# Incompatibility Alleles and Fertility of Typhula idahoensis

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## ABSTRACT

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Monokaryons representing the four mating types of 36 dikaryons of *Typhula idahoensis*, a tetrapolar species, were obtained from dikaryons from north central Washington. Efforts to obtain monokaryons representing all four mating types from dikaryons collected in Idaho and adjacent Utah failed. We conclude that this species is nearly asexual in Idaho and northern Utah. Twelve alleles of the A and 12 of the B locus were identified in Washington. These alleles were found in several combinations, suggesting interbreeding. When the few vigorous monokaryons from Idaho and Utah were paired with Washington testers, several alleles were common to both populations. Dikaryons from Idaho and Utah, even though almost sterile, usually were more virulent on Sprague winter wheat than were Washington isolates. Our data indicate that a functional sexual stage leads to reduced average virulence in a population.

Basidiospores of *Typhula idahoensis* Remsberg are capable of initiating snow mold of winter cereals under favorable conditions (4). Moreover, its functional sexual stage might give rise to isolates with increased virulence upon snow mold-resistant cultivars of winter wheat (*Triticum aestivum* L.). Preliminary to assessing the role of basiodiospores in the epidemiology of the disease, we studied the sexual competence and virulence of several isolates of *T. idahoensis* collected in scattered plateaus and mountain valleys of the Pacific Northwest.

Because isolates vary greatly in culture, we have been unable to identify clones by cultural characteristics as has been done with some *Fusarium* spp. (2). The lack of distinctive cultural types within geographic areas led us to select incompatibility alleles as markers for the identification of populations within the species. The present study presents the results of these efforts.

## MATERIALS AND METHODS

In previous years, mycelial cultures obtained from germinated sclerotia were subsequently increased and the sclerotia which resulted were placed outdoors in the fall for sporophore production (1). In 1975, however, leaves of diseased wheat plants and roadside grasses were placed outdoors and sporophores were obtained directly from sclerotia upon or within the leaves.

Basidiospores were obtained by suspending mature sporophores individually over Difco corn-meal agar (CMA) or potato-dextrose agar (PDA) at 10 C for 12-24 hr. Spores on the agar surface were suspended in 3 ml of tap water. The spore suspension was poured serially onto three dishes of CMA, the surplus liquid was decanted, and the inoculated dishes were incubated 4-10 days at 10 C in darkness. These dishes were examined periodically. When the germlings were easily visible at ×30 magnification, they were individually isolated.

Monokaryons were mated as previously described (1) to type them in terms of the four incompatibility alleles of each dikaryon. When all four were obtained, a representative of each mating class was maintained in culture for subsequent use. Clamp formation on normal hyphae was the criterion of compatibility. The A and B loci were arbitrarily assigned (3).

For the test of virulence, inoculum of *T. idahoensis* was produced on sterilized wheat kernels (5) at 10 C. Plants of vigorous early-seeded Sprague wheat (C.I. 15376) were dug from the field, transplanted into 15-cm diameter clay pots, and placed outdoors to recover from the transplanting. In late December, 1976, they were inoculated with fresh nondried sclerotia and incubated under layers of wet cotton at 0.5 C in darkness (5). After 60 days, the cotton was removed and the wheat plants were allowed to recover in a greenhouse maintained at 10 C under natural light for 30 days. The green weights of plants from five pots per treatment were averaged and virulence was judged on the basis of new growth (plant recovery) from the crowns.

## RESULTS

**Incompatibility alleles in Typhula idahoensis in Washington.**—Complete sets of four tester monokaryons were obtained from 36 dikaryons, 29 from Douglas County, six from Okanogan County, and one from Lincoln County, Washington (Table 1). The dikaryons originated from a geographic area about 115 km north to

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south and 125 km east to west. Only 12 A and 12 B alleles were detected among the 36 dikaryons. This is a small number considering that 72 A and 72 B alleles were theoretically retrievable. We conclude that the Washington population contains relatively few alleles and that considerable inbreeding has occurred within this isolated population.

**Typhula idahoensis from Idaho, Utah, Montana, and Wyoming.**—Attempts to obtain sets of tester monokaryons from dikaryons from the adjacent states failed. During the last 4 yr 1,933 germlings from spores of Idaho dikaryons, 1,078 from northern Utah, 88 from Montana, and 16 from Wyoming were isolated. More than 90% did not form visible growth, grew too poorly to work with, or died in culture. No set of four testers was obtained from the 3,115 germlings (no more than 40 from a single sporophore).

The number of germlings of *T. idahoensis* obtained from each state, listed by counties, is as follows. Idaho counties: Bear Lake, 76; Blaine, 104; Butte, 23; Camas, 201; Caribou, 66; Cassia, 28; Elmore, 24; Franklin, 491; Fremont, 1; Lemhi, 41; Madison, 94; Oneida, 28; Power, 51; and Teton, 453, totaling 1,933. Montana counties: Flathead, 60; Gallatin, 28, totaling 88. Utah counties: Box Elder, 1,078. Washington counties: Douglas, 809; Lincoln, 40; Okanogan, 181, totaling 1,036. Wyoming counties: Lincoln, 16. These numbers represent spores that germinated and grew well enough to be isolated as germlings. We have no estimate of those that did not germinate, but the number of nonviable spores must have been great.

TABLE 1. Dikaryons of the *Typhula idahoensis* isolates arranged systematically to illustrate the multiplicity of combinations of alleles of the A and B incompatibility alleles found in Washington

| Incompatibility alleles of loci A and B | | Dikaryon | |
|---|---|---|---|
| A | B | Culture | Geographic origin |
| 1,2 | 1,2 | 5999-5 | Mud Springs |
| 1,2 | 1,4 | 6274 | W[a] of Mansfield |
| 1,2 | 10,11 | 75-90-5 | W of Mansfield |
| 1,4 | 1,5 | 75-63a-1 | NE of La Fleur[b] |
| 1,6 | 1,6 | 75-87-1 | S of Waterville |
| 1,6 | 2,10 | 75-90-10 | W of Mansfield |
| 1,9 | 6.8 | 75-89-1 | N of Waterville |
| 1,12 | 6,10 | 75-92-5 | Dyer Hill |
| 2,5 | 1,2 | Black D | Dyer Hill |
| 2,5 | 5,7 | 75-95-5 | W of La Fleur[b] |
| 2,5 | 6,7 | 75-91-2 | NW of Mansfield |
| 2,6 | 1,8 | 75-77-1 | Farmer |
| 2,6 | 5,6 | 70-22 | Withrow |
| 2,7 | 1,2 | 75-63-5 | NE of La Fleur[b] |
| 2,7 | 1,5 | GOB | NE of La Fleur[b] |
| 2,8 | 2,4 | Cunfer 1 | N of Wilbur[b] |
| 2.8 | 4,7 | 75-92-6 | Dyer Hill |
| 2,8 | 5,9 | 75-88-5 | Mud Springs |
| 3,4 | 2,3 | 69-3M | NW of Mansfield |
| 3,5 | 1,2 | 6299-B | NW of La Fleur[b] |
| 3,7 | 6,9 | 75-85-1 | Mud Springs |
| 3,8 | 2,4 | 75-83-4 | Mud Springs |
| 3,8 | 2,4 | 75-84-3 | Mud Springs |
| 3,8 | 7,12 | 75-77-3 | Farmer |
| 4,5 | 3,9 | 75-90-9 | W of Mansfield |
| 4,9 | 7,8 | 75-93-5 | Dyer Hill |
| 5,6 | 2,8 | 75-88-2 | N of Waterville |
| 5,6 | 3,8 | E | Withrow |
| 5,12 | 10,12 | 75-91-4 | NW of Mansfield |
| 6,7 | 1,5 | 75-92-1 | Dyer Hill |
| 6,8 | 2,3 | 70-40 | NW of La Fleur[b] |
| 6,8 | 2,6 | 75-88-4 | N of Waterville |
| 6,9 | 1,2 | 75-92-2 | Dyer Hill |
| 8,9 | 1,3 | 75-85-3 | Mud Springs |
| 8,9 | 6,7 | PP-2 | N of Waterville |
| 10,11 | 3,6 | 75-86-2 | S of Waterville |

[a]N = north, S = south, E = east, W = west.
[b]La Fleur is in Okanogan County, Wilbur is in Lincoln County. All other locations are in Douglas County, Washington.

**Relation of Typhula idahoensis from Washington and T. idahoensis from Idaho and Utah.**—The morphology of sclerotia and sporophores, the nature of the attack on wheat, and results of dimon breeding tests (1) led us previously to assume that the fungus from Washington, Idaho, and Utah was the same species. The relative fertility of the Washington collections compared to collections from the other states led us to reconsider our identifications. To further identify our isolates, we paired the few vigorous monokaryons obtained from Idaho and Utah dikaryons with four tester monokaryons each of *T. idahoensis* and *T. ishikariensis* Imai to determine their compatibilities. [The reason for including monokaryons of *T. ishikariensis* in this trial is that this species occurs in this region, it can attack wheat, and it is morphologically quite similar to *T. idahoensis* (1).]

The monokaryons of Idaho and Utah collections were compatible with *T. idahoensis* testers; ie, they formed normal hyphae with regular clamp connections, and were

TABLE 2. Alleles of incompatibility loci A and B in monokaryons of *Typhula idahoensis* from Utah and Idaho that correspond to alleles found in Washington

| Monokaryon | Geographic origin | Alleles of incompatibility factors A and B | |
|---|---|---|---|
| | | A | B |
| 212-1 | Power County, Idaho | 1 | 7 |
| 3-1-10 | Box Elder County, Utah | 2 | 3 |
| 3-7-13 | Box Elder County, Utah | 2 | x[a] |
| 2-6-5 | Box Elder County, Utah | 4 | 3 |
| 302-2 | Blaine County, Idaho | 4 | 3 |
| 73-11-1 | Camas County, Idaho | 4 | 3 |
| Fairfield 44-1 | Camas County, Idaho | 4 | 3 |
| 303-b-9 | Camas County, Idaho | 4 | 3 |
| 73-10-1 | Camas County, Idaho | 4 | 6 |
| 303-b-1 | Camas County, Idaho | 4 | 7 |
| 303-b-11 | Camas County, Idaho | 4 | 7 |
| 2-10-7 | Box Elder County, Idaho | 4 | 7 |
| 8d(48-1)[b] | Lemhi County, Idaho | 5 | 6 |
| 208-1 | Oneida County, Idaho | 8 | 3 |
| 207-2 | Franklin County, Idaho | 9 | x |
| 109-b-1 | Teton County, Idaho | x | 1 |
| 208-2 | Oneida County, Idaho | x | 3 |
| 304-2 | Camas County, Idaho | x | x |

[a]The symbol × indicates the allele was not identified by the testers used (testers for A3, 10, 11, 12, and B4, 10, 11, 12 were not included in this test).
[b]The Lemhi County, Idaho, collection is approximately 400 km from the Washington *T. idahoensis* population. All other collections listed in this table were more distant.

incompatible with testers of *T. ishikariensis* (did not form vigorous hyphae with regular clamp connections).

To further test the relatedness of the Idaho and Utah materials with those of Washington and to obtain some idea as to the incompatibility alleles present in the former, Idaho and Utah monokaryons were paired with tester monokaryons capable of identifying eight of the A and eight of the B alleles found in Washington (Table 2). Of the possible 18 unknown A and B alleles, all but three alleles at each locus were identified by the selected Washington testers. The frequency of allele duplication suggests a common origin of the two populations.

**Virulence of Typhula idahoensis isolates on Sprague winter wheat.**—Sprague was inoculated with 48 isolates from Idaho, Utah, and Montana and 33 from Washington. The virulence of isolates from Washington (judged by the ability of Sprague to recover after attack) was less than that of isolates from the other states. The criterion applied to judge virulence was the average green weight after a 30-day recovery period. The most virulent group consisted of six isolates from Box Elder County, Utah, the least virulent group consisted of isolates from Douglas County, Washington (Table 3).

These data prove that the loss of fertility by *T. idahoensis* in Utah and Idaho was not accompanied by avirulence.

## DISCUSSION

The 12 A and 12 B alleles found in north central Washington were detected in various combinations with no marked intra-regional distribution, indicating that this population exists as an active, interbreeding unit (5). The unexpected partial or complete sterility of most collections from Idaho, Utah, and Montana prevented the accumulation of comparable information for those regions. The limited data obtained, however, indicated that *T. idahoensis* in these relatively isolated geographic areas has incompatibility alleles similar to those of Washington, suggesting that representatives of this species in the Pacific Northwest have a common origin.

We had difficulty with some Washington isolates, but compared to Utah and Idaho isolates, the sexual stage of Washington isolates was more competent. Assuming that all isolates of *T. idahoensis* in the Pacific Northwest have a common origin, what is the basis for the observed difference in fertility? Several hypotheses seem plausible, but we propose the following. Many plant pathogens may have begun with functional sexual stages that have either degenerated or been lost. Possibly, the north central Washington population was the "youngest" one studied; the present home of this species in Washington was either covered or severely disturbed by a continental ice sheet as recently as 12-15,000 years ago. The Idaho and Utah areas were not so disturbed. The Washington area was colonized subsequent to this ice sheet and sexually active forms may have had an advantage as colonists. If this hypothesis be correct, Washington populations descended from the population in adjacent nonglaciated areas, explaining the occurrence of common incompatibility alleles to the south and east. Thus, the Washington populations, too, will become increasingly asexual with time. The sexual stage, with its airborne spores, may be of greatest value to a colonist of new areas.

Our study of isolates from nature indicates that a functioning sexual stage in *T. idahoensis* resulted in greater variability in virulence and reduced mean virulence (Table 3). A greater range in virulence was found among 33 isolates from the area of relative fertility than was found among 48 isolates from the area of relative sterility. The recovery of plants as determined by green weights after attack was 34% for the sexually active population and 16% for the sexually inactive population. In an earlier study (5), 143 dikaryons were synthesized by mating monokaryons of a single moderately virulent isolate. These new $F_1$ dikaryons, unselected by nature, ranged in virulence from 0 kill of test plants to 100%. The average kill by the 143 dikaryons was 62%; the average kill by the parent was 78%. Thus, the present study with dikaryons from nature supports the earlier results obtained from synthesized dikaryons not selected by nature. Our conclusion is that virulence in *T. idahoensis* is governed by many genes (5), that a typical isolate is probably highly heterozygous (5), and that passage through the sexual stage produces a wide range of offspring, some more virulent than the parents, but more that are less virulent than the parents.

The frustrations involved in collecting sporophores, diluting spore showers, and transferring germlings that often failed to develop from the Idaho and Utah collections taught us that a fructification does not always result in production of functional basiodiospores. Development of sterility in what surely was a fertile higher basidiomycete at one time provides circumstantial evidence that sexual reproduction was once important but has now been largely abandoned by *T. idahoensis* in much of its present geographic range.

TABLE 3. Virulence of *Typhula idahoensis* isolates from the Pacific Northwest as judged by the recovery (green weight) of Sprague winter wheat after attack

| Geographic origin of isolates | Number of isolates per group | Green weight | |
|---|---|---|---|
| | | Mean (g) | Range (g) |
| Idaho, Montana, Utah, Wyoming[a] | 48[b] | 1.8 | 1.0- 2.8 |
| Washington[c] | 33[b] | 3.7 | 1.3- 6.8 |
| Control[d] | 0 | 11.0 | 8.5-13.3 |

[a]The total of 48 isolates in the group includes 36 from Idaho, three from Montana, six from Utah, and three from Wyoming.

[b]Each isolate is represented by the mean green foliage weight in grams of the wheat from five pots.

[c]The total of 33 isolates in the group includes 24 from Douglas County, and nine from Okanogan County.

[d]There were five sets of controls, five pots in each set.

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