>
<tr>
<td>Table 1.7 – Effect of root pruning on leaf nutrient content of 4th leaf ‘d’Anjou’ trees (2013) and spur nutrient content.</td>
<td>35</td>
</tr>
<tr>
<td>Table 2.1 – The effect of abscisic acid (ABA) rate on fruit set, fruit number, fruit weight, fruit yield and return bloom for 6-year-old ‘Bartlett’/OH x F 97 pear trees (Exp. 1) treated at 10 days after full bloom.</td>
<td>60</td>
</tr>
<tr>
<td>Table 2.2 – The effect of abscisic acid (ABA) rate on fruit production of 18 and 19-year-old ‘Bartlett’/OH x F 87 pear trees (Exp. 2 and 4) treated at 10 days after full bloom.</td>
<td>61</td>
</tr>
<tr>
<td>Table 2.3 – Effects of abscisic acid (ABA) rate on fruit set (fruit per cluster), return bloom, fruit number, fruit weight, and yield of 8-year-old ‘Bartlett’ OH x F 97 pear trees (Exp. 3) treated at 10 days after full bloom.</td>
<td>62</td>
</tr>
<tr>
<td>Table 2.4 – Effect of abscisic acid (ABA) rate on fruit quality measurements of 19-year-old ‘Bartlett’ OH x F 87 pear trees (Exp. 4). Fruit firmness (FF), soluble solids content (SSC) and titratable acidity (TA) were measured at harvest and after 60 days of cold storage at regular atmosphere with and without + 5 days of ripening.</td>
<td>63</td>
</tr>
</tbody>
</table>
LIST OF TABLES (CONTINUED)

Table 3.1 – Effects of shade (0%, 44%, 77%), and 125 ppm abscisic acid (ABA) on fruit set, tree yield (number and weight) and fruit weight of 10-year-old ‘Bartlett’/OH x F 87 pear trees.

Table 3.2 – Effects of shade (0%, 44%, 77%) and 125 ppm abscisic acid (ABA) on 10-year-old ‘Bartlett’/OH x F 87 fruit quality (FF, Fruit Firmness; SSC, soluble solids content; and, TA, titratable acidity) at harvest, after 60 days RA cold storage, and after 60 days RA cold storage + 5 days of ripening.
DEDICATION

I want to express gratitude to my wonderful wife Maren, for all the patience and support she has given through this process. I am also thankful for my two fun and energetic boys Orson and Ezra, for their love and their constant desire to play.
General Introduction

Managing crop load of pear in the Pacific Northwest (PNW) is of vital importance if producers are to optimize their production and profitability. In general, but within limits, tree density and early production are positively related; however, high-density plantings of ‘d’Anjou’, which lack sufficiently dwarfing rootstocks in the US, are difficult to manage due to excessive vigor and delayed production. These issues directly limit early returns on investment. The situation for ‘Bartlett’ pear is different. ‘Bartlett’ is relatively precocious, but tends to overset fruit. Fresh market ‘Bartlett’ pears need to achieve a minimum size, therefore, crop reduction is required (i.e., thinning). Thinning is achieved by hand, thus is costly and depends on the availability of labor. Production practices that reduce high labor inputs need to be developed.

The present project addresses historic and emerging crop management and labor issues associated with the two major pear cultivars produced in the US. For ‘d’Anjou’, root pruning was explored as a potential method to limit vigor and, consequently, promote early fruiting. Vegetative growth and fruiting are inversely related for most tree fruit species. Hence, if precocity of ‘d’Anjou’ can be increased with simultaneous vigor regulation, ‘d’Anjou’ could plausibly be established at higher tree densities facilitating better management and early returns on investment for producers. For ‘Bartlett’ we investigated the use of a natural plant hormone [abscisic acid (ABA)] to chemically thin pears. Uptake and activity of commercially available chemical thinners for pear are limited in the PNW by low temperatures during early fruit development. ABA reduces stomatal aperture and as a result, Pn, causing carbon deficits which, in-turn, may cause
fruit abscission. Due to its climatic insensitivity, ABA may be an ideal candidate for thinning in the PNW. In this study, we evaluated the effectiveness of root pruning and chemical thinning of ‘d’Anjou’ and ‘Bartlett’ pear trees, respectively, on vegetative and reproductive parameters.
Chapter 1: Root pruning reduces vegetative growth, improves return bloom and fruit set, and increases yield efficiency of young ‘d’Anjou’ pear trees

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Abstract

European pear orchards in the US are not established at high tree densities due to excessive scion vigor and low precocity. Dwarfing rootstocks are not commercially available to ameliorate these issues. Root pruning offers a mechanical approach to positively affect canopy growth and fruiting, yet, we are unaware of a comprehensive evaluation of root pruning on pear trees in the US. We, therefore, investigated the response of ‘d’Anjou’ pear trees to root pruning over multiple years at two separate sites: site one was a 6th-leaf, moderate-density (769 trees per ha) orchard; and, site two was a 4th-leaf, high-density (2,243 trees per ha) orchard. At both sites, root pruning was imposed at \textasciitilde10\% of full bloom at a distance of 45 cm from the trees on either one or two sides of the tree row and compared to untreated control trees. The depth of root pruning was 45 cm. Vegetative growth was consistently reduced relative to the severity of root pruning (i.e., single- vs. double-sided), though the magnitude of the response differed between sites partly as a function of tree age and/or planting density. For the 6th-leaf orchard, root pruning was applied in year one and not re-applied in years two or three. Year-one root pruning reduced shoot growth by 25\% and 38\% for one and two-sided treatments, respectively, compared to control plots; however, fruit set, yield and fruit
weight at harvest were all significantly reduced relative to pruning severity. In year two, return bloom, fruit set and yield were significantly improved for the double-sided root pruning treatment only, compared to control plots. Fruit weight, however, remained reduced by both root pruning treatments. Shoot elongation of one and two-sided root pruned trees remained reduced by 9% and 18% in year two and 3% and 4% in year three, respectively, relative to control plots. Harvested fruit number and yield in year three were highest for double-sided root pruned trees, but did not significantly differ from controls; fruit size remained smaller for root pruned treatments. At site two, seasonal shoot elongation of 4th leaf trees was reduced by two-sided root pruning ~ 20% and was unaccompanied by deleterious effects on fruit set, fruit growth, yield or fruit weight the year of root pruning. In year two, half the trees per replicate of all treatments remained unpruned, while the other half were re-pruned to the same level as in year one; additionally, half of the control plots were root pruned on both sides. Shoot growth of unpruned trees, irrespective of year one treatment severity, grew at equivalent rates as untreated control shoots throughout year two. Successive root pruning produced a roughly equivalent level of vigor reduction as achieved in year one, relative to the control. Return bloom, fruit set, yield, and yield efficiency were all improved (yield efficiency and return bloom significantly) for trees root-pruned on both sides the previous year, irrespective of whether or not they were root pruned again in year two, compared to controls and trees root pruned on one-side. Overall, a consistent improvement in the balance between vegetative and reproductive parameters occurred the year following root pruning, irrespective of tree age; however, a concomitant reduction in vigor, yield and fruit weight was observed when root pruning was applied to older trees at levels too
severe. Root pruning, therefore, should be a dynamic process applied early in the establishment phase of the orchard.

1.1 Introduction

Increasing production costs lower the profitability of tree fruit producers. A concomitant reduction in the supply of labor has incentivized producers to adopt labor-assist technologies and high-efficiency training systems (Ampatzidis et al., 2013; Ampatzidis and Whiting, 2013). For precocious systems, the duration of time to return the establishment costs shortens with increasing tree density, up to a point (Elkins et al., 2008; Robinson, 2008; Robinson and Lakso, 1991). High-density plantings are capable of greater light interception per unit land area, and come into production sooner compared to orchards established at lower tree densities (Ferree and Warrington, 2003). Importantly, light interception is positively related to dry matter production, and thus higher yields (Lakso, 1994; Robinson, 1992). Shortening the in-row planting distance is important given the inverse relationship between spacing and tree size due to root competition (Ferree et al., 1993; Robinson, 2011; Weber, 2001). By far, though, the most important component of the modern, high-density orchard system is the dwarfing rootstock.

Dwarfing rootstocks confer size control and induce precocity of the scion (Cummins and Aldwinckle, 1974; Fazio et al., 2014; Fischer, 1974). Several mechanisms are responsible for the effects of dwarfing rootstocks on vigor and bearing habits of fruit trees. Altered translocation of carbohydrates (Jones and Pate, 1976, 1984; Kamboj et al. 1997) and/or water (Webster et al., 2004), and auxin-mediated root metabolism (Lockard and Schneider, 1981) have been purported to alter hormone and
nutrient distribution in roots, which, in turn, reduce scion vigor. Anatomical anomalies in vascular tissue at or around the graft union caused an increased supply of carbohydrate and overgrowth around the graft union of sweet cherry (Olmstead et al., 2010). This led to an interruption in sap flow between the scion and rootstock and was correlated with tree size. Indeed, xylem anatomy and, hydraulic characteristics of rootstocks were strongly related to dwarfing-potential of a new series of peach rootstocks (Tombesi et al., 2011). In addition to physical and physiological modulation of scion behavior, recent evidence is accumulating for the role of molecular mechanisms in dwarfing, such as RNA transport across graft unions (Zhang et al., 2012) and differential gene expression in scion grafted to either dwarfing or non-dwarfing rootstocks (Prassinos et al., 2009). Nevertheless, for pear, there are few semi-dwarfing rootstocks commercially-available in the U.S. and none that adequately control scion vigor (Elkins et al., 2012). *Cydonia oblonga* L. (Quince) confers marked dwarfing on pear scions (Maas, 2006; Wertheim, 1998), but has limited use in the Pacific Northwest (PNW) U.S. due to purported poor freeze and fireblight resistance (Einhorn et al., 2011; Iglesias and Robinson, 2011; Webster, 1998). Hence, insufficient dwarfing in the pear germplasm limits the implementation of high-density orchard systems (Wertheim, 2002).

Root excision to dwarf plants has been employed for centuries. In the last few decades mechanized root pruning has been evaluated in modern orchards (see reviews by Ferree et al., 1992; Geisler and Ferree, 1984; Saure, 2007). There are several advantages of root pruning; the method is simple, mechanized, and hence, labor efficient, and can markedly alter plant behavior. Root pruning is extensively used in commercial apple and pear production in Italy, Belgium, and The Netherlands (S. Musacchi, T. Deckers, and F.
Maas, personal communication). Evaluations have been performed in other regions (Richards and Rowe, 1977; McCartney and Belton, 1992) but widespread adoption has been limited due to inconsistent tree response.

One of the challenges associated with root pruning is achieving a reduction in shoot growth without reducing the carbon supply necessary to optimize fruit set, yield and fruit weight (Poni et al., 1992). Ideally, the reduction in root volume should result in vegetative growth and improved fruit set. The former effect is proportional to the amount of roots removed and will be, in part, influenced by the tree’s ability to re-establish a root-to-shoot equilibrium (Poni et al., 1992). The latter effect is accomplished by relatively low sink strength of stressed, vegetative organs compared to reproductive processes. The subsequently greater sink strength of fruit for carbon might create a cycle that promotes reproductive processes over vegetative growth; a process that conforms to the well-established inverse relationship between scion vigor and fruiting (Westwood, 1993). Moreover, reduced shoot elongation should improve light distribution within canopies (Schupp and Ferree, 1988); a critical aspect for pear given the high sensitivity of fruit set and size to intra-canopy shade (Einhorn et al, 2012; Garritz et al., 1998; Kappel and Nielson, 1994).

Reduced vegetative growth was the most consistent response to root pruning for apple (Ferree, 1989, 1992; Ferree and Rhodus, 1993; Ferree and Knee, 1997; Geisler and Ferree, 1984; Kahn et al., 1998; McCartney and Belton, 1992; Sosna, 2002) and pear (Asin et al., 2007; Vercammen et al., 2005). Results diverge, however, for reproductive parameters: Fruit set, yield, and/or fruit weight of apple were reduced (Ferree, 1989; Schupp and Ferree, 1988, 1990), unaffected (Elfving et al., 1991), or improved (Khan et
al., 1998; Sosna, 2002). For pear, return bloom, fruit set and yield were improved (Asin et al., 2007; Maas, 2008; Yehia et al., 2011). Non-uniform experimental conditions among these studies would have contributed to the disparate results. For example, plant (species, cultivar, rootstock, tree age, root zone), cultural (timing and frequency of application, blade depth and orientation, severity of root removal), and environmental (soil moisture and nutrition, climatic conditions, etc.) factors all have a profound effects on tree response to root pruning (Ferree et al., 1992; Geisler and Ferree, 1984; Saure, 2007). Vercammen et al. (2005) proposed a simple decision-aid model for root pruning severity and timing that accounts for cultural factors such as fertilization and is primarily based on fruit bud quality and quantity in early spring.

‘D’Anjou’ pear comprises 58% of Oregon’s pear production (NASS; Moffitt, personal communication). ‘D’Anjou’ is a non-precocious and vigorous cultivar; low and moderate density plantings generally bear few fruits prior to 6 years after planting (Lombard and Westwood, 1976). Consequently, ‘d’Anjou’ planted in a high-density orchard system tends to be difficult to manage and is, therefore, labor intensive. Despite the lack of commercial, dwarfing pear rootstocks, new plantings are consistently established at higher densities, in part, due to success observed with modern apple production. Hence, root pruning may offer a tool to manage vigor and improve early production. A comprehensive evaluation of root pruning has not been performed on pear in the US. The objectives of the present study were to evaluate single-sided and double-sided root pruning of ‘d’Anjou’ pear trees for reduced vigor of extension shoots and simultaneous improvement of fruit set and yield. We also tested tree response to single
and successive season applications. We hypothesized that root pruning would reduce tree vigor as well as increase second-year bloom, fruit set and yield.

1.2 Materials and Methods

Planting material and experimental design

Experiments were performed at two commercial ‘d’Anjou’ pear orchards in the Hood River valley, OR, between 2012 and 2014. The root pruner comprised a 45 cm long steel shank, ~5° from the vertical mounted at the rear of a tractor. Pruning was performed at 4 km·h⁻¹ at a distance of 45 cm from the trees and applied to either one or two sides of the tree row. Comparisons were made to untreated control plots at each site. All other production practices were performed according to industry standards.

Exp. 1 A sixth-leaf ‘d’Anjou’/OH×F 87 orchard (3 x 5 m.; 769 trees/ha) was selected in Odell, OR (lat. 45.59° N, long. 121.52° W). Soil is a deep, well-drained coarse sandy loam. Treatments were arranged in a randomized complete block design with four, single-row replicates: Untreated control (UC); single-sided (1X) root pruned (RP); and, double-sided (2X) RP. Root pruning was only performed in 2012, but all variables were measured for three years on 10 contiguous trees selected at the time of root pruning.

Exp. 2 In 2013, a 4th leaf ‘d’Anjou’/OHxF 87 orchard (1.5 x 3 m; 2,243 trees/ha) was selected in Dee, OR (Long. 45.58° N, Lat. 121.64° W). Soil is a deep, well-drained fine sandy loam. Treatments were arranged in a randomized complete block design with four, eight-tree replicates: Untreated control (UC); single-sided (1X) RP; and, double-sided (2X) RP (Table 1.1). In 2014 (year 2), all treatment replicates, including the control, were divided in half (each half comprised of four contiguous trees); one half was
left untreated and the other half was root pruned to the same level as the preceding year (Table 1.1). For the former control plots of year one, half of the trees per plot remained as controls and the other half was subjected to 2X RP. The decision to apply 2X RP to untreated trees in year two was due to the insufficient vigor control observed for 1X RP trees in year one.

**Measurements and Procedures**

**Vegetative parameters.** Trunk circumference was measured 20 cm above the graft union and converted to trunk cross-sectional area (cm$^2$). Individual trees of each replicate were measured at full bloom and again at post-harvest. Extension shoots were randomly selected after emergence (25 shoots per replicate), numbered, and flagged. Shoots were selected at a height of approximately 1.5 m from the ground. Each shoot was measured from the growth ring separating current-season growth from one-year-old growth to the apex of the shoot. Measurements were taken weekly from full bloom through harvest and compiled as a seasonal growth curve. In year two, all flagged extension shoots from Exp. 2 were removed following the final measurement date. Nodes were counted on each shoot, and all leaves were counted and processed using a calibrated LI 3000 portable leaf area meter equipped with a 3050 transparent belt conveyer (LICOR Inc., Lincoln, NB) to generate total and average leaf area (LA; cm$^2$). In year two, shoot growth of trees in Exp. 1 was determined from a random sample of 20 shoots selected at the end of the season from low, mid and high positions in the canopy (1, 2 and 3 m height, respectively).

**Reproductive parameters.** Fruit set (%) was expressed as the number of fruit per flower cluster. Flower clusters (no fewer than 200) were counted on selected scaffold
limbs at ~80% full bloom. Fruits retained on each scaffold limb at ~ 35 days after full bloom (DAFB) were counted and divided by the total number of clusters to generate percent fruit set. In years two and three, return bloom was expressed as the percent of total spurs on preselected scaffold limbs with flower clusters. In Exp. 2, 25 fruit per replicate were randomly selected and tagged at ~10 mm diameter to determine fruit growth. Fruit diameter was measured weekly at the widest region of the fruit using a Cranston fruit gauge (Cranston Machinery Company, Inc., Oak Grove, OR, U.S.A.). Whole canopies were harvested in a single event when flesh pressure reached ~6.8 kg, based on commercial maturity standards. Yield efficiency (YE) was calculated as total tree yield (kg) divided by TCA (cm²). At harvest, 50 fruits were randomly sampled and individually weighed to determine average fruit weight. Fruit samples were then used in post-harvest tests below.

**Fruit quality.** Twenty fruits from the 50-fruit sample were immediately assessed for post-harvest quality following harvest. Fruit firmness (FF) was determined on two sites per fruit (opposite one another) after removing a ~5 cm² area of peel using a Fruit Texture Analyzer (Güss Manufacturing, Strand, South Africa). Determination of soluble solids concentration (SSC) and titratable acidity (TA) were performed on a composite juice sample with a digital refractometer (Model PR-101, ATAGO Co., LTD., Bellevue, WA) and automated titration system (Model DL15, Mettler-Toledo, LLC., Columbus, OH), respectively. A 20 mL sample (10 mL juice + 10 mL of DI H₂O) was titrated to an endpoint pH of 8.1 using 0.1 N NaOH. The remaining 30 fruit were stored at -1°C, regular air (RA) for approximately 60 ds (Chen and Mellenthin, 1981; Sugar and Einhorn, 2011). Upon removal from RA, half of the fruit were warmed to approximately
20°C and assessed for fruit quality as described above. The remaining 15 fruits per replicate were placed in a RA room and held at 20°C (±1°C) for 7 days (Gerasopoulos and Richardson, 1999) before evaluating fruit quality.

**Environmental and physiological measurements.** Light interception was measured by placing a ceptometer (ACUPAR LP80, Decagon Devices Inc., Pullman, WA, U.S.A.) on the ground beneath trees according to the methods of Wünsche et al. (1995). Briefly, the land area (m²) occupied by an individual tree (based on actual number of trees per ha) was divided into 15 measurements (spaced approximately 30 cm x 25 cm) and applied to three contiguous trees per replicate. Clear-sky photosynthetic active radiation (PAR) was recorded between each replicate (open alley; no shade) and used to calculate the percentage of incident light on the orchard transmitted to the orchard floor. Light interception (including the portion of light reflected in canopies) was calculated as: \( \text{PAR}_{\text{intercepted}} = 1 - \frac{\text{PAR}_{\text{transmitted}}}{\text{PAR}_{\text{clear sky}}} \). Measurements were recorded during clear, sunny day, once per year: 25 July 2013 (Exp. 1); 2 Aug. 2014 (Exp. 1); 5 Aug. 2013 (Exp. 2); 1 Aug. 2014 (Exp. 2).

Stem water potential (\( \Psi \)) was measured mid-season in Exp. 2 (1 July to 5 Aug. 2013 and 2 to 20 Aug. 2014). On day with bright, sunny conditions, six fully-developed, uniform leaves per replicate were selected at approximately 1.5 m height near the base of scaffold limbs. Leaves were enclosed in reflective bags ~1 h prior to excision from the tree. Water potential was determined on leaves immediately after removal from trees ±1 h from solar noon. Leaf petioles were freshly cut, sealed in a Model 610 pressure chamber (PMS Instrument Co., Albany, OR) and slowly pressurized until a film of water was observed at the cut surface of the petiole. The balancing pressure was recorded and
converted to tension (- MPa). Measurements were repeated weekly in 2013 and bi-weekly in 2014.

**Nutrient content.** Leaf nutrient content was determined for Exp. 2 in year one, from a sample of 50 fully-developed leaves from the midsection of current-season extension shoots for each plot on 25 July 2013 (Table 1.7). In year two, spurs were collected (comprised of leaves, fruits, and woody tissue) on 6 May 2014 to determine carryover effects of root pruning on nutrient concentrations necessary to support early growth and development (Table 1.7). Samples were collected at approximately 1.5 m height from four quadrants (cardinal directions) of the canopy. Nutrient analyses were performed by Brookside Laboratories (Brookside Laboratories Inc., New Bremen, OH). Briefly, nitrogen was analyzed by combustion analysis and minerals were digested in a microwave system using nitric acid and hydrogen peroxide. The digested solution was then analyzed on an ICP.

**Statistical Analyses.**

Data were analyzed using analysis of variance (ANOVA) and significance tested at P≤0.05. Treatment means were separated using Fisher’s protected least significant difference test (LSD). Arccosine transformations were performed on percent data and statistical tests were performed in R-studio statistical platform, using ‘agricolae’ statistical package (Mendiburu, 2014).

**1.3 Results**

**Vegetative parameters.** In 2012, shoot elongation of 6th leaf trees (Exp. 1) was reduced by 35% when 2X RP was applied, relative to the control (Fig. 1.1 A and B; Table 1.2). Shoot growth of the 1X RP was intermediate between 2X RP and control (i.e., 23%
reduced compared to control; Fig. 1.1 and Table 1.2). Extension shoot growth of both RP treatments did not fully recover to control levels in year two, despite no further root pruning. The carryover effect was 9% and 18% for 1X RP and 2X RP respectively, compared to the control. At the conclusion of the third year, effects were barely discernible (Table 1.2). For young trees, TCA was a better integrator of treatment effects on total vegetative growth. Root pruning markedly reduced TCA; 2X RP trees were 7% smaller than control plots in year one and 12% smaller than control plots at the end of year 3 (Table 1.2).

For Exp. 2, shoot extension of fourth-leaf trees (year 1) subjected to 2X RP, was reduced by 20% following the first year application, relative to control plots (Figure 1.1 C; Table 1.3). At the end of year one, 1X RP had only minor effects on shoot elongation relative to the control (i.e., 4% reduction). Interestingly, growth of shoots of 1X or 2X RP trees was similarly reduced throughout the first 40 DAFB (Fig 2.3), but growth recovery of 1X RP shoots occurred by ~ 50 DAFB. Comparatively, growth of 2X RP shoots ceased ~50 DAFB. In year two, a full recovery of shoot growth was observed for both RP levels when trees were not re-pruned in year two (Fig 1.1 C). In contrast, trees which received either consecutive years of root pruning, or those unpruned in year one and 2X RP in year two, had 20% shorter shoots than untreated control plots (Fig 1.1 C). No differences were detected for LA (average LA and total shoot LA) or node density among treatments (Table 1.3). TCA was reduced by both root pruned treatments at the end of year one by ~ 30% of control, albeit non-significantly (P= 0.077). TCA in year two was significantly reduced by 18% to 36% depending on the treatment (Table 1.3).
**Reproductive parameters.** In year one, bloom of 6th leaf trees (Exp. 1) was not negatively affected by root pruning, irrespective of the treatment level (i.e., 1X or 2X); however, fruit set of 2X RP trees was significantly reduced (i.e., ~30%) compared to control trees (Table 1.4). Poor fruit set of 2X RP trees translated to fewer fruits of smaller average fruit weight, which, in turn, produced significantly lower yields. Although a reduction of initial fruit set of 1X RP trees was not detectable, fruit number, yield and fruit weight were all significantly lower than control plots, but higher than 2X RP trees (Table 1.4).

In year two, root pruning significantly increased return bloom and fruit set (Table 1.4); the response of the latter was proportional to RP level. Fruit set and yield tended to be higher in 2X RP plots than either the control or 1X plots \((P=0.12 \text{ and } P=0.085,\) respectively). The combination of reduced TCA and increased yield led to improved YE for 2X RP trees by year two of Exp. 2 (Table 1.4). As in year one, fruit weight tended to be smaller for RP treatments, but was not significant. In year three, 2X RP trees tended to have greater return bloom and more fruit at harvest \((P=0.052)\) relative to the other treatments, despite a lower fruit set efficiency (Table 1.4). Interestingly, two years after applying root pruning, fruit weight of the root pruned treatments remained smaller than control fruits. As a consequence, yield and YE tended to be higher for 2X RP plots.

Contrary to the adverse effects of root pruning on production of 6th leaf trees during the initial year of Exp. 1, no significant differences were detected among treatments for fruit set, number of fruit, or yield in Exp. 2 (Table 1.5). Although yields were similar between control and RP treatments, smaller trees of RP plots tended to have higher YE than control plots, albeit non-significantly \((P=0.077)\). Average fruit weight of
root pruned trees, however, was ~13% smaller than control fruits (Table 1.5). In year two, return bloom was significantly increased by 2X RP only (c.a., ~37% higher than control plots). Fruit set, fruit number and yield were all highest for trees receiving 2X RP in year one (Table 1.5). YE of trees that received 2X RP in year one, irrespective of year two treatment, was nearly two-fold that of control plots (Table 1.5). Treatments which were not root pruned in year two had reduced fruit growth rate beginning ~130 DAFB (Fig. 1.2) and were significantly smaller at harvest (Table 1.5).

**Fruit quality.** There were no significant differences in fruit quality (FF, SSC, and TA) among treatments in either experiment at harvest or following two mo. of storage, with and without a seven day ripening treatment (Table 1.6). All fruit ripened to acceptable firmness (<2 kg) indicating that sufficient chill units had accumulated.

**Environmental, physiological and leaf nutrient measurements.** In Exp. 1, 2X RP plots had higher transmitted light by 25%, 10% and 6% in 2012, 2013 and 2014, respectively, relative to control plots (Table 1.2). In Exp. 2, root pruned did not affect PAR light transmittance in year one, but in year two all trees subjected to root pruned in year one had significantly lower light interception than control trees (Table 1.3).

Mid-season Ψ was not affected by root pruning in Exp. 2 in either year (Figure 1.2 A, B). In both years, low Ψ occurred when high temperature events corresponded with low soil moisture between irrigation events. Macro- or micro-nutrient contents of leaves (year 1) or whole spurs (year 2) from trees of Exp. 2 were not affected by root pruning (Table 1.7).
1.4 Discussion

Vegetative growth of ‘d’Anjou’ pear was consistently and positively affected by root pruning. Shoots of RP trees were significantly reduced in both trials. The effect of RP on shoot number was not determined but fewer water sprouts were visibly apparent in RP treatments compared to controls. The number of nodes and LA for the population of shoots sampled was not affected by RP. Decreased shoot growth, therefore, was a function of shorter internodes. Sauer (2007) proposed that root pruning reduced gibberellic acid (GA) levels in shoots in response to low cytokinin transported from roots. GA is associated with internode elongation. In fact, a similar growth reduction of ‘d’Anjou’ shoots occurred in response to the GA antagonist prohexadione-calcium (Einhorn et al., 2014; Pasa and Einhorn, 2014). The magnitude of growth control incurred by RP was dependent upon tree age and rate [1X or 2X]. At the end of the season, 6th leaf 2X RP trees had ~40% less shoot growth than controls, while 4th leaf 1X RP trees did not significantly differ from controls (Fig. 1.1 B; Table 1.3). For the latter, a recovery from growth retardation began ~50 dafb (Fig. 1.1 B), likely a function of root restoration. A similar recovery of shoot growth was shown for potted apple (Schupp and Ferree, 1990) and pear (Poni et al., 1992) trees. Recovery of shoot to root ratios of pear 50 d after root pruning was due to the combination of high rates of new root production and limited shoot growth (Poni et al., 1992); however, a direct comparison cannot be made between these studies given that potted pear trees in that study were grafted to Quince C rootstock.

While we can infer that RP caused a disruption in the hormone balance of trees which led to reduced vegetative growth (Saure, 2007), other mechanisms have been
proposed to explain the vegetative response to RP. Given the removal of roots from pruning one or both sides of the tree row, limited root exploration and an imbalanced shoot to root ratio immediately following RP could potentially lead to nutrient deficiencies (Tromp, 1983). Nutrient content of leaves, fruits, or woody tissues, however, have rarely been evaluated in root pruning studies. In apple, leaf nutrient levels were not influenced by root pruning (Schupp and Ferree, 1990). Vercammen et al. (2005) proposed supplementing RP ‘Conference’ pear trees with additional fertilization to preempt poor fruit size and quality, but direct comparisons of RP treatments with and without supplemental nutrition were not performed to support this practice. Nutrient content of ‘D’Anjou’ pear leaves sampled mid-season (i.e., 93 dafb) and whole spurs the subsequent early spring (37 dafb) did not indicate nutrient deficiencies from root pruning, irrespective of treatment level, and, therefore, would not likely contribute to the growth control associated with RP.

Regulation of growth via water stress is well understood. In fact, managing water to reduce pear vigor led to the development of regulated deficit irrigation (Chalmers, 1989; Marsal et al., 2000; Mitchell et al., 1984; 1986; 1989). Plausibly, growth regulation elicited by RP could be attributed to water stress. Potted apple plants showed reduced transpiration following RP, but the effect was transient lasting only 7 d (Schupp and Ferree, 1990). Poni et al. (1992) compared the effects of root pruning and deficit irrigation on plant water status and root and shoot growth of apple, pear, peach (Prunus persica L.), and grape (Vitis vinifera) and concluded that greater control of shoot growth from root pruning was attributed to lower gas exchange, but not Ψ. Given that we did not assess Ψ immediately after RP, we cannot eliminate water stress as a contributing factor
to the regulation of early-season shoot growth. Mid-season \( \Psi \) was not affected by RP (Figure 1.3, A or B), despite additional stress of high temperatures and low irrigation frequency; however, osmotic adjustment and/or root regrowth may have ameliorated plant water stress (Poni et al., 1992). There are difficulties, however, when using container studies to interpret physiological responses of trees in field trials, primarily due to challenges estimating the volume of soil occupied by roots. Prior to RP ‘d’Anjou’ trees in Exp.1, we excavated vertical trenches (2 contiguous trees per replicate, 9 m length by 45 cm depth) to evaluate the size and density of roots removed by RP, but the commercial orchard setting of the trial precluded a full excavation of trees to assess root density below the tree row. Pyrus communis root systems possess good anchorage (Rom and Carlson, 1987) via a pronounced vertical rooting system (Atkinson, 1980). Removal of smaller diameter roots in the top 45 cm of the soil profile by RP would reduce absorption of water, but might not undersupply the canopy given the depth of pear root systems, low evaporative demand (via moderate temperature and low canopy LA), and a deep, moist soil profile in early spring.

TCA of ‘d’Anjou’ trees was significantly decreased by RP irrespective of tree age as previously reported for apple (Ferree and Knee, 1997; Ferree and Rhodus, 1993) and pear (McArtney and Belton, 1992). A significant reduction in trunk growth implies that less carbon was available to support tree growth, despite selected shoots from RP trees possessing similar LA as controls. If LA was constant among treatments, a sufficient supply of photosynthates would exist to support trunk growth; especially in year one of both trials when the sink strength of fruit was not augmented by greater fruit set or number at harvest. Likely, total assimilates were reduced due to less shoot initiation
(Vercammen et al., 2005). Translocation of storage carbon would be considerably less
given that a portion of the root system was excised. Moreover, RP may have directly
compounded competition for carbon among sinks via new root production from cut roots
(Poni et al., 1992). These processes may, in part, have been responsible for reduced
‘d’Anjou’ fruit size generally observed throughout the experiments. Water or carbon
stress during the cell division period (i.e., for ‘d’Anjou’ ~60 d from bloom; Westwood,
1993) could affect early fruit size (Janssens et al., 2011; Marsal et al., 2000) since the
final fruit size of pome fruits is heavily dependent upon cell number (Curreti et al., 2013;
Goffinet et al., 1995). Ferree and Knee (1997) documented a decrease in fruit size by
June from root pruning. In our study, however, fruit growth was not adversely impacted
early or midway through the growing season by RP, suggesting that cell division was not
affected (Figure 1.2).

Root pruning also consistently improved return bloom of ‘d’Anjou’ trees. Pear
flowering and fruit set are highly sensitive to light (Chapter 3; Einhorn et al., 2012;
continued, significant increase in light penetration through RP canopies (Tables 1.2 and
1.3) may, partly, explain the consistently higher return bloom of RP trees (Asín and
Vilardell, 2008; Ferree et al., 1992; Khan et al., 1998; McArtney and Belton, 1992; Yehia
et al., 2011). Return bloom generally led to higher fruit set and/or fruits per tree as
similarly observed for ‘Le Conte’ pear trees RP at bud break and full bloom (Yehia et.
al., 2011). Reduced TCA, concomitant with an increase in the number of fruits per tree
resulted in higher YE, especially for ‘d’Anjou’ trees RP in their 4th leaf.
Root pruning has been shown to be influenced by application timing in apple (Schupp and Ferree, 1987, 1990). In pear, McArtney and Belton (1992) observed reduced shoot length throughout the entire canopy when RP was performed during dormancy, but only in the lower canopy when applications occurred at petal fall. Root pruning occurred at 80% full bloom in Exp. 1 in the present study resulting in severe reduction of vegetative growth and fruit set. In fact, reduced shoot length was observed throughout the canopy in both experiments when trees were root pruned at 80% full bloom. While shoot elongation was typically suppressed in the year of root pruning, growth recovered in subsequent years so long as root pruning was not reapplied. Schupp and Ferree (1990) and Ferree and Knee (1997) similarly reported reduced shoot elongation in the year of application with a notable recovery in subsequent years. As previously reported, ‘d’Anjou’ trees root pruned in their 6th leaf exhibited strong carry-over effects for multiple years demonstrating potential drawbacks of RP if too severe. These trees were not re-pruned given the negative effects observed in year one.

When root pruning was repeated in consecutive years, shoot growth and development responded similarly between years. Perhaps, subjecting ‘d’Anjou’ trees of the present study to annual root pruning for several additional years would have resulted in diminished vigor control as observed for apple trees RP nine consecutive years (Ferree and Knee, 1997). Good balance between vigor and productivity over multiple years of Exp. 2 was contrasted by strong negative effects on fruit set and size in Exp. 1 (Table 1.2). In the case of the latter, annual root pruning as performed by Schupp and Ferree (1988) would likely have compounded these negative effects over time.
In conclusion, RP reduced extension shoot growth and trunk diameter of ‘d’Anjou’ pear trees, regardless of tree age. A consistent improvement of return bloom in year two was observed from 2X RP in both experiments resulting in higher fruit set and generally greater yield. One-sided root pruning was less effective at improving production than 2X RP. Although the degree of vigor control varied depending on the experiment, higher YE of RP trees was generally observed. YE was increased in all RP trees, with an increase of up to 57% for ‘d’Anjou’ trees 2X RP in the 4th leaf. Higher YE is evidence of a positive and marked change. Root pruning should most likely be applied in the 3rd to 4th leaf and the decision to re-prune based on shoot balance, productivity and return bloom (Vercammen et al., 2005).
1.5 Cited Work


Table 1.1 - Treatment summary of Exp. 2 applied to 4th (2013) and 5th (2014) leaf ‘d’Anjou’ trees. Year one (2013) replicates were divided in half to accommodate year 2 (2014) treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Abbr.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2013</strong></td>
<td></td>
</tr>
<tr>
<td>Untreated control</td>
<td>UC</td>
</tr>
<tr>
<td>Single-sided root pruned</td>
<td>1X</td>
</tr>
<tr>
<td>Double-sided root pruned</td>
<td>2X</td>
</tr>
<tr>
<td><strong>2013</strong></td>
<td><strong>2014</strong></td>
</tr>
<tr>
<td>Untreated control</td>
<td>Untreated control</td>
</tr>
<tr>
<td>Untreated control</td>
<td>Double-sided root pruned</td>
</tr>
<tr>
<td>Single-sided root pruned</td>
<td>Untreated control</td>
</tr>
<tr>
<td>Single-sided root pruned</td>
<td>Single-sided root pruned</td>
</tr>
<tr>
<td>Double-sided root pruned</td>
<td>Untreated control</td>
</tr>
<tr>
<td>Double-sided root pruned</td>
<td>Double-sided root pruned</td>
</tr>
</tbody>
</table>

\(^2\)2013 Replicates consisted of 8 trees

\(^1\)For 2014 replicates were divided in half and randomly assigned stasis or repeated treatment
Table 1.2 – Effect of root pruning on trunk cross-sectional area (TCA), average annual shoot length and photosynthetic active radiation (PAR) interception of 6th leaf (2012), 7th leaf (2013) and 8th leaf (2014) ‘d’Anjou’ trees of Exp. 1. Root pruning was only applied in year one (2012). UC, 1X and 2X represent untreated control, single sided root pruned, and double sided root pruned treatments, respectively.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TCA (^z) (cm(^2))</th>
<th>Shoot length (^y) (cm)</th>
<th>PAR interception (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC</td>
<td>96.4 a(^x)</td>
<td>47.2 a</td>
<td>-</td>
</tr>
<tr>
<td>1X</td>
<td>94.1 ab</td>
<td>36.3 b</td>
<td>-</td>
</tr>
<tr>
<td>2X</td>
<td>89.8 b</td>
<td>30.7 c</td>
<td>-</td>
</tr>
<tr>
<td>Pr(&gt;F)</td>
<td>0.017(^w)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC</td>
<td>124.4 a</td>
<td>50.4 a</td>
<td>42.3 a</td>
</tr>
<tr>
<td>1X</td>
<td>118.7 a</td>
<td>45.7 b</td>
<td>41.1 a</td>
</tr>
<tr>
<td>2X</td>
<td>110.5 b</td>
<td>41.1 c</td>
<td>37.2 b</td>
</tr>
<tr>
<td>Pr(&gt;F)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>2014</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC</td>
<td>140.6 a</td>
<td>49.9</td>
<td>42.2</td>
</tr>
<tr>
<td>1X</td>
<td>132.6 b</td>
<td>48.3</td>
<td>41.9</td>
</tr>
<tr>
<td>2X</td>
<td>123.2 c</td>
<td>47.7</td>
<td>39.6</td>
</tr>
<tr>
<td>Pr(&gt;F)</td>
<td>&lt;.001</td>
<td>0.2342</td>
<td>0.1532</td>
</tr>
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</table>

\(^{x}\)Trunk cross-sectional area  
\(^{y}\)Random shoots selected from current year wood at approximately 1.5 m.  
\(^{w}\)Letters signify significant difference with LSD test, all values are means of 4 replicates, n=25  
\(^{w}\)Analysis of variance pr(>f).05
Table 1.3 – Effect of root pruning on trunk cross-sectional area (TCA), average shoot length, photosynthetic active radiation (PAR) interception, average node number per shoot and average leaf area of 4\(^{th}\) leaf (2013) and 5\(^{th}\) leaf (2014) ‘d’Anjou’ trees of Exp. 2. Treatments included, UC (untreated control); 1X (single sided root pruned); 2X (double sided root pruned); UC/UC (untreated control 2 consecutive years; UC/2X (untreated control in 2013 double sided root pruned in 2014); 1X/UC (single sided root pruned 2013, untreated in 2014); 1X/1X (2 consecutive years of single sided root pruned treatment); 2X/UC (double sided root pruned, untreated in 2014); 2X/2X (double sided root pruned treatment 2 consecutive years).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TCA(^z) (cm(^2))</th>
<th>Shoot length(^x) (cm)</th>
<th>PAR interception (%)</th>
<th>Nodes (node no./shoot)</th>
<th>Avg. leaf area (cm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2013</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC</td>
<td>40.8</td>
<td>53.2</td>
<td>41.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1X</td>
<td>27.5</td>
<td>51.3</td>
<td>40.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2X</td>
<td>30.8</td>
<td>42.2</td>
<td>40.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pr(&gt;F)</td>
<td>0.0773(^w)</td>
<td>0.0277</td>
<td>0.7617</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>2014</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC/UC</td>
<td>57.8 a</td>
<td>40.1</td>
<td>45.4 a</td>
<td>15.2</td>
<td>24.9</td>
</tr>
<tr>
<td>UC/2X</td>
<td>48.2 b</td>
<td>32.1</td>
<td>43.9 ab</td>
<td>12.6</td>
<td>26.7</td>
</tr>
<tr>
<td>1X/UC</td>
<td>37.7 c</td>
<td>40.6</td>
<td>42.8 bc</td>
<td>14.5</td>
<td>24.4</td>
</tr>
<tr>
<td>1X/1X</td>
<td>48.6 b</td>
<td>33.1</td>
<td>40.9 c</td>
<td>14.0</td>
<td>24.4</td>
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<td>2X/UC</td>
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<td>43.1 b</td>
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<td>25.3</td>
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<tr>
<td>2X/2X</td>
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<td>32.3</td>
<td>42.4 bc</td>
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<td>0.0585</td>
<td>&lt;.001</td>
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<td>0.7081</td>
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</tbody>
</table>

\(^{a}\)Trunk cross-sectional area  
\(^{b}\)Random shoots selected from current year wood at approximately 1.5 m.  
\(^{c}\)Letters signify significant difference with LSD test, all values are means of 4 replicates, n=25  
\(^{d}\)Analysis of variance Pr(>f).05
Table 1.4 – Effects of root pruning on return bloom and yield characteristics of 6th leaf (2012), 7th leaf (2013) and 8th leaf (2014) ‘d’Anjou’ trees of Exp. 1. Root pruning was only applied in year one (2012). UC, 1X and 2X represent untreated control, single sided root pruned, and double sided root pruned treatments, respectively.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit set (%)</th>
<th>Fruit wt. (g)</th>
<th>Yield (kg)</th>
<th>Fruit no. (fruit no./tree)</th>
<th>Return bloom (%)</th>
<th>YE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC</td>
<td>10 a&lt;sup&gt;x&lt;/sup&gt;</td>
<td>230 a</td>
<td>34 a</td>
<td>148 a</td>
<td>80.1</td>
<td>0.22</td>
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<tr>
<td>1X</td>
<td>10 a</td>
<td>205 b</td>
<td>25 b</td>
<td>122 b</td>
<td>71.7</td>
<td>0.23</td>
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<tr>
<td>2X</td>
<td>7 b</td>
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<td>21 c</td>
<td>106 c</td>
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<td>0.29</td>
</tr>
<tr>
<td>Pr(&gt;F)</td>
<td>&lt;.001&lt;sup&gt;y&lt;/sup&gt;</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>0.3938</td>
<td>0.1606</td>
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<tr>
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<tr>
<td>UC</td>
<td>8 b</td>
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<td>0.1654</td>
<td>0.0853</td>
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<td>0.0328</td>
<td>0.2441</td>
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<tr>
<td>2014</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC</td>
<td>24 a</td>
<td>251 a</td>
<td>29&lt;sup&gt;x&lt;/sup&gt;</td>
<td>114</td>
<td>49.9 b</td>
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</tr>
<tr>
<td>1X</td>
<td>21 ab</td>
<td>231 b</td>
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<tr>
<td>2X</td>
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<tr>
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<td>&lt;.001</td>
<td>0.0678</td>
<td>0.0518</td>
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</table>

<sup>x</sup>Letters signify significant difference with LSD test, all values are means of 4 replicates, n=25
<sup>y</sup>Analysis of variance pr(>f).05
<sup>a</sup>Avg. Yield in 2014 was estimated from
Table 1.5 – Effects of root pruning on return bloom and yield characteristics of 4th and 5th leaf ‘d’Anjou’ pear trees at high density. Means of 4 multi-tree replicates: UC (untreated control); 1X (single sided root pruned); 2X (double sided root pruned); UC/UC (untreated control 2 consecutive years; UC/2X (untreated control in 2013 double sided root pruned in 2014); 1X/UC (single sided root pruned 2013, untreated in 2014); 1X/1X (2 consecutive years of single sided root pruned treatment); 2X/UC (double sided root pruned, untreated in 2014); 2X/2X (double sided root pruned treatment 2 consecutive years).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit set (%)</th>
<th>Fruit wt. (g)</th>
<th>Yield (kg)</th>
<th>Fruit no. (fruit no./tree)</th>
<th>Return bloom (%)</th>
<th>YE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC</td>
<td>11</td>
<td>265.3</td>
<td>7.3</td>
<td>28</td>
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<td>0.18</td>
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<td>13</td>
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<td>6.1</td>
<td>27</td>
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<td>0.22</td>
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<tr>
<td>2X</td>
<td>13</td>
<td>233.7</td>
<td>8.3</td>
<td>34</td>
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<td>0.28</td>
</tr>
<tr>
<td>Pr(&gt;F)</td>
<td>0.7376(^{a})</td>
<td>0.1648</td>
<td>0.5608</td>
<td>0.4941</td>
<td>0.5621</td>
<td>0.0773</td>
</tr>
<tr>
<td>2014</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC/UC</td>
<td>70.0</td>
<td>253 b(^{d})</td>
<td>13.3 ab</td>
<td>53</td>
<td>34.3 b(^{x})</td>
<td>0.23 b</td>
</tr>
<tr>
<td>UC/2X</td>
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<td>235 c</td>
<td>14.3 ab</td>
<td>62</td>
<td>-</td>
<td>0.29 b</td>
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<td>1X/UC</td>
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<td>221 d</td>
<td>13.0 b</td>
<td>67</td>
<td>40.1 b</td>
<td>0.25 b</td>
</tr>
<tr>
<td>1X/1X</td>
<td>58.8</td>
<td>231 cd</td>
<td>13.1 ab</td>
<td>57</td>
<td>-</td>
<td>0.26 b</td>
</tr>
<tr>
<td>2X/UC</td>
<td>85.8</td>
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<td>20.4 ab</td>
<td>93</td>
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<td>0.54 a</td>
</tr>
<tr>
<td>2X/2X</td>
<td>85.4</td>
<td>274 a</td>
<td>22.4 a</td>
<td>85</td>
<td>-</td>
<td>0.54 a</td>
</tr>
<tr>
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<td>0.0314</td>
<td>0.0513</td>
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</tr>
</tbody>
</table>

\(^{a}\)Analysis of variance pr(>f).05

\(^{b}\)Letters signify significant difference with LSD test, all values are means of 4 replicates, n=25

\(^{d}\)Due to the division of replicates "Avg. bloom" for UC, 1X and 2X are the same.
Table 1.6 – Effect of root pruning on fruit firmness (FF), soluble solids content (SSC) and titratable acidity (TA) at harvest and after 60 days of RA storage with or without ripening of 8th leaf ‘d’Anjou’ (2014, Exp. 1) and 5th leaf ‘d’Anjou’ (2014, Exp. 2) fruits. UC (untreated control); 1X (single sided root pruned); 2X (double sided root pruned); UC/UC (untreated control 2 consecutive years; UC/2X (untreated control in 2013 double sided root pruned in 2014); 1X/UC (single sided root pruned 2013, untreated in 2014); 1X/1X (2 consecutive years of single sided root pruned treatment); 2X/UC (double sided root pruned, untreated in 2014); 2X/2X (double sided root pruned treatment 2 consecutive years).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Harvest FF (kg)</th>
<th>Harvest SSC (%)</th>
<th>Harvest TA (%)</th>
<th>+ 2 months RA FF (kg)</th>
<th>+ 2 months RA SSC (%)</th>
<th>+ 2 months RA TA (%)</th>
<th>+ 2 months RA + ripening FF (kg)</th>
<th>+ 2 months RA + ripening SSC (%)</th>
<th>+ 2 months RA + ripening TA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp 1.</td>
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</tr>
<tr>
<td>UC</td>
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<td>13.80</td>
<td>0.3604</td>
<td>6.29</td>
<td>14.00</td>
<td>0.2755</td>
<td>1.6</td>
<td>14.01</td>
<td>0.2534</td>
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<tr>
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<td>6.64</td>
<td>13.85</td>
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<td>1.7</td>
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</tr>
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<td>1.6</td>
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<tr>
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<td>0.9135</td>
<td>0.7402</td>
<td>0.5096</td>
<td>0.7680</td>
<td>0.2905</td>
<td>0.9403</td>
<td>0.7542</td>
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<tr>
<td>Exp 2.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>UC/UC</td>
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<td>13.23</td>
<td>0.3123</td>
<td>5.91</td>
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<td>0.3243</td>
<td>1.4</td>
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<tr>
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<td>13.03</td>
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<td>5.99</td>
<td>13.03</td>
<td>0.3209</td>
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<td>5.90</td>
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<td>0.3217</td>
<td>1.7</td>
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</tr>
<tr>
<td>2X/UC</td>
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<td>0.3107</td>
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<td>13.73</td>
<td>0.2673</td>
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<tr>
<td>2X/2X</td>
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</tbody>
</table>

*a2 months cold storage -1˚C, regular air
*b2 months cold storage at -1˚C followed by 7 days 20˚C, regular air.
*cAnalysis of variance pr(>f).05
Table 1.7 – Effect of root pruning on leaf nutrient content of 4th leaf ‘d’Anjou’ trees (2013) and spur nutrient content of 5th leaf ‘d’Anjou’ trees (2014) of Exp. 2 UC (untreated control); 1X (single sided root pruned); 2X (double sided root pruned); UC/UC (untreated control 2 consecutive years; UC/2X (untreated control in 2013 double sided root pruned in 2014); 1X/UC (single sided root pruned 2013, untreated in 2014); 1X/1X (2 consecutive years of single sided root pruned treatment); 2X/UC (double sided root pruned, untreated in 2014); 2X/2X (double sided root pruned treatment 2 consecutive years).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N (%)</th>
<th>P (%)</th>
<th>K (%)</th>
<th>Mg (%)</th>
<th>Ca (%)</th>
<th>S (%)</th>
<th>B (ppm)</th>
<th>Fe (ppm)</th>
<th>Ma (ppm)</th>
<th>Cu (ppm)</th>
<th>Zn (ppm)</th>
<th>Al (ppm)</th>
</tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>UC/UC</td>
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<tr>
<td>2X/2X</td>
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</table>

*Mid-season leaf tissue samples of 3rd leaf ‘d’Anjou’ pear 2013.
*Analysis of variance pr(>f).06
*Spring spur tissue samples of 4th leaf ‘d’Anjou’ pear 2014.
Figure 1.1 – Effects of root pruning on shoot growth of 6th leaf ‘d’Anjou’ of Exp. 1 (A), 4th leaf (B) and 5th leaf ‘d’Anjou’ of Exp. 2 (C). Means are from 4 replicates (n=25). UC (untreated control); 1X (single sided root pruned); 2X (double sided root pruned); UC/UC (untreated control 2 consecutive years; UC/2X (untreated control in 2013 double sided root pruned in 2014); 1X/UC (single sided root pruned 2013, untreated in 2014); 1X/1X (2 consecutive years of single sided root pruned treatment); 2X/UC (double sided root pruned, untreated in 2014); 2X/2X (double sided root pruned treatment 2 consecutive years).
Figure 1.2 – Effect of year one and year 2 root pruning treatments on ‘d’Anjou’ fruit growth. Data were taken on 5th leaf trees of Exp. 2. Means are from 4 replicates (n=20). UC (untreated control); 1X (single sided root pruned); 2X (double sided root pruned); UC/UC (untreated control 2 consecutive years; UC/2X (untreated control in 2013 double sided root pruned in 2014); 1X/UC (single sided root pruned 2013, untreated in 2014); 1X/1X (2 consecutive years of single sided root pruned treatment); 2X/UC (double sided root pruned, untreated in 2014); 2X/2X (double sided root pruned treatment 2 consecutive years).
Figure 1.3 – Effect of root pruning on stem water potential of 5th leaf ‘d’Anjou’ trees (A) and 4th leaf ‘d’Anjou’ trees (B) of Exp. 2. Means were derived from 4 replicates (n=4). UC (untreated control); 1X (single sided root pruned); 2X (double sided root pruned); UC/UC (untreated control 2 consecutive years; 1X/1X (2 consecutive years of single sided root pruned treatment); 2X/2X (double sided root pruned treatment 2 consecutive years; UC/2X (untreated control in 2013 double sided root pruned in 2014).
Chapter 2: Abscisic Acid (ABA) reduces photosynthesis but has inconsistent thinning efficacy for ‘Bartlett’ pear in Oregon

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Abstract

Post-bloom fruitlet thinning is required to facilitate consistent bearing and achieve commercially-acceptable fruit size of ‘Bartlett’ pear (*Pyrus communis*). In Oregon, temperatures are often unfavorable for optimal thinning during fruitlet development for many of the commercially available thinning agents; consequently, hand thinning is required to reduce fruit set, but the process is labor intensive. The thinning efficacy of the plant hormone abscisic acid (ABA) was evaluated in four separate ‘Bartlett’ pear orchards from 2012-2014. ABA was applied to whole canopies at variable rates [0-500 ppm] between bloom and ~12 mm fruit size. ABA reduced fruit set in three of four field trials. Depending on the rate, net photosynthesis (Pn) was transiently reduced 75% to 90% within one day of ABA application, but returned to ~80% of control levels within a week after application and fully recovered by ~ 14 days. When effective, ABA increased the proportion of blank and single-fruited spurs and had no negative effects on fruit weight, quality or return bloom. Optimal ABA thinning was achieved in combination with low daily solar radiation during the week of application.
2.1 Introduction

‘Bartlett’ pear trees are precocious and typically set fruits on about half the flowers of a raceme (van der Zwet and Childers, 1982). Therefore, fresh market ‘Bartlett’ pears require hand thinning to attain a marketable fruit size in the Pacific Northwest (PNW). As labor becomes more expensive and scarce, alternative solutions to hand thinning will be required (Wells et al., 1998).

Mechanized and/or chemical thinning strategies have been developed for tree fruits to minimize hand thinning (Williams and Edgerton, 1981). Mechanical thinning with string thinners has decreased fruit set and increased fruit size in peach (*Prunus persica*) (Reighard and Henderson, 2012; Schupp et al., 2008) and apple (Dennis, 2000; Ellis et al., 2010; Schupp and Kon, 2014). Such technology is well-suited for high-density systems comprised of planar architectures. Seehuber et al. (2010) found reduced fruit set and increased fruit weight in apple (*Malus domestica*) pruned to super spindles with a mechanical string thinner. To overcome potential tree injury, less rigorous mechanized pruning coupled with chemical thinning was recommended. There is also potential for damage to spurs and spread of fireblight, *Erwinia amylovora*, with string thinning (Ngugi and Schupp, 2009). Despite advances in mechanical thinning technology, current pear tree architecture in the PNW is not amenable to mechanical thinning.

Chemical agents as thinners are far more common in apple and pear (Wertheim, 2000), in part because of their ability to affect source-to-sink ratio and hormonal interactions (Bangerth, 2000) when applied at specific developmental phases. A disproportionate body of thinning literature pertains to apple, given the relatively high
percentage of carbon allocated to reproductive processes by precocious and dwarfing rootstocks. In order for fruits to meet their genetic growth potential, assimilate supply must meet demand (Lakso, 2008), but temporary deficits of available carbohydrates, when fruit demand for carbon is high (i.e., 10-12 mm fruitlet stage), augments competition and abscission of ‘weak’ fruits (Greene and Lakso, 2013; Lakso, 2008; Lakso and Robinson, 2012; Untiedt and Blanke, 2001). Thus, influencing carbon competition through hormone interactions and photosynthetic limitation thins fruit. The uptake and thinning activity of bio-chemicals on fruits, however, is highly dependent on temperature and relative humidity (RH) prior to, during and immediately after application (Luckwill and Lloyd-Jones, 1962; Stover and Greene, 2005; Williams, 1979). In apple, the primarily synthetic auxin, naphthaleneacetic acid (NAA), has been shown to be an effective fruit thinner; however field trials have resulted in mixed performance. The general unreliability of NAA and its amide NAAm are due to temperature and RH as mentioned above, limiting widespread adoption (Black et al., 1995; Edgerton and Haeseler, 1959). Carbaryl, a carbamate family insecticide has been a highly efficacious thinner of apple (Childers, 1978). Carbaryl is, however, toxic to pollinators (Westwood, 1978) and has been banned or severely restricted in several countries. Ethephon has been widely utilized for thinning during bloom but requires a specific developmental window to be effective (ovule length of 9 to 15 mm) (Childers, 1978; Wertheim, 1973). Combining ethephon with post-bloom thinners (NAA or 6-BA) has improved efficacy (Bound et al., 1998). Ethephon has also been trialed to thin fruitlets as well as flowers in apple, especially in periods of development when fruit are naturally sensitive and prone to drop (Child and Mapairoje, 1977; Veinbrants, 1979, Williams, 1973; Williams and
Edgerton, 1981). Relatively recently, the fertilizer ammonium thiosulfate (ATS), which thins by damaging stigmatic surfaces, reduced fruit set and improved yield and fruit weight of apple (Balkhoven-Baart and Wertheim, 1997). Mild phytotoxicity of young leaves from ATS application was observed by Byers (1999), but the compound appeared to be relatively safe for fruit. The cytokinin, 6-benzylaminopurine (6-BA), effectively thinned apple fruitlets (Ferree, 1996); however, 6-BA has been associated with asymmetric fruit (Greene, 1995). Further, thinning efficacy remains inconsistent in apple, due, in part, to difficulty attaining temperature optima for activity during applications (McArtney et al., 1995).

Pear fruitlet thinners have typically targeted post-bloom developmental stages. NAA has been shown to be as variable a thinner in pear as in apple (McArtney and Wells, 1995; Wertheim, 1973). Temperature dependency of NAA in pear is problematic (Wertheim, 2000). However, developmental timing also plays a role in efficacy. NAA spray application timings have been reported to be more effective at full bloom (FB) and petal fall (PF) in pear (Reginato and Gonzalez, 1998). NAD had greater post-bloom efficacy for ‘Bartlett’ than NAA (Lombard and Grim, 1966), but required temperatures exceeding 20° C, advanced maturity (e.g., pink end) and was phytotoxic at moderately low concentrations. Ethephon applications need to coincide with a specific developmental window (as outlined above in apple) for successful thinning (Bonghi et al., 2002; Childers, 1978; McArtney and Wells, 1995). 6-BA requires temperatures to remain consistently high throughout the active period for reliable and efficacious thinning (Asín et al., 2009; Curreti et al., 2010; Dussi and Sugar, 2010; Gimenez et al., 2009;
Maas and van der Steeg, 2011; Theron et al., 2010; Vilardell et al, 2005; Wertheim, 2000).

ABA is a natural plant hormone having varied cellular and molecular functions in plant development (Addicott and Praeger, 1983) and is most notably related to abscission processes and plant dormancy (Taiz and Zeiger, 2010). ABA facilitates stomatal movement, often as a result of drought stress (Cummins, 1971; Davies and Zhang, 1989; Trejo et al., 1993; Raschke and Hedrich, 1985; Rock, 2000). Meidner and Mansfield (1968) and Milborrow and Lund (1969) have demonstrated chloroplast as a major site of ABA synthesis; however, elevated apoplastic ABA around guard cells is responsible for stomatal closure (Hartung, 1983). Stomatal closure can limit severe water stress and preserve water by reducing transpiration (Kraalingen, 1990).

Exogenous ABA has potential for fruit thinning by eliciting stomatal closure and, hence, carbon deficits associated with fruit abscission (Lakso, 2008, Lakso and Robinson, 2012). Moreover, uptake of ABA at the leaf cuticle is insensitive to fluctuations in temperature and RH (Middelberg et al., 2014); factors influencing consistency of most currently available chemical thinners. Little research attention has been devoted to ABA until recently (Greene, 2012; McArtney et al., 2014) following the development of commercial formulation of ABA (ProTone, Valent BioSciences). Greene (2012) observed consistent thinning when ABA was applied to ‘Bartlett’ pear, both alone and in combination with 6-BA. Thinning effects were noted with early (full bloom to 10 mm fruit size) applications and low concentrations of ABA. Return bloom was not negatively impacted and thinning appeared reliable. Hand thinning ‘Bartlett’ pears is costly and time consuming. Given the potential of ABA to thin ‘Bartlett’, an evaluation of ABA in
the US pear production region is necessary. The primary objective of this project, therefore, was to evaluate the effectiveness and consistency of ABA as a thinner in ‘Bartlett’ pear. The secondary objective was to determine optimal ABA rate. A technical objective was to quantify and characterize the effects of ABA on photosynthesis.

2.2 Materials and Methods

Planting material and experimental design

Experiments were carried out at three ‘Bartlett’ pear orchards in Hood River and Parkdale, OR, 2012-2014. Concentrations of ABA (ProTone, Valent BioSciences, Walnut Creek, CA) were combined with a surfactant (Sil 100, Clariant Corp., Mount Holly, NC) at 0.1% and applied to drip between ~6:00 and 10:00 HR, approximately 10 DAFB, using a hydraulic pressurized spray gun (20 atm). All other production practices were performed according to industry standards.

Exp. 1 In 2012, an 8th leaf ‘Bartlett’/OHxF 87 orchard (3 x 5 m; 666 trees/ha) was selected in Hood River, OR at the Oregon State University Mid-Columbia Agricultural Research and Extension Center (long. 45.68, lat. 121.51). Soil is a Van Horn fine sandy loam. Treatments were arranged in a randomized complete block design with four, single-tree replicates (selected for uniformity): Untreated control; control + surfactant; and, 125, 250 and 500 ppm ABA plus surfactant. Applications were made at 10 days after full bloom (DAFB). No significant differences were observed between the control and control + surfactant for any of the measured variables; therefore, results are not shown for the control + surfactant and the treatment was omitted from all subsequent experiments.
Exp. 2 In 2013, an 18-year-old ‘Bartlett’/OHxF 87 pear orchard (2.5 x 5.5 m; 727 trees/ha) was selected in Hood River, OR at the Oregon State University Mid-Columbia Agricultural Research and Extension Center (long. 45.68, lat. 121.51). Soil is a Van Horn fine sandy loam. Treatments were arranged in a randomized complete block design with four, single-tree replicates. Treatments consisted of an untreated control, 50 ppm ABA, 100 ppm ABA, 150 ppm ABA and 200 ppm ABA.

Exp. 3 In 2013, a 10th leaf commercial ‘Bartlett’/OHxF 97 pear orchard (3 x 6 m; 556 trees/ha) was selected in Parkdale, OR (long. 45.29, lat. 121.34). Soil is a Hutson fine sandy loam. Treatments were arranged in a complete randomized design with four, two-tree replicates: Untreated control; 50 ppm ABA; 100 ppm ABA; 200 ppm ABA; and, 400 ppm ABA. Applications were made when fruits were ~12 mm (20 DAFB).

Exp. 4 In 2014, a new trial was established in the same orchard as described in Exp. 2 but treatments were applied to different trees not previously treated with thinning compounds. Treatments were arranged in a randomized complete block design with four, two-tree replicates: Untreated control; 50 ppm ABA; 100 ppm ABA; 200 ppm ABA; and, 400 ppm ABA.

Measurements and procedures

Flowering, fruiting and vegetative growth. Fruit set (%) was expressed as the number of fruit per flower cluster. Flower clusters (no fewer than 200) were counted on selected scaffold limbs at ~80% full bloom. Fruits retained on each scaffold limb at ~35 DAFB were counted and divided by the total number of clusters to generate percent fruit set prior to hand thinning. Return bloom was expressed as the percent of total spurs on preselected scaffold limbs with flower clusters. Between 45 and 55 DAFB, fruit were
hand-thinned according to industry standards. The total number of fruits per tree removed by thinning was recorded. Tree yield was determined by weighing all fruit per tree in a single harvest based on commercial maturity standards (i.e., fruit firmness). For Exp. 3, fruit set was recorded on all individual spurs from selected limbs by counting the fruit per spur prior to hand thinning. For Exp. 4, 50 fruits were randomly sampled and individually weighed to determine average fruit weight. Fruit samples were then used in post-harvest tests below.

The effect of ABA on shoot growth was evaluated in Exp. 1 on a population of 10 shoots per tree, selected at approximately 1.5 m height from the ground. Shoots were measured weekly from the shoot apex to the growth ring separating current season from one-year-old growth. In Exp.1 initial and final trunk circumference was measured 20 cm above the graft union prior to full bloom and post-harvest, respectively.

**Leaf gas-exchange.** For Exp. 2 and 3, gas exchange \[ A = \text{photosynthesis} (\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}); \text{gs} = \text{stomatal conductance} (\text{mmol} \cdot \text{m}^{-3} \cdot \text{s}^{-1}) \] was quantified using a PP systems Ciras-2 gas analyzer (PP systems, Amherst, MA) on all replications per treatment (four leaves per replication). Measurements were taken on the most recent fully mature leaves of current-season extension shoots at a height of ~1.5 m between 12:00-14:00 HR at approximately 3-4 day intervals. CO$_2$ was supplied to the cuvette at 385 ppm and measurements were recorded when leaf gas exchange reached steady-state in the cuvette (~60 s).

**Fruit quality.** In Exp. 4, fruit quality attributes of 20 fruit per plot were determined immediately following harvest. Thirty fruit per plot were stored at -1°C, regular air (RA) for 60 d. Of this sample, fruit quality attributes were determined on15
fruit per replicate after warming to room temperature (~20°C). The remaining 15 fruit were ripened for 5 d at 20°C then evaluated for fruit quality. At each measurement date, fruit firmness (FF) was measured on two sites per fruit (opposite one another) after removing a ~5 cm² area of skin using a Fruit Texture Analyzer (Güss Manufacturing, Strand, South Africa). Soluble solids (SSC) were determined using a digital refractometer (Model PR-101, ATAGO Co., LTD., Bellevue, WA) and titratable acidity (TA) was determined by titrating a juice sample of 10 mL in 10 mL of DI H₂O to an endpoint pH of 8.1 using 0.1 N NaOH with an automated titration system (Model DL15, Mettler-Toledo, LLC., Columbus, OH).

**Solar radiation.** Total incoming solar radiation (W· m⁻²) was recorded at 15 min intervals and collected from weather stations located ~100 km from all experimental orchard sites. Diurnal curves were generated from -5 to +15 days from application (dfa).

**Statistical analyses**

Data were analyzed using analysis of variance (ANOVA) and significance tested at P≤0.05. When significance was detected, treatment means were separated using least significant difference test (LSD). Arcosine transformations for percent data were done in Microsoft Excel and back transformed for presentation, all other tests were performed in R-studio statistical platform, using ‘agricolae’ statistical package (Mendiburu, 2014).

**2.3 Results**

**Flowering, fruiting and vegetative growth.** Depending on the year and/or experiment, ABA effects on fruit set and yield were highly variable (Tables 2.1-2.3). In half of the trials, fruit set decreased markedly and significantly with increasing ABA rate (Table 2.1 and 2.3). In Exp. 2, however, fruit set was not affected by ABA, irrespective
of rate (Table 2.2). In Exp. 4, fruit set was significantly reduced by ABA, but only at the higher rates (i.e., 200 and 400 ppm; Table 2.2) and not nearly to the magnitude observed in Exp. 1 and 3. The number of spurs that had no fruit or one fruit/spur was increased by ABA relative to control plots (Figure 2.2).

Fruit number at harvest was reduced significantly by ABA in 3 of 4 experiments. In Exp. 1, ABA reduced fruit number relative to rate with the highest concentrations (250 and 500 ppm) severely over thinning (Table 2.1). Fruit number was not affected in Exp. 2. Fruit number at harvest was significantly reduced in Exp. 3 for 200 and 400 ppm ABA only; lower concentrations tended to have reduced fruit number compared to controls (Table 2.3). The reduced fruit set observed in Exp. 1 and 3 resulted in less yield and fewer fruit at harvest, despite hand-thinning. In these trials, fruit weight increased with increasing ABA rate as a consequence of reduced crop load (Tables 2.1 and 2.3). In fact, in Exp. 1, shoot growth was significantly greater when treated with ABA, presumably due to the limited fruit sink (Fig. 2.1); though a rate response was not observed. Trunk growth was inversely related to crop load as influenced by ABA rate (Fig. 2.1 inset). In Exp. 2 and 4, differences in yield, fruit number and fruit weight were not detected among treatments (Tables 2.2).

**Leaf gas exchange and light.** Compared to controls, ABA significantly decreased photosynthesis of ‘Bartlett’ pear leaves, relative to rate (Fig. 3.3). Leaf Pn was markedly reduced (75% to 90%), immediately following ABA application compared to controls (Figure 3.3 A). By day four, however, Pn of 50 and 100 ppm ABA leaves recovered to ~75% of control levels. For these rates, a full recovery was observed by day eight. Both 200 and 400 ppm ABA required additional time to return to control levels.
(i.e., by day 15 and 20 for 200 and 400 ppm ABA, respectively). In Exp. 3, Pn measurements did not begin until 6 days after treatment due to instrument malfunction. However, Pn was significantly reduced for ABA rates > 100 ppm relative to the control (Fig 3.3 B). In fact, for 200 ppm ABA, the 20% reduction of Pn on day six was nearly equivalent to the reduction observed in Exp. 2 between days four and eight at 200 ppm ABA (Fig 3.3 A). By day 13 all treatment Pn levels were ~ 90% or above the control rate (Figure 3.3 B).

**Fruit quality.** There was no effect of ABA at any of the concentrations applied on ‘Bartlett’ pear quality, irrespective of concentration (Table 2.4). Harvest pressures (FF) indicated no direct ABA effects on fruit maturity. SSC and TA were also not affected by foliar ABA applications. A slight decrease in FF was observed following 60 days of RA, irrespective of treatment. ABA did not affect ripening following five days of 20°C; all fruit had good-eating quality (juicy, buttery texture).

**Solar radiation.** Diurnal solar radiation curves differed markedly among sites and years (Fig. 2.4). In Expt. 1 and 3, low solar radiation (~40% of cloud-free days; ~900 W \cdot m^{-2}) was observed during the initial 3 day from application (Fig. 2.4 A and C). Interestingly, ‘Bartlett’ fruit in these experiments showed the strongest response to ABA (Tables 2.1, 2.3). In contrast, 10 consecutive days of cloud-free, high solar radiation conditions following ABA applications occurred in Exp. 2 (Fig 2.4 B), a trial in which fruit were unresponsive to ABA (Table 2.2). In Exp. 4, low solar radiation occurred between days 4-6 following application of ABA (Fig 2.4 D); interestingly, ABA effects on fruit thinning were apparent, but subtle (Table 2.4).
2.4 Discussion

Application of ABA to ‘Bartlett’ resulted in inconsistent fruit thinning and only resulted in lower fruit numbers in two of four trials. These results contrast those reported by Greene (2012) whereby ABA applied to single limbs of mature ‘Bartlett’ pear trees, at a similar concentration range as reported herein, consistently thinned relative to rate. Recently, 250 ppm ABA, applied alone or in combination with 6-BA, was only partially effective for thinning apple fruits (McArtney et al., 2014). In that study, ABA reduced \( g_s \) and, presumably \( Pn \) by a similar magnitude given the strong, linear relationship between \( g_s \) and \( Pn \) of apple leaves (Lakso, 1994). The effect, however, was transient, a ~60% reduction of \( Pn \) relative to control plots was maintained for only a few days after application and diminished after ~7 days (McArtney et al., 2014). Pear leaves, in the present study, had markedly reduced carbon assimilation over the first few days following ABA treatment, then slowly recovered to Control levels after ~ one week (Fig. 3.3). The ABA active ingredient for the applied product ProTone (Valent) is the physiologically active form in-situ, thus the rapid metabolism by pear and apple. Consequently, ABA confers only mild carbon stress, and in pear, specifically, potentially little to none given the vast pools of storage carbon in above and below-ground organs which would be anticipated to ameliorate growth-limitation associated with transient perturbations of \( Pn \). This is loosely supported by the vast difference in tree size among the trials: Good thinning was achieved in trials comprised of <10 year-old trees (i.e., Exp. 1 and 3) while fruits of ~20-year-old trees (Exp. 2 and 4) were more-or-less insensitive to ABA, despite similar \( Pn \) response to ABA. In fact, the relative sink strength of fruit was observed for young trees of Exp. 1 treated with ABA; those trees
had significantly greater trunk and shoot growth (Fig. 3.1) given ample carbon available to vegetative organs without competition from fruit (Hansen, 1971; Quinlan and Preston, 1971; Teng et al., 1999). In the absence of dwarfing rootstocks, highly vigorous species such as pear pose a significant challenge to chemical thinners whose mode of action induces short-term carbon stress. Nevertheless, Greene (2012) reported excellent thinning activity of ABA when applied to limbs of mature ‘Bartlett’ pear trees on seedling rootstock.

We did not measure Pn in all experiments; however, effects of temperature and/or relative humidity on ABA uptake and activity are unlikely given that ABA penetration through the leaf cuticle is unaffected by temperature (Middelberg et al. 2014). Varying light levels, however, would be expected to alter tree response to ABA given the well-established effect of shade on pear fruit set (Einhorn et al., 2011; Garriz et al., 1997; Garriz et al., 1998; Kappel, 1989; Chapter 4). Indeed, diurnal solar radiation markedly differed among the individual trials (Fig. 3.4). High solar radiation prior to and after ABA application (as in Exp. 2; Fig. 3.4 B) would have led to a carbon surplus and thus limited thinning (Lakso, 2008; Lasko and Robinson, 2012). Conversely, overcast conditions (Exp. 1 and 3) may have produced carbon deficits and augmented the thinning action of ABA. It is unclear if an interaction between low light and ABA led to strong thinning of ‘Bartlett’ (Greene, 2012) given that solar radiation levels were not reported.

Applications of chemical thinners need to coincide with sensitive stages of fruit development. For both pear and apple, petal fall applications of ABA effectively thinned fruit but greater sensitivity of fruits to ABA was observed at 10-12 mm diameter timing (Greene, 2012; McArtney et al., 2014). In the present study, ABA at ~100-125 ppm was
an appropriate rate when applied at 10-12 mm timing, since, when effective, higher rates reduced yield excessively (Tables 3.1 and 3.2). Concentrations of 250-500 ppm of ABA facilitated a longer period of reduced photosynthesis, however, significant phytotoxicity and leaf abscission was observed when ABA concentration was ≥400 ppm, as similarly observed for apple (McArtney et al., 2014). These data limit the potential of applying higher rates when conditions that are not conducive to thinning exist.

In conclusion, ABA appears to thin ‘Bartlett’ pear by inducing a short-term carbon stress due to reduced Pn and partial stomatal closure. However, the effect lasted for several days and contributing biotic and abiotic factors (light and carbon reserves) potentially alter tree response to ABA. We are presently investigating the additive effects of shade and ABA on thinning action of ‘Bartlett’ pear. By forecasting solar radiation, it might be possible to enhance the reliability of using ABA as a commercial thinner during seasons, or in climates, where alternative thinning agents are limited by cool, cloudy conditions. Further development of ABA analogs for thinning is warranted (McArtney et al., 2014).
2.5 Works Cited


Table 2.1 – The effect of abscisic acid (ABA) rate on fruit set, fruit number, fruit weight, fruit yield and return bloom for 6-year-old ‘Bartlett’/OH x F 97 pear trees (Exp. 1) treated at 10 days after full bloom.

<table>
<thead>
<tr>
<th>ABA rate</th>
<th>Fruit set (%) control</th>
<th>Fruit no. z (fruit/tree)</th>
<th>Yield (kg/tree)</th>
<th>Fruit wt. (g/fruit)</th>
<th>Return bloom (%) control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0ppm ABA</td>
<td>-</td>
<td>158 a</td>
<td>69.1 a&lt;sup&gt;y&lt;/sup&gt;</td>
<td>213.7 b</td>
<td>-</td>
</tr>
<tr>
<td>125ppm</td>
<td>64 b</td>
<td>128 b</td>
<td>59.1 b</td>
<td>218.6 b</td>
<td>95 b</td>
</tr>
<tr>
<td>250ppm</td>
<td>9 c</td>
<td>17 c</td>
<td>8.2 c</td>
<td>230.3 a</td>
<td>103 ab</td>
</tr>
<tr>
<td>500ppm</td>
<td>0 d</td>
<td>0 d</td>
<td>n.d. x&lt;sup&gt;x&lt;/sup&gt;</td>
<td>n.d.</td>
<td>122 a</td>
</tr>
<tr>
<td>Pr(&gt;F)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001&lt;sup&gt;w&lt;/sup&gt;</td>
<td>0.0370</td>
<td>0.0412</td>
</tr>
</tbody>
</table>

<sup>x</sup>Fruit number after hand thinning.
<sup>y</sup>Letters signify significant difference with LSD test, all values are means of 4 replicates
<sup>x</sup>n.d.= no data, fruit did not remain on the tree for average yield or fruit weights.
<sup>w</sup>Analysis of variance pr(>f).05
Table 2.2 – The effect of abscisic acid (ABA) rate on fruit production of 18 and 19-year-old ‘Bartlett’/ OH x F 87 pear trees (Exp. 2 and 4) treated at 10 days after full bloom.

<table>
<thead>
<tr>
<th>ABA rate</th>
<th>Fruit/cluster (no.)</th>
<th>Yield (kg/tree)</th>
<th>Fruit no. (no./tree)</th>
<th>Fruit wt. (g/fruit)</th>
<th>Return bloom (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td><strong>Exp. 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0ppm</td>
<td>1.11</td>
<td>54.1</td>
<td>254</td>
<td>212.5 bcd</td>
<td>-</td>
</tr>
<tr>
<td>50ppm</td>
<td>0.95</td>
<td>64.3</td>
<td>277</td>
<td>231.0 ab</td>
<td>81</td>
</tr>
<tr>
<td>100ppm</td>
<td>0.67</td>
<td>50.2</td>
<td>227</td>
<td>220.2 bcd</td>
<td>102</td>
</tr>
<tr>
<td>150ppm</td>
<td>0.70</td>
<td>58.1</td>
<td>240</td>
<td>241.6 a</td>
<td>87</td>
</tr>
<tr>
<td>200ppm</td>
<td>0.81</td>
<td>59.5</td>
<td>257</td>
<td>229.5 ab</td>
<td>97</td>
</tr>
<tr>
<td>Pr(&gt;F)</td>
<td>0.0947w</td>
<td>0.0881</td>
<td>0.2327</td>
<td>0.0423</td>
<td>0.7412</td>
</tr>
<tr>
<td><strong>Exp. 4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0ppm</td>
<td>.75 a</td>
<td>140.8</td>
<td>663</td>
<td>220.5</td>
<td>- v</td>
</tr>
<tr>
<td>50ppm</td>
<td>.70 a</td>
<td>136.3</td>
<td>639</td>
<td>213.3</td>
<td>-</td>
</tr>
<tr>
<td>100ppm</td>
<td>.64 ab</td>
<td>145.7</td>
<td>611</td>
<td>205.7</td>
<td>-</td>
</tr>
<tr>
<td>200ppm</td>
<td>.59 b</td>
<td>141.2</td>
<td>618</td>
<td>201.0</td>
<td>-</td>
</tr>
<tr>
<td>400ppm</td>
<td>.41 c</td>
<td>150.1</td>
<td>633</td>
<td>208.7</td>
<td>-</td>
</tr>
<tr>
<td>Pr(&gt;F)</td>
<td>&lt;.001</td>
<td>-</td>
<td>0.3034</td>
<td>0.1392</td>
<td></td>
</tr>
</tbody>
</table>

\[^{\text{a}}\text{fruit per cluster before hand thinning}\]
\[^{\text{b}}\text{fruit number per tree after hand thinning}\]
\[^{\text{c}}\text{Letters signify significant difference with LSD test, all values are means of 4 replicates, n=25}\]
\[^{\text{d}}\text{Analysis of variance pr(>f).05}\]
\[^{\text{e}}\text{No return bloom data for 2015}\]
\[^{\text{f}}\text{Average yield for experiment 4 was estimated from average fruit weight and fruit per tree counts.}\]
Table 2.3 – Effects of abscisic acid (ABA) rate on fruit set (fruit per cluster), return bloom, fruit number, fruit weight, and yield of 8-year-old ‘Bartlett’ OH x F 97 pear trees (Exp. 3) treated at 10 days after full bloom.

<table>
<thead>
<tr>
<th>ABA rate</th>
<th>Fruit/cluster$^z$ (no.)</th>
<th>Avg. yield (kg/tree)</th>
<th>Fruit no.$^y$ (no./tree)</th>
<th>Avg. fruit wt. (g/fruit)</th>
<th>Return bloom (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0ppm</td>
<td>1.36 a$^x$</td>
<td>95.5 a</td>
<td>510 ab</td>
<td>196.1 b</td>
<td>-</td>
</tr>
<tr>
<td>50ppm</td>
<td>1.01 ab</td>
<td>82.9 a</td>
<td>439 b</td>
<td>193.8 b</td>
<td>92 b</td>
</tr>
<tr>
<td>100ppm</td>
<td>.77 b</td>
<td>76.7 a</td>
<td>349 bc</td>
<td>212.5 ab</td>
<td>89 b</td>
</tr>
<tr>
<td>200ppm</td>
<td>.25 c</td>
<td>51.5 b</td>
<td>244 cd</td>
<td>238.3 a</td>
<td>106 a</td>
</tr>
<tr>
<td>400ppm</td>
<td>.03 c</td>
<td>37.6 b</td>
<td>172 d</td>
<td>232.1 a</td>
<td>118 a</td>
</tr>
<tr>
<td>Pr(&gt;F)</td>
<td>0.04245$^w$</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>0.0391</td>
</tr>
</tbody>
</table>

$^z$fruit per cluster before hand thinning

$^y$fruit number per tree after hand thinning

$^x$Letters signify significant difference with LSD test, all values are means of 4 replicates, n=25

$^w$Analysis of variance pr(>f).05
Table 2.4 – Effect of abscisic acid (ABA) rate on fruit quality measurements of 19-year-old ‘Bartlett’ OH x F 87 pear trees (Exp. 4). Fruit firmness (FF), soluble solids content (SSC) and titratable acidity (TA) were measured at harvest and after 60 days of cold storage at regular atmosphere with and without + 5 days of ripening.

<table>
<thead>
<tr>
<th>ABA rate</th>
<th>Harvest</th>
<th>+ 2 months RA&lt;sup&gt;z&lt;/sup&gt;</th>
<th>+ 2 months RA + ripening&lt;sup&gt;y&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FF</td>
<td>SSC</td>
<td>TA</td>
</tr>
<tr>
<td></td>
<td>(kg)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>0ppm</td>
<td>8.2</td>
<td>12.7</td>
<td>0.31</td>
</tr>
<tr>
<td>50ppm</td>
<td>8.1</td>
<td>12.5</td>
<td>0.33</td>
</tr>
<tr>
<td>100ppm</td>
<td>8.3</td>
<td>12.8</td>
<td>0.32</td>
</tr>
<tr>
<td>200ppm</td>
<td>8.0</td>
<td>13.1</td>
<td>0.31</td>
</tr>
<tr>
<td>400ppm</td>
<td>8.3</td>
<td>13.9</td>
<td>0.28</td>
</tr>
<tr>
<td>Pr(&gt;F)</td>
<td>0.4332&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.1911</td>
<td>0.3168</td>
</tr>
</tbody>
</table>

<sup>x</sup>After chilling approximately 60 days at -1°C regular air.

<sup>y</sup>After ripening 5 days at 20°C regular air.

<sup>z</sup>Letters signify significant difference with LSD test, all values are means of 4 replicates, n=25

<sup>w</sup>Analysis of variance pr(>f).05
Figure 2.1 – Effect of abscisic acid (ABA) rate on 8-year-old ‘Bartlett’ pear shoot growth. In-set is the percent increase in trunk cross-sectional area between 20 and 171 days after full bloom.
Figure 2.2 – Effect of abscisic acid (ABA) rate on the percentage of spurs with varying fruit density for 9th leaf ‘Bartlett’ pear. Data are means of 4 replicates.
Figure 2.3 – Effect of abscisic acid (ABA) rate on single-leaf photosynthesis of 18-year-old ‘Bartlett’/OH x F 87 pear trees of Exp. 2 (A) and 19-year-old Bartlett / OH x F 87 trees of Exp. 3 (B) Data are means of 4 replicates (n=6).
Figure 2.4 – Diurnal solar radiation from -5 to 15 days from ABA application. (A) Hood River, OR 2012 (B) Hood River, OR 2013 (C) Parkdale, OR 2013 (D) Hood River, OR 2014. Data were collected from a weather station ~100 m from trial site.
Chapter 3: Shade and abscisic acid (ABA) effectively thin fruits of ‘Bartlett’ pear but are not additive

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Abstract

Previous experiments using the plant growth regulator abscisic acid (ABA) to thin ‘Bartlett’ pears resulted in transient stomatal closure, which, in turn, decreased net carbon assimilation. However, the effect of ABA on thinning was not consistently achieved. For individual trials where ABA effectively thinned ‘Bartlett’, a decrease in daily solar radiation within the first few days from application was concomitantly observed. Therefore, two levels of ABA (0 and 125 ppm) and three levels of shade (0%, 44% and 77%) were applied to ‘Bartlett’ pear trees to evaluate the relationship between ABA and shade on gas exchange and fruit thinning. The entire canopy of the trees was shaded immediately following applications of ABA (at petal fall) for a period of 15 day. Net photosynthesis (Pn) of single leaves was reduced and thinning was increased by shade but interactions between them were non-significant. A rate of 125 ppm ABA reduced Pn of control plots 10-fold (i.e. from 10 to ~0 μmol ·m⁻² ·s⁻¹) within hours of application, but a recovery to ~75% to 80% of control plot levels occurred by day three. ABA reduced fruit set ~35%, relative to untreated control plots. Moderate shade (44%) produced only a minor reduction of Pn (i.e., ~10% of control) and fruit set, compared to control plots. Increasing shade to 77%, however, produced greater thinning than 125 ppm ABA alone but the difference was not significant. Pn was severely reduced under 77% shade. A
minor additive effect on Pn from combination treatments of ABA and shade resulted in only a slight increase in the number of fruits abscised relative to shade alone. Yield was reduced by all ABA and Shade treatments compared to controls, but fruit weight and quality were unaffected. Overall, shading did not increase the efficacy of fruit thinning with ABA.

3.1 Introduction

‘Bartlett’ pear trees are precocious and prone to over-setting spurs and shoot tips with fruits. Dense clusters of fruit and/or heavy crop loads can result in a source-to-sink ratio imbalance leading to reduced fruit weight at harvest. Hence, thinning clusters to one or two fruits is common to attain the desired size for fresh markets (Dennis, 2000; Wells et al., 1998). There are two practical methods for thinning pears in the Pacific Northwestern (PNW) region of the United States: Chemical thinning and hand thinning (Lombard, 1968a). Hand thinning is labor intensive and typically not performed until ~40 to 50 days after full bloom (DAFB). By comparison, chemical thinning requires less labor, is less time consuming, and has the distinct advantage of occurring earlier in fruitlet development. However, the response of fruits to chemical thinning agents is notoriously unpredictable due to the high dependency of uptake and activity of the chemicals on climatic conditions before, during and after application (Stover and Greene, 2005).

The most commonly used bio-regulators for pear thinning are naphthaleneacetic acid (NAA), ethephon ((2-chloroethyl) phosphonic acid), and 6-benzylaminopurine (6-BA). NAA uptake is greater at higher temperature and relative humidity (RH) (Knoche and Bukovac, 2001). Ethephon is an effective bloom thinner but requires a specific
developmental window (ovule length of 9 to 15 mm) (Childers, 1978; McArtney and Wells, 1995). More recently, investigation of 6-BA to thin pears has shown promise (Asín et al., 2009; Curreti et al., 2010; Dussi and Sugar, 2010; Gimenez et al., 2009; Maas and van der Steeg, 2011; Theron et al., 2010; Vilardell et al, 2005; Wertheim, 2000). However, NAA and 6-BA, require optimum temperatures for activity (>20°C) between full bloom and 10 to 12 mm fruit size, which poses a significant challenge in Oregon’s Hood River Valley.

Abscisic acid (ABA) has been investigated for use as a post-bloom thinner in apple (Greene et al., 2011; McArtney et al., 2014) and pear (Chapter 2; Greene, 2012) with varying degree of success. ABA has the distinct advantage over alternative thinners of being insensitive to fluctuations in temperature and humidity (Baldocchi et al., 1987; BassiriRad and Radin, 1992; Middelberg et al., 2014). Subsequently, ABA is taken up relatively rapidly through the cuticle (Kang et al., 2010). Greene (2012) reported consistent thinning of ‘Bartlett’ pear trees in several experiments in the Northeastern U.S. However, marked variation in thinning efficacy among multiple trials was observed in the PNW (Chapter 3). In trials where ABA was found to be effective for thinning, a rate of 125 ppm ABA was adequate. Thinning was, however, positively related to rate for both pear and apple (Greene, 2012; Greene et al., 2011; McArtney et al., 2014; Chapter 3). When ABA was ineffective at 125 ppm, rates ≥ 250 ppm resulted in unacceptable levels of foliar phytoxicity and/or abscission depending on the study (Greene, 2012; Greene et al., 2011; McArtney et al., 2014; Chapter 3). The effect of ABA on fruit abscission was associated with reduced gas exchange of apple and pear leaves for a short period of time (1 to 2 days), followed by recovery of 75% to 80% of control levels over
the following week (McArtney et al., 2014; Chapter 3). Consequently, ABA caused only mild carbon deficit. Incidentally, we observed a clear, inverse relationship between diurnal PAR the week succeeding ABA application and ‘Bartlett’ fruit abscission over multiple years of field trials (Chapter 3). Reduced light intensity, therefore, seemed a prerequisite for ABA-induced thinning of ‘Bartlett’ pears, potentially because adequate reserve carbon of pear trees can offset transient deficiencies in net photosynthesis (Pn).

Childer (1979) concluded that of all the climatic and abiotic factors governing fruit set, growth, and development, light intensity was the most important. Solar radiation is one of two critical factors required to estimate apple tree carbon budgets in order to optimize timing and rate of thinning compounds (Lakso, 2008; Lakso and Robinson, 2012; Robinson et al., 2010). Indeed, several artificial shading experiments have demonstrated marked fruit drop of apple exposed to several days of shade around 10 mm fruit size (Basak, 2011; Byers et al., 1990, 1991; McArtney et al., 2004; Stopar et al., 2001; Yoon et al., 2011). For these studies ≥70% shade cloth was generally utilized. Combining shade with thinning agents to induce even greater carbon deficits than achievable with thinning agents alone have not always produced additive responses on fruit abscission (Lehman et al., 1987; McArtney et al., 2004); though, Byers et al. (1990) documented a significant additive effect of Carbaryl plus four days of 92% shade on apple fruit set. Shading has recently been evaluated, alone, as a sustainable alternative to chemical thinning of apple (Aliev et al., 2012).

Relatively less is known regarding the role of light on pear fruit set. Fruit set of ‘Bartlett’ pear was reduced roughly three-fold by 80% shade, but in this trial the duration of shade treatment was rather long (14–76 DAFB; Garriz et al., 1998). We
hypothesized that low light intensity decreases tree carbon available to support fruit 
growth and thereby will increase the sensitivity of fruits to ABA. Our primary objective 
was to determine the relationship between light intensity and ABA on gas exchange and 
fruit abscission of ‘Bartlett’ pear.

3.2 Materials and Methods

**Planting material and experimental design**

The present experiment was performed in a 10-year-old ‘Bartlett’ pear orchard (3 
x 5 m, 667 trees/ha) in 2014 located at Oregon State University’s Mid-Columbia 
Agricultural Research and Extension Center in Hood River, Oregon (Long. 45.68, Lat. 
121.51). Soil is a deep, well-drained fine sandy loam. The experimental design was a 
two-way factorial arranged in a randomized complete block design with two levels of 
ABA (0 and 125 ppm) and three levels of light (0, 44% and 77% shade). Twenty-four 
trees were selected for canopy uniformity and randomly assigned to 6 treatments 
comprised of four single-tree plots: Untreated control (0% Shade + 0 ppm ABA); 0% 
Shade + 125 ppm ABA (ProTone, Valent, Walnut Creek, CA); 44% shade + 0 ppm 
ABA; 44% shade + 125 ppm ABA; 77% shade + 0 ppm ABA; and, 77% shade + 125 
ppm ABA. ABA was applied at petal fall to runoff using a hydraulic spray gun. A 
surfactant (Sil 100, Clariant Corp., Mount Holly, NC) was added to ABA mixture at a 
concentration of 0.1%. Previous evaluation of the surfactant alone found no effects on 
the measured variables (Chapter 3). Shade structures were constructed from 2.5 cm 
diameter polyvinylchloride (PVC) pipe with three-way, 90° elbow fittings, to enclose 
whole tree canopies under shade cloth treatments (Agriculture Solutions, Strong, Maine). 
The dimensions of the structure were 3 x 3 x 4 m and allowing ~60 cm of space between
the perimeter of canopies and the shade enclosures (Fig. 3.1 and 3.2). PVC cages were anchored on three sides with steel fence posts. The percentage of shade inside each structure was directly measured using a ceptometer (described below under light measurements). Pre-fabricated shade structures installed within 6 h of ABA application and remained in place for 15 days.

**Measurements and procedures**

**Fruit set, yield and fruit quality.** Total flower clusters per tree were counted at 80% full bloom. At 35 DAFB, total fruits per tree were counted and divided by the total number of cluster per tree to express fruit set (%). Fruits were hand thinned (12 June, 2014) according to commercial standards (approximately 1-2 fruit spaced 15 cm apart) and all thinned fruits were counted (data not shown). Whole trees were harvested in one event when fruits reached commercial maturity (determined when flesh pressure of the block softened to ~8.6 kg). Tree yield was determined by weighing total fruits per tree. Average fruit weight was determined by averaging individual weights of 50 randomly sampled fruit.

Fifty fruit samples per plot were selected for fruit quality analysis (flesh firmness, FF; soluble solids content, SSC; and, titratable acidity, TA). Fruit quality attributes of 20 fruit per plot were determined immediately following harvest. Thirty fruit per replicate were stored at -1°C, regular air (RA) for 60 days. Of this sample, fruit quality attributes were determined on 15 fruit per replicate after warming to room temperature (~20°C). The remaining 15 fruit were ripened for 5 d at 20°C and then evaluated for fruit quality. At each measurement date, fruit firmness (FF) was determined using a Fruit Texture Analyzer (Güss Manufacturing, Strand, South Africa) on two sites per fruit (opposite one
another) after removing a ~5 cm² area of skin. Soluble solids content (SSC) was determined using a digital refractometer (Model PR-101, ATAGO Co., LTD., Bellevue, WA). Titratable acidity (TA) was determined by titrating a juice sample of 10 mL + 10 mL of DI H₂O to an endpoint pH of 8.1 using 0.1 N NaOH with an automated titration system (Model DL15, Mettler-Toledo, LLC., Columbus, OH).

**Single-leaf gas exchange.** Single leaf gas exchange [\( A = \) photosynthesis (\( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)); \( g_s = \) stomatal conductance (\( \text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \))] was quantified using a PP systems Ciras-2 gas analyzer (PP systems, Amherst, MA) on all replications per treatment (four leaves per replication). Measurements were taken on fully-mature leaves of current-season extension shoots at a height of ~1.5 m between 12:00-14:00 HR at approximately three day intervals. CO₂ was supplied to the cuvette at 385 ppm and measurements were recorded when leaf gas exchange reached steady state in the cuvette (~60 s).

**Light.** To determine actual percentage shade generated by the two shade cloth treatments, light interception was measured inside all shade enclosures with a ceptometer (ACUPAR LP80, Decagon Devices Inc., Pullman, WA, U.S.A.) at 10:00, 13:00 and 16:00 HR on a clear, sunny day. For each plot, the ceptometer was positioned at ~1.5 m above ground with the tip of the light bar touching the trunk. Light interception was recorded at each of the four cardinal directions per tree (i.e., n=4). Clear sky photosynthetic active radiation (PAR) was recorded between each plot and used to calculate % shade.
Statistical Analysis.

Data were analyzed using two-way analysis of variance (ANOVA). When the differences among the treatments were significant ($P \leq 0.05$) means were separated using Least Significant Difference (LSD) test. Aov model ($x \sim B_1 + B_2 + B_1* B_2$) and HSD functions of agricolae package were used for determining interactions between ABA and shade responses. Significant main effects were present for ABA and shade; interactions between factors were not significant. Arccosine transformations were performed for all percent data. All other tests were performed in R-studio statistical platform, using ‘agricolae’ statistical package (Mendiburu, 2014) and ‘ggplot’ (Wickham, 2009).

3.3 Results

Fruit set, yield and fruit quality. ABA reduced fruit set by 35%, but there was no significant effect of shade ($P=0.055$) or any interaction between ABA and shade on fruit set (Table 3.1). Fruit abscission tended to increase with increasing shade percentage. The highest level of shade (77%) led to a 42% reduction in fruit set relative to control plots. Crop load and yield (fruit number per tree and average weight per tree) were significantly reduced by ABA and shade, but only yield was significantly impacted by the interaction between ABA and shade (Table 3.1). Average fruit weight was significantly increased by ABA but not by shade or the interaction of factors. ‘Bartlett’ pears attained commercial harvest maturity, irrespective of treatment, as indicated by FF (Table 3.2). Differences in FF, SSC, and TA were not significant among treatments at harvest or following RA. All fruit softened and ripened to acceptable levels and were unaffected by ABA, reduced light intensity or the combination of factors.
**Solar radiation, single-leaf gas exchange and PAR.** Daily solar radiation was recorded from -5 to +15 days from ABA and shade application. The decision to start the experimental period was based on a 10-day forecast of clear, sunny conditions; however, clouds limited light on -1, +3 to 5, 9, and 10 days from application (dfa) of treatments (Fig. 3.3). Days 3-5 and 10 were partially sunny, as evident from sun-breaks near solar noon, as compared to the ~75% diurnal reduction of light on -1 and + 9 dfa. The level of shade beneath the moderate and heavy shade structures was 44% and 77% of clear-sky conditions but these levels differed depending on canopy position (Fig. 3.4). Intra-canopy shading increased linearly with canopy depth (Fig. 3.4). Treatment differences in the distribution of light intercepted within canopies were marked, but decreased with increasing canopy depth. Canopies under 44% shade intercepted ~45% less PAR at solar noon compared to controls in the outer 20 cm of the canopy. This difference, however, was only ~25% at mid canopy depth and nearly non-existent in the interior 10 cm (Fig 3.4). With the exception of the inner 20 cm of the canopy, where little differences were observed, PAR was reduced by ~65% in 77% shade canopies compared to controls. Differences in PAR levels within canopies were less pronounced in the afternoon. PAR, measured at the leaf surface, during single-leaf gas exchange measurements, was reduced proportionally to the level of shade (Fig. 3.5 A), as would be inferred from solar noon ceptometer measurement at 10 cm canopy depth (Fig. 3.4). With the exception of day three and five, when light was reduced to approximately the light saturation level for Pn (Einhorn et al., 2011), incident light was near the upper limit. Moderate shade (44%) reduced PAR below Pn light saturation on days three and five. PAR was markedly reduced throughout the entire experimental period by 77% shade cloth.
ABA reduced Pn to ~0 µmol·m\(^{-2}\)·s\(^{-1}\) one day after application, whether or not ABA was combined with shade (Fig. 3.5 B). Between days 3 and 11, Pn of ABA-treated leaves, in the absence of shading, recovered to ~75% to 80% of control plots, and fully recovered by day 14. Pn was reduced by shade treatments, but 44% shade led to only slight reductions in Pn (80% of control levels) on day one and three; otherwise, Pn was not reduced from control levels. For 77% shade, significant and marked reductions of control plot Pn were observed (i.e., 40% to 85%) depending on the day. The combination of 125 ppm ABA and shade had an additive effect on reducing Pn rate compared to either factor alone. Notwithstanding day one, when nearly no net gains in ABA-treated trees were recorded, the addition of 125 ppm ABA to moderate shade (44%) reduced Pn by 10% to 20% relative to Pn of moderate shade alone. Compared to ABA alone, the combination of ABA and 44% shade reduced Pn slightly (~10%), but the effect was not observed consistently throughout the experimental period. The combined effect of 77% shade and 125 ABA, interestingly, was not discernable from 77% shade alone after day one.

3.4 Discussion

In the present experiment, moderate shade (i.e., 44%) had little effect on Pn when introduced in the absence of ABA, most likely because Pn of pear leaves saturates at roughly 50% of full light (Einhorn et al., 2013). Control plot PAR was reduced from approximately 1800 to 1100 µmol·m\(^{-2}\)·s\(^{-1}\) for trees shaded 44% (i.e., still above light saturation); however, mid to inner canopy leaves of 44% shade-covered trees had a much reduced light environment (Fig. 3.4). Increasing shade to 77%, on the contrary, severely reduced PAR, Pn and fruit set. Heavy shade reduced fruit set by ~42% compared to
control plot (Table 3.1); a level similar to that reported in artificial shade experiments for apple (Aliev et al., 2012; Basak, 2011; Byers et al., 1990; Lehman et al., 1987; McArtney et al., 2004; Stopar et al., 2001).

ABA, alone, thinned ‘Bartlett’ fruit quite well, as similarly observed in previous experiments (Chapter 2; Greene, 2012). For trials in the PNW, a correlation existed between natural light levels in the field and thinning efficacy of ABA over multiple experiments (Chapter 2). During those trials, thinning was high when periods of low light (~20% to 30% of full sun) coincided with day one to five from ABA applications. In the present trial, days 3 to 5 from application were overcast, and, potentially, contributed to the positive effects of ABA on thinning (34% less fruit set than control plots). This would agree with our previous observations (Chapter 2) and suggest some threshold of shade is required for optimal ABA activity. Net photosynthesis following the application of ABA was severely reduced 24 to 48 h after treatment, but nearly recovered to control levels within a week after application (Fig 3.5; Chapter 2). This highly transient nature of ABA on pear Pn is corroborated by recent results of ABA activity on apple leaf gas exchange and fruit abscission (McArtney et al., 2014). The ABA analog [(+)-8’-acetylene ABA] had greater activity on gas exchange of apple leaves than S-ABA. Importantly, reduced gas exchange following ABA applications was detected despite little to no apple or pear fruit abscission, depending on the trial (McArtney et al., 2014; Chapter 2). These data indicate that variation in ABA-influenced fruit abscission (Chapter 2; McArtney, 2014) is likely not associated with differences in the uptake or activity of ABA due to environmental factors (Knoche and Petracek, 2013: Middleton et
al., 2014), but rather a function of reduced assimilate supply in combination with available carbohydrates.

The fact that ABA and shade treatments resulted in similar levels of fruit abscission, presumably by a similar mechanism, but did not result in additional thinning when combined is unclear. A weak interaction between shade and NAA or 6-BA-induced thinning of apple (P= 0.053) was documented by Stopar et al. (2001) using 90% shade. Lehman et al. (1987), however, using 73% shade did not observe an additive effect of shade and carbaryl. Given the short-term effect of ABA on Pn, potentially any interaction is limited to the first 3-5 d following application. The shading effect on fruit set may have occurred in the first week of shade, with additional time having a reduced role in abscission. We did not evaluate the effect of shade duration on thinning, but observed a strong fruit drop from only 2-3 d of shade. There may be a threshold of fruit drop; especially given that all treatments except for 0 ABA/44% Shade had roughly 40% to 50% less fruit set than the untreated control (0% Shade/0 ppm ABA). At higher rates, ABA may indeed regulate additional mechanisms, such as hormonal interactions that directly lead to fruit abscission.

Based on the current project, there is not sufficient evidence to recommend the use of ABA as a thinner for ‘Bartlett’ pear. Inconsistencies in thinning prevent predicted outcomes. We cannot completely rule out the interaction of shade and ABA, since cloudy conditions were prevalent during the first week of the experiment, limiting the 0% shade + 0 ppm ABA plots.
3.5 Works Cited


Table 3.1 – Effects of shade (0%, 44%, 77%), and 125 ppm abscisic acid (ABA) on fruit set, tree yield (number and weight) and fruit weight of 10-year-old ‘Bartlett’/OH x F 87 pear trees.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit setz (%)</th>
<th>Fruit no.y (no./tree)</th>
<th>Yield (kg/tree)</th>
<th>Fruit wt. (g/fruit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ppm ABA</td>
<td>31 a</td>
<td>185 a</td>
<td>38</td>
<td>205 b</td>
</tr>
<tr>
<td>125 ppm ABA</td>
<td>23 b</td>
<td>142 b</td>
<td>36</td>
<td>255 a</td>
</tr>
<tr>
<td>Shade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% Shade</td>
<td>30 a</td>
<td>183</td>
<td>41</td>
<td>231</td>
</tr>
<tr>
<td>44% Shade</td>
<td>31 a</td>
<td>154</td>
<td>35</td>
<td>231</td>
</tr>
<tr>
<td>77% Shade</td>
<td>20 b</td>
<td>153</td>
<td>34</td>
<td>227</td>
</tr>
</tbody>
</table>

Significance

<table>
<thead>
<tr>
<th></th>
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<th>Shade</th>
<th>ABA x Shade</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABA</td>
<td>0.0400</td>
<td>0.0239</td>
<td>0.1844</td>
</tr>
<tr>
<td>Shade</td>
<td>0.0405</td>
<td>0.2596</td>
<td>0.2258</td>
</tr>
<tr>
<td>ABA x Shade</td>
<td>0.6288</td>
<td>0.2651</td>
<td>0.0795</td>
</tr>
</tbody>
</table>

zFruit set was calculated from whole tree bloom and fruit counts before thinning.

yFruit no. following thinning

Letters signify significant difference with LSD test, all values are means of 4 replicates, n=100

wR-studio 'agricolae' HSD for main effects and interactions (p>.05)
Table 3.2 – Effects of shade (0%, 44%, 77%) and 125 ppm abscisic acid (ABA) on 10-year-old ‘Bartlett’/OH x F 87 fruit quality (FF, Fruit Firmness; SSC, soluble solids content; and, TA, titratable acidity) at harvest, after 60 days RA cold storage, and after 60 days RA cold storage + 5 days of ripening.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Harvest</th>
<th>After 60 days storage</th>
<th>60 days storage + 5 days ripening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FF (kg)</td>
<td>SSC (%)</td>
<td>TA (%)</td>
</tr>
<tr>
<td>2014</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABA</td>
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<tr>
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<td>8.3</td>
<td>12.7</td>
<td>0.31</td>
</tr>
<tr>
<td>125ppm ABA</td>
<td>8.1</td>
<td>12.8</td>
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<td>Shade</td>
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<tr>
<td>0% Shade</td>
<td>8.1</td>
<td>12.7</td>
<td>0.3</td>
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<tr>
<td>44% Shade</td>
<td>8.2</td>
<td>13.2</td>
<td>0.32</td>
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<tr>
<td>77% Shade</td>
<td>8.2</td>
<td>13.4</td>
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<td>ABA x Shade</td>
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60 days of cold storage at -1 C regular air.
60 days storage + 5 days ripening at 20 C regular air.
Analysis of variance p(r)>0.05.
Figure 3.1 – Randomized complete block design with 4 single-tree replicates selected for uniformity. Each row comprised 6 treatments: 1) Untreated control (0% shade, 0 ppm abscisic acid [ABA]); 2) 0% shade, 125 ppm ABA; 3) 44% shade, 0 ppm ABA; 4) 44% shade, 125 ppm ABA; 5) 77% shade, 0 ppm ABA; and, 6) 77% shade, 125 ppm ABA. Three border rows separated reps.

Figure 3.2 Single replicates of 77% (left) or 44% shade cloth (right). PVC shade enclosures measured 3 x 3 x 4 m (l x w x h).
Figure 3.3 – Diurnal solar radiation from -5 to 15 days from abscisic acid (ABA) application. Data were collected from a weather station ~100 m from trial site.
Figure 3.4. Average photosynthetic active radiation (PAR) intercepted throughout 10-year-old ‘Bartlett’/OH x F 87 canopies at 10:00, 13:00 and 16:00 HR under 0% (i.e., exposed canopies), 44% and 77% shade. Measurements were taken during one day using a ceptometer positioned at 1.5 m height. Abscisic acid (ABA) had no effect on PAR, therefore data are only provided for shade treatments. Data are means of 4 replicates. PAR was measured for each replicate at 4 cardinal positions (n=4).
Figure 3.5- Photosynthetic active radiation (PAR) (A) and single-leaf photosynthesis (B) of 10-year-old ‘Bartlett’/OH x F 87 pear trees during a 15-day shade treatment with and without 125 ppm abscisic acid (ABA). Day one measurements were made ~ 4 h after ABA was applied. Applications coincided with petal fall. Data are means of 4 reps (n=4). Asterisk at day 15 indicates the removal of shade covering.
General Conclusion

Management of crop load is vital to successful pear production, allowing growers to maximize young tree fruit production and minimize labor inputs. ‘Bartlett’ and ‘d’Anjou’ pears are important to the US pear market and especially to the PNW growing region. Both varieties are characterized by unique production challenges. ‘Bartlett’ pear is vigorous and precocious, often requiring bloom or fruitlet thinning for acceptable harvest size. ‘d’Anjou’ pear is vigorous and non-precocious, producing copious vegetative growth and little fruit in years following planting. Management and cultural practices can improve production and overcome these challenges to a degree; however, new techniques are needed to maximize the potential of a growing industry and high production pressures.

Root pruning was seen to be an efficient tool in reducing vigor of ‘d’Anjou’ pear, with consistently reduced vegetative growth over multiple sites and years. The extent of vigor reduction was difficult to predict and appeared to be at least partially dependent on tree age and size. Continued work in root pruning with pear is recommended including root analysis and carbon modeling. Generally reductions in fruit set and return bloom were not adverse and did not linger without continued root pruning. It is possible that including specialized fertilizer regimes with root pruning could increase bloom and fruit set while controlling vigor.

Abscisic acid (ABA) performed inconsistently as a thinner of ‘Bartlett’ pear, with varied effects across years and trial sites. Two of four ABA experiments were thinned excessively, removing all fruit at high rates and half of all fruit at moderate rates. In one site results were milder with little impact on fruit set and no effects on total yield or fruit
weight; and in one study no thinning effect whatsoever was seen. In years where effects were mild or non-existent, high light conditions in the active period were observed and conversely low light was observed in both years of high thinning efficacy. We hypothesized that ABA was more efficacious when accompanied with low light conditions because a more intense carbon deficit was likely to be produced.

In order to test this hypothesis another experiment was designed to evaluate the effects of ABA with shade on ‘Bartlett’ thinning. ABA alone reduced fruit set and number and shade alone reduced fruit set at higher rates (77% shade). While main effects were significant, interactions between shade and ABA were non-significant. Our results indicate that ABA likely thinned ‘Bartlett’ by reducing stomatal conductance, inducing a carbon deficit, which strengthened sink competition. However effects were not consistent and may not have been enhanced by low light conditions as previously thought.
Common Bibliography


