Managing Apple Crop Load and Diseases with Bloom Thinning Applications in an Organically Managed ‘Honeycrisp’/‘MM.111’ Orchard

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Additional index words. apple scab, cedar apple rust, Japanese knotweed, liquid lime sulfur, powdery mildew, quince rust

Abstract. Although demand for organic produce continues to increase in the mid-Atlantic, few apple (Malus × domestica Borkh.) growers in the region have adopted organic management practices due to the considerable disease, insect, and weed pressure, as well as the lack of effective crop load management tools. In this study, lime sulfur (LS) and Regalia® (R) were applied in different sequences (i.e., LS/LS, LS/R, R/R, and R/LS), each in a mixture with JMS Stylet-Oil, to chemically thin apple flowers in an organically managed ‘Honeycrisp’/‘MM.111’ orchard. There was also a nontreated control, a “grower standard” control (LS at 11 mm fruitlet diameter), and a hand-thinned control. The treatments were evaluated for their ability to reduce crop load, as well as to control powdery mildew [Podosphaera leucotricha (Ellis & Everh.) E. S. Salmon], cedar apple rust (Gymnosporangium juniperi-virginianae Schwein.), and quince rust (Gymnosporangium clavipes Cooke & Peck). All treatments reduced crop load compared with the nontreated control, and after the first application of LS or R, the number of fertilized king blooms was reduced and fertilization was prevented in all side blooms. All bloom thinning treatments had more fruit peel russet than the control and russet was more severe when LS was one of the applications. Bloom thinning applications of LS and R did not reduce powdery mildew leaf infection compared with the nontreated control. Cedar apple rust incidence was reduced by all bloom thinning treatments, though some lesions were detected in all treatments. There were minimal quince rust infections in any of the treatments, including the nontreated control. These results suggest that when LS and/or Regalia® are mixed with JMS Stylet-Oil and applied as bloom thinners, they can reduce crop load, and, as a secondary benefit, they can also decrease cedar apple rust incidence from infections that occur during bloom.

Received for publication 3 Oct. 2016. Accepted for publication 11 Jan. 2017.

Funding support was provided by a U.S. Department of Agriculture-Specialty Crop Block Grant administered by the Virginia Department of Agriculture and Consumer Services, Virginia Agricultural Experiment Station, and Virginia Tech’s Department of Horticulture. We thank Sierra Athey, Matt Borden, David Carbaugh, Allen Cochran II, Scott Kilmer, Abby Kowsalski, Taylor Mackintosh, Jared Repass, William Royston, Jr., Ashley Thompson and Jim Warren for their assistance with this experiment. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the authors and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

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product was caustic and potentially damaging to other floral organs. The disease control efficacy of Regalia® is reported to be from a unique mechanism of induced plant resistance that upregulates chalcone synthase and chalcone isomerase in the phenylpropanoid pathway and induces the production and accumulation of phytoalexins (Su et al., 2012). In a previous formulation, named Milsana® (Marrone Bio Innovations, Inc.), R. sachaenensis extracts were shown to decrease powdery mildew infection on cucumbers, tomatoes, begonias, and wheat (Daayf et al., 1995; Dik and van der Staay, 1995; Herger et al., 1988; Herger and Klingauf, 1990; Konstantinidou-Doltsinis et al., 2006; Konstantinidou-Doltsinis and Schmitt, 1998). Regalia® is currently labeled for use as a fungicide on many horticultural and agronomic crops, but its mode of action as a bloom thinner is unknown.

Among the many fungal diseases that require grower intervention in the mid-Atlantic, cedar apple rust (G. juniperi-virginiana) and quince rust (G. clavipes) are formidable barriers to organic apple production because they are not effectively controlled by organically approved fungicides such as sulfur and copper (Yoder et al., 2006). An experimental formulation of R. sachaenensis (MOI-106020) that was tested before the commercial release of Regalia® was found to provide cedar apple rust control (Halbrendt et al., 2010). Similarly, prior tests in Virginia indicated that control of rusts by a combination of Regalia® and JMS Stylet-Oil (JMS Flower Farms, Inc., Vero Beach, FL) provides effective control of cedar apple rust and after-infection control of quince rust (Peck et al., 2016; Yoder et al., 2015). Thus, we saw an opportunity to target two of the key barriers for organic apple production in the mid-Atlantic, namely reliable crop load and disease management.

The objective of this project was to test the disease control achieved while using different sequences of LS and/or Regalia®, each in combination with JMS Stylet-Oil, for bloom thinning. We hypothesized that these bloom thinning materials would aid disease control in an organically managed apple orchard.

Materials and Methods

The study was conducted in an 8-year-old ‘Honeycrisp’/‘MM.111’ apple orchard located at Virginia Tech’s Alson H. Smith, Jr. Agricultural Research and Extension Center in Winchester, VA (39°06' N, 78°17' W). Trees were spaced 3 m between trees and 7 m between rows. In 2015, the year of this experiment, only USDA-NOP approved products were applied to the orchard (Table 1). Before 2015, all trees had been managed conventionally. A row of border trees on the west side of the block, as well as four trees on each end of each row with treatment trees were not sprayed for the entire season. The experiment was conducted on the remaining four rows.

Table 1. Organic fungicides and insecticides applied to the ‘Honeycrisp’/‘MM.111’ apple orchard used for a bloom thinning experiment in 2015.

| Date            | Fungicides (rate/ha)                              | Target disease                  | Insecticides (rate/ha)                              | Target insect                  |
|-----------------|--------------------------------------------------|---------------------------------|----------------------------------------------------|--------------------------------|
| 18 Apr. (Pink) | Cueva 9.4 L + Double Nickel LC 2.3 L             | Scab, fire blight              | Entrain SC 0.6 L + Pyganic EC1.4L 2.9 L            | Codling moth (CM), orange fruit moth (OFM), plum curculio (PC) |
| 6 May (Petal fall) | Cueva 9.4 L + Double Nickel 2.3 L                | Scab, fire blight              | Scab, fire blight                                  |                                |
| 20 May          | Microthiol Dispers 9 kg                           | Powdery mildew                 | Madex HP 0.1 L + Deliver 0.5 kg                    | CM, OFM, leaf roller (LR)      |
| 27 May          | Microthiol Dispers 9 kg                           | Powdery mildew                 | Madex HP 0.1 L + Deliver 0.5 kg                    | LR, brown marmorated stink bug (BMSB) |
| 6 June          | Microthiol Dispers 9 kg                           | Powdery mildew                 | Deliver 0.5 kg + Pyganic 1.5 L                     | LR, BMSB, Japanese beetle (JB) |
| 18 June         | Microthiol Dispers 9 kg                           | Powdery mildew                 | Deliver 0.5 kg + Pyganic 1.5 L                     | CM, OFM, JB                    |
| 1 July          | Cueva 4.7 L + Double Nickel 2.3 L                 | Summer diseases                | Entrain SC 0.6 L + Madex HP 0.1 L + Deliver 0.5 kg | CM, OFM, JB                    |
| 15 July         | Cueva 4.7 L + Double Nickel 2.3 L                 | Summer diseases                | Summer diseases                                   | CM, OFM, AM, JB                |
| 31 July         | Cueva 4.7 L + Double Nickel 2.3 L                 | Summer diseases                | Summmer diseases                                  |                                |
| 6 Aug.          | Cueva 4.7 L + Double Nickel 2.3 L                 | Summer diseases                | Summmer diseases                                  |                                |

Three king bloom flowers and three side bloom flowers per block (12 total) were collected 24 h after the first application of LS or Regalia®. Flower samples were stored in a 5% sodium sulfitie (Amresco, Solon, OH) solution at 4 °C to halt pollen tube growth until histological examination as described by Embree and Foster (1999). Briefly, flowers were prepared for visualization by boiling in the sodium sulfitie solution 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 Bio-medicals, LLC, Solon, OH) stain in 0.067 M K2HPO4. Slides were then held in a dark cabinet for 24 h. Flower organs were visualized using a compound microscope (Nikon Optiphot, Melville, NY) at ×100 magnification with a high-pressure mercury vapor epi-ultraviolet light source (Zeiss HBO-50; Osram GmbH, Augsburg, Germany). Flowers were considered fertilized if at least one pollen tube reached the base of the style.

Two branches per tree were assessed on 3 June for crop load [number of fruit/cm2 branch cross-sectional area (BCSA)]. Disease incidence was rated on 10 shoots per replicate tree on 12 Aug. Leaf nodes were counted on each shoot, and missing leaves at each node position for the first 20 nodes were recorded. Fruit size and russet ratings were taken on 25 fruit per experimental unit on 20 Aug. Fruit peel russetting was evaluated by estimating the percent area affected on individual fruit. Fruit were rated U.S. Fancy or U.S. Extra Fancy if less than 15% of the surface area was russeted (U.S. Department of Agriculture, 2002).

Each treatment consisted of four single-tree replications in a completely randomized block design. Blocking was based on bloom density ratings that were recorded before the first treatment application in 2015. Statistical analysis was completed using analysis of variance (PROC ANOVA, SAS Institute Inc., Cary, NC), and post hoc mean separations were determined by Tukey’s Honestly Significant Difference or the Waller–Duncan K-ratio test. Flower fertilization data were arcsine square root
transformed before analysis, but are presented as untransformed means.

Results

A visual assessment of flowering in 2015 ranged between 20% and 30% of the greatest amount of flowering in that block. There were not enough trees with greater bloom density to use in the experiment. The ‘Honeycrisp’ pollen tube growth model was started at 1000 HR on 20 Apr. and thinning treatments were applied at 0800 HR on 27 Apr. and at 1600 HR on 29 Apr. (Fig. 1). The nontreated control had an average of 6.8 fruit/cm² BCSA (Table 2). All treatments reduced crop load compared with the nontreated control, and there were no differences in crop load among the treatments. Only the LS/LS treatments did not have greater fruit mass at harvest than the nontreated control. The LS/LS treatment had less fruit mass and smaller fruit diameter than the R/R and LSfruitlet treatments (the grower standard treatment), but had similar fruit size to the other treatments.

All four bloom thinning treatments had greater fruit russet ratings and less fruit in the U.S. Fancy or Extra Fancy grades than the nontreated control, LSfruitlet, and hand-thinned treatments, which all had similar levels of russet (Table 2). Among the four bloom thinning treatments, R/R had the lowest fruit russet rating and the most fruit in the U.S. Fancy and Extra Fancy grades.

The R/R treatment had greater return bloom (measured as the percent of spurs with flowers) in 2016 than the nontreated control, LS/LS, R/LS, LSfruitlet, and hand-thinned treatments, but was similar to the LS/R treatment (Table 2). Apart from the R/R treatment, the return bloom was very low and 2016 would be considered an “off year” in the biennial bearing cycle for this orchard.

Ninety-two percent of the nontreated control king flowers were fertilized, whereas 67% and 50% of the king flowers were fertilized after the first treatments of LS and Regalia®, respectively (Fig. 2). Twenty-five percent of the side bloom flowers on control trees were fertilized, whereas none of side bloom flowers were fertilized on trees treated with LS or Regalia®.

As indicated by the incidence of powdery mildew and cedar apple rust on the nontreated control trees, the test orchard had substantial levels of both diseases (Table 3). The hand-thinned treatment had a greater percentage of leaves with powdery mildew infection than the nontreated control, R/R, and LSfruitlet treatments. The percent of leaves infected with powdery mildew ranged from 10% to 22%, and was statistically greater for the hand-thinned treatment than the LS/LS, R/R, R/LS, and LSfruitlet treatments. The percent of leaves with cedar apple rust infection was greater on control and hand-thinned trees compared with all of the chemical thinning treatments (Table 3). The LSfruitlet treatment had less cedar apple rust lesions than the nonsprayed treatments. Minimal quince rust infection was recorded in this experiment and there were no treatment differences (data not shown). The four bloom thinning treatments had similar disease incidence.

The number of leaves per shoot was the same among treatments (Table 3). The LS/LS and the R/LS treatments resulted in a greater percent of defoliation in the first 20 leaves, compared with the control, R/R, and hand-thinned treatments.

Discussion

The overall goal of this study was to determine if LS and Regalia®, applied for bloom thinning, could sufficiently reduce crop load, as well as powdery mildew, cedar

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Table 2. Crop load, fruit mass and diameter, fruit finish (0 = no visible russet; 5 = severe russet), fruit above U.S. Fancy or Extra Fancy grades for russet, and 2016 return bloom (percentage of visible spurs with flowers) from successive applications of lime sulfur (LS) and/or Regalia® used during bloom in an organically managed ‘Honeycrisp’/‘MM.111’ apple orchard in 2015.

| Thinning treatment | First application | Second application | Crop load (fruit/cm² branch cross sectional area) | Fruit mass (g) | Fruit diam (mm) | Fruit russet rating (0–5) | Fruit graded as U.S. Fancy or Extra Fancy (%) | 2016 return bloom rating (%) |
|---------------------|------------------|--------------------|-----------------------------------------------|--------------|---------------|--------------------------|------------------------------------------|--------------------------|
| No treatment        | No treatment     | 6.8 a              | 166.6 c                                      | 60.5 d       | 2.2 a         | 74 a                     | 2 b                                      |                           |
| LS                  | LS               | 1.7 b              | 170.5 bc                                     | 61.6 ed      | 4.0 cd        | 16 c                     | 8 b                                      |                           |
| Regalia®            | Regalia®         | 3.3 b              | 195.6 a                                      | 64.0 ab      | 3.1 b         | 36 b                     | 25 ab                                    |                           |
| Regalia®            | Regalia®         | 2.6 b              | 185.3 ab                                     | 63.4 bc      | 3.7 c         | 20 bc                    | 46 a                                    |                           |
| LSfruitlet          | LSfruitlet       | 2.6 b              | 202.3 a                                      | 65.9 a       | 2.7 a         | 66 a                     | 3 b                                      |                           |
| Hand thinned        | Hand thinned     | 3.7 b              | 185.5 ab                                     | 62.6 bc      | 2.5 a         | 64 a                     | 2 b                                      |                           |

All bloom thinning applications were mixed with JMS Stylet-Oil. There was also a nontreated control, a “grower standard” control (LS mixed with JMS Stylet-Oil applied at 11-mm fruitlet diameter; LSfruitlet), and a hand-thinned control. Bloom thinning applications occurred on 27 Apr and 29 Apr. and were based on a ‘Honeycrisp’-specific, temperature-based pollen tube growth model, the grower standard application occurred on 11 May, and hand thinning occurred between 3 and 5 June.

*Mean separation within column by Tukey’s Honestly Significant Difference.

Waller–Duncan K-ratio t test (P = 0.05) using four single-tree replications.
apple rust, and quince rust infections, in an organically managed apple orchard. We evaluated blossom thinning chemicals as the only fungicide sprays applied during bloom. All of the chemical thinning treatments that we tested reduced crop load and cedar apple rust infection compared with the nontreated control. Regalia® and LS, when applied with JMS Stylet-Oil, provided similar blossom thinning results to each other, matching our previous research focused on blossom thinning applications when these materials were applied with the spreader/sticker Latron B-1956 (Yoder et al., 2013). As a bloom thinner, the mode of action for Regalia® is unknown, but our study suggests that Regalia® may inhibit fertilization by damaging floral organs. Similar to LS, Regalia® inhibited pollen tubes from reaching the base of the style and fertilizing the egg in side bloom flowers (Fig. 2). Although our experiment does not explain why Regalia® is caustic, we do provide evidence that Regalia® can reliably be used for bloom thinning in conjunction with the pollen tube growth model.

All chemical thinning treatments in this study 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 nontreated control treatment had 6.8 fruit/cm² BCSA, while the target crop load for mature ‘Honeycrisp’/‘MM.111’ trees is ≈5 fruit/cm² 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 LS applied on 29 Apr. (when the maximum temperature was 24 °C) resulted in lower crop load and higher fruit russet ratings than those of Regalia® treatments applied on the same day. LS is known to be phytotoxic and may result in several undesirable side effects, including increased russet and reduced photosynthesis, especially when applied during warmer temperatures (Holb et al., 2003; Hyre, 1939; McArtney et al., 2006; Palmer et al., 2003). Our results indicate Regalia® can be applied during bloom with lower phytotoxicity risk than LS.

Our study also suggests that selective use of Regalia® and JMS Stylet-Oil could decrease cedar apple rust infection in organic orchards. Cedar apple rust infection periods occurred on 14–15 Apr., 30 Apr. to 1 May, 1–2 May, 5–6 May, and 6–7 May. These infections were most likely suppressed by the bloom thinning treatments applied on 27 and 29 Apr. Thus, the cedar apple rust suppression may have been achieved by a combination of 13 d of postinfection activity (from 14 to 27 Apr.) and up to 8 d of protectant action (29 Apr. to 6–7 May). The findings in the present study support those of a previous study that showed postinfection suppression of quince rust by Regalia® mixed with JMS Stylet-Oil in a conventionally managed orchard (Yoder et al., 2015). However, timing fungicide applications according to the blossom thinning pollen tube growth model may miss cedar apple rust infection periods in some years and commercial apple growers should base crop load and disease management decisions on the specific conditions in their orchard each year. Furthermore, the LSfruitlet treatment in our study resulted in less cedar apple rust infection than the control and hand-thinned treatments suggesting that a later fungicide application timing may have had greater efficacy.

Bloom thinning applications of LS and Regalia® did not reduce mildew percent infection compared with the nontreated control (Table 3). However, border trees with no chemical pest control applications had 64% of leaves infected with mildew, compared with an average of 16% of leaves infected on treatment trees. Thus, while Su et al. (2012)

### Table 3. Powdery mildew (Podosphaera leucotricha) and cedar apple rust (Gymnosporangium juniperi-virginianae) incidence and the mean number of leaf nodes and the percent of missing leaves within the first 20 leaf nodes on the assessed branches after successive applications of lime sulfur (LS) and/or Regalia® used during bloom in an organically managed ‘Honeycrisp’/‘MM.111’ apple orchard in 2015.

| Foliar diseases | Fertilized flowers (%) | Infected leaves (%) | Lesions/leaf (no.) | Leaf nodes/shoot (no.) | Missing leaves/shoot (%) |
|-----------------|------------------------|--------------------|-------------------|------------------------|-------------------------|
|                 | No treatment           | Lime sulfur        | Regalia           |                        |                         |
|                 | King bloom             | Side bloom         |                   |                        |                         |
|                 | No treatment           | 15 ab              | 22 cd             | 27                     | 2 a                     |
|                 | LS                     | 16 abc             | 14 ab             | 27                     | 8 bc                    |
|                 | Regalia®               | 18 bc              | 15 bc             | 25                     | 5 b                     |
|                 | Regalia®               | 14 ab              | 13 ab             | 24                     | 2 a                     |
|                 | LSfruitlet             | 15 abc             | 14 ab             | 25                     | 10 c                    |
|                 | Hand thinned           | 10 a               | 9 a               | 25                     | 5 b                     |
|                 | Border thinned         | 22 c               | 22 d              | 27                     | 2 a                     |
|                 | 64                     | 19                 | 37                | 26                     | 1                       |

All bloom thinning applications were mixed with JMS Stylet-Oil. There was also a nontreated control, a “grower standard” control (LS mixed with JMS Stylet-Oil applied at 11-mm fruitlet diameter; LSfruitlet), and a hand-thinned control. Bloom thinning applications occurred on 27 Apr. and 29 Apr. and were based on a ‘Honeycrisp’-specific, temperature-based pollen tube growth model, the grower standard application occurred on 11 May, and hand thinning occurred between 3 and 5 June. Border trees were not treated with any thinning or pest control materials for the entire season, which is not part of the replicated experiment, and thus the data from these trees are provided only as a reference point to show the amount of disease pressure in the orchard.

*Mean separation within column by Waller–Duncan K-ratio t test (P = 0.05) using four single-tree replications and ten shoots per replication.*
reported that *R. sachalinensis* extracts reduced powdery mildew infection (as well as, several bacterial and other fungal diseases) on other horticultural crops, more research is needed to understand the circumstances (e.g., application timing and rate) that would lead to greater control of apple powdery mildew with Regalia® and other products with a similar a.i. and/or mode of action. Additionally, although there were nearly 3 weeks between the application of the second bloom thinning treatments and the elemental sulfur sprays, treatment differences in our study may not have been evident due to the elemental sulfur applications on all treatments during the early summer, which would have protected the trees and fruit from powdery mildew infection. Future studies should extend the time between the bloom thinning applications and other fungicides to determine if greater control can be achieved.

Through our results, we provide evidence that bloom thinning sprays of LS and Regalia® in combination with JMS Stylet-Oil can reduce crop load and decrease cedar apple rust infection. Thus, bloom thinning with these materials could also provide disease control, potentially reducing the need for at least one postbloom fungicide cover spray and leading to an overall reduction in the volume of chemicals used in an organic apple orchard. At an average cost of $100/ha/yr for a fungicide application (including material, labor, and machinery), reducing fungicide applications would have a direct economic impact on orchard profitability (Peck et al., 2010). Additionally, the use of two Regalia® applications instead of two LS applications reduced fruit russet down-grading by more than 50%, which would also result in increased profitability. However, timing of fungicide applications should not be completely contingent on the criteria being used for bloom thinning applications, and commercial growers should not assume that bloom thinning applications with LS or Regalia® will control cedar apple rust in years when infection periods occur much later than anthesis. Conversely, growers should be aware that bloom-time applications of LS or Regalia® for disease control can damage flowers and potentially reduce yields. In conclusion, our findings indicate that LS and the biofungicide, Regalia®, in combination with JMS Stylet-Oil, could provide after-infection control of cedar apple rust, reduce crop load, and possibly result in an overall reduction in the number of required chemical sprays.

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