Pollen Tube Growth Characteristics of Selected Crabapple Cultivars and Managing Apple (*Malus* x *domestica*) Crop Load and Early Season Diseases with Organic Bloom Thinning Chemicals

Candace N. DeLong

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
In
Horticulture

Gregory M. Peck
Keith S. Yoder
Richard E. Veilleux

January 14, 2016
Winchester, Virginia

Keywords: developmental biology, histology, lime sulfur, Regalia®, apple scab, cedar-apple rust

Pollen Tube Growth Characteristics of Selected Crabapple Cultivars and Managing Apple (*Malus* x *domestica*) Crop Load and Early Season Diseases with Organic Bloom Thinning Chemicals

Candace N. DeLong

ABSTRACT

Reducing apple (*Malus* x *domestica* Borkh.) crop load during bloom is a reliable option for increasing fruit quality and return bloom. In this thesis, multiple approaches to improving bloom thinning practices are discussed. The first project analyzed the pollen tube growth of several crabapple cultivars. Previous research had improved the use of bloom thinning chemicals, by coordinating the application timing with the pollen tube growth between pollination and fertilization. However, pollen tube growth rates have only been measured in a few genotypes. In Chapter 2, the pollen tube growth rates of five crabapple cultivars were measured in the styles of 'Fuji', 'Golden Delicious', and 'Pink Lady' flowers, at four temperatures 12, 18, 24 and 30°C. Complex relationships were found among paternal pollen tube growth, maternal cultivar, and temperature. Chapters 3 and 4 describe projects where organically-approved chemicals, including the biofungicide, Regalia®, were evaluated for their ability to simultaneously reduce crop load and decrease early season disease infection. These chemicals were applied in conventionally managed orchards (Chapter 3), and in an organically-managed 'Honeycrisp' orchard (Chapter 4). The number of chemicals approved for bloom thinning is limited, especially in the Eastern U.S. where lime sulfur and oil applications are not permitted during bloom. These studies indicate Regalia®, applied during bloom, can reduce crop load and provide early season disease control. The research presented in this thesis provides new knowledge that can be incorporated into crop load management practices in both conventional and organic apple orchards

Acknowledgements

I would like to thank my mom, Jackie, for making higher education a priority and for allowing me to believe I could be whatever I wanted to be. Thank you to my brother, Aaron, for adventuring with me on weekends and being a great listener. Thank you to the rest of my family and friends for the encouragement and support of my move away from home.

I would like to thank the Peck lab: Abby Kowalski, Ashley Thompson, Dave Carbaugh, Sierra Athey, and Taylor Mackintosh for helping me complete various tasks from pollinating to digging trees out of the ground. Most importantly, thank you for your companionship—it has meant the world to me.

Thank you to my advisor, Greg Peck, and committee member, Keith Yoder, for allowing me to study here in Winchester, I have learned more than I could imagine. Also thank you to Richard Veilleux for being on my committee.

I would like to thank Leon Combs, for teaching me how to visualize a pollen tube, and for the many hours spent assisting me with problem-solving and fixing broken equipment.

Thank you to the Yoder lab: Al Cochran, Billy Royston, Scott Kilmer, Matt Border, and Jared Repass for cooperating with my slow leaf counting, and answering all of my many questions.

Also, thank you to everyone at the Alson H. Smith, Jr. AREC and Virginia Tech Horticulture Department for allowing me to continue my education, and be a part of your community.

Funding

Funding for this work was provided in part by a U.S. Department of Agriculture-Specialty Crop Block Grant administered by the Virginia Department of Agriculture and Consumer Services, the Virginia Apple Research Program, the Virginia Agricultural Experiment Station, and the Hatch Program of the National Institute of Food and Agriculture, U.S. Department of Agriculture. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA or Virginia Tech and does not imply its approval to the exclusion of other products or vendors that also may be suitable. We also thank the following for their supporting this project: JMS Flower Farms, Marrone Bio Innovations, Miller Chemical & Fertilizer Corp., and Crocker's Fish Oil.

Table of Contents

List of Tables

List of Figures

Chapter 1: Literature Review

Introduction

The humid climate, unpredictable weather, and greater disease and insect pressure has caused growers in the apple (*Malus* x *domestica* Borkh.) producing Mid-Atlantic States of Virginia, Maryland, West Virginia, and Pennsylvania to hesitate before adopting certain management practices that are widely used in the Western U.S. (Byers, 1997; Greene, 2002). Managing crop load by chemically thinning during bloom is one of these practices. Many apple cultivars naturally crop in biennial and sometimes triennial bearing cycles; producing too many apples during the ‘on’ years, and too few apples during the ‘off’ years. Reducing fruit numbers during the ‘on’ year can prevent irregular bearing cycles. Crop load can be managed at multiple points throughout the growing season but the greatest positive impacts on fruit size and return bloom are gained by thinning during bloom (Batjer and Hoffman, 1951). The early removal of excess fruit increases the allocation of resources to the remaining fruit and the initiation of flower buds for the next season (Dennis, 2000).

Research previously completed at Virginia Tech’s Alson H. Smith, Jr. Agricultural Research and Extension Center in Winchester, VA has revealed a close relationship between the successful application of bloom thinning chemicals and the rate of pollen tube growth (Yoder et al., 2009; Yoder et al., 2013). From this research, a pollen tube growth model has been developed that can predict when to apply chemicals by incorporating real-time temperature, the average style length, and the pollen tube growth rate. Currently, this model is being successfully beta-tested in Washington State orchards. The risk of post-bloom frost and the higher phytotoxic risk of using caustic bloom chemicals in humid climates, has kept growers in the Eastern U.S.

from widely adopting bloom thinning practices. Precise crop load management tools are increasingly important, as more Eastern growers are converting from processing to fresh market fruit production.

Organic growers in the Mid-Atlantic region face additional challenges due to the limited number of chemicals labeled for thinning. Lime sulfur applied with oil has become the standard bloom thinning material used in the Western U.S., but the currently available lime sulfur products, specifically, the Miller Chemical and Fertilizer, LLC (Hanover, PA) formulation, are not labeled for bloom thinning or for application with oil in the Eastern U.S. Therefore, growers in the East are left without an organically approved bloom thinning chemical, and must rely on post-bloom chemicals or other methods of reducing crop load. In the past, crop load management may have been a low priority because of the limited number of approved chemicals and the lack of reliable methods. As additional organically approved chemicals are researched, blossom thinning could be used in Eastern organic orchards to improve overall fruit quality and decrease disease pressure.

Organic orchards in the Eastern U.S. are not as numerous as they are in the West due to increased insect and disease management challenges, but local organic apples are still in high demand (Ames, 2001; Hinman, 2011). Research is needed on improving organic management to increase organic production. Specifically, studies evaluating the use of different chemicals, or new management practices and techniques, would increase organic production in the region. One possibility would be to use fungicide applications for combined blossom thinning and disease control; therefore, reducing the total number of pesticide applications needed for profitable apple production.

Both basic and applied aspects of improving apple crop load management are discussed within this thesis. Chapter 2 discusses a project that evaluated the pollen tube growth characteristics of five crabapple cultivars and measured the relationships among pollen tube growth, maternal cultivar, and temperature. The work in Chapters 3 assessed the disease control, crop load reduction, and fruit finish provided by bloom thinning applications of Regalia® and lime sulfur in conventionally managed orchards. Chapter 4 further evaluated bloom thinning and disease protection of the same chemicals, Regalia® and lime sulfur, but in an organically managed 'Honeycrisp' orchard.

Pollination

Successful pollination is the first objective in crop load management. The formation of pollen grains is initiated in male meiotic cells inside microsporocytes located on the anther of the flower. These cells undergo meiosis and two rounds of mitosis, eventually forming two microgametes, or the sperm cells (Hong, 2005). Pollen grains are tricolpate, dehydrated, and contain lipid reserves allowing for germination and pollen tube growth (Jackson, 2003). Pollination occurs when pollen grains are transferred from the male anthers to the female stigma.

Apple cultivars generally are not self-compatible, so pollen must be transported from the anthers of one cultivar to the stigma of a compatible cultivar, usually by an insect pollinator. Even for cultivars that are self-compatible, pollinizers should be present in the orchard to ensure a full crop load. Honey bees (*Apis mellifera*) are the primary pollinators in apple orchards. As bees contact anthers, pollen grains attach to the bee and are taken to other flowers where they adhere to the sticky surface of the stigma. In the course of one day a bee can visit up to 5,000 flowers (Warmund, 2002). Honey bee pollinators are most active at temperatures above 55°F, and are inhibited by rainy or windy weather (Keogh et al., 2010). Wind has little to no significance in apple pollen dispersal (Free, 1964). Once a pollen grain lands on the stigma it is rehydrated by the stigmatic secretions and germination begins (Sedgley, 1990).

Germination, Pollen Tube Growth, and Fertilization

Pollen germination is related to cultivar compatibility and the temperature after pollination (Jackson, 2003). A mature pollen grain consists of two generative nuclei and a tube cell nucleus. These three nuclei are carried down the pollen tube through the style into the ovaries (Dennis, 2003). Under ideal conditions (daytime temperatures ranging from 18 to 24°C) it takes around 48 hours for the pollen tube to grow down the style and into the ovary (Yoder et al., 2009). Pollen tube growth is guided by specialized synergid cells in the female gametophyte that direct the pollen tube to ensure the sperm cells are properly delivered to the ovule (Kessler and Grossniklaus, 2011). A pollen tube grows through the mycropylar end of the ovule before entering and causing apoptosis of a synergid cell. The pollen tube then ruptures inside the ovule releasing the two generative nuclei (Berger et al., 2008). One nucleus unites with the egg cell producing a diploid zygote, the other nucleus joins with two polar nuclei to make a triploid nucleus that divides and forms the liquid endosperm. The zygote resulting from the sperm nuclei joined with the egg cell divides rapidly to form the embryo (Jackson, 2003). This process, found in flowering plants, is referred to as double fertilization because of the two sets of male and female gametes involved (Berger et al., 2008). Once fertilization occurs, the newly formed embryo continues to develop into a seed. Seeds initiate plant growth regulators that cause the hypanthium to expand into the flesh of the apple (Way, 1995).

Compatibility

Compatibility in the Rosaceae family is controlled by a S-RNase based gametophytic self-incompatibility system to prevent self-fertilization and thus inbreeding (De Nettancourt, 2001; Dondini, 2012; Shulaev et al., 2008). Incompatible pollen tubes are recognized in the style and only the most compatible pollen tubes continue to grow. Often incompatible pollen grains will grow slowly and stop before reaching the ovary (Jackson, 2003; Stösser et al., 1996). A single multi-allelic *S* locus recognizes self- and non-self pollen in apple (Wu et al., 2013). If the pistil and the pollen grain have two matching *S* loci they will be completely incompatible, if they have one matching loci they will be partially compatible, and if they have two different loci they will be fully compatible (Schneider et al., 2005). On a molecular level, an *S*-RNase gene is expressed in the pistils, and the pollen tube expresses an *S*-haplotype specific F-box gene. The protein products of these two genes recognize and inhibit self-pollen tubes and allow compatible pollen tubes to proceed down the style (Hedegus, 2006). Triploid cultivars are more often incompatible because they have a third allele that must be unmatched. Compatibility alleles should be taken into account in the orchard planning process to ensure successful cross-pollination.

Crop Load Management

Ensuring Pollination and Fertilization

Before altering crop loads growers must first ensure adequate pollination. Apple flowers typically have five carpels, with two ovules in each carpel. Theoretically, every ovule should be fertilized leading to an apple with ten seeds, one inside each ovule; however, apples can frequently be found with higher or lower seed numbers (Ramírez and Davenport, 2013). Apples with low seed numbers are often small, misshapen and will likely drop from the tree before they are commercially mature (Way, 1995). Planting multiple pollinizing cultivars in an orchard helps ensure each ovule is fertilized. Often in commercial orchards ornamental crabapples are used as pollinizers for solid blocks of a single fruit-bearing cultivar (Ko et al., 2010). Crabapples flower prolifically, thus providing more pollen than many fruit-bearing cultivars. They also bloom for longer periods of time and some selections and species offer natural disease resistance. Ideally, one pollinizer should be planted for every 20 fruit bearing trees (Peck and Merwin, 2010). Pollinizing cultivars should be carefully selected for bloom periods that overlap with the fruit bearing cultivars in the orchard.

Thinning

Apple producers have been managing crop load by thinning, or removing the excess blossoms or fruitlets, for thousands of years. Thinning improves fruit size and quality, increases tree cold hardiness, prevents breaking of limbs from excessive fruiting, and inhibits irregular bearing cycles (Dennis, 2000). Thinning can be completed at multiple points throughout the growing season. Dormant pruning can improve fruit size, quality, and color along with several other physical aspects of the tree, but the number of fruit per cluster or the space between clusters cannot be improved by pruning alone (Byers, 2003). Mechanical thinning is effective on peaches, but is not consistent enough on apples to be used as a stand-alone thinning method (Dennis, 2000; Kon et al., 2013). Manually removing blossoms or small fruitlets allows growers to select unblemished and healthy fruit, but is extremely costly and not a realistic method. Multiple techniques can be used to reduce fruit set, but the majority of apple thinning is completed with chemicals. Thinning chemicals can be applied from bloom until the fruit reach

25 mm (Schwallier, 1996). The characteristics of each apple cultivar must be considered before applying thinning chemicals. Some cultivars may produce no return bloom after a heavy crop load, while others can produce a substantial crop load regardless of the previous season (Byers, 2003). Apple cultivars with known biennial-bearing cycles may need to be more severely thinned to obtain a greater return bloom in the following year (Harley et al., 1934, 1942).

Post-Bloom Chemical Thinning

Historically, most thinning chemicals have been applied when the fruit reach seven to 12 mm (Williams & Edgerton, 1981). The majority of post-bloom chemical thinners are synthetic plant growth regulators that reduce fruit numbers by creating a carbohydrate stress on the tree from reduced photosynthesis, respiration, and carbohydrate movement into the fruit (Dennis, 2000). Some commonly used post-bloom chemical thinners include: carbaryl (1-naphthyl-N-methylcarbamate), naphthalene acetic acid (NAA), naphthaleneacetamide (NAD), cytokinin (6-benzyladenine), liquid lime sulfur (calcium polysulfide) applied with oils, and the ethylene precursor, ethrel (2-chloroethylphosphonic acid). Temporary decreases in photosynthesis, which creates plant stress, have been reported after applications of lime sulfur, NAA, NAD, and ethrel applied as post-bloom chemicals (McArtney et al., 2006; Untiedt and Blanke, 2001). When thinning chemicals induce stress, the plant increases production of 1-aminocyclopropane-1-carboxylic acid (ACC), which then creates an increase in ethylene, a plant growth regulator associated with abscission and fruit drop (Williams, 1989).

Chemical thinners have the greatest effect when photosynthesis is reduced by cloudy or hot weather (Greene, 2002). Weather conditions before chemical application are also important; when cool, cloudy, and rainy weather occur before chemical application the epicuticular wax on the leaves will be thinner, leading to a greater uptake of the endogenous thinning chemicals (Westwood et al., 1960). Warm, sunny weather before chemical application will make thinning more difficult because of rapid fruit growth and an increased waxy cuticle on leaves (Greene, 2002). Crop load can be reduced successfully using post-bloom thinners; however, many advantages are associated with applying thinners earlier in the growing season.

Bloom Thinning

Bloom thinning is the most effective way to increase fruit size and maximize return bloom (Batjer and Hoffman, 1951). The early application of bloom thinning chemicals allows for carbohydrate reserves to be allocated to fruit that will be maintained until the end of the season, instead of fruitlets that abscise or are removed (Lakso et al., 2001). Apple flower initiation begins during the preceding summer, and heavy crop loads can reduce the number of blossoms initiated, increasing the likelihood of a tree bearing cycle irregularity (Luckwill, 1970). Reducing crop load later in the growing season may improve fruit size and color, but has little effect on return bloom (Harley et al., 1942)

In the past two decades much research has been focused on finding useful chemicals for blossom thinning. Before 1990, Elgetol (sodium dinitro-o-cresylate), the first registered pollinicide, was used widely in the Western U.S. to inhibit fertilization (Byers, 2003). Elgetol was removed from the market in 1989 due to environmental issues and registration costs (Dennis, 2000). Several chemicals have been tested to replace Elgetol, including: ammonium thiosulfate (ATS), Endothall (7, oxabicyclo (2,2,1) heptane-2-3 dicarboxylic acid), Dormex (hydrogen cyanamide), liquid lime sulfur, petroleum, fish, and plant based oils, NAA, Thinex® (pelargonic acid), and Wilthin ® (sulfcarbamide) (Byers, 1997; Fallahi and Fallahi, 2006, Fallahi and Greene, 2010; Fallahi et al., 1997). The majority of chemicals used for bloom thinning are caustic and reduce fruit set by damaging floral organs. Some research has been completed on synthetic hormone sprays applied during bloom, but these chemicals have not been widely adopted (Burkholder and McCown, 1941; Greene, 2002; Jones et al., 1992). Today, lime sulfur applied with oil is the most effective blossom thinning agent in both organic and conventional orchards (Schmidt and Elfving, 2007). Lime sulfur may inhibit pollination and fertilization by reducing pollen tube growth, and by decreasing the rate of photosynthesis (He and Wetzstein, 1994; McArtney et al., 2006).

Weather, individual tree conditions, and specific cultivar ultimately determine the success of bloom thinning chemicals (Dennis, 2000). Historically, the ideal time to apply chemical bloom thinners has been subjective and is often referred to as an ‘art’ instead of a ‘science.’ To prevent fertilization of side blooms, caustic bloom thinners should be applied once the king bloom has been fertilized, but before fertilization of the side blooms (Fallahi and Willemsen, 2002; Greene, 2002; McArtney et al. 2006; Williams and Edgerton, 1981; Yoder et al., 2009). Applications are typically made between 70 and 90% bloom, but the exact time is difficult to determine (Byers, 1997; Fallahi et al., 1997; Williams et al., 1995). Bloom thinning chemicals applied too late are more likely to result in fruit marking or russet, and may have little to no thinning effect because too many flowers have previously been fertilized (Greene, 2002).

Using a Pollen Tube Growth Model to Predict Chemical Application Time

Fertilization of the king bloom can be more accurately predicted when the rate of pollen tube growth is known (Yoder et al., 2013). For more than a decade, researchers at the Alson H. Smith, Jr. AREC in Winchester, VA have been evaluating relationships between the rate of pollen tube growth and the effective use of bloom thinning chemicals. A temperature-based model that can determine when to apply bloom thinning chemicals using real-time orchard temperature, the rate of pollen tube growth, and the length of the style has been developed from this research. A rate of pollen tube growth was established for this model by measuring the pollen tube growth of the crabapple 'Snowdrift' at different temperatures. This model has been successfully tested in Washington State orchards. To use this model the average style length must be known, and the date and time of bloom for the last king flower intended to be retained as part of the crop is needed for starting the model. Hourly temperatures are used to calculate pollen tube growth. With this information the model can predict when to apply chemicals based on the percent of flowers that are estimated to be fertilized. Separate models have been produced to account for differences among commercially important cultivars. This model can account for two factors that have previously resulted in inconsistent blossom thinning results: temperature and cultivar differences (Yoder et al., 2013).

Negative Effects of Lime Sulfur

Bloom thinning applications of lime sulfur and oils are commonly used in both organic and conventional orchards in the Western U.S.; however, this chemical combination is not labeled for application during bloom in the Eastern U.S. (Peck and Merwin, 2010). Several studies in Eastern orchards have shown reduced fruit set, and increased fruit size with bloom applications of lime sulfur and oil (Noordijk and Schupp, 2003; Schupp and Robinson, 2001; Yoder et al., 2013). Despite the bloom thinning success of lime sulfur, many negative side effects result from lime sulfur use in orchards, including fruit russeting, phytotoxic burns on leaf tissue, premature fruit drop, and decreases in beneficial mite populations (Beers et al., 2009;

Holb et al., 2003; Holdsworth, 1972; MacHardy, 1996; Palmiter and Smock, 1945; Stopar, 2004). Higher rates of lime sulfur increase the thinning effect, but also increase damage to fruit and trees (Peck and Merwin, 2010; Yoder et al., 2013). Furthermore, lime sulfur has increased phytotoxic effects when applied during high humidity or temperatures greater than 26.7°C (Holb et al., 2003) Warm temperatures and high humidity are not uncommon during bloom in the Eastern U.S.

Alternative Organic Chemicals

Various alternate chemicals have been evaluated for organic crop load management. In Spain, applications of potassic soap and lime sulfur effectively thinned cider apple trees and increased return bloom (de la Fuente and Fernández-Ceballos, 2008). Myra et al. (2006) evaluated six different pollinicides suitable for organic production in Canada and found lime sulfur, and horticultural-vinegar reliably decreased pollen viability. A calcium-magnesium brine solution (NC99) has shown potential to reduce fruit set and increase fruit size (Schupp and Robinson, 2001). Oil emulsions can reduce fruit set, but applications made during full bloom result in increased fruit russet (Alegre and Alins, 2007; Stopar, 2004). Miller and Tworkoski (2010) found eugenol, an essential oil, to be an effective bloom thinner and though phytotoxic effects were found on plant tissues immediately after application, the damage was not visible after three to four weeks. Mechanical string thinners have shown some potential for use during bloom in apple orchards, but these machines are non-selective, cause damage to spur leaves, and show greater potential as a supplement to chemical thinning (Hehnen, et al., 2012; Kon et al., 2013; Schupp et al., 2008).

Regalia® (MBI-106020; Marrone Bio Innovations, Inc., Davis, CA), a biofungicide made from extracts of Giant Knotweed [*Reynoutria sachalinensis* (F. Schmidt) Nakai syn. *Polygonum sachalinense* F.Schmidt] reduced the number of pollen tubes growing to the base of the style and provided significant thinning when applied during bloom to 'Golden Delicious' trees in Winchester, VA (Yoder et al., 2013). In the same experiment lime sulfur applied with JMS Stylet-Oil or Crocker's Fish Oil resulted in greater crop load reductions, but also increased fruit russet compared to Regalia® treatments. The results found by Yoder et al. (2013) and observations of Regalia® applications damaging flower petals, indicate Regalia® could be an effective bloom thinning chemical for organic production and for orchards in the Eastern U.S.

Using Chemicals for Crop Load Reduction and Disease Control

Both lime sulfur and Regalia® are primarily labeled as fungicides, but research is limited on the disease control provided by bloom thinning applications of these chemicals. Sulfur is one of the first known chemicals used as a fungicide and insecticide, and the use of lime sulfur was first described in 1802 (Secoy and Smith, 1983; Tweedy, 1967). Lime sulfur was discovered as a possible bloom thinner during an evaluation of apple scab protection provided by different fungicide mixtures (Bagenal et al., 1925). Apple scab [*Venturia inaequalis* (Cooke) G. Winter], powdery mildew [*Podosphaera leucotricha* (Ell. & Ev.) E.S. Salmon], and the sooty blotch and fly speck complex [*Phyllachora pomigena* (Schwein.) Sacc. and *Schizothyrium pomi* (Mont. & Fr.) Arx.] can be efficiently controlled by lime sulfur (Peck and Merwin, 2010). Lime sulfur is especially useful in control of apple scab due to its post-infection 'kickback' activity (Hamilton and Keitt, 1928; Jamar and Lateur, 2007; Montag et al., 2005). Though lime sulfur is a useful fungicide, new bio-friendly chemicals are desirable for organic production due to the previously mentioned negative effects of lime sulfur on tree health, especially in warm, humid climates (Holb et al., 2003; MacHardy, 1996; Noordijk and Schupp, 2003; Palmiter and Smock, 1945; Stopar, 2004).

Regalia® is currently registered for use on both ornamental and food crops including tomatoes, peppers, leafy greens, cucurbits, strawberries, citrus and others (Marrone Bio Innovations, 2015a). Regalia® can protect against bacterial and fungal diseases such as powdery mildew, bacterial spot, downy mildew, and botrytis (Marrone Bio Innovations, 2015b). *R. sachalinensis* extracts have previously been formulated into a fungicide called Milsana® that was registered for use in the 1980s and 1990s. The new formulation, Regalia®, is reported to have better efficacy and a broader spectrum (Su et al., 2009; Su et al., 2012). When used as a fungicide, extracts of *R. sachalinensis* provide disease control by increasing the plant's natural defenses. *R. sachalinensis* treatments have resulted in increased amount of phytoalexins in crops; these anti-fungal compounds increased the resistance of wheat and cucumbers to powdery mildew in several studies (Daayf et al., 1997; Daayf et al., 2000; Fofana et al., 2002; McNally et al., 2003). *R. sachalinensis* extracts have also provided adequate control of powdery mildew on tomatoes and begonia (Herger et al., 1988; Konstantinidou-Doltsinis et al., 2006). Other characteristics involved in pathogen resistance have been observed from treatments of *R. sachalinensis*, including increases in proteins that contribute to disease resistance, elevated levels of defense signaling reactive oxygen species, and increases in cell wall thickness (Fofana et al., 2005; Randoux et al., 2006; Schneider and Ullrich, 1994; Su et al., 2012; Véchet et al., 2005; Wurms et al., 1999; Zavareh et al., 2007*)*. Unlike lime sulfur, *R. sachalinensis* extracts have not shown detrimental effects to beneficial mites (Hafez, 1999; Schuld et al., 2002)

Protection from disease requires year-round management from apple growers, especially in regions like the Mid-Atlantic U.S. where frequent rains and high humidity require a consistent coverage of protective sprays. Disease pressure from apple scab, powdery mildew, and cedar apple rust begins early in the growing season. Applications of lime sulfur or Regalia® applied as bloom thinners may be able to simultaneously reduce crop load and provide adequate control of early season diseases leading to a lower number of fungicide sprays being applied during bloom.

Literature Cited

Alegre, S. and G. Alins. 2007. The flower thinning effect of different compounds on organic 'Golden Smoothee®' apple trees. Acta Hort. 737:67-69.

Ames, G. 2001. Considerations in organic apple production. ATTRA's Organic Matters Series. Natl. Center Appropriate Tech.

Bagenal, N.B., W. Goodwin, E.S. Salmon, and W.M. Ware. 1925. Spraying experiments against apple scab. J. Min. Agr. 32:137-150.

Batjer, L.P. and M.B. Hoffman. 1951. Fruit thinning with chemical sprays. U.S. Dept. Agr. Circ. 867, Washington, D. C.

Beers, E.H., L. Martinez-Rocha, R.R. Talley, and J.E. Dunley. 2009. Lethal, sublethal, and behavioral effects of sulfur-containing products in bioassays of three species of orchard mites. J. Econ. Entomol. 102:324-335.

Berger, F., Y. Hamamura, M. Ingouff, and T. Higashiyama. 2008. Double fertilization – caught in the act. Trends Plant Sci. 13:437-443.

Byers, R. E. 1997. Effects of bloom-thinning chemicals on apple fruit set. J. Tree Fruit Product. 2:13-31.

Byers, R.E. 2003. Flower and fruit thinning and vegetative:fruiting balance. In: D.C. Ferree and I.J. Warrington (eds.). Apples: Botany, production and uses. CABI Publishing, Cambridge, MA.

Burkholder, C.L. and M. McCown. 1941. Effects of scoring and of a naphathyl acidic acid and amide spray upon fruit set and of the spray upon pre-harvest fruit drop. Proc. Amer. Soc. Hort. Sci. 38:117-120.

Daayf, F., A. Schmitt, and R.R. Belanger. 1997. Evidence of phytoalexin in cucumber leaves infected with powdery mildew following treatment with leaf extracts of *Reynoutria sachalinensis*. Plant. Physiol. 113:719-727.

Daayf, F., M. Ongena, R. Boulanger, I.E. Hadrami, and R.R. Bélanger. 2000. Induction of phenolic compounds in two cultivars of cucumber by treatment of healthy and powdery mildew-infected plants with extracts of *Reynoutria sachalinensis.* J. Chem. Ecol. 26:1579-1593.

de la Fuente, E.D. and A. Fernández-Ceballos. 2008. Thinning of organic apple production with potassic soap and calcium polysulfide at the north of spain. In: Boos, Markus (Ed.) Ecofruit - 13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing: Proceedings to the Conference from 18 February to 20 February 2008 at Weinsberg/Germany, pp. 319-323.

De Nettancourt, D. 2001. Incompatibility in incongruity in wild and cultivated plants. Springer Verlag, Berlin.

Dennis, F. G. 2000. The history of fruit thinning. Plant Growth Regulat. 31:1-16.

Dennis, F.G. 2003. Flowering, pollination and fruit set and development, p. 153-166. In: D.C. Ferree and I.J. Warrington (Eds.) Apples: Botany, production, and uses. CABI Publishing, Cambridge, MA.

Dondini, L. 2012. Gametophytic incompatibility in pome and stone fruits: Genes controlling S-locus. Acta Hort. 967:67-75.

Fofana, B., D.J. McNally, C. Labbé, R. Boulanger, N. Benhamou, A. Séguin, and R.R. Bélanger. 2002. Milsana-induced resistance in powdery mildew-infected cucumber plants correlates with the induction of chalcone synthase and chalcone isomerase. Physiol. Molec. Plant Pathol. 61:121-132.

Fofana, B., N. Benhamou, D.J. McNally, C. Labbé, A. Séguin, and R.R. Bélanger. 2005. Suppression of induced resistance in cucumber through disruption of the flavonoid pathway. Phytopathology 95:114-123.

Fallahi, E. and B. Fallahi. 2006. Application of blossom thinners for crop load management in apple, peach, and nectarine. Proc. Plant Growth Reg. Soc. Amer. Annu. Mtg. 33:87-90.

Fallahi, E. and D.W. Greene. 2010. The impact of blossom and postbloom thinners on fruit set and fruit quality in apples and stone fruit. Acta Hort. 884:179-187.

Fallahi, E. and K.M. Willemsen. 2002. Blossom thinning of pome and stone fruit. HortScience 37:474-477.

Fallahi, E., M.W. Williams, and W.M. Colt. 1997. Blossom thinning of 'Law Rome Beauty' apple with hydrogen cyanamide and monocarbonate dihydrogen sulfate. J. Tree Fruit Prod. 2:33-44.

Free, J. B. 1964. Comparison of the importance of insect and wind pollination of apple trees. Nature 201:726-727.

Greene, D.W. 2002. Chemicals, timing, and environmental factors involved in thinner efficacy on apples. HortScience 37:477-481.

Hafez, M.B., A. Schmidt, and S.A. Hassan. 1999. The side effects of plant extracts and metabolites of *Reynoutria sachalinensis* (F. Schmidt) Nakai and conventional fungicides on the beneficial organism *Trichogramma cacoecia* Marchal (Hymenoptera, Trichogrammatidae). J. Appl. Entomol. 123:363-368.

Hamilton, J.M. and G.W. Keitt. 1928. Certain sulfur fungicides in control of apple scab. Phytopathol. 18:146-147.

Harley, C. P., J.R. Magness, M.P. Masure, L.A. Fletcher, and E.S. Degman. 1942. Investigations on the cause and control of biennial bearing of apple trees. U.S. Dept. Agr. Tech. Bul. 792.

Harley, C. P., M.P. Masure, and J.R. Magness. 1934. Fruit thinning and biennial bearing in individual main leaders of yellow Newtown apples. Proc. Amer. Soc. Hort. Sci. 32:43-46.

He, Y. and H.W. Wetzstein. 1994. Pollen degeneration and related leaf development from fungicidal sprays applied during microspore development and shoot expansion. J. Hort. Sci. 69:975-983.

Hedegus, A. 2006. Review of the self-incompatibility in apple (*Malus* x *domestica* Borkh., syn.: *Malus pumila* Mill). Intl. J. Hort. Sci. 12:31-36.

Hehnen, D., I. Hanrahan, K. Lewis, J. McFerson, and M. Blanke. 2012. Mechanical flower thinning improves fruit quality of apple and promotes consistent bearing. Sci. Hort. 134:241-244.

Herger, G., F. Klingauf, D. Mangold, E.H. Pommer, and M.Scherer. 1988. Efficacy of extracts of *Reynoutria sachalinensis* (F. Schmidt) Nakai Polygonaceae, against fungal diseases, especially powdery mildews. Nachrichtenblatt des Deutschen PflanzenSchutzdienstes 40:56-60.

Hinman, T. and G. Ames. 2011. Apples: Organic production guide. ATTRA Natl. Sustainable Agr. Info. Serv.

Holb, I.J., P.F. De Jong, and B. Heijne. 2003. Efficacy and phytotoxicity of lime sulfur in organic apple production. Ann. Appl. Biol. 142:225-233.

Holdsworth, R.P. 1972. European red mite and its major predators: Effects of sulfur. J. Econ. Entomol. 65:1098-1099.

Hong, M. 2005. Molecular genetic analyses of microsporogenesis and microgametogenesis in flowering plants. Annu. Rev. Plant Biol. 56:393-434.

Jackson, J.E. 2003. Biology of apples and pears. Cambridge Univ. Press, Cambridge, MA.

Jamar, L. and M. Lateur. 2007. Strategies to reduce copper use in organic apple production. Acta Hort. 737:113-120.

Jones, K.M., S.A. Bound, and M.J. Oakford. 1992. Identifying optimum thinning time of red 'Fuji' apples. J. Hort. Sci. 67:685-694.

Keogh, R.C., A.P.W. Robinson, and I.J. Mullins. 2010. Pollination aware case study: Apple. Publication No. 10/109. p.8.

Kessler, S.A., and U. Grossniklaus. 2011. She's the boss: Signaling in pollen tube reception. Curr. Opin. Plant Biol. 14:622-627.

Ko, K., S.K. Brown, J.L. Norelli, G. Hrazdina, and H.S. Aldwinckle. 2010. In vitro pollen functionality of attacin-transgenic 'Royal Gala' apple plants and apples transformed with 1-aminocyclopropane-1-carboxylic acid synthase (ACS)-antisense vector. Plant Biosystems 144:778-783.

Kon, T.M., J.R. Schupp, H.E. Winzeler, and R.P. Marini. 2013. Influence of mechanical string thinning treatments on vegetative and reproductive tissues, fruit set, yield, and fruit quality of 'Gala' apple. HortScience 48:40-46.

Konstantinidou-Doltsinis, S., E. Markellou, A.M. Kasselaki, M.N. Fanouraki, C.M. Koumaki, A. Schmitt, A. Liopa-Tsakalidis, and N.E. Malathrakis. 2006. Efficacy of Milsana®, a formulated plant extract from *Reynoutria sachalinensis*, against powdery mildew of tomato (*Leveillula taurica*). BioControl 51:375-392.

Lakso, A.N., T.L. Robinson, M.C. Goffinet, and M.D. White. 2001. Apple fruit growth responses to varying thinning methods and timing. Acta Hort. 557:407-412.

Luckwill, L.C. 1970. The control and growth of fruitfulness of apple trees, p. 237-254. In: Luckwill L.C. and Cutting C.V. (eds.). Physiology of tree crops. Academic Press, New York, NY.

MacHardy, W.E. 1996. Apple scab: Biology, epidemiology, and management. American Phytopathological Society Press, St. Paul, MN.

Marrone Bio Innovations. 2015a. Disease and weed control products. 1 Jan 2016. <http://marronebioinnovations.com/biopesticides-3/biopesticides/sample-products/>.

Marrone Bio Innovations. 2015b. Regalia® Biofungicide: An effective, environmentally responsible biofungicide for better crop protection, better crop performance. 1 Jan 2016. <http://marronebioinnovations.com/ag-products/brand/regalia/>.

McArtney, S., J. Palmer, S. Davies, and S. Seymour. 2006. Effects of lime sulfur and fish oil on pollen tube growth, leaf photosynthesis and fruit set in apple. HortScience 41:357-360.

McNally, D., K.V. Wurms, C. Labbé, and R.R. Bélanger. 2003. Synthesis of C-glycosyl flavonoid phytoalexins as a site-specific response to fungal penetration in cucumber. Physiol. Molec. Plant. Pathol. 63:293-303.

Miller, S.S. and T. Tworkoski. 2010. Blossom thinning in apple and peach with an essential oil. HortScience 45:1218-1225.

Montag, J., I. Schreiber, and J. Schönherr. 2005. An in vitro study on the postinfection activity of hydrates lime and lime sulphur against apple scab (*Venturia inaequalis*). J. Phytopathol. 153:485-491.

Myra, M.T., C.G. Embree, S.V. Good-Avila, and V.K. Morton. 2006. Assessment of potential organic pollenicides as apple blossom thinners. Int. J. Fruit Sci. 6:35-52.

Noordijk, H. and J. Schupp, J. 2003. Organic post-bloom apple thinning with fish oil and lime sulfur. HortScience 38:690-691. (abstr.)

Palmiter D.H. and R.M. Smock. 1954. Effect of fungicides on McIntosh apple yield and quality: A five year study under Hudson Valley conditions 1949-1953. New York State Agr. Expt. Sta., Geneva, NY.

Peck, G.M. and I.A. Merwin. 2010. A grower's guide to organic apples. NYS IPM Publication No. 223.

Ramírez, F. and T.L. Davenport. 2013. Apple pollination: A review. Sci. Hort. 162:188-203.

Randoux, B., D. Renard, E. Nowak, J. Sanssené, J. Courtois, R. Durand, and P. Reignault. 2006. Inhibition of *Blumeria graminis* f.sp. *tritici* germination and partial enhancement of wheat defenses by Milsana. Phytopathology 96:1278-1286.

Schmidt, T.R. and R.C. Elfving. 2007. Crop load management of apple via induced plant stress. J. Am. Pomol. Soc. 61:167-169.

Schneider, S. and W.R. Ullrich. 1994. Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. Physiol. Molec. Plant Pathol. 45:291-304.

Schneider, D., R.A. Stern, and M. Goldway. 2005. A comparison between semi- and fully compatible apple pollinators grown under suboptimal pollination conditions. HortScience 40:1280-1282.

Schuld, M., D. White, B. Seddon, A. Ernst, and A. Schmitt. 2002. Tolerance of *Typhlodromus pyri* (Acari, Phytoseiidae) and *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) In: K.H. Kuck. P. Russell, and H. Lyr. (eds), Modern Fungicides and Antifungal Compounds III - 13th International Reinhardsbrunn Symposium, May 14-18, 2001; Verlag Thomas Mann GmbH, Gelsenkirchen pp. 411-417.

Schupp J.R. and T.L. Robinson. 2001. Organic blossom thinning of apple. HortScience 36: 497. (abstr.)

Schupp, J.R., T.A. Baugher, S.S. Miller, R.M. Harsh, and K.M. Lesser. 2008. Mechanical thinning of peach and apple trees reduces labor input and increases fruit size. Hort Tech. 18:660-670.

Schwallier, P. G. 1996. Apple thinning guide. Sparta, MI: Great Lakes Publishing Company.

Secoy, D.M. and A.E. Smith. 1983. Lineage of lime sulfur as an insecticide and fungicide. Bull. Entomol. Soc. Am. 29:18-23.

Sedgley, M. 1990. Flowering of deciduous perennial fruit crops. Hort. Rev. 12:223-264.

Shulaev, V., S.S. Korban, B. Sosinski, A.G. Abbott, H.S. Aldwinckle, K.M. Folta, A. Iezzoni, D. Main, P. Arús, A.M. Dandekar, K. Lewers, S.K. Brown, T.M. Davis,S.E. Gardiner, D. Potter, and R.E. Veilleux. 2008. Multiple models for Rosaceae genomics. Plant Physiol. 147:985-1003.

Stopar, M. 2004. Thinning of flowers/fruitlets in organic apple production. J. Fruit and Ornamental Plant Res. 12:77-82.

Stösser, R., W. Hartmann, and S.F. Anvari. 1996. General aspects of pollination and fertilization of pome and stone fruit. Acta Hort. 423:15-22.

Su, H., C. Morgan, B. Campbell, H. Huang, J. Hernandez, M.E. Koivunen, and P.G. Marrone. 2009. Efficacy of new formulations of Milsana®, conventional and organic Regalia®, in controlling cucumber powdery mildew (*Sphaeotheca fuliginea).* Phytopathol. 44:135-162.

Su, H., R. Blair, T. Johnson, and P. Marrone. 2012. Regalia® bioprotectant in plant disease management. Outlooks Pest Mgt. 23:30-34.

Tweedy, B.G. 1967. Elemental sulfur. In fungicides an advanced treatise 1. Pp. 119-145. (Ed.). D. C. Torgeson. Academic Press, New York, NY.

Untiedt, R. and M. Blanke. 2001. Effects of fruit thinning agents on apple tree canopy photosynthesis and dark respiration. Plant Growth Regulat. 35:1-9.

Věchet, L., J. Martinková, M. Šindelářová, and L. Burketová. 2005. Compounds of natural origin inducing winter wheat resistance to powdery mildew (*Blumeria graminia* f. sp. *tritici*). Ann. Rev. Phypathol. 18:259-288.

Warmund, M. R. 2002. Pollinating Fruit Crops. In U. o. M. Extension (Ed.), (Vol. G6001). <http://extension.missouri.edu/p/G6001>.

Way, R.D. 1995. Pollination and fruit set of fruit crops. Cornell Coop. Ext. Info. Bul. 237.

Westwood, M.N., L.P. Batjer, and H.D. Billingsley. 1960. Effects of environment and chemical additives on absorption of dinitro-o-cresol by apple leaves. Proc. Amer. Soc. Hort. Sci. 76:30-40.

Williams, M.W. 1989. Control of flowering, fruit set, and seed development in apple (*Malus* x *domestica* Borkh.) with chemical growth regulators. Acta Hort. 240:221-228.

Williams, M. W., S.A. Bound, J. Hughes, and S. Tustin. 1995. Endothall: a blossom thinner for apples. Hort Tech. 5:257-259.

Williams, M. W. and L.J. Edgerton. 1981. Fruit thinning of apples and pears with chemicals. Washington, D. C.

Wu, J., C. Gu, M. Awais Khan, J. Wu, Y. Gao, C. Wang, S.S. Korban, and S. Zhang. 2013. Molecular determinants and mechanisms of gametophytic self-incompatibility in fruit trees of Rosaceae. Critical Rev. Plant Sci. 32:53-68.

Wurms, K., C. Labbé, N. Benhamou, and R.R. Bélanger. 1999. Effects of Milsana and benzothiadiazole on the ultrastructure of powdery mildew haustoria on cucumber. Phytopathol. 89:728-736.

Yoder, K. S., G.M. Peck, L.D. Combs, and R.E. Byers. 2013. Using a pollen tube growth model to improve apple bloom thinning for organic production. Acta Hort. 1001:207-214.

Yoder, K., R. Yuan, L. Combs, R. Byers, J. McFerson, and T. Schmidt. 2009. Effects of temperature and the combination of liquid lime sulfur and fish oil on pollen germination, pollen tube growth, and fruit set in apples. HortScience 44:1277-1283.

Zavareh, A.H.J., A.S. Tehrani, and M. Mohammadi. 2007. Effects of leaf extract of *Reynoutria sachalinensis* on the defense responses of host in cucumber-powdery mildew interaction. J. Sci. Tech. Ag. Nat. Res. 11:299-307.

Chapter 2: Pollen Tube Growth Characteristics of Selected Crabapple Cultivars

Abstract

Reducing crop load improves fruit quality, and minimizes biennial bearing habits in apple (*Malus* x *domestica* Borkh.). Chemically reducing the number of viable flowers can be an efficient way to reduce crop load. Most bloom thinning chemicals are caustic, resulting in damage to floral organs, thus preventing fertilization. Although caustic materials have been shown to be effective pollenicides, their precise application timing is somewhat subjective. Understanding apple pollination and fertilization, specifically pollen tube growth rates, can help improve application timing, greatly improving chemical thinning efficacy and reliability. The pollen tube growth rate of the crabapple 'Snowdrift' has been incorporated into a physiological model used to reduce the subjectivity involved in bloom thinning. This model used real-time temperature, average style length, and pollen tube growth rate to predict proper chemical application time. However, the pollen tube growth rates of other pollinizing cultivars have not been evaluated. Under controlled pollination and growth conditions, the pollen tube growth rates of five crabapple cultivars were measured in the styles of three maternal cultivars at temperatures of 12, 18, 24, or 30°C. After 24 h, differences were found in the pollen tube lengths of paternal cultivars on all three maternal cultivars at 18, 24, and 30°C. 'Snowdrift' pollen tubes were often shorter than the pollen tubes of the other evaluated cultivars. After 24 h at 24 and 30°C, 'Indian Summer' and 'Thunderchild' pollen tubes reached the base of the style most frequently, and 'Snowdrift' pollen tubes the least frequently. Our findings reveal complex relationships between paternal and maternal cultivars, as well as the importance of temperature after pollination.

Introduction

Apple (*Malus* x *domestica* Borkh.) growers reduce crop loads, or the number of fruit per tree, to prevent biennial bearing cycles, and improve fruit size. Crop load management, including manually or chemically removing flowers and fruitlets, can be completed at multiple points during the year. Manually thinning fruitlets allows growers to select large, unblemished fruit, but it is not a desirable option for commercial growers because of the time required and expensive labor costs. Caustic chemicals and/or plant growth regulators can reduce fruit numbers anytime from bloom to 25 mm fruitlet size (Schwallier, 1996). Effectiveness of chemicals applied post-bloom is dependent on specific environmental and tree physiological conditions at the time of application (Lakso et al., 2006). Reducing crop load during bloom provides the greatest increases on fruit size and return bloom (Batjer and Hoffman, 1951; Greene, 2002). However, bloom thinning chemical application time is often subjective, and results may be inconsistent due to the stage of floral development, external environmental factors, and cultivar differences (Fallahi and Willemsen, 2002). A greater understanding of apple flower pollination and fertilization is essential for improving crop load management practices.

Apple pollination occurs when pollen grains are transferred from the anthers of one blossom to the stigma of another, often by a pollinator. Although some cultivars are self-fertile, cross-pollination leads to high rates of embryonic fertilization, and helps ensure a full crop load in commercial orchards. Once pollen grains reach the stigmatic surface, they are rehydrated by released secretions (Dennis, 2000; Jackson, 2003). Mediated by proteins, the pollen tubes then grows through the stigma and style, and into the ovaries (Ramírez and Davenport, 2013). Once the pollen tube reaches the ovary it enters through the micropyle and opens, releasing two generative nuclei into the ovule. Fertilization is complete when one generative nucleus has combined with the egg cell, and the second generative nucleus has combined with the embryo sac beginning seed formation (Dennis, 2000). Knowing the amount of time between pollination and fertilization can help growers determine when to apply bloom thinning chemicals. The majority of bloom thinning chemicals are caustic and prevent pollination or fertilization by destroying flower parts and inhibiting pollen germination and pollen tube growth (Yoder et al., 2009). Due to the caustic nature of these chemicals, they must be applied after a desired number of flowers have been fertilized, but before fertilization of all flowers (Fallahi and Willemsen, 2002; Greene, 2002; McArtney et al. 2006; Williams and Edgerton, 1981). Some bloom thinners may have additional modes of action; for example, lime sulfur can suppress photosynthesis creating whole plant stress (McArtney et al., 2006; Schmidt and Elfving, 2007; Schupp, 2006; Whiting and McFerson, 2006). The use of caustic bloom thinners is improved when the number of fertilized flowers can be accurately estimated,, using the length of the style and the rate of pollen tube growth (Yoder et al., 2013). Previous research has indicated temperature and maternal cultivar are among the factors affecting this growth rate (Yoder et al., 2009).

Flowering crabapples are often used as pollinizers in orchards due to longer bloom periods, lower maintenance, and greater pollen production (Fitzgerald, 2005). The pollen tube growth rate of the crabapple *Malus* ‘Snowdrift’ has been used to develop a pollen tube growth model that can predict the number of flowers fertilized by incorporating the average style length and the cumulative pollen tube growth which is calculated using the hourly temperature in the orchard (Yoder et al., 2013). However, research on the pollen tube growth rates of other crabapple cultivars is limited. The pollen tube growth of the crabapple *Malus mandshurica* ‘Manchurian’ has also been analyzed, but it has fallen into disfavor as a valuable pollinizer due to its high susceptibility to canker diseases (Yoder et al., 2009). Preliminary studies evaluating pollen tube growth through ‘Gala’ stigmas suggest differences may exist between crabapple cultivars (Combs, unpublished data). Analyzing the pollen tube growth rates of additional cultivars could be used to improve the application of blossom thinning chemicals, and contribute to the selection of pollinizers for orchards.

The purpose of this study was to compare the pollen tube growth rates of five crabapple cultivars: ‘Evereste’, ‘Indian Summer’, ‘Selkirk’, ‘Snowdrift’, and ‘Thunderchild’. Pollen from each crabapple cultivar was hand-pollinated onto ‘Fuji’, ‘Golden Delicious’ and ‘Pink Lady’ (‘Cripps Pink’) blossoms. The objectives were to (1) discover if pollen tube growth rates are different among crabapple paternal cultivars, (2) determine if growth rates differ in the styles of maternal cultivars, and (3) determine if differences exist at four different temperatures.

Materials and Methods

All trees were grown at the Alson H. Smith, Jr. Agricultural Research and Extension Center in Winchester, VA. Pollen used in this experiment was collected in spring of 2013 and 2014 from mature ‘‘Evereste’, ‘Indian Summer’, ‘Selkirk,’ ‘Snowdrift’, and ‘Thunderchild’ trees. Collected pollen was stored in a freezer at -12°C until use. Pollen from 2013 and 2014 was combined into one vial for each crabapple cultivar to reduce variability. Percent pollen germination was evaluated after 24 h at 21°C on a medium of agarose (10 g/L), sucrose (100 g/L) and boric acid (10 mg/L) using the methods of Yoder et al. (2009) and Williams and Maier (1977).

Mature ‘Pink Lady’/’M.9’, ‘Autumn Rose Fuji’/‘M.9’ and ‘Golden Delicious’/‘M.27’ trees grown in 19-L root bags (Lacebark Inc., Stillwater, OK) were selected as the maternal cultivars. Trees were removed from the orchard in Jan. 2015, placed in 19-L buckets, and kept in cold storage to accumulate sufficient winter chill hours to complete endodormancy. ‘Fuji’ and ‘Pink Lady’ trees were kept at 4.4°C for 400 h, and ‘Golden Delicious’ trees were kept at 4.4°C for 700 h. After chilling hours had been met, trees were moved into a greenhouse (average temperature 24°C) to force bloom.

Flowers for the experiment were selected during the late pink flower stage (when petals still covered the sexual organs). Forty-two flowers from each maternal cultivar were used for each temperature trial; seven flowers for each paternal cultivar and seven emasculated, non-pollinated flowers were used as a control. All side blooms and any extra flowers were removed to prevent self-pollination. Flowers were tagged to identify the pollen source, and a small sterilized artist’s brush was used to apply pollen directly to the stigma. Pollen was applied in a uniform way until pollen grains were visible on the stigma. Immediately after pollination, trees were placed into a Percival Intellus Control System (Percival Scientific, Perry, IA) growth chamber for 24 h with a diurnal light cycle of 12 h light and 12 h dark. For each maternal cultivar the experiment was repeated at four different air temperatures, 12, 18, 24, and 30°C. After 24 h in the growth chamber, flowers were removed from the tree in the order they were pollinated and placed into glass vials filled with a 5% sodium sulfite (Amresco, Solon, OH) solution.

Before histological examination, the vials containing the flowers were placed in boiling water for 20 min to soften tissues. The pistillate portions were then excised from each flower, and rinsed in distilled water. The styles were individually detached from the ovary with tweezers and a scalpel. The styles were stained with a water-soluble fluorescent solution containing 0.01% Aniline Blue dye (MP Biomedicals, LLC, Solon, OH) in 0.067 M K_2HPO_4 for pollen tube visualization then pressed between two microscope slides (Yoder et al., 2013). Slides were viewed with a Nikon Eclipse Ci microscope (Nikon, Tokyo, Japan) and a Nikon Intensilight C-HGFI (Nikon, Tokyo, Japan) fluorescent light source. Data collected for each individual style were similar to Yoder et al. (2009) and Yoder et al. (2013), including pollen germination/tube growth on the stigmatic surface (0 to 100% of visible stigmatic surface covered with pollen), number of pollen tubes penetrating through the base of the stigma, length of the longest pollen tube, style length, and the number of pollen tubes that grew to the base of the style.

The Kruskal-Wallis test of JMP (SAS Inst., Cary, NC) was used to separate pollen tube growth on non-pollinated control flowers from experimental flowers. Differences in paternal cultivar pollen tube growth length were determined using the Mixed Procedure of SAS (SAS Inst., Cary, NC). The fixed effects and interactions of the model included temperature (12, 18, 24, 30°C), maternal cultivar (‘Golden Delicious’, ‘Fuji’, ‘Pink Lady’), paternal cultivar (‘Evereste’, ‘Indian Summer’, ‘Selkirk’, ‘Snowdrift’, ‘Thunderchild’), maternal x paternal, and maternal x paternal x temperature. Due to previous research on the relationship between temperature and pollen tube growth (Yoder et al., 2009), and the highly significant interaction between maternal x paternal x temperature ($P<0.0001$), interactions maternal x temperature and paternal x temperature were left out of the model. A two-way analysis of variance was used to analyze differences between maternal cultivars, paternal cultivars and the maternal x paternal interaction at each temperature.

Differences in the success rate of paternal pollen tubes reaching the base of the style were determined using the Glimmix Procedure of SAS (SAS Inst., Cary, NC). The fixed effects included in the model were temperature (24, 30°C), maternal cultivar (‘Golden Delicious’, ‘Fuji’, ‘Pink Lady’), and paternal cultivar (‘Evereste’, ‘Indian Summer’, ‘Selkirk’, ‘Snowdrift’, ‘Thunderchild’). Interaction terms were not included in the model because they were not significant.

Results

Germination testing before experiment showed that ‘Thunderchild’ pollen had a germination rate of 90%, ‘Selkirk’ and ‘Indian Summer’ pollen had germination rates of 85% and 75%, respectively, and pollen from ‘Snowdrift’ and ‘Evereste’ each had a 65% germination rate (Table 2.1).

Pollen tube growth on non-pollinated control blossoms was significantly less than growth of paternal cultivars, regardless of maternal cultivar or temperature ($P < 0.0001$*) (Table 2.2). Due to this highly significant difference the pollen tube growth of the five paternal cultivars was analyzed without further comparison to control blossoms. No pollen tube growth was found on control blossoms during the 12°C trial. ‘Pink Lady’ control blossom styles had the longest pollen tube growth length at temperatures 18, 24, and 30°C. The average pollen tube growth length found on control blossoms did not exceed one third of the average style length.

All effects and interactions (temperature, maternal, paternal, maternal x paternal, maternal x paternal x temperature) were significant in the mixed model analyzing the average pollen tube growth length (Table 2.3). Two-way ANOVA of data from each individual temperature trial revealed significant maternal x paternal interactions at 18 and 30°C, but not for temperatures 12 and 24°C. An example of pollen tube growth in the style can be seen in Fig. 2.1.

No differences were found in paternal cultivar pollen tube length at 12°C regardless of maternal cultivar (Table 2.4 A and 2.4 B). The maternal x paternal interaction was not significant at 12°C so the mean pollen tube growth length in maternal cultivar styles and of paternal cultivars was analyzed separately (Table 2.3). With all paternal cultivars combined pollen tubes grew significantly further on ‘Pink Lady’ styles, and with the styles of all maternal cultivars combined ‘Selkirk’ and ‘Thunderchild’ pollen tubes grew further than pollen tubes of ‘Evereste’, ‘Indian Summer’, and ‘Snowdrift’ (Fig. 2.2 A and 2.2 B).

The average paternal pollen tube growth length was longer at 18°C compared to 12°C on all maternal cultivars (Table 2.4 C). In ‘Fuji’ styles, ‘Indian Summer’, ‘Selkirk’, and ‘Thunderchild’ pollen tubes grew on average over 1.5 mm further than ‘Evereste’ and ‘Snowdrift’ pollen tubes, and in ‘Golden Delicious’ styles, ‘Selkirk’ pollen tubes grew on average 1.6 mm further than ‘Evereste’ pollen tubes (Table 2.4 A). ‘Thunderchild’ pollen tubes grew significantly further than those of ‘Selkirk’ and ‘Evereste’ in ‘Pink Lady’ styles. ‘Thunderchild’ pollen tubes grew an average of 1.8 mm further in ‘Fuji’ styles than in ‘Golden Delicious’ styles and ‘Selkirk’ pollen tubes grew an average of 1.4 mm further in ‘Fuji’ styles compared to ‘Pink Lady’ styles (Table 2.4 B).

The maternal x paternal interaction was not significant for the 24°C trial indicating the pollen tube growth of paternal cultivars was similar across all three maternal cultivars (Table 2.3). On average, pollen tubes grew over 1 mm further in ‘Golden Delicious’ styles, and ‘Snowdrift’ pollen tube length was over 2 mm shorter than all other paternal cultivars (Fig. 2.2 C and 2.2 D).

In comparison to the 24°C trial, ‘Selkirk’ pollen tubes in ‘Golden Delicious’ styles, and ‘Thunderchild’ pollen tubes in ‘Fuji’ and ‘Pink Lady’ styles grew significantly further during the 30°C trial (Table 2.4 C). In ‘Fuji’ styles, ‘Thunderchild’ pollen tubes grew ≈ 2.5 mm further, and ‘Snowdrift’ pollen tubes grew ≈ 2.7 mm less than all other paternal cultivars (Table 2.4 A). ‘Indian Summer’ and ‘Selkirk’ pollen tubes grew significantly further than ‘Evereste’ and

‘Snowdrift’ pollen tubes in ‘Golden Delicious’ styles. In ‘Pink Lady’ styles the average pollen tube length of ‘Snowdrift’ was at least 2.3 mm less than the pollen tubes of all other paternal cultivars. ‘Snowdrift’ pollen tubes grew further in ‘Golden Delicious’ styles compared to ‘Fuji’ styles, and the average ‘Thunderchild’ pollen tube length was 1.3 mm further in ‘Fuji’ styles than in ‘Pink Lady’ styles (Table 2.3 B).

No pollen tubes reached the base of the style at 12°C. During the 18°C trial, ‘Thunderchild’ pollen tubes grew to the base of three ‘Pink Lady’ styles, and one ‘Fuji’ style, and a ‘Selkirk’ pollen tube grew to the base of a ‘Pink Lady’ style (data not shown). Twenty-nine percent of pollen tubes at 24°C and 44% of pollen tubes at 30°C reached the base of the style (Table 2.5). The maternal x paternal interaction was not significant indicating the percent of paternal pollen tubes reaching the base of the style was similar for paternal cultivar regardless of maternal cultivar or temperature (Table 2.3). Thirty-seven percent of ‘Pink Lady’ styles, 35% of ‘Golden Delicious’ styles, and 19% of ‘Fuji’ styles had pollen tubes growing to the base. ‘Indian Summer’ pollen tubes most frequently grew to the base of the style, a 32% increase over the success rate of ‘Snowdrift’ pollen tubes (Table 2.5).

Discussion

Yoder et al. (2013) noted as much as a threefold difference between the pollen tube growth rates of different apple cultivars. Our research, evaluating pollen from five crabapple cultivars found differences among the average pollen tube length in the styles of three commercially important cultivars, ‘Fuji’, ‘Golden Delicious’, and ‘Pink Lady’.

The three-way interaction term in our model (maternal x paternal x temperature $P<0.0001$*) suggests that paternal cultivar pollen tube growth rates are unique on different maternal cultivars, but this relationship is also altered with increasing temperature. We did not find trends in pollen tube growth that remained consistent throughout all four temperature trials. The changes in trends are most likely related to the optimum pollen tube growth temperature of the paternal cultivars. The pollen tube length of several paternal cultivars increased up to 24°C, but growth did not increase between the 24 and 30°C trials indicating the optimum growth temperature of these cultivars is less than 30°C. In contrast, ‘Thunderchild’ pollen tube growth increased during the 30°C trial on two of the three maternal cultivars indicating it has a higher optimal growth temperature.

Results from the 18 and 30°C trials had significant maternal x paternal interactions when temperature trials were evaluated separately. This interaction indicates paternal pollen tubes did not grow equal lengths in the styles of the three maternal cultivars. At 18°C this interaction is partly a result of the large differences in paternal cultivar pollen tube growth lengths in ‘Fuji’ styles, and much smaller differences in ‘Pink Lady’ and ‘Golden Delicious’ styles. In ‘Fuji’ styles at 18°C, ‘Thunderchild’ pollen tubes grew 2.7 mm further than ‘Snowdrift’ pollen tubes. This trend was also evident at 30°C where ‘Thunderchild’ pollen tubes grew 5.5 mm further than ‘Snowdrift’ pollen tubes in ‘Fuji’ styles. In comparison, the spread between the cultivar with the longest growing pollen tubes and the cultivar with the shortest growing pollen tubes at 30°C was only 3.9 mm in ‘Golden Delicious’ styles and 3.4 mm in ‘Pink Lady’ styles.

Although no overarching trends existed among maternal cultivars, paternal cultivars, and temperature certain pollen tube growth characteristics can be ascertained. At 12°C, ‘Thunderchild’ and ‘Selkirk’ pollen tubes grew further than those of ‘Indian Summer’, ‘Evereste’, and ‘Snowdrift’ (Fig 2.2 B). Most likely these differences were not significant when analyzed with data from all four temperatures combined because of the substantial increases in pollen tube growth at warmer temperatures. The increased growth of ‘Selkirk’ and

‘Thunderchild’ pollen tubes in colder temperatures may be related to the early bloom time and short phenology of these cultivars. During spring of 2015 in Winchester, VA, ‘Selkirk’ and ‘Thunderchild’ were the first of the five paternal cultivars to bloom and first bloom and petal fall occurred within a seven day period (Table 2.1).

The pollen tube growth length of ‘Snowdrift’ was most often significantly less than the other evaluated cultivars. This low rate of pollen tube growth could be related to the low germination rate of ‘Snowdrift’ pollen; however, ‘Evereste’ pollen had the same percent germination before the experiment. Evereste and ‘Snowdrift’ were the only two cultivars evaluated with white flowers (Table 2.1). These two cultivars had the lowest germination rates, the lowest percent of pollen tubes reaching the base of the style, and most often the shortest pollen tube growth length. Variability in the pollen tube growth rate of different colored flowers have been previously reported in the ornamental species, *Mirabilis jalapa,* where pollen tubes of red- and magenta-flowered plants grew further than yellow- and pink-flowered plants (Berardi et al., 2013). ‘Snowdrift’ pollen tube length decreased between the 24°C trial and the 30°C trial an average of 1.3 mm in ‘Fuji’ styles, 0.7 mm in ‘Golden Delicious’ styles, and 1.1 mm in ‘Pink Lady’ styles. Yoder et al. (2009) showed an increase in the pollen tube growth of ‘Snowdrift’ with increasing day/night temperatures up to 24/7°C, but they did not evaluate temperatures as high as 30°C. For the above mentioned reasons, ‘Snowdrift’ may not be the best pollinizer in areas with warmer temperatures during bloom.

Paternal cultivar pollen tubes often grew different lengths in the styles of each maternal cultivar evaluated. These findings may be primarily related to the maternal cultivar, instead of paternal pollen tube growth differences. For example, during the 24°C trial, pollen tubes from all paternal cultivars grew further on ‘Golden Delicious’ styles compared to ‘Fuji’ styles. The same result was found at 30°C, with the exception of ‘Thunderchild’ which grew equal lengths in ‘Golden Delicious’ and ‘Fuji’ styles. These findings are in agreement with previous research suggesting that pollen tube growth is not a consistent rate in the styles of all commercially important cultivars, and specific bloom thinning management strategies are needed for different cultivars (Yoder et al., 2013).

Pollen tube growth found on non-pollinated control blossoms is most likely the result of self-pollination during anther removal. Self-pollen can initiate tube growth but is often halted in the top third of the style by proteins formed when the *S- alleles* of the style and the pollen tubes are recognized as equal (Stösser et al., 1996).

The success rate of pollen tubes reaching the base of the style was not analyzed for temperatures 12 and 18°C due to the low numbers of pollen tubes reaching the base, which was expected based on previous research (Yoder et al., 2009). Although some differences were found in paternal cultivar success rate, these differences do not match up exactly with the mean length of the longest pollen tube data. Our results may indicate certain cultivars are more likely to grow to the base of the style. The mean pollen tube length of ‘Indian Summer’ and ‘Selkirk’ was not significantly different at 24 or 30°C, but ‘Indian Summer’ had a higher percentage of pollen tubes growing to the base of the style. ‘Selkirk’ pollen tubes that grew close to the base are not accounted for in the success rate data. Although 24 h was adequate for evaluating pollen tube growth length, future research should include a longer time period (i.e., 36 h) and several check points to obtain a more thorough representation of pollen tubes growing to the base of the style.

Successful application of blossom thinning chemicals is highly dependent on understanding the rate of pollen tube growth (Yoder et al., 2013). Selecting pollinizers with a known pollen tube growth rate could improve the efficiency of blossom thinning chemicals.

From this research we have revealed pollen tube growth is not equal for all cultivars, and the relationship between pollen tube growth, maternal cultivar and temperature is a complex phenomenon that involves both parents, as well as environmental conditions. This research and continued research on crabapple pollinizers can help determine the ideal application time for bloom thinning chemicals and help growers select pollinizing cultivars for their orchards.

Literature Cited

Batjer, L.P. and M.B. Hoffman. 1951. Fruit thinning with chemical sprays. U.S. Dept. Agr. Circ. 867, Washington, D. C.

Berardi, A.E., F.M. Frey, E.M. Denton, and J.H. Wells. 2013. Betalain color morphs exhibit differential growth, defensive ability, and pollen tube growth rates in *Miribilis jalapa* (Nyctaginaceae). Intl. Jour. Plant Sci. 174:1229-1238.

Dennis, F.G. 2000. The history of fruit thinning. Plant Growth Regul. 31:1-16.

Fallahi, E. and K.M. Willemsen. 2002. Blossom thinning of pome and stone fruit. HortScience 37:474-477.

Fitzgerald, T. 2005. Pollination in fruit trees. Wash. St. Spokane Co. Ext. Bul. C105.

Greene, D.W. 2002. Chemicals, timing, and environmental factors involved in thinner efficacy on apples. HortScience 37:477-481.

Jackson, J.E. 2003. Biology of apples and pears. Cambridge Univ. Press, Cambridge, MA.

Lakso, A.N., T.L. Robinson, and D.W. Greene. 2006. Integration of environment, physiology and fruit abscission via carbon balance modeling-implications for understanding growth regulator responses. Acta Hort. 727:321-326.

McArtney, S., J. Palmer, S. Davies, and S. Seymour. 2006. Effects of lime sulfur and fish oil on pollen tube growth, leaf photosynthesis and fruit set in apple. HortScience 41:357-360.

Ramírez, F. and T.L. Davenport. 2013. Apple pollination: A review. Scientia Hort. 162:188-203.

Schmidt, T.R and R.C. Elfving. 2007. Crop load management of apple via induced plant stress. J. Amer. Pomol. Soc. 61:167-169.

Schupp, J. 2006. Efficacy and physiological effect of oil/lime sulfur combination. Final project report #AH-03-308 to WA Tree Fruit Res. Comm.

Schwallier, P.G. 1996. Apple thinning guide. Sparta, MI: Great Lakes Publishing Company.

Stösser, R., W. Hartmann, and S.F Anvari. 1996. General aspects of pollination and fertilization of pome and stone fruit. Acta Hort. 423:15-22.

Whiting, M. and J. McFerson. 2006. Impact of cultural practices on apple canopy carbon physiology. Continuing project report #AH-04-412 to WA Tree Fruit Res. Comm.

Williams, M.W. and L.J. Edgerton. 1981. Fruit thinning of apples and pears with chemicals. U.S. Dept. Agr. Bul. 289, Washington, DC.

Williams, R.R. and M. Maier. 1977. Pseudocompatibility after self-pollinating of the apple Cox's Orange Pippin. J. Hort. Sci. 52:475-483.

Yoder, K., R. Yuan, L. Combs, R. Byers, J. McFerson, and T. Schmidt. 2009. Effects of temperature and the combination of liquid lime sulfur and fish oil on pollen germination, pollen tube growth, and fruit set in apples. HortScience 44:1277-1283.

Yoder, K.S., G.M. Peck, L.D. Combs, and R.E. Byers. 2013. Using a pollen tube growth model to improve apple bloom thinning for organic production. Acta Hort. 1001:207-214.

Table 2.1 Percent pollen germination, 2015 first bloom and petal fall dates, and the flower color of paternal cultivars, ‘Evereste’, ‘Indian Summer’, ‘Selkirk’, ‘Snowdrift’ and ‘Thunderchild’.

Paternal Cultivar	Percent pollen germination	2015 First bloom	2015 Petal fall	Flower Color
Evereste	65	22 Apr	4 May	White
Indian Summer	75	17 Apr	28 Apr	Pink
Selkirk	85	15 Apr	22 Apr	Pink
Snowdrift	65	22 Apr	4 May	White
Thunderchild	90	17 Apr	24 Apr	Pink

Table 2.2 Average length of the longest pollen tubes (mm) found on non-pollinated ‘Fuji’, ‘Golden Delicious’, and ‘Pink Lady’ blossoms, at temperatures 12, 18, 24, and 30°C and the average style length (mm).

Temp (°C)	Fuji		Golden Delicious		Pink Lady	
----	Pollen tube length^z	Style length	Pollen tube length^z	Style length	Pollen tube length^z	Style length
12	0.00	8.91	0.00	9.56	0.00	9.08
18	0.31	8.58	0.00	9.86	0.89	9.12
24	0.53	10.39	0.29	10.08	1.01	9.06
30	0.18	10.69	0.08	10.65	0.21	9.42

[z] Statistical analysis comparing average pollen tube growth length of paternal cultivars and pollen tubes found on non-pollinated control blossoms determined to be significantly different using a Kruskal-Wallis Test (P < 0.0001*). Length measurements represent 35 styles. Style length presented in this table is not statistically analyzed.

Table 2.3 Significance levels of main effects and interactions included in models used to analyze the mean length of the longest pollen tube and the percent of pollen tubes reaching the base of the style. The significance level of effects and interactions from each temperature trial are also presented. The percent of pollen tubes reaching the base of the style was analyzed with and without interaction terms.

Pollen tube measurement	Temp	Maternal	Paternal	Maternal x paternal	Maternal x paternal x temp
Mean length of longest pollen tube[z]	< 0.0001*	<0.0001*	<0.0001*	0.0194	<0.0001*
Analysis by temperature (°C)					
12	----	0.0002	<0.0001*	0.2122	----
18	----	0.0133*	<0.0001*	0.0010*	----
24	----	<0.0001	<0.0001*	0.2305	----
30	----	<0.0001	<0.0001*	0.0079*	----
Percent of pollen tubes reaching base of style[y]	0.0744	0.0560	0.0155*	0.8562	0.4496
Analysis without interaction	0.0057*	0.0008*	0.0001*	----	----

[z] Analysis completed using SAS Mixed Procedure
[y] Analysis completed using SAS Glimmix Procedure

Table 2.4 Mean paternal cultivar pollen tube growth length, in maternal cultivar styles, at four different temperatures, 12, 18, 24, and 30°C. Each mean represents pollen tube growth measurements in 35 styles. The same mean pollen tube growth length is represented in each table, but arranged so differences in (A) paternal cultivars, ‘Evereste’ (EV), ‘Indian Summer’ (IS), ‘Selkirk’ (SK), ‘Snowdrift’ (SD), ‘Thunderchild’ (TC), (B) maternal cultivars, ‘Fuji’, ‘Golden Delicious’, ‘Pink Lady’ and (C) temperatures can be visualized. Cultivars or temperatures not connected by the black boxes are significantly different. Differences considered significant at the $p \leq 0.05$ level.[z]

2.4 A

Temp (°C)	12			18				24				30				
Maternal	Paternal	Pollen tube length (mm)		Paternal	Pollen tube length (mm)			Paternal	Pollen tube length (mm)			Paternal	Pollen tube length (mm)			
Fuji	TC	1.5	■	TC	5.3	■		IS	6.9	■		TC	9.0	■		
	SK	1.4	■	SK	5.0	■		EV	6.3	■		IS	6.6		■	
	IS	1.2	■	IS	4.5	■		TC	6.2	■		SK	6.4		■	
	EV	1.2	■	EV	3.0		■	SK	6.1	■		EV	6.3		■	
	SD	1.1	■	SD	2.7		■	SD	4.8		■	SD	3.5			■
Golden Delicious	TC	1.8	■	SK	4.3	■		TC	8.6	■		SK	9.2	■		
	SK	1.6	■	TC	3.5	■	■	IS	8.4	■		IS	8.9	■		
	IS	1.2	■	IS	3.5	■	■	SK	7.8	■		TC	8.6	■	■	
	SD	1.1	■	SD	3.3	■	■	EV	7.8	■		EV	7.8		■	
	EV	1.0	■	EV	2.7		■	SD	6.0		■	SD	5.3		■	
Pink Lady	SK	1.8	■	TC	4.6	■		IS	7.5	■		IS	8.4	■		
	TC	1.7	■	IS	3.9	■	■	SK	7.3	■		SK	7.8	■		
	SD	1.6	■	SD	3.6	■	■	EV	6.5	■	■	TC	7.7	■		
	IS	1.6	■	SK	3.5		■	TC	6.1		■	EV	7.4	■		
	EV	1.4	■	EV	3.4		■	SD	6.1		■	SD	5.0		■	

2.4 B

Temp (°C)	12			18				24				30			
Paternal	Maternal	Pollen tube length (mm)		Maternal	Pollen tube length (mm)			Maternal	Pollen tube length (mm)			Maternal	Pollen tube length (mm)		
Evereste	PL	1.3	■	PL	3.4	■		GD	7.8	■		GD	7.8	■	
	FJ	1.2	■	FJ	2.7	■		PL	6.5		■	PL	7.4	■	
	GD	1.0	■	GD	2.9	■		FJ	6.3		■	FJ	6.3		■
Indian Summer	PL	1.6	■	FJ	4.5	■		GD	8.4	■		GD	8.9	■	
	FJ	1.2	■	PL	3.9	■		PL	7.5		■	PL	7.4	■	
	GD	1.2	■	GD	2.7	■		FJ	6.9		■	FJ	6.3		■
Selkirk	PL	1.8	■	FJ	4.9	■		GD	7.8	■		GD	9.2	■	
	GD	1.6	■	GD	4.3	■	■	PL	7.3	■		PL	7.8	■	
	FJ	1.4	■	PL	3.5		■	FJ	6.2		■	FJ	6.4		■
Snowdrift	PL	1.6	■	PL	3.6	■		PL	6.1	■		GD	5.3	■	
	GD	1.1	■	GD	3.3	■		GD	6.0	■		PL	5.0	■	■
	FJ	1.1	■	FJ	2.6	■		FJ	4.8	■		FJ	3.5		■
Thunderchild	GD	1.8	■	FJ	5.3	■		GD	8.6	■		FJ	9.0	■	
	PL	1.7	■	PL	4.6	■	■	PL	6.1		■	GD	8.6	■	■
	FJ	1.5	■	GD	3.5		■	FJ	6.1		■	PL	7.7		■

2.4 C

Maternal	Fuji					Golden Delicious						Pink Lady				
Paternal	Temp °C	Pollen tube length (mm)				Temp °C	Pollen tube length (mm)					Temp °C	Pollen tube length (mm)			
Evereste	12	1.2	■			12	1.0	■				12	1.4	■		
	18	2.9		■		18	2.7		■			18	3.4		■	
	24	6.3			■	24	7.8			■		24	6.5			■
	30	6.3			■	30	7.8			■		30	7.4			■
Indian Summer	12	1.2	■			12	1.2	■				12	1.6	■		
	18	4.5		■		18	3.5		■			18	3.9		■	
	24	6.9			■	24	8.4			■		24	7.5			■
	30	6.6			■	30	8.9			■		30	8.4			■
Selkirk	12	1.4	■			12	1.6	■				12	1.8	■		
	18	4.9		■		18	4.3		■			18	3.5		■	
	24	6.2			■	24	7.8			■		24	7.3			■
	30	6.4			■	30	9.2				■	30	7.8			■
Snowdrift	12	1.1	■			12	1.1	■				12	1.6	■		
	18	2.6		■		18	3.3		■			18	3.6		■	
	24	4.8			■	24	6.0			■		24	6.1			■
	30	3.5			■	30	5.3			■		30	5.0			■
Thunderchild	12	1.5	■			12	1.8	■				12	1.7	■		
	18	5.3		■		18	3.5		■			18	4.6		■	
	24	6.1		■		24	8.6			■		24	6.1			■
	30	9.0			■	30	8.6			■		30	7.7			■

Table 2.5 Percent of maternal cultivar styles with pollen tubes reaching the base, percent of styles with pollen tubes reaching the base at 24 and 30°C, and percent of styles with paternal cultivar pollen tubes reaching the base. Statistical analysis by the Glimmix Procedure of SAS.

Temperature	Percent of styles with pollen tube reaching base
30	44 a
24	29 b
Maternal Cultivar	
Pink Lady	37 a
Golden Delicious	35 a
Fuji	19 b
Paternal Cultivar	
Indian Summer	44 a
Thunderchild	39 ab
Selkirk	36 bc
Evereste	23 cd
Snowdrift	12 d

Fig 2.1 ‘Thunderchild’ pollen tube growth in a ‘Fuji’ style 24 h after pollination at a constant temperature of 24°C, visualized with water-soluble fluorescent solution containing 0.01% Aniline Blue dye in 0.067 M K_2HPO_4 and magnified 10x using a Nikon Eclipse Ci microscope and a Nikon Intensilight C-HGFI.

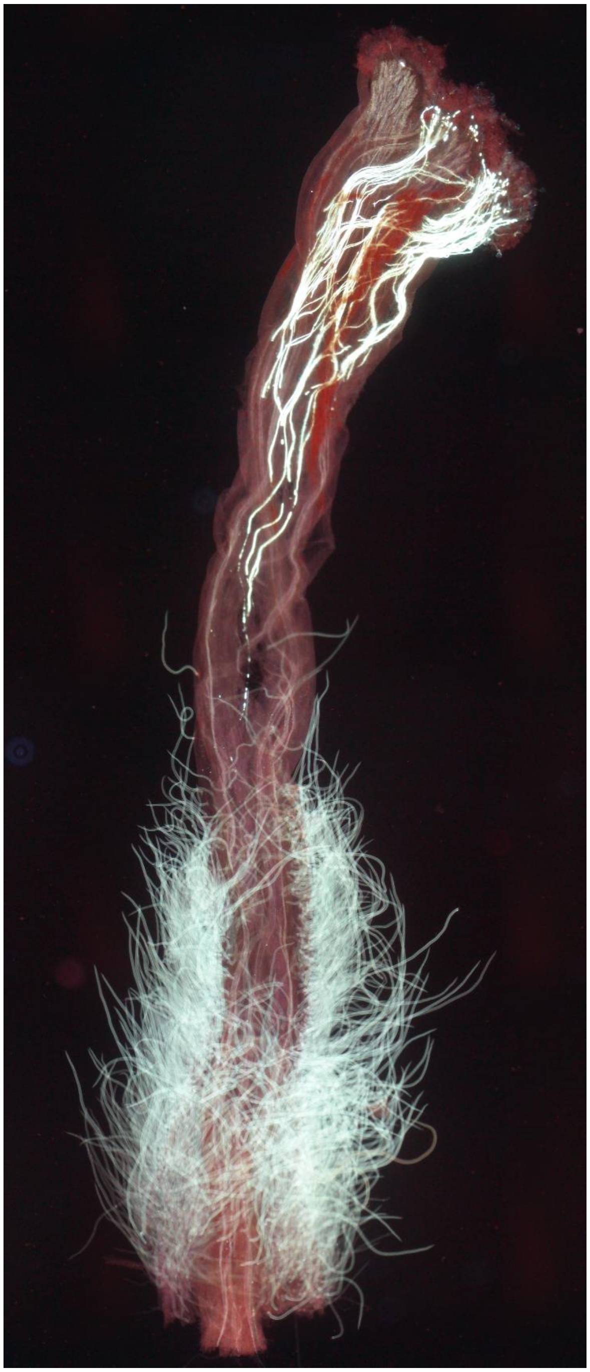
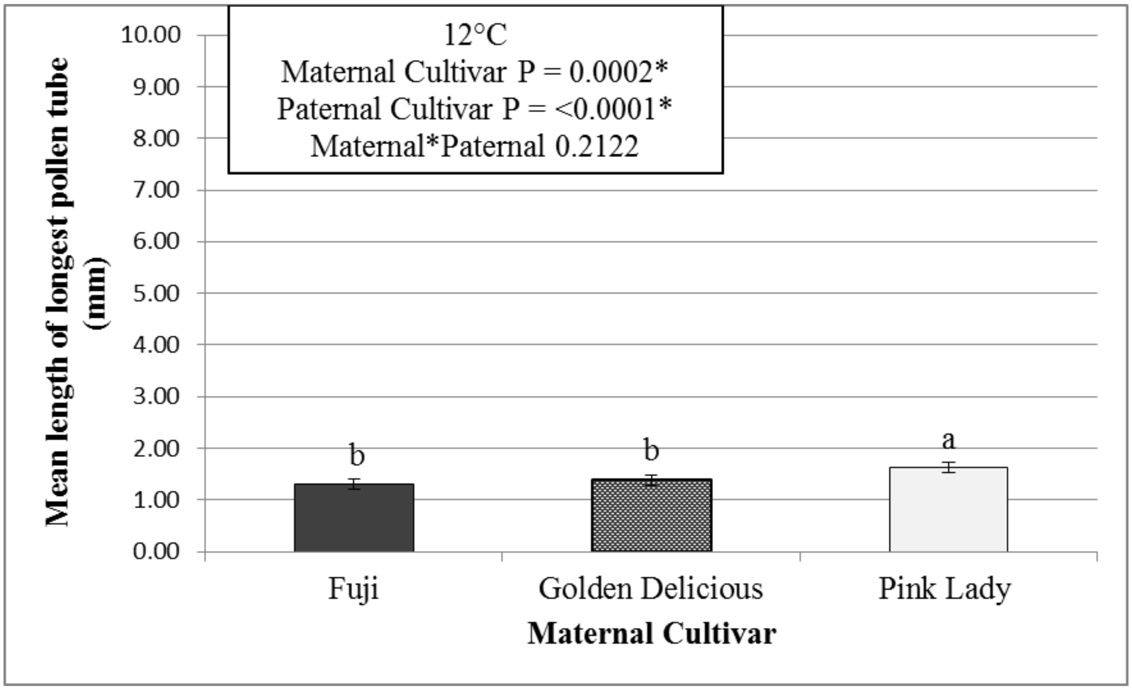
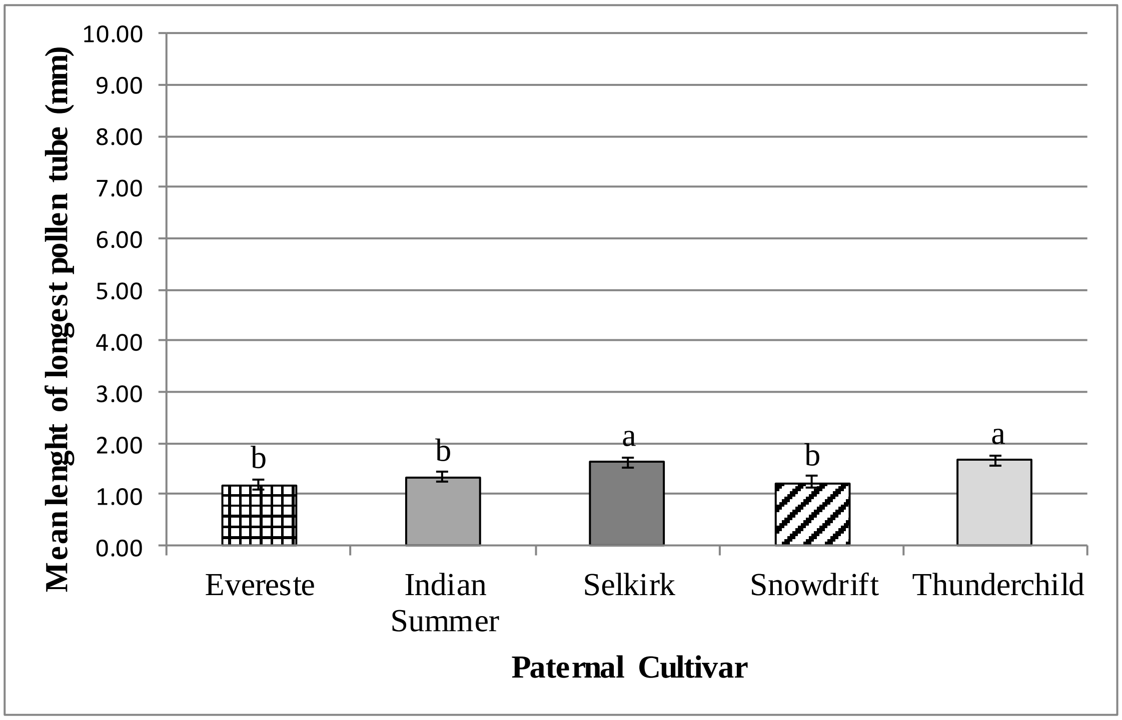


Fig. 2.2 Mean pollen tube growth length in ‘Fuji’, ‘Golden Delicious’, and ‘Pink Lady’ styles at (A) 12 and (C) 24°C , and mean pollen tube growth length of ‘Evereste’, ‘Indian Summer’, ‘Selkirk’, ‘Snowdrift’, and ‘Thunderchild’ at (B) 12°C and (D) 24°C.

2.2 A

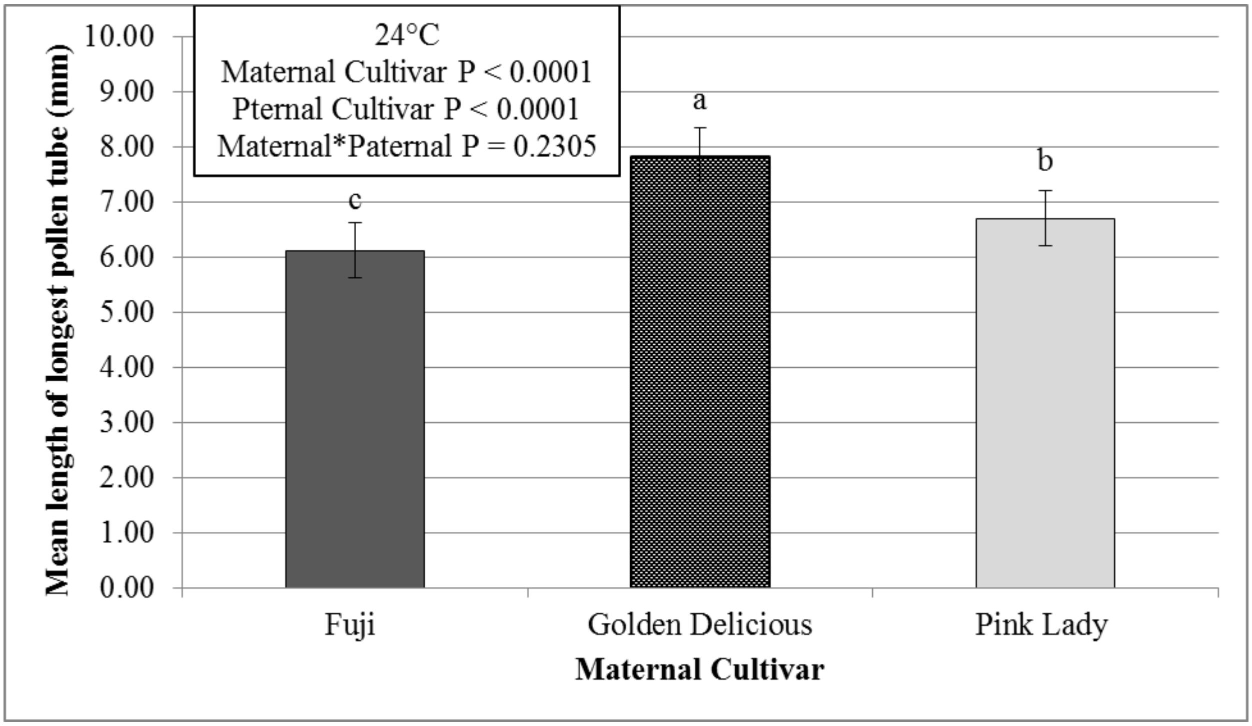


2.2 B

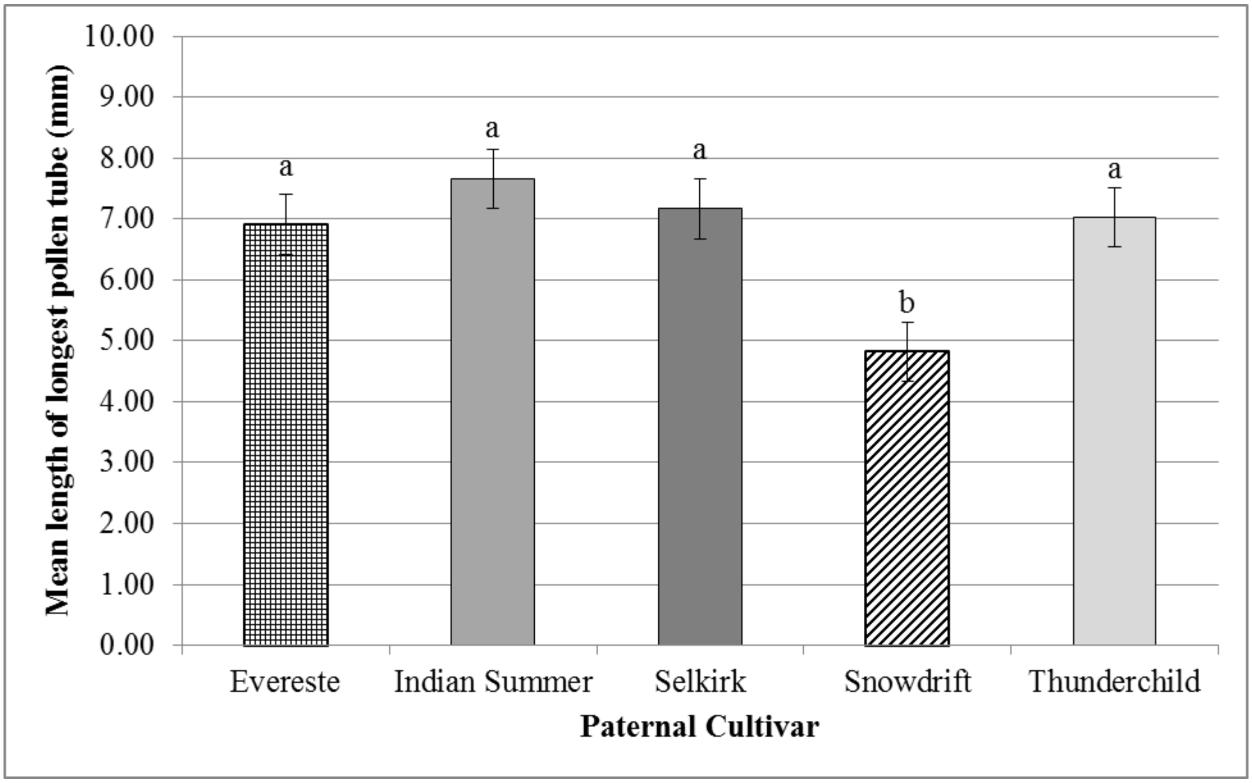


10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Meanlenght of longest pollen tube (mm)
b
b
a
b
a
Evereste
Indian Summer
Selkirk
Snowdrift
Thunderchild
Paternal Cultivar

2.2 C


24°C
Maternal Cultivar P < 0.0001
Pternal Cultivar P < 0.0001
Maternal*Paternal P = 0.2305
Mean length of longest pollen tube (mm)
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
c
a
b
Fuji
Golden Delicious
Pink Lady
Maternal Cultivar

2.2 D


Mean length of longest pollen tube (mm)
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
a
a
a
b
a
Evereste
Indian Summer
Selkirk
Snowdrift
Thunderchild
Paternal Cultivar

Chapter 3: Disease control and thinning efficacy of lime sulfur and Regalia® treatments applied during bloom

Abstract

Apple (*Malus* x *domestica* Borkh.) growers need management practices that will produce high quality fruit while minimize the number of chemicals required for adequate disease control and management practices. Potentially, chemicals applied for bloom thinning could replace a fungicide cover spray during this time period. Over four years, treatments of lime sulfur or Regalia®, an organically approved biofungicide, were evaluated for protection against apple scab [*Venturia inaequalis* (Cooke.) G. Wint.], powdery mildew [*Podosphaera leucotricha* (Ellis & Everh.) E. S. Salmon], and cedar-apple rust (*Gymnosporangium juniperi-virginiana* Schwein.), as well as, crop load reduction and fruit finish. Both lime sulfur and Regalia® reduced apple scab and cedar-apple rust during every year evaluated, except 2013. Treatments of Regalia® applied with JMS Stylet-Oil provided disease protection and crop load reduction similar to lime sulfur applied with JMS Stylet-Oil. We provide evidence that lime sulfur and Regalia®, applied as bloom thinners, could reduce chemicals used during bloom by combining two chemical spray functions; one for disease protection and one for crop load management.

Introduction

As consumer demand for apples (*Malus* x *domestica* Borkh.) from organic and low input systems increase, there is a need to develop management practices that reduce chemical use without sacrificing fruit quality. To decrease environmental concerns, published research has evaluated management techniques and spray programs that reduce the volume of chemicals used during the growing season to control apple scab [*Venturia inaequalis* (Cooke.) G. Wint.] (Ellis et al., 1998; Holb, 2008; Jamar et al., 2008, 2010; Trapman and Polfliet, 1997). However, reducing chemical inputs is especially difficult in the humid regions of the Mid-Atlantic United States where a large complex of diseases including apple scab, powdery mildew [*Podosphaera leucotricha* (Ellis & Everh.) E. S. Salmon], and cedar-apple rust (*Gymnosporangium juniperi-virginiana* Schwein.) require multiple well-timed fungicide applications into June. Apple scab is the most serious disease of apple in the Mid-Atlantic region, and losses of 50-90% could result if protective measures are not taken (Bengtsson et al., 2009; Holb, 2006; MacHardy, 1996). These early season diseases have traditionally been controlled with fungicide applications applied on a seven- to ten-day schedule once infection periods have occurred (Mills, 1944; Turechek et al., 2004).

Reducing apple crop load minimizes biennial bearing patterns, decreases disease pressure, and improves fruit color and size. Multiple opportunities to reduce crop load exist throughout the early growing season, but chemical thinning during bloom increases the above mentioned benefits, particularly return bloom (Batjer and Hoffman, 1951; Greene, 2002). Bloom thinning chemicals are often caustic and prevent pollination and fertilization by damaging reproductive organs (Fallahi and Greene, 2010; McArtney et al., 2006; Yoder et al, 2009). The timing of such chemicals is of high importance because only flowers fertilized before chemical application will develop into fruit (Fallahi and Willemsen, 2002; Williams and Edgerton, 1981;

Yoder et al., 2009, 2013b). Lime-sulfur (calcium polysulfide), a commonly used bloom thinner, is caustic, but can also increase the number of aborted fruitlets by suppressing photosynthesis, resulting in increased plant stress (Schmidt and Elfving, 2007; Schupp, 2006; Whiting and McFerson, 2006). Although an effective thinner, lime sulfur has many negative effects on tree health due to its phytotoxic properties (Burrell, 1945; Cromwell et al. 2011; Holb et al., 2003; Palmiter and Smock, 1954).

Lime sulfur is one of the first known materials to be used as an insecticide and fungicide (Secoy and Smith, 1983). Lime sulfur can protect against many common apple diseases including apple scab (Montag et al., 2005; Moore, 1957, 1961), and powdery mildew (Tweedy, 1967). Regalia®, a biofungicide released in 2009 by Marrone Bio Innovations, Inc. (Davis, CA) was developed from the extract of Giant Knotweed [*Reynoutria sachalinensis* (F. Schmidt) Nakai syn. *Polygonum sachalinensis* F. Schmidt] and can be used to replace fungicides; such as, strobilurins and sulfur in control of leaf spot diseases and powdery mildew (Su et al., 2012). Extracts of *R. sachalinensis* have previously been formulated into a fungicide known as Milsana® during the 1980s and 1990s. Disease control capabilities of *R. sachalinensis* extracts have been shown for powdery mildew of cucumbers, tomatoes, begonias, and wheat (Daayf et al., 1995; Dik and VanDerStraay, 1995; Herger et al., 1988; Herger and Klingauf, 1990; Konstantinidou-Doltsinis and Schmitt, 1998; Konstantinidou-Doltsinis et al., 2006). Regalia® applied with mineral oil has previously provided sufficient post-infection control of quince rust on ‘Golden Delicious’ trees in Winchester, VA (Peck et al., 2015). In addition, studies on extracts of *R. sachalinensis* have revealed no negative side effects to beneficial insects (Hafez et al., 1999, Schuld et al., 2002) and lower fruit russet ratings than lime sulfur (Yoder et al. 2013b). Using biofungicides, like Regalia®, provides multiple benefits over the use of synthetic fungicides, including a reduction in pathogen resistance and a decrease in synthetic fungicide residues in the environment. In previous evaluations, Regalia® applied during bloom caused burning of flower petals, and from this observation we decided to test the capabilities of Regalia® as a bloom thinner (personal communication T. Johnson, and K. Yoder). If effective, Regalia® could be used as an alternative to lime sulfur.

Over four years, we studied the disease control potential of bloom thinning treatments applied to ‘Ginger Gold’, ‘Jonagold’, ‘Fuji’, and ‘York’ trees in place of a fungicide treatment during bloom, and determined the crop load reduction and fruit finish resulting from these treatments. The main objectives of this study were to (i) evaluate the disease control of apple scab, cedar-apple rust, and powdery mildew provided by chemicals applied for bloom thinning on ‘Ginger Gold’, ‘York’, ‘Fuji’ and ‘Jonagold’ and (ii) determine crop load reduction by these treatments applied to ‘Ginger Gold’.

Materials and Methods

Treatment evaluations took place at the Alson H. Smith, Jr. Agricultural Research and Extension Center in Winchester, VA. Bloom thinning treatments were applied dilute to the point of runoff with a single nozzle handgun at 2068 kPa, and maintenance sprays were applied separately with a commercial airblast sprayer over all four years. Test trees included: ‘Ginger Gold’/’M.26’ planted in 1992, ‘Jonagold’/’M.26’ planted in 1992, ‘Fuji’/’M.9’ planted in 1995, and ‘Ramey York’/’M.9’ planted in 1999. Treatments were applied each year in a randomized complete block design with four replications. Trees used in the experiment were blocked based on similar bloom density. Products evaluated included lime sulfur (Miller Chemical & Fertilizer Corp., Hanover, PA), Crocker’s Fish Oil (Crocker’s Fish Oil, Quincy, WA), JMS Stylet-Oil

(JMS Flower Farms, Inc., Vero Beach, FL), Rally® (myclobutanil) (DowAgroSciences, Indianapolis, IN), Latron B-1956 (Phthalic glycerol alkyld resins) (DowAgroSciences, LLC, Five Points, CA), Syllit 3.4F (dodine) (Agriphar S.A., Ougree, Belgium) and Regalia® (Marrone Bio Innovations, Davis, CA). Untreated controls for each cultivar were not sprayed with any fungicides. Diseases developed from inoculum naturally present in the test area. Treatments varied among years due to tree availability at the research orchard. Russet ratings were determined based on a 0-5 scale with 0 representing fruit with no russet, and 5 representing severe fruit russet.

All percentage data were converted by the square root arcsin transformation for statistical analysis. Disease variables were analyzed using an analysis of variance (PROC ANOVA) and mean separations were determined using the Waller-Duncan k-ratio t-test (SAS Institute Inc., Cary, NC).

2011

In 2011, early season apple scab and cedar-apple rust pressures were moderate and powdery mildew pressure was heavy. Bloom thinning treatments consisting of combinations of lime sulfur, JMS Stylet-Oil, Crocker's Fish Oil, and Rally were applied between 19 Apr and 27 Apr (early bloom and petal fall) to 'Ginger Gold' trees. Follow up cover spray treatments of Rally 35.4 g/378.5 L dilute were applied as first-fifth cover sprays on 12 May, 20 May, 6 Jun, 21 Jun, and 5 Jul. Standard maintenance materials including acetamiprid (Assail), chlorantraniliprole (Altacor), imidacloprid (Provado), methidathion (Supracide), phosmet (Imidan), spinetoram (Delegate), and thiacloprid (Calypso) were applied on 19 Mar, 6 May, 20 May, 6 Jun, 16 Jun, 30 Jun, 13 Jul, and 28 Jul (Pfeiffer et al., 2011). Foliar data was recorded on ten terminal shoots from each of four single-tree replications on 17 Jun and represent averages of all leaves or sorted for the oldest ten leaves on each shoot. Fruit counts are means of 25-fruit samples from each of four single-tree replications on the tree at harvest 16 Jul. Crop load was evaluated before harvest on 14 Jul.

2012

Apple scab and cedar-apple rust disease pressures were moderate, and powdery mildew pressure was heavy in 2012. Bloom thinning treatments of combinations of lime sulfur, Regalia®, Latron B-1956, JMS Stylet-Oil, and Crocker's Fish Oil were applied between 3 Apr and 6 Apr (petal fall) to 'Ginger Gold' trees. Follow up treatments of Rally 35.4 g/378.5 L dilute were applied as first-fifth cover sprays over all treatments on 26 Apr, 3 May, 16 May, 30 May, and 19 Jun. Standard maintenance materials of acetamiprid (Assail), carbaryl (Sevin XLR), chlorantraniliprole (Altacor), esfenvalerate (Asana), imidacloprid (Provado), methomyl (Lannate), mineral oil (Damoil), phosmet (Imidan), spinetoram (Delegate), and thiacloprid (Calypso) were applied on 14 Mar, 30 Apr, 18 May, 31 May, 16 Jun, 2 Jul, 14 Jul and 22 Jul (Pfeiffer et al., 2012). Foliar data counts consisted of ten terminal shoots each from four single-tree replications taken on 3 Aug and represent averages of all leaves or sorted for just leaves 1-10 on each shoot. Fruit counts are means of 25-fruit samples from each of four single-tree replications at harvest on 8 Aug. Crop load was evaluated before harvest on 14 Jul.

2013

Apple scab, cedar-apple rust, and powdery mildew disease pressures were high in 2013. Bloom thinning treatments were applied to 'Jonagold', 'York', 'Fuji', and 'Ginger Gold'. Bloom thinning effects were evaluated for ‘Ginger Gold’ trees only, while disease control was evaluated on all cultivars. On ‘Ginger Gold’ and ‘Jonagold’ trees bloom thinning treatments were applied between 23 Apr and 1 May following the ‘Golden Delicious’ pollen tube growth model (Yoder et al., 2013b). Follow up treatments of with Rally 35.4 g + Microthiol Disperss 0.9 kg/378.5 L dilute were applied as cover sprays on 31 May, 14 June, 28 Jun and 16 Jul. Standard maintenance materials of acetamiprid (Assail), carbaryl (Sevin XLR), chlorantraniliprole (Altacor), imidacloprid (Provado and Admire Pro), permethrin (Perm-Up), phosmet (Imidan), spinetoram (Delegate), and thiacloprid (Calypso) were applied on 8 Apr, 14 May, 27 May, 8 Jun, 21 June, 4 Jul, 19 Jul, and 2 Aug (Pfeiffer et al., 2013). Foliar data represent counts of the first 10 leaves of 8 terminal shoots from each replicate tree on 1 Aug for ‘Ginger Gold’ and 7 Aug for ‘Jonagold’. ‘Ginger Gold’ crop load was evaluated before harvest on 29 Jul, and fruit counts and russet ratings are means of 25-fruit samples harvested on 19 Aug from each of four single-tree replications.

Bloom thinning treatments of combinations of lime sulfur, JMS Stylet-Oil, Regalia®, Latron B-1956, and Syllit 3.4 F were applied to ‘York’ and ‘Fuji trees between 26 Apr and 2 May following the ‘Fuji’ pollen tube growth model (Yoder et al., 2013b) . Follow up treatments of Arysta Captan 80WDG (N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide) 425.3g/378.5 L dilute were applied as cover sprays on 30 May, 12 Jun, 26 Jun, 10 Jul, 25 Jul, 7 Aug, and 21 Aug. Standard maintenance sprays of acetamiprid (Assail), carbaryl (Sevin SLR), chlorantraniliprole (Altacor), imidacloprid (Provado and Admire Pro), mineral oil (Biocover MLT), phosmet (Imidan), spinetoram (Delegate), and thiacloprid (Calypso) were applied on 4 Apr, 14 May, 27 May, 8 Jun, 21 Jun, 4 Jul, 22 Jul, 5 Aug, and 24 Aug (Pfeiffer et al., 2013).

Foliar data represents counts of the first 10 leaves of 8 terminal shoots from each replicate-tree taken on 14 Aug.

2014

During 2014, disease pressures were heavy for apple scab and cedar-apple rust, and moderate for powdery mildew. Bloom thinning treatments consisted of lime sulfur and Regalia®, both with JMS Stylet-Oil. Treatments on ‘Ginger Gold’ and ‘Jonagold’ trees were applied between 28 Apr and 5 May following the ‘Golden Delicious’ pollen tube growth model (Yoder et al., 2013b). Cover sprays of Rally 35.4 g + Microthiol Disperss 0.9 Kg/378.5 L dilute were made on 30 May, 11 Jun, 23 Jun, 7 Jul, and 21 Jul. Additional cover sprays were applied on 4 Aug, and 28 Aug to ‘Jonagold’ only. Standard maintenance materials of acetamiprid (Assail), chlorantraniliprole (Altacor), esfenvalerate (Asana), imidacloprid (Provado and Admire Pro), methomyl (Lannate), mineral oil (Biocover), phosmet (Imidan), dinotefuran (Scorpion), and spinetoram (Delegate) were applied on 1 Apr, 9 May, 23 May, 7 Jun, 20 June, 5 Jul, 18 Jul, 2 Aug, and 24 Aug (Pfeiffer et al., 2014). Foliar data represent counts of the first 10 leaves on 10 terminal shoots from each replicate tree on 4 Jun for ‘Ginger Gold’, and 19 Aug for ‘Jonagold’. ‘Ginger Gold’ crop load was evaluated on the tree on 23 Jun, and fruit russet ratings are means of 25-fruit samples from each of four single-tree replications taken after harvest on 19 Aug.

Bloom thinning treatments of lime sulfur and Regalia® plus JMS Stylet-Oil were applied to ‘York’ trees on 2 May and 6 May based on the ‘Golden Delicious’ pollen tube growth model (Yoder et al., 2013b). Follow up cover treatments of Captan (Agristar) 425.3 g/378.5 L dilute were applied on ‘York’ trees on 30 May, 11 Jun, 17 Jun, 2 Jul, 17 Jul, 8 Aug, and 28 Aug. Standard maintenance materials, mentioned in the previous paragraph, were applied to ‘York’ trees on 1 Apr, 13 May, 28 May, 7 Jun, 20 Jun, 5 Jul, 18 Jul, 2 Aug, and 24 Aug (Pfeiffer et al., 2014). ‘York’ foliar data represent counts of the first 10 leaves of 8 terminal shoots from each replicate tree on 18 Aug.

Results

Disease control provided by bloom thinning sprays

2011

Rally was the only treatment that did not reduce apple scab percent infection on leaves and fruit on ‘Ginger Gold’ in 2011(Table 3.1). Cedar-apple rust and powdery mildew infections were sufficiently reduced by all treatments.

2012

All materials reduced apple scab, powdery mildew, and cedar-apple rust percent infection on ‘Ginger Gold’ in 2012 (Table 3.2). Fruit harvested from Regalia®/Latron B-1956, and lime sulfur 3.8 L/JMS Stylet-Oil 3.8 L treatments (applied on 4 Apr and 6 Apr) had 26% higher scab infection than fruit from other treatments.

2013

In 2013, on ‘Fuji’, only lime sulfur/JMS Stylet-Oil (applied on 28 Apr and 2 May), Syllit, and lime sulfur/Crocker’s Fish Oil reduced apple scab percent infection on leaves 1-10 (Table 3.3). Lime sulfur/JMS Style-Oil and Regalia®/JMS Stylet-Oil reduced cedar-apple rust percent infection on leaves the greatest amount. On ‘Ginger Gold’ only Syllit reduced scab percent infection on leaves (Table 3.4). All treatments of lime sulfur or Regalia® reduced the percent of powdery mildew infection on leaves 1- 10, and on total leaf area, but only lime sulfur 7.6 L/JMS Stylet-Oil 7.6 L reduced the percent of powdery mildew infection found on fruit. All treatments reduced cedar-apple rust percent infection, but lime sulfur 7.6 L/JMS Stylet-Oil 7.6 L and Regalia/JMS Stylet-Oil reduced infection the greatest amount. On ‘Jonagold’, no treatments reduced apple scab percent infection on leaves, but Syllit, lime sulfur/JMS Stylet-Oil (applied on 24 Apr and 1 May), and Regalia®/Stylet-Oil reduced apple scab percent infection on fruit by more than 10% (Table 3.5). Treatments of lime sulfur 7.6 L/JMS Stylet-Oil 7.6 L and Regalia®/JMS Stylet-Oil reduced the number of cedar-apple rust lesions per leaf the greatest amount. On ‘York’, all treatments reduced apple scab infection except Regalia®/Latron B-1956, and all treatments provided adequate control of cedar-apple rust (Table 3.6).

2014

On ‘Ginger Gold’, ‘Jonagold’, and ‘York’, all treatments reduced the percent infection of apple scab and cedar-apple rust (Table 3.7 – 3.9). On ‘Ginger Gold’ lime sulfur reduced apple scab percent infection on leaves more than Regalia® 3.8 L/JMS Stylet-Oil 7.6 L, and lime sulfur/JMS Stylet-Oil reduced the percent of apple scab infection on fruit a greater amount than both Regalia®/JMS Stylet-Oil treatments (Table 3.7). On ‘Jonagold’, lime sulfur/JMS Stylet-Oil reduced powdery mildew percent infection on leaves (Table 3.8). On ‘York’ trees, lime sulfur/JMS Stylet-Oil treatments reduced apple scab percent infection on leaves a greater amount than Regalia/JMS Stylet-Oil (Table 3.9).

C*rop load estimates and russet ratings of treatments applied for bloom thinning of ‘Ginger Gold’ 2011-2014*

In 2011, lime sulfur 7.6 L/Crocker's Fish Oil 7.6 L (applied on 20, 22, and 27 Apr) reduced crop load the greatest amount (Table 3.10). All lime sulfur-related treatments increased the percent of russeted fruit and the percent of fruit area russeted, and treatments containing JMS Stylet-Oil had more area russeted than those with Crocker's Fish Oil. The 20 Apr application of lime sulfur/Crocker's Fish Oil was the only treatment with greater stem-end russet ratings than control.

In 2012, all treatments except lime sulfur/JMS Stylet-Oil (applied on 4 Apr and 6 Apr) reduced crop load by more than 60% (Table 3.11). Treatments of lime sulfur/JMS Stylet-Oil (applied on 3 and 5 Apr) increased fruit side russet ratings, and the percent of fruit area russeted post-harvest. Regalia®/Latron B-1956 resulted in the lowest stem-end russet rating.

In 2013, Regalia®/Latron B-1956 and Syllit, resulted in the lowest fruit russet ratings of all treatments, but did not reduce crop load (Table 3.12). Lime sulfur 7.6 L/JMS Stylet-Oil 7.6 L resulted in an 83% crop load reduction and the highest fruit russet rating among treatments.

In 2014, all treatments reduced crop load more than 40% (Table 3.13). Lime sulfur treatments resulted in higher fruit russet ratings than Regalia® treatments.

Discussion

The goals of this research were to assess disease control provided by lime sulfur and Regalia® treatments applied to 'Fuji', 'Ginger Gold', 'Jonagold', and 'York' trees during bloom, and to evaluate the blossom thinning efficacy of these treatments on 'Ginger Gold'.

With a few exceptions, treatments of lime sulfur or Regalia®, when applied with JMS Stylet-Oil, reduced apple scab, and cedar-apple rust infection on all evaluated cultivars. In 2013, a year with high disease pressure, all treatments reduced apple scab percent infection on 'Ginger

Gold' fruit, but only Syllit reduced apple scab percent on leaves (Table 3.7). The reduced activity of lime sulfur and Regalia® treatments on 'Ginger Gold' leaves in 2013 is most likely due to apple scab infection periods consisting of 138 wetting hours and 9.9 cm of rain between bloom thinning application time (24 Apr and 1 May) and the first cover spray of Rally (31 May). In addition, apple scab resistant to sterol-inhibiting (SI) fungicides was also present in the test plots in 2013, indicating cover spray applications of Rally may not have provided sufficient control during late spring infection periods. High levels of SI-resistant *V. inaequalis* have previously been reported in the Winchester, VA area (Marine et al., 2007; Yoder, 2006). The leaves of 'Ginger Gold', a cultivar highly susceptible to apple scab, were the most severely infected (Biggs et al., 2010). Some treatments applied to 'York' and 'Fuji' reduced the percent of apple scab infection on leaves; however, the cover treatments applied to these two cultivars consisted of Arysta Captan instead of Rally (Table 3.3 and 3.6). Although treatments did not reduce the percent of apple scab on 'Jonagold' leaves, percent infection was on average over 20% lower on 'Jonagold' compared to 'Ginger Gold' (Table 3.5).

In 2013, powdery mildew percent infection was not different from the control for any treatments applied to 'Fuji', 'Jonagold', or 'York'. These findings may be related to low levels of powdery mildew found overall on these cultivars. In 2013, leaves 1-10 of the 'Ginger Gold' control treatment trees, which had no fungicide applications, had a 44% mildew infection, compared to 11% on 'Jonagold', 4.6% on 'York', and 3.3% on 'Fuji'. A higher percentage of powdery mildew is expected on 'Ginger Gold' due to its increased susceptibility (Biggs et al., 2009).

Also in 2013, cedar-apple rust infection was reduced by all bloom thinning treatments on 'Fuji', 'Ginger Gold', and 'York'. On 'Jonagold' in 2013 all treatments reduced cedar-apple rust infection on leaves 1-10, except lime sulfur/JMS Stylet Oil (applied on 23 Apr and 1 May). This treatment was applied on 23 Apr. when the temperature was cooler (5.6°C) than on 24 Apr. when the other treatments were applied (15°C). Though concentrations of 3.8 L lime sulfur proved effective against cedar-apple rust during 2011 and 2012, a higher concentration may be needed when severe infection periods occur during the bloom period (e.g. 2013).

In 2012, 2013, and 2014, lime sulfur and Regalia® treatments, when applied with JMS Stylet-Oil, provided similar protection against early season diseases and always resulted in equivalent crop load reductions. When differences existed in disease control, Regalia®/JMS Stylet-Oil was more effective in control of cedar-apple rust, and lime sulfur/JMS Stylet-Oil was more effective in control of apple scab. Although not applied with JMS-Stylet Oil, Regalia® applied to ‘Golden Delicious’ trees with mineral oil as the surfactant, provided similar quince rust protection to conventional ‘grower standard’ fungicides (Peck et al., 2015). In prior evaluations, Regalia® did not reduce crop load as severely as lime sulfur (Peck et al, 2015, Yoder et al., 2013b). In these evaluations, Regalia® was applied exclusively with Latron B-1956 instead of JMS Stylet-Oil.

In our evaluations, Regalia® treatments provided better disease protection and reduced crop load in greater amounts when applied with JMS Stylet-Oil instead of Latron B-1956. JMS Stylet-Oil was not applied alone in our treatment evaluations, but JMS Stylet-Oil has previously reduced apple scab and cedar-apple rust infection when applied as a fungicide to ‘Stayman’, ‘Idared’, and ‘Ginger Gold’ trees (Yoder et al., 2013a). In accordance with our results, Yoder et al. (2013b) found greater crop load reduction from lime sulfur/JMS Stylet-Oil treatments than Regalia/Latron B-1956 treatments. Our results indicate greater effectiveness in crop load reduction and disease protection can be attained from Regalia® applied with JMS Stylet-Oil as the surfactant.

With few exceptions, every lime sulfur treatment increased fruit russet ratings. Lime sulfur is more phytotoxic, and results in increased amounts of russet when applied at temperatures higher than 26.6°C (Peck and Merwin, 2010). Regalia® treatments had greater russet rating than control on one occasion, in 2013 when Regalia® 3.8 L/JMS Stylet-Oil 3.8 L was applied on 24 Apr and 1 May (Table 3.12). In 2014, Regalia®, applied with 3.8 L or 7.6 L of JMS Stylet-Oil did not increase fruit russet (Table 3.13). When russet was increased in 2013, Regalia®/Stylet-Oil treatments were applied when maximum temperatures reached 24.4°C on 24 Apr and 19.4°C on 1 May. While in 2014, Regalia® treatments were made on 28 Apr and 5 May when temperatures reached 15 and 17.7°C, respectively. Future studies should evaluate russet incidence caused by Regalia® treatments applied on days with higher maximum temperatures.

Regalia® reduces disease infection by improving the plant's natural defenses (Su et al., 2012). Increased phytoalexins have previously been reported in plants after application of *R. sachalinensis* extracts (Daayf et al., 1995; Daayf et al., 1997; Daayf et al., 2000; Su et al. 2012). The blossom thinning mode of action of Regalia® is unknown, but damaged flower petals observed after Regalia® application indicate it may be caustic and can reduce crop load to some extent by damaging the plant's reproductive organs. However, it should be noted that no foliar injury resulted from Regalia® treatments.

Our results suggest bloom thinning treatments of lime sulfur and Regalia® could replace separate fungicide applications made during bloom, and Regalia® can provide similar bloom thinning results to lime sulfur without the increased levels of fruit russet. More research is needed to determine the optimum rate of chemicals and surfactants, and the application time that will most efficiently protect from diseases and reduce crop load. The number of chemicals available for blossom thinning is limited, especially for organic growers, and although lime sulfur is an option for both conventional and organic production, it has many negative side-effects including increased phytotoxicity and fruit russet. Regalia® should continue to be researched for use in apple orchards, especially on other cultivars and in different apple producing regions.

Literature Cited

Batjer, L. P. and M.B. Hoffman. 1951. Fruit thinning with chemical sprays. U.S. Dept. Agr. Circ. 867 Washington, D. C.

Bengtsson, M., E. Wulff, H.J. Lyngs Jorgensen, A. Pham, M. Lübeck and J. Hockenhull. 2009. Comparative studies on the effects of a yucca extract and acibenzolar-S-methyl (ASM) on inhibition of *Venturia inaequalis* in apple leaves. Eur. J. Plant Pathol. 124:187-198.

Biggs, A.R., G.W. Sundin, D.A. Rosenberger, K.S. Yoder, and T.B. Sutton. 2010. Relative susceptibility of selected apple cultivars to apple scab caused by *Venturia inaequalis*. Online. Plant Health Progress.

Biggs, A. R., K.S. Yoder, and D.A. Rosenberger. 2009. Relative susceptibility of selected apple cultivars to powdery mildew caused by *Podosphaera leucotricha*. Online. Plant Health Progress.

Burrell, A.B. 1945. Practical use of our newer knowledge of apple scab control. Proc. Of New York State Hort. Soc. 90:9-16.

Cromwell, M.L., L.P. Berkett, and H.M. Darby. 2011. Alternative organic fungicides for apple scab management and their non-target effects. HortScience 46:1254-1259.

Daayf, F., A. Schmitt, and R.R. Belanger. 1995. The effects of plant extracts of *Reynoutria sachalinensis* on powdery mildew development and leaf physiology of long English cucumber. Plant Dis. 79:577-580.

Daayf, F., A. Schmitt, and R.R. Belanger. 1997. Evidence of phytoalexins in cucumber leaves infected with powdery mildew following treatment with leaf extracts of *Reynoutria sachalinensis*. Plant Physiol. 113:719-727.

Daayf, F., M. Ongena, R. Boulanger, I.E. Hadrami, and R.R. Belanger. 2000. Induction of phenolic compounds in two cultivars of cucumber by treatment of healthy and powdery mildew-infected plants with extracts of *Reynoutria sachalinensis*. J. Chem. Ecol. 26:1579-1593.

Dik, A.J. and VanDerStraay. 1995. The effects of Milsana® on cucumber powdery mildew under Dutch conditions. Med. Fac. Landbouww. Rijksuniv. Gent. 59:1027-1039.

Ellis, M.A., D.C. Ferree, R.C. Funt, and L.V. Madden. 1998. Effects of an apple scab-resistant cultivar on use patterns on inorganic and organic fungicides and economics of disease control. Plant Dis. 82:428-433.

Fallahi, E. and D.W. Greene. 2010. The impact of blossom and postbloom thinners on fruit set and fruit quality in apples and stone fruit. Acta Hort. 884:179-187.

Fallahi, E. and K.M. Willemsen. 2002. Blossom thinning of pome and stone fruit. HortScience 37:474-477.

Greene, D.W. 2002. Chemicals, timing, and environmental factors involved in thinner efficacy on apples. HortScience 37:477-481.

Hafez, M.B., A. Schmitt, and S.A. Hassan. 1999. The side-effects of plant extracts and metabolites of *Reynoutria sachalinensis* (F. Schmidt) Nakai and conventional fungicides on the beneficial organism *Trichogramma cacoecia* Marchal (Hym., Trichogrammatidae). J. Appl. Ent. 123:363-368.

Herger, G., F. Klingauf, D. Mangold, E.H. Pommer, and M. Scherer. 1988. Efficacy of extracts of *Reynoutria sachalinensis* (F. Schmidt) Nakai (Polygonaceae) against fungal diseases, especially powdery. Nachrichtenbl Dtsch Pflanzenschutzdienst (Braunschweig) 40:56-60.

Herger, G. and F. Klingauf. 1990. Control of powdery mildew fungi with extracts of the giant knotweed, *Reynoutria sachalinensis* (Polyconaceae). Med. Fac. Landbouww Rijksuniv Gent. 55:1007-1014.

Holb, I.J., P.F. DeJong, and B. Heijne. 2003. Efficacy and phytoxicity of lime sulfur in organic apple production. Ann. Appl. Biol. 142:225-233.

Holb, I.J. 2006. Effect of six sanitation treatments on leaf litter density, ascospore production of *Venturia inaequalis* and scab incidence in integrated and organic apple orchards. Eur. J. Plant Pathol. 115:293-307.

Holb, I.J. 2008. Timing of first and final sprays against apple scab combined with leaf removal and pruning in organic apple production. Crop Prot. 27:814-822.

Jamar, L., B. Lefrancq, C. Fassotte, and M. Lateur. 2008. A during-infection spray strategy using sulphur compounds, copper, silicon and a new formulation of potassium bicarbonate for primary scab control in organic apple production. Eur. J. Plant. Pathol. 122:481-493.

Jamar, L., M. Cabelier, and M. Lateur. 2010. Primary scab control using a “during-infection” spray timing and the effect on fruit quality and yield in organic apple production. Biotechnol. Agron. Soc. Environ. 14:423-439.

Konstantinidou-Doltsinis, S. and A. Schmitt. 1998. Impact of treatment with plant extracts from *Reynoutria sachalinensis* F. Schmidt Nakai on disease severity of powdery mildew and yield in cucumber under Greek conditions. Crop Prot. 17:649-656.

Konstantinidou-Doltsinis, S., E. Markellou, A.M. Kasselaki, M.N. Fanouraki, C.M. Koumaki, A. Schmitt, A. Liopa-Tsakalidis, and N.E. Malathrakis. 2006. Efficacy of Milsana®, a formulated plant extract from *Reynoutria sachalinensis,* against powdery mildew of tomato (*Leveillula taurica*). BioControl 51:375-392.

MacHardy, W.E. 1996. Apple scab biology, epidemiology and management. The Amer. Phytopathol. Soc. Press, St. Paul, MN.

Marine, S.C., D.G. Schmale III, and K.S. Yoder. 2007. Resistance to Myclobutanil in populations of *Venturia inaequalis* in Winchester, Virginia. Online. Plant Health Progress.

McArtney, S., J. Palmer, S. Davies, and S. Seymour. 2006. Effects of lime sulfur and fish oil on pollen tube growth, leaf photosynthesis and fruit set in apple. HortScience 41:357-360.

Mills, W.D. 1944. Efficient use of sulphur dusts and sprays during rain to control apple scab. Cornell Extension Bull. 630:1-4.

Montag, J., I. Schreiber, and J. Schönherr. 2005. An in vitro study on the postinfection activity of hydrates lime and lime sulphur against apple scab (*Venturia inaequalis*). J. Phytopathol. 153:485-491.

Moore, M.H. 1957. Concentrate spraying of apple trees: Fungicidal efficiency of lime-sulphur. Ann. Appl. Biol. 45:11-18.

Moore, M.H. 1961. Concentrate spraying of apple trees: Dosage/volume relationships of lime-sulphur in disease control and spray damage. Ann. Appl. Biol. 49:254-266.

Palmiter, D.H. and R.M. Smock. 1954. Effect of fungicides on McIntosh apple yield and quality: A five-year study under Hudson Valley conditions 1945-1953. New York State Agr. Expt Stat. Geneva, NY.

Peck, G.M. and I.A. Merwin. 2010. A grower’s guide to organic apples. NYS IPM Publication No. 223.

Peck, G.M., L.D. Combs, C.N. DeLong, and K.S. Yoder. 2015. Precision apple flower thinning using organically-approved chemicals. Acta Hort. (in press)

Pfeiffer, D.G., J.C. Bergh, R.D. Fell, C.R.R. Hooks, C.S. Walsh, K.S. Yoder, A.R. Biggs, J.B. Kotcon, J.F. Derr, R.S. Chandran, M.J. Weaver, A. Brown, and J. Parkhurst. 2011. 2011 Spray Bulletin for Commercial Tree Fruit Growers. Ext. Pub. Num.: 456-419. VA Polytech. Inst. and State Univ., Blacksburg, VA.

Pfeiffer, D.G., J.C. Bergh, R.D. Fell, C.R.R. Hooks, G.M. Peck, C.S. Walsh, K.S. Yoder, A.R. Biggs, J.B. Kotcon, J.F. Derr, R.S. Chandran, M.J. Weaver, A. Brown, and J. Parkhurst. 2012. 2012 Spray Bulletin for Commercial Tree Fruit Growers. Ext. Pub. Num.: 456-419. VA Polytech. Inst. and State Univ., Blacksburg, VA.

Pfeiffer, D.G., J.C. Bergh, R.D. Fell, C.R.R. Hooks, G.M. Peck, C.S. Walsh, K.S. Yoder, A.R. Biggs, J.B. Kotcon, J.F. Derr, R.S. Chandran, M.J. Weaver, A. Brown, and J. Parkhurst. 2013. 2013 Spray Bulletin for Commercial Tree Fruit Growers. Ext. Pub. Num.: 456-419. VA Polytech. Inst. and State Univ., Blacksburg, VA.

Pfeiffer, D.G., J.C. Bergh, D.L. Frank, C.R.R. Hooks, G.M. Peck, K.S. Yoder, C.S. Walsh, A.R. Biggs, J.B. Kotcon, J.F. Derr, R.S. Chandran, M.J. Weaver, A. Brown, and J. Parkhurst. 2014. 2014 Spray Bulletin for Commercial Tree Fruit Growers. Ext. Pub. Num.: 456-419. VA Polytech. Inst. and State Univ., Blacksburg, VA.

Schmidt, T.R. and R.C. Elfving. 2007. Crop load management of apple via induced plant stress. J. Am. Pomol. Soc. 61:167-169.

Schuld, M., D. White, B. Seddon, A. Ernst, and A. Schmitt. 2002. Tolerance of *Typhlodromus pyri* (Acari, Phytoseiidae) and *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) to Milsana® and *Brevibacillus brevis* in integrated control systems. In: Dehne, H.-W., Gisi, U., Kuck, K.-H., Russell, P., and Lyr, H. (eds), Modern Fungicides and Antifungal Compounds III – 13th International Reinhardsbrunn Symposium, May 14-18, 2001; Verlag Thomas Mann GmbH, Gelsenkirchen. Pp. 411-417.

Schupp. J. 2006. Efficacy and physiological effect of oil/lime sulfur combinations. Final project report #AH-03-308 to Washington Tree Fruit Res. Comm.

Secoy, D.M. and A.E. Smith. 1983. Lineage of lime sulfur as an insecticide and fungicide. Bull. Entomol. Soc. Am. 29:18-23.

Su, H., R. Blair, T. Johnson, and P. Marrone. 2012. Regalia® bioprotectant in plant disease management. Outlooks on Pest Mgt. 23:30-34.

Trapman M. and C.M. Polfliet. 1997. Management of primary infections of apple scab with the simulation program RIMpro: Review of four years field trials. IOBC Bull. 20:241-250.

Turechek, W.W., J.E. Carroll, and D.A. Rosenberger. 2004. Powdery mildew of apple *Podosphaera leucotricha* (Ell. & Evherh.) Salm. Cornell Univ. Integrated Pest Mgt. Bul. #102GFSTF-D4.

Tweedy, B.G. 1967. Elemental sulfur. In fungicides an advanced treatise 1. Pp. 119-145. Ed. D. C. Torgeson. New York, USA: Academic Press.

Whiting, M. and J. McFerson. 2006. Impact of cultural practices on apple canopy photosynthesis and dark respiration. Plant Growth Reg. 35:1-9.

Williams, M.W. and L.J. Edgerton. 1981. Fruit thinning of apples and pears with chemicals. U.S. Dept. Agr. Bul. 289, Washington, DC.

Yoder, K. 2006. Orchard and laboratory testing of apple scab sensitivity to sterol-inhibiting fungicides. Virginia Fruit 41:25-30.

Yoder, K., R. Yuan, L. Combs, R. Byers, J. McFerson, and T. Schmidt. 2009. Effects of temperature and the combination of liquid lime sulfur and fish oil on pollen germination, pollen tube growth, and fruit set in apples. HortScience 44:1277-1283.

Yoder, K.S., A.E. Cochran II, W.S. Royston, S.W. Kilmer, A.G.F. Engelman, and L.A. Hickey. 2013a. Evaluation of mixed fungicide schedules on Stayman, Idared, and Ginger Gold apples, 2013. Plant Dis. Mgt. Rpt. Vol. 8:PF016.

Yoder, K. S., G.M. Peck, L.D. Combs, and R.E. Byers. 2013b. Using a pollen tube growth model to improve apple bloom thinning for organic production. Acta Hort. 1001:207-214

Table 3.1 Apple scab, powdery mildew, and cedar-apple rust infection levels by bloom thinning treatments applied to 'Ginger Gold'/'M.26' in 2011.[z][y]

Bloom treatment and rate/378.5 L	Bloom timing	Apple scab % infection			Powdery mildew % infection			Cedar-apple rust % infection	
		lvs 1-10	all lvs	fruit	lvs 1-10	all lvs	area	lvs 1-10	all lvs
No fungicide	----	29 c	26 d	88 c	48 b	72 b	46 b	12 b	6 b
Lime Sulfur 7.6 L + Crocker's Fish Oil 7.6 L	4/19, 4/22, & 4/27	7 a	8 ab	19 ab	21 a	36 a	5 a	0 a	0 a
Lime Sulfur 7.6 L + Crocker's Fish Oil 7.6 L	4/20, 4/22, & 4/27	8 a	6 a	18 ab	21 a	32 a	4 a	0 a	0 a
Lime Sulfur 7.6 L + Crocker's Fish Oil 7.6 L	4/22 & 4/27	10 ab	12 bc	33 b	22 a	34 a	5 a	0 a	0 a
Lime Sulfur 7.6 L + JMS Stylet-Oil 7.6 L	4/22 & 4/27	7 a	9 ab	15 a	21 a	33 a	4 a	<1 a	<1 a
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/22 & 4/27	6 a	8 ab	35 b	19 a	36 a	5 a	0 a	0 a
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L + Rally 40W 35.4 g	4/22 & 4/27	6 a	7 ab	24 ab	16 a	28 a	4 a	0 a	0 a
Rally 40W 35.4 g	4/22 & 4/27	18 b	19 cd	77 c	20 a	33 a	4 a	0 a	0 a

[z] Mean separation by Waller-Duncan K-ratio t-test (p=0.05). Four single tree replications.

[y] Foliar data represent counts of 10 terminal shoots from each replicate tree on 17 Jun.

Table 3.2 Apple scab, powdery mildew, and cedar-apple rust infection levels by bloom thinning treatments applied to 'Ginger Gold'/'M.26' in 2012.[z][y]

Bloom treatment and rate/378.5 L	Bloom timing	Apple scab % infection			Powdery mildew % infection			Cedar-apple rust % infection	
		lvs 1-10	all lvs	fruit	lvs 1-10	all lvs	area	lvs 1-10	all lvs
No Fungicide	----	64 b	62 a	73 c	33 b	61 b	23 b	22 b	23 b
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/3 & 4/5	40 a	51 a	12 a	15 a	29 a	3 a	0 a	0 a
Lime Sulfur 7.6 L + JMS Stylet-Oil 7.6 L	4/3 & 4/5	37 a	52 a	12 a	9 a	27 a	3 a	0 a	0 a
Lime Sulfur 7.6 L + Crocker's Fish Oil 7.6 L	4/3 & 4/5	33 a	48 a	12 a	14 a	32 a	3 a	0 a	0 a
Regalia 0.9 L + Latron B-1956 0.2 L	4/3 & 4/5	43 a	58 a	38 b	14 a	31 a	3 a	0 a	0 a
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/4 & 4/6	34 a	54 a	39 b	12 a	31 a	4 a	0 a	0 a

[z] Mean separation by Waller-Duncan K-ratio t-test (p=0.05). Four single tree replications.

[y] Foliar data represent counts of the 10 terminal shoots from each replicate tree on 3 Aug.

Table 3.3 Apple scab, powdery mildew, and cedar-apple rust infection levels by bloom thinning treatments applied to 'Fuji'/'M.9'in 2013.[z][y]

Bloom treatment and rate/378.5 L	Bloom timing	Apple scab % infection		Powdery mildew % infection	Cedar-apple rust % infection	
		lvs 1-10	fruit	lvs 1 -10	lvs 1-10	les/lf
No Fungicide	----	51 cd	3.0 b	3.3 a	65 e	4.3 b
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/28 & 5/2	26 ab	0.8 a	1.6 a	14 a	0.6 a
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/26 & 5/2	38 b-d	2.0 ab	1.3 a	16 a-c	0.5 a
Lime Sulfur 7.6 L + JMS Stylet-Oil 7.6 L	4/26 & 5/2	32 ab	1.7 ab	2.0 a	16 ab	0.6 a
Regalia 3.8 L + Latron B-1956 0.2 L	4/26 & 5/2	52 d	2.8 b	0.7 a	31 b-d	1.5 a
Regalia 3.8 L + JMS Stylet-Oil 0.2 L	4/26 & 5/2	36 a-c	1.4 ab	1.6 a	13 a	0.4 a
Syllit 3.4F 0.2 L	4/26 & 5/2	21 a	0.5 a	2.2 a	40 d	1.5 a
Lime Sulfur 3.8 L + Crocker's Fish Oil 3.8 L	4/26 & 5/2	26 ab	0.8 a	1.0 a	34 cd	1.2 a

[z] Mean separation by Waller-Duncan K-ratio t-test (p=0.05). Four single tree replications.

[y] Foliar data represent counts of the first 10 leaves of 8 terminal shoots from each replicate tree on 14 Aug.

Table 3.4 Apple scab, powdery mildew, and cedar-apple rust infection levels by bloom thinning treatments applied to 'Ginger Gold'/'M.26' in 2013.[z][y]

Bloom treatment and rate/378.5 L	Bloom timing	Apple scab % infection		Powdery mildew % infection			Cedar-apple rust % infection
		lvs 1-10	fruit	lvs 1 -10	area	Fruit	lvs 1-10
No Fungicide	----	48 b	68 c	44 c	4.2 c	10 b	67 c
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/23 & 5/1	46 b	41 b	21 a	2.4 a	5 ab	51 b
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/24 & 5/1	42 ab	37 b	21 a	2.2 a	4 ab	50 b
Lime Sulfur 7.6 L + JMS Stylet-Oil 7.6 L	4/24 & 5/1	45 b	44 b	20 a	2.1 a	1 a	32 a
Regalia 3.8 L + Latron B-1956 0.2 L	4/24 & 5/1	40 ab	35 b	18 a	1.8 a	7 ab	51 b
Regalia 3.8 L + JMS Stylet-Oil 3.8 L	4/24 & 5/1	51 b	47 b	28 ab	2.5 ab	4 ab	24 a
Syllit 3.4F 0.2 L	4/24 & 5/1	25 a	6 a	37 bc	3.7 bc	7 ab	45 b

[z] Mean separation by Waller-Duncan K-ratio t-test (p=0.05). Four single tree replications.

[y] Foliar data represent counts of the first 10 leaves of 8 terminal shoots from each replicate tree on 1 Aug.

Table 3.5 Apple scab, powdery mildew, and cedar apple rust infection levels by bloom thinning treatments applied to 'Jonagold'/'M.26' in 2013.[z][y]

Bloom treatment and rate/378.5 L	Bloom timing	Apple scab % infection		Powdery mildew % infection		Cedar-apple rust % infection	
		leaves	fruit	leaves	lf area	% lvs	les/lf
No Fungicide	----	11 ab	30 d	11 a	1.6 a	51 e	4.4 b
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/23 & 5/1	14 b	23 cd	6 a	1.0 a	43 de	5.5 b
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/24 & 5/1	12 b	11 bc	5 a	0.8 a	37 b-d	5.7 b
Lime Sulfur 7.6 L + JMS Stylet-Oil 7.6 L	4/24 & 5/1	17 b	10 b	8 a	1.3 a	28 ab	2.0 a
Regalia 3.8 L + Latron B-1956 0.2 L	4/24 & 5/1	12 ab	31 d	10 a	1.3 a	40 cd	9.1 c
Regalia 3.8 L + JMS Stylet-Oil 0.2 L	4/24 & 5/1	15 b	12 bc	8 a	1.2 a	21 a	1.7 a
Syllit 3.4F 0.2 L	4/24 & 5/1	6 a	1 a	8 a	1.4 a	32 bc	4.3 b

[z] Mean separation by Waller-Duncan K-ratio t-test (p=0.05). Four single tree replications.

[y] Foliar data represent counts of the first 10 leaves of 8 terminal shoots from each replicate tree on 7 Aug.

Table 3.6 Apple scab, powdery mildew, and cedar-apple rust infection levels by bloom thinning treatments applied to 'York'/'M.9' in 2013.[z][y]

Bloom treatment and rate/378.5 L	Bloom timing	Apple scab % infection		Powdery mildew % infection	Cedar-apple rust % infection	
		lvs 1-10	les/lf	% lvs	% lvs	les/lf
No Fungicide	----	65 d	10.7 c	4.6 a	93 c	11.4 b
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/28 & 5/2	42 bc	2.8 ab	2.3 a	60 b	6.1 a
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/26 & 5/2	30ab	1.2 ab	3.5 a	53 b	4.6 a
Lime Sulfur 7.6 L + JMS Stylet-Oil 7.6 L	4/26 & 5/2	33 a-c	1.9 ab	1.4 a	60 b	6.0 a
Regalia 3.8 L + Latron B-1956 0.2 L	4/26 & 5/2	55 cd	6.1 bc	3.5 a	62 b	5.7 a
Regalia 3.8 L + JMS Stylet-Oil 0.2 L	4/26 & 5/2	29 ab	1.9 ab	1.0 a	40 a	3.8 a
Syllit 3.4F 0.2 L	4/26 & 5/2	18 a	0.5 a	2.5 a	54 b	4.6 a

[z] Mean separation by Waller-Duncan K-ratio t-test (p=0.05). Four single tree replications.

[y] Foliar data represent counts of the first 10 leaves of 8 terminal shoots from each replicate tree on 14 Aug.

Table 3.7 Apple scab, powdery mildew, and cedar-apple rust infection levels by bloom thinning treatments applied to 'Ginger Gold'/'M.26' in 2014.[z][y]

Bloom treatment and rate/378.5 L	Bloom timing	Apple scab % infection		Powdery mildew % infection		Cedar-apple rust % infection		
		lvs	fruit	lvs	lf area	lvs	les/if	Fruit
No Fungicide	----	35 c	99 c	35 b	4 b	58 b	9.7 b	35 b
Lime Sulfur 7.6 L + JMS Stylet Oil 7.6 L	4/28 & 5/5	3 a	8 a	4 a	1 a	16 a	0.7 a	0 a
Regalia 3.8 L + JMS Stylet-Oil 3.8 L	4/28 & 5/5	7 ab	35 b	6 a	1 a	17 a	0.6 a	0 a
Regalia 3.8 L + JMS Stylet-Oil 7.6 L	4/28 & 5/5	9 b	42 b	3 a	1 a	21 a	0.5 a	0 a

[z] Mean separation by Waller-Duncan K-ratio t-test (p=0.05). Four single tree replications.

[y] Foliar data represent counts of the first 10 leaves of 10 terminal shoots from each replicate tree on 4 Jun.

Table 3.8 Apple scab, powdery mildew, and cedar apple rust infection levels by bloom thinning treatments applied to 'Jonagold'/'M.26' in 2014.[z][y]

Bloom treatment and rate/378.5 L	Bloom timing	Apple scab % infection		Powdery mildew % infection	Cedar-apple rust % infection	
		lvs	les/lf	lvs	lvs	les/if
No Fungicide	----	58 b	10.6 b	5.3 b	59 c	6.0 c
Lime Sulfur 7.6 L + JMS Stylet-Oil 7.6 L	4/28 & 5/5	27 a	1.3 a	0 a	17 b	1.1 b
Regalia 3.8 L + JMS Stylet-Oil 3.8 L	4/28 & 5/5	40 a	4.1 a	1.3 ab	10 b	0.5 ab
Regalia 3.8 L + JMS Stylet-Oil 7.6 L	4/28 & 5/5	40 a	2.0 a	1.0 ab	4 a	0.1 a

[z] Mean separation by Waller-Duncan K-ratio t-test (p=0.05). Four single tree replications.

[y] Foliar data represent counts of the first 10 leaves of 8 terminal shoots from each replicate tree on 19 Aug.

Table 3.9 Apple scab, and cedar-apple rust infection levels by bloom thinning treatments applied to 'York'/'M.9' in 2014.[zy]

Bloom treatment and rate/378.5 L	Bloom timing	Apple scab % infection		Cedar-apple rust % infection	
		lvs	les/lf	lvs	les/if
No Fungicide	----	92 d	23.3 d	81 b	15.9 b
Lime Sulfur 7.6 L + JMS Stylet-Oil 7.6 L	5/2 & 5/6	51a	2.8 a	42 a	3.3 a
Lime Sulfur 7.6 L + JMS Stylet-Oil 3.8 L	5/2 & 5/6	60 ab	3.0 a	44 a	3.7 a
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	5/2 & 5/6	63 b	3.7 a	31 a	2.8 a
Regalia 3.8 L + JMS Stylet-Oil 7.6 L	5/2 & 5/6	78 c	6.0 ab	34 a	3.3 a
Regalia 3.8 L + JMS Stylet-Oil 7.6 L	5/2 & 5/6	83 c	9.2 c	41 a	4.5 a
Regalia 1.9 L + JMS Stylet-Oil 3.8 L	5/2 & 5/6	80 c	8.1 bc	44 a	5.2 a

[z] Mean separation by Waller-Duncan K-ratio t-test (p=0.05). Four single tree replications.

[y] Foliar data represent counts of the first 10 leaves of 8 terminal shoots from each replicate tree on 18 Aug.

Table 3.10 Crop load and fruit finish following bloom thinning treatments applied to ‘Ginger Gold’/‘M.26’ in 2011.[z]

Bloom treatment and rate/378.5 L	Bloom timing	Percent of optimal crop load on tree	Difference in crop load from control (%)	Fruit finish assessments: Percent of fruits with side russet[y]	Fruit finish assessments: postharvest russet ratings: % fruit area russeted[x]	Fruit finish assessments: postharvest russet ratings: Stem-end russet (0-5)[x]
		14-Jul		14 Jul		
No Fungicide	----	96 b	0 b	4 a	0.8 a	1.1 a
Lime Sulfur 7.6 L + Crocker's Fish Oil 7.6 L	4/19, 22, & 27	66 ab	-31 ab	28 b	9.7 bc	1.7 ab
Lime Sulfur 7.6 L + Crocker's Fish Oil 7.6 L	4/20, 22, & 27	30 a	-69 a	34 b	7.0 b	2.3 b
Lime Sulfur 7.6 L + Crocker's Fish Oil 7.6 L	4/22 & 27	78 ab	-19 ab	29 b	7.2 b	1.8 ab
Lime Sulfur 7.6 L + JMS Stylet-Oil 7.6 L	4/22 & 27	66 ab	-31 ab	30 b	12.8 cd	1.8 ab
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/22 & 27	73 ab	- 24 ab	49 b	14.0 d	1.3 a
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L + Rally 40 WD 35.4 g	4/22 & 27	120 b	+25 b	45 b	12.6 cd	1.6 ab
Rally 40W 35.4 g	4/22 & 27	125b	+30 b	3 a	0.5 a	1.1 a

[z] Mean separation by Waller-Duncan K-ratio t-test. Four single tree replications.

[y] Fruit russet ratings are means of 25-fruit samples from each of four single-tree replications on the tree or [x] after harvest on 16 Jul.

Table 3.11 Crop load and fruit finish following bloom thinning treatments applied to 'Ginger Gold'/'M.26' in 2012.[z]

Bloom treatment and rate/378.5 L	Bloom timing	Percent of optimal crop load on tree	Difference in crop load from control (%)	Fruit finish assessments: Side russet rating (0-5)[y]	Fruit finish assessments: Stem-end russet (0-5)[y]
		14-Aug		8 Aug	8 Aug
No Fungicide	----	51.3 b	0 b	0.9 a	1.3 ab
Lime Sulfur 7.6 L + JMS Stylet-Oil 3.8 L	4/3 & 4/5	12.5 a	-76 a	1.8 b	1.6 b
Lime Sulfur 7.6 L + JMS Stylet-Oil 7.6 L	4/3 & 4/5	13.5 a	-74 a	1.9 b	1.5 b
Lime Sulfur 7.6 L + Crocker's Fish Oil 7.6 L	4/3 & 4/5	8.5 a	-83 a	1.8 b	1.7 b
Regalia 0.9 L + Latron B-1956 0.2 L	4/3 & 4/5	16.9 a	-67 a	0.7 a	1.0 a
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/4 & 4/6	32.5 ab	-37 ab	0.8 a	1.6 b

[z] Mean separation by Waller-Duncan K-ratio t-test (p=0.05). Four single tree replications.

[y] Fruit russet ratings are means of 25-fruit samples from each of four single tree replications. Harvested on 2 Aug and evaluated on 8 Aug after stored in cold storage for 6 days.

Table 3.12 Crop load and fruit finish following bloom thinning treatments applied to 'Ginger Gold'/'M.26' in 2013.[z]

Bloom treatment and rate/378.5 L	Bloom timing	Percent of optimal crop load on tree	Difference in crop load from control (%)	Postharvest russet rating (0-5)[y]
		29-Jul		19-Aug
No Fungicide	----	94 a	0 a	1.2 a
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/23 & 5/1	54 bc	-43 bc	1.8 b
Lime Sulfur 3.8 L + JMS Stylet-Oil 3.8 L	4/24 & 5/1	53 bc	-44 bc	2.6 b
Lime Sulfur 7.6 L + JMS Stylet-Oil 7.6 L	4/24 & 5/1	23 c	-76 c	3.0 c
Regalia 3.8 L + Latron B-1956 0.2 L	4/24 & 5/1	73 ab	-22 ab	1.4 ab
Regalia 3.8 L + JMS Stylet-Oil 3.8 L	4/24 & 5/1	55 bc	-41 bc	1.9 b
Syllit 3.4F 0.2 L	4/24 & 5/1	86 ab	-9 ab	1.5 ab

[z] Mean separations by Waller-Duncan K-ratio t-test (p=0.05). Four single tree replications.

[y] Fruit russet ratings are means of 25-fruit samples from each of four single-tree replicates after harvest.

Table 3.13 Crop load and fruit finish following bloom thinning treatments applied to ‘Ginger Gold’/‘M.26’ in 2014.[z]

Bloom treatment and rate/378.5 L	Bloom timing	Percent of optimal crop load on tree	Difference in crop load from control (%)	Postharvest russet rating (0-5)[y]
		23-Jun		19-Aug
No Fungicide	----	60 a	0 a	1.3 a
Lime Sulfur 7.6 L + JMS Stylet-Oil 7.6 L	4/28 & 5/5	25 b	-58 b	2.5 b
Regalia 3.8 L + JMS Stylet-Oil 3.8 L	4/28 & 5/5	34 b	-43 b	1.3 a
Regalia 3.8 L + JMS Stylet-Oil 7.6 L	4/28 & 5/5	36 b	-40 b	1.2 a

[z] Mean separations by Waller-Duncan K-ratio t-test (p=0.05). Four single tree replications

[y] Fruit russet ratings are means of 25-fruit samples from each of four single tree replicates after harvest

Chapter 4: Managing crop load and diseases with bloom thinning applications in an organically managed ‘Honeycrisp’/‘MM.111’ orchard

Abstract

Few growers have chosen to grow apples (*Malus* x *domestica* Borkh.) under organic management in Virginia due to considerable weed, insect, and disease pressure. In addition, little research has been completed on organic management in this region due to the low numbers of growers attempting to grow organic fruit; however, the demand for organic produce is increasing. Applications of bloom thinning chemicals were evaluated for their early season disease control of powdery mildew [*Podosphaera leucotricha* (Ellis & Everh.) E. S. Salmon], and cedar-apple rust (*Gymnosporangium juniperi-virginiana* Schwein.) in an organically managed ‘Honeycrisp’/‘M.M.111’ orchard. Labeled rates of lime sulfur or Regalia® were applied as flower thinning agents with application timing based on a ‘Honeycrisp’ specific, temperature-based pollen tube growth model. All treatments reduced crop load compared to the non-treated control, and after one application of lime sulfur or Regalia®, the number of fertilized king blooms was reduced and zero percent of side blooms had been fertilized. All treatments increased fruit peel russet, but russeting was the most severe when lime sulfur was applied as the second treatment. Cedar-apple rust percent infection was reduced by all treatments. These results suggest lime sulfur and Regalia® applied as bloom thinners can reduce crop load and decrease early season disease infection in organic orchards.

Introduction

Growing apples (*Malus* x *domestica* Borkh.) organically using the current USDA-National Organic Program scheme in Virginia can be very challenging due to the immense insect, disease, and weed pressure in this region (National Organic Program, 2016). Some of these challenges are unique to organic production due to the lack of effective materials and available techniques for organic growers. As the demand for organic products remains high and new tools and techniques become available for organic growers, there is a need to conduct research specifically for organic production. Through this project we targeted some of the key barriers, namely reliable crop load and disease management practices, that have prevented Virginia growers from adopting organic apple production methods.

Crop load management is essential for improving fruit size and color, preventing broken limbs due to the excess weight of fruit, and ensuring adequate return bloom. Reducing crop load, or thinning, can also decrease disease pressure by eliminating humid microclimates in the middle of fruit clusters, allowing for complete spray coverage of the developing fruitlets (Peck and Merwin, 2010). Thinning during bloom increases these benefits and decreases the likelihood of biennial bearing cycles by eliminating excess fruit before flower bud initiation takes place in the summer (Batjer and Hoffman, 1951; Greene, 2002).

In this experiment we evaluated bloom thinning treatments applied following the output from a ‘Honeycrisp’-specific pollen tube growth model. For nearly a decade, the Washington Tree Fruit Research Commission has funded apple pollen tube growth research at Virginia Tech. This research has culminated into an empirically derived model that can be used to improve the timing of apple bloom thinning applications (Yoder et al., 2013). The model uses hourly temperature data to calculate the amount of time between pollination and fertilization. This information is then used to allow a pre-set percentage of flowers to set fruit, while later blooming flowers are prevented from setting fruit by treatment with a caustic material. Liquid lime sulfur tank mixed with oil (either fish or a petroleum based product) has become the standard for apple tree bloom thinning (McArtney et al., 2006; Peck and Merwin, 2010). However, outside of Washington State, no lime sulfur products have been labeled for bloom thinning or for use with oil. For these reasons, additional bloom thinning products are needed for the Eastern U.S.

The organically-approved biofungicide, Regalia® (Marrone Bio Innovations, Inc., Davis, CA), made from the extracts of Giant Knotweed [*Reynoutria sachalinensis* (F. Schmidt) Nakai syn. *Polygonum sachalinense* F. Schmidt], has shown potential as a reliable bloom thinner on apple (Peck et al., 2015; Yoder et al., 2013; Chapter 3). Additionally, prior tests at the Alson H. Smith, Jr. Agricultural Research and Extension Center (AREC) indicate that control of rusts by a combination of Regalia® and JMS Stylet-Oil (JMS Flower Farms, Inc., Vero Beach, FL) is unique among the limited organic options for disease management (Peck et al., 2015; Chapter 3). Among the many fungal diseases that need to be managed in Virginia, cedar-apple rust (*Gymnosporangium juniperi-virginiana* Schwein.) and quince rust (*Gymnosporangium clavipes* Cooke & Peck) are formidable barriers to organic apple producers. Regalia®, currently labelled for use on several horticultural and row crops, may replace azoles, strobilurins and sulfur sprays for control of leaf spot diseases and powdery mildew, and is a useful alternative in areas of fungicide resistance (Su et al., 2012). *R. sachalinensis* extracts, in a previous formulation called Milsana®, have decreased powdery mildew infection on cucumbers, tomatoes, begonias and wheat (Daayf et al., 1995; Dik and VanDerStraay, 1995; Herger et al., 1988; Herger and Klingauf, 1990; Konstantinidou-Dolsinis and Schmitt, 1998; Konstantinidou-Dolsinis et al., 2006).

In this project, we tested the use of lime sulfur and Regalia® in different combinations to achieve a greater range of disease control in organic systems while also reducing crop load. This approach explored the use of registered rates of the fungicides, lime sulfur and Regalia®, to bloom thin apples and ultimately reduce the number of necessary fungicide applications.

Materials and Methods

This study was conducted on a mature plot of ‘Honeycrisp’/‘MM.111’ apple trees located at the Alson H. Smith, Jr. AREC in Winchester, Virginia. All trees had been managed conventionally before the start of this experiment in Spring 2015. Fifty king bloom flowers were collected and used to obtain an average style length. This data was used in a ‘Honeycrisp’ specific pollen tube growth model to determine treatment application timing (Yoder et al., 2013).

Bloom thinning treatments consisted of lime sulfur (Miller Chemical & Fertilizer Corp., Hanover, PA), or Regalia® (Marrone Bio Innovations, Davis, CA) applied at registered rates with JMS Stylet-Oil (JMS Flower Farms, Inc., Vero Beach, FL). An organic “grower standard” bloom thinning treatment of lime sulfur and JMS Stylet-Oil applied at 10 mm, a treatment of hand-thinned blossoms, and a non-treated control were also included in the experiment.

Each treatment consisted of four single-tree replications in a completely randomized block design. Blocking was based on bloom density ratings before the first treatment application. Treatments and maintenance sprays were applied dilute to runoff at 1723 kPa by an airblast sprayer. Two branches per tree were used for measurement of crop load, assessed on 3 Jun. Fruit size and russet ratings were taken on 20 Aug. Twelve king bloom flowers and 12 side bloom flowers per treatment were collected 24 h after one application of lime sulfur or Regalia®. Collected samples were preserved until histological examination in a 5% sodium sulfite

(Amresco, Solon, OH) solution to stop pollen tube growth. Flowers were boiled for 15 min to soften tissues. Carpels were then excised and pressed between two microscope slides with a water-soluble solution of 0.01% Aniline Blue (MP Biomedicals, LLC, Solon, OH) stain in 0.067 M K_2HPO_4 for pollen tube visualization. Slides were placed in a dark cabinet for 24 h before visualization with an epi-ultraviolet light using a Zeiss HBO-50 high pressure mercury vapor light source (OSRAM GmbH, Ausburg, Germany) and a Nikon Optiphot microscope (Mellville, NY) at 100x (Yoder et al., 2009, Yoder et al., 2013). Flowers were considered fertilized when pollen tube growth could be seen at the base of the style and in the ovary.

Statistical analysis was completed using analysis of variance (PROC ANOVA, SAS Inst. Inc, Cary, NC), and mean separations were determined by Tukey's Honestly Significant Difference or the Waller-Duncan K-ratio t-test.

Results

The 'Honeycrisp' pollen tube growth model was started on 20 Apr at 1000 hr and thinning treatments were applied on 27 Apr and 29 Apr (Fig. 4.1). Return bloom was low in this orchard and the non-treated control had 6.8 fruit per cm^2 branch cross sectional area (BCSA). All treatments reduced crop load compared to the untreated control, but there were no differences among the treatments (Table 4.1). The double lime sulfur application treatment at bloom had a similar fruit weight to the control, and all other treatments had greater fruit weight than those two treatments. The double Regalia® and lime sulfur/JMS Stylet-Oil at 10 mm treatments had greater fruit weight than the control and the double lime sulfur at bloom treatments. Fruit diameter was similar for the control, double lime sulfur at bloom, and hand-thinned treatment, and the lime sulfur/JMS Stylet-Oil at 10 mm had a greater diameter than those treatments. Fruit length was similar for the control and double lime sulfur at bloom, both of which had shorter fruit length than the other treatments. The double Regalia® treatment and lime sulfur/JMS Stylet-Oil increased fruit weight, diameter, and length compared to control. All chemical bloom thinning treatments resulted in increased russet. When lime sulfur was used as the second thinning treatment, russet was the most severe, and when Regalia® was applied as the second thinning treatment russet was the least severe.

Ninety-two percent of the control king flowers were fertilized, while 67 and 50% of the king flowers were fertilized after the first treatments of lime sulfur and Regalia®, respectively (Fig. 4.2). Twenty-five percent of the side bloom flowers on control trees were fertilized, while 0% of side bloom flowers were fertilized on trees treated with lime sulfur or Regalia®, respectively.

Powdery mildew disease pressure in the test area was typical for the region in 2015. The hand-thinned treatment had a greater percentage of leaves with powdery mildew infection than the control, double Regalia®, and 10 mm lime sulfur/JMS Stylet-Oil treatments (Table 4.2). The percent of leaf area infected with powdery mildew was very similar for all treatments, but statistically greater for the hand thinned treatment than the double lime sulfur, double Regalia®, lime sulfur/Regalia®, and 10 mm lime sulfur/JMS Stylet-Oil treatments.

Rust infection periods that were most likely affected by treatments occurred on 14-15 Apr, 30 Apr-1 May, 1-2 May, 5-6 May, and 6-7 May (Table 4.2). Quince rust infections begin during bloom and blossoms mostly escaped infection in 2015 (data not shown). Cedar-apple rust was consistently found on control and hand-thinned trees more so than any of the chemical thinning applications (Table 4.2). The 10 mm fruitlet application of lime sulfur/JMS Stylet-Oil had less cedar-apple rust lesions than the non-sprayed treatments.

The number of leaves per shoot was not different among treatments (Table 4.2). The double lime sulfur and the Regalia®/lime sulfur treatments resulted in the greatest percent defoliation in the first 20 leaves. The control, double Regalia®, and hand-thinned treatments had less defoliation than the other treatments.

Discussion

The overall goal of this study was to determine if lime sulfur and Regalia®, applied for bloom thinning could sufficiently reduce crop load as well as powdery mildew and cedar-apple rust infection in an organically-managed ‘Honeycrisp’/‘MM.111’ orchard. We evaluated blossom thinning chemicals as the only fungicide cover sprays applied during bloom. All chemical thinning treatments evaluated reduced crop load and cedar-apple rust infection compared to control.

Our results indicate Regalia® and lime sulfur when applied with JMS Stylet-Oil provide similar bloom thinning results; matching previous research on bloom thinning applications of these chemicals on ‘Ginger Gold’ trees (Chapter 3). Previously, when applied with the spreader/sticker Latron B-1956, Regalia® reduced crop load, and fruit from Regalia® treatments had lower russet ratings than fruit from lime sulfur treatments (Yoder et al., 2013). Regalia® and lime sulfur both inhibited pollen tubes from reaching the base of the style and fertilizing the egg in side-bloom flowers (Fig. 4.2). As a bloom thinner, the mode of action of Regalia® is unknown, but it may inhibit pollination and fertilization by damaging floral organs, indicating the pollen tube growth model could also be used for Regalia® applications (Peck et al., 2015; Yoder et al., 2013).

All chemical thinning treatments would be considered ‘over-thinned’ (too few fruit) in a commercial setting; however, the ‘Honeycrisp’ block used in this experiment had low levels of bloom at the start of the project. The non-thinned control treatment had 6.8 fruit/cm^2 BCSA, while the target crop load for mature ‘Honeycrisp’/‘MM.111’ trees is ≈5 fruit/cm^2 BCSA indicating the amount of thinning needed initially in this orchard block was minimal (Robinson et al., 2009). The chemical thinning treatments used in this experiment may not ‘over-thin’ in years when flower density is greater.

Treatments of lime sulfur applied on 29 Apr (max temp, 24°C) resulted in lower fruit/cm^2 BSCA, and higher fruit russet ratings than those of Regalia® treatments applied on the same day. Lime sulfur is phytotoxic and may result in several negative side effects, including increased russet, and reduced photosynthesis, especially when applied during warmer temperatures (Burrell, 1945; Cromwell, 2011; Hoffman, 1935; Holb et al., 2003; Hyre, 1939; McArtney et al., 2006; Palmer et al., 2003). Our results indicate Regalia® can be applied with lower phytotoxic risks o than lime sulfur.

Bloom thinning applications of lime sulfur and Regalia® did not reduce mildew percent infection compare to the non-treated control (Table 4.2). In past evaluations, these chemicals have reduced powdery mildew percent infection, but these results were not consistent over a four-year period (Chapter 3). *R. sachalinensis* extracts have been reported to reduce powdery mildew infection on a wide variety of horticultural crops (Su et al., 2012). Differences may not have been evident in our results due to the Microthiol Disperss applied on all treatments during the early summer for protection against powdery mildew (Table 4.3). Trees with no chemical pest control applications all season, located at the ends of the test blocks, were not used for analysis because they were not replicated within the blocks; however, these trees show the high mildew pressure in the area. Trees with no chemical control had 59 and 69% of leaves infected with mildew, compared to an average of 16% of leaves infected on treatment trees (Table 4.2).

These results add to previous reports of the effectiveness of Regalia® against cedar-apple rust infection (Chapter 3). Sulfur (Microthiol Disperss) has a general suppressive effect on mildew and can be widely used in organic production; however, rust diseases are more difficult to control. Selective use of Regalia®/JMS Stylet-Oil could decrease cedar-apple rust infection in organic orchards.

Through our results, we provide evidence that bloom thinning sprays of lime sulfur and Regalia® can reduce crop loads and decrease cedar-apple rust infection. Bloom thinning applications could take the place of a fungicide cover spray, leading to an overall reduction in the number of chemicals used. Our findings indicate the new biofungicide, Regalia®, could provide unique protection against damaging cedar-apple rust, reduce crop load during years of high return bloom, and result in an overall reduction in the number of required chemical sprays.

Literature Cited

Batjer, L. P. and M.B. Hoffman. 1951. Fruit thinning with chemical sprays. U.S. Dept. Agr. Circ. 867 Washington, D. C.

Burrell, A.B. 1945. Practical use of our newer knowledge of apple scab control. Proc. New York State Hort. Soc. 90:9-16.

Cromwell, M.L., L.P. Berkett, and H.M. Darby. 2011. Alternative organic fungicides for apple scab management and their non-target effects. HortScience 46:1254-1259.

Daayf, F., A. Schmitt, and R.R. Belanger. 1995. The effects of plant extracts of *Reynoutria sachalinensis* on powdery mildew development and leaf physiology of long English cucumber. Plant Dis. 79:577-580.

Dik, A.J. and VanDerStraay. 1995. The effects of Milsana® on cucumber powdery mildew under Dutch conditions. Med. Fac. Landbouww. Rijksuniv. Gent. 59:1027-1039.

Greene, D.W. 2002. Chemicals, timing, and environmental factors involved in thinner efficacy on apples. HortScience 37:477-481.

Herger, G., F. Klingauf, D. Mangold, E.H. Pommer, and M. Scherer. 1988. Efficacy of extracts of *Reynoutria sachalinensis* (F. Schmidt) Nakai (Polygonaceae) against fungal diseases, especially powdery mildews (in German). Nachrichtenbl Dtsch Pflanzenschutzdienst (Braunschweig) 40:56-60.

Herger, G. and F. Klingauf. 1990. Control of powdery mildew fungi with extracts of the giant knotweed, *Reynoutria sachalinensis* (Polygonaceae). Med. Fac. Landbouww Rijksuniv Gent. 55:1007-1014.

Hoffman, M.B. 1935. The effect of lime-sulfur spray on the respiration rate of apple leaves. Proc. Amer. Soc. Hort. Sci. 33:173-176.

Holb, I.J., P.F. DeJong, and B. Heijne. 2003. Efficacy and phytoxicity of lime sulfur in organic apple production. Ann. Appl. Biol. 142:225-233.

Hyre, R.A. 1939. The effect of sulfur sprays on the photosynthesis and transpiration of apple leaves. N.Y. Agr. Exp. Sta. Mem. 222.

Konstantinidou-Doltsinis, S. and A. Schmitt. 1998. Impact of treatment with plant extracts from *Reynoutria sachalinensis* (F. Schmidt) Nakai on disease severity of powdery mildew and yield in cucumber under Greek conditions. Crop Prot. 17:649-656.

Konstantinidou-Doltsinis, S., E. Markellou, A.M. Kasselaki, M.N. Fanouraki, C.M. Koumaki, A. Schmitt, A. Liopa-Tsakalidis, and N.E. Malathrakis. 2006. Efficacy of Milsana®, a formulated plant extract from *Reynoutria sachalinensis*, against powdery mildew of tomato (*Leveillula taurica*). BioControl 51:375-392.

McArtney, S., J. Palmer, S. Davies, and S. Seymour. 2006. Effects of lime sulfur and fish oil on pollen tube growth, leaf photosynthesis and fruit set in apple. HortScience 41:357-360.

National Organic Program. 2016. Organic Regulations. U.S. Dept. Agr., Washington, D.C. 25 Jan. 2016. <http://www.ams.usda.gov/rules-regulations/organic/>

Palmer, J.W., S.B. Davies, P. Shaw, and J.N. Wünsche. 2003. Growth and fruit quality of ‘Braeburn’ apple trees as influenced by fungicide programmes suitable for organic production. N.Z.J. Crop Hort. Sci. 31:169-177.

Peck, G.M. and I.A. Merwin. 2010. A grower’s guide to organic apples. NYS IPM Publication No. 223.

Peck, G.M., L.D. Combs, C.N. DeLong, and K.S. Yoder. 2015. Precision apple flower thinning using organically-approved chemicals. Acta Hort. (in press)

Robinson, T., S. Lopez, K. Iungerman, and G. Reginato. 2009. Crop load management for consistent production of Honeycrisp apples. NY Fruit Quart. 17:24-28.

Su, H., R. Blair, T. Johnson. and P. Marrone. 2012. Regalia® bioprotectant in plant disease management. Outlooks on Pest Mgt. 23:30-34.

Yoder, K.S., G.M. Peck, L.D. Combs, and R.E. Byers. 2013. Using a pollen tube growth model to improve apple bloom thinning for organic production. Acta Hort. 1001:207-214.

Table 4.1 Crop load, fruit weight and size, and chemical russet incidence from a test of bloom thinning chemical in an organically managed ‘Honeycrisp’/‘MM.111’ orchard in 2015.[v]

Bloom thinning treatment							
1st app. 27 Apr	2nd app. 29 Apr	Crop Load (fruit/cm^2 branch cross sectional area)[z]	Fruit Weight (g)[y]	Fruit Diameter (mm)[y]	Fruit Length (mm)[y]	Chemical Russet (%)[y]	Chemical Russet Rating (0-5)
No treatment	No treatment	6.8 a[x]	166.6 c[y]	60.5 d[y]	73.7 cd[y]	0 a[y]	2.2 a
LS + SO	LS + SO	1.7 b	170.5 bc	61.6 cd	73.3 d	75 cd	4.0 cd
LS + SO	Regalia + SO	2.6 b	185.3 ab	63.4 bc	76.1 bc	59 c	3.7 c
Regalia + SO	Regalia + SO	3.3 b	195.6 a	64.0 ab	77.7 ab	36 b	3.1 b
Regalia + SO	LS + SO	1.5 b	187.2 ab	63.2 bc	76.6 ab	81 d	4.2 d
LS + SO at 10 mm fruitlet size (organic standard)		2.6 b	202.3 a	65.9 a	78.8 a	0 a	2.7 a
Hand-thinned flowers on selected limbs[w]		3.7 b	185.5 ab	62.6 bcd	76.4 a	0 a	2.5 a

[z] Crop load measured on 03 June .

[y] Fruit size and russet measured on 20 Aug .

[x] Mean separation within column by Tukey’s Honestly Significant Difference or [y] Waller-Duncan K-ratio (p=0.05).

[w] Hand thinning occurred 3-5 June.

[v] Full bloom occurred on 25 Apr and petal fall occurred on 4 May

Treatment Code: LS+SO= Lime Sulfur 18.8 L + JMS Stylet-Oil 9.4 L/935 $L \cdot ha^{-1}$; Regalia + SO = Regalia 9.4 L + JMS Stylet-Oil 9.4 L/935 $L \cdot ha^{-1}$. Bloom thinning application dates for trts #1-4: 27 Apr, 29 Apr; 10 mm fruit size applied on 11 May.

Table 4.2 Disease control by bloom-thinning treatments applied to ‘Honeycrisp’/‘MM.111’.[w]

Bloom thinning treatment[yw]		Foliar diseases				Number of leaves	% defoliation,
		Powdery mildew		Cedar-apple rust			
1st app. 27 Apr	2nd app. 29 Apr	% leaves	% lf area	% leaves	lesions /lf	per shoot	first 20 lvs
No treatment	No treatment	15 ab	3 ab	22 c	0.5 b	27 a	2 a
LS + SO	LS + SO	16 a-c	3 a	14 ab	0.3 ab	27 a	8 bc
LS + SO	Regalia + SO	18 bc	3 ab	15 b	0.3 ab	25 a	5 b
Regalia + SO	Regalia +SO	14 ab	2 a	13 ab	0.3 ab	24 a	2 a
Regalia + SO	LS + SO	15 a-c	2 a	14 ab	0.3 ab	25 a	10 c
10 mm-fruit LS + SO (organic standard)		10 a	2 a	9 a	0.2 a	25 a	5 b
Hand-thinned flowers on selected limbs		22 c	3 b	22 c	0.5 a	27 a	2 a
No treatment all season- south end		59	22	35	1.3	24	1
No treatment all season- north end		69	15	38	1.4	27	1

[z] Column mean separation by Waller-Duncan K-ratio t-test (p=0.05). Four single-tree replications, ten shoots per replication, rated 12 Aug.

[y] Bloom thinning application dates for trts #1-4: 27 Apr, 29 Apr; 10 mm fruit size app. for #6, 11 May.

[w] Treatments applied dilute to runoff at 250 psi.

Treatment code: LS+SO= Lime Sulfur 7.6 L + JMS Stylet Oil 9.4 L/935 L·ha^{-1} L; Regalia + SO = Regalia 3.8 L + JMS Stylet Oil 3.8 L/378.5 L·ha^{-1}

Table 4.3 Organic maintenance application schedule for 'Honeycrisp'/'MM.111' in 2015.[z]

Date	Stage	Disease Control, Rate/ha	Target Disease	Insect control, Rate/ha	Target Pest
18 Apr	Pink	Cueva 9.4 L + Double Nickel 2.3 L	Scab, fire blight	None	
6 May	Petal Fall	Cueva 9.4 L + Double Nickel 2.3 L	Scab, fire blight	Entrust 0.6 L + Pyganic 1.4L 3 L + Therm X 0.3 L	Codling moth (CM), Oriental fruit moth (OFM) Plum curculio (PC)
20 May	1st Cover	Microthiol Disperss 9 kg	Mildew	Madex HP 0.1 L + Deliver 0.5 kg	CM, OFM, Leaf rollers (LR)
27 May	2nd Cover	Microthiol Disperss 9 kg	Mildew	Madex HP 0.1 L + Deliver 0.5 kg	LR, Brown marmorated stink bug (BMSB)
6 Jun	3rd Cover	Microthiol Disperss 9 kg	Mildew	Deliver 8 oz + Pyganic 1.5 L + Therm X 0.3 L	LR. BMSB, Japanese beetle (JB)
18 Jun	4th Cover	Microthiol Disperss 9 kg	Mildew	Deliver 8 oz + Pyganic 1.5 L + Therm X 0.3 L	CM, OFM, JB
1 Jul	5th Cover	Cueva 4.7 L + Double Nickel 2.3 L	Summer diseases	Entrust 0.6 L + Madex HP 0.1 L + Deliver 0.5 kg + Therm X 0.3 L	CM, OFM, JB, Apple maggot (AM)
15 Jul	6th Cover	Cueva 4.7 L + Double Nickel 2.3 L	Summer diseases	Deliver 0.5 kg	CM, OFM,
31 Jul	7th Cover	Cueva 4.7 L + Double Nickel 2.3 L	Summer diseases	Entrust 0.6 L + Pyganic 2.3 L	OFM, AM, JB
6 Aug	8th Cover	Cueva 4.7 L + Double Nickel 2.3 L	Summer diseases	None	

[z] Weed management with Suppress Herbicide EC, 28.3 L/467.2 L·ha^{-1} 23 May and 4 Jul.

Fig. 4.1 The ‘Honeycrisp’ specific pollen tube growth model. Temperature data were collected on site from a Virginia Agricultural Experiment Station mesonet weather station. Average style length = 9.0 mm.

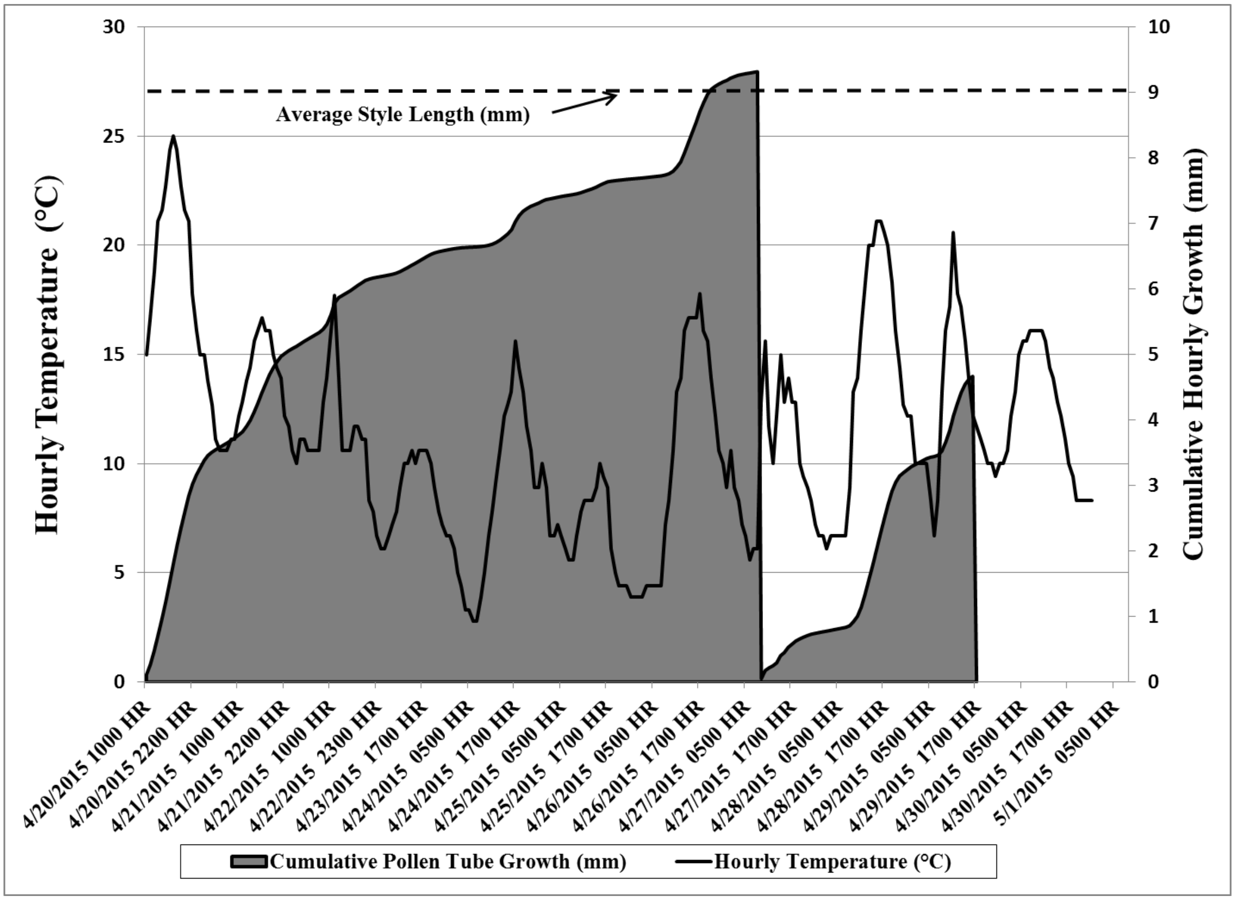
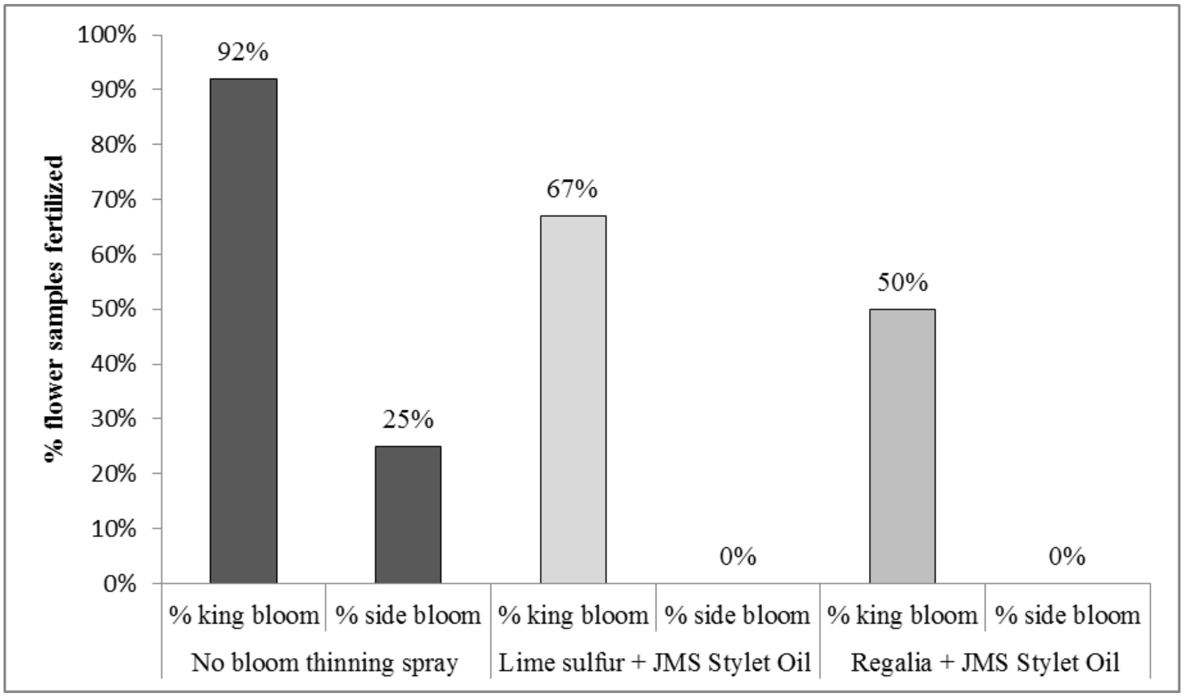


Fig. 4.2 Percent of fertilized king and side flowers 24 h after the first thinning application on 27 Apr.



Chapter 5: Conclusions

When growing apples, some of the most important management decisions a grower will make involve crop load management. As more fruit are being grown for the fresh market, the size and appearance of the apple is increasing in importance. Chemical thinning, including applications made during flowering, is the most efficient way to obtain market-quality apples, and eliminate biennial bearing cycles. Improving blossom thinning practices is dependent on research clarifying the developmental biology of the apple flower, and on evaluation of environmentally friendly chemicals. This thesis primarily explored interactions among paternal pollen tube growth, maternal cultivar, and temperature to determine if pollen tube growth rates differ among cultivars. Secondly, Chapters 3 and 4 presented research evaluating bloom thinning application of fungicides for crop load reduction and protection against common early season apple diseases.

This research revealed a complex relationship between paternal pollen tube growth, maternal cultivar and temperature. The pollen tube growth rates of paternal cultivars did not consistently follow trends related to maternal cultivar or temperature. Meaning, one paternal cultivar did not have the faster or slowest pollen tube growth under all growth conditions evaluated. However, ‘Snowdrift’, the cultivar used to develop the pollen tube growth model, consistently had a slower rate of pollen tube growth than other evaluated cultivars, especially cultivars ‘Indian Summer’, ‘Selkirk’, and ‘Thunderchild’. In addition, ‘Snowdrift’, had the slowest rate of pollen tube growth at 30°C. Only one year (2015) of data was considered reliable for this project. If reliable data had been collected from 2014, clearer trends may have been found in relationships between paternal cultivars, maternal cultivars, and temperatures. However, in all evaluations completed for this thesis, ‘Snowdrift’ had the slowest overall pollen tube growth rate.

Fungicidal bloom thinning chemicals decreased disease infection on cultivars ‘Jonagold’, ‘York’, ‘Ginger Gold’, and ‘Fuji’ and also reduced ‘Ginger Gold’ crop load. Most notably, Regalia®, a biofungicide, provided equal disease protection and crop load reduction to lime sulfur when applied with JMS Stylet-Oil. Regalia® treatments resulted in lower fruit russet ratings than lime sulfur treatments. Similar results were found when treatments of lime sulfur and Regalia® with JMS Stylet oil were applied to organically-managed ‘Honeycrisp’ trees in 2015. The lower russet ratings and decreased infection of cedar-apple rust provided by Regalia® treatments indicate Regalia® could be a useful chemical in organic orchards, especially in the Eastern U.S. where applications of lime sulfur and oil are not labeled for use during bloom.

Crop load management is essential to profitably growing apples, and thinning during bloom provides growers with a great number of benefits. The research presented in this thesis sought to bring more science to a management practice that is too often referred to as an “art.” Complex relationships were discovered between paternal pollen tube growth and maternal cultivar in an attempt to provide more information on the developmental biology of the apple in relation to the use of bloom thinning chemicals. Also, the research presented in the thesis indicates organic production, especially in the Eastern U.S., could be improved by the use of Regalia® as both a bloom thinner and a fungicide.

Appendix A: Evaluating the pollen tube growth characteristics of selected crabapple cultivars, 2014.

Introduction

The pollen tube growth of crabapple cultivars, ‘Evereste’, ‘Indian Summer’, ‘Selkirk’, ‘Snowdrift’, and ‘Thunderchild’, growing in the styles of ‘Fuji’, ‘Golden Delicious’, and ‘Pink Lady’at four different temperatures, 12, 18, 24, and 30 °C is presented in this appendix. The primary goal of this experiment was to determine the pollen tube growth rate differences, if any, of the selected crabapple cultivars.

Data presented in this appendix was collected using the same experimental design and cultivars as those presented in Chapter 2. We decided the data were not reliable enough for use in a publication due to the following reasons: blossoms from ‘Golden Delicious’ and ‘Pink Lady’ had a high number of styles with no visible pollen on the stigma, or pollen tube growth in the style, and pollen tubes longer than one-third of the style length were found on non-pollinated control blossoms. For example, out of 25 ‘Golden Delicious’ styles pollinated with ‘Snowdrift’ pollen, only three of them had any visible pollen tube growth after 24 h in the growth chamber at 12°C. These three styles do not provide an accurate representation of ‘Snowdrift’ pollen tube growth. Pollen tube growth found on non-pollinated control blossoms was most likely not self-pollen, indicating pollen from paternal cultivars may have ended up on control blossoms, or other experimental blossoms.

Materials and Methods

Pollen used in 2014 was collected from mature ‘Evereste’, ‘Indian Summer’, ‘Selkirk’, ‘Snowdrift’, and ‘Thunderchild’ trees at the Alson H. Smith, Jr. Agricultural Research and Extension Center during bloom of 2010 through 2013. Trees were removed from the orchard in Dec. 2013 and kept in cold storage to accumulate sufficient winter chill hours to break endodormancy. A general linear model procedure was used to determine differences in the average length of the longest pollen tube in relation to the total style length (SAS Inst., Cary, NC). Effects and interactions included in the model were: maternal cultivar, paternal cultivar, temperature, maternal cultivar x temperature, and paternal cultivar x temperature. All other materials and methods are presented in Chapter 2.

Results

In germination testing before experiment, ‘Thunderchild’ pollen had the highest germination rate of 90%, followed by ‘Evereste’ at 85%, ‘Selkirk’ at 70%, ‘Indian Summer’ at 60%, and ‘Snowdrift’ at 45%. In contrast to data collected in 2015, the three way interaction, maternal cultivar x paternal cultivar x temperature, was not significant for the 2014 data indicating pollen tubes of paternal cultivars grew similarly in the styles of maternal cultivars at all four temperatures (Table A.1). In addition, the interaction between maternal cultivar and paternal cultivar was not significant for the data collected in 2014. Interactions between maternal cultivar x temperature and paternal cultivar x temperature were both significant ($P < 0.0001$).

Pollen tubes in ‘Fuji’ styles grew a greater distance than pollen tubes in ‘Pink Lady’ styles (Fig. A.1). Pollen tubes from ‘Thunderchild’ and ‘Selkirk’ grew the furthest distance down the style across all temperatures analyzed and on all maternal cultivars (Fig. A.2).

Pollen tube growth in the styles of maternal cultivars was significantly different during the 18°C trial and the 30°C trial (Fig. A.3). After 24 h at 18°C, pollen tubes in ‘Fuji’ and ‘Golden Delicious’ styles grew further than pollen tubes in ‘Pink Lady’ styles. After 24 h at 30°C, pollen tubes in ‘Golden Delicious’ styles grew further than pollen tubes in ‘Pink Lady’ styles.

After 24 h at 12 or 18°C there were no differences in the pollen tube growth of paternal cultivars. At 12°C only ‘Selkirk’ pollen tubes grew significantly further than pollen tubes found on control blossoms (Fig. A.4). ‘Indian Summer’, ‘Selkirk’, and ‘Thunderchild’ pollen tubes grew significantly further than the pollen tubes in control blossoms after 24 h at 18°C. After 24 h at 24°C, ‘Selkirk’ and ‘Thunderchild’ pollen tubes grew a further distance down the style than ‘Evereste’, and ‘Snowdrift’ as well as pollen tubes found on control blossoms. At 30°C, ‘Thunderchild’ pollen tubes grew further than ‘Evereste’, and ‘Snowdrift’, while ‘Indian Summer’ and ‘Selkirk’ pollen tubes grew further than ‘Evereste’. ‘Evereste’ and ‘Snowdrift’ pollen tubes grew the same distance down the style regardless of temperature. ‘Indian Summer’ pollen tube growth did not increase until the 30°C trial. ‘Selkirk’ pollen tubes grew further down the style at 24°C than at 12 and 18°C trials while ‘Thunderchild’ pollen tubes grew further down the style at 24 and 30°C than at 12 and 18°C.

Discussion

Although pollen tube growth of paternal cultivars was not affected by maternal cultivar as represented by the non-significant maternal x paternal interaction, we did find differences among the five paternal cultivars. Overall, ‘Thunderchild’ and ‘Selkirk’ pollen tubes grew the furthest distance relative to style length, followed by ‘Indian Summer’. When the experiment was repeated in 2015, the same three cultivars had the longest pollen tube growth (Chapter 2).

Pollen tube growth was the longest on ‘Fuji’ blossoms during 2014, but when the experiment was repeated in 2015, pollen tube growth was longer on both ‘Golden Delicious’ and ‘Pink Lady’ compared to ‘Fuji’. However, our results from 2014 may not provide an accurate representation because of the many ‘Golden Delicious’ and ‘Pink Lady’ blossoms without visible pollen on the stigma during 2014.

True conclusions cannot be made from this data due to the large amount of missing data, and the pollen tube growth found on non-pollinated control blossoms. In 2015, when the experiment was repeated, less pollen tube growth was found on control blossoms, and the majority of experimental blossoms had sufficient pollen germination and tube growth down the style.

Table A.1 Significance of effects and interactions included in the GLM procedure used to determine differences in pollen tube growth length compared to style length.

Effect	P-Value
Maternal Cultivar	<0.0001
Paternal Cultivar	<0.0001
Temperature	<0.0001
Maternal x Paternal	0.2210
Maternal x Temperature	<0.0001
Paternal x Temperature	<0.0001

Fig. A.1 Average percent of the total style length covered by pollen tube growth on blossoms from maternal cultivars, 'Fuji', 'Golden Delicious', and 'Pink Lady'. (Data combined from all four temperature trials, and all five paternal cultivars).

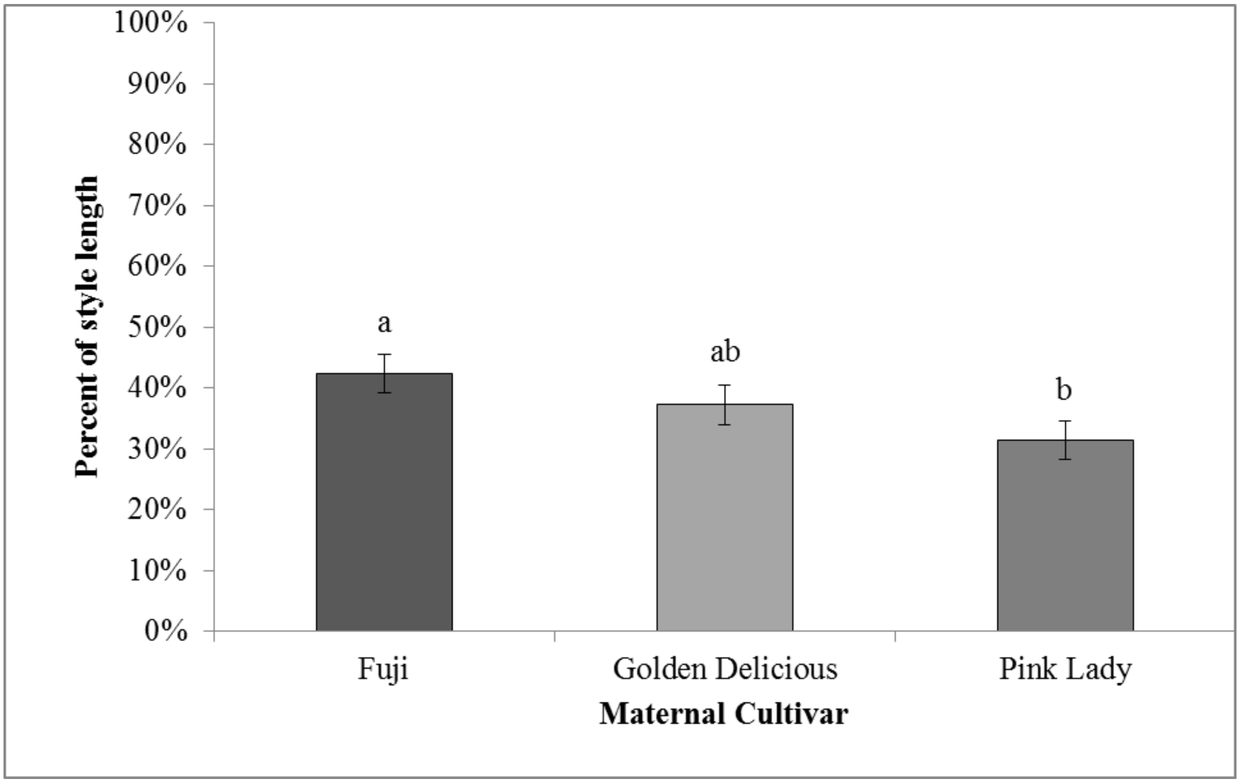


Fig. A.2 Average percent of the total style length covered by pollen tube growth of paternal cultivars ‘Evereste’, ‘Indian Summer’, ‘Selkirk’, ‘Snowdrift’, ‘Thunderchild’ and found in non-pollinated control blossom styles. (Data combined from all four temperature trials and all three maternal cultivars).

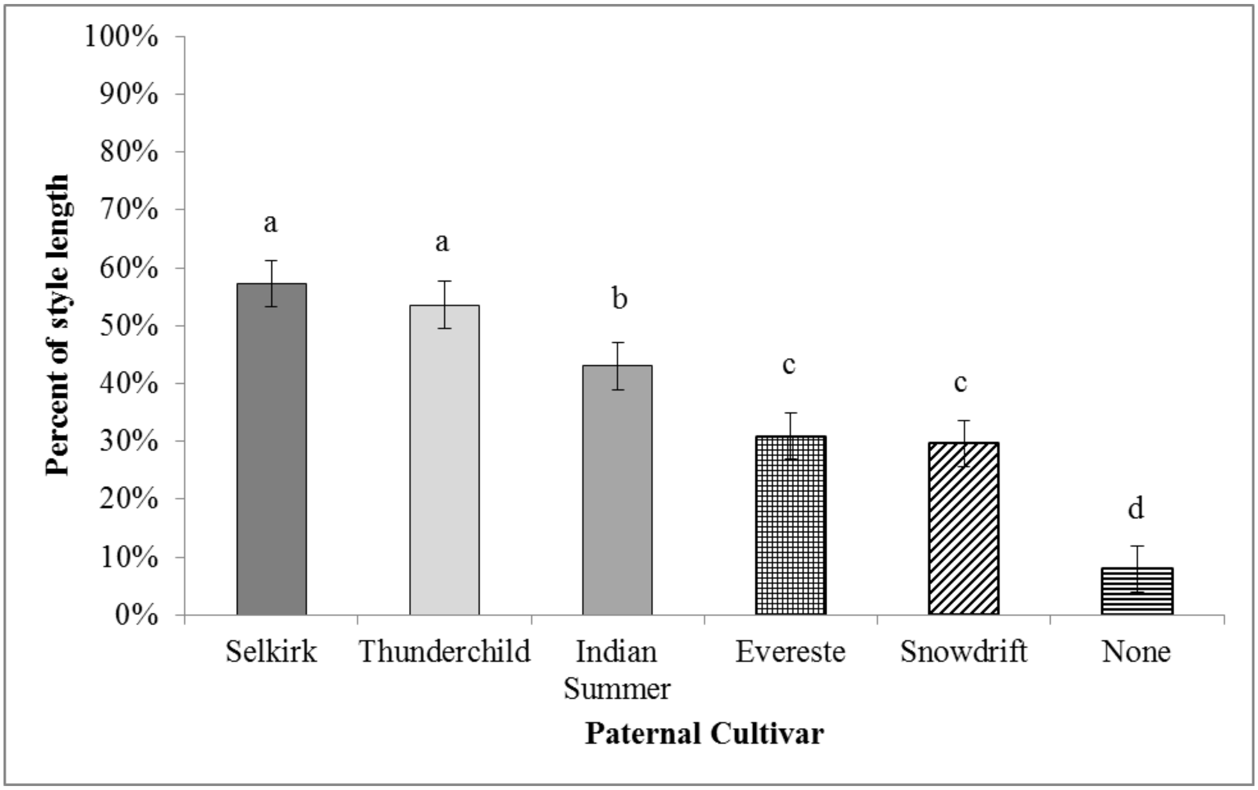


Fig. A.3 Average percent of the total style length covered by pollen tube growth on blossoms from maternal cultivars, ‘Fuji’, ‘Golden Delicious’, and ‘Pink Lady’ at temperatures 12, 18, 24, and 30°C. (Data combined from all five paternal cultivars).

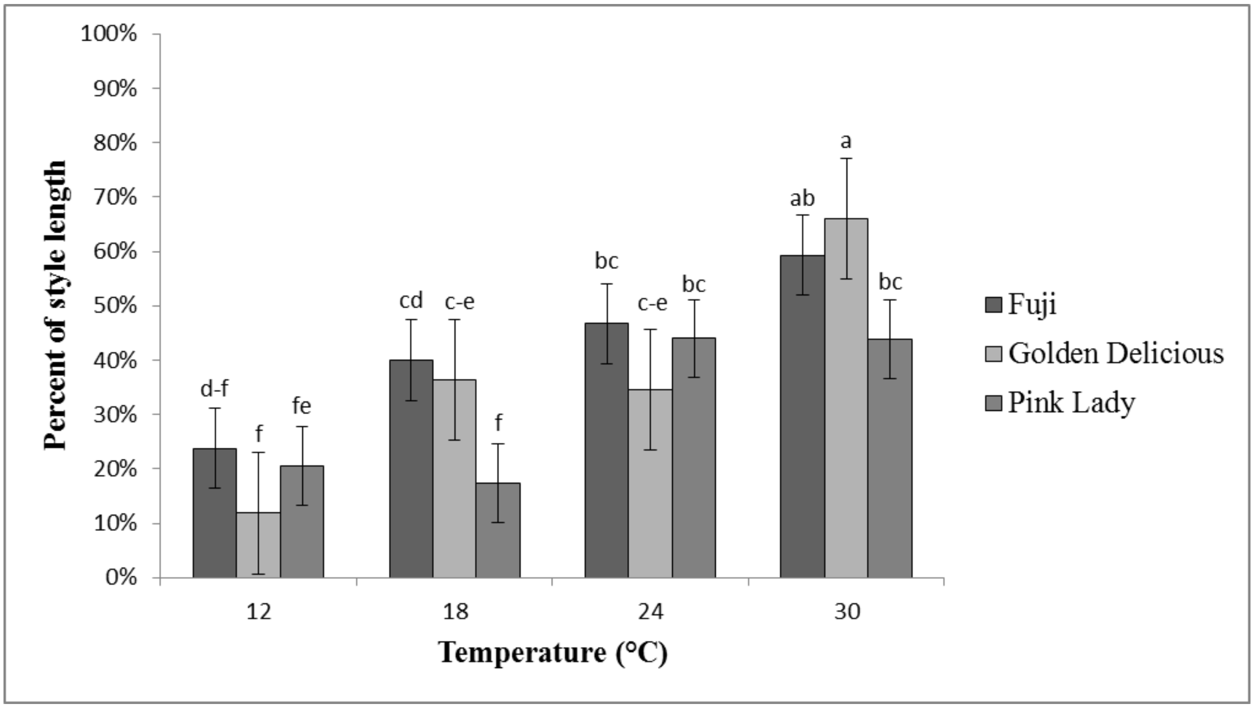


Fig. A.4 Average percent of the total style length covered by pollen tube growth of paternal cultivars ‘Evereste’, ‘Indian Summer’, ‘Selkirk’, ‘Snowdrift’, ‘Thunderchild’ and found in non-pollinated control blossom styles. (Data combined from all three maternal cultivars).