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TITLE: Coupling host resistance with the evaluation of biofungicides for the management of common foliar vegetable diseases in Pennsylvania.

PRINCIPLE INVESTIGATOR:

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INTRODUCTION:

Biofungicides, whether based on microbial or biochemical active ingredients, are a primary tool for vegetable disease management in organic production and are becoming commonly used as a part of conventional fungicide programs due reduced environmental and human safety risk as well as increased production flexibility in terms of reduced REIs and PHIs and potential market opportunities. In addition, due to the expedited EPA registration process, an increasing number are becoming available each year and marketed in trade magazines. For example, in a recent issue of American Vegetable Grower, five of the seven pest management advertisements were for biopesticides. In university trials, product efficacy is often evaluated on highly susceptible cultivars under conditions to promote high disease pressure as an "acid" test. Under these conditions when disease development is assessed regularly throughout the season, in general, biofungicides are most effective when disease pressure is low and then fail later in the season under higher levels of disease pressure. In these trials, the use of biofungicides is less frequently leveraged with the use of host resistance which is the scenario when they are most likely to be effective. Trials coupling host resistance and conventional programs with biofungicides were conducted in 2018 and 2019 for powdery mildew on pumpkin, downy mildew on cucumber, early blight on tomato and in 2019 for late blight on tomato. The methods and results of the 2019 trials are presented and then comparisons with the 2018 trials are summarized in the summary/conclusion. All trials were conducted at The Pennsylvania State University Russell E. Larson Agricultural Research Center in Pennsylvania Furnace, PA in Centre County.

METHODS AND RESULTS FOR EACH TRIAL:

PUMPKIN POWDERY MILDEW TRIAL

Methods: The field was plowed and fertilizer (N-P-K; 46-0-0 at 110 lb/A, 0-0-60 at 200 lb/A and 11-52-0 at 230 lb/A based on soil test results) was broadcast and incorporated on 9 May. Nineteen raised beds with 1.5-mil black-plastic mulch and a single row of drip-irrigation tape were formed in early Jun. Raised beds were 48-in. wide and 6-in. high and were laid on 13ft centers. For each set of three rows, the center row was an untreated guard row and the two outer rows were treatment rows. On 6 Jun, pumpkin cvs. Howden (susceptible) and Bayhorse Gold (moderate resistance) were direct-seeded in designated plots, two seeds per hole, spaced 36-in. apart, one row per bed. Plants were thinned to one plant per hole or missing plants were replaced with transplants in early Jul so that each plot consisted of six plants. Plots were single rows, 20ft long and separated in row by 22-ft of bare plastic. Fungicide treatments were replicated four times and arranged in a modified split-plot randomized complete block design with nine treatments being applied to cv. Howden and a subset of six treatments being applied to cv. Bayhorse Gold for a comparison of host resistance. Fungicide treatment was the whole block and cultivar the sub-plot. Cucumber beetle was managed with an application of Admire 2F applied with a Solo backpack sprayer (mixed 5 ml/1gal then drench applied 5.0 fl oz/plant) on 14 Jun and squash bugs with an application of Lambda (3.8 oz/A) plus PBO (4.8 oz/A) on 7 Aug. Weeds were managed with an application of Medal EC (1.33 pt/A) plus Profine 75DF (0.5 oz/A) and Round-up (1.5 pt/A) on 4 Jun. On 27 Jun and 5 Jul, the trial was spot sprayed with Scythe and Round-up respectively and supplemental hand weeding was conducted throughout the season. Plots were fertigated regularly (N-P-K; 20-20-20 at 7 lb/A) and at increasing intervals as the crop matured. Fungicide applications were made using a tractor mounted, CO₂powered sprayer with an offset-boom (R&D Sprayers, Opelousas, LA) traveling 2 mph and calibrated to deliver 52 gpa at 50 psi at the tank through six TX-18 hollow-cone nozzles on 20-in. centers on 25 Jul, 1, 8, 16 and 22 Aug. Powdery mildew severity was rated on a continuous scale of 0 to 100% based on the percentage of upper and lower leaf surface (rated separately) showing symptoms on six leaves per plot on 1, 10, 17, and 25 Aug and used to calculate area under the disease progress curve (AUDPC) values. Rainfall totals (in.) were 3.76, 2.83 and 3.16 for Jun, Jul, and Aug, respectively.



Figure 1. All fungicides were applied at the highest label rate. Vivando, Quintec and Torino were tank-mixed with Bravo Weather Stik (2.0 pt/A) plus Induce (0.125% v/v) when applied. The numbers in parentheses indicate the following fungicide applications dates: 1 = 25 Jul; 2 = 1 Aug; 3 = 8 Aug; 4 = 16 Aug and 5 = 22 Aug. As indicated by the letters at the top of the bars, programs containing Vivando, Quintec and Torino were most effective on the upper (black bars; lower case letters) and lower leaf surface (gray bars; upper case letters) for both cultivars Howden and Bayhorse Gold (P < 0.05).

Results: Natural inoculum was relied upon and symptoms were first observed on 22 Jul. Disease pressure became severe, with the untreated control plots in both cultivars reaching near 100% disease severity on both the upper and lower leaf surfaces by the end of the trial. There was a significant cultivar by treatment interaction so fungicide treatments were compared separately within each cultivar (Figure 1). Not surprisingly, disease was less severe on

cv. Bayhorse Gold compared to Howden across all treatment comparisons. All treatments significantly reduced powdery mildew on both the upper and lower leaf surface however, the programs which included targeted fungicides (Vivando, Quintec and Torino) were most effective by providing 90 to 100% powdery mildew control. Inclusion of the biorational products LifeGard (a.i. *Bacillus mycoides* isolate J) or OxiDate 2.0 (a.i. hydrogen dioxide and peroxyacetic acid) did not improve efficacy of the standard commercial rotation of Vivando, Quintec and Torino tank-mixed with Bravo Weather Stik. The two biorational programs also effectively managed powdery mildew on the upper leaf surface (80 to 90% control) but were less effective on the lower leaf surface providing between 20 and 35% control compared to the untreated plots. This is not surprising since these products are protectants, so plant coverage is critical for them to be effective.

CUCUMBER DOWNY MILDEW TRIAL

Methods: The field was plowed and fertilizer (N-P-K; 19-19-19 at 500 lb/A based on soil test results) was broadcast and incorporated on 9 May. Nineteen raised beds with 1.5-mil black-plastic mulch and a single row of drip-irrigation tape were formed in early Jun. Raised beds were 48-in. wide and 6-in. high and were laid on 9-ft centers. For each set of three rows, which were bordered by driveways, the center row was an untreated guard row and the outer rows were two treatment rows. Plots were single rows, 16-ft long and separated in row by 8-ft bare plastic buffers. The field was divided into two separate experiments but managed identically aside from individual plot treatments. On 7 Jun, trial 1 was direct seeded with cv. Darlington and trial 2 was direct seeded with cvs. Darlington (susceptible) and SV4719CS (intermediate resistance) two seeds per hole, spaced 24-in. apart, one row per bed in designated treatment plots. Guard rows of both trials were directed seeded with cv. Straight Eight. Fungicide treatments were replicated four times. Trial 1 was ranged in a randomized complete block design and trial 2 was arranged in a split-plot randomized complete block design with cultivar as the whole-plot and fungicide treatment as the sub-plot. Plots were thinned to one plant per hole or missing plants replaced with transplants in late Jun so that each plot consisted of eight plants. Cucumber beetles were managed with a soil application of Admire 2F applied with a Solo backpack sprayer (mixed 5 ml/1gal then drench applied 5.0 fl oz/plant) on 14 Jun and foliar applications of Baythroid (2.8 fl oz/A) on 2 Aug and Warrior (1.92 oz/A) on 14 Aug. Weeds were managed with an application of Curbit EC (3.0 pt/A), Profine 75DF (1 oz/A) and Round-up (1.5 pt/A) on 4 Jun that was activated with 0.5 in. of overhead irrigation on 5 Jun. On 27 Jun and 5 Jul, the trials were spot sprayed with Scythe and Round-up respectively and supplemental hand weeding was conducted throughout the season. Plots were fertigated regularly (N-P-K; 20-20-20 at 7 lb/A) and at increasing intervals as the crop matured. Fungicide applications were made using a tractor mounted, R&D CO₂ powered offset-boom sprayer traveling 2 mph and calibrated to deliver 52 gal/A at 52 psi at the tank through six TX-18 hollow-cone nozzles on 20-in. centers on 6, 14, 21 and 28 Aug. Downy mildew severity was rated on a continuous scale of 0 to 100% based on the percentage of the leaf surface showing symptoms on six leaves per plot on 17, 22, 26 Aug and 1 Sep. Whole plot disease severity was also assessed as the percentage of symptomatic leaves across the whole plot on each assessment date. Rainfall totals (in.) were 3.76, 2.83 and 3.16 for Jun, Jul, and Aug, respectively.

Results: Natural inoculum was relied upon and symptoms were first observed on 16 Aug. Disease pressure became severe, with the untreated control plots of both cultivars reaching nearly 100% disease severity when the trials were ended. This resulted in only four treatments applications so for several of the programs the complete program sequence scheduled was not applied. There was no statistical difference in disease development between the cvs. Darlington and SV4719CS, indicating that host resistance was ineffective in this trial, so the data was pooled prior to analysis. Including biopesticide(s) [LifeGard, Stargus (a.i. *Bacillus amyloliquefaciens* strain F727)and OxiDate 2.0 and OxiPhos 3.0 (a.i. hydrogen peroxide and phosphorous acid)] did not improve disease management over a commercial standard rotation of Ranman, Previcur Flex and Zampro tank mixed with Bravo Weather Stik; however this rotation was highly effective (nearly 100% control). Alternation between Actinovate (a.i. *Streptomyces lydicus* WYEC 108) and Elumin tank mixed with Bravo Weather Stik was statistically as effective as the programs including four applications of targeted fungicides. The program that started with two applications of Zonix (a.i. rhamnolipid surfactant) followed by Ranman was least effective. Had the trial last longer before the untreated controls reached 100% disease severity, applications of Ranman and Previcur Flex would have also been applied.



Figure 2. All fungicides were applied at the highest label rate. When applied Ranman, Previcur Flex, Zampro, Actinovate and Elumin were tank-mixed with Bravo Weather Stik (2.0 pt/A). The numbers in parentheses reflect the following fungicide applications dates: 1 = 6 Aug; 2 = 14 Aug; 3 = 21 Aug; and 4 = 28 Aug. As indicated by the letters at the top of the bars, all treatments except Zonix and Ranman were equally effective at managing downy mildew on individual leaves (black bars; lower case letters) and across the entire plot (gray bars, upper case letters) (P < 0.05). There was no difference between the cultivars Darlington and SV4719CS so the data was combined prior to analysis.

TOMATO EARLY BLIGHT TRIAL

Methods: The field was plowed and fertilizer (N-P-K, 46-0-0 at 185 lb/A and 11-52-0 at 145 lb/A based on soil test results) was broadcast and incorporated on 26 Jun. Tomato transplants cvs. Mountain Fresh Plus and BHN 964 were transplanted on 28 Jun. A starter fertilizer (N-P-K, 20-20-20 at 7 lb/A) along with Admire 2F (1.0 pt/A) was applied in the transplant water. Each plot was 12-ft-long and separated by a 5-ft break within the row and 5-ft between row centers. Untreated guard rows, planted with cv. Gem (susceptible processing cultivar) on 26 Jun, separated each treatment row. Each plot was planted with 8 transplants spaced 18-in. apart. Treatments were replicated four times and arranged in a split-plot randomized complete block design with cultivar as the whole plot and fungicide treatment as the subplot. Weeds were managed with an application of Medal EC (1.5 pt/A) and Omni Metribuzin 75DF (0.33 lb/A) on 27 Jun and Scythe (5%) was spot sprayed between the rows on 31 Jul. Plots were fertigated regularly (N-P-K, 20-20-20, 7.0 lb N/A) through a single row of drip irrigation tape laid on the soil surface adjacent to the plants. Fungicide applications were made using a tractor mounted, CO₂-powered offset-boom sprayer traveling at 3 mph and calibrated to deliver 30 gal/A at 32 psi through three TX-18 hollow-cone nozzles on 23, 31 Jul, 8, 16, 23, 30 Aug, 5 and 12 Sep. Unexpectedly, foliar late blight resulting from natural inoculum was observed on 10 Aug. Due to significant late blight disease pressure, the trial was reconfigured with three complete replicates and unneeded plots were burned down with Scythe (7%) on 14 Aug. The trial was maintenance sprayed with Ranman (2.75 oz/A) on 12, 19 Aug and 3 Sep, Orondis Opti A (3.0 oz/A) on 14 Aug and Zampro (14.0 oz/A) on 28 Aug and 11 Sep. Maintenance sprays were made with a R&D PTO Sprayer with five TX-18 nozzles. Early in the evening on 25 Jul, the guard rows were inoculated with a mix of three Pennsylvania Alternaria solani isolates (1.5 x 10⁴ spores/ml sprayed in three to four spots per 12 ft guard row using a handheld Hudson sprayer). Foliar early blight severity was evaluated on 17, 25 Aug, 1, 8, and 14 Sep by estimating the percent of early blight symptomatic foliage across the whole plot and area under the disease progress curve (AUDPC) was calculated. Rainfall totals (in.) were 2.83, 3.16, and 1.44 for Jul, Aug and 1 to 14 Sep, respectively.

Results: Disease severity developed slowly reaching near 50% in the untreated control plots by the end of the season. The late blight fungicide program was effective at slowing disease progression, so it did not interfere with early blight disease assessments. There was no significant effect of cultivar on early blight disease development, so the data was pooled across cultivar Mountain Fresh Plus and BHN 964 prior to analysis. Aside from weekly applications of Stargus tank mixed with Badge X2, the majority of treatments significantly reduced early blight compared to the untreated controls. Replacing Bravo Weather Stik in the commercial standard program

(alternating Bravo Weather Stik with Quadris tank mixed with Bravo Weather Stik) with the OMRI-listed fungicides, LifeGard or Double Nickel (a.i. *Bacillus amyloliquefaciens* strain D747) were equally as effective for managing early blight and replacing it with Champ statistically further reduced disease. The alternation of Fontelis and Regalia (a.i. extract of *Reynoutria sachalinensis*) tank mixed with Kocide 3000 was also equally as effective as Champ alternated with Quadris tank mixed with Bravo Weather Stik.



Figure 3. The fungicide rates are as follows: Champ (3.2 lb/A),), Quadris (5 fl oz/A), Bravo Weather Stik (Bravo WS 2 pt/A), Fontelis (1 pt/A), Regalia (4 qt/A), Kocide 3000 (1 lb/A), Aprovia Top (13.5 fl oz/A), OxiDate 2.0 64 fl oz/50 gal water), LifeGard (4.5 oz/100 gal), Double Nickel (0.55 lb/A), Cueva (0.5 gal/A), Stargus (2.0 qt/25 gal) and Badge X2 2.0 lb/A). The numbers in parentheses reflect the following fungicide application dates: 1 = 23 Jul; 2 = 31 Jul; 3 = 8 Aug; 4 = 16 Aug; 5 = 23 Aug; 6 = 30 Aug; 7 = 5 Sep and 8 = 12 Sep. There was no significant difference in the level of disease control obtained on the cv. Mountain Fresh or BHN 964 so the data were pooled together within fungicide treatment across cultivar prior to data analysis. The black bars represent percent early blight disease control on 14 Sep (P < 0.0001) and the gray bars represent disease control over the course of the whole season calculated based on area under the disease progress curves (P < 0.0001).

TOMATO LATE BLIGHT TRIAL

Methods: Tomato seedlings were transplanted on 28 Jun. Each plot was 12 ft long with 8 plants spaced 18 in. apart. There was a 5-ft unplanted area between plots within the row and 5 ft between row centers. Guard rows planted with processing cv. Gem separated the treatment rows. Treatments were replicated four times in a randomized complete block design. Unexpectedly, foliar late blight lesions resulting from natural inoculum (US-23) were observed on 12 Aug therefore fungicide treatments were started immediately. Applications were made on 12, 20 and 28 Aug using a tractor-mounted, R&D CO₂-powered side boom sprayer calibrated to deliver 30 gal/A at 32 psi at the tank through one center and two drop TX-18 nozzles. To ensure uniform disease distribution, a mixture of four isolates of *Phytophthora infestans* clonal lineage US-23, at a concentration of 2.3×10^4 sporangia/ml were inoculated within the guard rows on 15 Aug using a hand-held Hudson sprayer. Foliar late blight severity was evaluated on 17, 22, 25 Aug and 1 Sep based on the percentage of the plot with symptoms. Rainfall totals (in.) were 2.83 and 3.16 for Jul and Aug, respectively.

Results: Disease pressure resulting from natural and artificial inoculum was severe with the untreated control plots quickly reaching 100% severity 20 days after observing first symptoms. Late blight is most effectively managed preventatively. Initiating the fungicide applications when symptoms were first seen, which was not the intention, likely led to an overall reduction in efficacy since the targeted fungicide program still resulted in 25% foliar disease severity. Despite this observation and with one exception, the application of some type of fungicide

program significantly reduced late blight severity compared to the untreated control. Applications of Bravo Weather Stik in alternation with the biofungicides Stargus and Zonix were as effective as a straight Bravo Weather Stik program reducing disease severity by approximately 60% compared to the plots receiving no fungicide. The inclusion of LifeGard, a plant activator, as a tank-mix partner in a conventional commercial rotation of the targeted fungicides Tanos, Previcur Flex and Zampro or OxiDate 2.0 in a Bravo Weather Stik program did not improve the efficacy of the program which may have been the result of application timing. Late blight symptoms were not observed on cv. Defiant even under high disease pressure therefore only the data for cv. Mountain Fresh Plus is shown.



Figure 4. The fungicide rates are as follows: OxiDate 2.0 64 fl oz/50 gal), LifeGard (4.5 oz/100 gal), Zonix (0.8 oz/A = 500 ppm), Stargus (4.0 qt/25 gal), Regalia (4.0 qt/25 gal), and Badge X2 1.75 lb/A). All other products were applied at maximum labelled rates. The numbers in parentheses reflect the following fungicide application dates: 1 = 12 Aug; 2 = 20 Aug; and 3 = 28 Aug. Late blight did not develop on cv. Defiant so only data for cv. Mountain Fresh Plus is shown. The black bars represent the percent late blight disease control obtained from each fungicide program. Treatments were initiated after symptoms were observed and only 3 fungicide applications were made before the trial was ended due to 100% disease severity being reached in the untreated plots.

CONCLUSION/SUMMARY:

From the trials conducted in 2018 and 2019, it is clear depending on the crop and disease, augmenting conventional fungicide programs with select biorational or OMRI-approved products can still provide the same or similar level of disease control as the conventional program. The inclusion of products such as LifeGard and Stargus tank-mixed with the conventional fungicide program, in general, did not provide an added benefit when their applications were timed using IPM principles rather than a regular calendar spray program. Products such as these as well as Regalia and Double Nickel that have modes of action characterized as boosting plant defenses may be more effective when used at planting and then throughout the season. For diseases such as powdery mildew on pumpkin and early blight on tomato, fungicide programs that included biorational program. Keep in mind, when evaluating the incorporation of new products into your own fungicide programs, it is important to not only review the research-based results available to help select the most promising biofungicides and OMRI-approved products but also consider conducting your own on-farm comparisons. The efficacy of fungicide programs can also be dependent on farm-specific factors so conducting a side-by-side comparison in conjunction with regular scouting to monitor disease development are important for successful disease management.

BIOFUNGICIDE PROJECT BUDGET:

Hourly wages for summer help to assist in conducting proposed field work for		
12 wks at 24 to 25 hrs/wk at \$11.00/hr	\$3	3200.00
Fringe benefits (7.8%)	\$	249.92
Supplies to establish, maintain, and evaluate the replicated field trials	\$ 2	245.00
Travel (in-state)	\$	300.00

Duration of Project: April 1, 2019 to November 30, 2019

Total.....\$ 5994.92

Signature:

Bal K Guyino