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Title: On-farm evaluation of cucurbit powdery mildew fungicide resistance using a cucurbit seedling bioassay.

Principle Investigator:

Beth K. Gugino, Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802; (814) 865-7328; bkgugino@psu.edu.

Collaborators:

- Meg McGrath, Dept. of Plant Pathology, Cornell University LIHREC, 3059 Sound Ave., Riverhead, NY 11901; 631-727-3595; mtm3@cornell.edu.
- Andy Wyenandt, Dept. of Plant Biology and Pathology, Rutgers University, 121 Northville Road, Bridgeton, NJ 08302; 856-455-3100 ext. 4144; wyenandt@njaes.rutgers.edu.
- Steve Rideout, Dept. Plant Pathology, Physiology and Weed Science, Virginia Tech ESAREC, 33446 Research Drive, Painter, VA 23420; 757-414-0724 ext. 17; srideout@vt.edu.
- Kate Everts, Dept. of Plant Science and Landscape Architecture, University of Maryland LESREC; 27664 Nanticoke Road, Salisbury, MD 21801; 410-742-8780; keverts@umd.edu.
- Bob Mulrooney, Dept. of Plant and Soil Sciences; University of Delaware, 148 Townsend Hall, Newark, DE 19716; 302-831-4865; bobmul@udel.edu.

Introduction:

Resistance is a major issue confronting powdery mildew management in cucurbit crops. Fungicides are the main management tool. Mobile fungicides needed to effectively control powdery mildew are at risk for developing resistance. Growers often do not know they are using a fungicide affected by resistance, even when it is no longer effective, because they are using it in a program with other fungicides. Applying a fungicide that is inadequately effective because of resistance is not only a waste of money, and puts the crop at risk for disease control failure, but also facilitates resistance developing to other fungicides used. Alternating among fungicides in different chemical classes is the main practice for managing (e.g. delaying) resistance development.

The goal of this project was to determine sensitivity to resistance-prone fungicides for the powdery mildew pathogen in the Mid-Atlantic region (PA, NY, NJ, MD, VA and DE) and then use this information to guide regional fungicide recommendations. This was accomplished by using a simple cucurbit seedling bioassay that has been used successfully in PA, NJ and NY in the past. In 2008, pathogen sensitivity to fungicides, in particular to DMI fungicides and Pristine, on Long Island, NY, exhibited some differences compared to eastern PA, leading to different fungicide recommendations and thus revealing the need for local information on resistance. It is especially critical for growers to know the resistance status in their region in order to be able to select the most appropriate fungicides to use in a resistance management program with Quintec since its label was expanded in 2009 to include other cucurbit crops in addition to melon. Additionally, in 2008 and 2009 regional differences to boscalid resistance were detected. In NY

and PA in 2008, a few isolates collected at the end of the season were completely resistant to boscalid (able to tolerate 500 ppm, which is equivalent to a labeled rate). Again in 2009, a few isolates collected in NY and from fungicide trials in NJ were completely resistant to boscalid in lab assays while in PA reduced sensitivity was detected using the seedling bioassay in commercial fields. These differences reflect the variation in the occurrence of resistant strains and again the importance of understanding regional variation when recommending fungicides for managing cucurbit powdery mildew. It is also important for understanding sensitivity shifts within the pathogen population over time when multiple years of data are compared so when resistance is detected, it provides justification for Section 18 requests.

Here, we report our efforts during 2010 to address the following objectives.

Objectives:

The purpose of this project was to:

- 1. Determine the sensitivity of the powdery mildew pathogen to the active ingredients in the fungicides currently recommended in PA, NY, NJ, MD, VA and DE.
- 2. Incorporate results into fungicide recommendations.
- 3. Disseminate results and recommendations to growers via newsletters as well as summer and winter grower meetings.

Methods and Results:

Objective 1: Determine the sensitivity of the powdery mildew pathogen to the active ingredients in the currently recommended fungicides in PA, NY, NJ, MD, VA and DE.

A seedling fungicide sensitivity bioassay was conducted in three cucurbit fields in south central PA as well as in NY, NJ, MD, DE and the Eastern Shore of VA. Greenhouse grown pumpkin seedlings of a susceptible cultivar (e.g. Sorcerer or Howden) were treated with different fungicides at different doses based on the label rates as well as previously conducted bioassays and placed out in fields where early symptoms of powdery mildew were observed for at least 4 hours to collect spores. The bioassay seedlings were re-collected and maintained in a greenhouse. After approximately 7 to 10 days, the seedlings were evaluated for symptom development and/or severity. The fungicides and doses evaluated included thiophanate-methyl (Topsin M, FRAC code 1) at 50 ppm, trifloxystrobin (Flint, FRAC code 11) at 50 ppm, boscalid (Endura, FRAC code 7, an active ingredient in Pristine) at 50, 175 ppm and 500 ppm (label rate), myclobutanil (Rally, FRAC code 3) at 40, 80 and 120 ppm, triflumizole (Procure, FRAC code 3) at 80 and 120 ppm, tebuconazole (Folicure, Tebuzol, FRAC code 3) at 40 and 80 ppm, difenconazole (Inspire, FRAC code 3) at 40 ppm, and quinoxyfen (Quintec, quinoxfen, FRAC code 13) at 1 and 10 ppm. Grower fields were identified in collaboration with several county extension educators in each of the respective states or the bioassay was conducted on near-by research farms.

A seedling fungicide sensitivity bioassay was conducted in two to eight fields in each of five states between late July and mid-October 2010 for a total of 16 fields. In MD, VA, NJ and PA powdery mildew disease pressure was low in the fields assayed therefore making data interpretation more difficult.

Resistance to FRAC code 11 fungicides (trifloxystrobin – Flint at 50 ppm) was detected at a high level in the majority of fields assayed in NY, NJ, PA, MD, DE and VA. This is not surprising since resistance to this group is thought to be common and widespread. It was surprising however to find that resistance to FRAC code 1 fungicides was not detected in all the fields assayed in NY and that the proportion of the pathogen population that was resistant was substantially lower than what has been observed in previous years. This was not the case in fields assayed in the other states. Resistance to FRAC code 1 and code 11 fungicides is considered qualitative, thus increasing the application rate/dose will not improve control; the pathogen is either susceptible or resistant.

In NY, PA, DE and VA strains of the cucurbit powdery mildew pathogen detected were able to tolerate 500ppm boscalid (FRAC code 7), the active ingredient in Endura. Not surprisingly in those same fields, strains resistant to lower doses (175 and 50ppm) of boscalid were also detected. Strains able to tolerate the 500ppm concentration would be fully resistant to this chemistry in the field because it is within a range that reflects a field application at the highest label rate of Pristine. Due to cross-resistance, resistance to the pyraclostrobin (FRAC code 11), the other component of Pristine, is thought to already exist within the powdery mildew pathogen population. Despite these observations, in fungicide efficacy trial in PA, Pristine (18.5 oz/A) still significantly reduced season-long powdery mildew severity compared to the untreated control however control was improved when used in a program alternating with Procure (FRAC code 3) or with Procure and Quintec (FRAC code 13). In contrast, Pristine was only effective early in powdery mildew development in the fungicide efficacy trial conducted in NY.

Powdery mildew developed on all doses of myclobutanil (Rally, FRAC code 3) evaluated; however development was the least at the highest dose (120 ppm) in fields in NY, PA and DE where this dose was evaluated. Powdery mildew also developed on the majority of plants treated with the lowest dose of triflumizole (Procure, FRAC code 3) and to a lesser extent at the higher dose (120 ppm). Resistance to this group of fungicides is quantitative therefore the highest label rate of Procure (8 oz/A) is still recommended because it provides twice as much active ingredient as Rally at its highest label rate. In the 2010 pumpkin powdery mildew fungicide trial in PA, Procure (8 oz/A) is provided good powdery mildew control compared to the untreated control. On Long Island, NY, where four different FRAC code 3 fungicides were evaluated, cucurbit powdery mildew exhibited some variation in sensitivity to FRAC code 3 fungicides but was most sensitive to difenconazole (Inspire, FRAC code 3). Unfortunately, difenconazole was only evaluated using the powdery mildew bioassay in NY this past season.

All of the cucurbit powdery mildew populations assayed across the five states exhibited a very high level of sensitivity to quinoxyfen (FRAC code 13), the active ingredient in Quintec. There was only an extremely low level of detection of strains able to tolerate 1 and 10 ppm in each of the Mid-Atlantic states.

In MD and NY, powdery mildew sensitivity was monitored at the end of the season using an additional set of bioassay plants. By the end of the season, the sensitivity of the pathogen population is often related to season-long fungicide use and efficacy. In MD, the percent of powdery mildew colonies that developed on pumpkin leaves treated with 50 ppm boscalid (Endura, FRAC code 3) compared to the untreated control almost tripled from 30 Jul to 12 Oct.

For the leaves treated with 50 ppm thiophanate-methyl and 50 ppm trifloxystrobin, populations increased 5.5 and 4.5 times that of the untreated control by the end of the season. In NY, shifts in the pathogen population to a higher frequency of resistant strains in response to fungicide use were also detected and associated with efficacy of powdery mildew control. For example, in a Farm 6 (Table 2) were powdery mildew control was considered good throughout the season, the proportion of the population tolerating 500 ppm boscalid increased from 0% on 31 Aug to 11% on 21 Sept compared to Farms 9 and 10. In these fields where control was poor, the proportion of the population tolerating 500 ppm boscalid increased from 14 and 11% on 31 Aug to 70 and 52% on 21 Sept.

Current cucurbit powdery mildew recommendations: For cucurbit powdery mildew management, the current recommendation is to start applying mobile fungicides (Quintec, Pristine, and Procure) when the scouting threshold of 1 out of 50 older leaves showing symptoms has been reached. Early in the season there is less selection pressure for resistant powdery mildew strains because there are fewer spores to be exposed to the active ingredient. Later in the season switch to a protectant spray program. When applying mobile fungicides (Quintec, Pristine, or Procure) always tank mix with a protectant and alternate between modes of action or FRAC codes. Two consecutive applications of Quintec can be applied but there is a total crop limit of 4 applications. After initiating a spray program continue to scout the fields looking at both the upper and lower leaf surface to evaluate your level of powdery mildew control.

Objectives 2 and 3: Incorporate results into fungicide recommendations and disseminate results and recommendations to growers via newsletters as well as field meeting.

It is anticipated that information derived from these bioassays will be incorporated into winter meeting presentations in each of the states by each of the collaborating researchers.

Additional funding for the powdery mildew bioassays conducted in NY came from the Friends of Long Island Horticulture.

| | Active ingredient | Trade name | Dose (ppm) | Mid-Atlantic states | | | |
|--------------|------------------------|---------------------|---------------|------------------------|---|--|--|
| FRAC code | | | | Evaluated | Resistance detected at some level | | |
| 1 | Thiophanate- methyl | Topsin M | 50* | NY, NJ, PA, DE, MD, VA | NY (variable), NJ, PA, DE, MD, VA (very low) | | |
| 11 | Trifloxystrobin | Flint | 50* | NY, NJ, PA, DE, MD, VA | All states | | |
| | | | 50 | NY, NJ, PA, DE, MD, VA | NY, PA, DE, MD, VA (?) | | |
| 7 | Boscalid | Endura | 175 | NY, NJ, PA | All states | | |
| | | | 500* | NY, PA, DE, VA | All states | | |
| | Myclobutanil | Rally | 40 | NY, NJ, PA, DE, MD, VA | NY, PA, DE | | |
| 3 | | | 80 | NY, PA | NY, NJ, PA (very low) | | |
| | | | 120 | NY, NJ, PA, DE, VA | NY, PA, DE (very low), VA (?) | | |
| 2 | Triflumizole | D | 40 | NY, NJ, PA | NY, PA | | |
| 3 | | Procure | 80 | NJ, PA | Very low | | |
| 3 | Tebuconazole | Folicur, Tebuzol | 40 | NY, PA | NY, PA | | |
| 3 | Difenconazole | Inspire | 40 | NY | Not detected | | |
| 12 | | <u></u> | 1 | PA, NJ, DE, MD, VA | Very low to not detected | | |
| 13 | Quinoxyfen | Quintec | 10 | NY, NJ, PA, DE | Very low to not detected | | |

Table 1. The locations where powdery mildew populations estimated as tolerant to different concentrations (ppm) of fungicides in commercial pumpkin plantings or research farm trials in the mid-Atlantic region in 2010 using a pumpkin seedling bioassay.

* Dose corresponds to practical (field) resistance because a pathogen strain resistant to this dose would not be controllable in the field with a labeled rate of the fungicide.

Table 2. Percentage of powdery mildew populations estimated tolerant to different concentrations (ppm) of fungicides in commercial plantings of pumpkin and research plantings of squash and pumpkin on Long Island on a) 31 Aug 2010 and b) 21 Sept 2010.

| a) 31 Aug 2010 | FRAC code 7 | | FRAC code 3 | | | FRAC code 3 | FRAC code 13 | | |
|--------------------------|-------------|-----|--------------|--------------|----|--------------|--------------|------------|-----|
| | Endura | | Nova | | | Tebuzol | Quintec | | |
| | Boscalid | | Myclobutanil | | | Tebuconazole | Quinoxyfen | | |
| Bioassay location | 50 | 175 | 500 | 40 | 80 | 120 | 40 | 1 | 10 |
| Farm 5 | 48 | 21 | 24 | 30 | 42 | 6 | 24 | ND | 0 |
| Farm 6 | 11 | 0 | 0 | 11 | 4 | 7 | 74 | ND | 0 |
| Farm 7 | 9 | 12 | 0 | 3 | 5 | 0 | 6 | ND | 0 |
| Farm 9 | 100 | 100 | 14 | 25 | 25 | 0 | 14 | ND | 0 |
| Farm 10 | 79 | 53 | 11 | 21 | 37 | 2 | 79 | ND | 1 |
| Average | 49 | 37 | 10 | 18 | 23 | 3 | 39 | | 0.4 |
| b) 21 Sept 2010 | | | | | | | | | |
| | Boscalid | | | Myclobutanil | | | Tebuconazole | Quinoxyfen | |
| Bioassay location | 50 | 175 | 500 | 40 | 80 | 120 | 40 | 1 | 10 |
| Farm 5 | 35 | 44 | 48 | 3 | 0 | 0 | 25 | 1 | 0 |
| Farm 6 | 10 | 8 | 11 | 0 | 0 | 0 | 14 | 0 | 0 |
| Farm 7 | 47 | 11 | 25 | 1 | 0 | 0 | 2 | 16 | 2 |
| Farm 9 | 91 | 75 | 70 | 1 | 0 | 0 | 66 | 13 | 0 |
| Farm 10 | 71 | 31 | 52 | 9 | 0 | 0 | 54 | 5 | 1 |
| Average | 51 | 34 | 39 | 3 | 0 | 0 | 32 | 7 | 0.6 |

Budget for cucurbit project:

| Hourly wages (\$325 x 6 states) | \$3900 |
|--|--------|
| Supplies to conduct on-farm bioassays (\$75 x 6 states) | \$900 |
| Travel (4 to 6 trips to conduct bioassay per state @ \$100 x 6 states) | \$1200 |
| | |

Total... \$3000