Occurrence and Incidence of Metalaxyl Resistance in *Pseudoperonospora humuli* in the Pacific Northwest

R. E. KLEIN, Assistant Plant Pathologist, Department of Plant Pathology, Washington State University—Prosser, Prosser 99350

ABSTRACT

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Basal spikes, systemically infected with *Pseudoperonospora humuli,* were collected from hop (*Humulus lupulus*) plants in Oregon, Washington, and Idaho in 1992. Zoosporangial suspensions from each spike were tested for metalaxyl resistance in a floating leaf disk assay. Washington samples had an average EC-50 (the metalaxyl concentration which reduced the incidence of sporulation by 50%) of 0.15 μg/ml, while the average for Oregon samples was 74 μg/ml. None of the zoosporangial suspensions from 50 Washington basal spikes were metalaxyl resistant at 25 μg/ml metalaxyl, while six of 10 Idaho and 81 of 94 Oregon suspensions exhibited resistance. This is the first report of metalaxyl resistance in *P. humuli* in North America.

Hop (*Humulus lupulus* L.), a critical flavor and aroma constituent of beer and ale, is grown on approximately 16,500 ha in the Pacific Northwest states of Washington, Oregon, and Idaho. These areas comprise the total U.S. commercial production of hop and account for 29% of the global hop production.

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The most important fungal disease of hop in this area is downy mildew, caused by *Pseudoperonospora humuli* (Miyabe & Takah.) G.W. Wils. The disease perennates in the hop crown, from which systemically infected shoots (basal spikes) emerge in the spring and early summer (10). These shoots serve as the primary inoculum source, and the disease spreads to healthy leaves and shoots under appropriate environmental conditions. Yield loss occurs especially when the hop-bearing vines or sidearms (branches) become systemically infected, or when the hop cones themselves become infected prior to harvest (12).

Control of hop downy mildew prior to 1980 relied on sanitation, copper or Bordeaux mixture sprays, or other fungicides including zineb (9). The fungicide metalaxyl offered excellent control of basal spikes and virtually eliminated secondary infections following a single preemergent crown drench in Oregon (5) or a single foliar application after emergence in Washington. Recently, metalaxyl has failed to control hop downy mildew in Oregon and northern Idaho hop growing areas, and this study was undertaken to determine if metalaxyl-resistant strains of *P. humuli* are present in the Pacific Northwest and if resistance is the probable cause of reduced control by metalaxyl.

MATERIALS AND METHODS

A leaf disk assay similar to that described by Sozzi and Staub (14) was used to test for metalaxyl resistance. Leaf disks were cut from fully expanded leaves of the mildew-susceptible variety Late Cluster using a no. 8 cork borer. Disks were floated abaxial surface up in a 60 × 15 mm petri plate containing filter paper and 4 ml of distilled water with or without a metalaxyl amendment. A

commercial preparation of metalaxyl, Ridomil 2E, was used as the source of metalaxyl throughout these studies. Treated and nontreated leaf disks were incubated 18–24 hr at room temperature in the petri plates before inoculation.

Basal spikes were collected randomly from Oregon, Washington, and/or Idaho hop yards in 1992. Spikes were returned to the laboratory, and sporulation was induced by enclosing each individual spike in a plastic bag and incubating at 18 C overnight in the dark. Zoosporangia were harvested from sporulating leaves by vigorously shaking the leaves in distilled water. No attempt was made to standardize zoosporangial concentrations, but concentrations were determined with the aid of a hemacytometer; zoosporangial concentrations ranged from 0.2 to 1×10^6 zoosporangia per milliliter.

Zoosporangial suspensions were applied to each leaf disk as three 15-μl drops. Inoculated leaf disks were incubated at 18 C with a 15-hr photoperiod provided by fluorescent lights throughout the experiments. Approximately 24 hr after inoculation, the remaining inoculum drops were removed by aspiration. Leaf disks were examined microscopically for sporulation beginning 72 hr after inoculation and continuing daily for up to five additional days. Each inoculation site was scored either positive or negative for sporulation regardless of the extent, and the percentage of inoculated sites with sporulation was considered the incidence of sporulation.

The concentration of metalaxyl that effectively reduced the incidence of sporulation to 50% of that of the nontreated control (EC-50) was determined for nine spikes from three Oregon hop yards and five spikes from a Washington hop yard. Zoosporangia derived from each spike were inoculated onto four leaf disks floated on solutions containing 0, 0.1, 1, 10, and 100 μg/ml of metalaxyl in each of four petri plates (a total of 48 inoculation sites per metalaxyl concentration). The incidence of sporulation was determined, transformed using a log transformation, and plotted; and the EC-50 was calculated from the line of best fit for each spike.

Leaf disks were also combined by metalaxyl concentration for four Oregon and two Washington spikes 8 days after inoculation, and the zoosporangia were harvested by agitating in 5 ml of water. The number of zoosporangia per microliter was determined with a hemacytometer. The EC-50 was determined similarly to that described previously. The spikes were selected to show the broadest range of metalaxyl sensitivities in order to validate the incidence of sporulation data.

In addition to the above tests, 94 spikes from eight Oregon hop yards, 50 spikes from three Washington hop yards, and 10 spikes from one Idaho hop yard were tested for metalaxyl resistance in 1992. Between five and 21 basal spikes were collected from each yard, and three to four leaf disks floated on 0 and 25 μg/ml of metalaxyl were inoculated with zoosporangial suspensions from each individual spike.

Table 1. Incidence of sporulation 6 days postinoculation on hop leaf disks floated on four different metalaxyl concentrations and a nontreated control, and the EC-50[a] for inoculum collected from nine Oregon and five Washington hop downy mildew basal spikes. Sporulation incidence was the percentage of inoculation sites with sporulation

	Metalaxyl concentration (μg/ml)					EC-50
Spike	0	0.1	1	10	100	(μg/ml)
Oregon						
1	46	35	40	21	6	9
2	52	56	50	38	17	35
3	96	100	100	98	27	50
4	100	100	100	96	44	76
5	98	96	100	100	77	>100
6	100	100	100	100	100	>100
7	100	100	100	100	100	>100
8	100	100	100	100	100	>100
9	100	100	100	100	100	>100
Washington						
1	100	52	0	0	0	0.1
2	100	50	0	0	0	0.1
3	100	67	0	0	0	0.12
4	100	63	4	0	0	0.15
5	100	100	21	0	0	0.3

[a] The concentration of fungicide that reduced the sporulation incidence to 50% of the untreated control.

Table 2. Number of zoosporangia (per μl) collected 8 days postinoculation from leaf disks treated with different metalaxyl concentrations and inoculated with zoosporangial suspensions from four Oregon and two Washington hop downy mildew spikes. The EC-50 values were determined from these data. Spike numbers refer to the same spikes as in Table 1

	Metalaxyl concentration (μg/ml)					EC-50
Spike	0	0.1	1	10	100	(μg/ml)
Oregon						
4	60	141	81	90	26	85
5	231	288	304	248	119	>100
6	73	95	83	101	86	>100
7	99	59	58	71	28	35
Washington						
2	43	9	3	0	0	0.06
3	106	21	5	0	0	0.06

RESULTS

Two estimates of the EC-50 for *P. humuli* were obtained, one based on the reduction in the incidence of sporulation and the other based on the amount of sporulation. Results are shown in Tables 1 and 2. The sporulation incidence method overestimates the EC-50 relative to the sporulation quantity method, because the incidence method does not account for the density of sporulation. However, results from the two methods are positively correlated ($r = 0.84$, $n = 6$, $P < 0.05$) (13), and the sporulation incidence method is quicker and simpler. The metalaxyl EC-50s for *P. humuli* from the nine Oregon downy mildew spikes ranged from 9 to more than 100 μg/ml (mean = 74 μg/ml assuming 100 μg/ml for the six spikes with EC-50s > 100 μg/ml), while those from Washington ranged from 0.1 to 0.3 μg/ml (mean = 0.15 μg/ml) when estimated by sporulation incidence.

No resistant zoosporangia were detected in the 50 spikes collected in Washington, while over 80% of the 94 Oregon spikes were resistant (Table 3). There was an overall decrease in the incidence of infection in the 25-μg/ml metalaxyl treatment, but this is consistent with a range of EC-50s observed previously. Metalaxyl resistance also was detected in zoosporangia from six of 10 Idaho basal spikes.

DISCUSSION

Metalaxyl resistance commonly occurs in a number of Oomycetes (1,8), especially when metalaxyl is used exclusively, as it is in the U.S. hop-growing areas. Thus, it is interesting to speculate why the Oregon growing area has a seemingly well-adapted metalaxyl-resistant *P. humuli* population while Washington does not. Environmental conditions in the Willamette Valley of Oregon are conducive to downy mildew epidemics virtually every year, while conditions in the

Yakima Valley of Washington are rarely favorable for downy mildew development (6). The same varieties which are susceptible in Oregon are resistant in Washington, but varieties which are too susceptible to grow in Oregon are grown in the Yakima Valley. Consequently, the hectarage of hops treated annually with metalaxyl differs only slightly between the two growing areas. Thus, the occurrence of metalaxyl resistance in Oregon is unlikely to be related to the quantity of fungicide or the varieties involved, but more likely to the method of fungicide application, the environmental conditions, and the *P. humuli* populations.

In Oregon, metalaxyl is applied as a preemergent or early emergent crown drench (essentially a soil application) in the early spring. In Washington, metalaxyl is applied to the foliage of 15–30 cm tall hop plants before pruning and training (11). Because metalaxyl is applied to foliage in Washington, it could be mixed with copper; but growers did not begin doing this until recently, and prepackaged copper–metalaxyl mixtures only became available in 1993. Although hop downy mildew perennates in the subterranean hop crown, it is a foliar disease; and the Fungicide Resistance Action Committee for the phenylamide fungicides now recommends against soil application of fungicides for control of foliar pathogens (15).

Unfortunately, no data exist on the sensitivity of *P. humuli* populations to metalaxyl before the widespread use of the fungicide. The 1,000-fold difference in sensitivity between samples from the two states and the loss of control by metalaxyl in Oregon but not Washington indicates that metalaxyl resistance is the reason for the loss of control in Oregon.

Hop downy mildew first appeared in the Pacific Northwest in the 1930s. The number of different introductions of *P. humuli* is unknown, nor have biological differences been reported for *P. humuli* isolates in the United States. Presumably, a wide variety of races or variants occurs, similar to those reported for other Oomycetes (7). When metalaxyl resistance was detected in *Bremia lactucae,* it was initially detected only in isolates with particular virulence genes (2). Perhaps the *P. humuli* populations in the different growing areas vary, and the Yakima Valley population is less likely to develop competitive metalaxyl-resistant variants because of its genetic background.

Table 3. Incidence of metalaxyl-resistant hop downy mildew basal spikes from eight Oregon hop yards and the incidence of sporulation on leaf disks treated with 0 or 25 μg/ml of metalaxyl and inoculated with zoosporangia derived from metalaxyl-resistant spikes

Variety	Incidence of resistance[a]	Sporulation incidence[b] metalaxyl concentration (μg/ml)	
		0	25
Nugget	18/21	151/162	142/162
Nugget	18/19	149/160	141/160
Willamette	8/9	73/96	55/96
Willamette	9/9	93/108	71/108
Willamette	7/9	80/84	42/84
Willamette	7/9	83/84	56/84
Tettnang	2/6	24/24	12/24
Cascade	11/11	515/573	482/573
Total	81/94	1,168/1,291	1,001/1,291

[a] Number of spikes with resistant zoosporangia/total number of spikes tested.
[b] Incidence of sporulation was the percentage of inoculation sites with sporulation 8 days after inoculation.

Regardless of the factors which led to metalaxyl resistance, a monitoring program to detect the resistance in *P. humuli* is needed in Washington. A similar program will be needed in Oregon as well to determine if the incidence of metalaxyl resistance declines sufficiently to allow the limited use of this fungicide in the future, as has been done in potatoes (3). Metalaxyl resistance in *P. humuli* has also been detected in Germany (4) by a different assay system incorporating a spray tower to apply the fungicide. Leaf disk assays may also prove convenient in other hop-growing areas with fungicide-resistance problems.

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LITERATURE CITED

1. Cohen, Y., and Coffey, M. D. 1986. Systemic fungicides and the control of Oomycetes. Annu. Rev. Phytopathol. 24:311-338.
2. Crute, I. R. 1987. The occurrence, characteristics, distribution, genetics, and control of a metalaxyl-resistant pathotype of *Bremia lactucae* in the United Kingdom. Plant Dis. 71:763-767.
3. Dowley, L. J., and O'Sullivan, E. 1985. Monitoring metalaxyl resistance in populations of *Phytophthora infestans.* Potato Res. 28:531-534.
4. Hellwig, K., Kremheller, H. T., and Agerer, R. 1991. Untersuchen zur resistenz von *Pseudoperonospora humuli* (Miy. & Tak.) Wilson gegenuber Metalaxyl. Gesunde Pflanz. 43:400-404.
5. Hunger, R. M., and Horner, C. E. 1982. Control of hop downy mildew with systemic fungicides. Plant Dis. 66:1157-1159.
6. Johnson, D. A., Skotland, C. B., and Alldredge, J. R. 1983. Weather factors affecting downy mildew epidemics of hops in the Yakima Valley of Washington. Phytopathology 73:490-493.
7. Michelmore, R. W., Ilot, T., Hulbert, S. H., and Farrara, B. 1987. The downy mildews. Pages 653-679 in: Advances in Plant Pathology: Genetics of Plant Pathogenic Fungi. G. S. Sidhu, P. H. Williams, and D. S. Ingram, eds. Academic Press, London.
8. Morton, H. V., and Urech, P. A. 1988. History of the development of resistance to phenylamide fungicides. Pages 59-60 in: Fungicide Resistance in North America. C. J. Delp, ed. American Phytopathological Society, St. Paul, MN.
9. Romanko, R. R., Ogawa, J. M., Skotland, C. B., Horner, C. E., and Brooks, S. N. 1964. Hop downy mildew: A symposium. Mod. Brew. Age 66:45-52.
10. Skotland, C. B. 1961. Infection of hop crowns and roots by *Pseudoperonospora humuli* and its relation to crown and root rot and overwintering of the pathogen. Phytopathology 51:241-244.
11. Skotland, C. B., and Johnson, D. A. 1983. Control of downy mildew of hops. Plant Dis. 67:1183-1185.
12. Skotland, C. B., and Romanko, R. R. 1964. Life history of the hop downy mildew fungus. Wash. Agric. Exp. Stn. Circ. 433.
13. Sokal, R. R., and Rohlf, F. J. 1969. Biometry. W. H. Freeman and Co., San Francisco, CA.
14. Sozzi, D., and Staub, T. 1987. Accuracy of methods to monitor sensitivity of *Phytophthora infestans* to phenylamide fungicides. Plant Dis. 71:422-425.
15. Wade, M., and Delp, C. J. 1985. Aims and activities of industry's fungicide resistance action committee. EPPO Bull. 15:577-583.