Metalaxyl Resistance in MI isolates of *Pythium* spp. and the development of a seedling disease nursery

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Introduction

Michigan sugarbeet growers have expressed concern over poor stand establishment of the crop in recent years. In 1999-2000, we surveyed fields exhibiting stand problems for seedling disease. The usual seedling pathogens were found, including *Pythium* spp., *Aphanomyces cochlioides*, and *Rhizoctonia solani*., with *Pythium* spp. predominantly isolated from early planted beets (**Figure 1**). Since more and more MI growers are planting early, an improved understanding of Pythium seedling disease is important, especially the actual species causing this disease. Most seed in MI is treated with metalaxyl, a fungicide specific against *Pythium* and related Oomycete genera. Brantner and Windels (1998) found some metalaxyl tolerance in Minnesota *Pythium* isolates, raising the question of whether metalaxyl tolerance was also present in MI. The objective of this portion of our study was therefore to: 1) identify to species pathogenic *Pythium* isolated in the disease survey and 2) test them for tolerance to metalaxyl.

We also describe the first stages of the development of a disease nursery for Pythium and Aphanomyces seedling disease in MI. Establishment of disease nurseries has improved breeding efforts for many sugarbeet diseases. One difficulty in establishing a disease nursery is finding a suitable location with high concentrations of inoculum in the soil; efforts to inoculate seedlings or soil in field have been ineffective or impractical on a larger scale. A site on the Bean and Beet Research Farm, Saginaw, MI, historically provides poor stands of sugarbeets, despite a favorable soil texture for seedling emergence. Previous work has shown that soil at the site contains high populations of the pathogen *Pythium ultimum*, which causes early season seedling disease of sugarbeets, and of *Aphanomyces cochlioides*, which causes later season seedling disease. We initiated studies in 2000 to demonstrate the potential of this area as a seedling disease nursery.

Methods

Isolations and identifications of pathogenic *Pythium* spp. Pythium isolates were obtained as part of a larger overall survey for seedling disease pathogens, from fields identified as having seedling disease or stand problems by Monitor Sugar Co. or Michigan Sugar Co. personnel. Diseased seedlings were collected and *Pythium* spp isolated by standard methods. Seedlings were washed free of soil and incubated in dH_20 for 1-2 days then plated on corn meal agar amended with rifampacin and benomyl. *Pythium* isolates were also obtained from soil samples taken from the same fields using a bioassay procedure. Field soil was diluted 50% with a sterilized sandy loam-vermiculite

mix to prevent crusting and planted with 25 sugarbeet seeds (untreated or treated with metalaxyl) in 9 cm round pots. These were incubated at 15 or 25°C to mimic early and late-planting soil temperatures, and watered daily to encourage disease. *Pythium* spp. were isolated from diseased seedlings using the procedures outlined above.

To test pathogenicity and identify *Pythium* spp. obtained, a simple assay was developed: 4-5 surface-sterilized sugarbeet seeds and a Pythium isolate were co-plated on a 9 cm petri dish containing 1.2% water agar. In 4-5 days, pathogenic isolates caused lesions on the germinating seedlings and formed characteristic structures (oospores and zoosporangia) useful for identification.

Metalaxyl tolerance. Pathogenic isolates were tested for metalaxyl tolerance using the methods outlined in Brantner and Windels (1998) except that 6.0 cm petri plates were used for the metalaxyl dilution series which used less media and gave results in 1-2 days. EC_{50} values (the concentration at which fungal growth is inhibited 50%) were used for comparison between varieties and were obtained as in Branter and Windels (1998).

Test evaluating seed treatments in seedling disease nursery. A single seed lot of a sugarbeet variety (no commercial variety is known to have resistance against *Pythium* spp.) was subjected to various seed treatments (see **Table II**) and planted into the pathogen-rich soil ("bad ground") at the Bean and Beet Farm, Saginaw Co., MI in 2000. Plots were planted early and late in the spring (early April, early June) with eight replications for each treatment. The entire experiment was replicated on virgin soil ("good ground": not planted to beets in recent memory) nearby as a control. Both locations were planted on the same dates.

Results and discussion

Identifications of pathogenic *Pythium* spp. *P. ultimum* and *P. aphanidermatum* are thought to be the two main *Pythium* pathogenic to sugarbeet. While *P. ultimum* was most commonly isolated from the soil bioassay, several *Pythium* spp. were also isolated from diseased seedlings in the field and the soil bioassays: *P. dissotocum* and *P. myriotylum,* and one isolate each of *P. aphanidermatum* and *P. irregulare* (**Table I**). Interestingly, *P. ultimum* was not isolated directly from the field until later in the growing season, although very common in the soil in these fields. *P. dissotocum* was isolated from the field with some frequency, but not from the soil bioassay, and was also weakly pathogenic on the pathogenicity/identification assay plates. Other *Pythium* spp. were localized to single fields. This is the first isolation of *P. dissotocum* since the 1920's in MI, and the first isolation of *P. myriotylum* in MI.

Metalaxyl tolerance. Figure 2 shows the average metalaxyl tolerance of each species isolated (only one isolate of *P. aphanidermatum* and *P. irregulare* were tested); some isolates have a moderate level of tolerance to metalaxyl. Of note is the low tolerance (basically negligible) of *P. ultimum*. In combination with the results above, it is belived that metalaxyl seed treatments largely control *P. ultimum*, especially in early

plantings. *P. dissotocum*, a fairly weak pathogen, may be exploiting this available niche to some extent. Also noteworthy is the fact that (except in *P. ultimum* isolated from one field (see below)) all *Pythium* isolates with metalaxyl tolerance form zoospores: these spp. can create single or few-isolated "epidemics" more readily than *P. ultimum*.

Seedling disease nursery. Stand emergence was poor in the early season planting on the bad ground. **(Table II)** At the early planting date, Tachigaren-pelleted seed provided superior stand establishment to other treatments. At the later planting date, where Aphanomyces seedling disease is likely, no benefits were shown, probably due to the lack of excess soil moisture that facilitate pathogen movement and contribute to plant anoxia. There were no significant differences among any treatments on the good ground.

Table I shows that P. ultimum isolates from the "Nursery" field had some of the largest tolerances to metalaxyl observed. The seed treatment results indicated below indicate that Tachigaren may provide some cross protection against these isolates of Pythium ultimum, although a signifigant caveat is the presence of A. cochlioides in this field. This experiment will be repeated in 2001 with a very early planting date to minimize the influence of A. cochlioides.

Figure 1: Pathogen Incidence in Disease Survey 1999

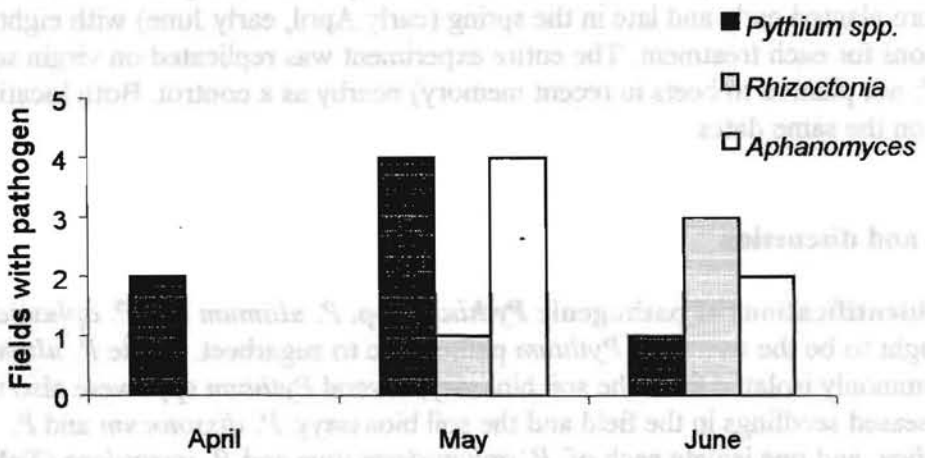

Figure 2: Metalaxyl Tolerance EC^{50} ug/ml of pathogenic *Pythium* spp.

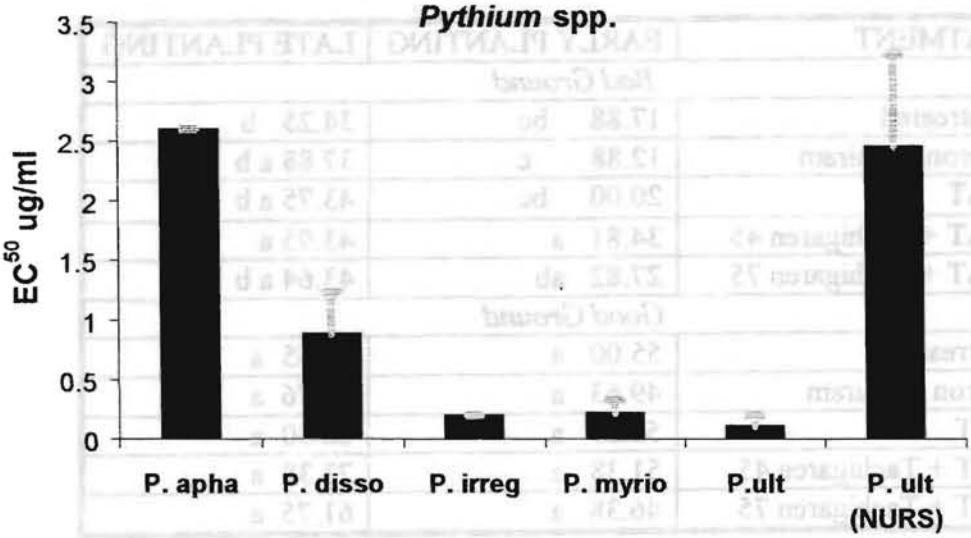

Table I: A. *Pythium* spp. isolated from field soil bioassay. Soil was sampled from 14 fields, pots were incubated at 15 or 25 C to mimic early and late planting soil temperatures. **B.** *Pythium* spp. isolated directly from diseased plants in the field.

A. (no. fields isolated from)		
	15 C	**25 C**
P. ultimum	5	10
P. irregulare	1	0
P. aphanidermatum	0	1
P. myriotylum	0	1

B. (no. fields isolated from)			
	April	**May**	**June**
P. ultimum	2	3	0
P. dissotocum	0	4	2
P. aphanimdermatum	0	1	0

Table II. Effect of seed treatment on stands (plants per 25 ft) of Early and Late planted beet seedlings on "good" or "bad" ground at B&B farm. Means with the same letter are

not significantly different at the 95% confidence level. Means are compared only within each ground/planting.

TREATMENT	EARLY PLANTING	LATE PLANTING
Bad Ground		
1 Untreated	17.88 bc	34.25 b
2 Apron + Thiram	12.88 c	37.88 a b
3 PAT	20.00 bc	43.75 a b
4 PAT + Tachigaren 45	34.81 a	43.95 a
5 PAT + Tachigaren 75	27.82 ab	43.64 a b
Good Ground		
1 Untreated	55.00 a	62.45 a
2 Apron + Thiram	49.63 a	68.76 a
3 PAT	56.25 a	68.50 a
4 PAT + Tachigaren 45	51.38 a	73.38 a
5 PAT + Tachigaren 75	46.38 a	61.75 a