Research

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Investigating Phenylamide Insensitivity in Wisconsin Populations of *Pseudoperonospora humuli*

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Abstract

Hop downy mildew caused by the oomycete *Pseudoperonospora humuli* is primarily managed with fungicides because commercial varietal resistance is unavailable. Mefenoxam, a phenylamide-class fungicide, is highly effective for systemic disease control but is at high risk for the development of pathogen insensitivity. Due to the recent expansion of hop production in Wisconsin, it was unknown if *P. humuli* could be managed with mefenoxam because insensitivity has been documented in other regions. During 2015 to 2017, isolates of *P. humuli* were collected from commercial yards throughout Wisconsin. Isolates were screened for insensitivity using a leaf disk assay with fungicide-amended water agar. Insensitivity was determined using a single discriminatory dose of 25 µg/ml of mefenoxam, and isolates were

considered insensitive if they exhibited at least 50% sporulation on amended media relative to nonamended water agar. Over all years, nearly 48% of all isolates were insensitive to mefenoxam. The incidence of insensitivity varied between individual hop yards, with most yards exhibiting a mixture of sensitive and insensitive isolates. At this time, mefenoxam-insensitive populations do not appear to be predominant in Wisconsin. Growers should monitor the effectiveness of mefenoxam applications in their hop yards and be responsive to changes in crop response.

Keywords: *Humulus lupulus*, *Pseudoperonospora humuli*, oomycetes, mefenoxam resistance, hops, downy mildew

Hops (*Humulus lupulus*) are perennial climbing vines grown for their oil-bearing cones, an important component in the flavoring, aroma, and preservation of beer (Neve 1991). Although Wisconsin (WI) was a historical leader in hop production during the 1860s, disease pressure, insect pests, and market factors drove production out of the state and to the Pacific Northwest (PNW) by the next decade (Barth et al. 1994). The PNW has remained the heart of commercial production to this day (George 2018). Over the past decade, hop production in WI has enjoyed a renaissance due to the surging popularity of microbreweries and the increased demand for locally sourced and regionally distinct ingredients.

Hop downy mildew, caused by the obligate oomycete pathogen *Pseudoperonospora humuli*, is the most widespread and severe disease affecting hops in WI and in many other hop-growing regions worldwide (Royle and Krehmiller 1981). This pathogen overwinters within the crown of the plant as well as the rhizome and dormant hop buds, emerging in the spring within symptomatic hop shoots known as basal spikes (Coley-Smith 1962; Salmon and Ware 1925; Ware 1929). These systemically infected shoots are typically

stunted, brittle, chlorotic in appearance, and have distinctly downward curling leaves (Coley-Smith 1962; Royle and Krehmiller 1981). Black or dark gray masses of sporangia occur on the undersides of these leaves when environmental conditions are favorable. Sporangia are dispersed by rain and wind, whereupon they may infect new plant material within the hop yard (Royle and Krehmiller 1981). Disease is favored by moderate temperatures (15 to 20°C), high humidity (>90% relative humidity), and prolonged periods of wetness (Royle and Krehmiller 1981). In WI, hop downy mildew occurs annually, and epidemics are common because disease risk remains elevated season-long given the highly promotive, humid weather and relatively frequent rain events.

Yield reductions occur when infections on the main growing vines or lateral, cone-producing shoots arrest further growth. Cones themselves may also become infected, which can lead to drastic reductions in overall quality and marketability (Royle and Krehmiller 1981). Further, severe disease reduces the health of the hop rhizome and crown and can have effects ranging from reduced yield and low vigor to plant death (Neve 1991; Skotland 1961; Williams et al. 1961). As hop yard acreage continues to expand in WI, hop downy mildew will likely increase as a significant production challenge.

Management efforts initiate with or near the first basal spike emergence in mid-April and continue through harvest. Disease management is achieved primarily through season-long fungicide applications, because varietal resistance is not durable enough to support healthy hop production without chemical inputs (Coley-Smith 1965; Hunger and Horner 1982; Skotland and Johnson 1983). Currently, there are about 15 registered fungicide active ingredients representing seven modes of action for control of downy mildew in hops in WI; however, most growers rely upon a smaller

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group of fungicides in building a season-long treatment program to limit disease (Colquhoun et al. 2018). Hop growers in WI presently rely upon frequent, calendar-based fungicide applications to manage this disease. Treatment programs often include 12 to 18 applications per season with modes of action that target *P. humuli* exclusively. Cultural practices such as bine stripping and crown pruning can help prevent the pathogen from infecting the plant at positions higher on the bine and reduce initial inoculum, respectively, but are employed sporadically and with varying quality in WI hop yards (Gent et al. 2012).

The phenylamide (PA) class of fungicides, including mefenoxam and its closely related enantiomer metalaxyl, has demonstrated a high level of control against *P. humuli* and other oomycete pathogens (Cohen and Coffey 1986; Davidse 1988; Gisi 2002; Hunger and Horner 1982). In PNW hop production systems, a single appropriately timed drench application of metalaxyl has been shown to provide nearly complete control of downy mildew (if the pathogen is sensitive) until conditions less favorable to disease prevailed (Hunger and Horner 1982). In WI, PAs (most commonly mefenoxam) are applied once or twice per yard per season if used at all. Currently, two PA fungicides, mefenoxam (Ridomil Gold SL, Syngenta) and metalaxyl (MetaStar and MetaStar 2E, LG International America) are registered for use on hops in WI. Application of these formulations, to date, has been somewhat limited due to expense, lack of knowledge regarding use, and concerns with control performance based on past fungicide resistance reports and control failures in the PNW. Growers are aware of the potential for strong control of downy mildew with PAs and are highly interested in the sensitivity status of current WI pathogen populations as they make disease management decisions. Although PA fungicides are costly, highly effective systemic control of this potentially devastating disease is a sound return on investment if the pathogen population is sensitive.

PA fungicides have been in use on many crops since their introduction in the 1970s, and detectable insensitivity to PAs has been shown to develop quickly following product registration, in some cases within the same growing season (Morton and Urech 1988; Russell 2005). Insensitivity to PAs in *P. humuli* populations was first detected in Germany in 1991 and in the PNW in 1994 (Hellwig et al. 1991; Klein 1994). As a result, hop growers in Oregon and Idaho were discouraged from using these fungicides and now rely upon other chemical modes of action such as the phosphorus acid fungicides and cymoxanil (Nelson et al. 2004). PA insensitivity of *P. humuli* populations has been studied in the PNW, at the onset of fungicide failures in the early 1990s and in the mid-2000s (Gent et al. 2008; Klein 1994). During the second of these investigations, Gent et al. (2008) discovered that despite limited use of PAs in the intervening years, insensitivity within PNW pathogen populations remained common, indicating that PA insensitivity is a relatively stable phenotype in *P. humuli*. That investigation also identified insensitivity to metalaxyl for the first time in Washington State, where hop downy mildew epidemics are less frequent and PA fungicides continued to be used (Gent et al. 2008). To our knowledge, the development of PA insensitivity in *P. humuli* has not been formally investigated in any other hop-growing regions of the United States.

Although hops have had a relatively short contemporary commercial significance in WI, many yards have been established by vegetatively propagated rhizomes or plantlets originating from the PNW, which has a history of PA insensitivity in the *P. humuli* population (Gent et al. 2008; Klein 1994; Nelson et al. 2004). Because *P. humuli* can persist systemically within the hop plant, including the rhizome, this pathogen may have been introduced via

infected planting material (Coley-Smith 1962; Ware 1926). Currently, there is no hop-specific certification process in place to ensure the cleanliness or disease status of the planting material.

To support growers' interest in using PAs, this research objective was developed to screen *P. humuli* isolates from multiple commercial hop yards within WI for insensitivity to mefenoxam. Results of this work were directly translated into location-specific, prescriptive downy mildew management programs in commercial hop yards.

Colony Isolate Collection

Basal spikes ($n = 152$) exhibiting active *P. humuli* sporulation were collected during 2015 to 2017. Collections were made from eight commercial hop yards in six counties throughout the state of WI, including one organic hop yard (Fig. 1). The collection of sporangia from an individual basal spike was considered a single colony isolate for the purpose of this analysis, in accordance with previous investigations (Gent et al. 2008). Isolates were collected from several hop varieties including Brewer's Gold, Cascade, Centennial, Challenger, Chinook, Crystal, Galena, Nugget, Southern Cross, Tettnanger, Triple Pearl, Willamette, and Zeus (Table 1). Isolate collection began in mid- to late April and typically ended in mid-July of each year, the time at which active *P. humuli* sporulation became difficult to find in abundance in commercially managed hop yards. Basal spikes were collected arbitrarily throughout the hop yard as disease conditions permitted. No more than one basal spike was collected from a single plant or adjacent plants to avoid collecting multiple clonal isolates. Upon collection, spikes were placed into plastic bags, sealed, and transported in a chilled cooler back to the laboratory, where they were immediately processed. Sporangia were harvested from each basal spike by selecting one to three leaves with sporangia and agitating them in approximately 20 ml of sterile water for 20 to 30 s using forceps. The sporangial suspension was then strained through several layers of cheesecloth to remove leaf fragments and debris. With the aid of a hemocytometer, the suspension was adjusted to a final concentration 10^4 sporangia/ml for use in leaf inoculations.

Dose Discrimination Assay

The dose discrimination assays were largely based on methods described by Gent et al. (2008) with some modifications. Standard 100-mm-diameter Petri plates were prepared with 10 ml of 1% water agar or 1% water agar amended with mefenoxam (25 μ g/ml) (Ridomil Gold SL, Syngenta). This discriminatory dose of 25 μ g/ml was demonstrated previously to distinguish between sensitive and insensitive isolates (Gent et al. 2008; Klein 1994). Although both mefenoxam and metalaxyl are registered for use in WI, mefenoxam was used for all subsequent assays because it is the more active enantiomer and is more commonly used in WI on hops for disease control. The fungicide was diluted with distilled deionized water and adjusted to pH 6.5 before being added to cooled, molten 1% water agar.

Leaves of the downy mildew-susceptible Nugget variety were collected from disease-free hop plants generated from clean tissue culture plantlets maintained in a greenhouse (University of Wisconsin–Madison King Hall Greenhouse Facility). Plants were grown in 3.78-liter pots and fertilized at every watering with Peters Professionals 20-10-20 Peat-Lite Special (JR Peters, Allentown, PA) to ensure vigorous, succulent growth. To minimize variability in disease susceptibility due to ontogenic resistance, only leaves positioned three to five nodes from the growing tip were used (Neve

1991). Leaves were surface sterilized with a 30-s dip in 70% ethanol followed by two 30-s rinses in sterile water and then patted dry with paper towels. Leaf disks were cut using a sterile 1-cm-diameter cork borer, and seven leaf disks were arranged on each plate with the abaxial leaf surface up. Plates were prepared 24 h before inoculation and maintained under refrigeration at 4°C.

After the collection of basal spikes and preparation of inoculum as described above, each leaf disk was inoculated by pipetting 10 µl of the sporangial suspension onto three discrete spots on each of 14 leaf disks, resulting in two duplicate plates (Supplementary Figure 1). This procedure took place both on plates amended with fungicide and those with nonamended water agar for each isolate. After inoculation, plates were sealed in clear plastic bags and left on the laboratory bench for 24 h. After 24 h, the inoculum droplet was removed via aspiration and the plates resealed in the bags. Plates were allowed to incubate on the laboratory bench for 5 to 7 days, whereupon each leaf disk was examined with a dissecting microscope at 200× magnification for incidence of sporulation (any amount of visible sporangiophores and sporangia).

The insensitivity determination was made as described by Gent et al. (2008). In brief, if the incidence of sporulation on fungicide-amended media was at least 50% of the incidence on nonamended media, the isolate was classified as insensitive. If, for a given isolate, more than 50% of inoculation sites on nonamended media showed

no sporulation, the isolate was excluded from further analysis to ensure viable isolates were used and to minimize effects due to poorly performing isolates. These thresholds were chosen to remain consistent with previous investigations and to allow some degree of comparability.

Frequency and Distribution of Mefenoxam Insensitivity

A total of 152 isolates were collected during 2015 to 2017 from eight different hop yards in six WI counties. One hundred eighteen isolates of the total 152 (77%) were collected in the months of May and June. Hop downy mildew severity varied between individual hop yards. Periods of favorable weather for disease development were frequent in all years of basal spike collection. Differences in severity were likely due to varying management practices and differing history of disease in each hop yard. Hop yards with well-developed, season-long fungicide programs generally had reduced downy mildew severity, and these growers were more likely to use mefenoxam. In general, disease severity was greatest from late May through June, and most basal spike collections occurred during this time, because collections were made based on availability of diseased plant material (Fig. 2). Basal spikes were collected from a wide variety of hop cultivars, although individual hop yards tended to have “hotspots” of increased downy mildew disease severity. This resulted in increased numbers of basal spike collections from

FIGURE 1

Pseudoperonospora humuli colony isolates were collected from commercial hop yards in six Wisconsin counties, shaded dark gray, throughout the state during 2015 to 2017.

certain hop varieties, including Centennial, Nugget, Tettnanger, and Zeus (Table 1).

To our knowledge, mefenoxam had never been applied in the Dane 2, Dane 3, Columbia, or Portage hop yards. Mefenoxam was applied in the Dane 1 hop yard in all years of sample collection (2015 to 2017), but the exact timing of application each year is unknown. Similarly, mefenoxam was applied in the Pepin yard in 2016 and 2017, but again the exact timing is unknown. Mefenoxam was applied in 2017 on April 30 in the Marathon hop yard; it was not applied in 2015 in that yard, and use in 2016 is unknown but likely. In the Dodge hop yard, mefenoxam was applied on May 28/29 in 2015 and twice in 2016, first on April 23/24 and again on May 30. It was also applied in 2017, but the application dates for that year are not known.

Out of all 152 isolates collected, 80 exhibited greater than 50% of inoculation sites with visible sporulation on nonamended media (Table 1). The remaining 72 isolates were excluded from analysis due to poor growth on control plates and are not described here. Of the 80 isolates further analyzed, nine were collected in 2015, 26 in 2016, and 45 in 2017. A total of 38 (47.5%) isolates over all years tested were insensitive to mefenoxam, whereas the remaining 42 (52.5%) isolates were sensitive. No insensitive isolates were detected in 2015, although only nine samples were collected that year from a single location and collection time. Twelve insensitive isolates were detected in 2016 (46.2% of isolates that year), and 26 were detected in 2017 (57.8% of 2017 isolates) (Table 2).

TABLE 1
List of *Pseudoperonospora humuli* isolates analyzed for sensitivity to the phenylamide fungicide mefenoxam^a

Yard ID	Date collected	Variety	Sensitivity status	Yard ID	Date collected	Variety	Sensitivity status
Dane 1*	8/27/2015	Zeus	S	Portage	6/8/2016	Nugget	IN
Dane 1*	8/27/2015	Zeus	S	Portage	6/8/2016	Centennial	IN
Dane 1*	8/27/2015	Zeus	S	Portage	6/8/2016	Centennial	IN
Dane 1*	8/27/2015	Zeus	S	Portage	6/8/2016	Centennial	IN
Dane 1*	8/27/2015	Zeus	S	Portage	6/8/2017	Centennial	IN
Dane 1*	8/27/2015	Zeus	S	Portage	6/8/2017	Centennial	IN
Dane 1*	8/27/2015	Zeus	S	Portage	6/8/2017	Centennial	IN
Dane 1*	8/27/2015	Zeus	S	Portage	6/8/2017	Centennial	IN
Dane 1*	8/27/2015	Triple Pearl	S	Portage	6/8/2017	Centennial	IN
Dane 1*	4/28/2016	Cascade	IN	Portage	6/8/2017	Centennial	IN
Dane 1*	4/28/2016	Chinook	S	Portage	6/8/2017	Nugget	IN
Dane 1*	5/31/2016	Tettnanger	S	Portage	7/13/2017	Centennial	IN
Dane 1*	5/31/2016	Tettnanger	S	Dodge*	6/23/2016	Galena	IN
Dane 1*	5/31/2016	Centennial	S	Dodge*	6/23/2016	Nugget	IN
Dane 1*	5/31/2016	Centennial	S	Dodge*	6/23/2016	Nugget	IN
Dane 1*	6/15/2016	Tettnanger	S	Dodge*	6/23/2016	Nugget	IN
Dane 1*	6/15/2016	Tettnanger	S	Dodge*	6/23/2016	Chinook	IN
Dane 1*	6/15/2016	Tettnanger	S	Dodge*	4/25/2017	Nugget	S
Dane 1*	6/15/2016	Tettnanger	IN	Dodge*	4/25/2017	Nugget	S
Dane 1*	5/23/2017	Tettnanger	IN	Dodge*	6/20/2017	Nugget	IN
Dane 1*	5/23/2017	—	IN	Dodge*	6/20/2017	Nugget	IN
Dane 1*	5/23/2017	Tettnanger	IN	Dodge*	6/20/2017	Nugget	IN
Dane 1*	5/23/2017	Southern Cross	S	Dodge*	6/20/2017	Nugget	S
Dane 1*	5/23/2017	Southern Cross	S	Dane 2	6/23/2016	Willamette	S
Dane 1*	6/14/2017	Tettnanger	IN	Dane 3†	5/2/2017	—	S
Dane 1*	6/14/2017	Tettnanger	IN	Dane 3†	5/18/2017	—	S
Dane 1*	6/14/2017	Tettnanger	IN	Marathon*	5/25/2017	Nugget	IN
Dane 1*	6/14/2017	Tettnanger	IN	Marathon*	5/25/2017	Nugget	S
Dane 1*	6/14/2017	Tettnanger	IN	Marathon*	6/22/2017	Centennial	IN
Dane 1*	6/14/2017	Tettnanger	IN	Marathon*	6/22/2017	Challenger	IN
Dane 1*	7/18/2017	—	S	Marathon*	6/22/2017	Challenger	IN
Pepin*	6/2/2016	Crystal	IN	Marathon*	6/22/2017	Centennial	IN
Pepin*	6/2/2016	Brewer's Gold	S	Marathon*	7/19/2017	—	S
Pepin*	6/21/2016	Brewer's Gold	S	Columbia	5/25/2017	Centennial	S
Pepin*	6/21/2016	Centennial	S	Columbia	6/29/2017	Centennial	S
Pepin*	6/21/2016	Crystal	S	Columbia	6/29/2017	Centennial	S
Pepin*	6/21/2016	Nugget	S	Columbia	6/29/2017	Centennial	S
Pepin*	6/6/2017	Nugget	IN	Columbia	6/29/2017	Centennial	S
Pepin*	6/6/2017	Nugget	S	Columbia	6/29/2017	Centennial	S
Pepin*	6/6/2017	Centennial	S	Columbia	6/29/2017	Centennial	S

^a This list does not include isolates excluded due to lack of sporulation on plates not amended with fungicide. S = sensitive; IN = insensitive; dagger (†) indicates an organically managed hop yard; and asterisk (*) indicates a hop yard with a known history of mefenoxam use.

2015

2016

2017

FIGURE 2

Number of isolates classified as sensitive or insensitive to mefenoxam in 2015, 2016, and 2017 over the course of the production season in Wisconsin. Values for each month represent the total number of sensitive and insensitive isolates combined over all locations where collections were made.

Insensitive isolates were detected in five out of the six (83.3%) counties sampled (all but Columbia) and in six of the eight (75%) hop yards in which collections were made. Five of the eight (62.5%) hop yards exhibited mixed mefenoxam sensitive/insensitive populations. Three hop yards, including an organic hop yard (Dane 3), had no insensitive isolates (Table 2). In one hop yard (Portage), all isolates collected tested as insensitive to mefenoxam (Table 2).

Samples were obtained over multiple years from four hop yards: Dane 1, Dodge, Pepin, and Portage. In the Dane 1 hop yard, the proportion of insensitive isolates collected increased over 3 years from 0% in 2015 to 20% in 2016 to 76.9% in 2017. The Dodge hop yard saw a decrease in the number of insensitive isolates from 100% in 2016 to 40% in 2017. The Pepin yard saw a moderate increase in insensitivity detections from 16.6% in 2016 to 33.3% in 2017. At the Portage yard, 100% of isolates collected tested as insensitive in both 2016 and 2017 (Table 2).

Conclusions and Implications for Future Disease Management

Isolates of the hop downy mildew pathogen were determined to be insensitive to mefenoxam at several commercial production locations in WI during 2015 to 2017. These results make it clear that varying frequencies of mefenoxam insensitivity exist within *P. humuli* populations in hop yards throughout the state. This is the first report of mefenoxam insensitivity in *P. humuli* in WI, and this research demonstrates that *P. humuli* insensitivity to PAs in the field is a risk in WI as it has been in other hop growing regions (Gent et al. 2008; Hellwig et al. 1991; Klein 1994).

In most WI hop yards investigated, *P. humuli* populations included both sensitive and insensitive phenotypes, consistent with previous investigations in hop and other crops (Gent et al. 2008; Gisi and Sierotzki 2008). In some hop yards where mefenoxam had been applied, such as the Dane 3 yard, the proportion of insensitive and sensitive isolates appeared to change over a given season or from one year to the next (Table 2). Detections of insensitivity become more likely after exposure to the target fungicide, because

the proportion of sensitive isolates is drastically reduced after fungicide application (Gisi and Cohen 1996; Williams and Gisi 1992). The phenotypic variability seen here may also be due in a large part to the timing of sample collections, because most isolates were collected after mefenoxam was applied to a hop yard earlier that season.

Despite mefenoxam applications being made in the Dane 1, Pepin, Marathon, and Dodge hop yards over multiple years, sensitive isolates were still detected in these hop yards later in the growing season after the fungicide application. This suggests that despite the selection pressure brought upon *P. humuli* populations with fungicide exposure, sensitive isolates persist in these hop yards, and complete dominance of insensitive *P. humuli* has not been established. Through the course of isolate collection in this work, downy mildew was observed at several hop yards where mefenoxam was routinely applied in the early season. With few exceptions, disease severity on basal hop growth tended to decrease over the course of the growing season in these hop yards. It should be noted, however, that other fungicide active ingredients with activity on oomycetes were also used in these yards up until harvest.

In the Portage hop yard, insensitive isolates were identified even though mefenoxam had never before been applied at this location (Table 2). This finding brings into question the source(s) of mefenoxam insensitivity in WI. Pathogen isolates insensitive to certain active ingredients have been identified in some systems even before such fungicides were used in the field (Daggett et al. 1993; Dekker 1976). A baseline analysis of PA sensitivity to mefenoxam prior to the start of use of this chemistry does not exist in WI or the PNW, but it is highly likely that some proportion of the “wild-type” population exhibited resistance, or partial resistance, to mefenoxam due to naturally occurring mutations (Gisi and Cohen 1996). Although natural mutations imparting fungicide resistance may be responsible for the detections of insensitivity in the absence of target fungicide documented here, introductions of insensitive pathogen from regions with a history of mefenoxam resistance may be another source, as previously discussed. Many WI hop yards, including the Portage hop yard discussed above, have been planted with rhizomes sourced from various suppliers in the PNW, and these rhizomes may have been harboring PA-insensitive *P. humuli*.

Alternatively, mefenoxam-insensitive inoculum could have been introduced to yards with no prior mefenoxam exposure from neighboring hop yards where mefenoxam is applied and insensitive strains may potentially be more numerous. To our knowledge, there have been no studies investigating the long-distance dispersal of *P. humuli*, but insight might be gained from its closely related sister species, *Pseudoperonospora cubensis*, causal agent of cucurbit downy mildew (Mitchell et al. 2011). Estimates of the mean distance of dispersal for *P. cubensis* are approximately 1,000 km, or about 621 miles (Ojiambo and Holmes 2011). Assuming that *P. humuli* has a similar movement capacity, this renders nearly any location in WI potentially accessible from any other single point source within the state. Susceptible hosts are readily abundant both within increasingly prevalent cultivated hop yards and hedgerows or other natural areas throughout the state, where feral or escaped hops are common, so movement may not be limited by the availability of host tissue (Ojiambo and Holmes 2011).

Recent work by Gent et al. (2019) showed a general lack of genetic variation among *P. humuli* isolates collected from multiple U.S. locations, including one isolate from WI, when compared with those collected in Oregon. This may suggest that *P. humuli* has been disseminated across the country from a focal introductory point and may further inform the origin of populations insensitive to

Year	Yard ID	n	Insensitive (%)
2015	Dane 1*	9	0 (0)
Total	–	9	0 (0)
2016	Dane 1*	10	2 (20)
	Dane 2	1	0 (0)
	Dodge*	5	5 (100)
	Pepin*	6	1 (16.6)
	Portage	4	4 (100)
Total	–	26	12 (46.2)
2017	Columbia	7	0 (0)
	Dane 1*	13	10 (76.9)
	Dane 3	2	0 (0)
	Dodge*	5	2 (40)
	Marathon*	7	5 (71.4)
	Pepin*	3	1 (33.3)
	Portage	8	8 (100)
Total	–	45	26 (57.8)
Total all years	–	80	38 (47.5)

^a Asterisk (*) indicates a hop yard with a history of mefenoxam application.

mefenoxam. Further research of this nature is necessary in hop-growing regions outside the PNW to clarify these relationships and elucidate the dispersal mechanisms at play. The results of this work indicated that the decision whether or not to use mefenoxam or related products will need to be made on a yard-by-yard basis, due to the differing resistance profiles of hop yards throughout the state. Future applications of mefenoxam will also need to be accompanied by careful monitoring of disease levels to ascertain if there is evidence of insensitivity. Gisi (1988) suggested that frequencies of insensitive isolates of 10 to 15% may result in control failures; our results indicated that this threshold has been reached in many hop yards. Growers are encouraged to carefully adhere to resistance management guidelines, which include use of PAs only on a preventative basis, adding a tank-mixed base protectant such as copper when using as a foliar application, and limiting the number of applications in a growing season, in addition to adherence to label requirements that inform maximum allowed dose and tank-mix directions (Williams and Gisi 1992). Although preventative fungicides cannot eradicate the systemic pathogen, preventative fungicide applications can limit symptom onset and pathogen sporulation, which can effectively manage disease. We also continue to promote the importance of using disease-free planting material to minimize pathogen spread and introduction to new hop yards or new areas of existing yards.

Currently, label directions for Ridomil Gold SL suggest a soil drench to crowns after pruning when hop shoots are 15 cm or less and/or a foliar application at the first sign of secondary infection; however, grower-reported usage patterns often differ from this. Improper application timing could account for inconsistencies in downy mildew control. Feedback from growers on performance of fungicides for hop downy mildew control indicates need for further education and research regarding fungicide application timing.

Insensitivity to mefenoxam and related active ingredients should continue to be monitored in WI as hop yard acreage continues to expand and use of PAs likely becomes more prevalent (Staub and Sozzi 1984). Growers using this group of fungicides should be aware of resistance risk and its mitigation with careful management and should consider field and crop histories when deciding upon mefenoxam use. Evidence suggests that when resistance management strategies are followed, PA-based products can continue to perform well at the farm level even where resistance has been detected (Williams and Gisi 1992). As hop acreage continues to expand in the Upper Midwest, there will be value in screening *P. humuli* populations for mefenoxam resistance within the region as a whole. As discussed above, further investigations into the relatedness of Upper Midwest *P. humuli* populations to those existing in the PNW may also be warranted. Such work may shed light on the origins of PA resistance in the Midwest and help inform future recommendations regarding the sourcing of plant material.

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Literature Cited

- Barth, H. J., Klinke, C., and Schmidt, C. 1994. The Hop Atlas: The History and Geography of the Cultivated Plant. Joh. Barth & Sohn, Nuremberg, Germany.
- Cohen, Y., and Coffey, M. D. 1986. Systemic fungicides and the control of oomycetes. *Annu. Rev. Phytopathol.* 24:311-338.
- Coley-Smith, J. R. 1962. Overwintering of hop downy mildew *Pseudoperonospora humuli* (Miy. and Tak.) Wilson. *Ann. Appl. Biol.* 50:235-243.
- Coley-Smith, J. R. 1965. Infection of hop rootstocks by downy mildew *Pseudoperonospora humuli* (Miy. & Tak.) Wilson and its control by early-season dusts. *Ann. Appl. Biol.* 56:381-388.
- Colquhoun, J. B., Gevens, A. J., Groves, R. L., Heider, D. J., Jensen, B. M., and Nice, G. R. W. 2018. Hops. Pages 120-131 in: *Commercial Vegetable Production in Wisconsin*. University of Wisconsin Extension, Madison, WI.
- Daggett, S. S., Gotz, E., and Therrien, C. D. 1993. Phenotypic changes in populations of *Phytophthora infestans* from Eastern Germany. *Phytopathology* 83:319-323.
- Davidse, L. C. 1988. Phenylamide fungicides: Mechanism of action and resistance. Pages 63-65 in: *Fungicide Resistance in North America*. C. J. Delp, ed. American Phytopathological Society, St. Paul, MN.
- Dekker, J. 1976. Acquired resistance to fungicides. *Annu. Rev. Phytopathol.* 14: 405-428.
- Gent, D., Adair, N., Knaus, B. J., and Grünwald, N. J. 2019. Genotyping-by-sequencing reveals fine-scale differentiation in populations of *Pseudoperonospora humuli*. *Phytopathology* 109:1801-1810.
- Gent, D., Nelson, M., and Grove, G. 2012. Association of spring pruning practices with severity of powdery mildew and downy mildew on hop. *Plant Dis.* 96:1343-1351.
- Gent, D. H., Nelson, M. E., and Grove, G. G. 2008. Persistence of phenylamide insensitivity in *Pseudoperonospora humuli*. *Plant Dis.* 92:463-468.
- George, A. 2018. 2017 Statistical Report. Hop Growers of America. https://www.usahops.org/img/blog_pdf/105.pdf.
- Gisi, U. 1988. Population dynamics in Peronosporales treated with phenylamide fungicides. Pages 66-71 in: *Fungicide Resistance in North America*. C. J. Delp, ed. American Phytopathological Society, St. Paul, MN.
- Gisi, U. 2002. Chemical control of downy mildews. Pages 119-159 in: *Advances in Downy Mildew Research*. P. T. N. Spencer-Phillips, U. Gisi, and A. Lebeda, eds. Kluwer Academic, Dordrecht, the Netherlands.
- Gisi, U., and Cohen, Y. 1996. Resistance to phenylamide fungicides: A case study with *Phytophthora infestans* involving mating type and race structure. *Annu. Rev. Phytopathol.* 34:549-572.
- Gisi, U., and Sierotzki, H. 2008. Fungicide modes of action and resistance in downy mildews. *Eur. J. Plant Pathol.* 122:157-167.
- Hellwig, K., Krehmeller, H. T., and Agerer, R. 1991. Untersuchungen zur Resistenz von *Pseudoperonospora humuli* (Miy. & Tak.) Wilson gegenüber Metalaxyl. *Gesunde Pflanzen* 43:400-404.
- Hunger, R. M., and Horner, C. E. 1982. Control of hop downy mildew with systemic fungicides. *Plant Dis.* 66:1157-1159.
- Klein, R. E. 1994. Occurrence and incidence of metalaxyl resistance in *Pseudoperonospora humuli* in the Pacific Northwest. *Plant Dis.* 78:161-163.
- Mitchell, M. N., Ocamb, C. M., Grünwald, N. J., Mancino, L. E., and Gent, D. H. 2011. Genetic and pathogenic relatedness of *Pseudoperonospora cubensis* and *P. humuli*. *Phytopathology* 101:805-818.
- 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.
- Nelson, M. E., Eastwell, K. C., Grove, G. G., Barbour, J. D., and Ocamb, C. M. 2004. Sensitivity of *Pseudoperonospora humuli* (the causal agent of hop downy mildew) from Oregon, Idaho, and Washington to fosetyl-Al (Aliette). *Plant Health Prog.* 5.
- Neve, R. A. 1991. Hops. Chapman and Hall, London, U.K.
- Ojiambo, P. S., and Holmes, G. J. 2011. Spatiotemporal spread of cucurbit downy mildew in the eastern United States. *Phytopathology* 101:451-461.
- Royle, D. J., and Krehmeller, H. T. 1981. Downy mildew of the hop. Pages 395-419 in: *The Downy Mildews*. D. M. Spencer, ed. Academic Press, London, U.K.
- Russell, P. E. 2005. A century of fungicide evolution. *J. Agric. Sci.* 143:11-25.
- Salmon, E. S., and Ware, W. M. 1925. On the presence of a perennial mycelium in *Pseudoperonospora humuli* (Miyabe & Takah.) Wils. *Nature* 116:134-135.
- 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.
- Skotland, C. B., and Johnson, D. A. 1983. Control of downy mildew of hops. *Plant Dis.* 67:1183-1185.
- Staub, T., and Sozzi, D. 1984. Fungicide resistance: A continuing challenge. *Plant Dis.* 68:1026-1031.
- Ware, W. M. 1926. *Pseudoperonospora humuli* and its mycelial invasion of the host plant. *Trans. Br. Mycol. Soc.* 11:91-107.
- Ware, W. M. 1929. Experiments on the production of diseased shoots by the hop downy mildew, *Pseudoperonospora Humuli* (Miy. et Takah.) Wils. *Ann. Bot.* 43:683-710.
- Williams, I. H., Roberts, J. B., and Coley-Smith, J. R. 1961. Studies of the dormant phase of the hop (*Humulus lupulus* L.). *Dep. Hop Res. Annu. Rep.* 1960:48-58.
- Williams, R. J., and Gisi, U. 1992. Monitoring pathogen sensitivity to phenylamide fungicides: Principles and interpretation. *EPPO Bull.* 22:299-306.