Role of Basidiospores as Propagules and Observations on Sporophores of Typhula idahoensis

Barry M. Cunfer and G. W. Bruehl

Experimental Aide and Professor, Department of Plant Pathology, Washington State University, Pullman 99163.

Portion of a Ph.D. thesis submitted by the senior author to Washington State University.
The support of the Washington State Wheat Commission is gratefully acknowledged.
Scientific Paper No. 3869, Project 796, College of Agriculture, Washington State University, Pullman.
Accepted for publication 27 July 1972.

ABSTRACT

Basidiospores of *Typhula idahoensis* serve as inoculum in inciting snow mold of wheat and barley, but are of lesser importance than sclerotia. Sporulation in the field reaches its peak in mid-November, just prior to the start of the winter snow cover. Moisture and cool temperatures are important factors governing time of sporulation; day-length appears appears to be unimportant. After sporulation is complete, hyphae emerge from overmature sporophores which grow like hyphae from sclerotia. Sporophore length increased, sporophore color became lighter, and fewer basidia matured as depth of a straw layer covering sclerotia increased from 0 to 2.5 cm. The importance of these results to taxonomy of *Typhula* spp. is discussed. Basidiospores survived from 52 to 67 days at -1 to 10 C in a water-saturated atmosphere. Spore germination occurred from -1 to 15 C; 10 C was optimum for germ tube elongation. *Typhula idahoensis* grew as a saprophyte only when competition from other microorganisms was minimal. It is rarely found as a saprophyte on straw in the field.

Phytopathology 63:115-120

Additional key words: Triticum aestivum, Hordeum vulgare.

Sclerotia are the major inoculum of *Typhula idahoensis* Remsberg and *T. incarnata*, both of which cause snow molds of winter cereals (2, 3). The role of the basidial stage is less well understood. Sprague (12, 13) and Sprague & Rainey (14) conducted limited basidiospore inoculation experiments with *T. idahoensis* in the field. Infection by basidiospores was not proved, but Sprague suggested that basidiospores caused infection but were of lesser importance than mycelium from sclerotia. Some other reports (6, 16) that basidiospores of *Typhula* species are infective agents are likewise unsubstantiated. Basidiospores of *T. incarnata* are functional but of minor importance in causing infection of wheat and barley (9, 15).

Basidiospore production by *T. idahoensis* occurs from late October until December during rainy or cloudy weather in northcentral Washington (12, 14). A similar pattern of sporulation by *Typhula* species pathogenic on winter cereals occurs in Japan (15), Scandinavia (16), and East Germany (9).

Since sporulation normally occurs just prior to snowfall, experiments were conducted to determine whether the basidial stage of *Typhula idahoensis* is able to incite snow mold of winter wheat and barley and to investigate the time of basidiospore production in the field, the effect of temperature on basidiospore germination and viability, and the ability of basidiospores and sclerotia to initiate saprophytic growth on wheat straw. A preliminary report of a portion of this study has been published (4).

MATERIALS AND METHODS.—Sclerotia-laden wheat leaves from plants inoculated with sclerotia of a composite of several *T. idahoensis* isolates were dried and stored at 25 C. In the fall, these sclerotia were placed outdoors. Basidiospores from sporophores produced from these sclerotia were used in all experiments.

Susceptible winter wheat, *Triticum aestivum* L. 'Burt' and 'Wanser', and winter barley, *Hordeum vulgare* L. 'White Winter', were used in inoculation experiments. Three plants/6-inch clay pot were grown in nonsterile Ritzville silt loam, a soil free of *Typhula idahoensis*. The hosts were seeded in early September and the plants were hardened outdoors.

Sporophore production.—Sclerotia were placed outdoors on soil in clay pots at ca. 10-day intervals beginning 8 September until 25 November at Pullman, Wash., in each of 2 years. Sporophore development was checked at 3- to 5-day intervals as long as weather conditions permitted.

Sporophore characters are important in species identification (11, 15), and light influences their size and color (8, 11). In the field, sporophores produced in open areas are smaller than those produced where the sclerotia are partly or entirely covered with plant debris or soil. Sporophores of varying dimensions resulted when induced partly or exclusively with ultraviolet light (8, 11). To determine the effect of depth of burial under straw and ultraviolet light on sporophore production, two experiments were conducted. Sclerotia placed on the soil surface in clay pots were covered with wheat straw to depths of 0, 0.5, 1, 1.5, and 2.5 cm. The dimensions of 50 mature sporophores/treatment were measured using a × 20 stereoscopic microscope. In order to compare ultraviolet-induced sporophores with those produced outdoors, *T. idahoensis* sclerotia were placed on moist sand in petri dishes and kept at 5 C. Five hr of ultraviolet light/day were supplied by an RS 275 sunlamp (General Electric Co.). The sclerotia were located diagonally from the light source at a distance of 130 to 150 cm.

Inoculation of wheat and barley with basidiospores.—In early December, plants were brought into a greenhouse. Some of the leaves were wounded with a scissors, and the plants were atomized with distilled water. Mature sporophores were severed from sclerotia, attached with adhesive tape to glass petri dish lids, and suspended several cm above the plants. In each instance, the number of sporophores was recorded. If any sporophore could not be accounted for at the end of the inoculation period, the pot was discarded. In this manner, any possibility of inoculum other than basidiospores, i.e., either sclerotia or the dikaryotic mycelium of the sporophores, was excluded. After placement of the sporophores over the plants, each pot was enclosed within a polyethylene bag secured with a rubber band. After storage at 5 C in the dark or 10 C in a greenhouse with natural light for 3 to 21 days, the bags and sporophores were removed from the pots. The plants were covered with moist cotton and incubated at 1 C for 90 to 140 days in snow mold chambers.

Basidiospore deposition, germination, and viability.—Thirty mature sporophores produced outdoors were placed on glass slides in petri dish moist chambers. After 48 hr at -1, 1, 5, 10, and 15 C, spore deposition was estimated at × 100 magnification.

We tested spore germination by streaking mature sporophores across 2% water agar on glass slides to release basidiospores. The slides were placed singly in petri dishes lined with moist filter paper and held at -1, 1, 5, 10, 15, and 20 C for 24, 56, and 72 hr in the dark. At -1 C, additional series were run for 96 and 144 hr, but the 24-hr sample was omitted. After incubation, the basidiospores were stained with cotton blue in lactophenol. Percent germination of at least 20 spores within several × 300 microscope fields/slide was counted. Germ tube lengths were measured with an ocular micrometer. Each treatment was replicated 12 times.

We also tested basidiospores for viability by placing mature sporophores on 18-mm^2 cover slips on hardware cloth squares in petri dishes lined with moist filter paper. After 24 hr at 10 C, the sporophores were removed, leaving the ejected spores firmly adhered to the cover slips. Dishes containing cover slips with basidiospores were moved to 5, 10, and 15 C. Some dishes for storage at 1 or -1 C were moved progressively from 5 C to 1 C to -1 C over a period of 24 hr to minimize shock due to rapid temperature changes.

At 4-day intervals, five cover slips/temperature were placed on potato-dextrose agar (PDA) so that the basidiospores were in direct contact with the agar. The plates were incubated at the temperatures at

TABLE 1. Average time of sporophore production by sclerotia of *Typhula idahoensis* placed outdoors at intervals during the autumns of 1970 and 1971

Date sclerotia placed outdoors	Number of days until		
	50% with stipes	25% of sporophores mature	50% of sporophores mature
8 Sept.	53 (31 Oct.)	63 (10 Nov.)	65 (12 Nov.)
15 Sept.	56 (10 Nov.)	59 (13 Nov.)	61 (15 Nov.)
25 Sept.	43 (7 Nov.)	48 (12 Nov.)	52 (16 Nov.)
5 Oct.	33 (7 Nov.)	38 (12 Nov.)	42 (16 Nov.)
15 Oct.	31 (15 Nov.)	35 (19 Nov.)	
25 Oct.	29 (23 Nov.)	36 (30 Nov.)	
5 Nov.	24 (29 Nov.)		
15 Nov.	10% of sclerotia with stipes by 30 Nov.		
25 Nov.	No stipes formed by 30 Nov.		

TABLE 2. Average dimensions of *Typhula idahoensis* sporophores produced outdoors from sclerotia under varying depths of straw and in the laboratory with 5 hr per day ultraviolet light at 5 C

Treatment	Stipe length (mm)	Head length (mm)	Total length (mm)	Head width (mm)	Stipe length/ head length
Outdoors					
0 cm (no straw)	2.5	2.9	5.4	0.4	0.8
0.5 cm straw	2.9	3.0	5.9	0.3	0.9
1.0 cm straw	3.8	3.4	7.2	0.3	1.1
1.5 cm straw	5.1	3.3	8.4	0.4	1.5
2.5 cm straw (with heads)[a]	6.0	3.9	9.9	0.3	1.5
2.5 cm straw (without heads)[b]	3.0		3.0		
Laboratory, 5 C					
Soil surface, ultraviolet light[b]	4.0	2.6	6.6	0.25	1.5

[a] 20 sporophores.
[b] 30 sporophores; all other treatments, except 2.5 cm straw (with heads), 50 sporophores.

which the basidiospores were stored with one exception. Basidiospores stored at -1 C were incubated at 1 C to allow more rapid growth. *T. idahoensis* was identified by the presence of clamp connections in the hyphae and production of typical sclerotia.

Saprophytic colonization of straw by sclerotia and basidiospores.—Clean, bright straw from healthy wheat collected shortly after harvest was cut into 3-cm lengths. A portion of clean straw was sterilized by placement with 0.5 ml of propylene oxide inside a tightly sealed 250-ml flask. Straw that had been in the field several months after harvest and was thoroughly infested with saprophytes also was used. These are hereafter referred to as "clean nonsterile", "clean sterile", and "infested straw". Samples of each straw type were placed in sterile flasks and a saturated atmosphere was maintained with sterile distilled water. The straws were inoculated with sclerotia or basidiospores. Either 10 fresh sclerotia were placed directly on the straws in each flask, or basidiospores from six to eight fertile sporophores were allowed to eject for 48 hr at 10 C onto the straw. The straws were incubated at -1, 1, 5, 10, and 15 C for 100 to 120 days and examined for sclerotia development. The 15-C treatment was omitted in the experiment using sclerotia. There were six replications/treatment.

RESULTS.—*Sporophore production*—In both years (Table 1), the first sporophores matured about 1 November. Sporophore production increased rapidly thereafter, reaching a peak from 12 to 30 November regardless of the time when the sclerotia were placed outside from 8 September to 25 October. A minimum of 35 days was required for sclerotia to produce mature sporophores. The most rapid fruiting occurred when sclerotia were put outdoors during October and optimum conditions persisted for several weeks. Once stipes began to form, sporophores matured within 1 week.

Snow and cold weather in early December virtually terminated sporophore formation and basidiospore production, but intermittent sporulation

occurred during periods of above-freezing temperatures. For example, dry sclerotia placed outdoors in mid-January sporulated during late February 1971.

In autumn 1971, sclerotia were put outside and kept hydrated by daily watering during September and October. Starting about 1 November, there was frequent rainfall and the sclerotia remained hydrated without watering. The watered sclerotia matured 4 to 8 days before those dependent on natural precipitation.

Sclerotia not covered with straw produced short, fawn- to light-brown sporophores, but as the depth of straw increased, the sporophores were longer and more etiolated (Table 2). Under 0.5 cm of straw, sporophore color remained normal but both stipe and head length increased. Most sporophores grew through openings between straws so that they were eventually exposed to full sunlight. At the 1-cm depth, sporophores were more elongate, and many which were completely covered by straw were white to cream-colored. Those which formed within an open space were nearly normal in color. All sporophores under 1.5 cm of straw were white, and the apices of the heads of many were tapered, brownish in color, and without mature basidia. Portions of all sporophores at 0 to 1.5 cm depth were fertile. At 2.5 cm, however, 30 of 50 sporophores produced long, thin stipes without heads. The stipes were rigid and dark brown from the base to near the tip, which was soft, tapered to a few strands of hyphae, and nearly white. The 30 sporophores which did mature were nearly twice the length of sporophores produced from sclerotia exposed to direct sunlight at the soil surface.

Although both stipe and head length increased with depth of cover, stipes elongated more than heads (Table 2). Sclerotia without straw cover and those from the 0.5-cm straw treatment had a length of stipe to length of head ratio of 0.8 and 0.9, respectively, whereas sclerotia buried under 1.5 and 2.5 cm straw had a stipe:head ratio of 1.5. Head width did not vary greatly, averaging 0.3 to 0.4 mm in all treatments. Sporophores induced by ultraviolet light but without straw cover had pure white heads and thin stipes that were dark brown at the base, fading to tan to nearly white at the base of the heads. The stipe- to head-length ratio was similar to that under 1.5 and 2.5 cm straw. Their over-all dimensions were somewhat less however.

Inoculation of hosts with basidiospores.—Sclerotia developed on wheat in 7/50 pots and on barley in 7/32 pots. There was no correlation between mold development and length of incubation at 5 and 10 C prior to incubation at 1 C. No sclerotia were observed, and plants remained healthy in 19 pots of wheat and 12 pots of barley used as noninoculated controls. Sclerotia were observed on barley in one pot after 89 days, but not on any wheat or other barley until 110 to 120 days after inoculation. Isolations were made from each pot with diseased plants. After confirming each isolate to be dikaryotic by observing clamp connections in the hyphae, we increased the isolates on sterilized wheat kernel medium (150 g wheat: 150 ml water). Sclerotia of these isolates were placed outdoors in September 1971. In each case, typical *T. idahoensis* sporophores developed. Another portion of sclerotia of each isolate was inoculated onto wheat plants and placed in a snow mold chamber. Typical snow mold symptoms and sclerotia resulted.

Basidiospore deposition, germination, and viability.—At 10 and 15 C, sporophores deposited a dense spore print. Spore deposition was somewhat less dense at 5 and 1 C, and at -1 C it was about 30% of that at 10 C. These results reflect the rate at which basidiospores mature and are ejected at each temperature, and they show that sporulation continued over a wide temperature range.

Basidiospores germinated over the entire range of temperature, from -1 to 20 C (Table 3). After 72 hr, 32 to 69% of the spores germinated over the range 1 to 15 C with a minimum germ tube length of 9 μ. Ten and 15 C gave the highest percent germination after 72 hr, but 10 C favored germ tube elongation. Even at -1 C, 89% of the spores had germinated by 144 hr with an average germ tube length of 27 μ. At 20 C, germination was much slower than at 15 C, and germ tubes were often thin and distorted. Basidiospores germinated from one or both ends of the spores. Septa formed in the germ tubes when they were only a few microns long, but no branching was seen during the time periods employed.

During the viability experiment, nearly all of the basidiospores germinated to produce short germ tubes upon the cover slips so that the surviving fungal material consisted of spores with germ tubes rather than ungerminated basidiospores. *Typhula idahoensis* colonies, identified by typical sclerotia and clamp connections, grew from basidiospore samples after storage up to 52 to 67 days at -1 to 10 C. At 15 C, viability lasted only 39 days. Because of the technique used, no estimate of the percent survival could be made.

Colonization of straw by basidiospores and sclerotia.—New sclerotia appeared on clean sterile and clean nonsterile straw 40 to 100 days after inoculation with basidiospores or sclerotia at -1 to 15 C. Sclerotia development was most rapid at 5 to 15 C. At 1 and -1 C, new sclerotia did not develop until 70 to 100 days after inoculation. The fungus was not recovered from infested straw inoculated with basidiospores or noninoculated straw at any temperature. When sclerotia were used as inoculum, only at 5 C did new sclerotia develop on infested straw. On clean nonsterile straw, the areas occupied by *T. idahoensis* remained bright, but portions not occupied were darkened by dematiacious fungi. It appeared as though where *T. idahoensis* became established, other fungi did not grow.

DISCUSSION.—The low percent infection (17% of inoculated plants) from basidiospores may be due to the low level of inoculum used, or it may reflect an inherent low inoculum potential of basidiospores. Even though less effective than sclerotia, the ability of basidiospores to serve as inoculum adds meaning to the other observations. In northcentral Washington,

the fall is cool with frequent light rains, particularly in November. Persistent snow cover usually begins in early December. Occasionally, however, snow cover may begin as early as 20 November. Basidiospore production reaches a peak in mid-November, just a few days or weeks before lasting snow cover. Moisture and cool temperatures are important in timing sporulation. Since sporophores mature within 35 days during optimal weather conditions, dry weather terminating in mid-October will still permit basidiospore inoculum to be present in the field prior to snowfall.

Ultraviolet light is essential for sporulation (11), and the effect of day-length might also be a factor in sporulation. Occasional sporulation of *Typhula* species in late winter was also observed by Remsberg (11). In a cool, damp climate, *T. gyrans* sporulates from May to October (10). *Typhula trifolii* sporulates in Scandinavia (16) in summer when moisture is plentiful and the temperature is below 20 C. The rapid response of sporophores to moisture and cool temperatures in the present study and sporulation of other species throughout the year indicates that day-length is unimportant.

The position of debris in relation to the sclerotium and light conditions affects the size and coloration of the positively phototrophic sporophores. Under deep cover, many sporophores are etiolated and many remain sterile. The light intensity required for sporophore development is very low. During much of the fall, heavy cloud cover filters out much ultraviolet light, and sclerotia buried under several cm of debris receive very little light. Taxonomists should be aware of the influence of light on sporophore size and color upon the identification of *Typhula* species.

The production of sporophores requires expenditure of much of the stored food within the sclerotium. If basidiospores are less effective than hyphae growing from sclerotia, as appears to be the case, then it would seem that much energy is wasted by the fungus. However, after a sporophore completes sporulation or if it does not mature, vegetative hyphae grow from all portions of the sporophore, and it is surrounded by a mass of aerial hyphae which could infect host tissue if contact is made. In this way, *T. idahoensis* sclerotia can make full use of their stored energy, producing spores to attack a substrate at a distance and dikaryotic hyphae to attack a proximal substrate. This observation is in agreement with Garrett's statement that "All organisms . . . can be seen to employ economy in the deployment of their reproductive resources" (7, p. 208).

Basidiospores and germ tubes survive at least 7 weeks between -1 and 10 C in a moist environment. It is noteworthy that basidiospores survive and germinate well at -1 and 1 C. The temperature at the soil surface, when mold is severe, is maintained at 0.5 C throughout winter under deep snow. Previous studies using sclerotia as inoculum showed that *T. idahoensis* can kill wheat at -1.5 C (2).

Sclerotia are rarely found on dead grasses or on straw of the previous year's crop in fields with severe snow mold (3). *Typhula idahoensis*, either as basidiospores or sclerotia, competes very poorly as a saprophyte on weathered wheat straw, which is the predominant plant residue after autumn rains have begun.

TABLE 3. Percent germination of *Typhula idahoensis* basidiospores and average germ tube length on 2% water agar at -1 to 20 C. Each treatment consisted of 12 replications with 20 to 25 spores observed/replication

	Incubation period, hr				
Temp (C)	24	56	72	96	144
		Germination, %			
-1		1.5	13	55	89
1		32	32		
5	24	43	46		
10	43	46	69		
15	46	37	68.5		
20	8	24	22		
		Average germ tube length, μ			
-1		1.4	2.7	5.3	27.2
1		7.3	12.7		
5	4.6	23.8	14.7		
10	6.1	28.6	34.0		
15	7.8	21.6	9.0		
20	5.6	4.6	5.3		

The time of host penetration and infection is still uncertain. Basidiospores germinate well over the range -1 to 15 C, and survive nearly 2 months at temperatures common in the field during winter. Infection could occur at any time during this period, either prior to or after snowfall. Tomiyama (15) determined that infection by *T. incarnata* does not begin until wheat leaves are weakened by exhaustion of food reserves. Lehmann (9) reported similar results with this species on winter barley. If a proper infection court is not established until leaves begin to deteriorate, basidiospores must survive to initiate infection.

The basidial stage of *T. idahoensis* is well-adapted to function during the fall and winter. Infection of wheat and barley by basidiospores has been demonstrated. Vegetative hyphae from sclerotia provide the major source of inoculum, but basidiospores also contribute to it. Races of the fungus have not been found (1, 9), but sexual recombination from a functioning basidial stage provides added potential for variation in this pathogen (4, 5).

LITERATURE CITED

1. BRUEHL, G. W. 1967. Lack of significant pathogenic specialization within Fusarium nivale, Typhula idahoensis, and T. incarnata and correlation of resistance in winter wheat to these fungi. Plant Dis. Reptr. 51:810-814.
2. BRUEHL, G. W., & B. CUNFER. 1971. Physiologic and

environmental factors that affect the severity of snow mold of wheat. Phytopathology 61:792-799.
3. BRUEHL, G. W., R. SPRAGUE, W. R. FISCHER, M. NAGAMITSU, W. L. NELSON, & O. A. VOGEL. 1966. Snow molds of winter wheat in Washington. Wash. Agr. Exp. Sta. Bull. 677. 21 p.
4. CUNFER, B. M. 1971. The role of the basidial stage in the life cycle of Typhula idahoensis. Phytopathology 61:889 (Abstr.).
5. CUNFER, B. M. 1972. Sexual incompatibility and aspects of the mono- and dikaryotic phases of Typhula idahoensis. Phytopathology :(in press).
6. EKSTRAND, H. 1955. Overwintering of winter cereals and forage grasses. Summary of the results and program for continued investigations [in Swedish, English summary]. Meddelande Växtskyddanst, Stockholm 67:1-125.
7. GARRETT, S. D. 1970. Pathogenic root-infecting fungi. Cambridge Univ. Press, Cambridge. 294 p.
8. LEHMANN, H. 1965. Untersuchungen über die Typhula-Fäule des Getreides. I. Zur Physiologie von Typhula incarnata Lasch ex Fr. Phytopathol. Z. 53:255-288.
9. LEHMANN, H. 1965. Untersuchungen über die Typhula-Fäule des Getreides. II. Zur Pathologie durch Typhula incarnata Lasch ex Fr. erkrankter Wirtspflanzen. Phytopathol. Z. 54:209-239.
10. MAC DONALD, J. A. 1934. The life history and cultural characteristics of Typhula gyrans (Batsch) Fries. Ann. Appl. Biol. 21:590-613.
11. REMSBERG, RUTH E. 1940. Studies in the genus Typhula. Mycologia 32:52-96.
12. SPRAGUE, R. 1952. Contribution to the life history and control of snow molds of winter wheat in Washington. Phytopathology 42:475 (Abstr.).
13. SPRAGUE, R. 1961. Epidemiology and control of snow mold of winter wheat in the Pacific Northwest of the United States. Recent Advances Bot. Sect. 5:540-541. Univ. of Toronto Press, Toronto.
14. SPRAGUE, R., & C. J. RAINEY. 1950. Studies on the life history of Typhula spp. on winter wheat in Washington. Phytopathology 40:969 (Abstr.).
15. TOMIYAMA, K. 1955. Studies on the snow blight disease of winter cereals [in Japanese, English summary]. Report 47, Hokkaido Nat. Agr. Exp. Sta. 234 p.
16. YLIMÄKI, A. 1969. Typhula blights of clovers. Ann. Agr. Fenniae 8:30-37.