Mefenoxam resistance within *Phytophthora* and *Pythium* isolates recovered from ornamental host plants from greenhouses and nurseries in Georgia

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Root and crown diseases, caused by the Oomycete pathogens, *Phytophthora* and *Pythium*, are the most common and damaging diseases affecting ornamental plants. The Oomycete pathogens are difficult to manage within greenhosues and ornamental plant nurseries. Management of root disease consists of irrigation management, avoiding overwatering and saturated rooting substrate, sanitation to reduce spreading pathogens into production, and the use of fungicide drenches, sprenches or sprays. The fungicides used to manage *Phytophthora* and *Pythium* are ineffective on other root and crown pathogens due to the physiological and morphological differences between Oomycetes and true fungi. Oomycetes are more closely related to brown algae than to fungi. Therefore, until recently the number of active ingredients available to use against *Pythium* and *Phytophthora* was very limited.

The most common material used was metalaxyl, the active ingredient in the fungicide, Subdue 2E®. Metalaxyl (Subdue 2E®) was introduced in the late 1970s for *Phytophthora* and downy mildew disease control. It was widely used in agronomic and horticultural crops primarily for *Phytophthora* management. In the late 1990's, Syngenta Corp., the manufacturer of Subdue 2E®, changed the active ingredient of the product to only include the active enantiomer, mefenoxam, and labeled the product as Subdue MAXX®. Both Subdue 2E® and Subdue MAXX® contained the same active ingredient, mefenoxam.

Resistance to mefenoxam (and metalaxyl) has been documented in numerous crops. Mefenoxam (and metalaxyl before it) is labeled as Ridomil® for agronomic crops. Several states, including PA, NY, NC, VA, and SC, have documented metalaxyl/mefenoxam resistance in *Phytophthora* and *Pythium* isolates obtained from ornamental plants. It is assumed that mefenoxam resistance is also present in Georgia, but we do not know for sure nor do we know how prevalent it may be within nurseries and greenhouses. The objectives of this study were to 1) identify *Phytophthora* and *Pythium* species recovered from ornamental plants within greenhouses and nurseries in Georgia, and 2) screen recovered isolates for sensitivity to mefenoxam fungicide to determine if resistant isolates exist in GA and how prevalent they are within each production facility.

Methods:

Root and crown rot symptomatic plant samples were collected from 17 ornamental production facilities in 2010-2011. Nine facilities specialized in container-grown woody ornamental shrubs and eight specialized in floriculture or herbaceous crops. A total of 152 plant samples were collected. Symptomatic roots, stems, and/or leaves were washed of growing substrate and plated onto dilute V8-PARP selective media to recover *Pythium* and/or *Phytophthora* isolates. Pure isolates were maintained on V8-juice agar at 22°C until identification and fungicide sensitivity screening. Isolate species identification was completed based upon morphological characteristics and DNA sequencing of the ITS region of each isolate.

To test for sensitivity to mefenoxam sensitivity, isolates were transferred to V8 agar plates containing 0, 10, and 100 ppm mefenoxam. Plates were incubated for up to 4 days in the dark at 22°C. The diameter of the hyphal growth of each isolate was measured per plate (3 replicate plates per isolate. The sensitivity testing for all isolates was repeated. To determine the level of sensitivity, the growth of each isolate at 10 and 100 ppm mefenoxam-amended medium was compared to the growth of the isolate on the non-amended medium (containing 0 ppm mefenoxam). Isolates growing greater

than 40% of the growth of the isolate on non-amended (0 ppm) medium were considered resistant to mefenoxam.

Results:

Out of the 152 plant samples, Oomycete root pathogens were recovered from 122 (80% of samples), of which *Phytophthora* species were recovered from 39 (26% of samples) and *Pythium* species were recovered from 83 (55% of samples). Either pathogen recovery was unsuccessful or a non-Oomycete pathogen was recovered from the remaining 20% of samples. *Phytophthora* species recovered included *Phytophthora nicotianae, P. pini, P. drechsleri, P. palmivora, P. undulata, P. citrophthora.* Several isolates are still unidentified *Phytophthora* species. Interestingly, *P. cinnamomi*, the species most commonly associated with root disease in ornamental production was not recovered. *Pythium* species identification is in progress.

Resistance to mefenoxam was identified within both *Phytophthora* and *Pythium* isolates recovered from ornamental greenhouses and nurseries. The presence of mefenoxam resistance varied across woody plant nurseries (Table 1) and floriculture/herbaceous plant greenhouses (Table 2). Of the 17 nursery/greenhouse facilities sampled, mefenoxam-resistant isolates were not recovered from only two facilities (12%). The remaining 88% of the facilities had either or both mefenoxam resistant *Phytophthora* or *Pythium* isolates. Three facilities had 100% of the isolates recovered were mefenoxam-resistant. In yet others, all *Phytophthora* isolates were sensitive to mefenoxam, but all *Pythium* isolates were resistant. In total, approximately 31% of all *Phytophthora* isolates and 59% of all *Pythium* isolates recovered were resistant to mefenoxam (Table 3). Of note is that 100% of the *P. palmivora* and *P. undulata* isolates were resistant to mefenoxam

Conclusions:

This study confirms the presence of mefenoxam resistant *Phytophthora* and *Pythium* species within ornamental nurseries and greenhouses in Georgia. The amount of resistance identified was higher than expected and higher than recent studies have found in other southeastern states. Mefenoxam resistance is not present in all facilities and this may be due to past mefenoxam (metalaxyl) fungicide usage. Fungicide use history data is being collected from the facilities sampled to determine if past usages of mefenoxam correlates with the degree of resistance currently present within the facility. In one instance, mefenoxam has not been used by the facility, yet a resistance *Phytophthora* isolate was recovered. It is possible that movement of plant material between production facilities such as occurs when plug or rooted cuttings are grown in one facility and then shipped to other facilities for finishing may also spread fungicide resistant isolates. Also, it is known that some *Phytophthora* species are unaffected by mefenoxam. All *P. palmivora* and *P. undulata* isolates were resistant to mefenoxam in this study. This could indicate that mefenoxam either has limited or no activity on these species. If this is so, then these isolates are not truly resistant to the fungicide in the same way as a species that is normally sensitive to the fungicide. Further testing of these isolates needs to be conducted.

With the amount of mefenoxam resistance identified in this study, facilities in which high levels of resistance is known to be present should consider using alternative fungicides to manage Oomycete root pathogens rather than relying on mefenoxam as control failures may occur. A more detailed sampling of facilities with high levels of resistant isolates needs to be conducted to determine the true risk posed by the resistant isolates.

Of particular interest is the species identified within this study. The species commonly assumed to be the most damaging and common on ornamental plants were not recovered. The pathogenicity of several of the recovered *Phytophthora* species is uncertain. Additional pathogenicity testing needs to be conducted to determine if these species are true pathogens of the ornamental plants. It is assumed that *Pythium* species identification will be as unexpected as *Phytophthora* species. Based upon initial

morphology evaluations, few of the recovered *Pythium* isolates are *P. aphanidermatum* or *P. ultimum*, which are often the two species most commonly associated with Pythium root rot disease in floriculture crops. Some of the isolates appear to be *P. irregulare*, which is also often associated with Pythium root rot disease. *Pythium* species are also known to be saprobes and although they were recovered from symptomatic tissue, they may not be the cause of the root disease. It is expected that pathogenicity testing will also have to be conducted on some of the *Pythium* species recovered.

Table 1: *Phytophthora* and *Pythium* isolates resistant to mefenoxam collected from woody ornamental hosts

Nursery	No. of *Phytophthora* isolates /No. of isolates recovered	% Resistant to Mefenoxam	No. of *Pythium* isolates /No. of isolates recovered	% Resistant to Mefenoxam
1	2/7	100	5/7	60
2	2/7	100	3/7	66.7
3	----	----	4/4	100
4	1/8	0	4/8	75
5	1/6	100	2/6	50
6	3/10	0	3/10	100
7	1/1	100	----	----
8	----	----	6/8	100
9	12/31	16.7	17/31	82.3
Total	**22/82**	**36.3**	**44/82**	**81.8**

Table 2. *Phytophthora* and *Pythium* isolates resistant to mefenoxam from floriculture and herbaceous ornamental hosts

Nursery	No. of *Phytophthora* isolates /No. of isolates recovered	% Resistant to Mefenoxam	No. of *Pythium* isolates /No. of isolates recovered	% Resistant to Mefenoxam
1	----	----	7/10	14.2
2	1/10	0	6/10	66.7
3	2/12	50	6/12	50
4	2/8	50	5/8	20
5	6/14	(66.7 MR)	7/14	28.6
6	2/7	100	2/7	50
7	----	----	5/5	0
8	4/4	0	----	----
Total	**17/70**	**23.5**	**38/70**	**31.6**

Table 3. Summary of *Phytophthora* and *Pythium* isolates resistant to mefenoxam from 152 ornamental plant samples

Host Plants	No. of *Phytophthora* isolates /No. of isolates recovered	% Resistant to Mefenoxam	No. of *Pythium* isolates /No. of isolates recovered	% Resistant to Mefenoxam
Woody	22/82	36.3	44/82	81.8
Herbaceous	17/70	23.5	38/70	31.6
All Plants	**39/152**	**30.8**	**82/152**	**58.5**