Cultural Variation Within *Typhula idahoensis* and *T. ishikariensis* and the Species Concept

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ABSTRACT

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By means of isolate × known monokaryon (dimon) pairings, 279 isolates from Washington, Idaho, Montana, Wyoming, and Utah were identified as *Tyhula idahoensis*, 123 were identified as *T. ishikariensis*, and 21 were unidentifiable. Nuclei of the latter group migrated into tester monokaryons of either species to form clamped hyphae. Both species were highly variable in culture. In general, *T. ishikariensis* grew faster, produced more pigment in the medium, and the rind cells of the sclerotia were more regular than those of *T. idahoensis*. No easy way was found to distinguish single isolates of these species in culture and they intergraded in all characters observed. A large collection of isolates provided a continuum of cultural types. We recognized no meaningful subgroups within either species. A large collection of isolates provided a continuum of cultural types.

Additional key words: snow molds, interspecies hybrids; taxonomy, splitting vs lumping.

In previous papers (2,3,6) we presented reasons for recognizing *Typhula idahoensis* Remsberg (15) and *T. ishikariensis* Imai (11) as species. Årsvoll and Smith (1) repeated some of our work and reduced *T. idahoensis* to a variety of *T. ishikariensis,* and established *T. ishikariensis* var. *canadensis* to accommodate a variant common in much of Canada. In order to assess the validity of these changes, these species were restudied.

Because of the difficulty of distinguishing them with certainty, especially in culture, we rely upon a dimon (dikaryon × monokaryon) mating system (3) as a major diagnostic tool. In this system a known monokaryon must permit nuclei from the unknown dikaryon to migrate through it. We assume this degree of general compatibility to be significant. Over 400 cultures were identified by dimon matings and their cultural characteristics were observed.

Cunfer (*unpublished*) produced vigorous interspecies dikaryons with monokaryons of a highly infertile *T. ishikariensis* isolate (Wa 69-7-3) and those of typical *T. idahoensis*. Christen presented genetic evidence that Wa 72-8, identified as *T. ishikariensis*, might be a natural interspecies hybrid (7). Cunfer (*unpublished*) and Christen (6,7) both proved that interspecies hybridization is possible, that some hybrids are vigorous and pathogenic, and that they could exist in nature in the sclerotial state whether or not they were fertile.

This paper describes the geographic distribution of the two species, and their growth and cultural characteristics.

MATERIALS AND METHODS

Sclerotia from the bottom of collection envelopes were surface-disinfested in a 1:1 mixture of 95% ethanol and a 6% sodium hypochlorite (Purex) solution for 30–60 sec and placed on Difco corn meal agar (CMA). We purposely used loose sclerotia in the envelopes because sclerotia of *T. ishikariensis* are usually superficial and tend to fall from host leaves (Fig. 1). Sclerotia were incubated at 10 C for 2 days on CMA to facilitate hydration, then were incubated at 1 C for an indeterminate time until growth was adequate for transfer. Over 700 new isolates were obtained. About 400 of these, plus isolates and monkaryons from previous studies, were used to study variation (Table 1).

All data are from isolates identified by dimon pairings performed as in previous studies (1–3). If nuclei of the donor dikaryon migrated 1 cm into the receptor monokaryon, the dikaryon was said to belong to the species of the monkaryon. Migration was considered positive if a mycelial plug of the monokaryon obtained 1 cm from the monokaryon-dikaryon union developed clamped hyphae upon transfer.

The new isolations were made in March–April 1978. Most of them were maintained on CMA at 5 C until November when the hyphae essentially had disappeared and the sclerotia were conspicuous. The sclerotia varied from very light to black. They were classified on the basis of intensity of sclerotial color and transferred to Difco potato dextrose agar (PDA) for rejuvenation at 10 C. Mycelial plugs 2×2 mm were removed near the leading edge of young mycelia and transferred to fresh PDA for growth rate determinations and observation of color development, with three replicates per isolate at 10 C.

RESULTS

Species distribution. The results of the present study, the most extensive use of identification by means of the dimon system attempted by us, are combined with results of an unpublished trial in Table 1. We discovered that *T. ishikariensis* was more widespread than suspected, and concluded that an adequate search

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would reveal *T. ishikariensis* in all areas of Washington and Idaho where *T. idahoensis* predominates. However, the totals in Table 1 do not reflect the true relative numbers of the two species. Selecting sclerotia free of host tissue favored isolation of *T. ishikariensis*.

In the two large dimon matings of the present study, nuclei of 279 isolates migrated only in tester monkaryons of *T. idahoensis*, nuclei of 132 isolates migrated only in tester monokaryons of *T. ishikariensis* and nuclei of 21 migrated in both. No migration was recorded from 57 isolates. In some pairings, antagonism prevented hyphae of mycelia from meeting a possible mate. Some of the non-mating isolates were not *Typhula* spp. (lacking clamped hyphae), a few were *Typhula* spp. other than *T. idahoensis* or *T. ishikariensis*, and three were synthesized *T. ishikariensis* × *T. idahoensis* dikaryons.

Cultural characteristics. *Growth rates.* Colony diameter measurements of 31 "hybrids" (= 10 synthesized hybrids + 21 unidentifiable isolates), 96 *T. ishikariensis*, and 277 *T. idahoensis* isolates after 16 days at 10 C on PDA are presented in Fig. 2A. *T. idahoensis* generally grew more slowly than *T. ishikariensis*, but growth rate did not distinguish these species because they overlapped. The "hybrids" were intermediate.

Pigmentation. Most isolates of *T. idahoensis* produced little pigment after 26 days on PDA at 10 C (Fig. 2B). Most isolates of *T. ishikariensis* produced brown to reddish brown pigments, but 27 isolates did not. Pigment production alone did not distinguish individuals of these species.

Zonation. Many cultures of both species produced sclerotia in concentric rings, but several isolates of both species produced sclerotia in no distinct pattern (Fig. 2C). Zonation did not distinguish the species. The sclerotia of several isolates of *T. idahoensis* were aggregated into a central mound (Fig. 3) at the point of inoculation. Such extreme mounding of sclerotia was not seen in any isolate of *T. ishikariensis*.

Sclerotial characteristics. *Sclerotial color.* The sclerotial color of isolates on CMA incubated 6 mo at 5 C varied from very light to black or near black. Only 11 of 258 cultures identified by the dimon mating as *T. idahoensis* formed black sclerotia, but 64 of 106 isolates of *T. ishikariensis* produced black sclerotia under these conditions (Fig. 2D). This cultural character gave the sharpest delineation between individuals of the two species.

Sclerotial rind cell pattern. Rind fragments were excised from moist sclerotia with razor blades, mounted in lactophenol, and the rind cell patterns examined with light microscopy. Of 34 *T. ishikariensis* isolates sampled, 22 had rather regular "parenchyma"-like patterns, 10 were semi-parenchyma-like, and two were highly irregular. Of *T. idahoensis*, 16 were highly irregular and four were semi-regular.

TABLE 1. The geographic origins of isolates of *Typhula idahoensis* = (*id.*) and of *T. ishikariensis* = (*ish*) identified by means of dimon matings

Origin	*Typhula* spp. *id.*	*ish.*	X ?[b]	Origin	*Typhula* spp. *id.*	*ish.*	X ?[b]
Washington counties				Idaho counties (continued)			
Douglas	94	10	6	Fremont	5	0	0
Grant	0	2	0	Idaho*	0	1	0
Kittatas*[a]	0	8	0	Lemhi*	12	1	0
Lincoln	4	4	5	Madison	6	1	0
Okanogan*	27	42	7	Oneida	22	0	0
Spokane	0	4	0	Power	3	0	0
Stevens	1	9	1	Teton	14	2	1
Idaho counties				Valley*	0	6	0
Bear Lake	6	0	0	Montana	8	1	0
Blaine*	5	10	0	Wyoming*	7	15	0
Bonneville	2	0	0	Utah	3	0	0
Butte*	3	0	0	Finland	0	2	0
Camas	9	1	1	Japan	0	6	0
Caribou	6	1	0	Norway	0	1	0
Cassia	12	0	0	Totals	279	132	21
Elmore	3	0	0				
Franklin	27	5	0				

[a] Asterisks mark collection sites containing plants in addition to winter wheat, usually clovers or lawns.
[b] X ? = Unidentifiable (nuclei migrated into testers of either species).

DISCUSSION

In 1975 Bruehl and Cunfer (2) wrote: "Since 1969 *Typhula* spp. pathogenic to winter wheat have been tentatively assigned to three species: *T. incarnata* with reddish-brown sclerotia and pink basidiocarps, *T. idahoensis* with black sclerotia and tan-brownish basidiocarps, and *T. ishikariensis* with black sclerotia and powdery white to gray basidiocarps. Observations of sclerotial rind patterns and basidiocarps led us to accept the validity of these species, but similarities between *T. idahoensis* and *T. ishikariensis,* which some authors place in synonymy (12,14), were so great we withheld publication of this paper on their morphology, physiology, and ecology until mating experiments established the degree of

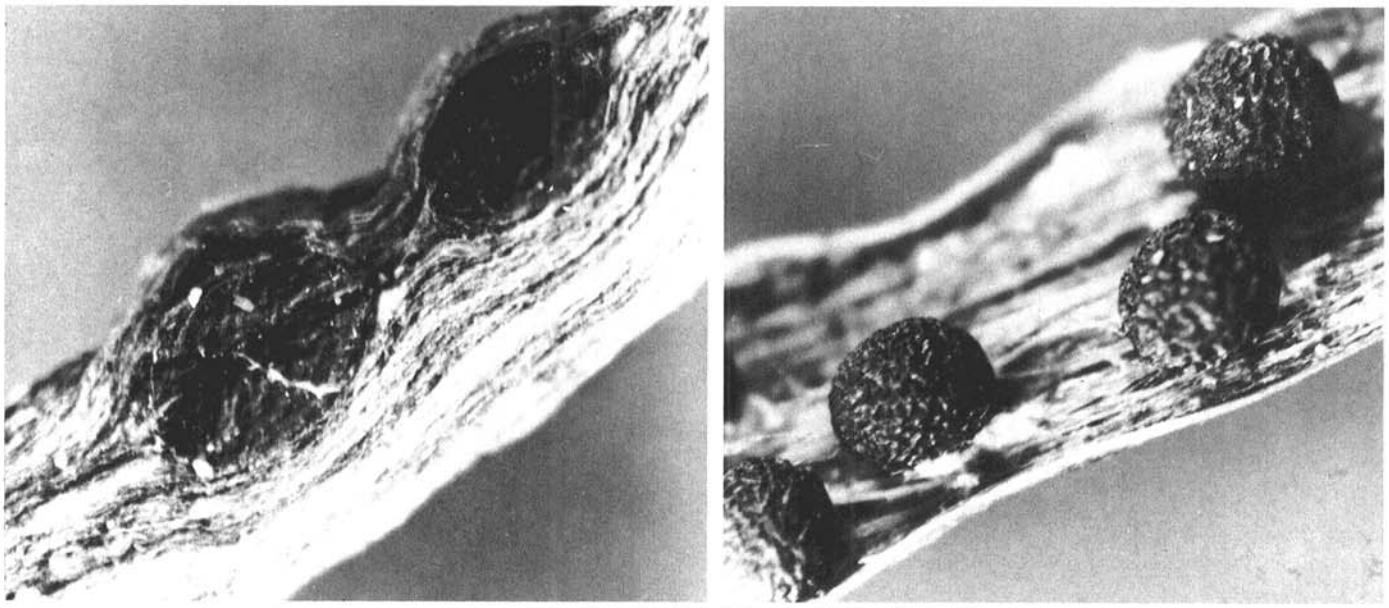

Fig. 1. Typical sclerotia of *Typhula idahoensis* (left) and of *T. ishikariensis* (right) formed on wheat leaves (×33). Sclerotia of *T. idahoensis* are frequently subepidermal and subspherical. Sclerotia of *T. ishikariensis* usually are superficial, easily dislodged when dry, and spherical.

compatibility among them. ...Because of the high degree of genetic separation...we accepted the above-mentioned species." Årsvoll and Smith (1) rejected the recognition of *T. idahoensis* and *T. ishikariensis* as separate species.

All our breeding trials to date indicate that *T. idahoensis* and *T. ishikariensis* are sufficiently close to hybridize, even though the hybrids are predominantly sexually incompetent (6,7). In spite of this closeness, dimon matings distinguish two large groups, one compatible with tester monokaryons of *T. idahoensis* and one compatible with tester monokaryons of *T. ishikariensis*. Dimon

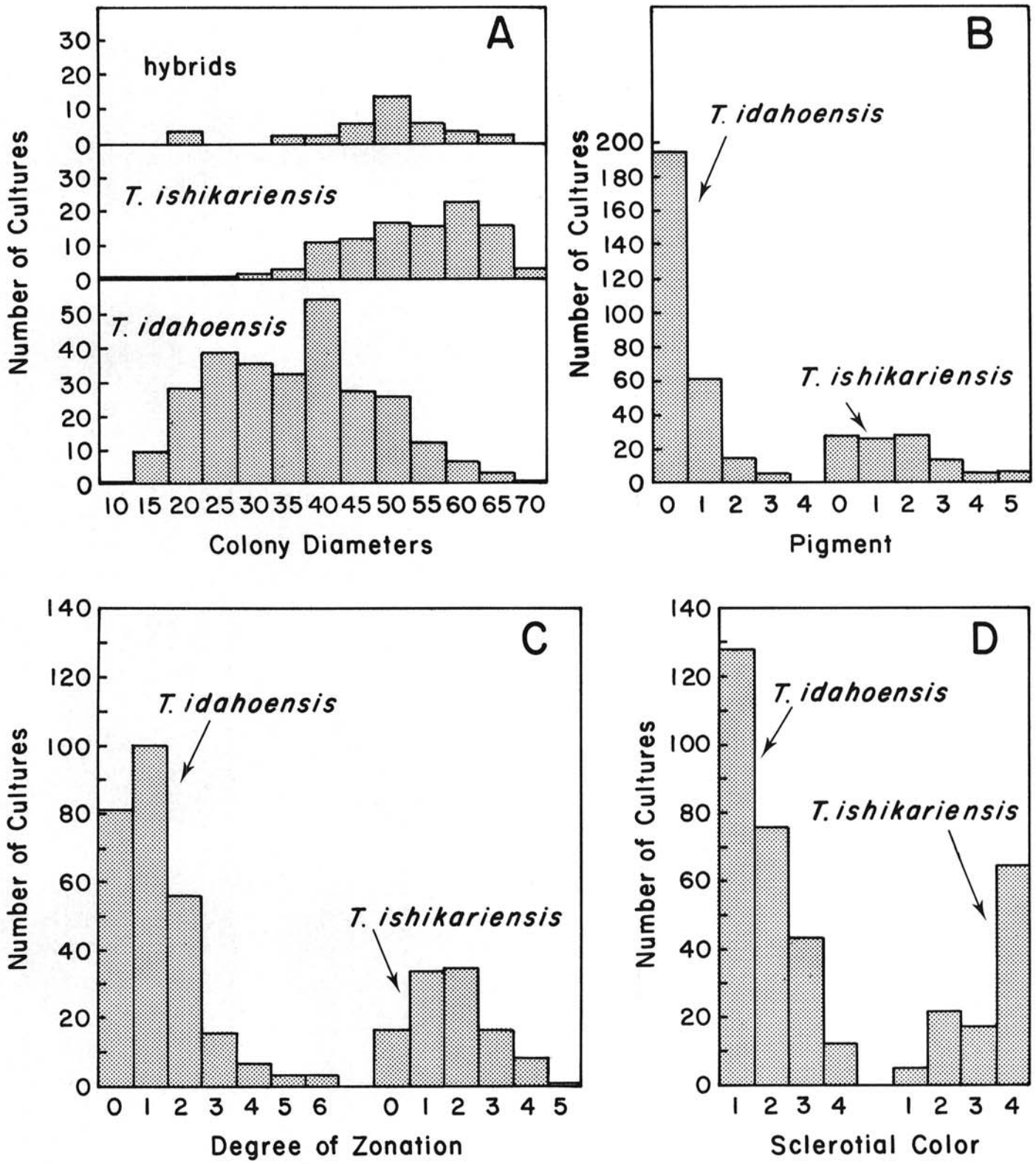

Fig. 2. *Typhula idahoensis* and *T. ishikariensis* compared for: **A,** radial growth (millimeters) on Difco potato dextrose agar at 10 C after 16 days in the dark; **B,** dark pigmentation of the same medium after 26 days (0 = little or no pigment, 5 = very dark reddish brown pigment in almost all of the medium); **C,** degree to which sclerotia are formed in rings (zones) on the agar surface (0 = clustered, scattered, no zonation, and 6 = six rings of sclerotia in a 90-mm-diameter dish); **D,** depth of color of sclerotia formed on Difco corn meal agar at 5 C after 6 mo (1 = light, 4 = very dark, black). The "hybrid" category (**A, top**) consists of 10 hybrid dikaryons synthesized in the laboratory plus field isolates that acted as nuclear donors to testers of both species. The latter group could contain natural hybrids and isolates of either species capable of forming hybrids. They are unidentifiable by the dimon method.

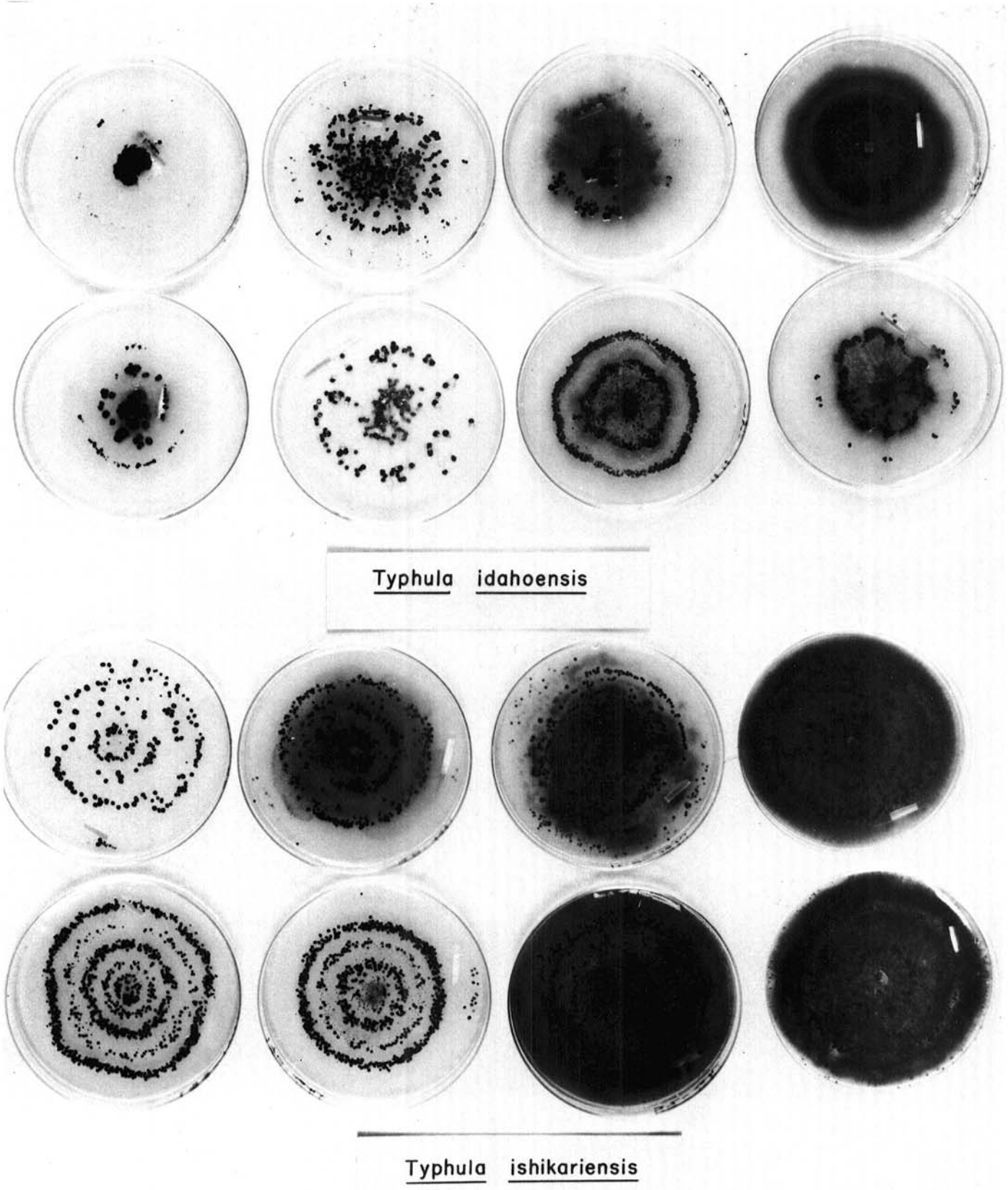

Fig. 3. Cultural variations within *Typhula idahoensis* (top) and *T. ishikariensis* (bottom) on Difco potato dextrose agar to 10 C. The central mound of sclerotia (top left) was observed only in *T. idahoensis*.

matings also give evidence of a smaller group of isolates compatible with testers of both species. This group could contain isolates that hybridize with the other species and it could contain actual hybrids. They are unidentifiable at the species level by the dimon method. Synthesized hybrids can be vegetatively vigorous, virulent, and they can readily form sclerotia (6).

Our studies, except for water relations (2), indicate that *T. idahoensis* and *T. ishikariensis* can be treated as distinct populations. They differ in growth rate on PDA, pigment production on PDA, color of sclerotia on old CMA dishes, distribution in nature, virulence (2,7), size of sclerotia in nature (2), the way sclerotia are produced in nature (2), sclerotial rind patterns, and the sporophores differ in size and color (2). Even so, as individual isolates it may not be possible to positively identify them by any single morphologic or physiologic characteristic. Some cultures of *T. ishikariensis* produce no pigment in culture; some cultures of *T. idahoensis* produce considerable pigment. A few cultures of *T. ishikariensis* produce sclerotia with highly irregular rind patterns. The dimon mating system is the surest way to identify these species.

According to Mayr (13, p. 8), Plate stated in 1914 that the ability of members of a species to recognize each other was important in taxonomy.

We believe that cultures of the same sexual species should be relatively fertile with each other and that ease of taxonomic identification is of secondary importance. Dobzhansky (9) commented that borderline situations are, on the whole, rare, that they may annoy classifiers, but that they are precious to evolutionists. He also warned that two morphologically indistinguishable species may well represent complete speciation. Even though isolate Wa 70-29 of *T. ishikariensis* was collected in an area dominated by *T. idahoensis*, it appears to be pure. Coexistence of two species in the same habitat is evidence of complete speciation.

We accepted the "biological" concept of species, that a sexual species consists of individuals that can share genetic materials with relative ease and regularity. Although much of *T. idahoensis* may be relatively asexual (4), we consider it (8) and *T. ishikariensis* to be sexual species. They can hybridize (6), but the two groups of organisms do not readily exchange genes.

Why labor so hard on so small a point as whether or not there are two species or one species with varieties? Holton, et al (10), asked, "which is the greater sin, naming too many species or naming too few?" In our opinion it is better to err by having too many than too few. Lumping can lead to loss of precision and of knowledge. Splitting can be subsequently proven unjustified, but it does not lead to loss of information.

At this point we cannot predict the importance of *T. idahoensis* vs *T. ishikariensis*, but we prefer to reduce the likelihood of workers identifying any black sclerotial *Typhula* sp. on wheat as *T. ishikariensis*. We do not recognize *T. ishikariensis* var. *canadensis* (1) even though we found similar isolates. Cultural characteristics within both species showed essentially a continuum of variation.

We recommend the use of *T. ishikariensis* sensu Årsvoll and Smith (1) by those who do not wish to attempt to separate these species.

Burnett (5) and Ullrich and Raper (16) stated that there were no known interspecies hybrids among the higher basidiomycetes. Interspecies hybridization surely occurs among these fungi. Why assume these organisms evolved without such interactions?

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