

### Factors Affecting the Effectiveness of Sclerotia of *Typhula idahoensis* as Inoculum

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#### ABSTRACT

Sclerotia of *Typhula idahoensis* were more effective in inciting snow mold of winter wheat when placed on the soil surface than when buried 0.5 or 1.0 cm in soil, and were not effective when buried 2, 5, or 10 cm in soil. Incorporating up to 80% coarse sand in the soil mixture did not affect the above results. When 37, 75, 150, 300, 625, 1,250, and 2,500 sclerotia/kg of soil were mixed randomly within four different soils, the disease ratings and sclerotium production were the same in each of the soils. Disease severity increased with inoculum density up to 300 sclerotia/kg of soil and leveled off; sclerotium

production increased with inoculum density up to 1,250 sclerotia/kg of soil. More sclerotia per infected plant were produced at 0.5 than at -0.5 C.

The percentage germination of sclerotia was higher on acidified cornmeal dextrose agar than on rinsed river sand with no exogenous nutrients, and was higher on sand at 10 than at 3 C.

When a dark fungus, believed to be *Cladosporium herbarum*, emerged from the sclerotia, *T. idahoensis* was seldom obtained.

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*Typhula idahoensis* Remsberg incites a serious snow mold of winter wheat in Douglas, Okanogan, and Chelan Counties, Wash. (3). The main survival and inoculum unit of the pathogen is the sclerotium. Mature sclerotia are chestnut brown to black, glo-

bose, and 0.5-2 X 0.5-0.9 mm (4, 15). Sclerotia are produced on leaves of affected wheat in late winter or early spring. During the cool, rainy weather of late October, November, and early December, the sclerotia germinate to form sporophores or mycelia. Al-

though airborne basidiospores are reported to infect wheat (16, 17, 18), they are probably not important as inoculum (3).

Other important snow mold fungi in Washington are *Typhula incarnata* Lasch ex Fr. and *Calonectria nivalis* Schaffnit [=*Fusarium nivale* (Fr.) Cesati] (17). *Typhula idahoensis*, *T. incarnata*, and *Fusarium nivale* may occur in the same field and on the same plant, but *T. idahoensis* usually predominates in such associations (3).

We herein report observations on the influence of depth of burial of sclerotia and relationships of soil texture and inoculum density to development of snow mold of wheat incited by *T. idahoensis*. The ability of sclerotia to germinate vegetatively in the absence of exogenous nutrients was also investigated.

**MATERIALS AND METHODS.**—*Experiments with wheat plants.*—Palouse silt loam (pH 6.2, 3.1% organic matter), Waha silt loam (pH 6.4, 6.2% organic matter), Ritzville silt loam (pH 6.7, 1.6% organic matter), and Touhey sandy loam (pH 6.6, 1.3% organic matter) from Whitman, Garfield, Adams, and Douglas Counties, Wash., respectively, were gathered from the 0- to 15-cm depth. The soils were passed through a 0.25-inch screen. They were collected each year just prior to use to avoid alterations of the natural microflora that might occur in storage. *F. nivale* and *T. incarnata* were present in all soils; *T. idahoensis* occurred only (in trace amounts) in the Touhey soil.

Sclerotia were separated from diseased wheat leaves collected at Fairfield, Camas County, Idaho, in May 1967, and were stored dry in an open container at 25 C. Their germination remained high (80-100%) during the 3-year period of these trials. Those germinating outdoors in the autumn gave rise to sporophores that identified the pathogen as *T. idahoensis* (15).

Nugaines winter wheat (C.I. 13968) was planted 1.5-2.5 cm deep in 6-inch clay pots in late August or early September. Sclerotia were added to the soil at planting time; they were counted when 60 or fewer were added per pot, or their number was estimated by weight when a greater number was used. In trials to determine the effectiveness of sclerotia beneath different depths of soil, 15 sclerotia/pot per depth were used in 1967-1968; 110 sclerotia/depth in 1968-1969; and 110 and 500 sclerotia/pot per depth in 1969-1970. In trials to determine the effectiveness of sclerotial populations randomly mixed within the soil as after tillage, 0, 37, 75, 150, 300, 625, 1,250, and 2,500 sclerotia/kg of soil were mixed uniformly within the upper 2.5 cm of soil in each pot. After seeding, the pots were embedded in sand outdoors. In early December, the young wheat plants were covered with moist cotton and placed in incubation chambers (3) at 0.5, -0.5, or -2 C for 95-126 days. Each treatment was replicated 4 times and eight -10 control pots to which no sclerotia were added were used in all experiments to assess the mold developing from inoculum already present in the soils.

The plants were taken from low-temperature incubation and the cotton was removed. The amount of leaf blight was estimated visually, sclerotia per plant

were counted in some trials, and the extent to which the plants recovered from a given treatment was determined by keeping them on a greenhouse bench in natural light at 10-15 C for 8 days and then weighing the green tissue present.

The amount of tissue blighted by *F. nivale* and *T. incarnata*, which were naturally present in all the soils, was subtracted from the totals of the blight scores to obtain a value that represented only *T. idahoensis* blight. The small amount of *T. idahoensis* blight that occurred naturally in the Touhey soil was likewise subtracted from the plants in that soil. When inoculum of *T. idahoensis* was abundant and on or near the soil surface, the data needed no adjustment as the test pathogen dominated under these conditions.

*Vegetative germination of sclerotia without exogenous nutrients.*—River sand was screened to remove particles larger than 0.7 mm, rinsed in tap water for 7 hr, and dried at 25 C. Fifty cc of dried sand were placed in a petri dish, and the surface was smoothed. The sand was then autoclaved for 20 min at 121 C, left at 25 C for several days, and autoclaved again. Fifteen ml of sterile, deionized water were added to the sand in each dish, and the dishes were autoclaved again at 121 C for 20 min. The sand was thoroughly wet, but there was no excess water. Sclerotia were immersed in a 1:1 solution of 6% sodium hypochlorite and 95% ethyl alcohol, plus two drops of polyoxyethylene sorbitan monolaurate (Tween 20) for 60 sec, rinsed for 5-10 sec in sterile water, and blotted. Two sclerotia were placed on the sand surface at opposite sides of the dish, and on opposite sides of acidified (pH 4.8) cornmeal dextrose agar (ACMDA). Dishes were enclosed in clear polyethylene bags, left at 25 C for 30 hr to allow the sclerotia to hydrate, and then placed at 3 or 10 C until mycelial growth was observed adjacent to sclerotia. Not all mycelia associated with surface-disinfested sclerotia were *T. idahoensis*; therefore, clumps of hyphae were transferred to potato-dextrose agar (PDA) and incubated at 3 or 10 C. Formation of *T. idahoensis* sclerotia on PDA proved that the parent sclerotium had germinated.

In 1968-1969, 25 sand cultures and 10 ACMDA controls were placed at each incubation temperature to observe the influence of exogenous food on germination. No attempt was made to exclude light, either from the intermittent illumination of the bulb in the incubator or from the fluorescent lights of the laboratory. In 1969-1970, one group of sclerotia was handled as before and an additional 25 sand cultures and 10 ACMDA controls/temperature were wrapped in black plastic immediately after the sclerotia were placed on the sand or ACMDA to exclude light. The dishes were incubated for 56 days at 3 C and 48 days at 10 C, after which hyphal transfers were made and incubation was continued with no attempt to exclude light.

**RESULTS.**—*Depth of burial of sclerotia.*—Sclerotia formed on the wheat plants when the inoculum was buried less than 2 cm deep, and more sclerotia were formed at 0.5 than at -0.5 C (Fig. 1). The great-

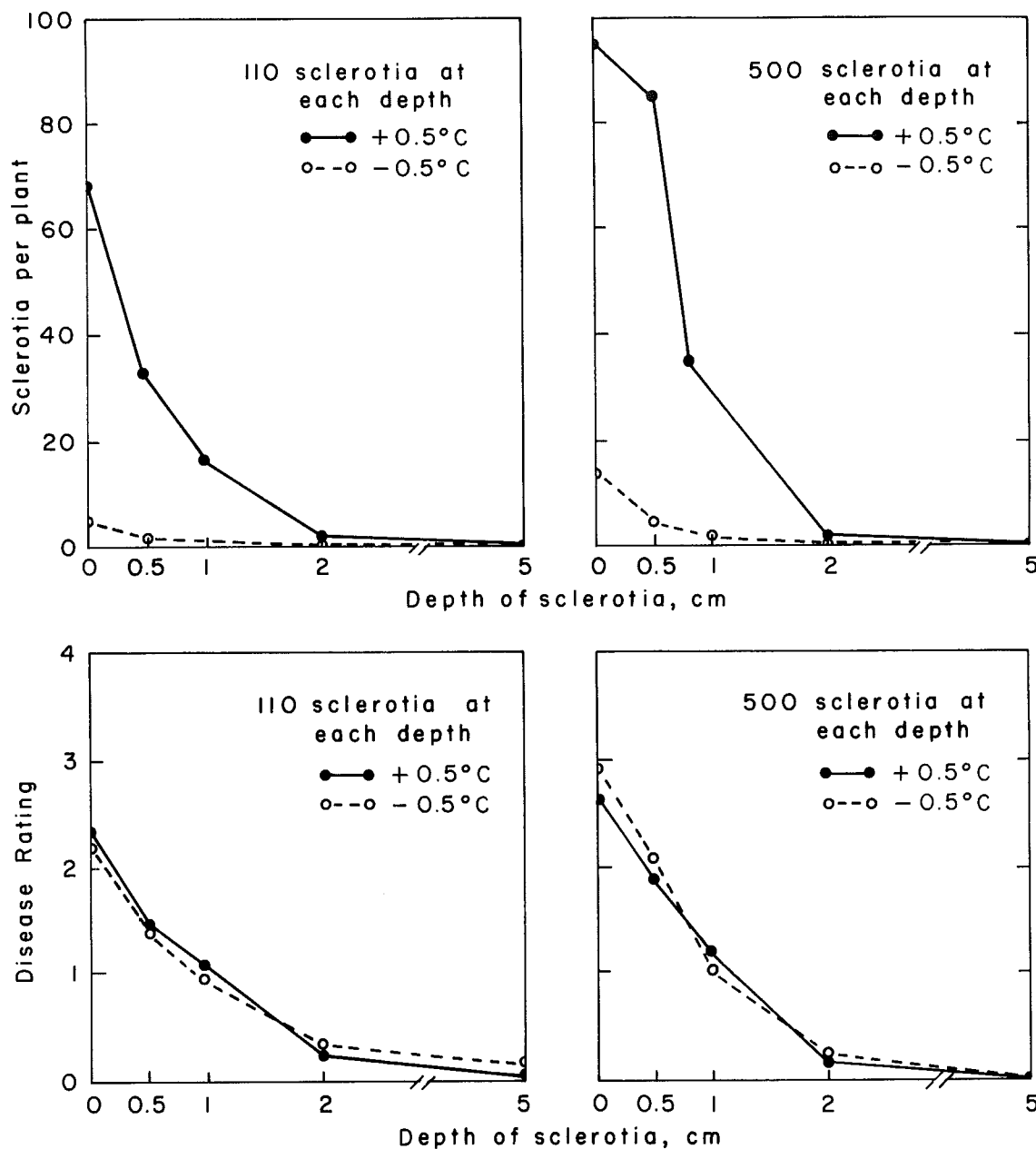


Fig. 1. Influence of depth of burial of 110 (left) and 500 (right) sclerotia of *Typhula idahoensis* per 6-inch clay pot on the number of sclerotia produced (top) and upon disease severity (bottom) on wheat plants incubated at 0.5 or -0.5 C for 15 weeks. Sclerotia incubated in three soils, four replicates of each. Data averaged, as there was no significant difference among soils. Number of sclerotia (top) = average of three plants each in each soil (nine plants). Disease rating (bottom): 0 = all leaves green; 1 = most leaves green; 2 = some green leaves; 3 = few green leaves; 4 = dead plant. Disease estimated after an 8-day recovery period on a greenhouse bench.

est number of sclerotia formed when inoculum was on the soil surface. No sclerotia were seen on roots, even when the inoculum was 10 cm deep in the soil. The infection observed in the Touhey soil when sclerotia were buried 2 cm or deeper was from natural inoculum.

Even though more and larger sclerotia formed at

0.5 than at -0.5 C, disease severity was the same at the two temperatures. No disease developed at -2 C (used in 1967-1968), and this temperature was not tested again.

*Soil texture.*—Because inoculum buried 2 cm or more was ineffective, coarse sand (particles 0.3-2 mm in diam) was added to soil (0, 10, 25, 50, and 80%

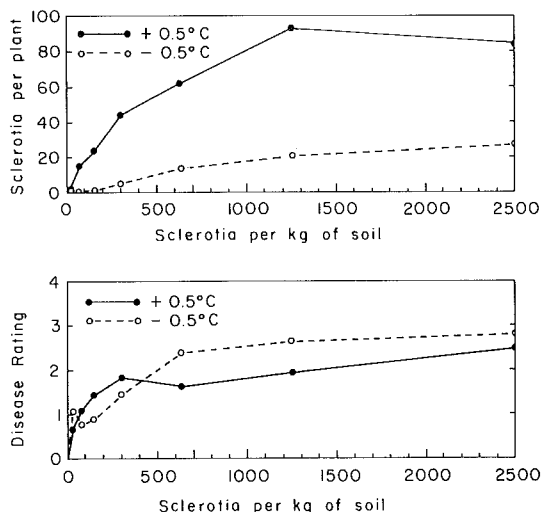


Fig. 2. Influence of numbers of sclerotia of *Typhula idahoensis* randomly mixed within the top 2.5 cm of soil in each 6-inch clay pot on sclerotia produced (top) and upon the disease severity (bottom) on winter wheat incubated for 15 weeks at 0.5 or  $-0.5$  C. Sclerotia incubated in three soils, five replicates of each. Data averaged as there was no significant difference among soils. Number of sclerotia (top) = average of three plants in each soil. Disease rating the same as in Fig. 1. Disease estimated after an 8-day recovery period on a greenhouse bench.

Fifteen, 30, 60, 120, 250, 500, and 1,000 sclerotia in the upper 2.5 cm of soil/6-inch clay pot is equivalent to 37, 75, 150, 300, 625, 1,250, and 2,500 sclerotia/kg of soil, respectively.

sand/soil by volume) to determine whether coarse-textured material facilitated infection from buried sclerotia. The soil-sand combinations were used to cover 110 sclerotia/pot 2 cm deep. Wheat seed was placed 1.5 cm below the surface. The plants were grown outdoors, brought in and covered with moist cotton, and incubated at 0.5 or  $-0.5$  C for 18 weeks.

Infection was sporadic and not related to the percentage of sand mixed with the soil. Inoculum 2 cm deep was almost ineffective, even when covered with soil containing 80% coarse sand.

**Inoculum density.**—The number of sclerotia produced by each diseased plant increased with increasing inoculum densities from 0 to 1,250 sclerotia/kg of soil at 0.5 C and then leveled off; at  $-0.5$  C, the number of sclerotia produced was smaller, but it increased with inoculum density from 0 to 2,500 sclerotia/kg of soil (Fig. 2, top). Disease severity increased rapidly with increasing inoculum densities from 0 to 300 sclerotia/kg of soil at 0.5 C, and less rapidly to 625 sclerotia/kg of soil at  $-0.5$  C (Fig. 2, bottom).

*F. nivale* blighted a significant amount of leaf tissue at lower inoculum densities (0-150 sclerotia/kg of soil) at 0.5 C, and up to the 300 sclerotia/kg of soil density in Waha silt loam. At all higher inoculum densities, *F. nivale* was suppressed by *T. idahoensis*.

Sclerotium production and disease severity are in-

dependent to a remarkable degree at  $-0.5$  C. The tendency for greater damage to the wheat plant at  $-0.5$  C than at slightly above freezing has been observed before (2), but has not been explained.

The recovery of plants, judged by taking fresh weights of all green tissue after incubation in the greenhouse, was highest for controls and declined to nearly zero in the 2,500 sclerotia/kg of soil inoculum density. The decline in green weights per unit inoculum was greatest between the zero and 300 sclerotia/kg of soil density. Plants incubated at 0.5 C during the disease development period recovered better than those incubated at  $-0.5$  C.

**Vegetative germination of sclerotia without exogenous nutrients.**—To explore the extent to which nutrients in the environment (host exudates?) might stimulate germination, sclerotia were germinated on washed sand and upon ACMDA. Out of 150 sclerotia, 37 germinated on sand at 3 C and 59 of 150 at 10 C by 151 days. On the agar medium, 56 of 60 germinated at 3 C and 41 of 60 at 10 C by 117 days. Germination in these trials averaged 32% on sand and 80% in the presence of nutrients. Sclerotia that received light when the incubators were opened and during observations and those kept in total darkness during incubation germinated equally.

Secondary sclerotia less than 0.2 mm in diam were formed by some of the sclerotia on moist sand after 90 days. Only one secondary sclerotium formed on any given sclerotium. They were viable when transferred to potato-dextrose agar (PDA). Although secondary sclerotia may be of value to some fungi (10, 19), they were too rare for significance in *T. idahoensis*.

After 150 days on sand, 48 sclerotia with no associated hyphae were transferred to PDA to determine their viability. Seven of the 48 sclerotia germinated.

A greenish-black fungus was obtained from 29 of 150 sclerotia on sand at 3 C and from 39 of 150 at 10 C. *T. idahoensis* was seldom obtained from sclerotia infested with this dark fungus. The mycelium of the greenish-black fungus was similar to *Cladosporium herbarum* Link ex Fr. It must have been within the sclerotium as it survived the surface disinfection.

**DISCUSSION.**—*T. idahoensis* primarily attacks leaves, and most of its sclerotia are formed on expanded leaves. In nature, in the absence of tillage, most sclerotia would stay on or near the soil surface, in the best position to attack the next crop of leaves that might be appressed to the soil surface by snow. Cultivation, however, mixed sclerotia with the soil, and the depth of burial experiments demonstrated that the fungus has not effectively adapted to having its sclerotia buried by tillage; when buried 2 cm deep or more, the sclerotia were ineffective. This fungus did not establish successful parasitism of roots or subterranean structures from deeply buried sclerotia. Sclerotia of *T. incarnata*, however, are formed most frequently below the soil surface between basal leaf sheaths and on roots. Also, in contrast, Lehmann (13) found that this fungus could establish parasitism from sclerotia buried 2 cm or deeper. *T. incarnata* is less restricted to aerial tissues than is *T. idahoensis*.

Sandy soils favor *Typhula* blights in Finland (9)

and in Germany (13), and Lehmann attributed this preference to sandy soils to reduced antagonism (12). Because the addition of up to 80% sand to soil by volume did not increase the effectiveness of sclerotia buried 2 cm deep, we agree with Lehmann. The sand altered the soil physically more than biologically, and it failed to alter disease relationships.

Deep burial in aerated soil does not kill the sclerotia, because Huber & McKay (8) reported that burial up to 20 cm for as long as 20 weeks had no effect on survival of *T. idahoensis*. Even though sclerotia of *Sclerotium rolfsii* germinate better on the soil surface than when buried 2 cm (1), Flados (5) attributed reduced growth through wet soil to antagonism rather than to reduced oxygen. Griffin & Nair (7) stated that low oxygen and high carbon dioxide levels reduced the germination of *S. rolfsii* sclerotia, but that this effect was insufficient to account for failure of buried sclerotia to germinate. Total exclusion of light apparently neither stimulated nor restricted vegetative germination of *T. idahoensis* sclerotia, so the reduced effectiveness of buried sclerotia cannot be attributed to lack of light.

An unknown dormancy mechanism probably contributes to the survival of sclerotia of *T. idahoensis*. Temperature, moisture, and oxygen conditions favor germination at least twice each year: during the cooling period in autumn and during the warming period of late winter or early spring. Yet field observations (3) prove that many sclerotia survive at least 3 years. All viable sclerotia do not germinate each time temperature, moisture, and oxygen conditions are favorable. Lehmann (12) reported that dormancy in sclerotia of *T. incarnata* is broken by drying, and he believed this to be the reason that new sclerotia did not germinate in the spring.

*T. idahoensis* sclerotia, considered in terms of Garrett's (6) grouping of sclerotia, resemble more those that act directly as the infective unit rather than those that support production of infectious spores (*Sclerotinia* spp., *Claviceps* spp., etc.). They are small, abundantly produced, and respond somewhat to food during germination.

Sclerotia in these studies were placed in or on soil at the time the wheat was planted, so microflora effects would be expressed only upon germination, hyphal growth, and infection of the wheat, not upon dormant sclerotia within or upon the soil during summers or between crop seasons unfavorable to mold. Possibly, the lack of evidence of a soil influence upon the above processes indicates a relative insensitivity of *T. idahoensis* to antagonism at low temperatures.

Sclerotia were produced when as few as 15 sclerotia were present within the upper 2.5 cm of soil in a 6-inch pot (37 sclerotia/kg of soil), suggesting that small amounts of inoculum are enough to perpetuate and perhaps increase the number of sclerotia in the soil under optimum conditions. Bruehl & Cunfer (2) estimated that 10,800-13,600 sclerotia/m<sup>2</sup> of soil surface are present on large wheat plants severely infected by *T. idahoensis* in the field. This is slightly more than 120 sclerotia in the top 2.5 cm of soil/pot

(300/kg), the inoculum density beyond which disease was not substantially increased at 0.5 C (Fig. 2). Lyda & Burnett (14) reported on optimum inoculum density of 125-625 sclerotia/kg of soil in work with *Phymatotrichum* root rot of cotton, and stated that higher inoculum densities gave less disease when sclerotia were uniformly mixed throughout the soil. However, Leach & Davey (11) found that the percentage of infection of sugar beets was closely correlated with the density of viable sclerotia of *Sclerotium rolfsii* in the soil before planting.

*T. idahoensis* was obtained at 10 C from more sclerotia germinated on sand than from sclerotia on ACMDA; at 3 C, *T. idahoensis* was obtained with greater frequency on ACMDA than on sand. Greater competition from *Cladosporium herbarum* at 10 C on agar medium may explain this difference. Although the dark mycelial fungus was abundant in this lot of sclerotia, observations by the junior author indicate that it is not commonly obtained from sclerotia of this species from most collections.

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