Research



Efficacy of Biological and Conventional Fungicide Programs for Foliar Disease Management on Pomegranate (*Punica granatum*) in Florida

Katia V. Xavier and Gary E. Vallad⁺

University of Florida, Gulf Coast Research and Education Center, Wimauma, FL 33598

Accepted for publication 18 May 2020.

Abstract

Pomegranate (*Punica granatum* L.) is an emerging alternative perennial crop in the southeastern United States. However, foliar diseases are present across this production area, causing almost 100% premature defoliation. A series of fungicide efficacy trials were conducted to evaluate biological, systemic, and contact fungicides at two locations in Florida, Plant City (cv. Angel Red) and Parrish (cvs. Christina, Azadi, Vikusnyi, Alsirinnar, Sakerdze, and Wonderful), for foliar disease management. Based on AUDPC, the fungicides Captan 80 WDG (captan, 78.2%), Penncozeb 75DF (mancozeb, 75%), Merivon (pyraclostrobin, 21.3% + fluxapyroxad, 21.3%), and Topsin 4.5 FL (thiophanate methyl, 45%) significantly reduced the percentage of foliar disease severity compared with biologicals consisting

California is the largest producer of pomegranate in the United States (U.S.) (NASS 2012). In the southeastern U.S., pomegranate has emerged as an alternative crop for growers dealing with devastating disease and pests on citrus and avocado (Castle et al. 2011). However, diseases are also limiting factors on pomegranate production. Leaf spots and blight, caused by *Colletotrichum* spp., *Dwiroopa punicae*, and *Pseudocercospora punicae*, are important foliar diseases occurring on pomegranate across the southeastern U.S. (Xavier et al. 2019a, 2019b). Together these pathogens can cause complete defoliation and fruit loss in the absence of an effective management strategy.

Integrated management strategies are recommended to reduce disease levels in the major pomegranate production areas (Jadhav and Sharma 2009). Cultural practices play an important role in disease management. The recommended field sanitation measures are pruning of suckers and infected plant parts followed by removal of infected material, as well as plant debris, from the orchard (Munhuweyi et al. 2016).

[†]Corresponding author: G. E. Vallad; gvallad@ufl.edu

Funding: Funding sources for this project were the U.S. Department of Agriculture's (USDA's) Agricultural Marketing Service and the FDACS through USDA-AMS-SCBGP-2012 award 92288 and USDA-AMS-SCBGP-2015 award 22899.

*The *e*-Xtra logo stands for "electronic extra" and indicates three supplementary tables are published online.

The author(s) declare no conflict of interest.

© 2020 The American Phytopathological Society

of Serenade OPTI and Tenet WP (Plant City) and the nontreated control (Plant City and Parrish cv. Wonderful). All treatments applied in a rotational program three times at bloom significantly reduced disease severity compared with the nontreated control in both locations. Rotational programs applied throughout the season also reduced disease severity compared with nontreated controls and repeated applications of neem oil. Foliar disease management is critical for long-term establishment of pomegranate as a viable economic crop in the southeastern United States.

Keywords: pesticides, integrated pest management, chemical control, biocontrol

There is limited information on the use of chemical and biological controls for the management of pomegranate diseases in the U.S. However, in subtropical and tropical countries where pomegranate is well adapted, more studies have been performed. For example, in India, where climatic conditions are similar to those in Florida, integrated management strategies include the application of chemical and biological controls (Munhuweyi et al. 2016; Thomidis 2014).

The use of preharvest chemical fungicides is especially important in areas with high relative humidity. In southwestern India the use of preharvest fungicides was reported to reduce the incidence of pomegranate fruit rot by more than 90% after the application of either the systemic fungicide propiconazole or a mixture of a systemic fungicide, carbendazim, and the contact fungicide mancozeb (Nargund et al. 2012). In another study conducted in India, the fungicides carbendazim, difenoconazole, captan, chlorothalonil, hexaconazole, iprobenfos, mancozeb, thiophanate-methyl, propineb, and propiconazole were effective in reducing disease levels when applied alone or in rotational programs in the field (Jadhav and Sharma 2009). Under field conditions, Ridomil Gold 68% WP (mefenoxam) was reported to reduce Alternaria alternata leaf spot by 90% compared with the nontreated control (Muthukumar and Udhayakumar 2015). Although fungicides are commonly used to control diseases of pomegranate worldwide, there are currently no conventional fungicides registered for preharvest disease control in the U.S.

Most of the studies to evaluate the efficacy of biocontrol agents and plant extracts have been performed in vitro. For instance, eight fungal species were tested as potential biopesticides against *A. alternata*, and results indicated that Trichoderma viride, T. hamatum, and Aspergillus niger showed the highest level of mycelial growth inhibition (Kadam et al. 2018). In addition, extracts of Allium sativum, Zingiber officinale, and Azadirachta indica showed the highest mycelial growth inhibition among 11 plant extracts tested (Kadam et al. 2018). The efficacy of biopesticides was also tested in vitro against Colletotrichum gloeosporioides. Once again, T. viride was the best in inhibiting mycelial growth, followed by T. harzianum and Pseudomonas fluorescens. The plant extracts from Datura stramonium and A. sativum showed more than 50% mycelial growth inhibition. In the same study, Bacillus subtilis showed the least inhibition of mycelial growth (Nargund et al. 2012). However, when Bacillus spp. were tested on fruits, it effectively retarded and controlled pomegranate fruit rot (Gajbhiye et al. 2013). Although multiple biopesticides are registered for use on pomegranate against foliar diseases in the U.S. (CDMS 2019), no information regarding their effectiveness in controlling preharvest diseases is available.

Recently, we reported the effect of three fungicide programs, Merivon (pyraclostrobin + fluxapyroxad), Luna Experience (fluopyram + tebuconazole), and a rotational program consisting of these two fungicides as well as Penncozeb 75DF (mancozeb), to control foliar and fruit diseases of pomegranate across the southeastern U.S. (Xavier et al. 2020). A request to expand the label of Merivon to include pomegranate is currently under consideration by the U.S. Environmental Protection Agency. The evaluation of additional fungicides will potentially help growers to develop an alternative fungicide program to control diseases on pomegranate across the southeastern U.S. Furthermore, the use of multiple fungicide chemistries in pomegranate can be essential in the management of fungicide resistance. Therefore, the objective of this work was to evaluate several biological and conventional fungicides in order to develop effective programs for disease management on pomegranate. This information is important in supporting labeling efforts necessary to provide the pomegranate industry in the southeastern U.S. with effective fungicide options for commercial production.

Fungicide Applications

Fungicide trials were established in 2017 at two pomegranate orchard sites in Florida. Tested biopesticides included Serenade OPTI (Bayer CropScience, Research Triangle Park, NC), containing the QST 713 strain of *B. subtilis* (26.2%); Tenet WP (Isagro USA, Morrisville, NC), containing *Trichoderma asperellum* (ICC 012) (2%) and *T. gamsii* (ICC 080) (2%); and neem oil (Lawn and Garden Products, Fresno, CA), containing clarified hydrophobic

extract of neem oil (70%). Tested conventional fungicides included Merivon SC (BASF, Research Triangle Park, NC), containing pyraclostrobin (21.3%) + fluxapyroxad (21.3%); Luna Experience SC (Bayer CropScience), containing fluopyram (17.6%) + tebuconazole (17.6%); Topsin 4.5 FL (United Phosphorus, King of Prussia, PA), containing thiophanate-methyl (45%); Penncozeb 75DF (United Phosphorus), containing mancozeb (75%); and Captan 80 WDG (Drexel Chemical Company, Memphis, TN), containing captan (80%) (Table 1). In addition, a nontreated control was included in each trial.

Fungicides were applied alone or within a series of rotational programs (Tables 2, 3, and 4) with a CO₂ backpack sprayer calibrated to deliver 46 liters/ha at 275.8 kPa. The first set of trials evaluated two consecutive foliar applications, 14 days apart, of Penncozeb 75DF, Captan 80 WDG, Topsin 4.5 FL, Serenade OPTI, Tenet WP, and Merivon treatments at bloom (Table 2). The second set of trials evaluated four at-bloom fungicide programs, consisting of three consecutive applications of Merivon, Topsin 4.5 FL, or Captan 80 WDG in different combinations and a seasonal program of Luna Experience (Table 3). The third set of trials evaluated a series of seasonal fungicide rotations beginning with at-bloom application of Merivon, followed by three applications of either Captan 80 WDG, Topsin 4.5 FL, neem oil, Serenade OPTI, or Tenet WP (Table 4).

Trials were initiated when pomegranate trees showed at least 20% flower bud break, on April 5 and 6 in Parrish and Plant City, respectively. In Plant City all the trials consisted of pomegranate cultivar Angel Red, with three replicate trees per treatment. In Parrish, due to a limit in available trees, each trial consisted of two separate cultivars with three replicate trees per cultivar for each treatment. Treatments, including a nontreated control, were arranged in a randomized complete block design. All trees used in this study were cultivated in a row spacing of 5.8 m and tree spacing of 2.7 m. Each orchard site had a history of high disease pressure, so trials relied on natural inoculum prevalent in the area.

Foliar Disease Assessment and Data Analysis

The most prevalent foliar diseases of pomegranate consist of leaf spots and blight caused by *Colletotrichum* spp., *D. punicae*, and *P. punicae*, as previously described (Xavier et al. 2019a, 2019b, 2019c). Foliar disease severity (leaf spotting and blighting) was assessed on each tree using the Horsfall–Barratt rating (Horsfall and Barratt 1945) every 2 weeks starting on April 19 and 20 in Parrish and Plant City, respectively. The Horsfall–Barratt rating was converted to a midpoint percentage for data analysis. Area under disease progress curve (AUDPC) was calculated based on foliar disease severity using the following formula: $\Sigma \{[(x_i + x_{i-1})/2](t_i - t_{i-1})\}$

| TABLE 1 Fungicides used in this study | | | | | | | | | |
|--|----------|--|--|------------------------|--|--|--|--|--|
| Product | Rate/ha | Active ingredient | Source | FRAC code ^z | | | | | |
| Merivon Xemium | 83.8 ml | Fluxapyroxad + pyraclostrobin | BASF Ag Products, Research Triangle Park, NC | 7 and 11 | | | | | |
| Penncozeb 75DF | 0.7 kg | Mancozeb | United Phosphorus, King of Prussia, PA | M3 | | | | | |
| Captan 80 WDG | 0.9 kg | Captan | Drexel Chemical Company, Memphis, TN | M3 | | | | | |
| Topsin 4.5 FL | 359 ml | Thiophanate-methyl | United Phosphorus, King of Prussia, PA | 1 | | | | | |
| Luna Experience | 203.5 ml | Fluopyram + tebuconazole | Bayer CropScience LP, Research Triangle Park, NC | 7 and 3 | | | | | |
| Neem oil | 622.3 ml | Neem oil | Lawn and Garden Products, Fresno, CA | NA | | | | | |
| Serenade OPTI | 0.23 kg | Bacillus subtilis | Bayer CropScience LP, Research Triangle Park, NC | BM 02 | | | | | |
| Tenet WP | 0.55 kg | Trichoderma asperellum + Trichoderma gamsii | Isagro USA, Morrisville, NC | 44 | | | | | |

^z FRAC codes from the Fungicide Resistance Action Committee (https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list/2020-finalb16c2b2c512362eb9a1eff00004acf5d.pdf?sfvrsn=54f499a_2). NA = not applicable.

in which x_i is the rating at the time of evaluation and $(t_i - t_{i-1})$ is the time between evaluations; the values are estimates of least squares means for treatments. AUDPC was calculated from midpoint percentages using the trapezoidal method (Jeger 2004).

The effect of the treatments on foliar disease severity and AUDPC was analyzed using a generalized linear mixed model (PROC GLIMMIX, SAS, version 9.4, SAS Institute, Cary, NC) with blocking as a random effect and fungicide program as a fixed effect in the model. Values of disease severity and AUDPC were log-normal transformed for statistical analyses but presented as actual means in the results and tables. Degrees of freedom were calculated using the Kenward–Roger method (Kenward and Roger 1997) and mean separation performed with Fisher's least square difference (LSD) method at the 95.0% level of confidence.

Effect of At-Bloom Fungicide Treatments

Based on foliar disease severity, significant differences were detected among the treatments beginning in May (week 4) and June (week 5) at Plant City and Parrish, respectively (Supplementary Table S1). Based on final disease severity and AUDPC, differences were observed among treatments applied on 'Christina' (P = 0.0206 and P = 0.0483, respectively), 'Wonderful' (P = 0.0027 and P = 0.0088, respectively), and 'Angel Red' (P < 0.0001 and P < 0.0001, respectively) (Table 2).

Based on final disease severity and AUDPC, the systemic fungicides, Merivon and Topsin 4.5 FL, provided the highest level of disease control in comparison with the nontreated control in both locations (Table 2). When applied on Wonderful, Angel Red, and Christina, the systemic fungicides provided up to 64, 92, and 92% disease control in comparison with the nontreated control, respectively, based on final disease severity (Table 2).

The contact fungicides, Captan 80 WDG and Penncozeb 75DF, provided 46 and 57% disease control, respectively, when applied on Angel Red (Table 2). On Wonderful, Penncozeb 75DF provided 75% disease control, based on the final disease severity; however, Captan 80 WDG was not effective in comparison with the non-treated control (Table 2). When applied on Christina, the contact fungicides were not effective in reducing foliar disease severity in comparison with the nontreated control (Table 2).

The biological fungicides, Tenet WP and Serenade OPTI, were not effective in reducing foliar disease severity in comparison with the nontreated control when applied on Angel Red, Wonderful, or Christina (Tenet WP only) (Table 2).

Effect of At-Bloom, Rotational Fungicide Programs

Significant differences in foliar disease severity were detected among fungicide treatments beginning in June (week 5) at both trial locations (Supplementary Table S2). Based on final disease severity and AUDPC, all treatments applied on Angel Red (P = 0.0001 and P < 0.0001, respectively) and 'Sakerdze' (P < 0.0001 and P < 0.00010.0001, respectively) had significant effects on foliar disease severity in comparison with the nontreated control (Table 3). Similar trends were observed when treatments were applied on 'Alsirinnar', based on final disease severity and AUDPC; however, due to the high variability there were no significant differences among the treatments (P > 0.1919 and P > 0.1039, respectively). Treatments including the three at-bloom fungicide rotation programs, the seasonal application of Luna Experience, and Merivon applied twice at bloom provided disease control that ranged from 84 to 92% and from 61 to 85% in Plant City and Parrish, respectively, in comparison with the nontreated control (Table 3).

Based on final disease severity, all treatments consisting of a rotational program among three fungicides were not significantly different from two at-bloom applications with Merivon or a seasonal application with Luna Experience (Table 3).

Effect of Postbloom Fungicide Applications with an At-Bloom Merivon Program

Based on foliar disease severity, significant differences were detected among the treatments beginning in June (weeks 4 and 5) at Plant City and Parrish (Supplementary Table S3). In Plant City, final disease severity ranged from 4.5 to 91.5%. All treatments significantly reduced disease severity compared with the nontreated control except for the neem oil program (Table 4). However, based on AUDPC, the season-long neem oil program significantly reduced foliar disease severity in comparison with the nontreated control when applied on Angel Red, with similar numerical trends, but not significant, observed on 'Azadi' and 'Vikusnyi' (Table 4).

TABLE 2

Effect of rotational programs applied twice at bloom on disease severity and area under the disease progress curve (AUDPC) in field trials performed at pomegranate orchards in Plant City and Parrish^w

| | | DS _f ^y | | | AUDPC ^z | | | |
|---|-------------------------|------------------------------|-----------|------------|--------------------|-----------|--|--|
| | Plant City Angel Red | Parrish | | Plant City | Parrish | | | |
| Treatments, rate (application) ^x | | Christina | Wonderful | Angel Red | Christina | Wonderful | | |
| Merivon, 83.8 ml/ha (1, 2) | 7.5 c | 4.5 b | 21.7 dc | 607 c | 558 b | 1,040 bc | | |
| Topsin 4.5 FL, 359 ml/ha (1, 2) | 7.5 c | 4.5 b | 31.2 bc | 565 c | 558 b | 1,015 bc | | |
| Captan 80 WDG, 0.9 kg/ha (1, 2) | 49.0 ab | 21.7 a | 45.8 ab | 1,279 b | 1,000 ab | 1,463 ab | | |
| Penncozeb 75DF, 0.7 kg/ha (1,2) | 39.5 b | 28.5 a | 15.3 d | 1,039 b | 963 ab | 852 c | | |
| Serenade OPTI, 0.23 kg/ha (1, 2) | 89.7 a | 9.0 ab | 45.8 ab | 2,837 a | 706 b | 1,379 ab | | |
| Tenet WP, 0.55 kg/ha (1, 2) | 86.5 a | 36.3 a | 62.5 a | 2,389 a | 1,067 ab | 2,080 a | | |
| Nontreated | 91.5 a | 42.7 a | 60.5 ab | 3,174 a | 1,557 a | 2,088 a | | |
| P > F | < 0.0001 | 0.0206 | 0.0027 | < 0.0001 | 0.0483 | 0.0088 | | |

^w Means followed by the same letter are not significantly different according to Fisher's LSD test ($\alpha = 0.05$).

^x Treatments were applied on April 5 and 6 (application 1) and April 19 and 20 (application 2) in Parrish and Plant City, respectively (corresponding with applications 1 and 2 shown in the table).

^y Final disease severity (DS_f) was evaluated on August 24.

^z AUDPC was calculated using the following formula: $\Sigma\{[(x_i + x_{i-1})/2](t_i - t_{i-1})\}$, where x_i is the midpoint percentage of the Horsfall–Barratt rating at each evaluation time and $(t_i - t_{i-1})$ is the time between evaluations.

In Parrish, based on the final disease severity, significant differences among the treatments were observed on the cultivars Azadi (P = 0.0082) and Vikusnyi (P = 0.0598) (Table 4). In both locations, up to 90% disease control was obtained by two at-bloom applications of Merivon followed by three seasonal applications of either a contact or systemic fungicide, in comparison with the nontreated control. Based on the AUDPC, the seasonal inclusion of Captan 80 WDG, Topsin 4.5 FL, Serenade OPTI, or Tenet WP with Merivon did not significantly improve disease control compared with the two at-bloom applications of Merivon alone (Table 4).

Conclusions and Implications

Although federal registration for the use of Merivon on pomegranate is under consideration, there are no chemical fungicides labeled for foliar disease control on pomegranate in the U.S. Furthermore, little information is available for biopesticides currently registered on pomegranate. Our prior research demonstrated the efficacy of at-bloom applications of Merivon for disease control on pomegranate (Xavier et al. 2020). Although Merivon is a formulation of two fungicides with different modes of action, additional fungicidal materials with differing modes of action are essential for managing the risk of fungicide resistance within pathogen populations. Results from this study indicate that fungicides such as Captan 80 WDG, Penncozeb 75DF, and Topsin 4.5 FL would be ideal rotational partners or alternatives to Merivon, whereas the biopesticides Tenet WP, Serenade OPTI, and neem oil were ineffective in reducing disease.

The contact fungicides, captan and mancozeb, have shown promise in reducing disease levels of pomegranate in India, which has the largest pomegranate production area in the world, comprising 143,000 ha in 2014 to 2015 (Chandra et al. 2010; Jain and Desai 2018). In the same study, thiophanate methyl, a single-site systemic fungicide from Fungicide Resistance Action Committee group 1, showed significant disease control, similar to our findings in Florida (Jadhav and Sharma 2009).

The use of biopesticides has shown promise in controlling fungal diseases on other fruit crop systems (Borges et al. 2018; Gurjar et al.

2012; Howell 2003). For example, T. harzianum was effective against the pathogen Botrytis cinerea on apple (Tronsmo 1991) and grape (Harman et al. 1996). B. subtilis (ACB-69) applied weekly reduced the amount of sweet orange postbloom fruit drop caused by C. acutatum by 47%, whereas the conventional fungicide applications of mancozeb + famoxadone followed by two applications of carbendazim at 15-day intervals showed reduction in fruit drop of only 28.7% (Kupper et al. 2012). The plant extract neem oil significantly reduced disease incidence of rusty spot, caused by Podosphaera leucotricha, on peach. However, the amount of disease control ranged from 24 to 34%, which is relatively low compared with 94% control for the systemic fungicide myclobutanil (Lalancette et al. 2013). In our study we also obtained relatively low foliar disease control using biopesticides in comparison with conventional fungicides. However, because the evaluated biopesticides consistently provided some level of control at the two locations, they have the potential to be used in an integrated program with conventional fungicides, or as an option for organic production.

Resistant populations of *C. acutatum* to quinone outside inhibitors have recently been reported in strawberry in Florida (Forcelini and Peres 2018). Because populations of *C. acutatum* from pomegranate and strawberry can cross-infect (Xavier et al. 2019b), there is a potential risk for the population from pomegranate to become resistant to pyraclostrobin, one of the active ingredients in Merivon. To minimize the risk of *C. acutatum* from pomegranate developing resistance to pyraclostrobin, it is important to include other effective fungicides with different modes of action within a rotational program. In this work the rotational programs with three fungicide applications at bloom or throughout the season were statistically the same as two at-bloom applications of Merivon. Despite the potential cost increase to growers, the rotational program, which requires the use of more fungicides, may effectively manage pathogen resistance.

Previously, we demonstrated that Merivon (pyraclostrobin + fluxapyroxad), Luna Experience (fluopyram + tebuconazole), and a rotational program consisting of these two fungicides as well as Penncozeb 75DF (mancozeb) were effective at controlling pomegranate

Effect of rotational programs applied three times at bloom on disease severity and area under the disease progress curve (AUDPC) in field trials performed at pomegranate orchards in Plant City and Parrish^w DS_f ^y AUDPC^z Parrish Parrish Plant City Plant City Treatments, rate (application)^x Angel Red Alsirinnar Sakerdze Angel Red Alsirinnar Sakerdze Merivon, 83.8 ml/ha (1, 2) 7.5 b 15.3 4.5 b 614 c 662 614 b Merivon, 83.8 ml/ha (1); Captan 80 WDG, 0.9 kg/ha (2); 12.2 b 21.2 6.0 b 661 c 859 601 b Topsin 4.5 FL, 359 ml/ha (3) Merivon, 83.8 ml/ha (1); Captan 80 WDG, 0.9 kg/ha (2); 13.8 b 6.0 b 24.8752 bc 876 600 b Merivon, 83.8 ml/ha (3) Merivon, 83.8 ml/ha (1); Topsin 4.5 FL, 359 ml/ha (2); 9.0 b 10.7 4.5 b 677 c 604 614 b Merivon, 83.8 ml/ha (3) Luna Experience, 203.5 ml/ha (1, 4, 6) 15.3 b 15.3 4.5 b 886 b 762 530 b 94.7 a Nontreated 33.2 31.2 a 4,084 a 1,185 a 1,466 0.0001 0.1919 P > F< 0.0001< 0.0001 0.1039 < 0.0001

TABLE 3

^w Means followed by the same letter are not significantly different according to Fisher's LSD test ($\alpha = 0.05$).

^x Treatments were applied on April 5 and 6 (application 1), April 19 and 20 (application 2), May 3 and 4 (application 3), May 17 and 18 (application 4), and July 12 and 13 (application 6) in Parrish and Plant City, respectively (corresponding with applications 1 to 4 and 6 shown in the table).

^y Final disease severity (DS_f) was evaluated on August 24.

^z AUDPC was calculated using the following formula: $\Sigma\{[(x_i + x_{i-1})/2](t_i - t_{i-1})\}$, where x_i is the midpoint percentage of the Horsfall–Barratt rating at each evaluation time and $(t_i - t_{i-1})$ is the time between evaluations.

TABLE 4

Effect of rotational programs applied throughout the season, starting at bloom, on disease severity and area under the disease progress curve (AUDPC) in field trials performed at pomegranate orchards in Plant City and Parrish^w

| | DS _f ^y | | AUDPC ^z | | | |
|---|------------------------------|---------|--------------------|------------|----------|----------|
| | Plant City Angel Red | Parrish | | Plant City | Parrish | |
| Treatments, rate (application) ^x | | Azadi | Vikusnyi | Angel Red | Azadi | Vikusnyi |
| Merivon, 83.8 ml/ha (1, 2) | 7.5 bc | 31.2 ab | 12.2 bc | 656 c | 1,253 bc | 701 bc |
| Merivon, 83.8 ml/ha (1, 2); Captan 80 WDG, 0.9 kg/ha (4, 5, 6) | 7.5 bc | 21.7 bc | 7.5 bc | 719 c | 968 c | 607 c |
| Merivon, 83.8 ml/ha (1, 2); Topsin 4.5 FL, 359 ml/ha (4, 5, 6) | 4.5 c | 10.7 c | 6.0 c | 551 c | 888 c | 608 c |
| Merivon, 83.8 ml/ha (1, 2); Serenade OPTI, 0.23 kg/ha (4, 5, 6) | 9.0 b | 37.5 ab | 12.2 bc | 686 c | 1,489 bc | 659 bc |
| Merivon, 83.8 ml/ha (1, 2); Tenet WP, 0.55 kg/ha (4, 5, 6) | 9.0 b | 26.8 bc | 15.3 abc | 656 c | 1,453 bc | 782 bc |
| Neem oil, 622.3 ml (1, 2, 4, 5, 6) | 63.7 a | 54.2 a | 34.8 ab | 1,984 b | 2,112 ab | 1,485 ab |
| Nontreated | 91.5 a | 75.2 a | 58.0 a | 3,174 a | 3,090 a | 2,422 a |
| P > F | < 0.0001 | 0.0082 | 0.0598 | < 0.0001 | 0.0217 | 0.0201 |

^w Means followed by the same letter are not significantly different according to Fisher's LSD test ($\alpha = 0.05$).

^x Treatments were applied on April 5 and 6 (application 1), April 19 and 20 (application 2), May 17 and 18 (application 4), June 14 and 15 (application 5), and July 12 and 13 (application 6) in Parrish and Plant City, respectively (corresponding with applications 1 to 2 and 4 to 6 shown in the table). ^y Final disease severity (DS_f) was evaluated on August 24.

^z AUDPC was calculated using the following formula: $\Sigma \{ [(x_i + x_{i-1})/2](t_i - t_{i-1}) \}$, where x_i is the midpoint percentage of the Horsfall–Barratt rating at each evaluation time and $(t_i - t_{i-1})$ is the time between evaluations.

fruit and foliar diseases. Furthermore, we demonstrated the importance of at-bloom fungicide applications for disease management on pomegranate (Xavier et al. 2020). In this study we are reporting the effect of other conventional fungicides as alternatives in fungicide programs to manage pomegranate diseases across the southeastern U.S. The use of multiple fungicide chemistries will be essential for the management of fungicide resistance in this region, especially if pomegranate is to be planted on a commercial scale in the future. Results from the current study further emphasize the importance of at-bloom applications for effective disease control, which were similar to the two rotational programs beginning at bloom, based on final disease severity. These results are in agreement with our previous study (Xavier et al. 2020).

In previous studies we reported that the most common foliar diseases, including leaf spots and blight, are caused by Colletotrichum spp., D. punicae, and P. punicae. In the present study we demonstrated the efficacy of several fungicides for the management of these three foliar diseases of pomegranate. In order to minimize the threat of developing fungicide-resistant pathogen populations, we recommend that growers apply these products within a rotational program beginning at bloom, and in conjunction with other integrated practices to reduce inoculum levels within the orchard. The registration of Merivon on pomegranate is expected in 2020, but the registration of additional conventional fungicides will take time. Although the evaluated biopesticides performed poorly, relative to conventional fungicides, their performance may be improved with increased application frequency, rates, and other integrated disease management practices. We have reported that fungal diseases are limiting factors to pomegranate production in the southeastern U.S. when control strategies are not applied (Xavier et al. 2020). Thus, similar to other tropical and subtropical regions where pomegranate is widely grown, an integrated approach incorporating cultural practices and the use of conventional fungicides could be an effective disease management program for commercial production in the southeastern U.S.

Acknowledgments

We are thankful to Scott Hughes, Steven Kalb, and Phanio M. E. L. Lawson Hellu for technical assistance; Carl and Martha Sutherland and Pacific Tomato Growers for giving us access to their pomegranate orchards for these trials; and Dr. Leandro G. Cordova for valuable input on the performed statistical analyses. Administrative support for this project came from the Florida Specialty Crop Foundation (FSCF) with input from the Florida Pomegranate Association (FPA). Authors have full responsibility of the contents presented in this manuscript, which do not necessarily represent the official views of the USDA, FDACS, FSCF, or FPA.

Literature Cited

- Borges, D. F., Lopes, E. A., Moraes, A. R. F., Soares, M. S., Visôtto, L. E., Oliveira, C. R., and Valente, V. M. M. 2018. Formulation of botanicals for the control of plant-pathogens: A review. Crop Prot. 110:135-140.
- Castle, W. S., Baldwin, J. C., and Singh, M. 2011. Pomegranate in Florida for commercial enterprises and homeowners. Pages 33-40 in: Proc. Annu. Mtg. Florida State Hort. Soc. 2011.
- CDMS. 2019. Crop Data Management Systems. https://www.cdms.net. Accessed on November 18, 2019.
- Chandra, R., Jadhav, V. T., and Sharma, J. 2010. Global scenario of pomegranate (Punica granatum L.) culture with special reference to India. Fruit Veg. Cereal Sci. Biotechnol. 4:7-18.
- Forcelini, B. B., and Peres, N. A. 2018. Widespread resistance to QoI fungicides of Colletotrichum acutatum from strawberry nurseries and production fields. Plant Health Prog. 19:338-341.
- Gajbhiye, M., Sathe, S., Marathe, R., and Deshmukh, R. 2013. Antifungal Bacillus subtilis AFB22 from pomegranate with potential to control fruit rot. Res. J. Biotechnol. 8:26-35.
- Gurjar, M. S., Ali, S., Akhtar, M., and Singh, K. S. 2012. Efficacy of plant extracts in plant disease management. Agric. Sci. 3:425-433.
- Harman, G. E., Latorre, B., Agosin, E., San Martin, R., Riegel, D. G., Nielsen, P. A., Tronsmo, A., and Pearson, R. C. 1996. Biological and integrated control of Botrytis bunch rot of grape using Trichoderma spp. Biol. Control 7: 259-266.
- Horsfall, J., and Barratt, R. 1945. An improved grading system for measuring plant diseases. (Abstr.) Phytopathology 35:655.
- Howell, C. R. 2003. Mechanisms employed by Trichoderma species in the biological control of plant diseases: The history and evolution of current concepts. Plant Dis. 87:4-10.

- Jadhav, V., and Sharma, K. 2009. Integrated management of diseases in pomegranate. Paper presented at: souvenir and abstracts 2nd International Symposium on Pomegranate and Minor Including Mediterranean Fruits, UAS Dharwad.
- Jain, K., and Desai, N. 2018. Pomegranate the cash crop of India: A comprehensive review on agricultural practices and diseases. Int. J. Health Sci. Res. 8:315-336.
- Jeger, M. J. 2004. Analysis of disease progress as a basis for evaluating disease management practices. Annu. Rev. Phytopathol. 42:61-82.
- Kadam, V. A., Dhutraj, D., Pawar, D., and Patil, D. 2018. Bio efficacy of bio agents and botanicals against *Alternaria alternata* (Fr.) Keissler causing leaf spot of pomegranate. Int. J. Curr. Microbiol. Appl. Sci. 7:1146-1155.
- Kenward, M. G., and Roger, J. H. 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53:983-997.
- Kupper, K. C., Corrêa, F. E., de Azevedo, F. A., and Da Silva, A. C. 2012. Bacillus subtilis to biological control of postbloom fruit drop caused by Colletotrichum acutatum under field conditions. Sci. Hortic. (Amsterdam) 134:139-143.
- Lalancette, N., Furman, L. A., and White, J. F. 2013. Management of peach rusty spot epidemics with biorational fungicides. Crop Prot. 43:7-13.
- Munhuweyi, K., Lennox, C. L., Meitz-Hopkins, J. C., Caleb, O. J., and Opara, U. L. 2016. Major diseases of pomegranate (*Punica granatum* L.), their causes and management—A review. Sci. Hortic. (Amsterdam) 211:126-139.
- Muthukumar, A., and Udhayakumar, R. 2015. Bioefficacy studies of new fungicide molecules (Ridomil Gold 68% WP) against leaf spot and fruit rot/ spot of pomegranate. Bioscan 10:1859-1862.

- Nargund, V., Jayalakshmi, K., Benagi, V., Byadgi, A., Patil, R., Melgarejo, P., and Valero, D. 2012. Status and management of anthracnose of pomegranate in Karnataka State of India. Options Méditerr. Sér. A: Sémin. Méditerr. 103: 117-120.
- National Agricultural Statistics Service (NASS). 2012. Census of Agriculture. U.S. Department of Agriculture, Washington, DC.
- Thomidis, T. 2014. Fruit rots of pomegranate (cv. Wonderful) in Greece. Australas. Plant Pathol. 43:583-588.
- Tronsmo, A. 1991. Biological and integrated controls of *Botrytis cinerea* on apple with *Trichoderma harzianum*. Biol. Control 1:59-62.
- Xavier, K., KC, A., Crous, P., Groenewald, J., and Vallad, G. 2019a. Dwiroopa punicae sp. nov. (Dwiroopaceae fam. nov., Diaporthales), associated with leaf spot and fruit rot of pomegranate (Punica granatum). Fungal Syst. Evol. 4:33-41.
- Xavier, K., KC, A., Peres, N. A., Deng, Z., Castle, W. S., Lovett, W., and Vallad, G. E. 2019b. Characterization of *Colletotrichum* species causing anthracnose of pomegranate in the Southeastern United States. Plant Dis. 103:2771-2780.
- Xavier, K., KC, A., and Vallad, G. E. 2019c. Diseases of Pomegranate (*Punica granatum*) in Florida. PP349. University of Florida Institute of Food and Agricultural Sciences, Gainesville, FL. https://edis.ifas.ufl.edu/pdffiles/PP/PP34900.pdf. Accessed November 20, 2019.
- Xavier, K., KC, A., and Vallad, G. E. 2020. Fungicide application timing essential for the management of leaf spot and fruit rot on pomegranate (*Punica granatum* L.) in Florida. Plant Dis. 104:1629-1637.