PAECILOMYCETES LILACINUS, A COLONIST OF ASPERGILLUS FLAVUS
SCLEROTIA BURIED IN SOIL IN ILLINOIS AND GEORGIA

D. T. WICKLOW

Northern Regional Research Center, ARS/USDA.
1 1815 North University Street, Peoria, Illinois 61604

AND

D. M. WILSON

Department of Plant Pathology, University of Georgia.
Coastal Plains Research Station, Tifton, Georgia 31793

Sclerotia of Aspergillus flavus Link: Fr. are an important source of inoculum in the disease cycle of this aflatoxin-producing fungus in maize (Zea mays L.) fields (Wicklow et al., 1984; Wicklow and Wilson, 1986). We have been investigating the survival of A. flavus sclerotia that were buried in sandy soils at the University of Illinois River Valley Sand Field, Kilbourne, Illinois, and the Coastal Plains Research Station, Tifton, Georgia. This allows us to contrast survival in a temperate versus near-subtropical latitude on a similar soil type. An earlier study revealed that all the A. flavus sclerotia survived overwintering while buried 6 months in Georgia soils (Wicklow, 1987) and we wanted to determine the fate of sclerotia while buried in soil up to 3 years in Illinois and Georgia without a susceptible host crop (e.g., peanuts, maize) in order to apply this information in planning crop rotation sequences.

Sclerotia were produced by culturing individual A. flavus strains on autoclaved corn kernels at 50% (w/w) moisture for 28 days at 28 C. The sclerotium-producing strains included: Aspergillus flavus var. flavus NRRL 6541, from corn, North Carolina; NRRL 13048, and NRRL 13892, from corn, Tifton, Georgia; NRRL 6556, from soil, Canton, Illinois; Aspergillus flavus var. parasiticus NRRL 13005, from microarthropod, beech forest, Michigan; NRRL 6433, from corn, North Carolina; NRRL 13539, from peanuts, Georgia; NRRL 13006, from maize, South Africa. Sclerotia were harvested from the molded corn kernels according to the method described by Wicklow (1987). After air-drying at room temperature the sclerotia produced by individual strains of A. flavus were blended into separate lots of air-dried field soil collected from each of the two field sites to give approximately 18 sclerotia per gram of soil. Eighteen stainless steel “teaballs” were filled with 60 grams of soil containing sclerotia harvested from a single strain of A. flavus var. flavus or A. flavus var. parasiticus. The individual teaballs were fastened together with nylon fishing line, labeled, buried (October 1986), and the site marked with a flag. Teaballs filled with soil from a particular field site were buried only at that field site. At 6-month intervals, three teaballs were recovered for each sclerotium-producing strain in both Georgia and Illinois. Individual sclerotia were recovered from the soil inside each teaball by wet-sieving and flotation as described by Wicklow et al. (1984). To test for viability, 30 randomly selected sclerotia were disinfected by shaking for 2 min with 0.25% sodium hypochlorite, rinsed twice with 5 ml sterile distilled water, and then transferred to Petri dishes containing potato dextrose agar. After 5–10 da incubation at 25 C, the plates were examined for A. flavus var. flavus or A. flavus var. parasiticus colony growth as evidence of sclerotium viability (D. T. Wicklow and D. M. Wilson, unpubl. data). In addition, we isolated and identified fungi other than A. flavus that had colonized or parasitized individual sclerotia.

Paecilomyces lilacinus (Thom) Samson was re-
TABLE I

<table>
<thead>
<tr>
<th>Fungal strain</th>
<th>Illinois</th>
<th>Georgia</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. flavus var. flavus</em></td>
<td>1%*</td>
<td>3%*</td>
</tr>
<tr>
<td>NRRL 6541</td>
<td>1%*</td>
<td>3%*</td>
</tr>
<tr>
<td>NRRL 13048</td>
<td>1%*</td>
<td>18%*</td>
</tr>
<tr>
<td>NRRL 6556</td>
<td>3%*</td>
<td>7%*</td>
</tr>
<tr>
<td>NRRL 13892</td>
<td>2%*</td>
<td>1%*</td>
</tr>
<tr>
<td><em>A. flavus var. parasiticus</em></td>
<td>3%*</td>
<td>11%*</td>
</tr>
<tr>
<td>NRRL 13005</td>
<td>3%*</td>
<td>11%*</td>
</tr>
<tr>
<td>NRRL 6433</td>
<td>2%*</td>
<td>2%*</td>
</tr>
<tr>
<td>NRRL 13866</td>
<td>2%*</td>
<td>22%*</td>
</tr>
<tr>
<td>NRRL 13006</td>
<td>1%*</td>
<td>10%*</td>
</tr>
</tbody>
</table>


* Within treatments, *P. lilacinus* colonization values assigned the same letter (a, b, or c) do not differ significantly (P = >0.05), whereas values showing different letters are significantly different (P = <0.05). For contrasts involving the same strain buried in Illinois vs. Georgia, values assigned the letter y are significantly different (P = <0.05).

Colonization of sclerotia by *A. flavus* var. *flavus* and *A. flavus var. parasiticus*, both isolated from agricultural soils in The Netherlands (Samson, 1974), was recorded from up to 18% of the sclerotia produced by strains of *A. flavus* var. *flavus* and up to 22% of the sclerotia from strains of *A. flavus* var. *parasiticus* that were buried for 18 months in Georgia soil (Table I). Colonization by *P. lilacinus*, of an equivalent set of *A. flavus* sclerotia buried 18 months in Illinois never exceeded 3%. Mean *P. lilacinus* colonization values for sclerotia of individual *A. flavus* strains were compared within each treatment by analysis of variance (ANOVA). Individual comparisons between means were done by planned t-tests of least squares means where a significant overall F-test (P = <0.05) was found in the ANOVA. Among the sclerotia plated from Georgia, *P. lilacinus* accounted for 78% (66 of 85) of the fungal colonists from the total of 720 sclerotia plated. This result strongly suggests mycoparasitism and not saprophytic colonization of damaged or killed sclerotia. Why sclerotia produced by some strains of *A. flavus* (e.g., NRRL 13048, NRRL 13539) are more readily colonized by *P. lilacinus* than others is not known.

*Paecilomyces lilacinus* strains isolated from *A. flavus* sclerotia have been deposited in the ARS Culture Collection at the Northern Regional Research Center. These include Illinois isolates from *A. flavus var. flavus* sclerotia = NRRL 13875, NRRL 13876 and *A. flavus var. parasiticus* sclerotia = NRRL 13872, NRRL 13873, NRRL 13874; Georgia isolates from *A. flavus var. flavus* sclerotia = NRRL 13868, NRRL 13869, NRRL 13870, NRRL 13871 and *A. flavus var. parasiticus* sclerotia = NRRL 13866, NRRL 13867. Several of our isolates produced synnemata on potato dextrose agar slants as has been reported for fresh isolates from agricultural soils in The Netherlands (Samson, 1974).

To determine if *P. lilacinus* can parasitize *A. flavus* sclerotia we distributed freshly harvested sclerotia produced by several of the *A. flavus* strains listed above onto moist autoclaved sand in individual Petri dishes. We then dripped 1.0 ml of a 1 x 10⁴ conidial suspension of *P. lilacinus* NRRL 13875 over the sclerotia in one-half of each dish. Dishes were incubated for 10 days at 25 °C and the sclerotia examined under the dissecting microscope for evidence of sporogenous germination (yellow-green conidial heads) and/or colonization by *P. lilacinus*. *Paecilomyces lilacinus* colonized and rotted *A. flavus* sclerotia, as evidenced by the entire sclerotium surface being covered with the pink conidial heads of *P. lilacinus*. These sclerotia were soft and presumably rotted as contrasted with uncolonized and ungerminated, hard, sclerotia.

Wicklow (1987) showed that all *A. flavus var. flavus* NRRL 6541 sclerotia that were buried for 6 mo in Tifton series loamy sand (Georgia) remained viable and free from fungal colonists. In the present study *P. lilacinus* probably colonized the sclerotia during the summer months since optimal growth of the fungus is reported to occur at 20–30 °C and the fungus does not grow at temperatures of 5 °C and below (Pitt, 1973). Stack et al. (1988) reported that sclerotia may be colonized by *Gliocladium sp., Paecilomyces variotii* Bain or *Trichoderma sp.*, all of which were able to render *A. flavus* sclerotia non-viable or suppress germination. *Paecilomyces variotii* was introduced into soil prior to solarization for the control of peanut diseases (Stack et al., 1988). The fungus grew well in soil at 30 °C under a wide range of soil moisture levels (-0.1 to -10.0 bars matric potential) and readily colonized sclerotia of *A. flavus*. The colonized sclerotia failed to germinate when the temperature of the soil returned to 25 to 30 °C.

*Penicillium lilacinum* Thom ( = *P. lilacinus*) was first isolated as a colonist of another fungus.
a basidiocarp of Polystictus, by Tubaki (1955). *Paecilomyces lilacinus* was reported to be a mycoparasite by Karhuvaara (1960) who isolated “strain No. 80” from the sclerotium of *Sclerotinia sclerotiorum* (Liben) de Bary. In a companion study, *P. lilacinus* strain No. 80 rotted sclerotia of *Sclerotinia trifoliorum* (Erikss., *Sclerotinia borealis* Bubak & Vleugel, and *Claviceps purpurea* (Fr.) Tulasne (Makkonen and Pohjakallio. 1960). The sclerotia, harvested from cultures grown on agar medium, or in the case of *C. purpureum* from ears of infected rye, were inoculated with *P. lilacinus* conidia and incubated (20 C) on autoclaved quartz sand in Petri dishes. We know of no other examples implicating *P. lilacinus* as a colonist/mycoparasite of another fungus (Domsch et al., 1980: Hawksworth, 1981). *Paecilomycetes lilacinus* has long been recognized as a parasite of insects (Samson, 1974). More recently, attention has focused on the ability of *P. lilacinus* to parasitize eggs of the root knot nematode (Jatala, 1986; Dube and Smart, 1987). It therefore comes as no surprise that *P. lilacinus* can degrade chitin (Okafor, 1967). The present research has identified *P. lilacinus* as a mycoparasite with the potential for biocontrol of *A. flavus* sclerotia in soil.

**ACKNOWLEDGMENTS**

This research was supported in part by a Specific Cooperative Agreement No. 58-5114-7-1001 between the University of Georgia Research Foundation and ARS, and by State Hatch funds allocated to the Illinois and Georgia Agricultural Experiment Stations. We acknowledge the generous assistance offered by Mr. Stan Sipp, Superintendent, Illinois River Valley Sand Field (Illinois Agricultural Experiment Station), Department of Agronomy, University of Illinois. We thank Dr. Terry Nelson (Biometrician, NRRC, ARS) for performing the statistical tests.

Key Words: *Paecilomyces lilacinus*, mycoparasitism, sclerotia, *Aspergillus flavus*

**LITERATURE CITED**


