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Pythium Species and Population Identification Using DNA Markers

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BACKGROUND

Many species of the fungus, *Pythium*, cause damping off, and rots of roots, stems, and cuttings. In roots, some species found are very weak parasites while others are beneficial because they attack other disease-causing fungi rather than the plant roots. Thus, it is very important to accurately identify the species associated with plants. *Pythium* can be found in soil carried in from outside the greenhouse, in peat or soilless potting mixes, and in pond and stream water. It can be brought into the greenhouse on infected cuttings or plugs purchased from another greenhouse. Once in the greenhouse, it survives in crop debris and soil on or under benches. It is important to know where the disease causing *Pythium* originated so control measures can be aimed at that source. This means that we must be able to tell for example, whether the *Pythium ultimum* causing crop losses is the same or different from the *Pythium ultimum* we might find in soil under the bench or in plants purchased from a supplier.

MATERIALS NEEDED

Pythium was isolated from commercial greenhouse samples sent to Penn State and PA Dept. of Ag. clinics. *Pythium* was also isolated from locations within 3 commercial greenhouses where it caused crop losses. For comparison isolates of known identity were obtained from world culture collections. The identities of all isolates were initially based on microscopic characters. DNA of over 400 isolates was extracted for analysis. The DNA base sequences were determined for the ribosomal DNA region which is known to be unique to a species. It cannot, however, be used to tell one population from another within a species. To try to separate one population from another, AFLP was used to examine *P. aphanidermatum, P. irregulare,* and *P. ultimum*. The sensitivity of isolates to mefenoxam (Subdue Maxx) and propamocarb (Banol) was tested.

RESULTS

While using microscopic methods to identify species of *Pythium* was very difficult, the ribosomal DNA region sequences proved to be highly accurate in species determinations. *P. aphanidermatum, P. irregulare,* and *P. ultimum* were the species most commonly causing greenhouse crop losses from 1996 through 2001 (Table 1). Within a given greenhouse, disease causing species were found in several locations. AFLP separated the *P. ultimum* populations and the *P. irregulare* populations, but could not separate *P. aphanidermatum* populations. *P. irregulare* caused 57% of all *Pythium* cases. *P. aphanidermatum* caused 77% of poinsettias *Pythium* root rots. *P. ultimum* was responsible in 12% of the cases. Close to 40% of the *P. aphanidermtum* and *P. irregulare* isolates were resistant to mefenoxam. . While 35% of all isolates were resistant to propomocarb, 12% were resistant to both fungicides (Table 1).

CONCLUSIONS

Pythium causes major losses in a wide variety of greenhouse crops. Using DNA sequences, we can now accurately identify which species are causing those losses and can identify specific populations within *P. irregulare* and *P. ultimum*. A method other than AFLP must

be found to separate *P. aphanidermatum* populations. Fungicide resistance to 2 totally different chemicals, Mefenoxam and propamocarb, appears to be a widespread problem.

IMPACT TO THE INDUSTRY

1. Obtain an accurate diagnosis of which species of *Pythium* is involved. 2. Ask the lab to determine whether the *Pythium* is resistant to Subdue Maxx or Banol if planning to use them on the crop. 3. If *Pythium* is an ongoing problem, sample the potting mix, irrigation water from ponds or streams, and soil under benches to be tested for *Pythium*. 4. Newly purchased plants should be examined for *Pythium* rot symptoms.

Table 2. Fungicide resistance.

Pythium species	Total samples/ #Subdue resistant/ #Banol resistant/ # resistant to both
aphanidermatum	32 / 1 / 5 / 2
cylindrosporum	1 / 1 / 1 / 1
dissotocum	4 / 1 / 3 / 1
group F	1 / 0 / 1 / 0
heterothallicum	1 / 1 / 1 / 1
irregulare	57 / 21 / 11 / 7
splendens	1 / 1 / 1 / 1
ultimum	13 / 1 / 6 / 1

Table 1. Clinic samples in Pennsylvania, 1996-2000

Sample	No. samples-Species
Adiantum, fern	1-*P. irregulare*
Antirrhinum snapdragon	1-*P. cylindrosporum* 3-*P. irregulare* 1-*P. ultimum*
Aquilegia	1-*P. irregulare*
Athyrium, fern	1-*P. irregulare*
Begonia	1-*P. ultimum*
Bellis	1-*P. irregulare*
Bidens	1-*P.myriotylum*
Bocopa	1-*P. irregulare* 1-*P. ultimum*
Chrysanthemum	3-*P. irregulare* 1-*P. ultimum*
Cordyline	1-*P. irregulare*
Cyclamen	1-*P. irregulare*
Dianthus	1-*P. myriotylum*
Digitalis	1-*P.myriotylum*
Euphorbia poinsettia	30-*P. aphanidermatum* 7-*P. irregulare* 2-*P. ultimum*
Gerbera	1-*P. irregulare*
Helianthemum	1-*P. irregulare*
Heuchera	2-*P. irregulare*
Impatiens	2-*P. irregulare*
Lilium, Easter lily	1-*P. ultimum*
Lupinus	1-*P. irregulare*
Mattuccia, fern	1-*P. ultimum*
Ocimum basilicum	1-*P. irregulare*
Osmunda, fern	1-*P. irregulare*
Pelargonium ivy geranium	1-*P. dissotocum*
Pelargonium zonal geranium	1-*P. aphanidermatum* 2-*P. dissotocum* 1-group F 1-*P. heterothallicum* 15-P. *irregulare* 3-*P. myriotylum* 2-*P. ultimum*
Petunia	1-*P.dissotocum*
Pulmonaria	1-*P. irregulare*
Ranunculus	1-*P. irregulare*
Rosa, miniature rose	1-*Pythium sp.*
Salvia	1-*P. ultimum*
Schlumbergera	1-*P. aphanidermatum* 1-*P. irregulare*
Sedum	2-*P. irregulare*
Thymus	1-*P. irregulare*
Verbena	1-*P. ultimum*
Viola	1-*Pythium sp.*
Zinnia	1-*P. irregulare*
Unused potting soil	3-*P. irregulare* 1-*P. splendens* 1-*P. sylvaticum*
Irrigation water	4-*P. irregulare* 1-*P. sylvaticum*
TOTAL	120

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