Sexual Incompatibility and Aspects of the Mono- and Dikaryotic Phases of Typhula idahoensis

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ABSTRACT

Typhula idahoensis has tetrapolar incompatibility with multiple alleles. Seven A and six B incompatibility alleles were recovered from six field collections. Several alleles at both loci were common to more than one collection. Tetrapolar incompatibility with six alleles at each locus was found in three field collections of *T. incarnata*, confirming previous results with this species. Interspecies matings were noncompatible in every case, supporting the established species designations.

At least 99% of the basidiospores are uninucleate when ejected from the basidium, but subsequent mitotic divisions may occur prior to germination.

Monokaryotic isolates and incompatible pairings of monokaryons either produce no sporophores or produce sterile sporophores, whereas field-collected dikaryotic isolates and compatible matings of monokaryons produce fertile sporophores. As a group, dikaryons grew faster in vitro than monokaryons, but there was much variability in growth rate among isolates of both genetic conditions. Optimum growth of mono- and dikaryons was at 10 and 15 C. There was no correlation between mycelial growth rate and virulence to wheat among monokaryons but virulence increased with increase of growth rate among dikaryons. The importance of the results to pathogenicity of the basidial stage on cereals is discussed.

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The sexuality of several *Typhula* spp. has been investigated and all are heterothallic (5, 7, 8, 11). *Typhula erythropus* (5, 6) and *T. incarnata* Lasch ex Fr. (11, 13) are tetrapolar, with two loci. Røed (11) demonstrated multiple alleles at both loci in *T. incarnata*.

Tomiyama (13) found that monokaryotic sclerotia of *T. incarnata* placed outdoors produced lobed, shortened, sterile sporophores. *Typhula trifolii* monokaryons produced both sporophores and basidiospores which were similar to those from dikaryons, except for their smaller size (8). Basidiospores from both types of thalli were uninucleate, as were basidiospores of *T. gyrans* (7).

Monokaryons of *T. incarnata* grew more slowly and their growth rates were more variable than those of dikaryons (13). Also, they were usually slower to infect wheat and rye. Remsberg (10) reported optimum growth of *T. idahoensis* Remsberg dikaryons on potato-dextrose agar (PDA) at 9-12 C with 18 C maximum. In another study (4), 5 C was slightly better than 10 C for growth on a malt-yeast-glucose agar and 20 C was maximum.

Typhula idahoensis and *T. incarnata* are major snow mold pathogens on winter wheat in the Pacific Northwest. *Typhula idahoensis* has spherical black sclerotia 0.5 - 2.0 mm in diam and tan sporophores 5- to 10-mm high. *T. incarnata* produces chestnut brown sclerotia 0.5 - 4.5 mm in diam and pink to red sporophores 3.4 - 30 mm high. This paper reports sexual incompatibility, cultural characteristics, sporophore production, in vitro growth rates, and virulence of monokaryons and dikaryons of *Typhula idahoensis*. Sexual incompatibility of *T. incarnata* was also

TABLE 1. Intra- and inter-isolate matings of four tester monokaryons from each of six *Typhula idahoensis* dikaryotic isolates and two additional monokaryons. Compatibility is represented by +, incompatibility by 0.

		Incompatibility loci and alleles																																	
		A_1B_1	A_2B_2	A_1B_2	A_2B_1	A_1B_1	A_2B_2	A_1B_2	A_2B_1	A_1B_1	A_2B_2	A_2B_1	A_1B_2	A_3B_3	A_4B_4	A_3B_4	A_4B_3	A_3B_3	A_5B_5	A_3B_5	A_5B_3	A_6B_6	A_2B_5	A_6B_5	A_2B_6	A_7B_6	A_7B_6									
		Monokaryons																																	
		P1-20	P1-14	P1-1	P1-4	D2-4	D2-8	D2-3	D2-2	D4-9	D4-4	D4-5	D4-8	E-7	E-9	E-1	E-2	F-4	F-5	F-6	F-3	G-1	G-6	G-2	G-3	P3-1	P3-2									
A_1B_1	P1-20	0																																	
A_2B_2	P1-14	+	0																																
A_1B_2	P1-1	0	0	0																															
A_2B_1	P1-4	0	0	+	0																														
A_1B_1	D2-4	0	+	0	0	0																													
A_2B_2	D2-8	+	0	0	0	+	0																												
A_1B_2	D2-3	0	0	0	+	0	0	0																											
A_2B_1	D2-2	0	0	+	0	0	0	+	0																										
A_1B_1	D4-9	0	+	0	0	0	+	0	0	0																									
A_2B_2	D4-4	+	0	0	0	+	0	0	0	+	0																								
A_1B_2	D4-5	0	0	0	+	0	0	0	+	0	0	0																							
A_2B_1	D4-8	0	0	+	0	0	0	+	0	0	0	+	0																						
A_3B_3	E-7	+	+	+	+	+	+	+	+	+	+	+	+	0																					
A_4B_4	E-9	+	+	+	+	+	+	+	+	+	+	+	+	+	0																				
A_3B_4	E-1	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0																			
A_4B_3	E-2	+	+	+	+	+	+	+	+	+	+	+	+	0	0	+	0																		
A_3B_3	F-4	+	+	+	+	+	+	+	+	+	+	+	+	0	+	0	0	0																	
A_5B_5	F-5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0																
A_3B_5	F-6	+	+	+	+	+	+	+	+	+	+	+	+	0	+	0	+	0	0	0															
A_5B_3	F-3	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	0	0	0	+	0														
A_6B_6	G-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0													
A_2B_5	G-6	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	0	0	+	+	0													
A_6B_5	G-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	+	0	0	0											
A_2B_6	G-3	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	+	+	0	0	+	0										
A_7B_6	P3-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	0	0									
A_7B_6	P3-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	0	0	0								

investigated. Some taxonomic implications from these studies are also discussed.

MATERIALS AND METHODS.—*Mating experiments.—Typhula idahoensis* and *T. incarnata* sclerotia were collected from molded wheat plants from several locations in Washington and placed outdoors at Pullman. From each field collection of sclerotia a mature sporophore growing from a sclerotium was suspended over PDA. Single basidiospores were isolated by micromanipulation, transferred to PDA tubes, and stored at 1C.

The six sporophores (dikaryotic isolates) and the number of monobasidiospore isolates (monokaryons) from each were: P1, 19; D2, 8; D4, 12; E, 10; F, 10; G, 10. Sporophores P1, D2, D4, E, and F were from sclerotia collected near Waterville, Douglas County, Washington. Sporophores D2 and D4 were from two sclerotia of the same collection; Sporophore G was from a sclerotium collected near Grand Coulee, Lincoln County, Washington. Similar to most Hymenomycetes, dikaryons of *T. idahoensis* and *T. incarnata* have two nuclei per cell and clamp connections. Monokaryons have cells with transverse septa lacking clamps, and with one nucleus per cell.

Pairings were made by placing hyphae from two monokaryons 1 cm apart in a petri dish containing PDA. After 10-30 days at 10 C, hyphae from the junction of the colonies were stained with cotton blue in lactophenol

and examined at ×562 magnification. Clamp connections in the hyphae indicated a compatible reaction, and their absence, an incompatible reaction.

Three *T. incarnata* sporophores and the locations where sclerotia were collected were: K, Hartline, Grant County; L, Leahy Junction, Douglas County; and M, Wilbur, Lincoln County. Ten monobasidiospore isolates per sporophore were paired in all combinations following the procedures used for *T. idahoensis* and examined for clamp connections.

Four mating types were identified from each group of monokaryons of the six *T. idahoensis* and three *T. incarnata* dikaryons. Four tester isolates, representing each of the four mating types per dikaryon, were selected and mated in all combinations within each species. Two additional monokaryons, P3-1 and P3-2, from another sporophore of *T. idahoensis* collection P were also mated to tester monokaryons of the other isolates. Sclerotia of *T. idahoensis* compatible cross P1-4×D4-5 (designated isolate PD) were placed outdoors and 10 monobasidiospore isolates (F_2 progeny) from one of the resultant sporophores were paired in all combinations. One tester isolate from each of the four mating types identified was backcrossed to the P1 and D4 tester isolates. Lastly, *T. idahoensis* dikaryon P1 testers were paired in all combinations with the testers of the three *T. incarnata* dikaryons.

To determine the nuclear condition of basidiospores, mature sporophores were placed on glass slides in petri dish moist chambers. After 24 and 48 hr at 10 C, samples of the ejected basidiospores were stained with Giemsa stain (12). The number of nuclei per spore was counted at ×594 magnification.

Sporophore production.— Sclerotia from field-collected isolates, monokaryons, and compatible and incompatible pairings of monokaryons were produced on a sterilized wheat kernel medium (150 g wheat: 150 ml water) or a sand-bran medium (1). The sclerotia were placed outdoors on soil in September. By mid-November, sporophore production was in full progress and samples were collected for sporophore measurement.

Growth rate of monokaryons and dikaryons.— The linear growth rate of four dikaryotic, field-collected sclerotial isolates, 12 monobasidiospore isolates from sporophores produced by these sclerotia, and eight compatible matings of monokaryons were compared. A 1.0 mm^2 portion of hyphae from the edge of a 12- to 15-day-old culture of each isolate, grown at 10 C, was transferred to a petri dish containing 25 ml PDA. There were eight replications per treatment. After incubation at −1, 1, 5, 10, 15, and 20 C in the dark for 20 days, colony diam was measured.

Effect of nuclear condition and growth rate on disease development.— Four pots, each containing three 12-week-old plants of winter wheat, *Triticum aestivum* L. cultivars 'Burt', 'Moro', and 'Wanser', were inoculated with 50 cc of fresh wheat kernel medium containing sclerotia. The isolates used were four monokaryons, four synthesized dikaryons, and one field-collected dikaryon. The plants were covered with moist cotton and incubated at 1 C for 48 days. Following incubation, the pots were placed in a 10 C greenhouse with natural light and the fresh weight of all living above-ground plant material and the number of dead plants was determined after 15 days of regrowth.

RESULTS. — *Mating experiments.* — The selected monokaryons from each of the six sporophores were arbitrarily assigned to one of four distinct incompatibility groups, showing that *T. idahoensis* has tetrapolar incompatibility. From the monokaryons of each of the six sporophores, four tester monokaryons representing each incompatibility group were selected and paired in all combinations (Table 1). Dikaryons P1, D2, and D4 all have incompatibility alleles A_1, A_2, B_1, and B_2. Tester monokaryons of dikaryons E and F were compatible with the testers of the above three dikaryons. The pairings between testers of isolates E and F gave incompatible reactions in seven of 16 pairings. These results indicated that dikaryons E and F have the alleles A_3 and B_3 in common. For the other allele, dikaryon E has A_4B_4 and dikaryon F has A_5B_5. Dikaryon G testers produced incompatible

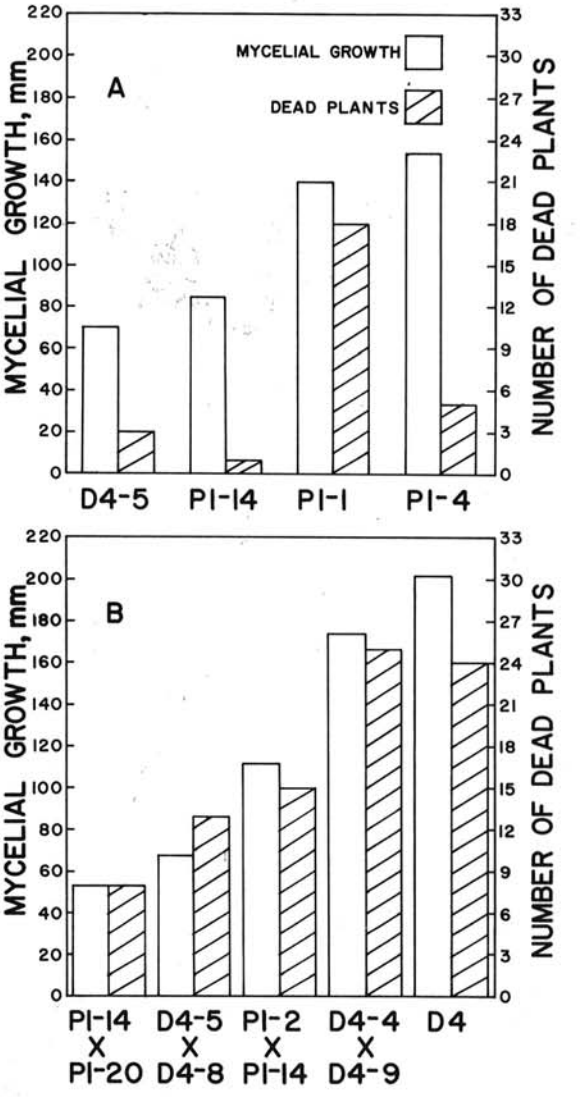

Fig. 1. Colony diameter of *Typhula idahoensis* after 20 days on potato-dextrose agar at different temperatures. Parent dikaryons x-x; dikaryons from mated monokaryons •-•; monokaryons Δ-Δ.

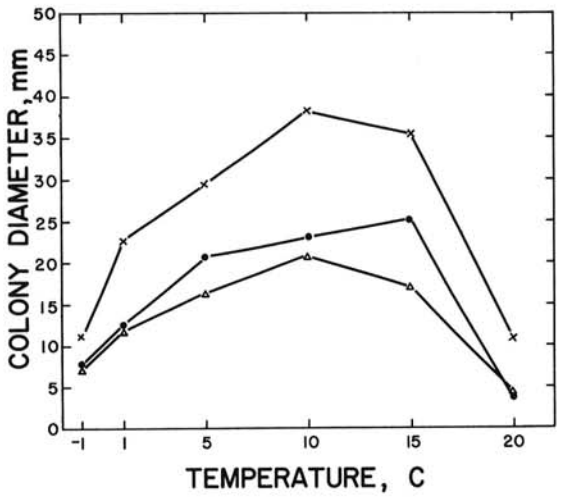

Fig. 2-A, B. Relation of growth rate in vitro at 1 C for 20 days to virulence of *Typhula idahoensis* on wheat. **A)** monokaryons and **B)** dikaryons. Thirty-six inoculated plants per treatment and incubated for 48 days.

reactions that were consistent with the allele assignments A_6B_6, A_2B_5, A_6B_5, and A_2B_6. The two P3 monokaryons were assigned alleles A_7B_6. Thus, in the six collections, seven A alleles and six B alleles were identified.

Alleles A_1B_1, A_2B_2, and their recombinants were identified in the F_2 progeny of isolate PD in backcrosses to the tester monokaryons of the parent dikaryons.

Typhula incarnata is also tetrapolar. Inter-isolate matings of the tester monokaryons produced compatible reactions in every instance. The three dikaryons were assigned alleles A_1B_1 to A_6B_6.

Attempted matings between the testers of the three *T. incarnata* dikaryons and *T. idahoensis* dikaryon P1 testers were noncompatible in every case.

In compatible matings, nuclear migration into monokaryotic hyphae was limited mostly to hyphae within 1.0 cm of the line of junction of the two colonies. Beyond that point, older hyphae remained monokaryotic. The dikaryon was stable when hyphae from the junction of the two compatible monokaryons was transferred to fresh medium.

Nuclear condition of basidiospores.— Twenty-four hr after basidiospore deposition, 99% of 5,136 spores had one nucleus; 1% had two nuclei per spore. Forty-eight hr after deposition, 86.4, 12.9, and 0.7% of 3,291 ungerminated basidiospores had one, two, and three nuclei, respectively. The change from the uninucleate condition is presumed to be the result of mitotic divisions. Basidiospores germinated whether they had one or more nuclei and all cells of the hyphae contained one nucleus per cell.

Sporophore development outdoors. — Seven monokaryons produced sterile sporophores and four others produced none. In some monokaryons only dark brown-black stipes 0.5 - 3.0 mm in length were formed. In three monokaryons, P1-1, D2-3, and D4-5, sporophores formed which were often greatly branched and bore many dentate projections at the apices. They were similar to those figured by Tomiyama (13) of monokaryotic sporophores of *T. incarnata*. The colors of these abnormal sporophores varied from creamy-white to the fawn color of normal sporophores. Because of the frequent lobing the range of dimensions was variable (0.7-5.0×0.2-2.8 mm). The average dimensions were 1.0×2.2 mm. Sclerotia from nine incompatible pairings produced only sterile sporophores. Sclerotia from four compatible matings produced typical fertile sporophores.

Cultural characters of monokaryons and dikaryons. — After 20 days on PDA at −1 to 20 C, monokaryons and dikaryons of *Typhula idahoenis* differed primarily in sclerotia production. Dikaryons produced sclerotia 1–2 mm in diameter that often coalesced into mounds. Most monokaryons produced fewer sclerotia than did dikaryons and the sclerotia from monokaryons were about one-third to two-thirds the size of dikaryotic sclerotia. Some monokaryons produced no or only a few sclerotia, whereas others produced sclerotia nearly the same size as dikaryons. In all isolates, sclerotium development was greatest at 10 and 15 C. Sclerotia were produced at 5 C by most dikaryons but by only a few monokaryons. At 1 C, only a few isolates of both genetic conditions had mature or immature sclerotia. At −1 C, no isolates produced sclerotia in the 20-day period.

Both monokaryons and dikaryons were generally hyaline. Occasional monokaryotic isolates from sporophore G produced a deep-brown pigment. Colony color was constant from −1 to 15 C. At 20 C, the mycelium in all cultures was very dense and creamy-white to dark brown. Few sclerotia developed at 20 C and these were usually quite small.

Growth rate in vitro. — For all isolates, 10 and 15 C were optimum for mycelial growth (Fig. 1). Between 15 and 20 C, growth declined rapidly. From 10 to −1 C growth decreased such that at −1 C growth was only slightly faster than at 20 C. From −1 to 20 C, field-collected dikaryons generally grew faster than monokaryons and dikaryons synthesized from monokaryons. Growth rates varied widely within each group; however, and there was frequent overlapping of individual isolates among groups. For example, the fastest-growing monokaryons, P1-1 and P1-4, grew as fast as some of the fastest-growing synthesized dikaryons and these nearly equaled the slowest-growing field-collected dikaryon. Seven of twelve monokaryons grew faster than the slowest synthesized dikaryons over the 5-15 C range.

Relation of growth rate to virulence. — Among monokaryons, there was no correlation between growth rate and virulence (Fig. 2-A). The fastest-growing monokaryotic isolate, P1-4, and two isolates which grew only half as fast at 1 C (P1-14 and D4-5), were equally pathogenic. Among dikaryons (Fig. 2-B), there was a general increase in number of dead plants as growth rate increased. Dikaryons, as a group, were more virulent than monokaryons. However, just as in the growth rate experiment, monokaryon P1-1 was as virulent as several dikaryons. Fresh weights of plant regrowth also reflected these results. Similar results were obtained with each of three wheat cultivars. All control plants remained healthy.

Monokaryons that produced few sclerotia in culture did likewise on wheat, but those that produced small sclerotia in vitro produced sclerotia on wheat that were indistinguishable from those of dikaryotic isolates.

DISCUSSION.—*Typhula idahoensis* has tetrapolar incompatibility just as do other *Typhula* species (5, 6, 11, 13) and the majority of other Hymenomycetes (9, 14) which have been studied. Dikaryons D2 and D4, derived from the same field collection, have identical alleles for incompatibility as does dikaryon P1, collected 0.3 km from D2 and D4. Dikaryon F, on the other hand, collected on the opposite side of the road from P1, has a different pair of incompatibility alleles, as do monokaryons P3-1 and P3-2 which were taken from the same collection as P1. In contrast, dikaryon E, collected 14.5 km east of the above collections, has allele A_3B_3 in common with dikaryon F. Dikaryon G, collected 80 km east of all other dikaryons, has allele A_2 (which is also found in dikaryons P1, D2, and D4), and allele B_5, also found in dikaryon F. In addition, dikaryon G and monokaryons P3-1 and P3-2 have allele B_6 in common. Not one collection of this relatively small sample has alleles which it does not share with at least one other collection. This indicates that the basidial stage has been active, permitting intermixing of genetic material. Outcrossing is highly favored. These data concur with results of basidiospore inoculations by the author (3), that the basidiospores function in the life cycle of *T. idahoensis*.

The sexual incompatibility of *Typhula incarnata* from Washington is identical to that found in Japan (13) and Norway (11). The fungus has tetrapolar incompatibility with multiple alleles. No incompatibility alleles are common to any of the three collections which cover the same geographic area as that sampled for *T. idahoensis*. A larger sample may reveal some common alleles. Since *Typhula incarnata* is widespread throughout the northern regions of the earth and several sets of alleles are known from Europe (11), collections from much wider geographic locations should be studied as a means of taxonomic comparison and a better understanding of the genetics of the fungus.

When *T. idahoensis* and *T. incarnata* monokaryons were mated, a noncompatible reaction occurred each time. Thus, the genetic data support morphological data (2, 10), that these species are distinct.

As with *T. incarnata* (13) and most Hymenomycetes (9), normal fruiting by *T. idahoensis* is associated with the dikaryon. *Typhula trifolii* (8) is the only *Typhula* species in which fertile monokaryotic fruiting bodies occur and it is the only species known to produce basidiospores in aseptic culture.

Mycelial growth rate influences virulence but the two are not dependent upon one another. A correlation between growth rate and virulence is evident among dikaryotic isolates. All field-collected isolates grew faster than synthesized dikaryons. For a newly synthesized dikaryon from compatible basidiospores or the union of a monokaryon and a dikaryon to compete, it must invade a plant as quickly as hyphae from sclerotia. Therefore, rapid mycelial growth is favored in nature.

Monokaryons or newly formed dikaryons should have the greatest chance for survival where sclerotial inoculum is very low or absent. Based on growth rate and virulence data, some monokaryons should survive in nature. However, in over 300 isolations from field-collected sclerotia, none without clamp connections has been found. Similar results have been found for *T. incarnata* (13).

These studies prove that the basidial stage of *Typhula idahoensis* increases the potential for variation. Outcrossing is favored in both *Typhula idahoensis* and *T. incarnata*. Much variability exists for mycelial growth rate and virulence among monokaryons and dikaryons. Monokaryotic hyphae must re-establish the dikaryon to compete successfully for substrate.

LITERATURE CITED

1. BRUEHL, G. W., R. SPRAGUE, W. R. FISCHER, M. NAGAMITSU, W. L. NELSON, and O. A. VOGEL. 1966. Snow molds of winter wheat in Washington. Wash. Agric. Exp. Stn. Bull. 677. 21 p.
2. CORNER, E. J. H. 1950. A monograph of Clavaria and allied genera. Oxford Univ. Press, London. 740 p.
3. CUNFER, B. M., and G. W. BRUEHL. 1973. Role of basidiospores as propagules and observations on sporophores of Typhula idahoensis. Phytopathology 63:115–120.
4. DEJARDIN, R. A., and E. W. B. WARD. 1971. Growth and respiration of psychrophilic species of the genus Typhula.Can. J. Bot. 49:339–347.
5. KNIEP, H. L. 1920. Über morphologische und physiologische Geschlechtsdifferenzierung. (Untersuchungen an Basidiomyzeten). Verh. phys.-med. Ges. Würzburg. 46:1-18.
6. LEHFELDT, W. von. 1923. Über die Entstehung des Paarkernmycels bei heterothallischen Basidiomyceten. Hedwigia 64:30-51.
7. MACDONALD, J. A. 1934. The life history and cultural characteristics of Typhula gyrans (Batsch) Fries. Ann. Appl. Biol. 21:590-613.
8. NOBLE, MARY. 1937. The morphology and cytology of Typhula trifolii Rostr. Ann. Bot. 1:67-98.
9. RAPER, J. R. 1966. Genetics of sexuality in higher fungi. The Ronald Press Co., New York. 283 p.
10. REMSBERG, RUTH E. 1940. Studies in the genus Typhula. Mycologia 32:52-96.
11. RØED, H. 1969. On the relationship between Typhula graminum Karst. and Typhula incarnata Lasch ex Fr. Friesia 9:219-225.
12. ROGERS, J. D. 1965. The condidial stage of Coniochaeta ligniaria: morphology and cytology. Mycologia 57:368-378.
13. TOMIYAMA, K. 1955. Studies on the snow blight disease of winter cereals [in Japanese, English summary]. Report 47, Hokkaido Nat. Agric. Exp. Stn. 234 p.
14. WHITEHOUSE, H. L. K. 1949. Multiple-allelomorph heterothallism in the fungi. New Phytol. 48:212-244.