Taxonomic Relationships Among Typhula Species as Revealed by Mating Experiments

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ABSTRACT

Typhula idahoensis, T. incarnata, T. ishikariensis, T. trifolii, and an unknown *Typhula* sp. all had tetrapolar incompatibility, but no distinction could be made between A and B incompatibility factors.

Partial sterility was common within natural dikaryons of *T. idahoensis,* occasional within *T. ishikariensis,* and rare in *T. incarnata.* Monokaryons did not give rise to fertile basidiocarps.

Interspecific pairings of monokaryons of *T. incarnata, T. trifolii,* and an unknown *Typhula* species were incompatible with each other and with *T. idahoensis* and *T. ishikariensis.* The latter two species mated to a limited extent, but most of the hybrid offspring were presumed incapable of survival in nature. Mating experiments support continued recognition of these species.

Typhula monokaryons are dikaryotized by donor dikaryons of the same species. Pairing dikaryons with monokaryons (di-mon matings) is thus useful in identifying *Typhula* isolates. Our procedure for using di-mon matings in identifying *T. idahoensis, T. incarnata,* and *T. ishikariensis* is given.

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Corner (3, 4) compiled descriptions of *Typhula* spp., made keys for their identification, and encountered overlaps and difficulties, with many forms intermediate in one criterion or another. Reliance upon descriptions of species found in Japan, Europe, and the United States of America (USA) with little interchange of specimens has contributed to the problem. Jamalainen (9), for instance, after reading the descriptions of *Typhula borealis* Ekstrand (7) from Sweden and of *T. idahoensis* Remsberg (18) from USA, concluded that these fungi represent only one species and applied the earlier name, *T. idahoensis.* McDonald (15) reviewed the literature and concluded that *T. borealis, T. idahoensis,* and *T. ishikariensis* Imai (8) are synonymous, with *T. ishikariensis* having priority. Corner (3) recently listed *T. ishikariensis* as a possible synonym of *Typhula erythropus* Fr. McDonald (15) stated that suspected synonymy between species would not be resolved until specimens from different sources are compared culturally, morphologically, and pathogenically by one investigator.

Rφed (19) has used infertility as the basis of species distinction in *Typhula.* Working with cultures of *Typhula graminum, T. itoana* from Canada, *T. incarnata* from Japan, and *Typhula* species from Norway, Rφed reached the conclusion that *T. itoana, T. graminum,* and *T. incarnata* were the same species. Cunfer (5) found that *T. incarnata* would not mate with *T. idahoensis,* and confirmed that they are different species. Because the original description of *T. idahoensis* (18) was based on material from this region, we have attempted to test the general applicability of infertility as a basis of species distinction. The present paper reports experiments on the incompatibility systems of *T. idahoensis, T. incarnata, T. ishikariensis,* and *T. trifolii;* on nuclear migration; and the failure to differentiate the A and B incompatibility factors in any species. We attempt to lay the foundation for a di-mon (dikaryon × monokaryon) mating system of taxonomy within the genus.

MATERIALS AND METHODS.—Cultures of natural dikaryons were obtained from single sclerotia. Sclerotia were treated with a mixture of commercial bleach (5.25% sodium hypochlorite) and 95% ethanol (1:1, v/v) for 1 minute, planted on Difco corn meal agar (CMA), and incubated at 10 C. To increase germination, some sclerotia were soaked in water for 2 hours at 1 C and a small portion of the rind was sliced away prior to disinfestation. Our studies included seven cultures of *T. idahoensis* (six from Washington, one from Idaho), seven cultures of *T. ishikariensis* (two each from Washington, Japan, and Finland, and one from Idaho), one culture of *T. trifolii* from Finland, and 16 cultures of *T. incarnata* (six from Washington, nine from Idaho, and one from Montana). Stock cultures were maintained on potato-dextrose agar (PDA) slants at 6 C. Large numbers of sclerotia used to produce basidiocarps were produced on a sand, bran, dextrose medium (2) at 10 C.

Basidiocarps were induced outdoors by placing sclerotia on the surface of an unsterilized soil and sand mixture in 15.2-cm diameter clay pots in September. A piece of cheesecloth was secured over the top of each pot to allow water and light to enter and prevent the scattering of sclerotia from pot-to-pot. Pots were then sunk in sand beds to within 3 cm of the top to reduce desiccation. Basidiocarps were collected from late October into January.

Basidiospores were obtained by securing individual basidiocarps with tape to the inside of a petri dish lid and allowing spores to shower for 12 hours onto the surface of PDA at 10 C in the dark. The spore-laden portion of agar was removed and shaken in about 3-4 ml of sterile tap water in a culture tube. The spore suspension was spread sequentially over the surface of three CMA dishes. Any surplus liquid was poured off the last dish. Plates were incubated at 10 C for 3 (*T. incarnata*) to 4 or 5 days (*T. idahoensis, T. ishikariensis*), after which time individual germlings were located at ×20-30 magnification, cut from

the agar and transferred, four per dish, to CMA, and incubated at 10 C. After 8-14 days, but before mycelial contact, the presumed single-spore cultures were examined at ×150 magnification for clamp connections. Cunfer (5) previously determined that all dikaryons in *T. idahoensis* have clamp connections, and that all monokaryons have unclamped hyphae. We used the absence of clamps to identify monokaryons in all species. Monokaryons were maintained on either CMA or PDA at 6 or 10 C.

Nuclei were stained by modifications of the Giesma technique (20).

EXPERIMENTS AND RESULTS.—*Intraspecific mating.*—1) Tetrapolar incompatibility.—Single basidiospore isolates from a dikaryotic isolate were tested for mating type by inoculating hyphae from two monokaryons 1 cm apart in a petri dish containing CMA. Pairings were made in all combinations, colonies were allowed to grow together at 10 C, and 3 to 4 days after contact, a 2 × 3 mm inoculum block ("clamp" piece) was removed from the union and placed on unoccupied medium in the same petri dish. Following 4 days incubation at 10 C, growth from the clamp piece was examined for clamp connections. Clamp connections were evidence of compatibility. Monokaryons derived from each dikaryotic isolate were thus classified by mating type, and a representative of each of the four mating types was saved as a tester for subsequent studies.

The species studied are reported to be heterothallic (5, 10, 12, 14, 16, 19, 21) and tetrapolar (5, 10, 12, 19, 21). Our results confirmed this. One dikaryon of an unidentified species was for the most part secondarily homothallic in that it produced many multinucleate basidiospores that gave rise to clamped hyphae, but it was found to be tetrapolar when the few monokaryons that were produced were paired.

2) Sterility.—Many dikaryons do not produce basidiocarps, produce few basidiospores, produce mostly spores that do not germinate, or produce spores that germinate, but fail to develop further. In some cases 50-100 germlings were transferred and either none or only a few, usually poorly growing, monokaryons were obtained. Our experience supports the complexity of sterility as emphasized by Lemke (13). In general, dikaryons of *T. incarnata* and the one dikaryon of *T. trifolii* were fertile, producing many basidiospores and vigorous monokaryons. Sterility of some type was common in *T. idahoensis* and less common in *T. ishikariensis*.

3) Migration of nuclei within compatible matings.—Migration of nuclei within pre-existing hyphae is critical for the success of the di-mon (dikaryon × monokaryon) matings that we propose as a taxonomic tool. No cytological observations were made to determine whether nuclei migrated through the septal pores or whether the septa were broken down prior to passage and reformed subsequent to nuclear passage.

In many cases, a dikaryon, as determined by regular formation of clamps, had formed at the distal margins of paired monokaryons at the time when clamp pieces were being examined, thus indicating "rapid" migration. Some monokaryons were good receptors of nuclei, some were good donors of nuclei, some were good receptors and donors, and a few were poor as receptors and donors. For example, tester 5 of *T. incarnata* dikaryon 6313 is a normal tester when sampled at the union of compatible combinations; however, it is a poor receptor of "compatible" nuclei. In 28 compatible matings, no clamps were observed on its growing margin 18 days after pairing (about 13 days after contact). Nuclei of other monokaryons were obviously impeded in this tester. In contrast, the nuclei of tester 5 moved rapidly through the hyphae of 15 of its 28 compatible mates, as clamps were present at their extremities. Tester 5 is a better donor than it is a receptor.

Tester 11 of dikaryon 6305, in contrast, was an excellent donor and receptor. Its nuclei moved to the hyphal ends in 29 of 30 compatible pairings, and its hyphae received nuclei in 29 of 30 compatible pairings. The only monokaryon with nuclei did not migrate to the culture extremities of tester 11 was that of tester 5 described above.

A final example to show the extremes found among monokaryons as receptors or donors of nuclei is within *T. incarnata* dikaryon 6317. Test pairing 12 × 3 was fully compatible when observed at the union, yet migration was not detected at 1 cm on either side of the union; i.e., neither monokaryon allowed significant passage of nuclei of its mate. Both A and B alleles differed (A≠ B≠), yet nuclear migration varied significantly among matings. Apparently, neither the A nor B factor alone controls nuclear migration.

Examples were presented only for *T. incarnata,* but similar differences in nuclear migration occurred in *T. idahoensis, T. ishikariensis,* and the unidentified dikaryon. Good nuclear migration was observed in testers from our one *T. trifolii* dikaryon.

4) Failure to distinguish A and B factors, and the lack of nuclear migration in common A, differing B combinations.—Prior to about 1950, A and B factors of tetrapolar incompatibility in the higher Basidiomycetes were designated arbitrarily (17, pages 68-72). At that time it became apparent that, in some fungi, A factors governed initiation of clamp formation and the B factors governed the migration of nuclei. Subsequent studies enabled students of several fungi to assign A or B to the factors in a definite rather than arbitrary manner.

In attempts to identify A and B factors by function in *Typhula,* we were unable to establish regular formation of false clamps nor were we able to associate colony types with differing A, common B (A≠ B=) or common A, differing B (A=B≠) pairings. As a consequence, we made exploratory pairings in an effort to detect nuclear migrations in presumed common A, differing B pairings. If an assumed A1B1 monokaryon anastomosed with an A1B2 monokaryon and nuclear migration occurred, the heterokaryon (A1B1 + A1B2) should form a dikaryon if challenged with A2B1. The original A1B1 monokaryon would not form a dikaryon with A2B1. Such pairings should enable one to detect nuclear migrations in illegitimate matings even though there is no visible evidence for such migrations.

Exploratory pairings of the testers of eight *T. incarnata,* seven *T. idahoensis,* four *T. ishikariensis,* and of two unknown dikaryons were incubated 2 weeks at 10 C after hyphal contact had occurred. Inoculum pieces 2× 2 mm were removed 1 cm from either side of the union. These pieces were then placed on CMA and challenged

TABLE 1. Mating reactions of tester monokaryons of four *Typhula* spp.; + = compatible, − = incompatible, s = spurious matings. *Typhula trifolii* and isolate 6621 are from Finland, isolate J-4 is from Japan. All others are from the USA

Species, isolates, and tester		Species, isolate, and tester monokaryons																									
		T. trifolii				*T. ishikariensis* 6621				*T. ishikariensis* J-4				*T. ishikariensis* Id-8g				*T. idahoensis* 5999-5				*T. idahoensis* C-1				*T. incarnata* 66-9			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
T. trifolii	1	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	2	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	3	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	4	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
T. ishikariensis 6621	1	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	2	−	−	−	−	−	−	+	−	+	+	+	+	+	+	+	+	−	−	s	s	s	−	−	−	−	−	−	−
	3	−	−	−	−	−	+	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	s	−	−	−	−	−
	4	−	−	−	−	+	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
J-4	1	−	−	−	−	+	+	+	+	−	−	−	+	+	+	+	+	−	−	−	−	−	−	−	s	−	−	−	−
	2	−	−	−	−	+	+	+	+	−	−	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	3	−	−	−	−	+	+	+	+	−	+	−	−	+	+	+	+	−	−	−	−	−	−	−	s	−	−	−	−
	4	−	−	−	−	+	+	+	+	+	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
Id-8g	1	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	−	−	−	−	s	−	s	s	−	−	−	−
	2	−	−	−	−	+	+	+	+	+	+	+	+	−	−	+	−	−	−	s	s	−	s	s	−	−	−	−	−
	3	−	−	−	−	+	+	+	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	4	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	s	−	s	s	s	−	−	−	−
T. idahoensis 5999-5	1	−	−	−	−	s	s	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	s	s	−	−	+	−	+	+	+	+	−	−	−	−
	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+	−	−	−	−	−
	4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	s	+	−	−	−	−	+	+	−	−	−	−	−
C-1	1	−	−	−	−	−	−	s	−	s	−	−	−	s	−	s	s	+	+	+	+	−	−	−	+	−	−	−	−
	2	−	−	−	−	−	−	−	−	−	−	−	−	−	s	s	−	+	+	+	+	−	−	+	−	−	−	−	−
	3	−	−	−	−	−	−	−	−	s	−	−	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−
	4	−	−	−	−	s	−	−	−	−	−	−	−	−	s	s	s	−	+	+	−	+	−	−	−	−	−	−	−
T. incarnata	1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
	2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
	4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−

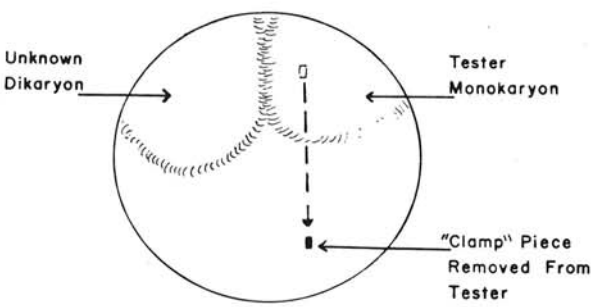

Fig. 1. Dikaryon paired with monokaryon. Monokaryon sampled 1 cm from the union. Sample ("clamp" piece) placed in same dish to avoid labeling and handling errors. Clamp connections on new growth from the clamp piece is evidence of nuclear migration and is considered proof that the tester and unknown are of the same species.

with appropriate testers to detect nuclear migration. A dikaryon was obtained only once (*T. ishikariensis* dikaryon Id. 8 g, testers 27 × 30). This sole dikaryon out of pairings of testers of 21 dikaryons, with six exploratory tests for each, probably resulted from an overgrowth (or intrusive growth) rather than from nuclear migration. We conclude that nuclear migration only occurs in A≠B≠ heterokaryons.

5) Monokaryons dikaryotized in the presence of spore showers.—Approximately 25 sclerotia-forming monokaryons, including some *T. incarnata, T. idahoensis,* and *T. ishikariensis,* were increased on sand, bran, dextrose medium (2) at 10 C and placed outdoors along with the dikaryons. Dikaryons and monokaryons were in separate, but adjacent, sand beds. Two *T. incarnata* monokaryons produced misshapen sterile basidiocarps like those described by Tomiyama (21) and Cunfer (5), and a third produced fertile basidiocarps. The fertile basidiocarp consisted of hyphae with clamps, so it

was presumably dikaryotized by air borne basidiospores.

Fertile basidiocarps were produced by "monokaryons" of two different *T. idahoensis* dikaryons. It was suspected that the monokaryons had been dikaryotized by air borne basidiospores. Monokaryons from these basidiocarps were mated with the testers of the parent dikaryon, and each dikaryon contained one A and B allele of monokaryon parent and one "foreign" A and B allele, proving that dikaryotization and meiosis has occurred. Presumably, dikaryotization was by air borne basidiospores. Many hyphae emerge from sclerotia in autumn. They ramify in the soil surrounding the sclerotium and anchor it (14) so that if a basidiocarp was produced, the structure would be held vertical. The basidiospores may contact the monokaryotic hyphae upon or in the soil with subsequent nuclear migration within and dikaryotization of the receptor mycelium. Cunfer (5) found that when monokaryotic sclerotia of *T. idahoensis* were incubated in isolation, no fertile basidiocarps were produced.

Interspecific pairings.—1) Species integrity.—In 1972-73, four testers of each of two dikaryons of *T. incarnata,* two *T. idahoensis,* two *T. ishikariensis,* and one unknown species were paired in all possible combinations. Neither *T. incarnata* nor the unknown gave any indication of compatibility with any other species. This confirmed Cunfer's (5) earlier report that *T. incarnata* would not mate with *T. idahoensis.* Matings of a sort, however, occurred in some combinations of *T. idahoensis* × *T. ishikariensis.* The products of these crosses were abnormal. Hyphae possessed a few scattered clamp connections, and growth by the mating product was slower than that of the parent monokaryons. It is questionable whether such offspring would survive in nature. We refer to the matings between *T. idahoensis* and *T. ishikariensis* as "spurious." We believe the two species are closely related, but that significant genetic separation exists between them.

In 1972-73 *T. ishikariensis* from Idaho and Washington was identified by us on the basis of agreement with the published description (8). To confirm our diagnosis, we obtained sclerotia of *T. ishikariensis* from Hokkaido, Japan, and from Finland for comparison with local materials. We also obtained *T. trifolii* from Finland for use in interspecies trials to determine more surely the relationships among the dikaryons.

T. trifolii and *T. incarnata* gave no evidence of mating with each other or with any other species (Table 1). *T. ishikariensis* from Japan was compatible with cultures labeled *T. ishikariensis* from Finland and from the USA, confirming their identification. We do not know how far apart the Finnish isolates 724 and 6621 (Table 1) were

TABLE 2. Some mating responses of meiotic products of di-mon matings in *Typhula ishikariensis* as determined by reaction with testers of their parents

Monokaryons from di-mon	Parent monokaryons							
	Dikaryon 70-7-b testers				Dikaryon 724 testers			
724/59[a]	59	60	61	62	1	2	3	4
1	−	+	−	+	+	−	+	−
2	+	+	+	+	−	−	+	−
3	−	−	+	−	+	+	+	+
	Dikaryon 8g testers				Dikaryon 724 testers			
724/30[b]	27	28	29	30	1	2	3	4
1	+	+	−	−	+	−	+	−
2	−	+	−	−	+	+	+	+
3	+	+	+	+	−	−	+	−
	Dikaryon 8g testers				Dikaryon 724 testers			
70-5/28[c]	27	28	29	30	1	2	3	4
1	+	−	−	+	−	+	−	+
2	−	−	+	+	+	+	−	−
3	+	+	+	+	−	+	−	−

[a]Parental dikaryon 724 (from Finland), parental monokaryon 59 derived from dikaryon 70-7-b (from Washington), monokaryons 1, 2, and 3 produced from this combination.
[b]Dikaryon 724 from Finland, monokaryon 30 derived from Idaho 8g.
[c]Dikaryon 70-5 from Washington, monokaryon 28 from Idaho 8 g.

TABLE 3. Expected incompatibility combinations of di-mon mating products in *Typhula* spp. when tested against the testers of both parents when no incompatibility factor in either stock is duplicated in the unknown

Monokaryon products of the di-mon	Dikaryon A testers				Dikaryon B testers			
	1	2	3	4	1	2	3	4
	Arbitrary genotypes of testers							
	A1B1	A1B2	A2B1	A2B2	A3B3	A3B4	A4B3	A4B4
A1B1[a]	−	−	−	+	+	+	+	+
A1B3	−	−	+	+	−	+	−	+
A3B1	−	+	−	+	−	−	+	+
A3B3	+	+	+	+	−	−	−	+

[a]The theoretical di-mon consisted of the A1B1 nucleus of dikaryon A, and the A3B3 nucleus of the unknown dikaryon B.

TABLE 4. Identification of unknown dikaryons of *Typhula* spp. using di-mon (dikaryon × monokaryon) pairings. + = good dikaryon formed; − = no dikaryon; s = "spurious" mating (1). The latter should be read as negative

Unknown dikaryon	Tester monokaryons				
	T. idahoensis		*T. ishikariensis*		*T. trifolii*
	10[a]	65[a]	2[a]	27[a]	1[a]
WA6298	−	s	+	+	−
WA6268	+	+	−	−	−
WA70-29 (straw)	s	−	+	+	−
WA70-31-d	+	+	−	−	−
WA70-36	−	−	+	+	−
WA70-38	+	+	−	−	−
WA72-8	−	−	+	+	−
ID73-7-2	+	+	−	−	−
ID112-b	−	−	+	+	−
Utah 2-3-2	−	+	−	−	−

[a] *T. idahoensis* tester monokaryons were of Washington origin, *T. ishikariensis* tester 2 is of Finland origin and tester 27 is of Idaho origin. *T. trifolii* tester is from a Finland dikaryon.

collected, but they shared one mating-type allele. Japanese isolate 3 (Table 1) had one allele in common with both Finnish isolates, and Japan 4 shared an allele with Finland 724. Finland 6621 shared an allele with Washington 70-7-b. The duplication of incompatibility factors within this small sample of widespread geographic origin is not considered to be evidence of recent interbreeding.

The mating similarities among foreign and USA testers of *T. ishikariensis* were further emphasized by the occurrence of spurious matings between all *T. ishikariensis* isolates, with the exception of Japan 3, and *T. idahoensis*.

Identification of Typhula species by di-mon matings.—Because of difficultures in identifying some *Typhula* spp. via morphological criteria (1), and because it is not always easy to obtain monokaryons, we resorted to dikaryon × monokaryon (di-mon) pairings as a means of identification. Hyphal anastomosis followed by migration of nuclei from a dikaryon into and through the hyphae of a monokaryon (16) and the ability to identify a dikaryon by the presence of clamp connections (5), suggested the practical use of di-mon matings as a taxonomic tool (17, pages 166-171) (Fig. 1).

1) Preliminary di-mon trials.—In the first experiment, 29 dikaryons were paired with eight monokaryotic testers representing the four mating types of one *T. idahoensis* and one *T. ishikariensis* dikaryon. Each unknown dikaryon thus required eight petri dishes. Sixteen dikaryons were compatible with at least one of the four *T. idahoensis* testers, and 10 were compatible with a least one tester of *T. ishikariensis*. Three of the unknown dikaryons were not compatible with any of the testers. Two of latter were *T. incarnata* dikaryons included as checks of the technique, and one dikaryon remains an unknown. This experiment also showed that tester monokaryons differ in their ability to serve as recipients of nuclei. The tester monokaryon should be vigorous enough to compete in a dish with the unknown dikaryon, and it should favor nuclear migration.

In a second experiment, 85 unknown dikaryons obtained from diseased wheat and grasses were paired with two tester monokaryons of each species, thus reducing the dishes required to four per unknown dikaryon. Forty-nine dikaryons were identified as *T. idahoensis,* 22 as *T. ishikariensis,* one mated with both, and 13 did not mate with either. In no case did dikaryons of other *Typhula* spp. mate in this experiment.

2) Spurious matings.—*T. idahoensis* and *T. ishikariensis* tend to mate, with nuclear migration sufficient to be detected 1 cm from the meeting of the two mycelia. Hyphae arising from the clamp piece of such matings grow slowly and with only a few clamp connections. These matings, insofar as species determination is concerned, are considered spurious, and should be read as negative. Only matings which produce abundant, normal clamp connections and "normal" dikaryotic hyphae should be read as positive.

3) Intrusive growth.—The results of di-mon matings are invalid as evidence of cytological compatibility if hyphae of the dikaryon merely grow into the monokaryotic mycelium. If the dikaryon grew 1 cm or more into the monokaryon, it would be recovered and the clamp piece would be read positive. To prove that donor nuclei entered the receptor hyphae, meiotic products of some di-mon matings were obtained and their incompatibility alleles determined.

Sclerotia resulting from di-mon matings were increased in culture and incubated outdoors in the autumn to produce basidiocarps. Single basidiospore isolates (monokaryons) were collected and paired with the four mating type testers of the "unknown" dikaryon parent and the four testers of the dikaryon from which the monokaryon parent was derived. Therefore, incompatibility readings would determine the origin of the meiotic products (Table 2). The results presented in Table 2 prove that the monokaryons accepted nuclei from the dikaryons, and that the meiotic products are not the result of intrusive growth. However, the expected recombinants resulting from random assortment of the incompatibility factors at meiosis are not produced.

The expected incompatibility relationships of meiotic products of di-mon matings tested against testers of the monokaryon and dikaryon parents bearing no common incompatibility factors are presented in Table 3. One compatible (+) and three incompatible (−) reactions with testers of a given dikaryon indicate recovery of both alleles of a parent haploid nucleus. Two compatible and two incompatible reactions indicate the recovery of one parental allele. Four compatible reactions thus indicate the recovery of two different alleles from the other parent nucleus. These reactions are the expected results of normal tetrapolar matings (Table 2, 3). The putative recombinants of di-mon 724/59 recorded in Table 2 suggest new incompatibility relationships that were not specified by the parental alleles. If it is assumed that the incompatibility alleles of all three nuclei somehow recombined to give rise to meiotic products from a single basidiocarp, it is possible to see numerous mating reactions in the resulting monokaryons. Alternatively, also it is possible that (i) somatic recombination in the vegetative hyphae may have occurred prior to fruit body production, or (ii) intragenic or intergenic crossing over may be occurring to produce new or recombinant specificities. Further studies will seek to clarify this situation.

Table 4 summarizes tests for the identification of unknown dikaryons of *Typhula* using di-mon (dikaryon × monokaryon) pairings. Note that these di-mon tests (Tables 2, 4) involved dikaryons of *T. ishikariensis* from Finland (724), Washington (70-7-b), and Idaho (8g). These *T. ishikariensis* isolates were fully compatible, not only producing normal clamp connections in the di-mon test, but also producing normal, vigorous meiotic products (monokaryons).

5) Final test of di-mon matings as a taxonomic tool.—Before proposing di-mon matings as a taxonomic tool, we tested local dikaryons against testers of local (USA) and foreign (Finland and Japan) origin. Only species with black sclerotia (*T. idahoensis, T. ishikariensis,* and *T. trifolii*) were included because these present the greatest taxonomic problems to the pathologist. A sample of the results (Table 4) illustrates the ability of foreign testers to identify USA dikaryons. Supposedly, USA testers would be equally capable of identifying foreign dikaryons.

DISCUSSION.—Although sclerotia of *Typhula* spp. are occasionally disseminated with clover (11) and grass seed (*Phleum pratense* L.) from Bonners Ferry, Idaho, (Bruehl et al., *unpublished*), their successful establishment in this manner must be an infrequent event. We suspect that *T. ishikariensis* is a circumpolar species which was widely spread before agriculture. Consequently, the genetic system of *Typhula* spp. must be conservative since long-separated populations, such as *T. ishikariensis* from the USA, Japan, and Finland, are compatible. Rϕed (19) made similar observations on *T. incarnata* from the USA and Norway.

Our ability to synthesize a few *T. idahoensis* × *T. ishikariensis* hybrids, and their ability to form sclerotia, made us suspect that natural hybrids may exist in nature. However, the high degree of sexual incompetence of such hybrids would make them functionally "imperfect," with the few compatible matings being vegetatively perpetuated as dikaryons. The vast majority of *T. idahoensis* × *T. ishikariensis* hybrids are not vegetatively competent. Hybrids grow very slowly and many form no sclerotia on laboratory media, or the sclerotia are usually few and very small. We believe this is sufficient evidence, along with morphological differences (1), to maintain both species as taxonomic units.

All species studied were either completely or almost completely interspecifically sterile, supporting them as species as described by the original authors. One important omission exists. No specimens of *T. borealis* from Sweden were studied. It is probable that *T. borealis* and *T. ishikariensis* are synonymous (1, 15), but this decision should depend upon both morphological and mating data.

No detailed cytological observations were made, but attempts to distinguish between A and B incompatibility factors were unsuccessful. It appears that only A≠ B≠ combinations form clamps and permit nuclear migration. Nuclear migration was more effectively prevented within incompatible matings of *T. idahoensis* and *T. ishikariensis* (intraspecific) than between (interspecific) the two species. The A and B factors enforce a powerful control on cytological phenomena.

The dikaryotization of monokaryons by airborne basidiospores helps explain why monokaryotic sclerotia have not been found in nature (5). Noble (16) reported fertile, basidiospore-producing monokaryotic sporophores in *T. trifolii*. If attempts are made to confirm Noble's report, precautions must be taken to incubate monokaryotic sclerotia in isolation (5).

Even though basidiospores can function in nature, vigorous monokaryons can infect a host (6, 22), monokaryons can dikaryotize each other, and dikaryons can dikaryotize monokaryons (16), field observations of areas where *T. idahoensis* is common provide good evidence that the main propagule is the sclerotium (2).

It would be impractical to use mating as a taxonomic tool on a large scale, such as following an extensive collecting trip, if monokaryons of each collection were required. It is not impractical, however, to obtain a few vigorous sclerotia-forming monokaryons that can be maintained in culture for use as testers. Obtaining dikaryotic mycelia (germinating the sclerotia) of the new collections and pairing them with known monokaryotic testers is no more laborious than many other taxonomic methods. We recommend di-mon matings as an aid to be used along with morphology in species identification.

We now have "testers" of *T. incarnata, T. idahoensis, T. ishikariensis, T. trifolii,* and of an unknown species. Pathologists around the world could assemble testers of all important species, and their interchange and use could introduce a new level of taxonomic precision (confidence) within the genus.

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