Saprophytic Ability of Typhula incarnata, T. idahoensis, and T. ishikariensis

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ABSTRACT


Laboratory-grown sclerotia of Typhula idahoensis, T. incarnata, and T. ishikariensis germinated on the soil surface without exogenous food. All three species colonized live (green) wheat leaf tissue on the soil surface but not dead leaf or stem pieces. Live wheat leaves within the soil were colonized most aggressively by T. incarnata and least aggressively by T. idahoensis. Hyphae of all three species grew farther from sclerotia over the surface of Ritzville silt loam than over the surface of Palouse silt loam, indicating that soil type is a factor in hyphal growth on soil. When excised from the sclerotia, hyphae of all three species grew slightly on Ritzville silt loam but not on Palouse silt loam. Hyphae of T. incarnata grew farther over soil when excised from the sclerotia than the other species. These three fungi are poor saprophytes in nature and depend on parasitism for existence.

Typhula idahoensis Remsberg, T. incarnata Lasch ex Fr., and T. ishikariensis Imai are soilborne pathogens that incite snow molds of grasses and some dicotyledonous plants. Several reports allude to their strong saprophytic ability. Huber and Anderson (10) reported that organic amendments provided a food base for saprophytic growth of T. idahoensis, and Huber and Hankins (11) reported the same for T. incarnata. In Japan, T. incarnata colonized both green and dead leaves of orchard grass, whereas T. ishikariensis colonized only green leaves (16). Matsumoto and Araki (15) suggested that the saprophytic ability of T. incarnata could explain its widespread distribution in Japan. Reports of strong saprophytic ability contrast with observations that sclerotia occur rarely on dead grass or residual straw of previous crops in fields in Washington despite severe snow mold on green wheat (Triticum aestivum L.) (3). Weathered wheat straw from previous crops, the predominant plant residue in autumn, is rarely colonized by Typhula spp. in Washington (5).

The objectives of this study were to determine the ability of sclerotia of Typhula spp. to initiate saprophytic growth on dead, mature wheat leaves and straw, to determine the degree of mycelial growth on natural soil, and to determine the influence of live, green wheat leaves on germination of sclerotia on soil.

MATERIALS AND METHODS

Production of sclerotia. Sclerotia of three isolates of T. incarnata, T. idahoensis, and T. ishikariensis obtained from diseased wheat in Washington were produced by transferring mycelial fragments from Difco potato-dextrose agar (PDA) cultures in water blanks onto autoclaved wheat kernels. The wheat kernels were prepared in 2-L flasks by mixing 225 cm³ of dry wheat grain with 150 ml of water and autoclaving the mixture for 1 hr at 121 C. The inoculated kernels were incubated in darkness at 10 C for 60 days. Flasks were shaken periodically during incubation to permit even distribution and development of mycelium. After sclerotia developed, the wheat kernels were dried and mature sclerotia were separated from them by screening. Germination of these sclerotia on PDA was almost 100%.

Germination of sclerotia on soil. Ritzville silt loam (pH 7.3) from Lind, WA, was adjusted to three moisture contents by weight (i.e., 9.4, 12.2, and 16.7%, which approximates -3, -2, and -1.5 bars water potential, respectively). Ten sclerotia of each isolate along with three pieces of green leaves (3 cm long) of Daws winter wheat grown in the greenhouse were placed at random on the surface of soil in 85-mm-diameter petri dishes. Soil in petri dishes without leaf pieces served as controls. The dishes were sealed with Parafilm to minimize moisture loss during incubation. After incubation for 11 days at 5 C, the percentage of germination of sclerotia was determined visually. Hyphae from several sclerotia of each species were removed and identified as Typhula spp. on the basis of the presence of clamp connections. Each treatment was replicated nine times.

Colonization of wheat leaves and straw. No sclerotia, or 4, 15, 45, or 90 sclerotia (= 0, 40, 150, 450, or 900 sclerotia per kilogram of soil, respectively), of each isolate were added to widemouthed glass jars (473-ml capacity) containing 100 g of moist (-3 bars) Ritzville silt loam. The jars were shaken to distribute the sclerotia within the soil. Dead (necrotic) winter wheat leaves were obtained from mature plants collected from the field in August; live (green) leaves were obtained from Daws plants grown in the greenhouse. Two leaf pieces were buried about 1.5 cm deep, and three leaf pieces were placed on the soil surface before the lids were tightened on the jars. Each treatment was replicated three times. After incubation for 106 days at 5 C in the dark, the percentage of colonization of leaf materials was determined visually on the basis of the number of leaves on which sclerotia formed. Because there were no significant differences in colonization among isolates within a Typhula sp., the data for isolates were combined.

The percentage of colonization of field-grown straws of Daws winter wheat was determined by placing two 5-cm-long stem pieces in jars containing 100 g of Ritzville silt loam (=3 bars) amended with 900 sclerotia per kilogram of soil of either T. incarnata, T. idahoensis, or T. ishikariensis per jar. Jars without sclerotia served as controls. Before the lid on each jar was tightened, one wheat straw was buried about 1.5 cm deep and one was placed on the soil surface. Each treatment was replicated 19 times. After incubation for 105 days at 5 C in the dark, the number of straws on which sclerotia formed was determined visually.

Hyphal growth from sclerotia on soil. Twenty sclerotia of T. incarnata, T. idahoensis, or T. ishikariensis were spaced separately on the surface of either Ritzville silt loam or on Palouse silt loam (pH 6.4) at about -2 bars water potential in 140-mm-diameter petri dishes. Soil pH was determined in 0.01 M CaCl₂. Parafilm was used to seal the petri dishes to preserve moisture, and incubation was at 5 C.

The radial growth of mycelium of T. incarnata was measured after 23 days, then 10 sclerotia on each soil were severed from their soils.
mycelia with a razor blade and removed with forceps. Mycelial growth was measured again 12 and 39 days after removal of the sclerotia.

Radial growth of mycelium of *T. idahoensis* and *T. ishikariensis* was determined after 35 days at 5°C. Ten sclerotia of each species on each soil type were likewise removed from their mycelia at this time.

Mycelial growth after separation from the sclerotia was measured 27 days later.

**RESULTS**

**Mycelial growth of sclerotia of *Typhula* spp. on soil.** Germination of *T. incarnata*, *T. idahoensis*, and *T. ishikariensis* sclerotia ranged from 98 to 100%, 94 to 97%, and 96 to 99%, respectively, for all treatments. The wheat leaves and soil moisture contents had no effect on germination.

**Colonization of wheat leaves and straw by *Typhula* spp.** As inoculum density increased, colonization of live (green) leaves on and within the soil increased (Fig. 1). *T. incarnata* colonized live leaves on or below the soil surface with greater frequency (*P = 0.05*) at the lower inoculum densities than did either *T. idahoensis* or *T. ishikariensis*. No dead leaves on or within the soil were colonized by any *Typhula* spp. except by one isolate of *T. ishikariensis* at 900 sclerotia per kilogram of soil that colonized a single dead leaf on the soil surface on which a single sclerotium formed. No mature straws of winter wheat on or below the soil surface were colonized by any *Typhula* sp.

**Hyphal growth on soil.** Hyphae of *T. incarnata* grew faster and farther on both Ritzville silt loam and on Palouse silt loam than hyphae of *T. idahoensis* or *T. ishikariensis*. The growth rate of *T. idahoensis* was slightly slower than that of *T. ishikariensis* on both soils (Table 1). Hyphae of all species grew faster on Ritzville silt

**TABLE 1. Radial hyphal growth from sclerotia of *Typhula* spp. on the soil surface before and after excision of hyphae from the sclerotia.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Ritzville silt loam</th>
<th>Palouse silt loam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23 Days 35 Days 62 Days</td>
<td>23 Days 35 Days 62 Days</td>
</tr>
<tr>
<td><em>T. incarnata</em></td>
<td>Excised 16.9* 28.9 31.4 12.5* 12.5 12.5</td>
<td>Attached 15.2 34.7 54.3* 10.9 24.0* 24.0*</td>
</tr>
<tr>
<td><em>T. idahoensis</em></td>
<td>Excised 15.3* 18.9</td>
<td>Attached 14.1 29.0* 10.6 16.4*</td>
</tr>
<tr>
<td><em>T. ishikariensis</em></td>
<td>Excised 17.1* 21.0</td>
<td>Attached 15.8 31.1* 11.7 16.9*</td>
</tr>
</tbody>
</table>

* T. incarnata excised from the sclerotia at 23 days, *T. idahoensis* and *T. ishikariensis* excised from sclerotia at 25 days.

* Vertical pair of data followed by an asterisk differ at the *P = 0.05* confidence level.

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**Fig. 1.** Percentage of colonization of green (live) and dead wheat leaves on the soil surface and buried in jars at 5°C by *Typhula incarnata*, *T. idahoensis*, and *T. ishikariensis* from sclerotia mixed with the soil.
The greater growth of Typhula spp. on Ritzville silt loam than on Palouse silt loam may be related to the degree of microbial activity in the two soils. The Palouse silt loam with a higher organic matter content (3.1%) should support greater microbial activity than the Ritzville silt loam with less organic matter (1.6%). Lehmann (12, 13) reported that fine sandy loams favored snow mold in Finland and Germany and attributed greater growth on light soils to less microbial antagonism in such soils.

*T. incarnata* has a wider geographic range than the other two species (3), and it infested leaf material beneath the soil surface with greater frequency. The ability to attack buried live leaves (Fig. 1) is evidence of greater adaptation to well-aerated, subsurface conditions. Its greater radial growth on soil (Table 1) should increase the competence radius of its sclerotia (9), another advantage. The greater radial growth on soil of hyphae attached to the sclerotia (Table 1) than when excised is evidence of translocation within the hyphae.

Huber and Hankins (11) found that snow mold severity increased when winter wheat was clipped from 23 November to 3 December and the leaf clippings were left on the soil surface. They concluded that these organic amendments provided a base for saprophytic growth. In our opinion, this does not prove significant saprophytic ability. Green wheat leaves could remain alive for some time under the cool, humid conditions of late fall, delaying colonization by strict saprophytes.

Huber and Anderson (10) reported that adding ground wheat straw and other organic materials to the soil surface before snowfall increased both Typhula spp. and Fusarium niveum (Fr.) Cesati, G. W. Bruehl (unpublished) repeated their experiments in the field in Douglas County on a soil dominated by *T. idahoensis* and obtained no response.

Sclerotia of *T. idahoensis* have been found on stones and wooden staves among disced wheat (3), but this did not prove that stones and wooden staves were substrates. Mycelium from parasitized wheat grew over these objects under the snow and formed sclerotia on them sustained by substances obtained through parasitism. Finding sclerotia on a substrate (dead straw in nature) does not always prove a nutritional relationship.

**LITERATURE CITED**


