INFLUENCE OF A MECHANICAL STRING THINNER
ON THE PHYSIOLOGY OF APPLE

A Thesis in
Horticulture
by
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ABSTRACT

The number of chemical thinning compounds available to apple growers is expected to be reduced in the future. Many of these compounds are not compatible with organic apple production, and hand thinning is a labor intensive and expensive process. The goals of this project were to test the efficacy of the mechanical string thinner in the Mid-Atlantic region, and to determine the mode of action of the string thinner. Trials conducted in 2010 and 2011 at Penn State’s Fruit Research and Extension Center in Biglerville, PA, tested six levels of thinning severity treatments (spindle speeds) to determine an effective range on field grown ‘Buckeye Gala’/M.9 apple trees. Treatments were applied to the same trees for two consecutive years to determine any cumulative effects of using the string thinner on apple. A linear reduction in blossom clusters per limb cross-sectional area, individual blossom number per spur, and leaf area per spur occurred as the level of thinning severity increased. The string thinner reduced cropload as thinning severity increased, but injury to spur leaves early in the season limited any benefit of increased fruit size. Since cropload was reduced as thinning severity increased, fruit quality was enhanced. Increased thinning severity treatments had no effect on return bloom, but increased annual trunk growth. Spindle speeds of 180 - 210 rpm provided the best overall thinning response and minimized injury to spur leaves. Further work will be required to determine if applying mechanical thinning treatments at earlier phenological stages may reduce the injury to spur leaves. A greenhouse trial was conducted on potted ‘Buckeye Gala’/M.9 apple trees to further elucidate the mode of action of the string thinner. Mechanical thinning treatments increased the rates of ethylene evolution of shoot leaves, and aminoethoxyvinylglycine (AVG) effectively reduced the rates of ethylene production, and prevented the string thinner-induced wound ethylene response. Net photosynthesis, vegetative growth, and dry matter accumulation
was not impacted by thinning severity treatment or AVG. Future work will be required to
determine the influence of wound induced ethylene on fruitlet abscission of apple.
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Chapter 1

Introduction

Background

_Malus x domestica_ (Borkh.) flowers abundantly in most years, and an excess of these flowers set fruit. Reducing the number of fruits or blossoms is an essential practice to assure that acceptable size, color, and fruit quality is achieved (Wertheim, 2000). The deliberate reduction of blossoms and/or fruit to enhance fruit quality and provide an annual crop is referred to as thinning. Until recently, the methods for managing cropload on apple have been limited to hand thinning and chemical thinning.

Hand thinning, which is the physical removal of blossoms or fruitlets, often results in larger fruit at harvest. Timing of hand thinning plays an important role in final fruit size. Blossom thinning is advantageous since the reduction of the reproductive sinks resulted in an increase in mean fruit weight, when compared to later hand thinning treatments (McArtney et al., 1996). While hand thinning is an effective means of improving fruit size and quality, it is difficult to complete early enough in the growing season to have a positive impact on return bloom and alternate bearing. Thinning reduces the number of fertilized embryos that are present on the tree, which emit phytohormones that inhibit flower bud formation for the next year (Westwood, 1993). To receive maximum benefit of return bloom, hand thinning must be accomplished within 40 days of full bloom. Bajter et al. (1957) hypothesized that maximal benefit of fruit size increase due to hand thinning occurred at or before 40 days after full bloom (DAFB), with diminishing benefits up to 95 DAFB. Hand thinning is a very labor intensive process, and growers report that obtaining a labor force in agriculture is becoming increasingly
difficult (Gasperini, 2012). Therefore, hand thinning is typically reserved for high value cultivars with excessive croploads after insufficient chemical thinning. The utilization of hand thinning as a primary means of cropload management may only be feasible on small acreage, since it is a time sensitive and labor intensive task.

The use of chemical thinners on apple has been intensely studied and documented since the 1940’s, when dinitro-o-cresylate (DNOC) and naphthalene acetic acid (NAA) were shown to have properties that ultimately reduce the fruit set of apple (Dennis, 2000). Factors such as consistency, phytotoxicity, and environmental sustainability have reduced the number of chemicals and chemical combinations that can be utilized to thin apples. Currently there are four classes of chemical thinners that can be utilized in a chemical thinning program in the United States: 1) plant growth regulators, 2) insecticidal carbamates, 3) photosynthetic inhibitors, and 4) caustic blossom thinners. Plant growth regulators make up the majority of the thinning options, and include two synthetic auxins, 1-naphthaleneacetic acid (NAA) and naphthaleneacetamide (NAAm), a cytokinin 6-benzyladenine (6-BA), and the ethylene producing compound ethephon [(2 – chloroethyl)-phosphonic acid]. Two carbamate insecticides, carbaryl (1-naphthyl methylcarbamate), and oxamyl (oximino oxamyl) have mild thinning activity and can be used in conjunction with other products or alone when less thinning is required. Despite the range of efficacy and variety of products available for chemical thinning, the mode of action of many of these products is poorly understood (Byers, 2003). An understanding of the mode of action of a given thinner is important when considering its application. Since both environmental factors and cultural practices play important roles in efficacy of chemical thinners, variable results often occur from season to season (Stover and Greene, 2005). The number and diversity of chemical compounds that can legally be applied in a conventional thinning program is expected to be
reduced in the United States. Carbaryl can harm pollinators and beneficial insects, and has been banned in the European Union (EU) (Anon, 2006). The EU is also considering limiting the use of ethephon, due to concerns about acceptable levels of residues on fruit (Anon, 2009). NAA and NAAm are being phased out in some European countries (Veal, 2011). Since conventional growers often utilize combinations of the aforementioned chemicals in their thinning programs, there are concerns about the viability of chemical thinning programs in the future. Researchers are currently seeking alternative thinners, but the testing of these products, determination of the efficacy, dose recommendations, sustainability, and high cost of registering potential products are all potential barriers for adoption. Since thinning is one of the most important annual decisions for apple growers worldwide, it is imperative to provide growers with a variety of options in all possible windows of thinning.

Most of the products utilized as chemical thinners are not compatible with the specifications of organic production systems. The lone exceptions are some blossom thinners. Blossom thinners are very desirable for small fruited cultivars and fruit producing regions in cool climates (McArtney et al., 1996). The first chemical blossom thinner was DNOC (Elgetol), and it was discontinued in 1989 as a thinning product due to expensive re-registration and environmental concerns (Dennis, 2000). Researchers have been working to find a suitable replacement for Elgetol with mixed success. Many of the compounds tested to replace DNOC yield inconsistent results and detrimentally injure the foliage (Miller and Tworkoski, 2010). When applying caustic blossom thinners, it is desirable to allow a small proportion of the flowers to become fertilized before application of the blossom thinner. This small window for application can make it difficult to determine and conduct application dates in large commercial orchards. Drying time of caustic compounds has a direct influence on the efficacy of the application of the
product, and rainfall can reduce efficacy or reactivate the compound on the tree. One of the more promising blossom thinners, liquid lime sulfur (LLS) + fish oil emulsion (FO), is an organic product that injures floral tissue. The mode of action of LLS + FO is debated, as there have been reports that lime sulfur inhibits pollen germination, pollen germ tube growth, and fertilization and/or inhibits photosynthesis (McArtney et al., 2006, and Yoder et al., 2009). LLS + FO has been one of the better organic blossom thinning products available (Tory Schmidt, personal communication). Unfortunately, growers in the Mid-Atlantic apple production region are not able to utilize this product due to insufficient labeling for thinning purposes. For apple growers to remain competitive, sustainable and environmentally sound thinning products must be developed (Dennis, 2000). Aside from LLS + FO, there are some caustic products that are available to organic growers, such as acetic acid, sodium chloride, soybean oil, and eugenol (2-methoxy-4(2-propenyl)phenol) (Stopar, 2008; Miller and Tworkoski, 2010). All of these products have potential as organic thinning options of apple, but the inconsistency of results, lack of proprietary exclusivity and concerns regarding phytoxicity have limited interest in registering these compounds as thinners.

Mechanical methods of thinning fruit trees have been described in the literature, and in some cases, have been adopted by the stone fruit industry. High pressure spray guns, tree shakers and club thinning, rope thinners, drum shakers, and string thinners are capable of generating a thinning response in stone fruits and some nut crops (Dennis, 2000). Since many of these practices may injure spurs and vegetative structures, there has been limited adoption of mechanical thinning practices in apple. This poor adoption rate is attributed to two primary factors: the phenological characteristics of apple as compared to stone fruit, and the potential to spread the pathogen *Erwina amylovora* (Burrill).
Stone fruits flower before vegetative leaf tissue is present. Thus, the flowering and fruit set of stone fruit relies heavily on stored carbohydrates. In the case of apple, primary spur leaves emerge before anthesis. While these primary spur leaves have a relatively small amount of leaf area, they are very efficient at photosynthesis. Ferree and Palmer (1982) showed the importance of spur leaf area on young fruit development and retention. Ngugi and Schupp (2009) demonstrated that mechanical thinners can also be an efficient vector of fireblight if the pathogen is present in the orchard. Precautions were advised when using the string thinner on apple, such as the use of predictive models to forecast the risk of infection, avoiding use of the string thinner in blocks that have a history of fireblight, and the prophylactic use of an antibiotic post-treatment if conditions are conducive for fireblight infection following thinning treatment (Ngugi and Schupp, 2009).

Recent investigations in mechanical thinning on apple have utilized two different thinning machines (Bertschinger et al., 1998; Damerow et al., 2007, Schupp, et al. 2008). Trials with a single spindle string thinner as described in Bertschinger et al. (1998), have shown encouraging results. Thinning with the Darwin, utilizing fast ground speeds (9-12km/h) was observed to limit injury to foliage, reduce fruit set by 25 %, and enhanced return bloom compared to the control (Weibel et. al, 2008). A 50% increase in mean fruit weight, enhanced fruit color, and a substantial reduction in follow up hand thinning time was demonstrated by Sinatsch et al. (2010). The impacts of spur leaf reduction due to mechanical thinning have been examined by Solomakhin and Blanke (2010). However, leaves were only considered damaged if 1/3 or more of the lamina was removed. At 320 rpm with a 3 rotor string thinner, it has been proposed that less than 8% of leaves were injured while providing acceptable thinning efficacy (Solomakhin and Blanke 2010). The timing of treatment ranges from pink to full bloom. Thinning at earlier
stages of blossom development may cause significant losses in yield, and thinning near petal fall
causes superficial injury to the fruit (Blanke, 2011). Trials in Germany with the 3 rotor thinner
resulted in a 25 g increase in mean fruit weight, reduced yield, and enhance packout by 20%
when compared to an unthinned control (Veal et al, 2011). The thinner has been reported to
enhance fruit quality characteristics with certain levels of thinning severity. The existing body of
literature of use of the string thinner is limited geographically, since most of the research on
mechanical thinning of apple has occurred in Europe. In the Mid-Atlantic region, the terrain is
not conducive to operating at the tractor speeds that are utilized in the above trials. To determine
if this technology is suitable for our region, we must utilize slower tractor speeds and determine
a matching spindle rotation that will provide acceptable results. Schupp et al., (2008), showed
that the string thinner reduced fruit set, increased fruit weight, reduced yield, and reduced hand
thinning time, at ground speed of 4.0 km/h and spindle speed of 245 rpm. Using the same
treatment in later trials resulted in over-thinning (J.R. Schupp, unpublished data).

In peach, the thinning effect of the mechanical string thinner is a result of blossom removal
(Schupp et al., 2008). However, we do not have a complete understanding of the mode of action
of the string thinner on apple. Yields and fruit size distribution in previous trials were
inconsistent over a three year period (Schupp et al., 2008 and J.R. Schupp, unpublished data). If
photosynthetically-active leaf tissue is sufficiently reduced, then the potential of carbohydrate
production of the tree is reduced, potentially resulting in the premature abscission of
reproductive sinks.
Photosynthetic Inhibition Resulting from Reduced Spur Leaf Area

Reduction of primary spur leaf tissue and shoot leaves can lead to extensive fruitlet abscission (Ferree and Palmer, 1982). Primary spur leaves are the main contributor of metabolites such as photosynthates early in the season. “June drop,” which is a naturally occurring wave of fruitlet abscission, is attributed to the competition between fruitlets for metabolites. June drop can be amplified by weather conditions, such as temperatures exceeding 16.7°C with 2-3 days of cloud cover (Byers, 2003). Shading or reducing photosynthesis caused fruitlet abscission shortly after bloom, between 10mm and 20 mm fruitlet diameters (Byers, 1990). Ferree and Palmer (1982) showed that a 50% or 100% reduction in spur leaves resulted in significant fruit drop as compared to the control treatment and reduced the calcium concentration of the fruit, since spur leaves are important in Ca and cytokinin allocation. Yuan and Greene (2000) suggested that a minimum of two spur leaves on a girdled spur per developing fruitlet is required to support the fruit to maturity. However, fruitlets with low leaf to fruit ratios had a temporary reduction in growth rate early in the season, resulting in smaller fruit at the end of the season. When outsourcing of local carbohydrates was prevented via girdling, a minimum of 16 leaves is required to maintain fruitlet growth rate per fruitlet.

It is well documented that the first 4-6 weeks after full bloom is the most important period for fruitlet cell division. Cell number, not cell size, is the primary factor for achieving large fruit size (Westwood et al., 1967). Spur leaves and stored reserves are the physiological sources for the developing fruitlets during this 4-6 week time frame, and injury to spur leaves may have a negative impact on fruit set. There is not an adequate understanding of the relationship between string thinning and spur leaf damage. Since it is inevitable that a number of spur leaves will
become injured after the string thinner has been utilized, an understanding of the thresholds of such damage must be determined.

The retention of spur leaves and bourse shoots increases fruit set (Ferree and Palmer, 1982). Bourse shoots are considered as vegetative sources rather than sinks near the end of fruit set, because the increased photosynthetic tissues can maintain the number of fruitlets that remain after the two waves of fruit drop (Abbott, 1960). Abbott (1960) showed that the bourse shoot is a strong competitor early in the season. However, the limbs that had bourse shoots adjacent to the fruiting spur had a larger fruit diameter later in the season. Shoot tip removal reduces fruitlet abscission, and increases yield (Quinlan and Preston, 1971). Since the action is non-selective, the string thinner may sever shoot tips, entire shoots and spurs, and may injure the remaining vegetative spur leaf tissue.

**Stress-Induced Ethylene**

Schnieder (1977) proposed that a reduction of carbohydrates is the first step in abscission of immature apple. There is thought to be a hierarchy of fruitlets within a cluster, as some fruitlets have a higher abscission potential than others. Fruit that originate from early opening blossoms have higher sink strength and have a lower abscission potential, and have dominance over fruitlets that are derived from later opening flowers (Botton et al, 2011). Fruit that are strong sinks are somewhat impervious to exogenous ethylene application since they contain a higher number of fertilized embryos and potentially a higher number seeds and carbohydrates. Conversely, fruitlets that do not have strong sink strength are prone to abscission if environmental conditions do not favor carbohydrate production, or if an additional stress is imposed. After the reduction in carbohydrate allocation to the fruit, an abscission signal is
produced by the cortex and the seed, which translates this signal to the abscission zone. If the stream of carbohydrate is high, then it is expected that the level of auxin in the embryo(s) of the fruit is high (Brown, 1997). This is followed by a reduction in fruit growth and an outward flux in endogenous auxin from the fruit. With this outward flux of auxin, the fruitlet becomes more sensitive to ethylene. Stress or treatment with ethylene can expedite the transition to stage II (Brown, 1997). Any naturally produced ethylene or stress may initiate abscission of the fruitlet once it is in stage II. If so, ethylene cannot impose abscission of fruit if adequate endogenous auxin is present. Despite the fact that high endogenous auxin is associated with resistance to abscission, the artificial auxin NAA is utilized as a thinner. The mode of action of NAA as a chemical thinner has been disputed since the 1970’s, as ethylene production and a reduction of carbohydrates to the fruitlets have both been confirmed. A study using immature green fruitlets by Zhu et al. (2000) confirmed that ethylene is involved in fruitlet abscission post-NAA application, by utilizing an ethylene inhibitor paired with NAA. Expression of ethylene receptor genes in the apple cortex and the abscission zone was increased by NAA. The relative number of receptor genes were was decreased in NAA trees that received a prophylactic application of aminoethoxyvinylglycine (AVG, commercial name ReTain) (Zhu et. al, 2008).

Ethylene biosynthesis in higher plants can be regulated by a number of physiological and environmental factors. Ethylene production can be initiated by a stress, such as mechanical wounding. After wounding, the conversion of S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) plays an integral role in controlling the production of stress induced ethylene, and auxin induced ethylene (Yang and Hoffman, 1984). The key enzyme in this process, 1-aminocyclopropane-1-carboxylate synthase (ACS) is essential to the ethylene pathway. The ACS enzyme can be controlled by commercially available growth
regulators. AVG is a potent inhibitor of ethylene production (Saltveit, 2005). It inhibits the activity of pyridoxal phosphate requiring enzyme, such as ACS, and prevents conversion to ACC (Huai et. al, 2001). Induction of wound ethylene is quite rapid when compared to exogenous hormonal induction (20 minutes compared to 2-3 hr respectively) (Saltveit and Dilley 1978). Studies on Phaseolus vulgaris L. ‘Pinto’ leaf tissue showed most of the ACC is formed 15 minutes after wounding (Konze and Kwiatkowski, 1981). A prophylactic or concurrent application of AVG prevented ethylene induction on wounded leaf tissue (Konze and Kwiatkowski, 1981). In apples, a 250 mg·L⁻¹ whole tree application of AVG resulted in a significant reduction of abscission rates and ethylene production of a fruit and leaf tissue when combined with an NAA treatment (Zhu et al., 2000).

Ethylene has been dismissed as the mode of action of the string thinner, since there was no measurable difference in ethylene concentration between hand torn leaf tissue and intact control vegetative tissues of pear and apple (Kong et al., 2009). This inference was based on a preliminary trial with a small sample size and a static ethylene collection system with a large volume. Aside from the aforementioned pilot study, there has been little documentation of the influence of mechanical thinning on ethylene production on leaf tissue of apple, and the potential of wound induced ethylene on fruit set and/or abscission of blossoms or immature fruitlets. However, it has been inferred that wound ethylene contributes to fruitlet abscission of mechanically thinned trees (Dorogoni, 2008). Monitoring fruitlet abscission patterns, measuring ethylene injured leaf tissue, and the use of an ethylene inhibitor may provide an opportunity to determine if wound induced ethylene is contributing to the inconsistent results that we have witnessed in the past three years.
Hypotheses

The purpose of this study is to elucidate the mode of action of the string thinner. This study will be conducted to test the following hypotheses:

1) The mode of action of the string thinner is a consequence of removal of blossoms early in the season, and reduction of fruit set corresponds with the level of thinning severity.

2) The mode of action of the string thinner is a reduction in the rate of photosynthesis resulting from reduced spur leaf area of the tree.

3) The mode of action of the string thinner is stress induced ethylene, as a result of the injury of leaf tissue.
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Chapter 2

Influence of mechanical thinning severity treatments on vegetative and reproductive tissues, fruit set, yield, and fruit quality of apple

Introduction

Apple (*Malus x domestica* (Borkh.)) trees bloom abundantly in most years, and an excess of these flowers set fruit. Reducing the number of fruits or blossoms is an essential practice to assure that acceptable size, color, and fruit quality is achieved (Wertheim, 2000). The deliberate reduction of blossoms and/or fruit to enhance fruit quality and encourage annual cropping is referred to as thinning. Until recently, the methods for managing apple cropload have been limited to hand thinning and chemical thinning.

Hand thinning, which is the physical removal of blossoms or fruitlets, often results in larger fruit at harvest (Dennis, 2000). Timing of hand thinning plays an important role in final fruit size (McArtney et al., 1996). Blossom thinning was advantageous since the reduction of the reproductive sinks resulted in an increase in mean fruit weight, when compared to later thinning hand thinning treatments (McArtney et al., 1996). While hand thinning is an effective means of improving fruit size and quality, it is difficult to complete early enough in the growing season to have a positive impact on return bloom and alternate bearing. Thinning reduces the number of fertilized embryos that are present on the tree, which emit phytohormones that inhibit flower bud formation for the next year (Westwood, 1993). To maximize the benefits of return bloom, hand thinning must be accomplished within 40 days of full bloom. Bajter et al. (1957) hypothesized that maximal benefit of fruit size increase due to hand thinning occurred at or before 40 days after full bloom (DAFB), with diminishing benefits up to 95 DAFB. Hand thinning is a very
labor intensive process, and growers report that obtaining a labor force in agriculture is becoming increasingly difficult (Gasperini, 2012).

The use of chemical thinners on apple has been intensely studied and documented since the 1940’s, when dinitro-o-cresylate (DNOC) and naphthalene acetic acid (NAA) were shown to have properties that ultimately reduce the fruit set of apple (Dennis, 2000). Factors such as consistency, phytotoxicity, and environmental sustainability are all important factors that have reduced the number of chemicals and chemical combinations that can be utilized to thin apples. Currently there are four classes of chemical thinners that can be utilized in a chemical thinning program in the United States: 1) plant growth regulators, 2) insecticidal carbamates, 3) photosynthetic inhibitors, and 4) caustic blossom thinners. Despite the range of efficacy and variety of products available for chemical thinning, the mode of action of many of these products are poorly understood (Byers, 2003). An understanding of the mode of action of a given thinner is important when considering its application. Since both environmental factors and cultural practices play important roles in efficacy of chemical thinners, variable results often occur from season to season (Stover and Greene, 2005).

The number and diversity of chemical compounds that can legally be applied in a conventional thinning program is expected to be reduced in the United States. Carbamate insecticides, such as carbaryl, can harm pollinators and beneficial insects, and have been banned in the European Union (EU) (Anon, 2006). The EU is also considering limiting the use of ethephon [(2 – chloroethyl)-phosphonic acid], due to concerns about acceptable levels of residues on fruit (Anon, 2009). Chemical thinning uses of NAA and its amide salt, napthaleneacetamide, (NAAm) are being phased out in some European countries (Veal, 2011). Since conventional growers often utilize combinations of the aforementioned chemicals in their
thinning programs, there are concerns about the viability of chemical thinning programs for the future. Researchers are currently seeking alternative thinners for growers, but the testing of these products, determination of the efficacy, dose recommendations, sustainability, and registration of potential products are all potential barriers for adoption. For apple growers to remain competitive, sustainable and environmentally sound thinning products must be developed (Dennis, 2000).

Mechanical methods of thinning fruit trees have been described in the literature, and in some cases, have been adopted by the stone fruit industry. High pressure spray guns, tree shakers and club thinning, rope thinners, drum shakers, and string thinners are capable of generating a thinning response in stone fruits and some nut crops (Dennis, 2000). Since many of these practices injure spurs and vegetative structures, there has been limited adoption of mechanical thinning practices in apple. This poor adoption rate is attributed to two primary factors: the phenological characteristics of apple as compared to stone fruit, and the potential to spread the fire blight pathogen *Erwina amylovora* (Burrill).

Stone fruits flower before vegetative leaf tissue is present. Thus, the flowering and fruit set of stone fruit relies heavily on stored carbohydrates. In the case of apple, primary spur leaves emerge before anthesis. While these primary spur leaves have a relatively small amount of leaf area, they are very efficient at photosynthesis. Ferree and Palmer (1982) showed the importance of spur leaf area on young fruit development and retention. As illustrated by Ngugi and Schupp (2009), mechanical thinners can also be an efficient vector of fire blight if present in the orchard. Precautions are advised when using the string thinner on apple, such as the use of predictive models to forecast the risk of infection, avoiding use of the string thinner in blocks that have a
Recent investigations in mechanical thinning on apple have utilized two different thinning machines (Bertschinger et al., 1998; Damerow et al., 2007, Schupp, et al., 2008). Trials with a single spindle string thinner as described by Bertschinger et al. (1998) have shown encouraging results. Thinning with the Darwin, utilizing fast ground speeds (9-12km/h) limited injury to foliage, reduced fruit set by 25 %, and enhanced return bloom when to the control (Weibel et. al, 2008). A 50% increase in mean fruit weight, improvement in fruit color, and a substantial reduction in follow up hand thinning time was demonstrated by Sinatsch et al. (2010). The impacts of spur leaf reduction due to mechanical thinning have been examined by Solomakhin and Blanke (2010). However, leaves were only considered damaged if 1/3 or more of the lamina was removed. At 320 rpm with a 3 rotor string thinner, it has been proposed that less than 8% of leaves were injured while providing acceptable thinning efficacy (Solomakhin and Blanke, 2010). The timing of application ranges from pink to full bloom. Thinning at earlier stages of blossom development may cause significant losses in yield, and thinning near petal fall causes superficial injury to the fruit (Blanke, 2011). Trials in Germany with the 3 rotor thinner resulted in a 25 g increase in mean fruit weight, reduced yield, and enhance packout by 20% when compared to an unthinned control (Veal et al, 2011). The thinner has been reported to enhance fruit quality characteristics with certain levels of thinning severity. The existing body of literature regarding string thinners is limited geographically, since most of the research on mechanical thinning of apple has occurred in Europe. In the Mid-Atlantic region, the terrain is not conducive to operating at the tractor speeds that are utilized in the above trials. To determine
if this technology is suitable for our region, we must utilize slower tractor speeds and determine a matching spindle rotation that will provide acceptable results.

Reduction of primary spur leaf tissue and shoot leaves can lead to extensive fruitlet abscission (Ferree and Palmer, 1982). Primary spur leaves are the main contributor of metabolites such as photosynthates early in the season. “June drop,” which is a naturally occurring wave of fruitlet abscission, is attributed to the competition between fruitlets for metabolites. June drop can be amplified by weather conditions, such as temperatures exceeding 16.7°C with 2-3 days of cloud cover (Byers, 2003). Shading or reducing photosynthesis caused fruitlet abscission shortly after bloom, between 10mm and 20 mm fruitlet diameters (Byers et al., 1990). Ferree and Palmer (1982) showed that a 50% or 100% reduction in spur leaves resulted in significant fruit drop as compared to the control treatment and reduced the calcium concentration of the fruit, since spur leaves are important in Ca and cytokinin allocation. Yuan and Greene (2000) suggested that a minimum of two spur leaves on a girdled spur per developing fruitlet were required to support the fruit to maturity. However, fruitlets with low leaf to fruit ratios temporarily reduced the fruitlet growth rate early in the season, resulting in smaller fruit at the end of the season. When outsourcing of local carbohydrates was prevented via girdling, a minimum of 16 leaves was required to maintain fruitlet growth rate per fruitlet (Yuan and Greene, 2000). It is well documented that the first 4-6 weeks after full bloom is the most important period for fruitlet cell division. Cell number, not cell size, is the primary factor for achieving large fruit size (Westwood et al., 1967). Spur leaves and stored reserves are the physiological sources for the developing fruitlets during this 4-6 week time frame, and injury to spur leaves may have a negative impact on fruit set. Documentation of the severity of spur leaf damage will be an important determinant of the overall reaction of the tree to the thinning
procedure. Since it is inevitable that a number of spur leaves will become injured after the string thinner has been utilized, an understanding of the thresholds of such damage must be determined. The purpose of this study is to evaluate the efficacy of a single spindle string thinner on apple in the Mid-Atlantic region, and determine an optimal range of spindle speeds. We will also be testing the following hypotheses: the mode of action of the string thinner is a consequence of removal of blossoms early in the season, and reduction of fruit set corresponds with the level of thinning severity.

**Materials and Methods**

**Thinning severity experiment.** Experiments were conducted in the spring of 2010 and 2011 at the Penn State Fruit Research and Extension Center in Biglerville, PA on five-year-old ‘Buckeye Gala’/M.9 apple trees at 1.2 x 4.6 m spacing. The trees were trained to a vertical axis system, with a narrow cone canopy architecture with an average tree height of ~4.0 m and canopy width at the lower scaffolds of ~1.5m. The experiment was a randomized complete block design with four replications. The experimental unit was an eight tree plot. The first and last trees in each plot were not used for data collection, as they may have been subjected to an irregular thinning treatment. Two data trees were selected within the center six trees of the eight tree plot to ensure consistent treatment and tree uniformity. Two to five limbs per data tree were flagged based on the following criteria: 1) the total number of blossom clusters per data tree was equal to or greater than 50 and 2) the limbs were not subjected to a heading cut in the previous season. Prior to treatment, the initial number of blossom clusters per flagged limb was recorded, limb circumferences of the selected limbs were measured 3 cm away from the trunk to calculate limb
cross-sectional area (LCSA), and trunk circumferences were measured on the trees 30 cm above the graft union, and initial trunk cross-sectional area (TCSA) was calculated.

Treatments were applied as follows: 1) unthinned control, 2) string thinned at 180 rpm, 3) string thinned at 210 rpm, 4) string thinned at 240 rpm, 5) string thinned at 270 rpm, and 6) string thinned at 300 rpm. Spindle speeds were verified with a digital tachometer prior to treatment (Albuquerque, Inc. model CDT-1000HD, Briarwood, New York). All treatments were applied at full bloom with a Darwin PT-250 mechanical string thinner (Figure 2-1) (Fruit-Tec, Deggenhauserertal, Germany). A description of the machine is provided in Schupp, et al., (2008). The string thinner was attached to the front of a tractor via a hydraulic fork lift, and forward speed was constant at 4.8 km/h. A helical string pattern comprised of 90 strings was utilized based on the results of a preliminary trial (Appendix A). The helical string pattern was created by utilizing all 30 plates with three 60 cm strings attached to each plate. Six of the nine strings on each plate were removed to assure that one string is present at each position along the spindle (Figure 2-2). The height and the angle of the string thinner can be modified by the operator, though this was unnecessary for our treatments since the tree architecture was uniform, and the canopy was narrow. To obtain an understanding of the cumulative effects of mechanical thinning of apple, the above treatments were applied to the same plots both years.

**Fruitlet abscission, fruitlet growth, fruit set, and harvest.** On the same day that the treatments were applied, post-treatment blossom cluster counts were recorded to determine the number of entire clusters that was removed by the string thinner. Eight days after treatment, individual fruitlet counts were recorded on each of the flagged limbs. Fruitlets were counted at 3-4 day intervals to observe fruitlet abscission patterns of all treatments. Once the fruitlet numbers remained constant for a period of 1 week, final fruit set was assessed on the flagged limbs. In
2011, mean fruitlet size was measured at each date that the fruitlet counts were conducted. Ten fruitlets per plot (240 fruitlets total) were randomly selected and measured with a set of digital calipers. Fruitlets were measured equatorially at their widest point.

In addition to the mean fruitlet size measurements, five spur complexes were flagged per plot to determine fruitlet growth rate over time. These clusters were selected based on the criteria that they were not lateral blossom clusters and contained a minimum of five fruitlets. Since king flowers do not have a distinct advantage over laterals in fruitlet development in ‘Gala’ (Ferree et al., 2001), the five fruitlets were selected randomly within a given cluster. There was no attempt to include or disregard king blossoms in our subsample. Small pieces of colored electrical tape were loosely placed around the peduncle for purposes of identification for future sampling.

Fruitlet diameter measurements occurred on the same days as fruitlet counts.

In both years of this study, a secondary flush of bloom occurred after treatment. We documented this phenomenon in 2010 by counting the number of blossoms present on flagged limbs that were used to observe fruitlet abscission patterns. The secondary flush blossoms were placed into one of three phenological categories: 1) pink, 2) full bloom, and 3) petal fall. This count occurred on 14 May 2010, over one month after treatment. In 2010, there was an outbreak of wooly apple aphids (Eriosoma lanigerum) in our plots. It appeared that there was a relationship between the number of wooly apple aphids and thinning treatment. The number of wooly apple aphid colonies per plot was estimated by a four minute count of the six center trees of each plot. Counts were conducted on 16 June 2010.

Since the string thinner is 2.5 m in height and the canopy was 3.0 m, the distal end of the trees did not receive the thinning treatment in 2010. To preserve the structural integrity of the central
leader, this portion of the canopy was hand thinned with a hand-held cropload adjustment tool, the MAFCOT 6 Equilfruit Disk. The use of this hand thinning gauge was to assure that all central leaders were thinned consistently. Tree height was reduced to 3.4 – 3.7 m in 2011 in an effort to manage tree height and assure that the entire canopy received the string thinner treatments. The selected data trees were harvested twice in both years of the study, to adhere to commercial maturity standards. The first harvest was the primary harvest in both years. Whole tree yields, mean fruit weight, and fruit size distribution were determined with an electronic fruit sorter (Durand Wayland, LaGrange, GA). The dollar value of harvested fruit was calculated with the following prices provided by the packer.com for the Appalachian District (dollar value/kg): 2010 prices: Processing – $0.11; Bags – $1.25; 100 count – $1.26; 88-80 count – $1.34. In 2011 the prices were as follows: Processing – $0.13; Bags – $1.47; 100 count – $1.42; 88-80 count – $1.52. Once the trees were in endodormancy, the trunk diameters and subsequent TCSA were recorded. Return bloom was quantified in 2011 by comparing the number of blossom clusters per LCSA on 4-5 limbs per plot.

Quantifying impacts of thinning severity treatments on vegetative and reproductive tissues.
Two trees from the interior six trees of each plot were designated to be used in the leaf area removal study. The trees selected for quantifying leaf and blossom removal were not utilized for the above counts. Immediately after treatment, 10 spurs were excised from two year old wood in each plot. These excised spurs were stored in polyethylene bags at 0 C° until they were analyzed. The number of flowers, leaves, and leaf area per spur were recorded. When counting the number of flowers per spur, some bourse shoots were terminated with a raceme. In this case, each flower was counted, since they were perfect and had potential to be pollinated. Conversely, if the ovary was visibly injured by the string thinner, it was assumed to be incapable of fertilization and was
not counted. A LI-COR 3100 leaf area meter (LI-COR, Lincoln, NE), was used to measure the average leaf area per spur for each treatment. When counting the number of leaves per spur and determining leaf area, floral bracts, petioles, and leaves that were not fully expanded were discarded. These vegetative organs are not considered to be significant contributors to the photosynthetic capacity of the spur or the leaf area of the spur. The trees in each plot that were manipulated via spur removal for the aforementioned leaf analysis were not utilized for any other purpose throughout the experiment.

**Postharvest fruit maturity and quality.** A sample of 20 fruit from each plot was selected for fruit quality analysis. All of the fruit utilized in postharvest fruit quality analysis ranged between 125-175 g. Fruit firmness was measured with a Güß GS-14 penetrometer (QA Supplies, LLC, Norfolk, VA). Juice samples were collected and tested for soluble solids concentration with a digital refractometer (Atago model PR-32 alpha, Bellevue, WA). Fifteen mL juice samples were extracted with an industrial juicer, and tested for pH and titratable acidity (malic acid equivalents) with an automatic minititrator and pH meter (Hanna Instruments model HI-84432, Woonsocket, RI).

In 2011, further postharvest work was completed to attain a better understanding of increased spindle speed on fruit maturity. A 20 fruit sample from each plot were cut at the equator and dipped in an iodine solution. Based on the staining patterns, each fruit was assigned a value from 1-8 in accordance with the Generic Cornell Starch-Iodine Index Chart for apples (Blanpied and Silsby, 1992).

An additional 10 fruit sample was used to quantify the fruit internal ethylene concentration. Internal ethylene of the whole fruit samples was collected via vacuum extraction (Saltveit, 1982).
A plexi-glass box was partially filled with water and connected to a vacuum pump. Apples were submerged underwater assuring that air pockets did not reside on the calyx end of the fruit. The apex of five plastic funnels fitted with rubber septa was submerged underwater. Care was taken to ensure that extraneous pockets of air did not reside on any of the surfaces of the fruit or the interior of the funnel when the apples were secured in the bell of the funnel. The water-filled funnel was orientated so that the apex of the funnel was at the surface of the water and the bell was completely submerged. The top of the plexi-glass box was positioned on the container and a vacuum of 1.03-1.38 MPa was briefly placed on the entire container, until several milliliters of internal gas was expressed from each fruit and collected at the apex of the funnel. At this time, the pump was turned off and a relief valve was opened to release the vacuum on the container.

One milliliter gas samples were extracted from the headspace of the funnel and injected into a gas chromatograph (Shimadzu GC-8A, Columbia, MD) with a 1/8 in. stainless steel column packed with alumina (Supelco, Bellefonte, PA).

An additional 20 fruit sample of 125-175 g fruits from each plot was collected to analyze for mineral content. A 1.0 cm equatorial slice of fruit tissue was made, and four circular plugs were cut with a 1.0 cm cork borer. The circular plugs of cortical tissue were taken right next to the epidermis of the fruit. The 120 cortical plugs from each treatment and replicate combination were placed in bags as a composite sample, and dried in a freeze dryer (Labconoco, Kansas City, MO). After the samples were dried for 72 h, they were ground with an electric coffee grinder until the tissue was macerated to <20 mesh. To inhibit rehydration of the samples, they were individually bagged and sealed in a glass jar with a desiccant. Samples were sent to the Penn State Agricultural Analytical Services Lab (University Park, PA) for mineral analysis. Mineral
content of the samples was analyzed for P, K, Ca, Mg, Mn, Fe, Cu, B, Al, Zn, Na, and S via automated hot block acid digestion (Huang and Schulte, 1985).

While ‘Buckeye Gala’ is a highly blushed strain of Gala, visual differences between treatments required investigation. Percent of fruit surface with red blush of 20 fruit was calculated via digital image analysis (Winzeler and Schupp, 2011). A spectrophotometer (Konica Minolta model CM2600D) was used to quantify lightness, chroma, and hue on the red and green fruit peel of each sample fruit.

**Statistical analysis** The PC version of SAS (version 9.3; SAS Institute, Cary, NC) was used to carry out all statistical analysis. Linear and quadratic relationships of plot means were conducted via the mixed procedure (PROC MIXED).

**Results**

**Initial impact on reproductive and vegetative tissues.** There was an increase in the number of blossom clusters (spurs) removed as spindle speed (rpm) was increased (Table 2-1). The number of blossoms and leaves on spurs that remained was reduced linearly as spindle speed increased. To account for the removal of spur leaves and spur leaf area of blossom clusters that were removed during treatment, mean cluster data was used as a multiplier to provide an estimate for leaf number and leaf area per limb cross-sectional area. In 2010, the use of this multiplier strengthened the existing negative linear relationship.

**Fruitlet abscission.**

There were two major concomitant waves of fruit drop in all treatments (Figure 2-3). In general, the control treatment had a higher abscission rate than the majority of the mechanical thinning
treatments. The major period of drop in 2010 appears to have reached a peak almost immediately after bloom. Mean fruit size data was not collected during fruit set in 2010. Conversely, in 2011 the main period of drop occurred between 9.1mm and 15.5mm fruit size. This corresponds with the period that immature fruitlets are very sensitive to stress.

The secondary flush of bloom increased as thinning severity increased (Figure 2-4; Table 2-2). Likewise, the number of wooly apple aphid colonies increased as thinning severity increased (Figure 2-5).

**Thinning severity impacts on cropload, yield, fruit weight and fruit size.** Fruit set, cropload, yield, and yield efficiency decreased linearly as thinning severity increased in both years. (Table 2-3). In 2010 fruit weight increased as thinning severity increased. In 2011, there was not a significant relationship between thinning severity and fruit weight. In both years, the most severe thinning treatments resulted in over a 50% reduction in yield.

The mechanical thinning treatments did not create an upward shift in the fruit size distribution when compared to the control (Figure 2-6). Mechanical thinning treatments did not increase the number of large fruit. The only modest gains of fruitlet size are present in the largest size categories. Table 2-4 further illustrates the trends of fruit size and market value. The market value of processing apples (0-6.35cm) and bagged apples (6.35-7.74 cm) decreased as thinning severity increased. In both years, the majority of apples in our experiment were in the size class of bagged fruit. There were no significant relationships between the market value of fruit in large size categories (7.94-8.89 cm). Fruit in the larger size categories (7.94-8.89 cm) made up only a small amount of the market value, as large fruit made up approximately 0.4% of the market value
in 2010 and 13% of the market value in 2011. The total market value decreased as thinning severity increased.

**Effect of mechanical thinning treatments on postharvest quality.** As thinning severity increased, there was a linear increase in fruit firmness (Table 2-5). Additionally, soluble solids and titratable acidity increased as the severity of mechanical thinning treatment increased. The sugar to acid ratio decreased in both years however, a linear relationship was only significant in 2011. Fruit calcium concentration (Ca) decreased as thinning severity increased. This reduction in fruit Ca in mechanical thinning treatment was substantial in both years, as the reduction of Ca concentration ranged from 3% to 30% that of the untreated control in both years depending on treatment. Starch rating was negatively related to increased thinning severity. Internal ethylene concentration was not affected by treatment.

In 2010, apple blush was not affected by severity treatment (Table 2-6). There was a quadratic relationship on percent blush as thinning severity treatments increased in 2011 with a maximum at 240 rpm. Mild and moderate thinning severities had more blush than the other treatments. Similarly, these mild to moderate treatments had less green background color. The spectral analysis indicates that moderate thinning severity treatments can result in fruit with more intense blush characteristics.

Return bloom in 2011 had a positive trend as thinning severity treatments increased; however, this relationship was not significant (Table 2-7). Trunk growth increased as thinning severity increased in both annual and cumulative analysis. When comparing the most severe treatment to the unthinned control, there was a 40% more annual trunk growth in 2010. This trend also occurred in the 2011 trunk analysis (24%) and the cumulative trunk growth analysis (30%)
Discussion

There are four variables that can impact the level of mechanical thinning severity: timing of treatment, spindle speed, string number, and tractor speed. Researchers have tested the timing of mechanical thinning treatment ranging from tight cluster to petal fall (Blanke, 2011; Sinatsch et al. 2010 Stadler et al., 1996; Veal et al., 2011). The Fruit-Tec Darwin string thinner operating manual has separate suggestions for timing of thinning application in North America and Europe. Trials in North America have occurred at pink (Schupp et al., 2008), and at full bloom (Hehnen et al., 2012), although the manufacturer of the string thinner recommends treatment between tight cluster and pre-pink. European recommendations are to use the string thinner ranging from pink (which results in higher yield loss) to full bloom (Blanke, 2011). Thinning later than full bloom may cause damage to the fruit (Stadler et al., 1996).

Use of spindle speed to modify thinning severity has been investigated by several workers (Damerow et al., 2007; Dorigoni et al., 2008; Dorigoni et al., 2010; Hehnen et al., 2012; Kong et al., 2009; Solomakhin and Blanke, 2010). In these trials, increased spindle speed resulted in an increase in thinning severity. In many cases, the researchers have investigated combinations of spindle speed and tractor speed. Our trial isolated one variable that contributes to thinning severity (spindle speed), and kept the other factors constant.

All publications regarding the string thinner have utilized the maximum number of strings in their trials, or do not mention the number of strings used. We selected a string number/pattern that reduced the total possible number of strings by two thirds. A preliminary trial on ‘Cripps Pink’ showed that more strings resulted in an unacceptably high level of thinning severity (Appendix A). Using a faster tractor speed decreases the amount of contact that the
string thinner has with the tree, and reduces the overall thinning severity. Some trials utilized relatively fast tractor speeds ranging from 6-12 km/h (Dorigoni et al., 2008; Dorigoni et al., 2010; Kong et al., 2009; Sinatsch et al., 2010; Solomakhin and Blanke, 2010; Strimmer and Kelderer, 1997; Veal et al., 2011; Weibel et al., 2008), while others have utilized speeds at similar or lower tractor speeds (2.5 km/h – 5km/h) when compared to our study (Damerow et al., 2007; Hehnen et al., 2012; Kong et al., 2009; Schupp et al., 2008; Solomakhin and Blanke, 2010; Stadler et al., 1996; Strimmer and Kelderer, 1997; Veal et al., 2011). Since the terrain in the Mid-Atlantic is quite variable, we were unable to safely operate a tractor at the fast ground speeds recommended in the European literature. Tractor speed and spindle speed recommendations have been developed by the manufacturer of the string thinner. The tractor speeds vary between 6-14 km/h, and the spindle speeds vary from 200 rpm to 320 rpm (Fruit-Tec). Based on the manufacturer recommendations, we should have utilized a spindle speed of \(\approx 228\) rpm at 3km/h on ‘Gala’. This level of severity was within the mid-range of treatments selected in this study.

To the best of our knowledge, cumulative effects of mechanical thinning treatments have not been documented (aside from return bloom). Most studies have been one year evaluations (Damerow et al., 2007; Hehnen et al., 2012; Kong et al., 2009; Schupp et al., 2008; Sinatsch et al., 2010; Solomakhin and Blanke, 2010; Stadler et al., 1996; Strimmer and Kelderer, 1997), and multi-year studies tested the effect of mechanical thinning treatments on multiple cultivars or utilized different plots (Dorigoni et al., 2008; Dorigoni et al., 2010; Veal et al., 2011; Weibel et al., 2008). Since apple is a perennial crop, and mechanical treatments affect the balance of vegetative and reproductive growth, we tested the impact of severity treatments over two
consecutive years. While our results have been relatively consistent, it is clear that there are carry over effects from the previous year’s treatment.

The removal of entire blossom clusters (spurs) has been described as an occasional occurrence, and only typical of the first use within the orchard (Veal et al., 2011). In our study the string thinner did remove more blossom clusters in 2010 when compared to 2011 (Table 2-1). Formal documentation of blossom cluster removal has occurred in other studies (Damerow et al. 2007; Sinatsch et al. 2010). Increased spindle speeds removed a higher amount of blossom clusters, and the most severe treatment (2.5 km/h at 320 rpm), removed 25 blossom clusters per tree (Damerow et al. 2007). Conversely, Sinatsch et al. (2010) reported no differences between severity treatments and the level of spur removal. In our study, the removal of blossom clusters increased linearly as the level of thinning severity increased. In 2010, 10 blossom clusters were removed per limb in the most severe treatment (300 rpm). High levels of thinning severity removed fruiting spurs that were located on two and three year old wood, and encouraged the development of fruit on the periphery of the canopy. Lateral blooms, which often produce inferior fruit, remained after thinning treatment. By removing spurs the string thinner was inadvertently selecting for lateral blossom clusters, the persisting fruit did not have the genetic potential to form large fruit. This may in part explain why increasing the thinning via spindle speed did not improve fruit size (Table 2-3).

Removal of individual blossoms was previously documented (Solomakhin and Blanke, 2010). All severity treatments removed less than 1 blossom from each blossom cluster in the recommend range of treatments (Solomakhin and Blanke, 2010). We had similar results at low spindle speeds: 180-210 rpm (Table 2-1). As spindle speed was increased, the rotating strings had more contact with the canopy, resulting in increased blossom removal. Similarly, Kong et al.
(2009) and Strimmer and Kelderer (1997) observed damage to leaves, bark, and wood as the string thinner passed through the orchard. Shoot and bud damage increased with higher spindle speeds (Solomakhin and Blanke, 2010). Damage to the bark, shoots, and buds was also observed in our trial, but was not formally quantified. However, the increase in wooly apple aphid colonization as thinning severity increased indirectly shows an increase in bark damage (Figure 2-5). When above ground, wooly apple aphids are often observed on wounded bark or in the axils of leaves. Increased populations of wooly apple aphids have been observed after mechanical thinning treatment (Bertschinger et al., 1998). Since there was an increase in wooly apple aphids as thinning severity increased, we can assume that there was an increase in bark damage.

Our study is the first to use excised spurs to determine the impact of thinning severity treatments on spur leaf number and spur leaf area. In 2010, leaf number and leaf area were strongly reduced in a linear fashion (Table 2-1). Minor reductions of spur leaf area occurred at 180 and 210 rpm (~9-15% reduction, respectively). In 2011, leaf area was reduced in a quadratic manner. These lower thinning treatments (180-210rpm) enhanced the leaf area per spur and in 2011. This demonstrated that low-moderate levels of thinning severity had a positive influence on spur quality when compared to severe thinning treatments that damaged spurs, or to the loss of spur quality that resulted from over-cropping of ‘Buckeye Gala’. The impact of mechanical thinning severity on spur leaves has been documented (Kong et al., 2009; Solomakhin and Blanke, 2010). There was a significant increase in leaf injury, as 10-42% of ‘Gala’ leaves and 15-32% of ‘Golden Delicious’ leaves were injured per limb, pending upon the level of thinning severity (Solomakhin and Blanke, 2010). However, leaves were only considered to be injured if more than 1/3 of the lamina was removed. With a similar set of
treatments to Solomakhin and Blanke (2010), Veal (2011) and Damerow et al. (2007) reported that a maximum of 8% of leaves were injured in the most severe treatments. To account for all removal of spur leaf area of thinning severity treatments, we estimated leaf area removal per cm$^2$ of LCSA. In 2010, leaf area/cm$^2$ was reduced by 16 – 74 %, depending upon the level of treatment, with a linear decrease in leaf area/cm$^2$ as thinning severity increased. In 2011, the level of injury to spur leaves not as severe, with the most severe treatment removing 46% of leaf area/cm$^2$. Low spindle speed improved spur quality, as 180 rpm had a 20 % increase in spur leaf area/cm$^2$ when compared to the control. McArtney et al. (1996) observed that trees subjected to hand thinning treatments applied at full bloom had poor spur quality when compared to later hand thinning treatments in the following year. He suggested that blossom thinning stimulated the growth of the primary bourse shoot. In the year after treatment, weaker blossom clusters originated from a secondary bourse shoot that originated from the original spur, while the primary bourse shoot had developed into a vegetative shoot. However, this does not explain the vast difference in spur quality observed in the control treatments between two the years. We applied our treatments at full bloom in both years of our study. Spur leaf development and flower bud development do not necessarily correspond with each other in a given year. The differences in spur quality may be an effect of the temperature and light conditions of each year.

Mechanical thinning has been shown to reduce fruit set, the number of fruit per tree, and cropload of apple when compared to control treatments (Damerow et al., 2007; Dorigoni et al., 2008; Dorigoni et al., 2010; Hehnen et al., 2012; Kong et al., 2009; Schupp et al., 2008; Sinatsch et al., 2010; Solomakhin and Blanke, 2010; Stadler et al., 1996; Strimmer and Kelderer, 1997; Weibel et al., 2008). Cropload declined linearly as thinning severity increased in both years of our study (Table 2-3). In 2010, control trees had a cropload of 13.8 fruit/cm$^2$ of TCSA, which is
considered supraoptimal (Table 2). Spindle speeds of 240-270 rpm provided a range of croploads that fell within the ideal range of a commercial crop for ‘Gala’. In 2011, the unthinned control had a mean crop density of 7.6 fruit/cm² of TCSA. This is nearly an ideal cropload to produce a commercial crop. All thinning treatments in 2011 resulted in over-thinning of the trees. This was a result of a period of carbon stress, due to extended low light conditions that occurred at 16-21 DAFB (Figure 2-3). The peak of fruitlet abscission occurred as mean fruit size was 9.1 – 15.5 mm in diameter. The peak of physiological stress occurs as fruit are near 9-12 mm, since vegetative shoots are competing with fruit at this time, rather than contributing to carbohydrate production (Abbott, 1962; Byers, 1982). While mechanical thinning can provide very predictable rates of blossom removal, environmental conditions ultimately dictate fruit set. The uncertainty of final fruit set is a valid concern for growers considering blossom thinning. In peach blossom thinning trials, the goal is to do partial cropload adjustment with the blossom thinner to reduce the risk of over-thinning (Schupp, et al, 2008). This suggests that the lower spindle speeds we tested may be a valid strategy when thinning high-value apple cultivars.

‘Gala’ was chosen for this study because it is a small-fruited cultivar that might benefit from early thinning. Despite removing a high percentage of reproductive sinks early in the season, mean fruit weight did not increase substantially in 2010 or 2011. While an increase in fruit weight was observed in 2010, the practical implications of fruit weight increase are not apparent (Table 2-3). A modest 28 g increase in fruit weight was the highest margin of increase in any measurement of yield in our two-year trial. Several authors have shown that there are increases in fruit weight or fruit size as thinning severity increases (Damerow et al., 2007; Dorigoni et al., 2008; Dorigoni et al., 2010; Hehnen et al., 2012; Kong et al., 2009; Schupp et al., 2008; Sinatsch et al., 2010; Solomakhin and Blanke, 2010; Veal et al., 2011). However, many of
these authors presented data that included follow-up hand thinning as part of the treatment. Spur leaves are important to fruit growth (Ferree and Palmer, 1982), and injury to spur leaves was shown to slow fruitlet development early in the season (Yuan and Greene, 2002). Since fruit size was not increased in our trial, we propose that the injury of spur leaves impacted the final fruit size of apple. The expected advantage of using a blossom thinner is that intra-fruit competition is reduced early in the season, but this was not demonstrated in this trial. The combination of injury to spur leaves and removal of 2-3 year old spurs offset much of the competitive advantage of blossom thinning treatments, especially at the highest spindle speeds.

Researchers have observed reductions in yield as thinning severity was increased (Dorigoni et al., 2008; Dorigoni et al., 2010; Hehnen et al., 2012; Kong et al., 2009; Schupp et al., 2008; Solomakhin and Blanke, 2010). However, some researchers have shown that there is no significant reduction in yield as thinning severity is increased, and in some cases, there is not a discernable pattern between thinning severity and yields (Damerow et al., 2007; Dorigoni et al., 2008; Sinatsch et al., 2010; Strimmer and Kelderer, 1997; Veal et al., 2011). Our trials illustrated that as thinning severity increases, there is a sharp linear reduction in yield (Table 2-3). A minor reduction in yield may be acceptable if fruit size was increased to an economically stimulating level. There are incentives in the market for growers to produce large ‘Gala’; however, none of the mechanical thinning treatments substantially enhanced fruit size. Using the mechanical thinner at any level of severity reduced the market value of ‘Buckeye Gala’. Stover et al. (2001) reported that decreases in profitability via chemical thinning in a given year are quite typical, due to the reduction in yields. Thinning practices are put into place to increase fruit size, fruit quality, and manage biennial bearing of apple. Since ‘Gala’ is not prone to biennial bearing, the control trees were able to set a commercial crop in both years of our study.
Fruit quality parameters generally benefitted from increased thinning severity (Table 2-5). While increased thinning severity increased fruit firmness in our study, mechanical thinning did not improve fruit firmness in other experiments (Hehnen et al., 2012; Kong et al., 2009; Solomakhin and Blanke, 2010). Since cropload was reduced as thinning severity increased, fruit maturity would be expected to be slightly accelerated. However, lower competition among fruit may have delayed maturity, which resulted in increased firmness. Soluble solids were also increased as thinning severity increased, which confirms the results of Kong et al. (2009) and Solomakhin and Blanke (2010). In 2011, starch ratings and internal ethylene measurements were taken to help confirm the inconsistent relationships observed between firmness and soluble solids in 2010. ‘Gala’ is a low ethylene apple, and there was no relationship between internal ethylene concentration and thinning severity treatments in 2011. Since all treatments had low-moderate croploads in 2011, relationships were not detected. Some cultivars, such as ‘Gala’, do not produce climacteric levels of ethylene production until they are harvested. The “tree effect” may have contributed to the low and inconsistent results that were observed in regard to the internal ethylene analysis (Lin and Walsh, 2007). The fruit were measured within two days of harvest, and according to Larrigaudiere et al. (1997), it takes 4-7 days after harvest to show climacteric levels of ethylene production. As thinning severity increased the result was a firmer, sweeter, more acidic, and starchier (less mature) fruit.

The reduction in calcium content of harvested fruit in both years can be explained by research done by Ferree and Palmer (1982). The reduction of spur leaf tissue has negative impacts on calcium allocation to fruit. If this experiment were conducted on a variety that was more sensitive to calcium deficiencies or in an orchard that does not judiciously apply foliar Ca each year, perhaps the reduction of Ca could have negative consequences on the storage potential.
and quality of the fruit. Alternatively, fruit from trees with low croploads are prone to Ca deficiencies.

Fruit blush was enhanced at 180-210 rpm in 2011 (Table 2-6). Mechanical thinning has been shown to improve blush (Dorogoni, 2010). Conversely, Hehnen et al. (2012) showed thinning severity did not have an influence on the proportion of ‘Buckeye Gala’ fruit in any commercial color grade. This is not surprising, as ‘Buckeye Gala’ is a highly blushed strain. Likewise, mechanical thinning treatments did not improve fruit color of ‘Elstar’ (Strimmer and Kelderer, 1997).

Return bloom was increased by utilizing mechanical thinning treatments in several studies (Dorogoni et al., 2010; Strimmer and Kelderer, 1997; Weibel et al., 2008). Dorogoni et al. (2010) showed improvement in return bloom across 5 different cultivars. Hand thinning treatments at full bloom increase fruit size and maximize return bloom in the following year (McArtney, 1996). In other studies, there were no differences in return bloom between control treatments and mechanically thinned trees in the year after treatment (Damerow et al., 2007; Dorogoni et al., 2008; Hehnen et al., 2012). While there was a positive numerical trend in return bloom as thinning severity increased, there was no relationship between thinning treatment and return bloom (Table 2-7). The high rates of spur removal observed in the previous season may have had a negative influence on return bloom. The reduced number of potential flowering spurs in severe thinning treatments may have offset any benefit of removing blossoms early in the season. Our study utilized an annual bearing variety, which was able to support a full crop in the following year. If a treated variety had a pronounced biennial bearing habit, perhaps thinning severity may have had a positive influence on return bloom.
Summary

The aim of this work has been to determine the effects of a range of thinning severity, and to identify an appropriate spindle speed for use on ‘Gala’ in the Mid-Atlantic Region. The mechanical thinner is an efficient tool at consistently removing flowers at bloom. Severe treatments, 240-300 rpm, resulted in removal of entire spurs and a drastic reduction in spur leaf area. This damage to spur leaves is the most negative consequence of string thinning, as it has a direct influence on fruit size, fruit retention, and fruit calcium. My data indicates that treatments of 180-210 rpm minimized spur leaf injury, and provided the best overall thinning response. Fruit blush and color parameters all improved with spindle speeds of 180-210 rpm. In years of heavy fruit set, the level of thinning achieved at these lower intervals is insufficient. In these years, mechanical thinning treatments should be supplemented with other thinning methods. Hand thinning or chemical thinning treatments in combination with early string thinning applications have shown promise in enhancing fruit size in apple (Damerow et al., 2007; Dorigoni et al., 2008; Dorigoni et al., 2010; Schupp et al., 2008; Sinatsch et al., 2010; Veal et al., 2011). Hand thinning times were reduced as mechanical thinning severity increased (Damerow et al., 2007; Sinatsch et al., 2010). Thus, the string thinner has potential in organic plantings, where thinning options are quite limited. In conventional orchards, combinational treatments of mechanical thinning at bloom followed by a post-bloom treatment of 6-BA+NAA outperformed mechanical thinning treatment alone (Dorogoni, 2010). Combinational treatments have been attempted in Pennsylvania trials, but low fruit set was observed in all treatments, including the control (Schupp, unpublished data). As the list of choices available in chemical thinning programs is likely to be reduced in the future, alternative treatments must be developed. The use
of mechanical thinning treatments in combination with other sustainable thinning treatments will be pursued in future trials.
Literature Cited


Anon. 2009. Review of the existing maximum residue levels (mrls) for ethephon. European Food Safety Journal. 7:1347.


Figure 2-1. The Darwin PT-250 (Fruit-Tec, Deggenhauserertal, Germany)
Figure 2-2. The helical string pattern. A total of 90 strings were attached to the 2.5 m spindle.
Table 2-1. Effect of mechanical string thinning treatments on reproductive and vegetative tissues of 'Buckeye Gala'/M.9 apple trees

<table>
<thead>
<tr>
<th>Thinning treatment (rpm)</th>
<th>Clusters removed/ (per spur)</th>
<th>No. of blossoms per sp ur</th>
<th>No. of leaves per spur</th>
<th>No. of leaves per LCSA</th>
<th>Leaf area (cm²) per spur</th>
<th>Leaf area (cm²) per LCSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>5.3</td>
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<td>200</td>
<td>65.2</td>
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<tr>
<td>180</td>
<td>3.4</td>
<td>4.8</td>
<td>8.6</td>
<td>157</td>
<td>59.3</td>
<td>1122</td>
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<td>210</td>
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<td>4.4</td>
<td>8.2</td>
<td>106</td>
<td>55.2</td>
<td>713</td>
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<td>3.5</td>
<td>7.1</td>
<td>83</td>
<td>36.9</td>
<td>420</td>
</tr>
<tr>
<td>270</td>
<td>9</td>
<td>3.3</td>
<td>7.4</td>
<td>60</td>
<td>41.4</td>
<td>336</td>
</tr>
<tr>
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<td>10.5</td>
<td>2.7</td>
<td>7.3</td>
<td>65</td>
<td>36</td>
<td>317</td>
</tr>
</tbody>
</table>

Significance

Linear: <0.0001, <0.0001, <0.0001, <0.0001, 0.002, <0.0001
Quadratic: 0.0012, 0.001, 0.687, 0.0878, 0.0728, 0.0452

--- 2010 ---

<table>
<thead>
<tr>
<th>Thinning treatment (rpm)</th>
<th>Clusters removed/ (per spur)</th>
<th>No. of blossoms per sp ur</th>
<th>No. of leaves per spur</th>
<th>No. of leaves per LCSA</th>
<th>Leaf area (cm²) per spur</th>
<th>Leaf area (cm²) per LCSA</th>
</tr>
</thead>
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<td>10.2</td>
<td>115</td>
<td>26.9</td>
<td>229</td>
</tr>
<tr>
<td>210</td>
<td>1.2</td>
<td>4.5</td>
<td>9.0</td>
<td>95</td>
<td>22.5</td>
<td>170</td>
</tr>
<tr>
<td>240</td>
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<td>4</td>
<td>8.8</td>
<td>86</td>
<td>19.8</td>
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<td>4.1</td>
<td>8.9</td>
<td>67</td>
<td>19.3</td>
<td>122</td>
</tr>
<tr>
<td>300</td>
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<td>3.3</td>
<td>8.3</td>
<td>50</td>
<td>16.9</td>
<td>103</td>
</tr>
</tbody>
</table>

Significance

Linear: 0.0004, <0.0001, 0.077, 0.1659, 0.24, 0.1184
Quadratic: <0.0001, 0.021, 0.071, 0.0055, 0.0051, 0.0089

1 Means of six observations.
1 LCSA=Limb cross-sectional area (cm²)
* Means of 80 harvested spurs per treatment (n=6).
* These values were attained by multiplying leaf no. or leaf area per spur by the number of clusters remaining per LCSA. Leaf area and leaf no. per spur did not account for the reduction in leaf area and leaf no. from the blossom clusters that were removed entirely.
Figure 2-3. Effects of mechanical thinning on fruitlet abscission per LCSA in 2010 and 2011 (A and B, respectively). Measurements began 8 days after treatment (full bloom) and were conducted until final fruit set. Average fruitlet diameter was determined by measuring 10 randomly selected fruitlets per plot (n=24).
Figure 2-4: Images of an observed secondary flush of bloom. As thinning severity increased, the number of secondary blooms increased. The secondary flush in bloom occurred on resting spurs (A), and at the terminus of bourse shoots (B).
Table 2-2: Effect of six levels of severity treatments on the occurrence of a secondary flush of bloom of 'Buckeye Gala'/M.9 in 2010

<table>
<thead>
<tr>
<th>Thinning treatment (rpm)</th>
<th>No. of blossoms at pink</th>
<th>No. of blossoms at full bloom</th>
<th>No. of blossoms petal fall</th>
<th>Total no. of blossoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>180</td>
<td>0.3</td>
<td>0</td>
<td>1.3</td>
<td>1.5</td>
</tr>
<tr>
<td>210</td>
<td>0</td>
<td>4</td>
<td>7.5</td>
<td>11.5</td>
</tr>
<tr>
<td>240</td>
<td>0.5</td>
<td>5</td>
<td>6.5</td>
<td>12</td>
</tr>
<tr>
<td>270</td>
<td>4.3</td>
<td>13</td>
<td>13.3</td>
<td>30.5</td>
</tr>
<tr>
<td>300</td>
<td>3.3</td>
<td>9.8</td>
<td>15.5</td>
<td>28.5</td>
</tr>
</tbody>
</table>

Significance
Linear  0.0388  0.0066  0.0208  0.0064
Quadratic  0.0405  0.0544  0.1564  0.0512

*Number of blossoms at different phenological stages on 5/14/2010.

Blossoms counts were conducted on pre-selected data limbs on two trees per plot (n=24).
Figure 2-5: The effect of mechanical string thinning treatments on the number of *Eriosoma lanigerum* colonies in 2010. A linear increase in *Eriosoma lanigerum* colonies was observed as thinning severity increased on 16 June 2010 (p-value = 0.0012). The number of colonies was determined in a 4 minute count of a six tree plot (n=24).
Table 2-3. Fruit set, crop density, yield per plot, yield efficiency, and mean fruit weight of six mechanical string thinning treatments on 'Buckeye Gala'/M.9 apple trees.

<table>
<thead>
<tr>
<th>Thinning treatment (rpm)</th>
<th>Fruit set (fruit/LCSA)(^z)</th>
<th>Crop density (fruit/TCSA)(^y)</th>
<th>Yield per tree (kg)(^x)</th>
<th>Yield efficiency (kg/cm(^2))(w)</th>
<th>Fruit weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>11.2</td>
<td>13.8</td>
<td>34.99</td>
<td>1.6</td>
<td>121</td>
</tr>
<tr>
<td>180</td>
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<td>9.6</td>
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<td>1.2</td>
<td>127</td>
</tr>
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<td>128</td>
</tr>
<tr>
<td>240</td>
<td>3.9</td>
<td>7.8</td>
<td>23.72</td>
<td>1.1</td>
<td>134</td>
</tr>
<tr>
<td>270</td>
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<td>7.8</td>
<td>20.61</td>
<td>0.9</td>
<td>137</td>
</tr>
<tr>
<td>300</td>
<td>2.3</td>
<td>4.6</td>
<td>16.33</td>
<td>0.7</td>
<td>149</td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0073</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.0589</td>
<td>0.2423</td>
<td>0.0047</td>
<td>0.0149</td>
<td>0.1251</td>
</tr>
<tr>
<td>2011</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6.7</td>
<td>7.6</td>
<td>26.53</td>
<td>1.2</td>
<td>170</td>
</tr>
<tr>
<td>180</td>
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<td>174</td>
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<tr>
<td>210</td>
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<td>3.5</td>
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<td>0.6</td>
<td>179</td>
</tr>
<tr>
<td>240</td>
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<td>2.6</td>
<td>11.56</td>
<td>0.5</td>
<td>173</td>
</tr>
<tr>
<td>270</td>
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<td>11.39</td>
<td>0.5</td>
<td>177</td>
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<tr>
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<td>Significance</td>
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<td></td>
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<td></td>
</tr>
<tr>
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<td>0.7775</td>
<td>0.1970</td>
<td>0.9533</td>
<td>0.8402</td>
</tr>
</tbody>
</table>

\(^z\)Means of four observations
\(^y\)LCSA= Total number of fruit per cm\(^2\) limb cross-sectional area.
\(^x\)TCSA= Total number of fruit cm\(^2\) trunk cross-sectional area.
\(^w\)Mean yield of a two tree plot (n=24).
\(^v\)Yield efficiency = yield (kg) / cm\(^2\) trunk cross-sectional area.
Figure 2-6. Fruit size distribution of six mechanical thinning treatments in 2010 and 2011 (A and B, respectively). The data are expressed as mean weight (kg) of Buckeye Gala of a two tree plot (n=24, 48 trees total) in nine size classes (g).
Table 2.4. Crop value\(^{2,3}\) of Buckeye 'Gala'/M.9 fruit of six mechanical string thinning treatments\(^{3,4}\)

<table>
<thead>
<tr>
<th>Thinning treatment (rpm)</th>
<th>&lt; 6.35 cm ($328)</th>
<th>6.35 – 7.94 cm ($328)</th>
<th>7.94 – 8.26 cm ($328)</th>
<th>8.26 – 8.89 cm ($328)</th>
<th>Total ($328)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.53</td>
<td>58.61</td>
<td>0.07</td>
<td>0</td>
<td>61.22</td>
</tr>
<tr>
<td>180</td>
<td>2.08</td>
<td>57.93</td>
<td>0.07</td>
<td>0</td>
<td>60.08</td>
</tr>
<tr>
<td>210</td>
<td>1.63</td>
<td>47.49</td>
<td>0.15</td>
<td>0</td>
<td>49.27</td>
</tr>
<tr>
<td>240</td>
<td>1.09</td>
<td>46.62</td>
<td>0.15</td>
<td>0.09</td>
<td>47.95</td>
</tr>
<tr>
<td>270</td>
<td>0.99</td>
<td>38.18</td>
<td>0.29</td>
<td>0.35</td>
<td>39.81</td>
</tr>
<tr>
<td>300</td>
<td>0.35</td>
<td>36.12</td>
<td>0.6</td>
<td>0.09</td>
<td>37.15</td>
</tr>
</tbody>
</table>

Significance

| Linear | 0.001 | 0.0277 | 0.0936 | 0.0661 | 0.0174 |
| Quadratic | 0.0751 | 0.1801 | 0.0641 | 0.2066 | 0.155 |

--- 2011 ---

| 0 | 0.5 | 67.16 | 3.71 | 1.35 | 72.72 |
| 180 | 0.11 | 47.02 | 2.99 | 2.07 | 52.19 |
| 210 | 0.23 | 34.12 | 3.49 | 2.08 | 39.92 |
| 240 | 0.05 | 26.51 | 3.99 | 2.88 | 33.43 |
| 270 | 0.13 | 26.02 | 3.32 | 2.6 | 32.08 |
| 300 | 0.08 | 14.65 | 3.09 | 2.26 | 20.08 |

Significance

| Linear | 0.0082 | <0.0001 | 0.7684 | 0.3245 | <0.0001 |
| Quadratic | 0.4566 | 0.1237 | 0.9917 | 0.8535 | 0.1506 |

\(^{2}\)Estimates of the market value of packed fruit for the Appalachian Region was collected from www.thepacker.com. Juice apple price estimates were given by a local fruit processing plant (Knouse Foods, Biglerville, Pa).

\(^{3}\)Mean U.S. dollar values presented for 5 observations.

\(^{4}\)Data presented in terms of experimental unit (two-tree plot).
Table 2-5. Postharvest fruit quality and maturity indices: firmness, soluble solids, titratable acidity, sugar to acid ratio and Ca concentration of 'Buckeye Gala'/M.9 apples

<table>
<thead>
<tr>
<th>Thinning treatment (rpm)</th>
<th>Fruit firmness (N)</th>
<th>Soluble solids (%)</th>
<th>Titratable Acidity (%)</th>
<th>Sugar to Acid Ratio (ss:ma)</th>
<th>Fruit Ca conc. (mg•L⁻¹)</th>
<th>Starch Rating (1-8)</th>
<th>Internal Ethylene (mg•L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>73.1</td>
<td>14.6</td>
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<td>49:1</td>
<td>339</td>
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<td>—</td>
</tr>
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<td>—</td>
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<tr>
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<td>—</td>
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<td>—</td>
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<tr>
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*Means of 7 observations

Sample size of 20 fruit per plot. All fruit selected for postharvest analysis were 125-175 g.

Percent malic acid was selected since it is the most abundant acid in apple.
Table 2-6. Blush analysis and spectral analysis of the peel of 'Buckeye Gala'/M.9 subjected to six mechanical string thinning treatments

<table>
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<tr>
<th>Thinning Treatment (rpm)</th>
<th>% Blush</th>
<th>L</th>
<th>chroma</th>
<th>hue°</th>
<th>L</th>
<th>chroma</th>
<th>hue°</th>
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<tr>
<td>0</td>
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<td>40.75</td>
<td>39.23</td>
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<td>35.62</td>
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<td>38.79</td>
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<td>33.77</td>
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<td>0.0354</td>
<td>0.0031</td>
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Table 2-7. Return bloom and annual trunk growth of 'Buckeye Gala'/M.9 subjected to six levels of thinning severity treatments

<table>
<thead>
<tr>
<th>Thinning Treatment (rpm)</th>
<th>Return bloom (Bl/LCSA)</th>
<th>Return bloom (Bl/LCSA)^2</th>
<th>Trunk Growth (cm2)</th>
<th>Trunk Growth (cm2)</th>
<th>Cumulative Trunk Growth</th>
</tr>
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<tbody>
<tr>
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<td>2</td>
<td>4.7</td>
<td>6.6</td>
</tr>
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<td>240</td>
<td>11.3</td>
<td>20.2</td>
<td>2.3</td>
<td>5.7</td>
<td>8</td>
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<tr>
<td>270</td>
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<td>2.4</td>
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<tr>
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<td>12.9</td>
<td>16.7</td>
<td>2.7</td>
<td>5</td>
<td>7.7</td>
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</tbody>
</table>

Significance

- Linear: 0.0778 0.4349 0.0006 0.0168 0.0006
- Quadratic: 0.8039 0.9116 0.0803 0.3067 0.787

^Bl/LCSA=Number of blossom clusters per limb cross-sectional area (cm^2)
Chapter 3. Influence of mechanical string thinning treatments and aminoethoxyvinylglycine (AVG) on ethylene evolution and net photosynthesis of potted apple

Introduction

Mechanical string thinners have been successfully used to thin peach trees (Schupp et al., 2008) and this technology shows promise for adjusting crop load on apple trees. Apple blossoms arise from a mixed bud, with spur leaves present. Unlike peach, subtending leaves are present when string thinning treatments are applied (Schupp et al. 2008). Apple flowers are attached to the spur by a long pedicel, so the velocity of the strings must be fast enough to clip the flowers cleanly. These two factors lead to considerable wounding of leaf tissue, which, in addition to the physical removal of flowers, is thought to explain the effect on fruit set (Dorigoni et al., 2008). Two thinning mechanisms that may be implicated are wound ethylene or reduced photosynthesis from partial leaf removal. The purpose of this study was to obtain more information on the mechanisms of fruit abscission following leaf injury caused by string thinning apple trees.

Wound-Induced Ethylene

Ethylene biosynthesis in higher plants can be regulated by a number of physiological and environmental factors. Ethylene production can be initiated by a stress, such as mechanical wounding. After wounding, the conversion of S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) plays an integral role in controlling the production of stress induced ethylene (Yang and Hoffman, 1984). The key enzyme in this process, 1-aminocyclopropane-1-carboxylate synthase (ACS) is essential to the ethylene pathway. The ACS enzyme can be controlled by commercially available growth regulators.
Aminoethoxyvinylglycine (AVG, commercial name ReTain) is a potent inhibitor of ethylene production (Saltveit, 2005). It inhibits the activity of pyridoxal phosphate requiring enzymes, such as ACS, and prevents conversion of SAM to ACC (Huai et. al, 2001). Induction of wound ethylene is quite rapid when compared to exogenous hormonal induction (20 minutes compared to 2-3 hr respectively) (Saltveit and Dilley, 1978). Studies on Phaseolus vulgaris L. ‘Pinto’ leaf tissue showed most of the ACC is formed 15 minutes after wounding (Konze and Kwiatkowski, 1981). A prophylactic or concurrent application of AVG prevented ethylene induction on wounded leaf tissue (Konze and Kwiatkowski, 1981). In apples, a 250 mg·L⁻¹ whole tree application of AVG resulted in a significant reduction of abscission rates and ethylene production of a fruit and leaf tissue when combined with an NAA treatment (Zhu et al., 2008).

A trial was designed to simulate string thinning injury, but there was no measurable difference in ethylene concentration between hand torn leaf tissue and intact control vegetative tissues of pear and apple (Kong et al., 2009). Aside from the aforementioned study, there has been little documentation of the influence of mechanical thinning on ethylene production from leaf tissue of apple. However, it has been inferred that wound ethylene contributes to fruitlet abscission of mechanically thinned trees (Dorogoni, 2008).

**Effect of partial defoliation on net photosynthesis**

Partial defoliation has been shown to have two effects on net photosynthesis (Pn) of Rosaceous crops: 1) a compensatory increase in Pn, or 2) a reduction in Pn. High rates of defoliation (90%) increased leaf Pn of potted greenhouse apple trees within two days of treatment (Zhou and Quebedeaux, 2003). Conversely, removal of up to 10% of the leaf area did not influence Pn of apple leaves (Hall and Ferree, 1976). Pn of spur leaves was not altered by spur leaf removal (Ferree and Palmer, 1982). Net CO₂ assimilation was decreased with 10% or
greater leaf area removal of cherry 1 day after treatment, however recovery occurred within 4
days after treatment (Layne and Flore, 1992). A compensatory increase in Pn occurred 2-3 weeks
after treatment (Layne and Flore, 1992). The type of injury and level of damage incurred to leaf
tissue can influence Pn. Ferree and Hall (1981) demonstrated that the rate of Pn decreased as the
number of cuts made to leaf tissue increased, though the level of leaf area removed was
negligible. The influence of mechanical thinning treatments on Pn has not been studied.
Mechanical thinning non-selectively removes spur leaf area, and may cause abrasions of the
lamina without removing leaf area. Both of these actions may affect Pn.

**Objectives:**

1) Determine the effect of mechanical thinning treatments on ethylene evolution.

2) Determine the effect of mechanical thinning treatments on Pn of apple.

**Materials and Methods**

Sixty dormant ‘Buckeye Gala’/ M.9 trees were planted in 10.85 L pots with 3 parts
Sunshine #1 mix(Sun Gro, Bellevue, WA) and 1 part perlite; 3:1 (v:v) in the spring of 2011.
Trees were headed approximately 1.2 m above the graft union to stimulate bud break at a height
adaptable to future mechanical thinning treatments. All trees were watered and fertilized as
needed. Most of the one year old trees were precocious, and all blossoms were removed.
Multiple shoots were maintained on each tree to assure that vegetative tissues would be present
for analysis post-treatment. Prior to treatment, trunk circumference was measured at 30 cm
above the graft union. Since trees were relatively uniform in trunk diameter and size, the
treatments were assigned in a completely random design. Temperature (C˚) and
photosynthetically active radiation (PAR, μmol photons/m²/second) was recorded with a data
logger (Hobo Micro Station, Onset Computer Corporation, Bourne, MA) at 10 minute intervals from green tip (14 May 2011) until the experiment was terminated (4 Aug 2011) (Appendix B).

Mechanical thinning treatments were applied at 4.8 km/h forward speed with a helical 90 string pattern, as described in Chapter 2. Since the trees were small and flexible, a support structure was required. A high-tensile steel wire was stretched between two tractors until taut, and the trees were temporarily affixed to the wire for support during all mechanical thinning treatments (Fig 3-1). Approximately 30 minutes prior to mechanical thinning treatment, an application of aminoethoxyvinylglycine (AVG, Retain; Valent Biosciences, Libertyville, IL) at 250 mg·L⁻¹ + 0.0125% Silwet-77 silicone surfactant (Loveland Industries, Loveland, CO) was applied half of the trees. The surfactant does not induce ethylene evolution (Zhu et al., 2000).

Treatments were applied as follows: 1) untreated control, 2) AVG, 3) mechanically thinned at 210 rpm, 4) mechanically thinned at 210 rpm + AVG, 5) mechanically thinned at 270 rpm, and 6) mechanically thinned at 270 rpm + AVG. One set of 30 trees (six treatments with five reps) was utilized for the wound induced ethylene sampling and the 2nd identical set of 30 trees was utilized for Pn measurements. Measurements of trunk growth, shoot growth, leaf area, and dry weights occurred on both sets of trees.

Immediately after treatment, nine leaves per tree were excised and evenly distributed between three 50 mL disposable centrifuge tubes. Petioles were submerged in 2 mL of distilled deionized water. Shoot leaves selected for this study were fully expanded and located near the mid-section of the shoot. If trees received mechanical thinning treatments, then leaves that reflected the level of injury were selected (i.e. leaves were damaged). A rubber septum was placed over the open end of the tube to inhibit gas exchange and permit repeated sampling. After
collection, all of the airtight centrifuge tubes were submerged in a cooler filled with 30 °C water. A digital thermometer was placed in the cooler to monitor temperature. The cooler was sealed with tape to prevent leakage and reduce any chance of temperature fluctuation. The samples were transported to University Park, PA for gas chromatography analysis. Upon arrival, the samples were transferred to an incubator set at 30 °C. The samples were incubated for 6 hours. One mL gas samples were extracted from the headspace of the disposable plastic centrifuge tubes with a disposable plastic syringe at 6 h, 12 h, and 24 h after string thinner treatments. The gas samples were analyzed with a gas chromatograph (Hewlett Packard 6890 Series, Agilent Technologies, Inc., Santa Clara, CA) with a 1/8 in. stainless steel column packed with alumina (Supelco, Bellefonte, PA).

Pn was measured with a portable infrared gas analyzer fitted with an LED light source (1000 μmol m⁻² s⁻¹) (Ciras-1, PP Systems, Amesbury, MA). Three leaves per tree were measured immediately after treatment and every day after treatment for 4 days. Leaves selected for measurements were fully expanded and were located at the medial portion of the shoot. Measurements were taken approximately at the same time each morning. Three shoots per tree were flagged and measured at weekly intervals. Trunk diameters were measured 30 cm above the graft union, and the point of measurement was marked with a wax pencil for consistency. Trunks were measured on the date of treatment, one week after treatment, and two weeks after treatment.

Trees were destructively harvested one and two weeks after treatment to record any differences in the dry weight of plant tissues due to thinning severity treatment. The 30 trees utilized for Pn measurements were harvested one week after treatment (28 July 2011), and trees utilized for ethylene analysis were harvested two weeks after treatment (4 Aug 2011). Plant tissues were separated into three categories: leaf tissue, current season’s shoots, and structural
wood. All leaves were removed, and whole tree leaf area was recorded with a leaf area meter (LI-COR 3100, Lincoln, NE). After leaf area was recorded, the leaves were placed into a drying oven. Samples remained in the drying oven until sample weight was consistent for a period of 3 days. The same drying procedure was carried out on shoots and structural wood. Structural wood was removed at the graft union and dried accordingly.

**Statistical Analysis**

Since some measurements occurred on the same plant tissues over a period of time, the REPEATED statement of SAS’s MIXED procedure was used to analyze ethylene evolution, phothosynthesis, shoot growth, and trunk growth, using time as a treatment factor (Barden and Marini, 1998). Simple effects were calculated for ethylene evolution using SLICE. Main effects and interactions were determined via SAS MIXED for leaf area and dry weights.

**Results**

The three main effects - spindle speed, AVG treatment, and time – were statistically significant ($P \leq 0.05$) for ethylene evolution (P-values for main effects and interactions are not presented). Increased spindle speed resulted in higher rates of ethylene evolution from leaf samples, while AVG treatment reduced ethylene production. As time after treatment increased, the level of ethylene production decreased. After 6 hours of incubation, increased spindle speed resulted in increased ethylene evolution in leaves treated with AVG (50% increase) and leaves that were untreated (64% increase) (Table 3-1). After 12 hours of incubation, increased spindle speed resulted in increased ethylene evolution of leaves that did not receive ethylene application; however, AVG treated leaves at 12 h were unaffected by increased spindle speed. The rate of ethylene production was uniformly low at all levels of thinning severity after 24 hours of
incubation. AVG application reduced ethylene evolution of leaves that were damaged by the mechanical thinner (210 and 270 rpm). There was a negative linear relationship between incubation time and ethylene evolution of leaves subjected to 270 rpm and AVG treatment. Ethylene evolution of leaves subjected to string thinner treatment was not influenced by incubation or AVG treatment.

String thinning at two spindle speeds, with or without AVG treatment, did not influence Pn (Table 3-2). Pn declined as time after treatment increased. Shoot growth and trunk growth increased as time increased (P<0.0001), however spindle speed and AVG treatment had no influence on shoot growth, nor were there any interactions. The AVG X day and rpm X day interactions were significant for trunk growth, in that trees treated with AVG had a slower rate of trunk growth over time when compared to untreated trees, while trees that were not mechanically thinned grew at a faster rate when compared to trees treated with 270 rpm.

Spindle speed reduced leaf area on both dates of analysis (Table 3-3). Two weeks after thinning treatment, trees did not compensate for removed leaf area. AVG treatment had no influence on leaf area, and the interaction of rpm X AVG on leaf area was insignificant. Dry weights of leaves were influenced by spindle speed one week after treatment. However, spindle speed had no influence on leaf dry weight two weeks after treatment. Dry weights of shoots and wood were not influenced by spindle speed, AVG treatment or rpm X AVG. There were no main effects of treatments or their interactions on total above-ground dry weight.

Discussion
Some leaves appeared to be initially unwounded after treatment, but shortly after treatment, brown lesions appeared (Figure 3-2). This was not observed in the field study, and has not been mentioned in the literature. The light conditions of the greenhouse likely promoted thin cuticle development, which likely made the leaves more susceptible to mechanical injury. Kong et al. (2009) used hand tearing of leaf tissue to simulate the injury created by the mechanical thinner and observed no difference in ethylene evolution among treatments. The bruising of leaf tissue that occurs in non-selective mechanical thinning is not adequately simulated by tearing or cutting of leaf tissue.

As spindle speed increased, there was an increase in ethylene evolution. Documentation of an increase in wound induced ethylene as a result of mechanical string thinning treatment had not occurred prior to this study, though it was alluded to by other authors. Cherry leaves wounded with a paper punch peaked in ethylene evolution 4 h after treatment and decreased to negligible levels 24 h after treatment (Layne and Flore, 1992). This is very similar to the results of our study, as the 6 h incubation had the highest level of ethylene evolution across treatments, and generally diminished over time. Layne and Flore (1992) suggested that wounds created by mechanical injury tend to heal quickly, and that by 24 h the wounds had healed. Our results demonstrate that increased spindle speeds result in an increase of wound induced ethylene on shoot leaves of container-grown apple trees. Increased spindle speeds increased leaf injury of field grown apple trees (Kong et al., 2009; Solomakhin and Blanke, 2010). As the level of leaf area removal increased, there was an increase in the ethylene evolution in cherry (Layne and Flore, 1992). AVG is a potent inhibitor of ethylene synthesis. Konze and Kwiatkowski (1981) demonstrated that a prophylactic application of AVG on bean reduced the rate of ethylene evolution to that of the control. We achieved similar results with AVG in our trial. AVG has
been tested as a means of increasing fruit set of apple (Greene, 1981). Practical use of this inhibitor to control unwanted fruit abscission outside of experimental conditions is limited. AVG increased the number of fruit that persist after spring or fall application, but reduced yield and lower fruit weight was observed (Greene, 1981). Exogenous application of ethephon reduced Pn of some plant species (Taylor and Gunderson, 1986), but Pn of apple was not affected by high concentrations of ethephon (up to 4000 ppm) (Dozier and Barden, 1971). This supports our finding that wound ethylene caused by string thinning had no effect on Pn. Use of the AVG treatments effectively reduced ethylene evolution of wounded trees to the rate of the control.

Even though we were able to detect measurable quantities of ethylene in this study, the amount of wound ethylene generated in our study was very low. Many papers that discuss wound ethylene report findings in nL while our results are in pL. Induction of wound ethylene is a very rapid process (28-29 min. in etiolated pea stems) (Saltveit and Dilley, 1978). Perhaps the relatively long incubation periods in our study led to the relatively low concentrations observed in our study, since the rate of ethylene evolution is standardized by h (hours of incubation). However, cherry leaves wounded with a leaf punch produced measurable quantities of ethylene until 24 h after injury (Layne and Flore, 1992).

Plant species, method of defoliation, and level of leaf area removal can influence the timing of compensatory enhancements or depression of Pn. Some plants, such as bean, demonstrate photosynthetic compensation within 2-3 days of defoliation (von Caemmerer and Farquhar, 1984). Low rates of cherry leaf area removal (10%) resulted in a reduction in net CO₂ assimilation one day after treatment. Simulated hail damage led to a reduction in Pn 15 minutes after treatment (Tartachnyk and Blanke, 2002). Hall and Ferree (1976) found that Pn was unaffected by a 10% removal of individual apple leaves, but higher rates of leaf removal resulted
in a reduction of Pn. In a later study Ferree and Hall (1981), found that the amount of cut leaf surface influenced the reduction of Pn, rather than the amount of leaf area removed. Perhaps our treatments did not exceed a threshold of cut leaf surface. We measured Pn for four days after wounding, and our results show that Pn was not influenced by spindle speed. Heat stress in the greenhouse prevented reliable Pn measurements taken shortly after treatment, as temperatures in the greenhouse exceeded 36 °C. Photosynthetic inhibition from stomatal closure occurs in woody temperate species between 35-40 °C (Berry and Bjorkmann, 1980).

Trunk growth and shoot growth was reduced as the level of leaf area removal increased in sour cherry (Layne and Flore, 1992). Their trees were allowed to grow for approximately 10 weeks in the greenhouse setting after treatment. Our experiments were terminated 1-2 weeks after treatment. Flore and Irwin (1983) only observed a reduction in TCSA of apple trees after >20% of the leaf area was removed. Our most severe treatment (270 rpm) removed over 20% of the leaf area, and trunk growth was influenced by this rate of leaf area removal.

We observed conflicting results between whole tree leaf area and the dry weight of leaf tissue. While there was no observed compensation of leaf area, dry weight accumulation demonstrated a level of compensation two weeks following treatment. Total above ground dry weight was not influenced by any of our treatments. The level of leaf area removal may not have had substantive impact on Pn and dry weights of young potted trees. Dry weight accumulation decreased as the level of leaf area removal increased in sour cherry (Layne and Flore, 1992). Compensation was observed in mulberry trees subjected to decapitation, remaining leaves had increased photosynthetic activity, greater chlorophyll content and mesophyll cell enlargement after treatment, and compensation was increased with axillary bud removal (Satoh et al, 1977). In potted apple, removal of 50% of the leaf area only reduced dry weight accumulation by 40%
(Maggs, 1964). This indicates with extremely severe defoliation treatments, a level of photosynthetic compensation occurred.

**Summary**

The purpose of this work has been to two-fold: to determine the influence of mechanical thinning treatments on ethylene efflux and Pn of apple. Increased thinning severity resulted in an increase in ethylene evolution, and AVG treatments were effective in inhibiting ethylene evolution of potted greenhouse trees. Pn was not affected by spindle speed or AVG. Vegetative growth and dry matter accumulation was not affected by spindle speed or AVG. Whole tree photosynthesis was likely influenced in this trial, due to the high levels of leaf area removed in this study and the maintenance of Pn at the level of the untreated control. Future work should be directed at understanding the influence of wound induced ethylene on fruit set of apple in the field.
**Literature Cited**


Figure 3-1: Potted trees were temporarily supported with a high tensile wire stretched between two tractors. Tree clips were used to affix the trees to the wire. All thinning treatments were applied with the PT-250 Darwin string thinner.
Table 3-1. Interaction means showing the effect of spindle speed, AVG treatment and time of sampling on ethylene evolution (pL·g fw leaf tissue\(^{-1}\)·h\(^{-1}\)) of container-grown 'Buckeye Gala'/M.9 apple trees. P-values were obtained with the slice option of SAS.

<table>
<thead>
<tr>
<th>AGV treatment (HAT)</th>
<th>Hours after</th>
<th>Spindle speed (rpm)</th>
<th>Significance (P&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AGV</td>
<td>0</td>
<td>210</td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
<td>3.8</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2.7</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>2.2</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Significance (P&gt;F) H(_{0}): 6=12=24</td>
<td>0.614</td>
<td>0.208</td>
</tr>
<tr>
<td>No</td>
<td>6</td>
<td>5.6</td>
<td>13.8</td>
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<td>12</td>
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<td>8.3</td>
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<tr>
<td></td>
<td>24</td>
<td>3.1</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>Significance (P&gt;F) H(_{0}): 6=12=24</td>
<td>0.308</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>H(_{0}): AVG = No AVG, 6 HAT</td>
<td>0.410</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>H(_{0}): AVG = No AVG, 12 HAT</td>
<td>0.355</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>H(_{0}): AVG = No AVG, 24 HAT</td>
<td>0.697</td>
<td>0.660</td>
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</table>


Table 3-2. Main effects and interactions of spindle speed, AVG treatment, and time on net photosynthesis, shoot length and trunk growth\(^1\)

<table>
<thead>
<tr>
<th>AVG</th>
<th>rpm</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
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</thead>
<tbody>
<tr>
<td>Yes</td>
<td>0</td>
<td>12.95</td>
<td>10.22</td>
<td>8.84</td>
<td>8.54</td>
<td>32.20</td>
<td>35.50</td>
<td>41.10</td>
<td>9.4</td>
<td>10.0</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>210</td>
<td>13.15</td>
<td>10.85</td>
<td>9.92</td>
<td>7.81</td>
<td>33.60</td>
<td>37.90</td>
<td>43.40</td>
<td>9.6</td>
<td>10.1</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>270</td>
<td>12.74</td>
<td>12.12</td>
<td>9.96</td>
<td>8.74</td>
<td>36.50</td>
<td>37.90</td>
<td>44.40</td>
<td>10.1</td>
<td>10.5</td>
<td>10.6</td>
</tr>
<tr>
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<td>11.86</td>
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<td>9.67</td>
<td>35.00</td>
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<td>40.20</td>
<td>8.7</td>
<td>9.9</td>
<td>11.6</td>
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<td>10.89</td>
<td>9.63</td>
<td>8.41</td>
<td>36.00</td>
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<td>12.6</td>
</tr>
<tr>
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<td>270</td>
<td>13.87</td>
<td>10.90</td>
<td>9.85</td>
<td>9.29</td>
<td>34.40</td>
<td>36.30</td>
<td>41.40</td>
<td>9.2</td>
<td>9.7</td>
<td>11.2</td>
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| Significance | rpm       | 0.6010 | 0.7222 | 0.6419 |
|             | AVG       | 0.5028 | 0.8514 | 0.6978 |
|             | day       | <0.0001 | <0.0001 | <0.0001 |
|             | rpm X AVG | 0.4811 | 0.7290 | 0.3706 |
|             | AVG X day | 0.9241 | 0.0816 | 0.0025 |
|             | rpm X day | 0.9937 | 0.1795 | 0.0178 |
|             | AVG X day X rpm | 0.8558 | 0.8523 | 0.9407 |

\(^1\)Treatment means of five replicates
Table 3-3. Main effects and interactions of spindle speed and AVG treatment on leaf area dry weights of above ground tissues of potted ‘Buckeye Gala’/M.9 harvested on two dates.

<table>
<thead>
<tr>
<th>AVG</th>
<th>rpm</th>
<th>Leaf Area (cm²)</th>
<th>Dry weight (g) 28 July</th>
<th>Leaf Area (cm²)</th>
<th>Dry weight (g) 4 Aug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leaves</td>
<td>Shoots</td>
<td>Wood</td>
</tr>
<tr>
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<td>7558</td>
<td>65</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>210</td>
<td>6627</td>
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<td>6207</td>
<td>56</td>
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<td>58</td>
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<td>7702</td>
<td>66</td>
<td>33</td>
<td>57</td>
</tr>
<tr>
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<td>210</td>
<td>7109</td>
<td>62</td>
<td>32</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>270</td>
<td>5828</td>
<td>49</td>
<td>31</td>
<td>56</td>
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</tbody>
</table>

Significance

<table>
<thead>
<tr>
<th>rpm X AVG</th>
<th>rpm</th>
<th>AVG</th>
<th>rpm</th>
<th>AVG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0017</td>
<td>0.0019</td>
<td>0.6549</td>
<td>0.8080</td>
</tr>
<tr>
<td></td>
<td>0.8000</td>
<td>0.4075</td>
<td>0.7315</td>
<td>0.5252</td>
</tr>
<tr>
<td></td>
<td>0.5510</td>
<td>0.4513</td>
<td>0.5163</td>
<td>0.7681</td>
</tr>
</tbody>
</table>

*Treatment means of 5 replicates

*Total dry weights of above ground tissues
Figure 3-2. Injury observed shortly after mechanical thinning treatments. While the string thinner was observed to reduce leaf area, small abrasions were observed on leaf tissue following treatment.
Chapter 4.

Summary

In Chapter 1, our research objectives were stated as follows:

The purpose of this study is to elucidate the mode of action of the string thinner. This study will be conducted to test the following hypotheses:

1) The mode of action of the string thinner is a consequence of removal of blossoms early in the season, and reduction of fruit set corresponds with the level of thinning severity.

2) The mode of action of the string thinner is a reduction in the rate of photosynthesis resulting from reduced spur leaf area of the tree.

3) The mode of action of the string thinner is stress induced ethylene, as a result of the injury of leaf tissue.

Hypothesis 1 was addressed in Chapter 2. The simple action of blossom removal clearly plays a major role in the mode of action of the string thinner. As the level of thinning severity increased, fruit set directly corresponded with the level of thinning severity in both year of this study. We have no doubt that physical blossom removal is a factor in the mode of action of the string thinner. Hypotheses 2 and 3 were addressed in Chapter 3. Pn of shoot leaves had no relationship with mechanical thinning treatments. Wound induced ethylene evolution increased as the level of thinning severity increased, but the amount of ethylene evolved was very minute. Blossoms can be very sensitive to exogenous ethylene, and abscission can occur within hours of exposure. We did not monitor the dynamics of blossom abscission in our experiment or measure ethylene evolution of shoots or blossoms that were damaged by the string thinner. However, it is unlikely that reduced Pn or wound induced ethylene have a major role in the mode of action of
the string thinner. Based on the observations of Chapters 2 and 3, we propose that the high rates of leaf spur leaf removal reduced whole tree photosynthesis and may have contributed to the thinning action of the mechanical string thinner.

Another goal of this research was to determine if mechanical thinning of apple was an effective method of cropload management in the Mid-Atlantic region. In Chapter 2, excessive injury to spur leaves was observed, which had negative consequences on final fruit size. The pending adoption of mechanical thinning of apple as a cropload management practice will hinge on the reduction of spur leaf injury. Our work investigated three of the four factors that are known to influence the level of thinning severity of mechanical thinning treatments. Investigation of spindle speed was a major component of Chapter 2, string number was discussed in Appendix A, and the influence of tractor speeds on thinning severity were also evaluated (data not presented). Timing of thinning application was not investigated in this project. Our treatments were applied at full bloom in both years of this study. Other workers in Europe have investigated the effect of timing on mechanical thinning efficacy of apple, and application in the range of pink to full bloom has been accepted as the heuristic for time of treatment. We observed substantially higher rates of injury to leaves than reported in other studies that occurred in Europe. While our treatments were applied at the same phenological stage as many other studies, our treatments imposed excessive damage to the tree. The cool maritime climate of Germany is quite different than that of Biglerville, PA. Spur leaf development is not in sync with that of the reproductive tissues. The timing of thinning application may have to be reconsidered to reduce injury to spur leaves. Rather than basing timing of treatment on the level of phenological development of flower clusters, perhaps timing should be based on the development of spur leaves. Further work is required to determine if altering the time of application of string thinning
treatment will reduce the injury to spur leaves. Other workers are conducting trials to achieve a means of selective mechanical thinning of apple. While these technologies may be the desired goal, non-selective mechanical string thinning should be further investigated and the system should be optimized.

This research focused on the application of a range of mechanical thinning treatments to a small fruited cultivar. The structures of the experiments were selected to determine an effective range of thinning treatments. The bulk of papers regarding mechanical thinning of apple have designed their experiments to separate the means of various mechanical thinning treatments, rather than evaluate relationship of thinning severity on selected response variables. There is little benefit in showing that the spur leaf area removal 270 rpm is different than 210 rpm. Conversely, modeling the relationship of thinning severity permits workers to select spindle speeds based response variables. Simply put, the wrong questions are being asked at the beginning of many mechanical thinning studies. P-values of quadratic and linear relationships were presented in this document, to show the relationship thinning severity on an array of response variables. Regression models were not presented in our study.

An effective range of treatments was identified in Chapter 2. Blossom thinning with a string thinner may be an attractive option for organic growers who have limited options in the realm of cropload management of apple. Combinations of mechanical thinning with hand thinning treatments may be an appropriate option for organic growers. The mechanical thinner has been shown to significantly reduce hand thinning in apple. Combinational treatments of mechanical thinning treatment paired with hand or chemical thinner treatments should be evaluated in the future.
Appendix A

Previous mechanical thinning studies at the Penn State Fruit Research and Extension Center suggested that we would have to use a slow forward speed, due to the local terrain. Slower ground speeds and the use of the Darwin with its full complement of strings resulted in excessive thinning and spur removal on apple (J. R. Schupp, unpublished data). Schupp, et al. (2008) reported effective thinning of ‘GoldRush’ apple with 648 strings and 4.0 km/h forward speed. After 2009, Fruit Tec altered the string design to increase string life and improve penetration of the canopy. Using the new style of strings, 90 strings at 4.0 km/h was reported to provide an appropriate level of thinning in peach (Baugher et al., 2010). To evaluate the effects of spindle speed, we needed to establish a string pattern that would provide an appropriate level of thinning when operating the tractor at the maximum safe forward motion. The objectives of this study were to:

1) To determine the relationship of string number and thinning severity.
2) To select a string number for use in the thinning severity experiment.

Materials and Methods

A preliminary trial was conducted in the spring of 2010 at the Penn State Fruit Research and Extension Center in Biglerville, PA on five-year-old ‘Cripps Pink’/M.9 apple trees at 1.2 x 4.6 m spacing. The trees were trained to a vertical axis system, with a narrow cone canopy architecture with an average tree height of ~4.0 m and canopy width at the lower scaffolds of ~1.5m. The experiment was a randomized complete block design with five replications. The experimental unit was a five tree plot. The first and last trees in each plot were not used for data collection, as they may have been subjected to an irregular thinning treatment. Two data trees
were selected within the center three trees of the five tree plot to ensure consistent treatment and tree uniformity. One tree from the interior three trees of each plot was designated to quantify the removal of reproductive and vegetative tissues. Two to three limbs per data tree were flagged based on the following criteria: the total number of blossom clusters was equal to or greater than 50, and the limbs were not subjected to a heading cut in the previous season. Prior to treatment, the initial number of blossom clusters per flagged limb was recorded, limb circumferences of the selected limbs was measured 3 cm away from the trunk to calculate limb cross-sectional area (LCSA).

All treatments were applied at full bloom with a Darwin PT-250 mechanical string thinner (Fruit-Tec, Deggenhauserertal, Germany). Forward speed and spindle speed were held constant in all treatments (4.8 km/h and 240 rpm, respectively). Treatments were applied as follows: 1) unthinned control, 2) 90 strings, 3) 180 strings, and 4) 270 strings.

Immediately after treatment, blossom clusters were counted and recorded. After treatment, 10 spurs were excised from two year old wood in each plot. These excised spurs were stored in polyethylene bags at 0 C˚ until they were analyzed. The number of flowers, leaves, and leaf area per spur were quantified. When counting the number of flowers per spur, some bourse shoots were terminated with a raceme. In this case, each flower was counted, since they were perfect and had potential to be pollinated. Conversely, if the ovary was visibly injured by the string thinner, it was assumed to be incapable of fertilization and was not counted. A LI-COR 3100 leaf area meter (LI-COR, Lincoln, NE), was used to determine the average leaf area per spur for each treatment. When counting the number of leaves per spur and determining leaf area, floral bracts, petioles, and leaves that were not fully expanded were discarded. These vegetative organs are not considered to be significant contributors to the photosynthetic capacity of the spur.
or the leaf area of the spur. The tree in each plot that were manipulated via spur removal for the
aforementioned leaf analysis were not utilized for any other purpose throughout the experiment.

Fruit set was determined after June drop. A one bushel sample was collected at harvest to
determine mean fruit weight.

Results

<table>
<thead>
<tr>
<th>Thinning treatment (string no.)</th>
<th>Clusters removed per LCSA&lt;sup&gt;y&lt;/sup&gt;</th>
<th>No. of blossoms per spur&lt;sup&gt;x&lt;/sup&gt;</th>
<th>No. of leaves per LCSA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of leaves per spur&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Leaf area per LCSA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Leaf area per spur&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Fruit set (fruit/cm&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>Fruit weight at harvest (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>4.6</td>
<td>8.2</td>
<td>73.2</td>
<td>47.4</td>
<td>420.2</td>
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<td>170.0</td>
</tr>
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<td>18.2</td>
<td>99.2</td>
<td>2.7</td>
<td>186.5</td>
</tr>
</tbody>
</table>

Significance

| Linear                        | <0.0001          | <0.0001         | 0.0005          | 0.0004          | 0.0002          | 0.0003          | 0.0055          | 0.0037          |
| Quadratic                     | 0.1267           | 0.0019          | 0.5604          | 0.7042          | 0.0218          | 0.0741          | 0.3976          | <0.0001         |

<sup>x</sup> Means of six observations.
<sup>y</sup>LCSA=Limb cross-sectional area (cm<sup>2</sup>)
<sup>a</sup>Means of 80 harvested spurs per treatment (n=4).
<sup>b</sup>These values were attained by multiplying leaf no. or leaf area per spur by the number of clusters remaining per LCSA. Leaf area and leaf no. per spur did not account for the reduction in leaf area and leaf no. from the blossom clusters that were removed.

There was an increase in damage of reproductive and vegetative tissues as thinning
severity increased. Fruit set decreased as string number increased, and fruit weight had a positive
quadratic relationship. Using 180 strings resulted in the highest fruit weight, but there was less
than a 4 g difference in mean fruit weight across trees subjected to mechanical thinning.

As string number increased, the level of thinning severity increased. To avoid excessive
injury and over-thinning, we utilized 90 strings in our field study.
Mean daily temperature (C˚) and PAR (µmol photons/m²/second) from a data logger placed in the greenhouse.