PA Vegetable Marketing and Research Program – PA Vegetable Growers Association
Final Report
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Project Title: Optimizing management of cucumber downy mildew to currently used fungicides and production methods

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Objectives of research proposal: Cucurbit downy mildew, caused by *Pseudoperonospora cubensis* (Berk. et Curt.) Rost., is one of the most important diseases of cucurbitaceous crops. Widespread cucumber crop failures have occurred throughout the Eastern half of the U.S. since 2004. New strains of the downy mildew pathogen introduced in 2004 are resistant to commonly used fungicides, and have overcome the genetic resistance of cucumber cultivars. It is our goal in this project to assess fungicide resistance in *P. cubensis*, predict disease spread and develop improved, economically and environmentally appropriate IPM approaches. The objectives of this research are:

1) assess the relative sensitivity of the downy mildew pathogen to fungicides commonly used to manage the disease using a greenhouse assay we have developed recently;

2) determine the relationship between *P. cubensis* spore counts, plant canopy for ground and trellis production, and outbreak of disease; and

3) develop an IPM approach to downy mildew management in cucumbers.

Materials and Methods:

1) Assessing the relative sensitivity of the downy mildew pathogen to fungicides commonly used:

Nine products were evaluated for efficacy against *Pseudoperonospora cubensis***:** Previcur Flex, Presidio, Ranman, Mancozeb, Bravo Weather Stik, Gavel, Tanos, Zampro, Ridomil Gold (Table 1).

Cucumber seeds (var. Lafayette) were planted individually in 4” square pots filled with Fafard potting mix in a greenhouse at the Ohio Agriculture Research and Development Center (OARDC) in Wooster, OH, on August 2. The experiment was set up in a complete block design

with four single plant replications of each fungicide treatment. All 10 treatments per block were placed in a 15 cell-carrying tray for easy transport to and from the downy mildew spore inoculation field. Seeded pots were hand watered and fertilized with 10-10-10 every 3 to 4 days from germination through the end of the experiment. When the second seedling leaf was fully expanded on September 4, fungicide treatments were applied to the top surface of both the first and second true leaves. Each leaf received 2.4 ml sprays from a bottle mister containing the fungicide at the label rate with a water carrier equivalent to 55 gallons per acre. On September 5, the treated Lafayette cucumber seedlings were transported outside the greenhouse in Wooster to a cucumber field to be exposed to downy mildew, and the remaining seedlings were transported in a covered truck to cucumber field at the Muck Crops Research Station in Willard, OH and squash field at the North Central Agricultural Research Station in Fremont, OH to be exposed to downy mildew at those locations. After field exposure for 24 hours, all seedlings were returned to the greenhouse in Wooster to monitor downy mildew development and phytotoxicity on the leaves 3, 5, 7 and 10 days after field exposure using a scale of 0-100% foliage affected. Analysis of variance was performed for experiment using the generalized linear mixed models procedure with SAS statistical software and means were separated by the least significant difference (LSD) test.

Table 1. Treatments and rates

Treatment	Rate
Previcur Flex	1.2 pt/A
Presidio	4 fl oz/A
Ranman	2.75 fl oz/A
Mancozeb	210 grams/100 L
Bravo Weather Stik	2 pt/A
Gavel	2.0 lb/A
Tanos	8 oz/A
Zampro	0.8 L/ha
Ridomil Gold	2 pt/A
Non-treated control	-

2) Determining the relationship between *P. cubensis* spore counts, plant canopy for ground and trellis production, and outbreak of disease:

The experiment was conducted at the Ohio Agricultural Research and Development Center's Muck Crops Agricultural Research Station in Celeryville, OH on Linwood muck soil (pH 5.6). The field was disked on 30 Apr. The fertilizer potassium (300 lb/A K_2O), phosphorous (150 lb/A P_2O_5), nitrogen (200 lb/A NH_4NO_3) was incorporated and the field was plowed on 1 May. Seeds cv. slicing cucumber cultivar 'Dasher II' were sown on 6 May into 72-cell plug trays containing Scott's 360 Metro seedling mix. The field was disked, leveled, and raised beds were prepared on 5 Jun. The herbicide Makaze (22 fl oz/A) was applied and incorporated into the test field on 23 Apr and 23 May and the herbicides Curbit EC (3 pt/A) and Command 3 ME (1pt/A) on 5 Jun. Cucumber seedlings were transplanted on 5 Jun. Each production method plot consisted of 5 rows and each row with 10 plants spaced 2 ft apart on 6 ft centers. Two Burkard 7 day & 24 hour Hirst spore samplers were purchased and placed within plots on trellises and another within a traditional cucumber planting on the ground on Jul 10. Downy mildew spore counts were determined for the two different plant canopies beginning in July 24 and continue through the end of the study. The insecticides Mustang Max (4 fl oz/A) and Sevin XLR Plus (1 qt/A) were applied on 12 Jun; 19, 26 Jun; 3, 15, 22 and 29 Jul; 5 Aug, respectively. The field was cultivated on 20 Jun, 12 and 16 Aug and hand weeded on 24 and 26 Jun and 3, 18, 22 Jul and 1 and 16 Aug. The severity of downy mildew on foliage was evaluated on 24, 31 Jul and 7, 14 and 21 Aug using a scale of 0-100% foliage affected.

Cucumber fruits from all plants of each treatment and production method row were harvested, counted and weighed on 24 and 31 Jul and 7, 14 and 21 Aug. Average maximum temperatures for 5-30 Jun, Jul and 1-21 Aug were 79.1, 81.2 and 77.5 ºF; average minimum temperatures were 63.4, 62.9 and 56.9 ºF; and total rainfall amounts were 2.8, 9.2 and 2.3 in., respectively.

3) Developing an IPM approach to downy mildew management in cucumbers:

The experiment was conducted at the Ohio Agricultural Research and Development Center's Muck Crops Agricultural Research Station in Celeryville, OH on Linwood muck soil (pH 5.6). The field was disked on 30 Apr. The fertilizer potassium (300 lb/A K_2O), phosphorous (150 lb/A P_2O_5), nitrogen (200 lb/A NH_4NO_3) was incorporated and the field was plowed on 1 May. Seeds cv. slicing cucumber cultivar 'Dasher II' were sown on 6 May into 72-cell plug trays containing Scott's 360 Metro seedling mix. The field was disked, leveled, and raised beds were prepared on 5 Jun. The herbicide Makaze (22 fl oz/A) was applied and incorporated into the test field on 23 Apr and 23 May and the herbicides Curbit EC (3 pt/A) and Command 3 ME (1pt/A) on 5 Jun. Cucumber seedlings were transplanted on 5 Jun. Treatments were arranged in a randomized complete block design with four replications. Each treatment consisted of one row with 10 plants spaced 2 ft apart on 6 ft centers. Treatment rows were alternated with non-treated ground production guard rows. Stakes and trellis string were placed into the trellis production plots on 2 Jul. Downy mildew was first observed on 24 Jul (disease severity 3.8% in the non-treated control). Treatments were applied using a tractor mounted 3.0 hitch (hydraulic attached) motor driven sprayer (40 psi, 29.1 gal/A, 2 mph) beginning 16 Jul and ending 21 Aug for a total of six applications for those applied on a 7-day schedule and four applications for those applied on a 10-day schedule. The insecticides Mustang Max (4 fl oz/A) and Sevin XLR Plus (1 qt/A) were applied on 12 Jun; 19, 26 Jun; 3, 15, 22 and 29 Jul; 5 Aug, respectively. The field was cultivated on 20 Jun, 12 and 16 Aug and hand weeded on 24 and 26 Jun and 3, 18, 22 Jul and 1 and 16 Aug. The severity of downy mildew on foliage was evaluated on 24, 31 Jul and 7, 14 and 21 Aug using a scale of 0-100% foliage affected. Cucumber fruits from all plants of each treatment and production method row were harvested, counted and weighed on 24 and 31 Jul and 7, 14 and 21 Aug. Average maximum temperatures for 5-30 Jun, Jul and 1-21 Aug were 79.1, 81.2 and 77.5 ºF; average minimum temperatures were 63.4, 62.9 and 56.9 ºF; and total rainfall amounts were 2.8, 9.2 and 2.3 in., respectively. Analysis of variance was performed using the general linear models procedure with SAS statistical software and means were separated using Fisher's least significant difference test.

Results:

1) Assessing the relative sensitivity of the downy mildew pathogen to fungicides commonly used:

There were significant differences in downy mildew pressure between three locations (Table 2). Downy mildew pressure was high for plants located in Celeryville (Table 3), reaching 63.8% and in Wooster (Table 4), reaching 57.5% foliage affected in the water control. However, downy mildew pressure was low in Fremont (Table 5), reaching 11.3% foliage affected in the water control. All treatments significantly reduced severity of downy mildew compared to the untreated control for all three locations. Ten days-long disease severity was most suppressed by treatments with Presidio, Ranman, Mancozeb, Bravo Weather Stik, Gavel

and Zampro for plants located in Celeryville and Wooster. However, downy mildew severity at the end of the experiment and disease progress were higher for the cucumber plants treated with Ridomil Gold, Previcur Flex and Tanos than the other treatments. No phytotoxicity was observed in treated cucumber plants.

Table 2. Effect of different treatments on severity of downy mildew disease on cucumber plants located in Celeryville, Wooster and Fremont

Location	Downy mildew[z]	
	% disease (15 Sep)	AUDPC[y]
Celeryville……..	16.4 a[x]	68.8 a
Wooster……..	11.8 b	20.2 b
Fremont……..	1.8 c	4.8 c
P value	<.0001	<.0001

[z]Disease ratings and area under the disease progress curve (AUDPC) were based on the percent foliage affected.
[y]AUDPC values were calculated according to the formula: $\sum([(x_i+x_{i-1})/2](t_i-t_{i-1}))$ where x_i is the rating at each evaluation time and (t_i-t_{i-1}) is the number of days between evaluations.
[x]Values are the means of four replicate plots; treatments followed by the same letter within a column are not significantly different at $P \leq 0.05$. Means were separated using the least significant difference test.

Table 3. Effect of different treatments on severity of downy mildew disease on cucumber plants located in Celeryville

Treatment and rate	Downy mildew[z]	
	% disease (15 Sep)	AUDPC[y]
Previcur Flex 1.2 pt/A ……..	31.3 c[x]	152.5 b
Presidio 4 SC 4 fl oz/A ……	0.0 e	0.0 d
Ranman 400SC 2.75 fl oz/A……	0.0 e	0.0 d
Mancozeb 210 grams/100 L……	0.0 e	0.0 d
Bravo Weather Stik 6SC 2 pt/A…….....	0.3 e	0.4 d
Gavel 75DF 2.0 lb/A……..	0.1 e	0.2 d
Tanos 8 oz/A……..	24.4 d	90.9 c
Zampro 0.8 L/ha…….....	0.9 e	2.3 d
Ridomil Gold 2 pt/A……	43.8 b	175.6 b
Non-treated control……	63.8 a	266.3 a
P value	<.0001	<.0001

[z]Disease ratings and area under the disease progress curve (AUDPC) were based on the percent foliage affected.
[y]AUDPC values were calculated according to the formula: $\sum([(x_i+x_{i-1})/2](t_i-t_{i-1}))$ where x_i is the rating at each evaluation time and (t_i-t_{i-1}) is the number of days between evaluations.
[x]Values are the means of four replicate plots; treatments followed by the same letter within a column are not significantly different at $P \leq 0.05$. Means were separated using the least significant difference test.

Table 4. Effect of different treatments on severity of downy mildew disease on cucumber plants located in Wooster

Treatment and rate	Downy mildew[z]	
	% disease (15 Sep)	AUDPC[y]
Previcur Flex 1.2 pt/A ……	11.3 cd[x]	44.0 c
Presidio 4 SC 4 fl oz/A ……..	0.0 d	0.0 d
Ranman 400SC 2.75 fl oz/A……..	0.0 d	0.0 d
Mancozeb 210 grams/100 L……..	0.0 d	0.0 d
Bravo Weather Stik 6SC 2 pt/A…….	0.1 d	0.2 d
Gavel 75DF 2.0 lb/A……	0.1 d	0.4 d
Tanos 8 oz/A……	17.5 c	54.1 c
Zampro 0.8 L/ha……....	0.3 d	0.4 d
Ridomil Gold 2 pt/A……	31.3 b	100.9 b
Non-treated control……	57.5 a	211.9 a
P value	<.0001	<.0001

[z]Disease ratings and area under the disease progress curve (AUDPC) were based on the percent foliage affected.
[y]AUDPC values were calculated according to the formula: $\sum([(x_i+x_{i-1})/2](t_i-t_{i-1}))$ where x_i is the rating at each evaluation time and (t_i-t_{i-1}) is the number of days between evaluations.
[x]Values are the means of four replicate plots; treatments followed by the same letter within a column are not significantly different at $P \leq 0.05$. Means were separated using the least significant difference test.

Table 5. Effect of different treatments on severity of downy mildew disease on cucumber plants located in Fremont

Treatment and rate	Downy mildew[z]	
	% disease (15 Sep)	AUDPC[y]
Previcur Flex 1.2 pt/A ……	2.4 b[x]	5.5 b
Presidio 4 SC 4 fl oz/A ……...	0.0 c	0.0 c
Ranman 400SC 2.75 fl oz/A……...	0.0 c	0.0 c
Mancozeb 210 grams/100 L……	0.0 c	0.0 c
Bravo Weather Stik 6SC 2 pt/A……..	0.3 c	0.2 c
Gavel 75DF 2.0 lb/A…….....	0.1 c	0.2 c
Tanos 8 oz/A……..	1.9 bc	4.8 bc
Zampro 0.8 L/ha……....	0.1 c	0.2 c
Ridomil Gold 2 pt/A……...	2.3 b	7.8 b
Non-treated control……	11.3 a	29.3 a
P value	<.0001	<.0001

[z]Disease ratings and area under the disease progress curve (AUDPC) were based on the percent foliage affected.
[y]AUDPC values were calculated according to the formula: $\sum([(x_i+x_{i-1})/2](t_i-t_{i-1}))$ where x_i is the rating at each evaluation time and (t_i-t_{i-1}) is the number of days between evaluations.
[x]Values are the means of four replicate plots; treatments followed by the same letter within a column are not significantly different at $P \leq 0.05$. Means were separated using the least significant difference test.

2) Determining the relationship between *P. cubensis* spore counts, plant canopy for ground and trellis production, and outbreak of disease:

Downy mildew appeared naturally on Jul 24 (disease severity 2.5% in ground production and 1.5% in trellis production plants). Downy mildew disease severity reached 100% in ground production plants and 95% in trellis production plants by the end of the experiment (Table 6). Trellis production method applied plots had numerically high marketable number (85%) and weight (97%) than ground production methods applied plots (Table 7). Cucumber fruit were naturally infected with *Phytophthora capsici* by the first harvest (24 Jul). Trellis production yielded numerically low number (33%) and weight (18%) of *P. capsici* infected fruit and low number (23%) and weight (27%) of culls than ground production plants.

Burkard 7 day & 24 hour Hirst spore samplers were placed in the plots on Jul 10. The drums inside the spore traps were changed every seven days. The drum with coated transparent plastic tape was brought back to the lab in the carrying box. The Melinex tape on the drum was lifted from the corner using forceps and then starting point was laid to the Perspex block. The block is marked at 48 mm intervals, representing lengths of 24 hours, the tape was cut using fine pointed scissors and sections were mounted on the microscope slide and stained with lactophenol cotton blue solution for 15 min. The cover slip was placed in position and the edges were sealed with nail polish. The slides were placed under microscope and the entire tape was examined for downy mildew spores. Spores were counted and logged into a data sheet under specific day for particular production method. Counting one drum of slides took approximately 3-4 hours.

Downy mildew appeared on Jul 24 (disease severity 2.5% in ground production and 1.5% in trellis production plants). Four days before the confirmation of the foliar disease, spore counts were showing two digit numbers and the confirmation day was showing three digit numbers for trellis and ground production methods (Table 8).

Table 6. Effect of production methods on severity of downy mildew disease on cucumber plants

Production methods	Downy mildew				
	% disease (24 Jul)	% disease (31 Jul)	% disease (7 Aug)	% disease (14 Aug)	% disease (21 Aug)
Ground……	2.5	30	65	95	100
Trellis……..	1.5	20	45	90	95

Table 7. Effect of production methods on yield on cucumber plants

Production methods	Marketable fruit		Phytophthora fruit rot		Culls	
	(no./plot)	(Ton/A)	(no./plot)	(Ton/A)	(no./plot)	(Ton/A)
Ground……	251	33.8	66	5.4	80	8.9
Trellis……..	465	66.7	44	4.4	61	6.5

Table 8. Downy mildew spore trap daily totals (counts/m^3/day) for different production methods

Date	Ground	Trellis
10-Jul	0	0
11-Jul	0	0
12-Jul	0	0
13-Jul	0	0
14-Jul	1	1
15-Jul	2	0
16-Jul	2	2
17-Jul	2	3
18-Jul	0	1
19-Jul	3	3
20-Jul	12	17
21-Jul	13	10
22-Jul	17	27
23-Jul	30	40
24-Jul	106*	112*
25-Jul	110	130
26-Jul	142	197
27-Jul	230	252
28-Jul	132	145
29-Jul	18	87
30-Jul	62	58
31-Jul	17	27
1-Aug	18	13
2-Aug	12	18
3-Aug	8	7
4-Aug	22	37
5-Aug	5	2
6-Aug	7	5
7-Aug	10	25
8-Aug	38	15
9-Aug	35	13
10-Aug	30	8
11-Aug	10	3
12-Aug	52	15
13-Aug	42	12
14-Aug	23	8
15-Aug	3	2
16-Aug	2	10
17-Aug	3	8
18-Aug	3	6
19-Aug	2	8
20-Aug	1	4
21-Aug	3	2

*Downy mildew was first observed on the foliage on this date.

3) Developing an IPM approach to downy mildew management in cucumbers:

Downy mildew appeared naturally and disease pressure moderate to high. The effects of treatment on downy mildew severity (final rating and AUDPC) were significant. There were significant interactions between treatment and production method in end of season disease severity and AUDPC. There were no significant differences in downy mildew severity and AUDPC between trellis production and ground production plots (Table 9). Downy mildew severity at the end of the experiment and disease progress throughout the season were higher in plants treated with Bravo Weatherstick 6SC alone than in those treated with Presidio 4SC + Bravo WeatherStik 6SC alternated with Ranman 400SC + Bravo WeatherStik 6SC. There was no difference on plants between the 7- or 10-day application schedules of Presidio 4SC + Bravo WeatherStik 6SC alternated with Ranman 400SC + Bravo WeatherStik 6SC and Bravo WeatherStik 6SC alone in downy mildew severity at the end of the experiment or in disease progress.

Cucumber fruit were naturally infected with *Phytophthora capsici* by the first harvest (24 Jul). Trellis production yielded lower number of *P. capsici* infected fruit than ground production plants. There were no significant differences in the number of *P. capsici* infected fruit among the treatments or interactions between treatment and production method. Trellis production method applied plots had higher marketable yield than ground production methods applied plots (Table 10). However, trellis production method applied plots produced significantly more culls than ground production method applied plots. There were significant differences in marketable yield among treatments.

Table 9. Effect of different treatments and production methods and their interactions on severity of downy mildew disease and yield on cucumber plants

Fixed effects*	Downy mildew		Phytophthora fruit rot (no./plot)	Culls (no./plot)	Marketable yield (ton/A)
	% disease (21 Aug)	AUDPC			
Treatment P values……	≤0.0001	≤0.0001	0.3692	0.0620	0.0136
Production method P values……..	0.2560	0.4416	0.0547	0.0372	0.0256
Treatment X Production method P values……	0.0471	0.0173	0.2157	0.0247	0.0120

*Statistical analyses were performed using linear mixed model with treatment, production method and treatment x production method as fixed variables.

Table 10. Effect of different treatments and production methods on severity of downy mildew disease and yield on cucumber plants

Treatment and rate/A	Production methods	Downy mildew[x]		Phytophthora fruit rot (no./plot)	Culls (no./plot)	Marketable yield (ton/A)
		% disease (21 Aug)	AUDPC[w]			
Bravo Weather Stik 6SC 2 pt (1-4)[z]	Ground	88.8 bc[v]	1040.5 b	14.8 a	17.5 bcd	7.9 cde
Bravo Weather Stik 6SC 2 pt (1-6)[y]	Ground	87.5 c	1103.4 b	24.0 a	13.0 d	3.9 e
Presidio 4SC 4 fl oz + Bravo Weather Stik 6SC 2 pt (1,3) alt Ranman 400SC 2.75 fl oz + Bravo Weather Stik 6SC 2 pt (2,4)[z]	Ground	50.0 d	566.7 c	22.3 a	29.5 a-d	11.5 a-d
Presidio 4SC 4 fl oz + Bravo Weather Stik 6SC 2 pt (1,3,5) alt Ranman 400SC 2.75 fl oz + Bravo Weather Stik 6SC 2 pt (2,4,6)[y]	Ground	48.8 d	540.8 c	36.0 a	32.0 a-d	11.4 a-d
Non-treated control	Ground	96.3 a	1807.3 a	11.0 a	16.0 cd	7.1 de
Bravo Weather Stik 6SC 2 pt (1-4)[z]	Trellis	87.5 c	1024.6 b	13.3 a	40.3 a	13.4 abc
Bravo Weather Stik 6SC 2 pt (1-6)[y]	Trellis	86.3 c	1073.6 b	14.0 a	34.0 abc	8.5 b-e
Presidio 4SC + Bravo Weather Stik 6SC 2 pt (1,3) alt Ranman 400SC 2.75 fl oz + Bravo Weather Stik 6SC 2 pt (2,4)[z]	Trellis	50.0 d	643.7 c	14.3 a	37.5 ab	13.9 ab
Presidio 4SC 4 fl oz + Bravo Weather Stik 6SC 2 pt (1,3,5) alt Ranman 400SC 2 pt + Bravo Weather Stik 6SC 2 pt (2,4,6)[y]	Trellis	43.8 d	506.2 c	15.3 a	47.8 a	15.4 a
Non-treated control	Trellis	95.0 a	1710.6 a	8.0 a	32.8 a-d	10.6 a-d
P value		0.0471	0.0173	0.2157	0.0247	0.0120

[z]Application dates for treatments applied on an ~10-day schedule were: 1= 16 Jul; 2=26 Jul; 3= 5 Aug; 4= 15 Aug.
[y]Application dates for treatments applied on an ~7-day schedule were: 1= 16 Jul; 2=23 Jul; 3= 30 Jul; 4= 6 Aug; 5= 14 Aug; 6= 21 Aug.
[x]Disease ratings and area under the disease progress curve (AUDPC) were based on percent foliage affected.
[w]AUDPC values were calculated according to the formula: $\sum([(x_i+x_{i-1})/2](t_i-t_{i-1}))$ where x_i is the rating at each evaluation time and (t_i-t_{i-1}) is the number of days between evaluations.
[v]Values are the means of four replicate plots; treatments followed by the same letter within a column are not significantly different at P≤0.05.