Hybridization of Typhula ishikariensis and T. idahoensis

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ABSTRACT

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Monokaryons from eight dikaryons each of *Typhula ishikariensis* and *T. idahoensis* were paired in 992 interspecific combinations. The 992 pairings resulted in 147 subculturable dikaryons, 124 of which produced sclerotia. Eighty-eight of the dikaryons produced regular clamp connections, but most of these 88 interspecific dikaryons grew slower than intraspecific dikaryons. Sporophores were produced by 13 of the 88 hybrid dikaryons; four of the 13 were fertile and yielded monokaryons. The spores from the four fertile hybrids germinated poorly and produced few vigorous monokaryons. The preponderance of incompatibility in these matings convinces us that the two species are sufficiently separate to merit retention as distinct species. The less vigorous hybrid dikaryons were avirulent, whereas some of the most vigorous interspecific dikaryons were as virulent as field dikaryons of either species. Most hybrid dikaryons produced few sclerotia on diseased leaves. The F_1 interspecific dikaryons were often as virulent on red clover as *T. ishikariensis*, the more virulent species on that host. Virulence on wheat was not closely linked with virulence on clover. Some hybrids' vigor and virulence make their survival in nature a likelihood.

In Washington and Idaho, *Typhula idahoensis* Remsberg dominates winter wheat in former grasslands and *T. ishikariensis* Imai is common in former forested areas (2). *T. ishikariensis* (4,13) attacks dicotyledonous plants to a greater extent than does *T. idahoensis*. The two species differ morphologically but intergrade, making visual identification difficult (1,3).

The dried sclerotia of both species appear black on dead, dried host materials, but sclerotia of *T. idahoensis* are larger and less spherical than those of *T. ishikariensis* and fewer are produced superficially on leaf laminae (2). The sclerotial rind cells of *T. idahoensis* are more irregular in shape than those of *T. ishikariensis*. The sporophores of *T. idahoensis* are smaller and darker, usually brownish, whereas those of *T. ishikariensis* are whitish. McDonald (10) combined both species under *T. ishikariensis*, and Årsvoll and Smith (1) agreed with McDonald. Årsvoll and Smith (1) erected *T. ishikariensis* var. *idahoensis* to accommodate *T. idahoensis*.

Both species are tetrapolar and multiallelic for incompatibility. Interspecific matings are infrequent and usually abnormal (3), but interspecific dikaryons have been synthesized from monokaryons of a normal isolate of *T. idahoensis* from Douglas County, Washington, and an infertile isolate of *T. ishikariensis* from Stevens County, Washington; the hybrids were virulent on winter wheat and formed sporophres (B. M. Cunfer, *unpublished*). The present study was initiated to determine the fertility, vigor, and virulence of interspecific hybrids.

MATERIALS AND METHODS

Interspecific mating. Monokaryons were obtained from isolates of *T. idahoensis* from Douglas (5999-5, 69-3M, 6274, 70-22, PP-2), Okanogan (6299-B, GOB), and Lincoln (C-1) counties, Washington, and from isolates of *T. ishikariensis* from Spokane (Wa 70-5, Wa 72-8) and Stevens (Wa 70-7a) counties, Washington, from Valley (MCGC, Id 73-1-1, Id 73-3-1) and Lemhi (Id 8g) counties, Idaho, and from Flathead County (Mo-2), Montana. Thirty-two monokaryons of eight isolates of *T. idahoensis* and 31 monokaryons of eight isolates of *T. ishikariensis* were paired in all possible interspecific as well as intraspecific combinations. Pairings were made on a Difco cornmeal agar (CMA) dish and incubated at 10 C. After the hyphae had grown together, a 2×3 mm block (clamp piece) was removed from the union and transferred to an unoccupied portion of the dish. The clamp piece was later examined for hyphae bearing clamp connections. Subcultures of promising dikaryotic (clamped) growth were made for further study. Colony diameters on CMA after 21 days at 10 C were recorded for most of the successful matings. Growth of a subculture on Difco potato dextrose agar (PDA) was also measured at 21 days for 42 interspecific F_1 dikaryons tested for virulence.

Sporophore production. The interspecific dikaryons were grown for sclerotia production at 10 C on a cellophane-covered medium consisting of 15 g of malt extract (BBL Division of Bioquest), 2.5 g of Difco Bacto peptone, 0.5 g of Difco Bacto yeast extract, 15 g of Difco agar, and 1 L of distilled water (MPYA). Sclerotia were placed on sterilized sand in Styrofoam cups. The cups were covered with four layers of cheesecloth, and sclerotia were incubated outdoors and kept moist, beginning in late September 1976. Sporophores were collected from late October through December. Monokaryotic isolates were obtained by taping individual basidiocarps to the inside of a petri dish lid (3) and allowing spores to shower onto a drop of water on water agar. Dilution technique was used to spread the spores over several CMA plates. Germlings were transferred to individual 60 × 15 mm dishes of CMA and incubated at 10 C. Monokaryons were identified by the absence of clamp connections.

Virulence trails. Forty-six interspecific F_1 dikaryons were tested for virulence on wheat during 1975–1976 and 1976–1977. During 1976–1977, 20 interspecific F_1 dikaryons were tested on red clover. Nugaines winter wheat (*Triticum aestivum* L. em. Thell.) was seeded 2 October 1975 at a rate of six seeds per clay pot (15.3 cm diameter) and 13 September 1976 at a rate of 10 seeds per pot in a mixture (1:1, v/v) of Palouse silt loam and sand. Dollard red clover (*Trifolium pratense* L.) was planted 28 August 1976 at a rate of 12 seeds per pot. The pots were sunk in sand beds, and the plants were grown outdoors under natural conditions. Inoculum was produced by transferring dikaryotic mycelia into sterilized (121 C for 50 min) 0.95-L (1-qt) jars containing either 225 cm^3 of dry wheat kernels moistened with 150 ml of water (9) or 250 ml of a presoaked mixture of dry wheat kernels, vermiculite, and water (1:1:1.2, v/v) with incubation in darkness at 10 C for 1–2 mo. Inoculation dates were 26 November 1975 and 25 November 1976; 40–50 cm^3 of fresh inoculum was applied over the plants and soil surface, irrespective of degree of sclerotial development. All plants were incubated at 0.5

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C under wet cotton for 50 days in darkness. Percentage of kill and reduction in fresh green weight of plants were determined after the wheat had recovered for 4 wk at 10–15 C under natural lighting in the greenhouse. Red clover was rated visually for damage at the time of removal from the incubation chamber.

RESULTS

Intraspecific compatibility. For the most part, the 31 monokaryons from eight isolates of *T. ishikariensis* paired in all intraspecific combinations mated normally. No compatible pairings occurred between isolates that shared a common allele, but a few pairings failed to produce hyphae with clamps even though compatibility was expected. The 32 monokaryons from eight isolates of *T. idahoensis* mated normally in intraspecific combinations.

Interspecific compatibility. Eighty-eight of the 992 interspecific pairings produced vigorous dikaryotic colonies from the clamp pieces. Most of these involved five testers, four from *T. ishikariensis* isolate Wa 72-8 and one from *T. idahoensis* isolate 6299-B. *T. idahoensis* 6299-B-10 mated with all testers of Wa 72-8, producing dikaryons comparable in vigor to those of intraspecies crosses, with average colony diameters of 74 mm after 21 days. When 6299-B-10 mated with other *T. ishikariensis* testers, the mean colony diameter was 43 mm (Table 1). Dikaryons from Wa 72-8-25 × the 32 *T. idahoensis* testers had a mean colony diameter of 33 mm. An additional 102 combinations developed somewhat vigorous dikaryotic hyphae, resulting in 190 subculturable dikaryons. The 147 dikaryons that remained alive on CMA were transferred to MPYA. Other pairings produced indefinite or no mating reactions.

Nuclear migration in interspecific pairings. Dikaryotization was usually from *T. ishikariensis* hyphae into *T. idahoensis* hyphae. Dikaryotization of *T. idahoensis* monokaryon 6299-B-10 hyphae usually was complete in interspecific pairings, with clamp connections formed at the colony margins. Clamp formation at the colony margins also occurred in some other interspecific combinations with *T. idahoensis* monokaryons, and clamp connections were present to a lesser degree in hyphae of the other compatible *T. idahoesis* monokaryons. Clamp connections were rare on *T. ishikariensis* hyphae and were restricted to the mating junction, except for 13 pairings in which reciprocal dikaryotization to colony margins was also evident.

Virulence of interspecific F_1 dikaryons. Of 42 F_1 interspecific dikaryons tested on wheat in the four- to five-leaf stage, 15 were virulent, 7 were moderately virulent, 6 were weakly virulent, and 14

TABLE 1. Colony diameter of *Typhula ishikariensis* dikaryons and *T. ishikariensis* × *T. idahoensis* dikaryons grown for 21 days on cornmeal agar at 10 C in the dark

T. ishikariensis monokaryons	× *T. ishikariensis* monokaryon Wa 72-8-25[a] (intraspecific) Growth (mm)	× *T. idahoensis* monokaryon 6299-b-10[b] (interspecific) Growth (mm)
Wa 70-5-16	60	40
-17	70	34
-18	74	39
-19	73	24
Wa 70-7a-20	63	46
-21	76	45
-22	71	47
-23	72	47
Wa 72-8-24	I[c]	74
-25	I[c]	75
-26	I[c]	64
-27	I[c]	80
Id 8g-32	75	47
-33	74	35
-34	78	43
-35	73	51
MCGC-38	73	48
-39	54	39
-40	71	33
Id 73-1-1-41	I[c]	55
-42	I[c]	60
-43	74	46
-44	72	56
Id 73-3-1-45		51
-46	70	30
-47	75	59
-48	60	40
Mo-2-49	54	40
-50	69	34
-51		42
-52	59	37

[a]Colony diameter was 40 mm.
[b]Colony diameter was 52 mm.
[c]Incompatible due to common incompatibility alleles.

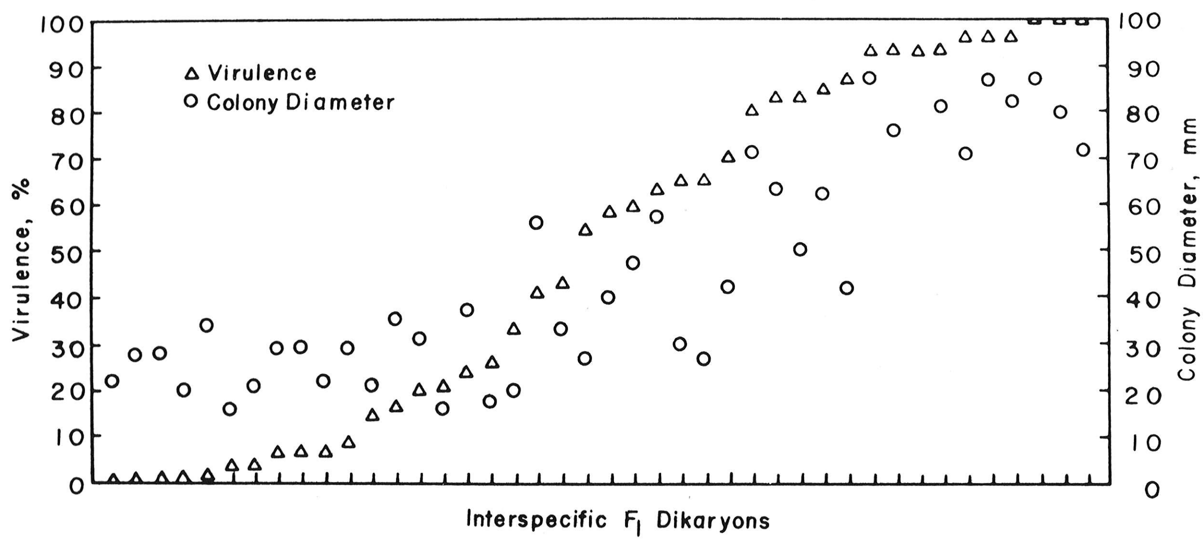

Fig. 1. Virulence and growth in culture of 42 *Typhula ishikariensis* × *T. idahoensis* F_1 dikaryons. Virulence was determined by the percentage of reduction in green weight of inoculated/noninoculated Nugaines wheat after 50 days in the dark at 0.5 C, followed by a recovery period of 4 wk in the greenhouse at 10–15 C. Colony diameter was measured in millimeters after 21 days on potato dextrose agar at 10 C in the dark. For comparison, green weight of wheat was reduced 100% after incubation with *T. ishikariensis* field dikaryon Wa 70-7a and 93% after incubation with *T. idahoensis* field dikaryon 5999-5.

were avirulent in the 1975–1976 test (Fig. 1). Interspecific dikaryons Wa 72-8-25 × 6299-B-10 and Wa 72-8-25 × 6274-16 formed sclerotia as abundantly on diseased leaves as did virulent dikaryons of either species, but the other interspecific dikaryons formed few or no sclerotia. The slow-growing interspecific dikaryons were avirulent (Fig. 1). Three of the four dikaryons synthesized between monokaryons of KF-69-3 and P1 dikaryons were virulent and produced sclerotia on diseased hosts (B. M. Cunfer, *unpublished*). During 1976–1977, wheat plants were larger, with five tillers, and recovery was greater than during 1975–1976, but the results were similar.

Fourteen interspecific dikaryons caused severe leaf and stem damage, four caused more leaf than stem damage, and two caused no damage on red clover. Some sclerotia were present on plants in crosses of Wa 72-8 monokaryons × 6299-B-10 in our study and in crosses of monokaryons of KF-69-3 × monokaryons of P1 in Cunfer's study. *T. ishikariensis* field dikaryons formed sclerotia on leaves and had the maximum virulence rating of 3, based on a scale of 0–3, with 0 signifying slight or no damage and 3 indicating severe leaf and stem damage. *T. idahoensis* field dikaryons formed fewer sclerotia in the leaves, and the plants were less affected, with virulence ratings of 1 or 2.

Fertility of interspecific F_1 dikaryons. One hundred twenty-four of our interspecific dikaryons and Cunfer's four interspecific dikaryons of KF-69-3 and P1 parentage produced sclerotia on MPYA, and the sclerotia were placed outdoors for fruiting. Only 17 interspecific dikaryons formed sporophores, and vigorous monokaryons were produced by six of these (Table 2). The other 11 crosses formed 45 sporophores that either did not produce showers or produced small showers of spores that did not germinate.

The percentage of spore germination was 64% for KF-69-3-1 × P1-2 and 71% for KF-69-3-1 × P1-4 (the mean count from five sporophores of each hybrid, 100 spores minimum per sporophore). However, only 23 and 39%, respectively, of these germlings actually developed.

DISCUSSION

The general infertility of *T. idahoensis* × *T. ishikariensis* hybrids indicates significant genetic isolation (2,3). Only 19% of the 992 interspecific pairings resulted in culturable dikaryons. Most interspecific hybrids (i) were less vigorous than intraspecific dikaryons, (ii) were not virulent, (iii) produced few or no sclerotia on wheat plants, and (iv) did not produce abundant or fertile sporophores. Some interspecific hybrids, however, were vigorous and pathogenic and, even though highly infertile, could exist in nature in the vegetative, sclerotial state.

Årsvoll and Smith (1) mated two *T. idahoensis* cultures (5999-5 and 6274) from Washington, three *T. ishikariensis* var. *canadensis* (1) cultures from Canada, and four *T. ishikariensis* isolates from Norway and concluded that recognition of two species was not justified. *T. idahoensis* from Washington was completely compatible with *T. ishikariensis* var. *canadensis*. *T. idahoensis* and *T. ishikariensis* var. *canadensis* were incompatible with three of the *T. ishikariensis* isolates from Norway; the fourth (15/73) was incompatible with *T. ishikariensis* var. *canadensis*, compatible with *T. idahoensis*, and incompatible with the other three *T. ishikariensis* isolates from Norway. Except for the unusual behavior of isolate 15/73, their results confirm ours. Possibly, their isolate 15/73 is similar to isolate Wa 72-8 of our study.

Årsvoll and Smith (1) reported that offspring of *T. ishikariensis* var. *canadensis* × *T. idahoensis*, *T. idahoensis* var. *canadensis* × *T. ishikariensis*, and *T. idahoensis* × *T. ishikariensis* produced normal, fertile sporophores. Unfortunately, no data were presented on the viability, vigor, or mating behavior of the meiotic products from these crosses. We suspect that *T. ishikariensis* var. *canadensis* × *T. idahoensis* offspring will be vigorous and fertile.

The general capability of *T. idahoensis* hyphae to be dikaryotized by *T. ishikariensis* nuclei, whereas *T. ishikariensis* hyphae were seldom reciprocally dikaryotized, was a fairly consistent difference between monokaryons of these two species. Unilateral nuclear migration probably results from a reaction of the migrating nucleus with a cytoplasmic substance in the recipient hyphae (8).

In the smut fungi, species are delineated on morphology and host family specialization (6). Species hybrids have been made in the smut fungi, ie, cells fused, dikaryons were pathogenic, hybrid teliospores formed in the host plant, and teliospores germinated (5). The fact that some monokaryons of *Typhula*, eg, 6299-B-10 and all monokaryons of dikaryon Wa 72-8, can mate with another species or that sporadic combinations of monokaryons mate should not militate against the use of mating experiments in support of morphology and host range in determining the validity of species.

T. idahoensis occurs primarily on winter cereals and grasses (12); *T. ishikariensis* has a broader host range, including dicotyledonous plants (2,7,13). *T. ishikariensis* was described primarily from red clover and only rarely from wheat and grasses (7). Tomiyama (13) reported that *T. ishikariensis* did not increase in the field on winter wheat during seven consecutive crops, even though wheat is susceptible. In contrast, *T. ishikariensis* increased on winter rape (*Brassica campestris* L.). *T. ishikariensis* blighted 12% of the wheat in the final year of the above experiment, whereas, it started year one at 18% (K. Tominyama, *unpublished*). In the final year, 67% of the rape was blighted. Winter wheat alternated with rape reduced the disease on rape.

The major winter wheat areas of Washington and Idaho are subject to chronic snow mold and are so dry that few crops other than winter wheat are economical. Winter wheat is the only crop on most of the land and is alternated with summer fallow. In the drier areas of Washington and Idaho, *T. idahoensis* is prevalent, usually accounting for at least 70% of the dead plants, even though *T. incarnata* and *Fusarium nivale* are common and widespread. *T. idahoensis* increases on wheat; this is in contrast to Tomiyama's (13) experience in Japan with *T. ishikariensis*. In Washington and Idaho, *T. ishikariensis* was reported (2) primarily from formerly forested areas, whereas *T. idahoensis* was reported primarily from former grasslands. The former forest lands, now cultivated, have occasionally produced clovers and alfalfa (*Medicago sativa* L.) because of greater rainfall. Clovers and alfalfa reduce *T. idahoensis* (11). These species may differ in real host range in nature more than is indicated by inoculation experiments.

Interspecific F_1 dikaryons with normal growth can be virulent on wheat. Virulence on wheat in *T. idahoensis* is controlled by many genes on chromosomes other than the two chromosomes that carry the incompatibility loci (9). For an interspecific dikaryon to be virulent, the other chromosomes must be sufficiently similar to enable genes controlling virulence to interact. *T. ishikariensis* is more virulent on red clover than *T. idahoensis*. Most F_1 dikaryons were equal to *T. ishikariensis* in virulence on red clover. Virulence on red clover was not closely associated with virulence on wheat, however.

The level of reproductive isolation between *T. idahoensis* and *T. ishikariensis* observed in this study, evident in the low production of fertile sporophores by F_1 dikaryons, low percentage of basidiospore germination, and low percentage of vigorous monokaryons recovered from the interspecific

TABLE 2. Number of vigorous monokaryons produced from germlings of interspecific F_1 dikaryons of *Typhula ishikariensis* × *T. idahoensis*

Dikaryons	Sporophores tested (no.)	Germlings obtained (no.)	Vigorous monokaryons (no.)
Wa 72-8-24 × 5999-5-3	4	18	13
Id 73-1-1-43 × 5999-5-4	8	6	4
Mo-2-49 × GOB-29	8	194	56
Mo-2-49 × PP-2-40	4	18	5
KF-69-3-1 × P1-2	17	400	92
KF-69-3-1 × P1-4	12	310	121
Total	53	946	291

F_1 dikaryons, supports the retention of the two species. The species are related, however, and natural hybridization is possible.

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