Absence of Sporidesmin Production by Twelve Texas Isolates of *Pithomyces* spp.

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Twelve isolates of *Pithomyces* spp. from Texas were tested for sporidesmin toxin production, using both high-performance and thin-layer chromatography techniques. None of the Texas isolates produced the toxin under the conditions used. A control toxigenic New Zealand isolate, *Pithomyces chartarum* strain C, was grown simultaneously under the conditions tested and was found to produce sporidesmin in all cases.

Sporidesmin, a mycotoxin produced by *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis, is the causative agent of pithomyctotoxicosis (facial eczema), a disease which affects sheep and cattle grazing summer and fall pastures in New Zealand, Australia, and, less frequently, South Africa (2, 8). The presence of *P. chartarum* was recorded in Britain in 1962 (4, 7), but based on field population studies, it was postulated that this fungus was not responsible for photosensitivity outbreaks in sheep in that country (6). A photosensitizing syndrome similar to pithomyctotoxicosis has also been observed in Texas, and *P. chartarum* has been isolated from seven counties within the state (10). However, a previous attempt to detect sporidesmin in cultures of *P. chartarum* isolated from pastures in the Gulf Coast Plain of Texas, on which cattle displaying photosensitivity lesions grazed, was unsuccessful (12).

More recently, frequent outbreaks of a photosensitizing disease have occurred in sheep and goats grazing Kleingrass (*Panicum coloratum* L.) pastures in West Texas (3, 11). The etiological agent for this syndrome is yet to be determined. This study, therefore, was undertaken in an attempt to determine whether Texas isolates of *Pithomyces* spp. are capable of producing sporidesmin and whether the production of sporidesmin may be involved in the manifestation of the photosensitizing outbreaks in West Texas.

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**MATERIALS AND METHODS**

**Fungi.** Twelve isolates of *Pithomyces* spp. were obtained from a variety of sources in Texas. The isolates and their sources are presented in Table 1. Pure cultures of the fungi were cultivated on V-8 juice agar (9) slants or plates at ambient temperature and transferred monthly. A pure culture of a highly toxic New Zealand strain, *P. chartarum* strain C (NZ-C), was kindly donated by M. E. diMenna (Ruakura Soil Research Station, Hamilton, New Zealand) and was cultured as previously described. Stock cultures of all isolates were stored on V-8 juice slants under oil in the refrigerator.

**Culturing techniques.** The three substrates chosen for preliminary comparisons of sporidesmin production included ryecorn (*Secale cereale* L.), Kleingrass straw, and V-8 juice agar. The ryecorn (35 g per flask) and Kleingrass straw (10 g per flask) cultures were grown in 500-ml Erlenmeyer flasks. The flasks were filled with tap water and soaked overnight; then the excess water was decanted, and the flasks were autoclaved at 15 lb/in² (121°C) for 15 (Kleingrass straw) or 30 (ryecorn) min. After inoculation, all cultures were incubated for 30 to 35 days at ambient temperature (range, 20 to 30°C) in total darkness or under normal fluorescent lighting near a window out of direct sunlight. Ryecorn was used in subsequent experiments, since it proved to be superior.

**Treatments.** In an attempt to enhance sporulation or sporidesmin production, or both, the effects of four parameters (substrate, light, temperature variation, and mechanical stress) were tested (see Table 2). The temperature variation study was conducted for 42 days, during which time cultures were incubated at 7.6 ± 3.1°C in a cool incubator for 14 h nightly and transferred to a warm incubator at 24.6 ± 0.9°C for 10 h daily in an attempt to simulate natural diurnal temperature changes. The cultures were grown in the dark throughout this study. The mechanical-stress treatments involved the use of a heated spatula to cut up the cultures in situ at weekly intervals after a predetermined incubation period. All treatments, with the exception of the temperature variation study, were conducted both in the dark and in the light, as described earlier.

**Harvesting and extraction techniques.** Cultures which sporulated sufficiently to enable harvesting of an adequate quantity of spores were wet harvested and extracted according to the procedure of Halder et al. (5). For cultures that did not sporulate readily, the contents of the flasks were extracted in situ by following a scaled-down version of the extraction procedure.
TABLE 1. Strain and source of Pithomyces isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Species</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZ-C</td>
<td>P. chartarum</td>
<td>New Zealand</td>
</tr>
<tr>
<td>WRAT-Tx5</td>
<td>P. chartarum</td>
<td>Kleingrass, a San Angelo, Tex.</td>
</tr>
<tr>
<td>WRAT-Tx6</td>
<td>P. chartarum</td>
<td>Kleingrass, a San Angelo, Tex.</td>
</tr>
<tr>
<td>WRAT-Tx7</td>
<td>Pithomyces spp. b</td>
<td>Ryegrass, East Central Tex.</td>
</tr>
<tr>
<td>WRAT-Tx8</td>
<td>P. chartarum</td>
<td>Johnson grass, a Bryan, Tex.</td>
</tr>
<tr>
<td>WRAT-Tx9</td>
<td>P. chartarum</td>
<td>Bermuda grass, a Yoakum, Tex.</td>
</tr>
<tr>
<td>WRAT-Tx10</td>
<td>P. chartarum</td>
<td>Bermuda grass, a Yoakum, Tex.</td>
</tr>
<tr>
<td>WRAT-Tx11</td>
<td>P. chartarum</td>
<td>Bermuda grass, a Yoakum, Tex.</td>
</tr>
<tr>
<td>WRAT-Tx12</td>
<td>P. chartarum</td>
<td>Bermuda grass, a Yoakum, Tex.</td>
</tr>
<tr>
<td>WRAT-Tx13</td>
<td>P. chartarum</td>
<td>Bermuda grass, a Yoakum, Tex.</td>
</tr>
<tr>
<td>WRAT-Tx14</td>
<td>P. chartarum</td>
<td>Bermuda grass, a Yoakum, Tex.</td>
</tr>
<tr>
<td>WRAT-Tx15</td>
<td>Pithomyces spp. b</td>
<td>Bermuda grass, a Yoakum, Tex.</td>
</tr>
<tr>
<td>WRAT-Tx16</td>
<td>Pithomyces spp. b</td>
<td>Ryegrass, East Central Texas</td>
</tr>
</tbody>
</table>

a Panicum coloratum L.
b Species descriptions will appear elsewhere (manuscript in preparation).
c Lolium perenne L.
d Sorghum halepense (L.) Pers.
e Cynodon dactylon (L.) Pers.


Detection. The detection and quantitation of sporidesmin were determined by high-performance liquid chromatography, as described by Halder et al. (5), and confirmed by thin-layer chromatography.

RESULTS

Sporidesmin production by the Pithomyces isolates under the various incubation treatments, as measured by high-performance liquid chromatography, is summarized in Table 2. The absence of sporidesmin was confirmed by thin-layer chromatography. Only the New Zealand isolate, NZ-C, was found to produce sporidesmin to varying degrees under all of the incubation treatments tested (Table 2). Substrate variation appeared to exert the greatest influence on sporidesmin production by strain NZ-C, and of the three substrates tested, ryecorn was the best substrate for production of sporidesmin. In addition, mechanical stress appeared to have a stimulatory effect on sporidesmin production, whereas temperature variation effects were largely ineffective. Differences between light and dark effects in the various incubation treatments were generally insignificant with regard to sporidesmin production.

Although the Texas isolates exhibited good vegetative growth in all of the treatments undertaken, no sporidesmin production was detected from any of these isolates under any of the treatments tested. Sporulation was a distinct problem with the Texas isolates with the exception of one isolate, WRAT-Tx6. This isolate grew well and sporulated profusely under all treatments tested and, in general, produced more spores than the NZ-C strain. The various treatment regimens were initiated to test parameters that could enhance sporulation, thereby increasing the likelihood of toxin production. Of those treatments, it was found that mechanical stress, using a sterile heated spatula to break up cultures grown in the light, produced the best response. In one study, all of the isolates tested under this treatment sporulated more heavily when the cultures were grown in the light, but were mostly unresponsive when grown in the dark. No enhancement in sporulation was achieved in subsequent studies in which oxygen, nitrogen, or carbon dioxide was periodically blown over undisrupted cultures grown in the light. It therefore appeared that a combination of light and mechanical stress was necessary to initiate sporulation by the Texas isolates.

DISCUSSION

The wide range in yields of sporidesmin by strain NZ-C under the influence of the various treatments is in agreement with studies conducted by diMenna et al. (1) in which it was suggested that the sporidesmin-producing capabilities of P. chartarum strains may be influenced more by treatment than by strain differences. Of these treatment effects, it appears from this study that substrate alone has the greatest influence on sporidesmin production. Substrate effects also appear to influence sporulation ability, as noted by an ancillary study in which some Texas isolates were grown in petri plates on two different agar media under ultraviolet light. All isolates grown on V-8 juice agar under ultraviolet light were stimulated to sporulate, whereas the same isolates grown on Sabouraud agar under ultraviolet light grew well vegetatively but failed to sporulate.

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A general relationship between numbers of spores formed by cultures of *P. chartarum* and amount of sporidesmin produced was noted by diMenna et al. (1). This relationship does not hold true for the Texas isolates, none of which produced sporidesmin regardless of sporulation capabilities. The lack of sporidesmin production by all of the Texas isolates under the various treatments is suggestive of a genetic incapability rather than of treatment or substrate effects. The virulence of one Texas isolate, WRAT-Tx6, was further borne out by a feeding trial in which rabbits fed culture material of the isolate for 6 weeks showed neither ill effects nor significant weight loss.

The WRAT-Tx6 isolate presents an interesting case. Although it produced no sporidesmin under the conditions tested, it grew well and sporulated prolifically. It produced an abundance of spores under all test conditions, sporulated significantly within 3 to 4 days of inoculation compared with 7 to 8 days for strain NZ-C under the same conditions, and, in general, outyielded the NZ-C isolate in spore production.

The problem of poor sporulation by the Texas isolates was overcome with limited success by subjecting cultures grown in the light to mechanical stress, using a heated spatula to cut up the mycelium-ryecorn mixture at predetermined intervals. As with many other fungi, the effects due to this injury provided the "stress" necessary to stimulate sporulation. A first cutting between days 10 to 17 of incubation, repeated weekly thereafter, was found to induce the best sporulation.

The prevalence of *P. chartarum* in pastures in which photosensitivity outbreaks have occurred is extremely low. That, in addition to the fact that no sporidesmin was detected from strains isolated thus far in Texas (including the problem pastures), leads to the conclusion that there is little probability that the etiological agent for the photosensitivity disease afflicting sheep grazing Kleingrass pastures in West Texas is *Pithomyces* related.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


