Cornell Cooperative Extension
Eastern NY Commercial Horticulture Program

Organic Management of Brassica Diseases: Efficacy Study of Biorational Pest Control Materials

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2020 Annual Report

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I. Background

Brassica crops, like broccoli, are susceptible to a number of plant pathogens. Black Rot (BR), Downy Mildew (DM) and Alternaria Leaf Spot/Head Rot (ALS), are among the most common and destructive diseases of brassica crops grown in the Northeast. Production of marketable organic brassica crops in the Hudson Valley is limited by these pervasive diseases despite grower efforts to implement best cultural practices like crop rotation and improving air flow in crops by adjusting planting spacing. A number of low-risk "biorational" products are allowed in organic production and are labeled to manage these diseases. One of the obstacles organic growers face in managing the three diseases is discerning which, if any, of these disease control materials are effective.

With support from the Hudson Valley Farm Hub (HVFH) in 2020, Cornell Cooperative Extension Eastern New York Commercial Horticulture (ENYCHP) regional vegetable specialists Ethan Grundberg and Teresa Rusinek began to evaluate nine biorational fungicides on broccoli produced at HVFH where BR, DM and ALS have all posed persistent production challenges. The 2020 trial was designed to generate data to help answer the following research questions:

1. What impact, if any, do pre-transplant applications of Badge X2 (Copper Oxychloride, Copper Hydroxide) or LifeGard (Bacillus mycoides isolate J) have on foliar disease severity observed over the production season when compared to broccoli plants in an untreated control that received no pre-transplant fungicide/bactericide?
2. Is there any synergistic disease suppression effect of combining pre-transplant biorational product applications with in-field biorational applications?
3. How well can each of the nine biorational products work alone to suppress BR, DM, and ALS in broccoli when compared to an untreated control that received no in-season fungicide/bactericide applications?
4. Can statistically significant differences in marketable broccoli yield be observed in a small-plot randomized complete block design trial intended to primarily evaluate disease severity?
5. What trends in disease severity reduction can be observed from a single year product screening trial in order to inform the development of season-long biorational programs?

The 2020 trial results used to help answer these questions will be discussed in the following sections.

II. Methods

Trial Design

The researchers used a randomized complete block design with four replicates and ten treatments for this trial. Each treatment plot was 15 bed-feet long and consisted of three rows of broccoli. The three pre-transplant biorational treatments were randomized and applied at the row level within each 15 bed-foot long plot. The ten field applied biorational treatments were randomized and applied at the plot level over each of the three rows in each 15 bed-foot

long plot. The statistical analyses for the data were completed using a generalized linear model to evaluate the row-level pre-transplant treatments, the plot-level field treatments, and the interaction between the pre-transplant and in-field treatments for statistically significant difference at $p<0.05$. Multiple comparisons of treatment means were made by applying a post-hoc Tukey's HSD at $p<0.05$.

Propagation

'Emerald Crown' broccoli was seeded in 128-cell propagation trays by the HVFH staff on June 18, 2020 using Vermont Compost Fort Lite compost-based potting mix. The seedling trays were removed from the greenhouse on July 14, 2020 to harden off and to receive the pre-plant biorational product application.

Pre-Transplant Biorational Application

The trial design included three pre-transplant biorational application treatments. On July 14, 2020, the 'Emerald Crown' seedlings were removed from the greenhouse and divided into three equal sets of nine trays. One set of trays, the untreated control (UTC) was not sprayed with a biorational product. The second set of trays was sprayed with the induced systemic resistance (ISR) biological product LifeGard (*Bacillus mycoides* isolate J) manufactured by Certis at the labeled greenhouse dilution of 4.5 oz per 100 gallons of solution. The third and final set of trays was sprayed with the copper product Badge X2 (Copper Oxychloride, Copper Hydroxide) at the maximum labeled greenhouse use rate of 2.4 pounds per acre. Both biorational applications were made with a CO_2–powered research backpack sprayer connected to a boom equipped with three turbo twinjet nozzles (TTJ60-10004) spaced 19.5 inches apart.

Field Establishment

The broccoli seedlings were left to harden off for an additional three days after the application of pre-transplant biorational treatments. The trial field was fertilized and tilled by HVFH in a plot that had been planted with broccoli and experienced severe ALS pressure in the fall of 2019. The trial field was dibbled with a water wheel transplanter on July 17, 2020 to mark three rows per five-foot wide bed with 12-inch in-row spacing between each plant. Each 15-bed foot long plot was marked with field stakes after dibbling and a three-foot buffer was left between each plot in the same replicate. First, broccoli seedlings from the untreated pre-transplant trays were planted by hand in a randomized row position (left, center, or right) in each plot. Once the untreated seedlings had been planted, the same process was followed to hand transplant the LifeGard treated broccoli plants and then Badge X2 treated starts. Drip irrigation was installed immediately after transplanting was complete.

Field Biorational Application

Each 15 bed-foot long plot received its first field biorational product application six days after transplanting on July 23, 2020. The field applications of biorationals were all made with a CO_2–powered research backpack sprayer connected to a boom equipped with four turbo twinjet

nozzles (TTJ60-10003) spaced 15 inches apart and calibrated on July 20, 2020 to deliver the equivalent volume of 43.46 gallons per acre. Plots were sprayed on a seven-day schedule for seven consecutive sprays with the final application occurring on September 4, 2020. Each plot was treated with the same biorational product mixed with the OMRI-listed spreader-sticker adjuvant Attach (Pinene (terpene) Polymers, petrolatum, a-(p-Dodecylphenyl)-Omega-hydroxypoly (oxyethylene)) at the highest labeled rate of 16 fl oz per 100 gallons of spray solution for all seven applications. The untreated control plot was not sprayed with any biorational products, but was sprayed weekly with water and Attach at the same rate referenced above in order to maintain consistent leaf wetness across all treatments. The full list of biorational product treatments applied in the field and their application rates can be found in table 1 below.

Table 1: Field Applied Biorational Product Treatments

Treatment	Product	Mfg	Active Ingredient	Application Rate
1	Regalia	Marrone	Extract of Reynoutria sachalinensis	2 qts/acre
2	LifeGard	Certis	Bacillus mycoides isolate J	4.5 oz/100 gal
3	Cueva	Certis	Copper Octanoate (Copper Soap)	2 gal/acre
4	Badge X2	Gowan	Copper Oxychloride, Copper Hydroxide	1.8 lbs/acre
5	Trilogy	Certis	Clarified Hydrophobic Extract of Neem Oil	1% v//v
6	Double Nickel 55 LC	Certis	Bacillus amyloliquefaciens strain D747*	6 qts/acre
7	Stargus	Marrone	Bacillus amyloliquefaciens strain F727* cells and spent fermentation media	3 qts/acre
8	Serenade	Syngenta	QST 713 strain of Bacillus subtilis	4 qts/acre
9	PerCarb	BioSafe	Sodium Carbonate Peroxyhydrate	3 lbs/acre*
10	Untreated Control		None	None

Flea Beetle Management

Since the trial field had been planted with brassica crops in 2019 as well, the broccoli seedlings experienced extreme flea beetle feeding pressure after transplant. HVFH made two applications of Entrust SC (spinosad) at 6 fl oz/acre for flea beetle suppression to the entire trial field independently of the biorational product sprays.

Foliar Disease Severity Data Collection

Foliar disease severity was evaluated in two ways during the trial period. First, the total percent of the plant foliage per plot that was green and healthy was estimated on five dates (7/23, 7/29, 8/28, 9/4, and 9/11/2020). Second, three intensive evaluations were conducted on seven randomly selected plants per row per plot to estimate the percent of the total leaf surface with visible ALS, BR, and DM symptoms per plant. Those evaluations were used to calculate the Area Under the Disease Progress Curve (AUDPC), which is commonly used to evaluate the rate at which diseases are spreading and/or how well fungicides are slowing the spread of foliar diseases in the field over multiple sampling dates.

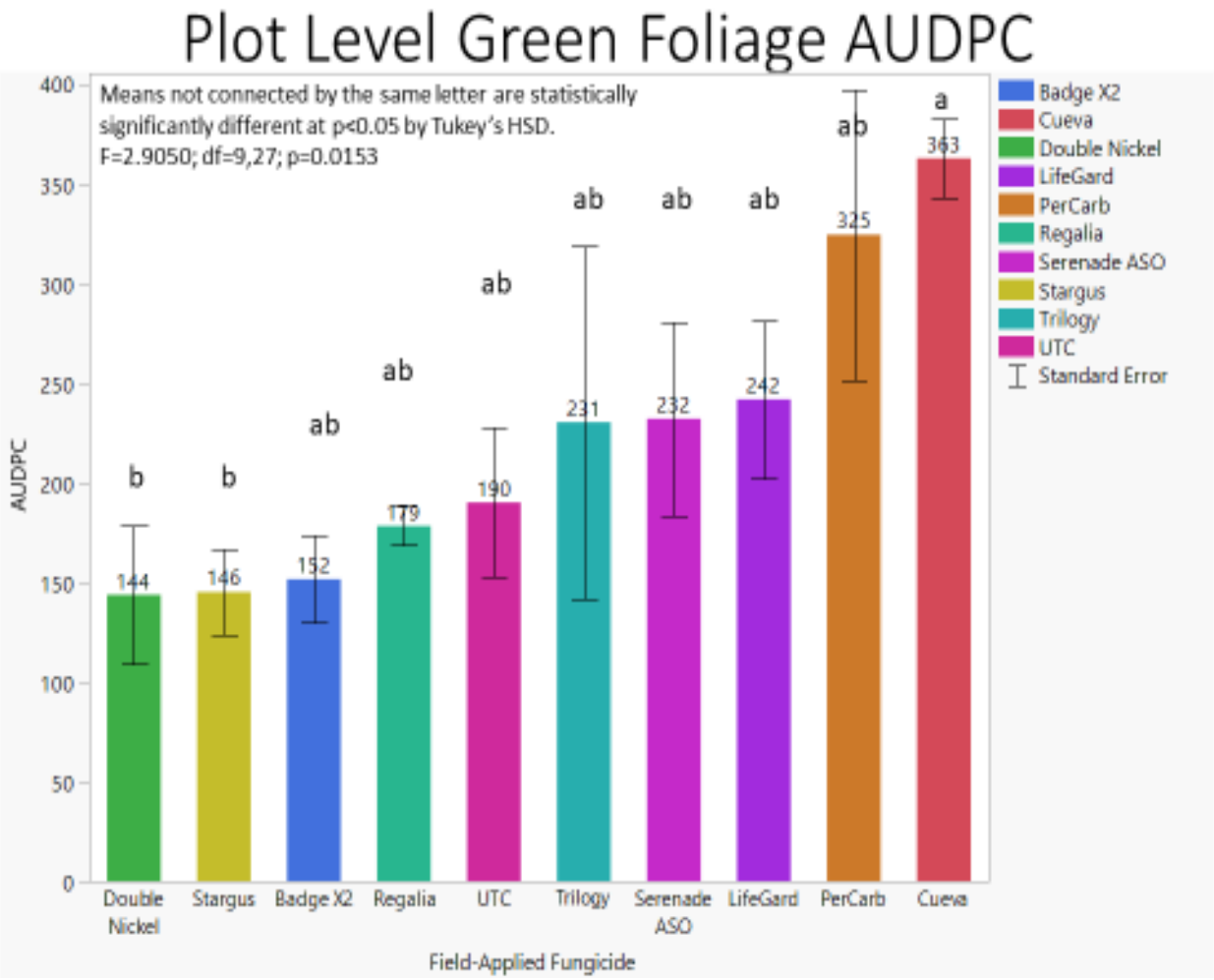
Harvest Data Collection

Seven randomly selected crowns of broccoli per each of the three rows in each plot were harvested and evaluated on 9/11 and 9/13/2020. Stems below the crown were uniformly trimmed to six-inches in length before being weighed using an Ohaus Catapult 1000 compact bench scale with 0.02 lb resolution. Each crown was also measured across the widest diameter using a Fowler 54-100-512-BT electronic calipers with 0.0005"/0.01mm resolution before having the total area of the crown with visible disease symptoms approximated. Finally, each crown was then classified as either marketable (maximum of 10% of the crown with visible disease symptoms) or unmarketable.

III. Results

Percent Green Foliage by Plot

The five plot-level estimates of the percent of total broccoli foliage that was healthy and unaffected by disease symptoms were subtracted from 100 then combined into a single value to calculate an AUDPC for statistical analysis. It is important to note that these plot-level observations do not account for the interaction of the pre-transplant biorational treatments with the in-field fungicide applications. Nevertheless, the data show a statistically significant effect of the in-field biorational treatment on the AUDPC values generated form the green foliage estimates. In this case, the lower numbers had healthier/greener foliage while the treatments with higher values had less healthy and/or more diseased foliage.

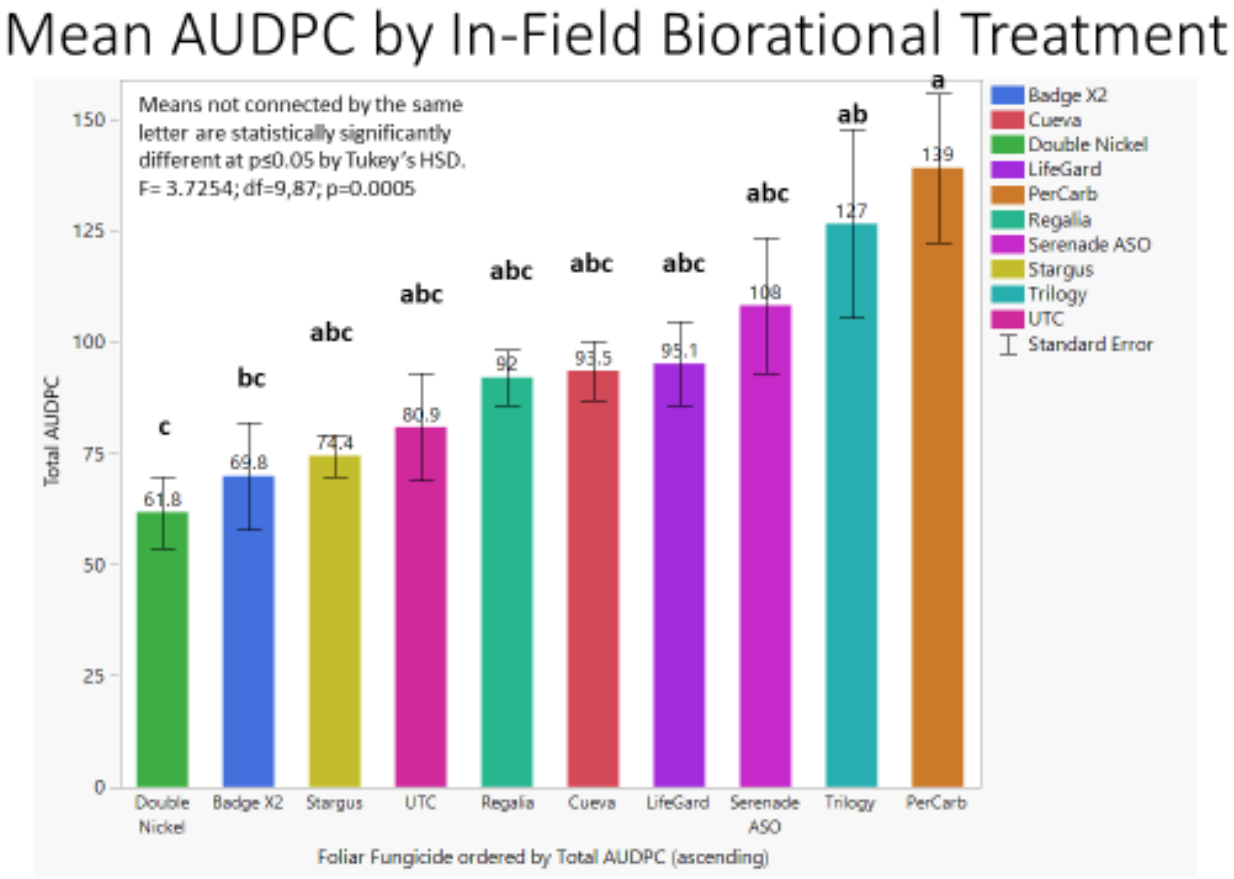


Though there are clear trends towards healthier foliage in the plots treated with Double Nickel, Stargus, and Badge X2, only the Double Nickel and Stargus plots were statistically significantly different from Cueva, which was numerically the worst treatment. However, the more rigorous plant disease evaluations discussed in the AUDPC section below show that the reduction in green foliage in the Cueva plots was mostly due to phytotoxicity from the soap-based copper product rather than higher disease pressure. Similarly, the PerCarb and Trilogy plots both suffered from high levels of phytoxicity damage on the broccoli foliage.

Row-Level AUDPC

The three intensive disease severity evaluations of seven plants per row in each plot were used to calculate AUDPC values and to evaluate the statistical significance of the pre-transplant biorational treatments, the in-field biorational treatments, and the interaction between the pre-transplant and in-field biorational treatments. Neither the pre-transplant biorational treatments (p=0.9369) nor the interaction between the pre-transplant and in-field biorational

applications (p=0.9998) were statistically significant at the 95% confidence interval. However, the in-field biorational treatments did have a statistically significant effect on the calculated AUDPC values (p=0.0005).



Similar to the plot level AUDPC calculations derived from the green foliage estimates, the numeric differences in disease severity between treatments did not translate into clear statistically significant separation between them after a post-hoc Tukey's HSD. Only the Double Nickel and Badge X2 treatments were significantly different from the worst treatment, PerCarb, at $p<0.05$. The untreated control plots, which received applications of only water and the adjuvant Attach, were not statistically significantly different from either the numerically worst or best treatments.

It is also important to note that the primary disease contributing to the AUDPC in 2020 was ALS. There was no DM detected in the trial plot prior to harvest in mid-September and very low levels of BR in the plots.

Percent of Broccoli Crown with Disease Symptoms

A similar statistical model to that described above in the AUDPC section that evaluated the effect of the pre-transplant biorational application, the in-field biorational applications, and the interaction between the two was used to determine the impact on the mean percent of

broccoli crowns covered with disease symptoms at harvest. While only the in-field biorational treatments had a statistically significant effect on the foliar disease severity AUDPC values, the interaction term between the pre-transplant biorational treatments and the in-field biorational treatments was significant in the crown disease evaluation at the 95% confidence interval ($F=1.7134$, $df= 18,780$, $p=0.0324$). Unfortunately, there was no separation between the combinations of pre-transplant and in-field treatments at $p<0.05$ after applying a post-hoc Tukey's HSD. When the less conservative Fisher's Protected LSD post-hoc test was applied, there was some separation between treatments.

Level											Least Sq Mean
PerCarb,UTC	A	B									50.644923
PerCarb,LifeGard	A	B	C	D	E						42.414250
Serenade ASO,UTC	A	B	C		E						39.586881
PerCarb,Badge X2			C	D	E	F					38.129228
LifeGard,LifeGard	A		C	D		F	G				36.746461
LifeGard,UTC	A	B	C	D	E	F	G	H			34.538171
Stargus,Badge X2	A	B	C	D	E	F	G	H	I	J	32.537899
Serenade ASO,Badge X2	A	B	C	D	E	F	G	H	I	J	30.260476
Stargus,LifeGard	A	B	C	D	E	F	G	H	I	J	28.672654
UTC,UTC	A	B	C	D	E	F	G	H	I	J	28.176497
Trilogy,Badge X2	A	B	C	D	E	F	G	H	I	J	27.212109
Cueva,UTC	A	B	C	D	E	F	G	H	I	J	27.102269
LifeGard,Badge X2		B			E			H	I	J	26.389059
Regalia,UTC	A	B	C	D	E	F	G		I		26.195570
UTC,LifeGard	A	B	C	D	E	F	G	H	I	J	26.082134
Stargus,UTC			C	D	E	F	G	H	I	J	25.946292
Trilogy,UTC			C	D	E	F	G	H	I	J	25.220923
Serenade ASO,LifeGard				D		F	G	H	I	J	24.365045
Cueva,Badge X2			C	D	E	F	G	H	I	J	23.432709
UTC,Badge X2			C	D	E	F	G	H	I	J	22.937907
Double Nickel,LifeGard			C	D	E	F	G	H	I	J	22.667358
Double Nickel,Badge X2			C	D	E	F	G	H	I	J	21.173965
Double Nickel,UTC			C	D	E	F	G	H	I	J	20.860367
Trilogy,LifeGard			C	D	E	F	G	H	I	J	20.726133
Cueva,LifeGard			C	D	E	F	G	H	I	J	20.413209
Badge X2,LifeGard						F	G	H	I	J	18.081901
Badge X2,UTC							G	H	I	J	16.868549
Regalia,LifeGard								H		J	16.099728
Regalia,Badge X2								H		J	16.020316
Badge X2,Badge X2									I	J	14.908718

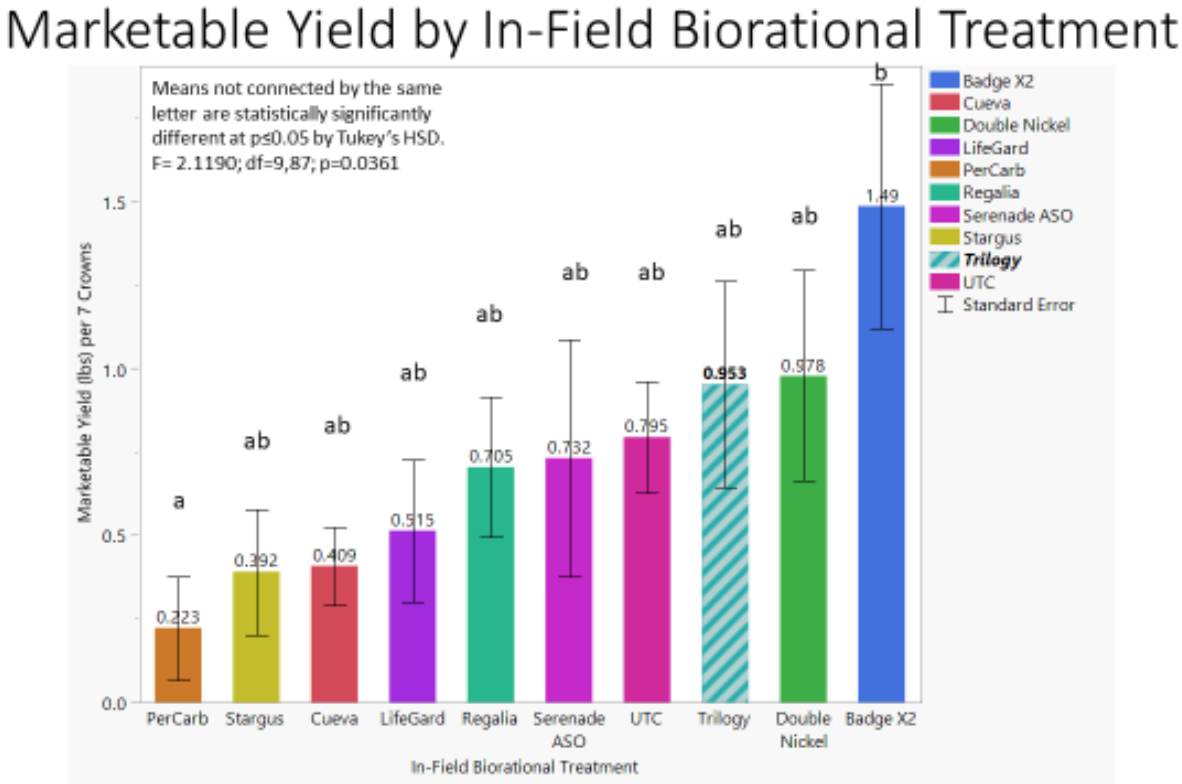
Levels not connected by same letter are significantly different.

As can be seen from the connecting letters report above, most of the combinations of treatments were not significantly different from each other. However, the treatment combinations that included in-field applications of PerCarb and LifeGard tended to have higher

levels of disease on the crowns at harvest compared to broccoli that was sprayed with Badge X2, Regalia, and Double Nickel in the field.

Yield

Using the same statistical model described above, it was determined that the effects of pre-transplant biorational treatment (p=0.9578) and the interaction term (p=0.8688) on marketable yield in pounds per seven broccoli crowns were not statistically significant. The effect of in-field biorational treatment, however, was statistically significant (p=0.0361).



As can be seen in the graph above, only the Badge X2 treatment was statistically significantly different from the treatment with the lowest mean marketable yield, PerCarb.

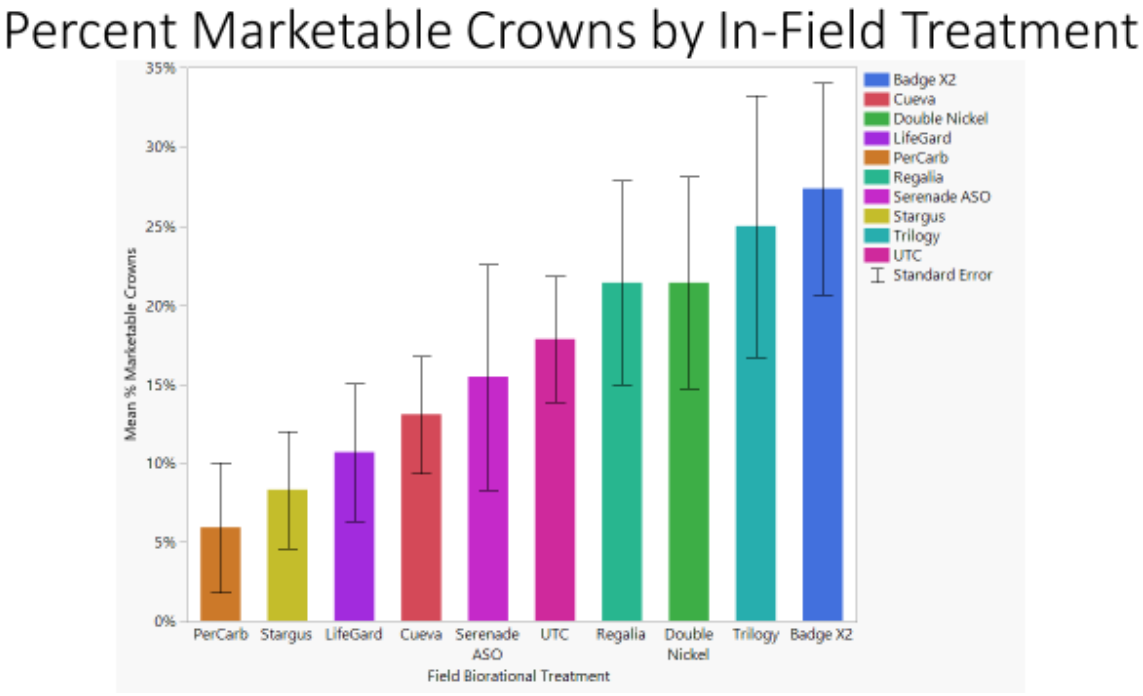
IV. Discussion

Pre-Transplant Biorational Treatments

There was no clear benefit to applying LifeGard or Badge X2 to the broccoli seedlings prior to transplanting in this trial. While the early biorational applications may be effective at reducing the severity of black rot infestations, BR pressure was very low in the trial field in 2020 (possibly due to the dry conditions during the broccoli production period). However, given the extremely high ALS pressure in the trial field, Rusinek and Grundberg have decided to drop the pre-transplant biorational treatments from future trial designs in order to focus on evaluating a broader selection of in-field biorational program treatments targeting ALS and DM.

ALS Suppression

As was mentioned above in the AUDPC results section, ALS was overwhelmingly the primary disease affecting the trial broccoli in 2020. Under these high pressure conditions, none of the biorational products alone provided acceptable levels of suppression. While the broccoli receiving seven sequential applications of Badge X2 and Double Nickel on a seven-day schedule did tend to have fewer disease symptoms and more yield, those treatments still failed to produce over 25% marketable crowns on average (there was no statistically significant effect of pre-transplant treatment, in-field treatment, or the interaction between the two on percent marketable crowns).



The results from the 2020 screening trial support what both private industry and researchers have concluded about managing other diseases with biorational products: biorationals alone are typically insufficient to manage intense disease pressure, especially difficult to control pathogens like those that cause ALS. Instead, more researchers and growers are experiencing greater success managing plant diseases with biorationals by using those products in rotation with more aggressive chemistries and as tank-mix companions to other products. The products that tended to perform better in suppressing ALS in terms of lower AUDPC values and lower percent of the broccoli crown with disease symptoms at harvest, such as Badge X2, Double Nickel, Regalia, and Stargus may be even more efficacious when used in rotation or as tank mix components.

BR and DM Management

Unfortunately, the BR and DM pressure in the 2020 trial field was too low to draw any conclusions about the potential for the biorational products evaluated to manage these diseases. Though BR is a sporadic disease that can be difficult to study in field trialing, DM severity generally increases later in the season when heavier dew sets and lower temperatures create a more favorable environment for the water mold pathogen to spread.

Disease Severity and Crop Yield

Though the inverse relationship between disease severity and crop yield is readily understood by commercial growers, data collected from small plot research trials often fails to support the common sense notion that less marketable yield will result from higher disease pressure. In the 2020 trial, for example, the neem oil product Trilogy had the second highest AUDPC, but the second highest percent of marketable crowns. The PerCarb treated broccoli did have the highest AUDPC and lowest marketable yield, but there are few other clear trends to emerge from the yield data.

PerCarb Rates

At the conclusion of the trial, it was discovered that the rate of PerCarb that was used in the 2020 trial was incorrect. The labeled rate calls for a 3 pound per 100-gallon dilution, but the researchers incorrectly used a 3 pound per acre rate. This resulted in almost a doubling of the labeled concentration of PerCarb and may explain the serious phytotoxicity observed in the PerCarb-treated plots and overall poor performance of the product in the trial.

LifeGard

The induced systemic resistance (ISR) product LifeGard is not intended to be used as a stand-alone product to manage plant diseases. Nevertheless, the researchers wanted to include the

product in the 2020 biorational screening to confirm that it's suggested use as a component in a biorational product rotation would be most appropriate.

V. Ongoing Research

The 2020 trial was intended to inform the development of more robust season-long biorational programs built by rotating through different chemistries and tank-mixing compatible products. Given the trends toward lower disease severity in plots treated with Badge X2, Regalia, Stargus, and Double Nickel, those products will be included in several different trial programs in 2021. The researchers will also include several newly released OMRI-listed biorational products in the 2021 trial, including Theia (*Bacillus subtilus* strain AFS032321), Howler (*Pseudomonas chlororaphis* strain AFS009), and Oso (polyoxin D zinc salt). The team has developed a total of 22 biorational programs to evaluate with the goal of collecting data to help answer the following research questions and potentially inform a strategic reduction in the number of copper applications per season while maintaining satisfactory disease suppression:

1. Can the addition of a biofungicide or biorational fungicide to a weekly copper application significantly reduce alternaria, black rot, and/or downy mildew severity? If so, does the reduction in disease severity translate to a significant increase in marketable yield compared to the use of copper alone? If so, is the increase in marketable yield large enough to justify the additional expense of adding the biofungicide to the copper applications?

	Program 1*	Program 2	Program 3	Program 4	Program 5	Program 6	Program 7	Program 8	Program 9
Week 1	UTC	Badge	Badge + Regalia	Badge + LifeGard	Badge + Stargus	Badge+ Double Nickel	Badge+OSO	Badge + Howler	Badge + Theia
Week 2	UTC	Badge	Badge + Regalia	Badge + LifeGard	Badge + Stargus	Badge+ Double Nickel	Badge+OSO	Badge + Howler	Badge + Theia
Week 3	UTC	Badge	Badge + Regalia	Badge + LifeGard	Badge + Stargus	Badge+ Double Nickel	Badge+OSO	Badge + Howler	Badge + Theia
Week 4	UTC	Badge	Badge + Regalia	Badge + LifeGard	Badge + Stargus	Badge+ Double Nickel	Badge+OSO	Badge + Howler	Badge + Theia
Week 5	UTC	Badge	Badge + Regalia	Badge + LifeGard	Badge + Stargus	Badge+ Double Nickel	Badge+OSO	Badge + Howler	Badge + Theia
Week 6	UTC	Badge	Badge + Regalia	Badge + LifeGard	Badge + Stargus	Badge+ Double Nickel	Badge+OSO	Badge + Howler	Badge + Theia
Week 7	UTC	Badge	Badge + Regalia	Badge + LifeGard	Badge + Stargus	Badge+ Double Nickel	Badge+OSO	Badge + Howler	Badge + Theia

2. Can alternating applications of copper with a biofungicide or biorational fungicide provide a significant reduction in disease severity compared to a program of every other week copper applications alone? If so, does the reduction in disease severity translate to a significant increase in marketable yield compared to the use of alternate week copper applications alone? If so, is the increase in marketable yield large enough to justify the additional expense of adding the alternate week biofungicide to the program?

	Program 10	Program 11	Program 12	Program 13	Program 14	Program 15	Program 16	Program 17	Program 18
Week 1	None	Regalia	LifeGard	Stargus	Double Nickel	Oso	Howler	Theia	PerCarb
Week 2	Badge	Badge	Badge	Badge	Badge	Badge	Badge	Badge	Badge
Week 3	None	Regalia	LifeGard	Stargus	Double Nickel	Oso	Howler	Theia	PerCarb
Week 4	Badge	Badge	Badge	Badge	Badge	Badge	Badge	Badge	Badge
Week 5	None	Regalia	LifeGard	Stargus	Double Nickel	Oso	Howler	Theia	PerCarb
Week 6	Badge	Badge	Badge	Badge	Badge	Badge	Badge	Badge	Badge
Week 7	None	Regalia	LifeGard	Stargus	Double Nickel	Oso	Howler	Theia	PerCarb

3. Can Regalia alternated with tank mix applications of copper and a biofungicide/biorational provide a comparable level of disease suppression to weekly applications of the tank mix combinations (blue programs)?

	Program 19	Program 20
Week 1	**Regalia**	**Regalia**
Week 2	**Badge + Stargus**	**Badge + Double Nickel**
Week 3	**Regalia**	**Regalia**
Week 4	**Badge + Stargus**	**Badge + Double Nickel**
Week 5	**Regalia**	**Regalia**
Week 6	**Badge + Stargus**	**Badge + Double Nickel**
Week 7	**Stargus**	**Double Nickel**

4. Can copper be eliminated from late fungicide applications (weeks 6 and 7) in order to reduce the risk of phytotoxicity on broccoli crowns without sacrificing the disease suppressing performance of the fungicide programs when compared to programs including late copper applications (blue programs)? Does spraying a stand alone biofungicide/biorational late improve disease suppression and marketable yield compared to programs with no fungicide applications in weeks 6 and 7?

	Program 21	Program 22
Week 1	Badge+ Double Nickel	Badge+ Double Nickel
Week 2	Badge+ Double Nickel	Badge+ Double Nickel
Week 3	Badge+ Double Nickel	Badge+ Double Nickel
Week 4	Badge+ Double Nickel	Badge+ Double Nickel
Week 5	Badge+ Double Nickel	Badge+ Double Nickel
Week 6	NONE	Double Nickel
Week 7	NONE	Double Nickel

The target transplant date for the 'Emerald Crown' broccoli will be moved three weeks later in the season in 2021 in an effort to try to increase the probability of detecting DM in the trial plots and evaluating the biorationals for DM management.