Baseline Sensitivity of Cucurbit Powdery Mildew (*Podosphaera xanthii*) to the Fungicide Quinoxyfen in New York State

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Abstract:

Managing fungicide resistance is an essential component of managing powdery mildew in cucurbits because fungicides with excellent activity on the lower surface of leaves are at risk for resistance developing due to their single-site mode of action and the fungus causing powdery mildew has a track-record of developing resistance to these types of fungicides. Quinoxyfen is a new active ingredient for powdery mildew. Data on the sensitivity of a pathogen to new chemistry is needed before the fungicide is widely used to serve as baseline information to document shifts in pathogen sensitivity, to provide evidence that control failures occurring in the future could be due to resistance, and to illustrate the amount of variation present in the population. Baseline sensitivity to quinoxyfen (ai in Quintec) was examined for 15 isolates of the pathogen in 2004 that varied in sensitivity to QoI and DMI fungicides. In 2005, 21 isolates were screened for their sensitivity to quinoxyfen. All isolates were collected from a research pumpkin field in Long Island where Quintec was used in some plots. A leaf-disk assay with fungicide-treated disks on media in Petri dishes was used to assess quinoxyfen sensitivity. The isolates tested exhibited variable sensitivities to quinoxyfen, with the highest concentration tolerated ranging from 0.01 to 10 ppm. All isolates tolerated 0.01 ppm, but showed a reduction in growth at this concentration when compared to growth at 0 ppm.

Background and Justification:

Managing fungicide resistance is an essential component of managing powdery mildew in cucurbits. Powdery mildew is the most common foliar disease of cucurbits, occurring every year throughout the Northeast. White powdery fungal growth develops on both surfaces of leaves and on stems. Resistant varieties are available, but application of fungicides continues to be the main management practice. It is critical to control powdery mildew on the lower surface, where this disease develops best. This is best accomplished with fungicides that are systemic, translaminar, or highly volatile. Unfortunately, products developed with these properties have had a high risk of developing resistance due to their single-site mode of action. The cucurbit powdery mildew fungus has demonstrated a high potential for developing resistance. It has developed resistance to all three fungicide classes at-risk for resistance that are currently registered in NY for its management: benzimidazoles, demethylation inhibitors (DMIs, FRAC group 3), and quinone outside inhibitors (QoI, aka strobilurins, FRAC group 11).

An important first step in fungicide resistance management is determining the sensitivity to the active ingredient among individuals of the target pathogen before the fungicide is widely used. With quantitative resistance, as occurs with DMI fungicides, this baseline sensitivity data can be used in the future as a benchmark for assessing whether shifts in sensitivity are occurring before the degree of shift results in control failure. If shifts appear to be occurring, then the appropriate management response would be to use higher rates and reduce the number of applications to

slow further resistance development. If control failure occurs in the future in commercial fields where the new fungicide was used, it will be possible to determine whether fungicide resistance is a possible cause by comparing sensitivity of isolates from those fields to the baseline sensitivity data. It would be valuable to determine if any variation in baseline sensitivity detected among isolates is associated with resistance to other fungicide groups (eg QoIs and DMIs). Both positive and negative associations are possible based on findings with other pathogens. Discovering a negative association would be especially valuable.

Quintec (Dow Agro-Sciences, LLC) is a new fungicide currently registered in the US for grapes, hops and cherry. It has an active ingredient, quinoxyfen, that is in a new chemical class (FRAC group 13). While full federal registration is not anticipated until 2007 for Quintec, it did receive exemptions (crisis and emergency) to allow use for cucurbit powdery mildew in NY in 2004 and again in 2005.

Objective:

1. Examine baseline sensitivity of the cucurbit powdery mildew fungus to quinoxyfen.

Procedures:

Isolates of the powdery mildew fungus, *Podosphaera xanthii*, were collected from pumpkin research fields at the Long Island Horticultural Research and Extension Center in Riverhead, NY, during the 2004 and 2005 growing seasons. Fungicide efficacy experiments conducted in these fields included treatments with Quintec: it was tested alone in 2004 and in combination fungicide programs in both years. The 2004 isolates used in the study were selected from a larger group of isolates based on their sensitivity to QoI and DMI fungicides.

The assay procedure has been used in several previous studies (McGrath et al 1996). It entailed spraying fungicide solutions of various concentrations on squash seedlings at the cotyledon stage. Six concentrations of quinoxyfen ranging from 0 to 10 ppm were applied in 2004. Quinoxyfen concentrations included: 0, 0.01, 0.1, 1, 5, and 10 ppm. In 2005, only three concentrations of quinoxyfen were used: 0, 1, and 5 ppm. Fungicide was applied using a DeVilbiss bottle attached to a compressed air source (20 psi). When the leaves were dry, disks were cut out with a number nine cork borer (9 mm diameter) and put on water agar media in Petri dishes. Spores of the 35 fungal isolates (10-20 conidia) were transferred to the leaf disks at each fungicide concentration. Once powdery mildew developed well on the nontreated leaf disks (30-50% coverage), which took about 10 days at 24°C, the percent of each disk with powdery mildew growth (severity) was estimated. The fungal isolate was considered able to tolerate a fungicide at a specific concentration if it was able to grow and produce spores on two out of three disks. When no growth was evident with the unaided eye, disks were examined under a dissecting microscope to determine if there was any growth and to confirm presence of inoculum.

Results and Discussion:

Quinoxyfen baseline sensitivity varied among powdery mildew isolates. In 2004, all isolates tolerated 0.01 ppm, exhibiting some decrease in severity on these disks compared to 0 ppm (Table 1). Forty percent of the 15 isolates collected in 2004 were able to tolerate and grow at 1 ppm quinoxyfen and 27% tolerated 10 ppm (Table 1a). No relationship was seen between quinoxyfen sensitivity and the date that each isolate was collected in 2004, or whether Quintec (22.6% quinoxyfen) had been applied to the research plot where the isolate was collected (Table 1b). There was also no relationship found between quinoxyfen sensitivity and QoI sensitivity (Table 1c) or DMI sensitivity (Table 1d) in 2004. In 2005, 80% of the 20 isolates tested grew at 1 ppm and 50% tolerated 5 ppm quinoxyfen (Table 2). There appears to be a possible shift in

sensitivity from 2004 to 2005; however, in 2004, isolates were collected at the start of powdery mildew development as well as after fungicides were applied, whereas in 2005 all isolates were collected late in the season after all fungicide applications for the efficacy experiment. Comparing isolates collected at the end of the season (20 Sep or later) reveals greater similarity between the 2 years. For the five isolates collected after fungicide treatments were completed in 2004, 100% tolerated 1 ppm and 60% tolerated 10 ppm. There appears to be an association between Quintec treatment and quinoxyfen sensitivity for the 2005 isolates but not the 2004 isolates. Isolates collected in 2005 from research plots where Quintec was applied tended to tolerate higher concentrations of quinoxyfen. No relationship was seen between quinoxyfen sensitivity and QoI sensitivity or DMI sensitivity again in 2005 (Table 2).

Field experiments carried out in 2004 and 2005 have demonstrated that Quintec (22.6% quinoxyfen) is providing excellent and consistent control of powdery mildew in multiple states and on multiple cucurbit crops. Quintec was the best product evaluated in fungicide efficacy trials in New York and North Carolina. In 2004 it provided 99% and 98% control on the upper and lower leaf surfaces, respectively, when applied at 4 fl. oz/A to pumpkin in New York (McGrath, 2005) and 100% overall control in acorn-type winter squash at both 6 and 12 oz/A in North Carolina (Holmes, 2005). In an efficacy trial conducted in California in 2004 on muskmelon, Quintec at 12 oz/A provided 97 and 92% control of powdery mildew on the upper and lower leaf surfaces, respectively, and at 6 oz/A control was 93% and 87% on the upper and lower leaf surfaces. The degree of control obtained with both rates was statistically equal to the best treatment in the trial, Procure 480SC 8 fl oz/A, which provided 100% and 97% control, respectively. In 2005, Quintec at 6 oz/A (82% control) also performed at the same statistical level as Pristine 18.5 oz/A (92%), which was the most effective treatment in the trial conducted in California on muskmelon (Turini, 2006).

Conclusions:

Variation in tolerance of the 35 isolates of *P. xanthii* to quinoxyfen demonstrates that there may be a risk of resistance development in the pathogen population to this fungicide over time. The range in sensitivity illustrates the potential of the population to shift towards insensitivity. No relationship was found between quinoxyfen sensitivity and whether Quintec was applied to the research plot prior to the isolate collection in 2004, and there was no evident relationship between when an isolate was collected and its sensitivity to quinoxyfen; which suggests that the risk of fungicide resistance development, at least on a short-term time scale, is minimal. However, the association between Quintec treatment and quinoxyfen sensitivity found for the 2005 isolates suggests pathogen response to selection pressure from new chemistry can occur during a growing season.

It is also important to note that this study did not find any relationship between quinoxyfen sensitivity and QoI sensitivity or DMI sensitivity. In contrast, sensitivity to QoI and DMI fungicides appears to be related. For example, in 2002, the first year that QoI resistance was detected in the US, 80% of QoI-resistant isolates tested were also moderately resistant to DMIs. Consequently, using either type of fungicide will likely select for strains that are resistant to both groups of fungicides. A common strategy for managing resistance is to alternate among fungicides in different chemical classes. Clearly this alternating strategy will not be very effective with QoI and DMI fungicides, and would be expected to actually promote further development of DMI resistance, which is the exact opposite goal of this resistance management strategy! Quinoxyfen is a valuable fungicide to rotate into a powdery mildew management program because it should help slow resistance development in powdery mildew populations to other high-risk fungicides. Additionally, Quintec has proven to be a highly effective fungicide, providing excellent control throughout a season. This is an important characteristic for a fungicide used in an alternation program. The more effective a fungicide, the smaller the

pathogen population that remains after treatment, and thus the lower the chance that strains will be present with resistance to another fungicide used in the rotation.

Resistance to fungicides is an ongoing problem in plant disease control. Continually monitoring the sensitivity of *P. xanthii* to quinoxyfen over time is expected to reveal shifts in the pathogen's sensitivity to this fungicide before resistance develops to the point of control failure thereby providing an opportunity to make changes in fungicide recommendations, which should extend the useful life of the fungicide. Perhaps if resistance monitoring had been in place for QoI fungicides, control failure due to resistance could have been avoided.

Literature Cited:

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Table 1a. Sensitivity to fungicides of *Podosphaera xanthii* isolates collected in 2004 sorted by Quintec sensitivity.

Isolate	Date Collected	Quintec Applied	Quintec Sensitivity	Nova Sensitivity	Flint Sensitivity
4f	8-Aug	No	VS	S	R
15j	22-Aug	No	VS	MR	R
29c	8-Aug	No	S	MS	S
2g	22-Aug	Yes	S	MS	S
16g	22-Aug	Yes	S	S	R
1f	22-Aug	Yes	S	MR	R
29w	22-Sep	No	S	MR	R
1x	22-Sep	Yes	S	MS	MR
29b	8-Aug	No	MS	S	S
6f	8-Aug	No	MS	S	S
4d	8-Aug	No	MS	MS	S
16f	22-Aug	Yes	MS	S	S
9z	22-Sep	No	MS	MR	S
6x	22-Sep	No	MS	MR	R
31z	22-Sep	Yes	MS	MS	MR

VS Very Sensitive: growth at only the lowest rate of Quintec tested (0.01 ppm).
S Sensitive: growth up to 1 ppm Quintec with no growth at 10 ppm, no growth at 20 ppm Nova, or no growth at 50 ppm Flint.
MS Moderately Sensitive: growth at all rates of Quintec tested (0, 0.01, 0.1, 1, 5, and 10 ppm) or limited growth at 20 ppm Nova.
MR Moderately Resistant: good growth at 50 ppm Nova or limited growth at 50 ppm Flint.
R Resistant: good growth at 50 ppm Flint.

Table 1b. Sensitivity to fungicides of *Podosphaera xanthii* isolates collected in 2004 sorted by date collected and whether Quintec was applied.

Isolate	Date Collected	Quintec Applied	Quintec Sensitivity	Nova Sensitivity	Flint Sensitivity
29b	8-Aug	No	MS	S	S
6f	8-Aug	No	MS	S	S
4d	8-Aug	No	MS	MS	S
29c	8-Aug	No	S	MS	S
4f	8-Aug	No	VS	S	R
15j	22-Aug	No	VS	MR	R
16f	22-Aug	Yes	MS	S	S
2g	22-Aug	Yes	S	MS	S
16g	22-Aug	Yes	S	S	R
1f	22-Aug	Yes	S	MR	R
9z	22-Sep	No	MS	MR	S
29w	22-Sep	No	S	MR	R
6x	22-Sep	No	MS	MR	R
1x	22-Sep	Yes	S	MS	MR
31z	22-Sep	Yes	MS	MS	MR

VS Very Sensitive: growth at only the lowest rate of Quintec tested (0.01 ppm).
S Sensitive: growth up to 1 ppm Quintec with no growth at 10 ppm, no growth at 20 ppm Nova, or no growth at 50 ppm Flint.
MS Moderately Sensitive: growth at all rates of Quintec tested (0, 0.01, 0.1, 1, 5, and 10 ppm) or limited growth at 20 ppm Nova.
MR Moderately Resistant: good growth at 50 ppm Nova or limited growth at 50 ppm Flint.
R Resistant: good growth at 50 ppm Flint.

Table 1c. Sensitivity to fungicides of *Podosphaera xanthii* isolates collected in 2004 sorted by Nova sensitivity.

Isolate	Date Collected	Quintec Applied	Quintec Sensitivity	Nova Sensitivity	Flint Sensitivity
4f	8-Aug	No	VS	S	R
16g	22-Aug	Yes	S	S	R
29b	8-Aug	No	MS	S	S
6f	8-Aug	No	MS	S	S
16f	22-Aug	Yes	MS	S	S
29c	8-Aug	No	S	MS	S
2g	22-Aug	Yes	S	MS	S
1x	22-Sep	Yes	S	MS	MR
4d	8-Aug	No	MS	MS	S
31z	22-Sep	Yes	MS	MS	MR
15j	22-Aug	No	VS	MR	R
1f	22-Aug	Yes	S	MR	R
29w	22-Sep	No	S	MR	R
9z	22-Sep	No	MS	MR	S
6x	22-Sep	No	MS	MR	R

VS Very Sensitive: growth at only the lowest rate of Quintec tested (0.01 ppm).
S Sensitive: growth up to 1 ppm Quintec with no growth at 10 ppm, no growth at 20 ppm Nova, or no growth at 50 ppm Flint.
MS Moderately Sensitive: growth at all rates of Quintec tested (0, 0.01, 0.1, 1, 5, and 10 ppm) or limited growth at 20 ppm Nova.
MR Moderately Resistant: good growth at 50 ppm Nova or limited growth at 50 ppm Flint.
R Resistant: good growth at 50 ppm Flint.

Table 1d. Sensitivity to fungicides of *Podosphaera xanthii* isolates collected in 2004 sorted by Flint sensitivity.

Isolate	Date Collected	Quintec Applied	Quintec Sensitivity	Nova Sensitivity	Flint Sensitivity
29b	8-Aug	No	MS	S	S
6f	8-Aug	No	MS	S	S
16f	22-Aug	Yes	MS	S	S
29c	8-Aug	No	S	MS	S
2g	22-Aug	Yes	S	MS	S
4d	8-Aug	No	MS	MS	S
9z	22-Sep	No	MS	MR	S
1x	22-Sep	Yes	S	MS	MR
31z	22-Sep	Yes	MS	MS	MR
4f	8-Aug	No	VS	S	R
16g	22-Aug	Yes	S	S	R
15j	22-Aug	No	VS	MR	R
1f	22-Aug	Yes	S	MR	R
29w	22-Sep	No	S	MR	R
6x	22-Sep	No	MS	MR	R

VS Very Sensitive: growth at only the lowest rate of Quintec tested (0.01 ppm).
S Sensitive: growth up to 1 ppm Quintec with no growth at 10 ppm, no growth at 20 ppm Nova, or no growth at 50 ppm Flint.
MS Moderately Sensitive: growth at all rates of Quintec tested (0, 0.01, 0.1, 1, 5, and 10 ppm) or limited growth at 20 ppm Nova.
MR Moderately Resistant: good growth at 50 ppm Nova or limited growth at 50 ppm Flint.
R Resistant: good growth at 50 ppm Flint.

Table 2. Sensitivity to fungicides of *Podosphaera xanthii* isolates collected in 2005 sorted by Quintec sensitivity.

Isolate	Date Collected	Quintec Applied	Quintec Sensitivity	Nova Sensitivity	Flint Sensitivity
R4 T6 A	20-Sep	No	VS	S	MR
R2 T1 B	3-Oct	No	VS	S	MR
R1 T1 B	3-Oct	No	VS	MS	R
R3 T2 A	20-Sep	Yes	S	MR	R
R2 T4 A	20-Sep	Yes	S	MR	R
R1 T5 A	20-Sep	No	S	MS	R
R2 T5 A	20-Sep	No	S	S	MR
R2 T6 A	20-Sep	No	S	MR	R
R3 T5 A	20-Sep	No	S	MS	R
R3 T5 B	3-Oct	No	S	S	R
R1 T5 B	3-Oct	No	S	MS	R
R3 T6 B	3-Oct	No	S	S	MR
R4 T1 A	20-Sep	No	MS	MS	R
R2 T2 A	20-Sep	Yes	MS	MS	MR
R1 T2 A	20-Sep	Yes	MS	S	R
R2 T2 A	20-Sep	Yes	MS	MS	MR
R4 T4 A	20-Sep	Yes	MS	MS	MR
R1 T2 B	3-Oct	Yes	MS	S	R
R1 T4 B	3-Oct	Yes	MS	S	R
R1 T6 B	3-Oct	No	MS	MS	R

VS Very Sensitive: no growth at the lowest rate of Quintec tested (1 ppm).
S Sensitive: growth up to 1 ppm Quintec with no growth at 5 ppm or limited growth at 20 ppm Nova.
MS Moderately Sensitive: growth at all rates of Quintec tested (0, 1, and 5 ppm), no growth at 20 ppm Nova, or no growth at 50 ppm Flint.
MR Moderately Resistant: good growth at 50 ppm Nova or limited growth at 50 ppm Flint.
R Resistant: good growth at 50 ppm Flint.