



Phenotypic characterization of *Phytophthora* from North Carolina greenhouse ornamentals

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INTRODUCTION

North Carolina is the fifth-largest producer of bedding and garden plants and potted flowering plants in the United States. Within North Carolina, the greenhouse and nursery industry is the third largest agricultural industry¹. Root rot, crown rot, and foliar blight, caused by several species of *Phytophthora*, are common disease problems on a wide range of ornamental crops. In 2007-2008, a survey of greenhouse facilities in North Carolina was carried out to determine the *Phytophthora* species present and the floriculture crops being affected. Isolation from symptomatic plant species resulted in 164 isolates of *Phytophthora* from thirteen host species at eleven locations. Using morphology and ITS sequencing, isolates were identified primarily as *P. nicotianae* (59%), *P. drechsleri* (23%), *P. cryptogea* (9%), and *P. tropicalis* (4%). Surprisingly, 64% of *Phytophthora* isolates were insensitive to 1 µg a.i./ml of mefenoxam (Subdue Maxx), one of the most widely used fungicides for *Phytophthora* in the floriculture industry. Additional phenotypic characterization was conducted to provide information for disease management strategies.

OBJECTIVES

- The objectives of this study were:
- To determine the effective concentration of mefenoxam providing 50% growth inhibition (EC50) for *Phytophthora* isolates that grew at 1 µg a.i./ml mefenoxam.
 - To characterize the mating type of heterothallic *Phytophthora* isolates.

MATERIALS AND METHODS

- ### 50% Growth Inhibition (EC50)
- Isolates within a species were divided into groups based on location, host, and initial mefenoxam sensitivity.
 - Up to three isolates per group were grown on commercial agar plates containing mefenoxam concentrations appropriate for regression analysis.
 - Groups 1 and 2: 0.1, 0.2, 0.5, and 1 µg a.i./ml
 - Group 3: 100, 200, and 500 µg a.i./ml
 - Groups 4-12: 200, 500, and 1000 µg a.i./ml
 - Hyphal growth was measured perpendicularly when growth on the non-amended control plate reached the edge (Figure 1).
 - The percent growth inhibition was determined and converted to a probit.
 - A regression analysis was run using the probits and log mefenoxam concentration. The resulting equation was used to interpolate the EC50².

Mating Type

- Isolates of heterothallic species were paired with A1 and A2 tester isolates on V8-juice agar amended with β-sitosterol or rapeseed-extract malt agar³ (Figure 4).
- Plates were incubated at room temperature in the dark and observed weekly for the formation of oospores (Figure 5).



Figure 1. Growth and measurement of *Phytophthora* on mefenoxam-amended plates.

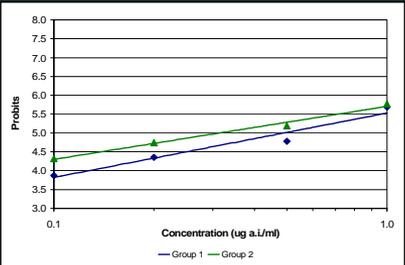


Figure 2. Log-probit plot of *P. tropicalis* isolates that showed intermediate sensitivity to mefenoxam at 1 µg a.i./ml.

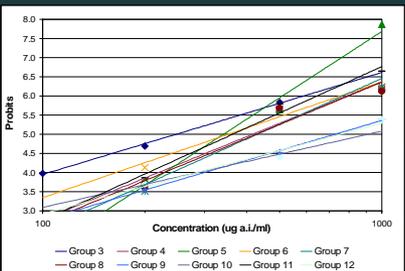


Figure 3. Log-probit plot of *P. drechsleri* and *P. nicotianae* isolates that showed complete insensitivity to mefenoxam at 1 µg a.i./ml.

Species	Location	Host	Mating type
<i>P. cryptogea</i>	B	Gerbera daisy	A1
	C	<i>Evolvulus</i>	A2
	E	Gerbera daisy	A2
	I	Verbena	A1
<i>P. drechsleri</i>	J	Dusty miller	A2
	B	Gerbera daisy	A1
<i>P. nicotianae</i>	G	Gerbera daisy, <i>Vicia</i>	A1
	A	Dusty miller, Gerbera daisy	A1
	Fa	Petunia	A1
	Fb	<i>Calibrachoa</i>	A1
	Fc	Gardenia	A2
<i>P. tropicalis</i>	G	<i>Vicia</i>	A1
	H	Annual vinca	A2
	H	<i>Euphorbia</i> 'Bonfire'	A1
	I	Gerbera daisy, Verbena	A2
<i>P. tropicalis</i>	K	<i>Calibrachoa</i>	A1
	B	Gloxinia	A2
	Fa	Verbena	A1
	K	Polihos	A2

Table 1. Location, host plant, and mating type of *Phytophthora* species collected from NC greenhouses.

RESULTS

50% Growth Inhibition (Figures 2 and 3)

EC50 estimates for *P. tropicalis* isolates were 0.49 (Group 1) and 0.31 (Group 2) µg a.i./ml. *Phytophthora drechsleri* isolates exhibited the greatest mefenoxam resistance with EC50 estimates of 341, 727, 910, and 755 µg a.i./ml (Groups 5, 9, 10, and 12, respectively). Isolates from Groups 9, 10, and 12 were collected from the same location. EC50 estimates for *P. nicotianae* isolates were 247, 415, 353, 429, 427, and 363 µg a.i./ml (Groups 3, 4, 6, 7, 8, and 11, respectively). Interestingly, isolates of *P. drechsleri* Group 10 and *P. nicotianae* Group 11 were collected from the same epidemic but have significantly different EC50 estimates.

Mating Type (Table 1)

Both the A1 and A2 mating types of *P. cryptogea*, *P. nicotianae*, and *P. tropicalis* were found in NC greenhouse facilities. At two locations, both mating types of *P. nicotianae* were collected but not from the same host plant. Only the A1 mating type of *P. drechsleri* was found.



Figure 4. Example of mating type pairing.



Figure 5. *Phytophthora cryptogea* oospore formed on rapeseed-extract malt agar.

CONCLUSIONS

- Phenotypic heterogeneity exists among isolates of *Phytophthora* species collected from NC greenhouse facilities.
- Resistance to mefenoxam is present in isolates of several *Phytophthora* species, illustrating the need for alternative chemicals and active resistance management programs.
 - Phytophthora tropicalis* EC50 estimates suggest that insensitive isolates may be emerging in the greenhouse industry.
 - Differences between EC50 estimates from *P. drechsleri* and *P. nicotianae* isolates collected from the same epidemic may indicate a tendency of some *Phytophthora* species to develop resistance more readily than others.
- The presence of both mating types of *P. cryptogea*, *P. nicotianae*, and *P. tropicalis* in the NC greenhouse industry allows for the potential of sexual reproduction with the movement of plant material between facilities.

LITERATURE CITED

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The authors gratefully acknowledge funding provided by USDA ARS Floriculture and Nursery Research Initiative and the Fred C. Gloeckner Foundation. The first author is very appreciative to APS Foundation and Dr. Don Mathre for the APS Foundation Don E. Mathre Student Travel Award which assisted her in attending the 2009 Annual APS meeting.