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

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ABSTRACT

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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 cm^3 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 $CaCl_2$. 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

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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.

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

	Hyphal growth (mm)					
	Ritzville silt loam			Palouse silt loam		
Species	23 Days	35 Days	62 Days	23 Days	35 Days	62 Days
T. incarnata						
Excised	16.9[a]	28.9	31.4	12.5[a]	12.5	12.5
Attached	15.2	34.7	54.3*[b]	10.9	24.0*	24.0*
T. idahoensis						
Excised	···	15.3[a]	18.9	···	11.4[a]	11.4
Attached	···	14.1	29.0*	···	10.6	16.4*
T. ishikariensis						
Excised	···	17.1[a]	21.0	···	12.3[a]	12.3
Attached	···	15.8	31.1*	···	11.7	16.9*

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

[b] Vertical pairs of data followed by an asterisk differ at the $P = 0.05$ confidence level.

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

RESULTS

Myceliogenic germination 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

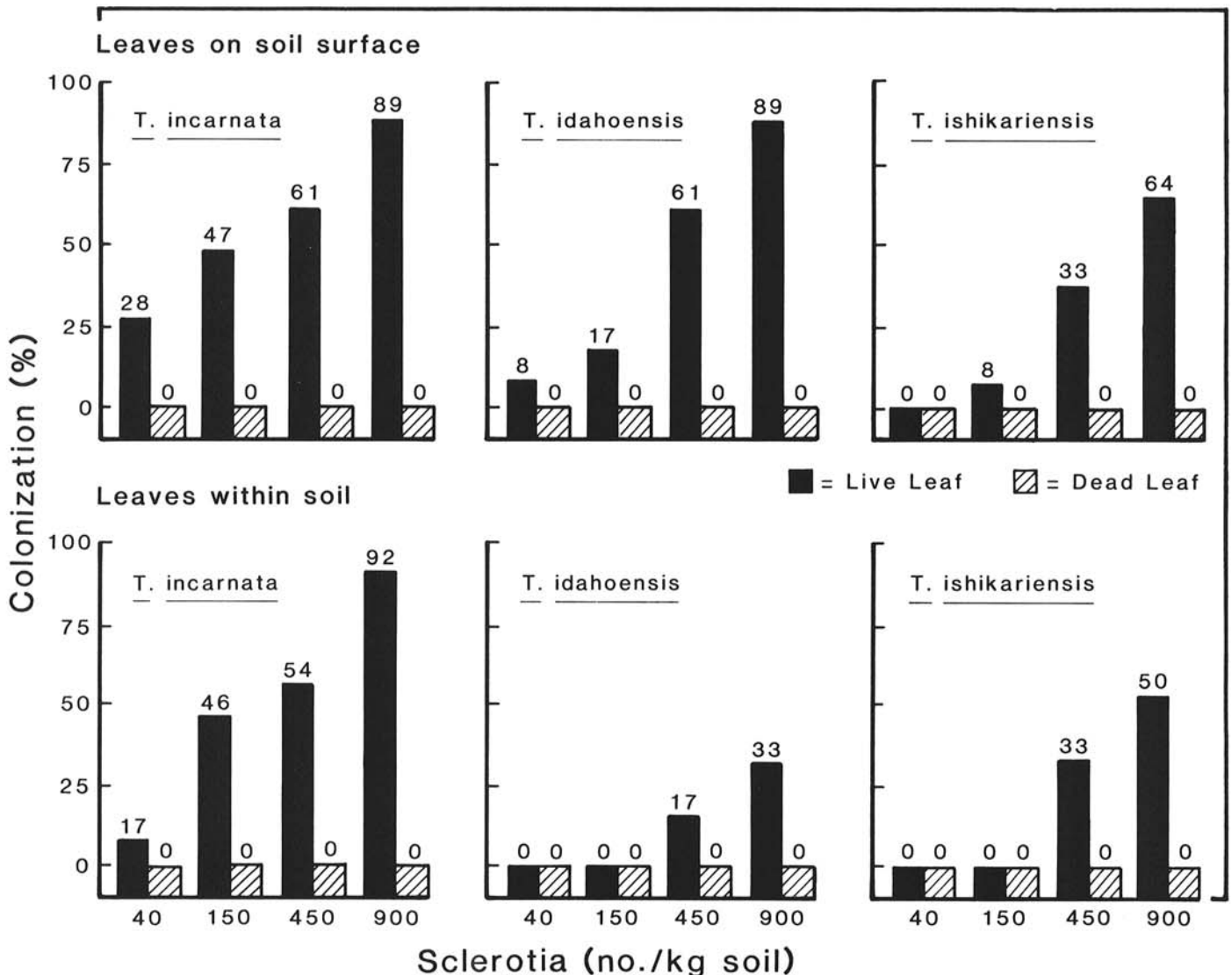

Fig. 1. Percentage of colonization of green (live) and dead wheat leaves on the soil surface and buried in jars at 5 C by *Typhula incarnata, T. idahoensis,* and *T. ishikariensis* from sclerotia mixed with the soil.

loam than on Palouse silt loam. After 62 days, hyphae attached to sclerotia had grown farther than hyphae not attached to sclerotia.

DISCUSSION

The sclerotia of this study were produced in the laboratory, and they lacked endogenous dormancy because almost 100% germination occurred on PDA. This was considered advantageous in trials to determine the ability of these fungi to colonize living or dead substrate. Davidson and Bruehl (6) found that natural sclerotia of *T. idahoensis* germinated on washed sand without added exogenous foods, and many workers have incubated natural sclerotia on sand or soil outdoors in the autumn to produce basidiocarps. Therefore, fungistasis does not prevent germination of all sclerotia whether they are collected from the field or produced in the laboratory. Davidson and Bruehl (6) reported 32% germination on washed river sand and 80% germination of the same lot of natural sclerotia of *T. idahoensis* on acidified cornmeal agar, but we believe the data obtained from laboratory-produced sclerotia are reliable for purposes of these experiments.

Although plant materials stimulate germination of some sclerotia (1,8,14,17), almost 100% germination of the sclerotia of these trials occurred on soil whether or not green leaves were present. Because germination was nearly 100% in the checks, it was impossible to determine whether the presence of plant materials stimulated germination. However, no chemotropic growth of mycelium from sclerotia toward green wheat leaves was observed. Lehmann (12) also reported that germination of sclerotia of *T. incarnata* did not depend on carbon and nitrogen sources, host plants, or other stimulants.

Detiffe et al (7) placed sclerotia of *T. incarnata* 0–8 cm from the bases of barley plants in the field in Belgium. The closer the sclerotia were to the base of the plant the sooner infection occurred and the more severe the disease. The exact distance the fungus grew over the surface of the soil is not known, however, if lower leaves were in contact with the soil.

The three *Typhula* spp. did not colonize and multiply on dead leaves or straw. The dead leaves used in this study were collected in early August from mature plants before harvest. In nature, wheat residues are already colonized by many saprophytes by October and November, when the sclerotia germinate in nature, further reducing the likelihood of their colonization by *Typhula* spp. Cunfer and Bruehl (5) also reported that *T. idahoensis* competed poorly as a saprophyte on weathered straw.

In contrast to our results, Matsumoto and Sato (16) reported that *T. incarnata* colonized autoclaved leaves of *Dactylis glomerata* L. (orchard grass) buried in natural soil by the Cambridge method (4) of estimating competitive saprophytic ability at both 10 and 1 C. *T. idahoensis* (= their *T. ishikariensis* biotype B [15]) and *T. ishikariensis* (= their *T. ishikariensis* biotype A) colonized autoclaved grass leaves at 0 C. In their system, the fungi were grown in a sand-wheat bran medium for 21 days at 10 C. This inoculum was mixed with the soil in varying proportions, and the leaf blades were introduced immediately. In our study, germinable sclerotia were the inoculum. It is probable that by the time the sclerotia in our study germinated, faster competitors had occupied the dead wheat leaves and straw that we used as substrate. We believe that the Cambridge method exaggerates the competitive saprophytic abilities of these fungi, especially if live active hyphae are introduced with the inoculum. *T. incarnata* is favored by weakened, senescent leaves (15,16,18), but even limited life of the leaves favors parasites over saprophytes.

The poor saprophytic capability of *Typhula* spp. was further indicated by the reduced growth on soil of hyphae excised from sclerotia (Table 1). These hyphae had limited ability to use soil nutrients for growth. For example, the total radial growth of hyphae removed 39 days earlier from sclerotia and of hyphae not removed from sclerotia of *T. incarnata* on Ritzville silt loam was 31.4 and 54.3 mm, respectively. In contrast, *Rhizoctonia solani* Kühn, which has considerable saprophytic ability, grew almost as far on soil whether its mycelium was attached to a food source or not (2).

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 nivale* (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 nursery stakes among diseased wheat (3), but this did not prove that stones and wooden stakes 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.

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