Sensitivity of *Pseudoperonospora humuli* (the Causal Agent of Hop Downy Mildew) from Oregon, Idaho, and Washington to Fosetyl-Al (Aliette)

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Abstract

Failures of fosetyl-Al (Aliette) to control hop (*Humulus lupulus*) downy mildew, caused by *Pseudoperonospora humuli*, have recently occurred in northern Idaho and Oregon. To determine if resistance of the pathogen to the fungicide has developed, leaf disk assays were conducted to compare sensitivity of *P. humuli* isolates from the different U.S. hop-growing regions to isolates from a research yard where exposure to fosetyl-Al had not occurred for at least 10 years. Dose response curves of transformed data were linear. The fosetyl-Al concentration effective against 50% of the *P. humuli* isolates (ED$_{50}$) from each location was estimated from the linearized data. The ED$_{50}$ values indicate that fosetyl-Al was about one-third as effective against *P. humuli* isolates from commercial hop yards in northern Idaho and Oregon and about one-half as effective against isolates from southern Idaho compared to isolates from the research yard. Commercial yards in Washington were similar to the research yard.

Introduction

Over 26 million kg of hops (*Humulus lupulus*), comprising about 28% of the world’s production and estimated to be worth over $113 million, are grown on about 12,000 ha in the Pacific Northwest states of Washington, Oregon, and Idaho. Downy mildew, caused by *Pseudoperonospora humuli* (Miyabe & Takah.) Wilson, is a serious disease in the region. This disease has resulted in significant shifts in production from high rainfall areas to the more arid Yakima Valley of Washington State and Treasure Valley of Idaho. Although downy mildew can be a periodic threat to production in the Yakima Valley (4), growers consider the disease relatively manageable. Higher rainfalls in the hop-growing regions of Oregon’s Willamette Valley and northern Idaho result in annual problems with downy mildew. As a result, only downy mildew-tolerant cultivars are grown in these regions.

Perennation of *P. humuli* occurs in infected perennial hop rootstock (crowns) from which infected buds develop into systemically infected shoots (primary basal spikes) in the spring. Zoosporangia that develop on these spikes serve as the primary inoculum spreading the disease to other shoots (that develop into secondary spikes; Fig. 1) and leaves (6,8). The foliar lesions soon dry and are of minor importance to the epidemic, but the secondary spikes harbor the pathogen throughout the growing season (10). Significant yield losses can occur when cones, the main bines (stems; Fig. 2), or cone-bearing side-arms
(branches) become infected. Crown rot and subsequent plant death can be a consequence of infection of highly susceptible cultivars (9,10).

Management of hop downy mildew is based on sanitation and sprays of copper and other fungicides (2,5,9). In the early 1980s, a single application of metalaxyl (Ridomyl) provided season-long control of hop downy mildew (2). By the early 1990s, strains of P. humuli resistant to metalaxyl had developed in Oregon and northern Idaho (5). Since that time, control of this pathogen has primarily relied on fosetyl-Al (Aliette) and more recently cymoxanil (Curzate). In recent years, growers in Oregon and northern Idaho have observed that fosetyl-Al applications have often not provided satisfactory control of hop downy mildew.

Leaf disk assays are reliable indicators of fungal sensitivity, including P. humuli, to fungicides (5,11). Diminished sensitivity of P. humuli isolates from commercial hop yards to fosetyl-Al in leaf disk assays would suggest the development of field tolerance to this fungicide. The objective of this study was to compare fosetyl-Al sensitivity of P. humuli isolates from the Oregon and northern Idaho hop-growing regions to that of P. humuli isolates from a research yard at Washington State University at Prosser (WSU) that have not been exposed to fosetyl-Al during the 10 years prior to initiation of this study. Isolates of P. humuli from the Washington and southern Idaho hop-growing regions were also evaluated.

Leaf Disk Assays

A modified version of the leaf disk assay described by Klein (5) was used to test for fosetyl-Al resistance. Stock suspensions of fosetyl-Al (pH = 6.5) were prepared in double-distilled water (ddH$_2$O) and added to molten 1% Bacto-agar (Difco Laboratories, Detroit, MI). The resulting suspension was poured into 60 × 15 mm polystyrene Falcon 1007 Petri dishes (Becton Dickinson and Co., Franklin Lakes, NJ). In 1999 and 2000, fosetyl-Al was added as Aliette 80% WDG (Aventis, currently Bayer Crop Sciences, Research Triangle Park, NC) to obtain final concentrations of 0, 200, 400, 800, and 1600 ppm active ingredient. In 2002 and 2003, final fosetyl-Al concentrations were 0, 100, 200, 400, 800, and 1600 ppm. About 24 h prior to inoculation, leaf disks 1 cm in diameter were excised from fully expanded hop leaves (three to five nodes from the growing tip) (cv. Symphony) with a No. 6 cork borer, and seven disks were placed in each dish with the abaxial surface facing up. Due to possible differences in susceptibility to infection of individual leaves (e.g., maturity), all fungicide concentrations for a single spike utilized disks from a common set of leaves. Plates containing leaf disks were placed under fluorescent lights in incubators (18°C; 16-h photoperiod).
Primary or secondary downy mildew spikes (spikes) were collected from WSU and commercial hop yards in Washington, Oregon, and Idaho. To reduce the possibility of evaluating isolates that might have arisen from the same infection, no more than one spike was collected from a single plant. Spikes were collected from plants selected as randomly as disease distribution allowed within each hop yard. To induce sporulation, spikes were sprayed with ddH$_2$O, enclosed in plastic bags with stems in a beaker of ddH$_2$O, and incubated overnight at room temperature (~ 20°C) in the dark. Zoospores were harvested by shaking each spike in 15 to 20 ml ddH$_2$O and straining through several layers of cheesecloth.

Leaf disks were inoculated by pipetting 12 µl of the zoosporangial suspension onto each of three spots on each disk. In 1999 and 2000 zoosporangia from each spike were placed onto three sets of seven leaf disks for each fosetyl-Al concentration (63 inoculation sites). In 2002 and 2003 zoosporangia from each spike were inoculated onto two sets of seven leaf disks (42 inoculation sites). Inoculated leaf disks were incubated at 18°C under artificial illumination with a 16-h photoperiod. After a 16-to-24-h incubation period, residual inoculum was removed from the inoculation sites by aspiration with a pipette tip attached to a vacuum pump. After 5 to 7 days of additional incubation, leaf disks were examined and the inoculation sites with sporulating _P. humuli_ colonies were determined by visual inspection. Total numbers of spikes evaluated were 45 from at least 8 Oregon hop yards (11 in 1999 and 34 in 2002), 12 from 8 northern Idaho hop yards (all in 2000), 25 from 3 Washington hop yards (all in 2002), and 20 from 4 Idaho hop yards (3 in 2002 and 17 in 2003). Twenty-five spikes were evaluated from the WSU hop yard (12 in 1999, 5 in 2000, and 7 in 2002).

Data were analyzed by probit analysis (1) using the probit procedure of SAS (7) with years as replicates, adjusting for natural mortality, and using a normal distribution to model response probabilities. The estimated dose effective against 50% of the _P. humuli_ isolates (ED$_{50}$) in each of the states was compared. Differences in the effectiveness of fosetyl-Al against _P. humuli_ were determined to exist if there was no overlap in the 95% confidence limits of the estimated ED$_{50}$ from the different states/regions. To help eliminate significant impacts due to condition of the spike or leaf disks (e.g., maturity), results from spikes with greater than 25% mortality at 0 ppm fosetyl-Al were excluded from the analysis and are not included in numbers of spikes evaluated from each location.

**Sensitivity to Fosetyl-Al**

Analysis indicated that the data were adequately described by the normal distribution (Pearson chi-square $P \leq 0.001$) for all states or regions in all years. The probability of _P. humuli_ not growing was significantly ($P \leq 0.001$) and positively related to fosetyl-Al concentration. Parameter estimates for each state or region are provided in Table 1. Estimated ED$_{50}$ values for each region (Fig. 3) show that _P. humuli_ isolates from northern Idaho and Oregon were similar and least sensitive to fosetyl-Al with ED$_{50}$ values of 163.3 ppm and 187.2 ppm, respectively. Isolates of _P. humuli_ from Washington commercial hop yards and from the WSU research hop yard were also similar and were most sensitive to fosetyl-Al, having ED$_{50}$ values of 52.1 ppm and 55.8 ppm, respectively. Isolates of _P. humuli_ from southern Idaho were intermediate in sensitivity to fosetyl-Al with an estimated ED$_{50}$ of 95.9 ppm.
Table 1. Parameter estimates of *Pseudoperonospora humuli* collected from the indicated states or regions.

<table>
<thead>
<tr>
<th>State or region</th>
<th>Y intercept</th>
<th>Slope (log 10 dose)</th>
<th>Estimate of natural (control) response</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSU</td>
<td>-4.163</td>
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<tr>
<td>N. ID</td>
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<td>2.348</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Analyses were conducted using the Probit procedure of SAS using years as replicates, adjusting for natural mortality and using a normal distribution to model response probabilities.

Summary

Sozzi and Staub (11) demonstrated that assays utilizing potato leaf disks, detached leaves, or whole plants are reliable for monitoring phenylamide sensitivity and are useful predictors of the presence of fungicide resistance in the field. Differences in fitness of resistant and susceptible isolates are unlikely to influence these test results. In addition, hop leaf disk assays have been effective in previous fungicide sensitivity evaluations of *P. humuli* isolates (5). In the present study, leaf disk assays revealed the presence of *P. humuli* isolates resistant to fosetyl-Al in Oregon and northern Idaho. The ED$_{50}$ values for those isolates were about three times greater than those observed for isolates from the WSU research yard where hops had not been exposed to fosetyl-Al for at least 10 years prior to initiation of these trials. The estimated ED$_{50}$ values for *P. humuli* resistant to fosetyl-Al in Oregon and northern Idaho are twice as high as for the WSU hop yard. Commercial yards in Washington appear to be similar in sensitivity to the WSU hop yard.

Fungicide application records for individual hop yards included in this study are not available to researchers. However, it is known that fosetyl-Al use in Oregon and northern Idaho has been much more frequent and widespread than in more arid regions of Washington and southern Idaho because of frequent downy mildew due to the wetter conditions common in the hop-growing regions of Oregon and northern Idaho. Selection pressure for fungicide-tolerant strains of *P. humuli* in Oregon and northern Idaho is probably due to the nearly exclusive use of a single fungicide combined with environmental conditions conducive to disease development. Resistance of *P. humuli* to metalaxyl has been reported in Oregon and northern Idaho (5) as well as Germany (3). In the arid Yakima Valley of Washington, conditions are less conducive for downy mildew development. Conditions favorable for severe epidemics occurred only in 9 of 28 years (4). Over time, this should result in fewer life cycles, fewer fungicide applications, and less opportunity for the pathogen to develop tolerance. Consequently, populations of *P. humuli* resistant to fosetyl-Al appear
to emerge less rapidly in the Yakima Valley than in other areas. Although Idaho is climatically similar to the Yakima Valley, P. humuli populations in southern Idaho appear to be more tolerant to fosetyl-Al. It is likely that strains of P. humuli resistant to fosetyl-Al have been introduced to southern Idaho by planting infected rootstock obtained from northern Idaho or Oregon. The hop-growing region of southern Idaho is much smaller, more compact, and managed by significantly fewer growers than the Yakima Valley production area resulting in greater reliance on planting material from outside the local area. It must be noted that due to difficulty in finding downy mildew in commercial hop yards in Washington and southern Idaho during the course of this study, results are based on a sample from only three and four hop yards, respectively. In the case of Washington this represents a very small percentage of the total number of hop yards while in southern Idaho this represents a much larger percentage of overall production. As such, results from Washington may not accurately represent all growing areas in the state and must be viewed with caution. Results from Oregon and northern Idaho are assumed reliable due to the larger number of hop yards tested.

There is no information in the literature regarding fosetyl-Al sensitivity of P. humuli prior to commercial use of the fungicide. However, isolates from hop yards in Oregon and northern Idaho where fosetyl-Al fails to adequately control downy mildew have been compared to isolates from a research hop yard that has not been exposed to fosetyl-Al. This evaluation has provided the best opportunity available to investigate the loss of disease control by fosetyl-Al in some hop yards. Data obtained in this study indicate that P. humuli has acquired tolerance to fosetyl-Al after several years of nearly total reliance on this one chemical by commercial producers.

Acknowledgments

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Literature Cited