

Detection of mefenoxam-insensitive populations of *Pseudoperonospora humuli* in Wisconsin Hop Yards

Michelle E. Marks and Amanda J. Gevens

Department of Plant Pathology, University of Wisconsin - Madison



Introduction

- Hops have become a significant specialty crop in Wisconsin (WI) and other Midwestern U.S. states
- Downy mildew caused by *Pseudoperonospora humuli* has been a persistent and increasing disease due to relatively high humidity and frequent precipitation during the production season in this region
- Phenylamide fungicides, including mefenoxam and metalaxyl, have been shown to be highly effective in controlling downy mildew, however, this class is highly prone to pathogen resistance development and insensitivity within *P. humuli* populations has been documented in other hop growing regions^{1,2}
- Use of phenylamide fungicides in WI has been limited due to 1) knowledge of resistance in other states, 2) concern of disease control failure, and 3) expense
- Our objective was to screen *P. humuli* populations from WI hops yards to inform prescriptive use of phenylamide fungicides to growers for improved downy mildew control statewide**

Results

Year	County	Yard	# Isolates	Insensitive (%)
2015				
	Dane	1	9	0 (0)
Total	-	-	9	0 (0)
2016				
	Dane	1	10	2 (20)
	Dane	2	1	0 (0)
	Dodge	1	5	5 (100)
	Pepin	1	6	1 (16.6)
	Portage	1	4	4 (100)
Total	-	-	26	12 (46.2)
2017				
	Columbia	1	7	0 (0)
	Dane	1	12	10 (83.3)
	Dane	3	2	0 (0)
	Dodge	1	5	2 (40)
	Marathon	1	6	5 (83.3)
	Pepin	1	3	1 (33.3)
	Portage	1	7	7 (100)
Total	-	-	42	25 (59.5)



Table 1. Summary of downy mildew mefenoxam insensitivity assay results for 2015-2017

Materials & Methods

- A leaf disk assay modified from Gent (2008)¹ was used to evaluate the phenylamide sensitivity status of WI *P. humuli* populations in 2015, 2016, and 2017
- Isolates of *P. humuli* were collected from basal spikes from 8 hop yard locations in 6 counties (Table 1, Fig. 2)
- Leaf disks sourced from susceptible 'Nugget' hop plants maintained in a disease-free greenhouse on the campus of University of Wisconsin-Madison; disks applied to media 24 hr prior to pathogen inoculation (Fig. 3)
- Petri plates prepared with 10 ml of 1% water agar (control) or 10 ml 1% water agar amended with 25 µg/ml of mefenoxam (Ridomil Gold® SL, Syngenta) (Fig. 4)
- Total number of sporulating sites counted on leaf disks after 5-7 days incubation under ambient laboratory conditions
- Isolate determined as 'insensitive' or resistant if pathogen sporulation incidence on fungicide-amended plates ≥ 50% of incidence on control plates
- An isolate was excluded from analysis if less than 50% of sites showed sporulation on non-amended media

Figure 2 (right). A. Intact spike showing *P. humuli* sporulation on leaf undersides. B. Sporangia were removed from hop leaves by agitating infected leaves in ~20 ml of water. C. Sporangial suspensions were adjusted to 10⁴ sporangia/ml prior to inoculation on leaf disks.

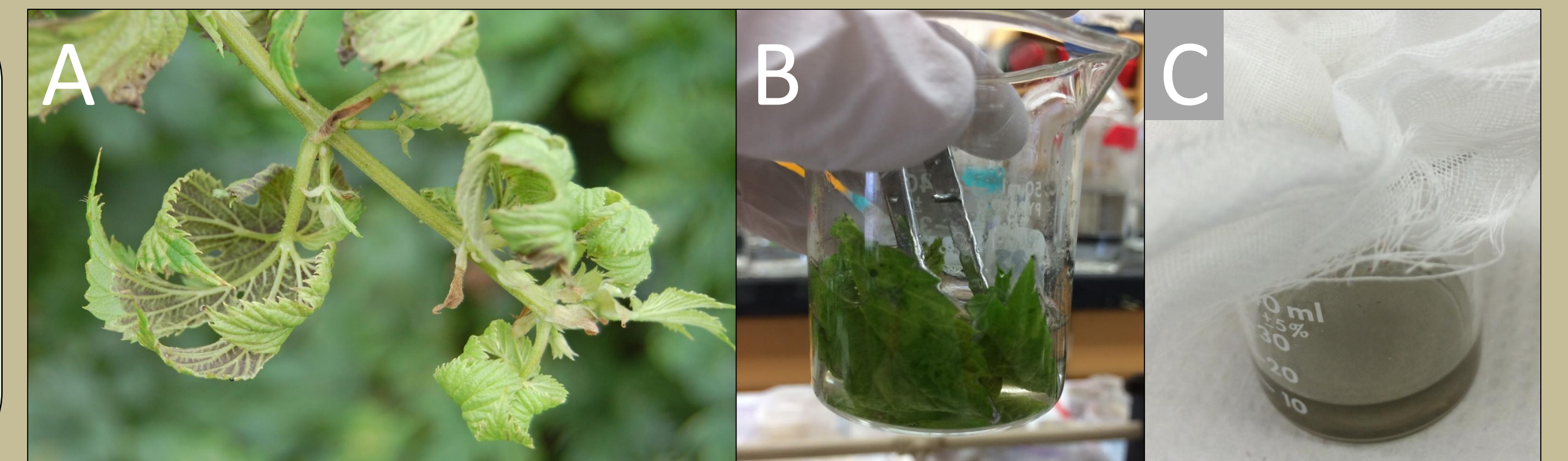


Figure 3 (left). A. Leaf disks 1 cm in diameter were cut with a cork borer. B. Seven leaf disks were arranged on each plate. C. Test for each isolate consisted of two non-fungicide amended control plates and two fungicide-amended plates.

Figure 4 (right). Inoculation procedure. A. Each leaf disk inoculated with 10 µl of inoculum in 3 locations. B. Seven leaf disks x 2 plates x 3 inoculate sites per disk = 42 replication sites per treatment. C. Inoculum droplet removed via aspiration 24h after inoculation.



Conclusions

- A total of 77 isolates sporulated sufficiently for further analysis over 3 years of this investigation**
- 40 of these isolates tested as sensitive to mefenoxam, 37 isolates tested as insensitive to mefenoxam**
- Insensitive isolates were detected even in yards with no history of phenylamide use, and were detected in all counties sampled except Columbia County**
- Recommendations for prescriptive use of phenylamide fungicides will need to be made on a site-by-site basis**

References

- Gent, D. H., Nelson, M. E., Grove, G. G. 2008. Persistence of phenylamide insensitivity in *Pseudoperonospora humuli*. *Plant Dis.* 92:463-468.
- Hunger, R. M., Horner, C. E. 1982. Control of hop downy mildew with systemic fungicides. *Plant Dis.* 66:1157-1159.

Acknowledgements

Many thanks to our WI grower cooperators for supporting our research endeavors. Additional thanks goes to members of the Gevens lab and to Dr. David Gent of Oregon State Univ. for their assistance with many technical aspects of this project. Funding for this research comes, in part, from a North Central Regional SARE Project for Research & Extension and a DATCP administered WI Specialty Crop Block Grant for hops research and development.



Figure 1 (right). Number of sensitive and resistant isolates detected in each of six counties where collections were made in 2015-2017. See map above for county locations.

