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Persistence of Metalaxyl Insensitivity in *Pseudoperonospora humuli*

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ABSTRACT

Metalaxyl insensitivity has been reported previously in isolates of *Pseudoperonospora humuli*, causal agent of hop downy mildew, but persistence of metalaxyl insensitivity in this pathogen has not been studied. One hundred fifty four hop shoots systemically infected with *P. humuli* (spikes) were collected from yards in Idaho, Oregon, and Washington and assayed for phenylamide fungicide sensitivity using a leaf disk assay. Metalaxyl and mefenoxam sensitivity was determined from inoculum derived from whole spikes and monosporic isolates in assays of a single discriminatory dose (25 ppm) and dilution series of metalaxyl. Mefenoxam insensitivity was detected in 11 of 13 hop yards and 31 of 74 (42%) spike isolates sampled in 2005. Metalaxyl insensitivity was detected in 7 of 9 yards and 52 of 80 (65%) spike isolates assayed in 2006, including four hop yards in Washington. Among monosporic isolates of *P. humuli*, metalaxyl insensitivity was detected in 4 of 11 hop yards and 8 of 37 (22%) of isolates. Preliminary analysis of ED_{50} values indicates metalaxyl insensitivity is prevalent among isolates of *P. humuli* in the U.S. and high levels of insensitivity have persisted in the population since the original report of insensitivity in 1992. Further studies to determine quantitative changes in the sensitivity of the population are ongoing.

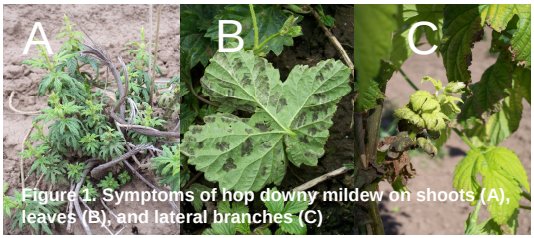

Figure 1. Symptoms of hop downy mildew on shoots (A), leaves (B), and lateral branches (C)

MATERIALS AND METHODS

Collection of basal spikes and maintenance of isolates. One hundred fifty four basal spikes were collected from commercial hop yards from the major hop growing regions in Oregon, northern Idaho, and Washington from 2005-2007. Monosporic isolates were obtained and maintained *in vitro* on detached hop leaves.

Fungicide assays. Fungicide sensitivity assays were conducted using a modification of the methods reported by Kline (1994) and Nelson et al. (2004) with metalaxyl (Ridomil 2E; Syngenta Crop Protection, Greensboro, NC) or mefenoxam (Ridomil Gold; Syngenta). For assays involving metalaxyl, plates were amended with 0, 0.1, 1, 10, 50, 100, and 1000 ppm or 0, 0.01, 0.1, 1, 10, 50, 100, and 500 ppm metalaxyl when performing assays to determine the effective dose that inhibited sporulation on 50% of the inoculation sites (ED_{50}). In discriminatory dose assays, the media was amended with 0 and 25 ppm metalaxy or mefenoxam (Klein, 1994). The concentrations of fungicides used in discriminatory dose assays was derived from previous studies (Klein, 1994) for consistency.

Fungicide assays were conducted with inoculum derived from homogeneous (monosporic) and heterogeneous (entire basal spikes) sources. For assays involving spike isolates of *P. humuli*, spikes were induced to sporulate and resulting sporangia were used as inoculum. In assays with monosporic isolates, inoculum was harvested from heavily infected leaves and adjusted to 10^4 sporangia/ml. After 7 to 11 days of incubation, leaf disks were examined and the incidence of inoculation sites with sporulating colonies of *P. humuli* was determined.

Statistical analysis. For studies involving a single discriminatory dose of a fungicide, an isolate was considered resistant if the incidence of sporulation on leaf disks placed on the amended media was at least 50% of the incidence of sporulation on leaf disks placed on non-amended media. To calculate ED_{50} values, a simple regression model was fit through the linear portion of the sigmoidal dose-response curve, and the model was used to calculate the ED_{50} value in a spreadsheet. Results were greater than 50% mortality was observed in the non-amended treatment were eliminated to minimize impacts due to the condition of the isolate or leaf disks.

Table 1. Incidence of metalaxyl resistance among monosporic isolates of *Pseudoperonospora humuli*.

State	Grower	Yard number	Cultivar[a]	*n*	Resistant (%)[b]
ID	1	ID-2	HMF	4	3 (75)
ID	1	ID-3	HMF	4	2 50)
ID	1	ID-5	HMF	1	0 (0)
ID	1	ID-6	HMF	2	0 (0)
WA	9	WA-3	CTZ	1	0 (0)
WA	10	WA-5	CTZ	4	1 (25)
WA	10	WA-6	CTZ	2	2 (100)
WA	8	WA-1	CTZ	14	0 (0)
WA	12	WA-8	Cluster	1	0 (0)
WA	13	WA-9	Cluster	1	0 (0)
WA	14	WA-10	Cluster	3	0 (0)
Total	…	…	…	37	8(21.6)

[A] HMF = Hallertauer Mittelfrüe 102. CTZ = cultivars Columbus, Tomahawk, and Zeus.
[b] Isolates were considered resistant to metalaxyl if the incidence of sporulation on inoculated leaf disks placed on water agar amended with 25 ppm metalaxyl was at least 50% of the incidence of sporulation on leaf disks on non-amended media.

Table 2. Phenylamide sensitivity among spike isolates of *Pseudoperonospora humuli*.

State	Grower	Yard	Cultivar[b]	*n*	Resistant (%)
Mefenoxam[a]					
ID	1	ID-1	Saazer 72	2	2 (100)
OR	2	OR-1	Glacier	4	3 (75)
OR	3	OR-2	Mt. Hood	5	2 (40)
OR	3	OR-3	Willamette	3	1 (0)
OR	4	OR-4	Nugget	9	5 (55.6)
OR	4	OR-5	Nugget	10	8 (80)
OR	5	OR-6	Nugget	3	0 (0)
OR	5	OR-7	Willamette	3	1 (0)
OR	6	OR-8	Crystal	9	2 (22.2)
OR	6	OR-9	Cascade	12	3 (25)
OR	7	OR-10	Millennium	7	2 (28.6)
OR	7	OR-11	Glacier	5	2 (40)
WA	8	WA-1	Chelan	2	0 (0)
Total	…	…	…	74	31 (41.9)
Metalaxyl[a]					
ID	1	ID-2	HMF	9	6 (66.7)
ID	1	ID-3	HMF	7	5 (71.4)
ID	1	ID-4	Saazer 72	5	4 (80)
WA	8	WA-1	Chelan	15	0 (0)
WA	9	WA-3	CTZ	5	2 (40)
WA	9	WA-4	CTZ	22	22 (100)
WA	10	WA-5	CTZ	2	0 (0)
WA	10	WA-6	CTZ	3	1 (33.3)
WA	11	WA-7	CTZ	12	12 (100)
Total	…	…	…	80	52 (65)

[a] Isolates were considered resistant to mefenoxam or metalaxyl if the incidence of sporulation on leaf disks placed on water agar amended with 25 ppm mefenoxam or metalaxyl was at least 50% of the incidence of sporulation on leaf disks placed on non-amended media. [b] HMF = Hallertauer Mittelfrüe 102.

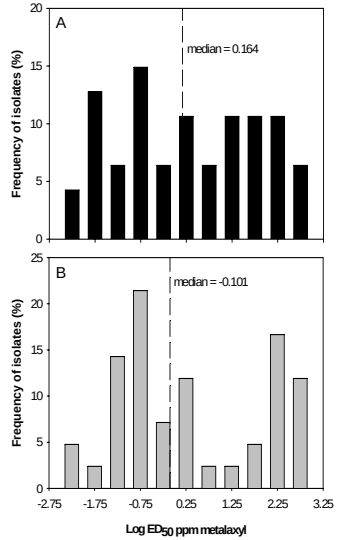

Figure 2. Frequency distribution of metalaxyl ED_{50} values among basal spike (A) and monosporic (B) isolates of *Pseudoperonospora humuli*.

RESULTS AND CONCLUSIONS

- Mefenoxam insensitivity was detected in 11 of 13 hop yards and 31 of 74 (42%) of spike isolates sampled in 2005.
- Metalaxyl insensitivity was detected in 7 of 9 yards and 52 of 80 (65%) spike isolates assayed in 2006 (Table 2). Phenylamide insensitive strains of *P. humuli* were confirmed for the first time in Washington State, and were present at a very high incidence within yards where insensitivity was detected.
- Among monosporic isolates of *P. humuli*, metalaxyl insensitivity was detected in 4 of 11 hop yards and 8 of 37 (22%) of isolates (Table 1).
- Frequency distributions of ED_{50} values indicates a bimodal distribution of metalaxyl sensitivity, with some isolates possessing high levels of insensitivity (Figure 2). Distributions of ED50 values varied between assays with heterogeneous and homogeneous sources of inoculum.
- Phenylamide insensitivity remains prevalent among isolates of *P. humuli* in the Pacific Northwest despite the near absence of phenylamide fungicides in the past 15 years.
- Phenylamide insensitivity appears to be more stable in *P. humuli* than other oomycete pathogens, perhaps because of perennation of the pathogen in hop crowns and thus enhanced survival of insensitive strains (2,6).

LITERATURE CONSULTED

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