Sexual Incompatibility and Virulence in Typhula idahoensis

R. K. Kiyomoto and G. W. Bruehl

Former Research Assistant and Professor, respectively, Department of Plant Pathology, Washington State University, Pullman 99163. Present address of senior author, Del Monte Corporation, P.O. Box 36, San Leandro, CA 94577.

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ABSTRACT

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Mating-type frequency determined for 35 to 687 monokaryons from three to 13 sporophores of four *Typhula idahoensis* field dikaryons revealed tetrapolar incompatibility in all isolates, and each sclerotial isolate yielded only four mating types. Matings of tester monokaryons from the four field dikaryons revealed five A alleles and four B alleles. Tests for virulence of six *T. idahoensis* field dikaryons on four winter wheats that differ in resistance revealed great differences in virulence on a given wheat. Since pathogen isolates showed no differential virulence to host cultivars, there is no evidence for a gene-for-gene host-pathogen relationship. The genetic basis of virulence was tested by forming 129 dikaryons from 28 monokaryons of field dikaryon 5999-5. Host survival following inoculation with these F_1 progeny indicated that the parental dikaryon was heterozygous for several genes which determine virulence. A complete spectrum of virulence was observed in F_1 and F_2 dikaryons. All monokaryons were avirulent. Only a weak correlation was found between dikaryon growth rate on culture media and virulence on the winter wheat cultivar Nugaines. Because all F_1 and F_2 dikaryons that were tested contained four chromosomes carrying the same complement of incompatibility genes, it is likely that incompatibility genes are not closely linked to virulence genes.

Typhula idahoensis Remsberg, an important snow mold fungus, is well suited for genetic studies because monokaryons and dikaryons can be grown on culture media and the sexual stage can be produced in the laboratory or under natural conditions in the autumn (7, 10). Heterokaryosis can be inferred from variation among the progeny derived from a single sexual fruiting body. Dikaryons formed by compatible monokaryons produce clamp connections (6). Because incompatibility between monokaryons is determined by complementary factors at two loci (6), the incompatibility alleles are convenient genetic markers. Basidiospores have a limited potential to serve as inoculum for the fungus; however, the presence of common incompatibility alleles among field dikaryons of *T. idahoensis* collected from Douglas County, Washington, has been interpreted as evidence that the sexual stage has been active (7).

Data on the virulence of the monokaryotic phase of *Typhula* are limited. Monokaryons of *T. incarnata* Lasch ex Fr. usually attack wheat and rye leaves more slowly than dikaryons, and they grow more slowly than dikaryons on artificial media (11). In *T. idahoensis* Cunfer (6) found no correlation between mycelial growth rate and virulence to winter wheat among monokaryons, but virulence increased with increased growth rate among dikaryons. Dikaryons of *T. idahoensis* generally were more virulent than monokaryons, but the limited data suggested that monokaryon virulence could approach the virulence of dikaryons (6).

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In low-temperature chamber studies (5), field isolates of *T. incarnata, T. idahoensis,* and *Fusarium nivale* (Fr.) Ces. have given little evidence of pathogenic specialization on winter wheats (2, 3). Such results would be expected if resistance to these fungi is polygenic (5). The present work investigated the sexual incompatibility and virulence of field dikaryons of *T. idahoensis,* attempted to determine whether genes for virulence in *T. idahoensis* are few or many and dominant or recessive, and studied the relationship of monokaryon and dikaryon virulence to in vitro growth rates on culture media.

MATERIALS AND METHODS

Determination of mating types.—Mating-type frequencies were determined for four field dikaryons to evaluate the genetic purity of single-sclerotium isolations from field collections. Dikaryotic cultures of *T. idahoensis* collected from Douglas and Okanogan Counties, Washington, were obtained from single sclerotia (= field dikaryons). Additional sclerotia were produced from each field dikaryon by growth on a sand, bran, and dextrose medium in darkness at 10 C (5). Basidiocarp formation was induced by placing sclerotia outdoors in late September (4). Single basidiospore cultures were obtained from individual basidiocarps secured with tape to the inside of the lid of a petri dish and allowing basidiospores to shower onto water agar. Spores were collected for approximately 10 hours in darkness at 10 C. Spore-laden portions of agar were removed, and

shaken in 5 ml of distilled water, and the spore suspension was spread sequentially onto three dishes of solid corn meal yeast extract agar (CMYEA) medium consisting of BBL corn meal agar (Becton, Dickinson and Co., Cockeysville, Maryland) supplemented with yeast extract (0.2%). The pH was adjusted with 1N NaOH to 6.5. After approximately 7 days in darkness at 10 C, germinated basidiospores (observed under × 30 magnification) were transferred individually to separate CMYEA dishes and allowed to grow in darkness at 10 C. After 1-2 weeks, putative monokaryons were examined for hyphal clamp connections. Cultures containing clamp connections and monokaryons that grew extremely slowly were discarded.

Monokaryons were obtained from four different field dikaryons (Table 1). Mating type was determined by mating at least 12 monokaryons from a single sporophore in all possible combinations. From these, monokaryon testers of each of the four possible mating types from each sporophore were selected, and testers were mated with all monokaryons from other basidiocarps of the same field dikaryon. All pairings between monokaryons were made on CMYEA according to methods described by Cunfer (6). In *T. idahoensis,* A and B incompatibility factors are designated arbitrarily because we know of no way to distinguish them (4). Crosses were made in all possible combinations between tester monokaryons of each field dikaryon to distinguish common incompatibility alleles.

Pathogenic interactions.—Six *T. idahoensis* field dikaryons were tested for virulence on four winter wheats that differ in snow mold resistance. Sclerotial inoculum was produced on wheat kernel medium in 0.95-liter (1-quart) jars after 40-60 days of incubation in darkness at 10 C (9). Approximately 40-50 cm^3 of fresh inoculum (wheat kernels and sclerotia) were used to inoculate each pot of host plants. Ten pots of each wheat were inoculated with each field dikaryon. Hosts included the resistant winter wheats C. I. 9342 and C. I. 14106, the moderately resistant wheat Moro (C. I. 13740), and the susceptible wheat Nugaines (C. I. 13968). Seeds were planted 3-4 cm deep in a mixture (1:1, v/v) of Palouse silt loam and sand in 15.3-cm diameter clay pots, eight seeds per pot. Wheats were cold-hardened outdoors in sand beds for 60 days before inoculation. After incubation for 50 days in darkness at 0.5 C, plants were transferred to the greenhouse where they were allowed to recover at 10-15 C under natural light. Recovery was evaluated after 30 days by scoring the number of surviving plants per pot.

TABLE 1. Number of sporophores used to obtain monokaryons and number of monokaryons recovered from five field dikaryons of *Typhula idahoensis*

Field dikaryons	Number of sporophores	Number of monokaryons
6258	8	0
5999-5	3	58
6299-B	5	35
69-3M	6	124
6274	13	687

Inheritance of virulence and growth rate.—The inheritance of virulence in *T. idahoensis* was tested by forming 129 F_1 dikaryons from 28 monokaryons of mating types A_1B_1 and A_2B_2 of field dikaryon 5999-5. Monokaryons and dikaryons were tested for virulence on a minimum of 55 Nugaines plants which had been cold-hardened under natural conditions for approximately 50 days. The inoculation and incubation procedures were conducted as described elsewhere (5).

Radial growth on solid media was studied by growing 26 of the 28 monokaryons on Difco corn meal agar (Difco, Detroit, Michigan) supplemented with yeast extract (0.2%) and on Difco potato-dextrose agar. A series of F_1 dikaryons formed from the 5999-5 monokaryons was similarly studied. Dishes were inoculated with 3-mm diameter circular plugs of agar taken from the edge of colonies grown on water agar for 27 days at 10 C. All inoculations were in duplicate, and incubation was in darkness at 10 C.

The synthesized $(A_1B_1)/(A_2B_2)$ dikaryons from monokaryons of field dikaryon 5999-5 have four chromosomes in common because the incompatibility loci are not linked. By forming dikaryons from monokaryons of a single field dikaryon, the importance

TABLE 2. Frequency of incompatibility alleles among monokaryons from *Typhula idahoensis* field dikaryons 5999-5, 6274, 6299-B, and 69-3M

Mating-type loci and incompatibility alleles[a]	Monokaryons from *Typhula idahoensis* field dikaryons							
	5999-5		6274		6299-B		69-3M	
	Number	Frequency	Number	Frequency	Number	Frequency	Number	Frequency
A_1B_1	14	0.24	163	0.24	0	0	0	0
A_1B_2	13	0.22	0	0	0	0	0	0
A_1B_3	0	0	170	0.25	0	0	0	0
A_2B_1	14	0.24	175	0.25	0	0	0	0
A_2B_2	17	0.29	0	0	0	0	0	0
A_2B_3	0	0	179	0.25	0	0	0	0
A_3B_1	0	0	0	0	11	0.31	0	0
A_3B_2	0	0	0	0	7	0.20	0	0
A_4B_1	0	0	0	0	11	0.31	0	0
A_4B_2	0	0	0	0	6	0.17	35	0.28
A_4B_4	0	0	0	0	0	0	26	0.21
A_5B_2	0	0	0	0	0	0	34	0.27
A_5B_4	0	0	0	0	0	0	29	0.23
Total monokaryons	58		687		35		124	

[a]A,B, denote mating-type loci; 1,2, etc. subscripts denote incompatibility alleles.

of the mating-type chromosomes in determining virulence and growth rate and the dominant or recessive nature of the genes can be determined.

Segregation of virulence was studied in six monokaryons derived from a 5999-5 F_1 dikaryon. Host plants were hardened under natural conditions for 80 days prior to inoculation and incubated in darkness at 1 C for 60 days.

RESULTS

Determination of mating types.—Each field dikaryon yielded four mating types in approximately equal frequencies (Table 2). Deviation from frequencies expected among mating types of field dikaryon 6299-B was probably due to the small sample (only 35 monokaryons) studied. The sample sizes (i.e., number of monokaryons) in Tables 1 and 2 reflect the relative ease with which monokaryons were obtained. Isolate 6274 produced basidiocarps readily, its basidiocarps gave dense spore showers, and its basidiospores germinated and produced vigorous monokaryons in 1 week. Monokaryons of other isolates often required 2 weeks to produce visible growth from basidiospores. Sporophores of isolate 6258 produced spore showers, but no monokaryons were obtained from washings of the agar block bearing the initial spore shower. Dikaryons were produced on the 6258 spore shower block itself, however.

Mating reactions of four tester monokaryons from field dikaryons 5999-5, 6274, 6299-B, and 69-3M revealed five A and four B incompatibility alleles (Tables 2, 3). Field dikaryons 5999-5 and 6274 differed in only one incompatibility allele, and dikaryons 6274 and 69-3M had no incompatibility alleles in common. Each dikaryon had alleles in common with at least two of the three other field dikaryons.

Pathogenic interactions.—Field dikaryons differed widely in virulence on a given wheat (Table 4), but all

TABLE 3. Intra- and inter-isolate mating-type monokaryons from each of four *Typhula idahoensis* field dikaryons

Monokaryon source	Monokaryons	Incompatibility loci and alleles[a]	5999-5 Monokaryons				6274 Monokaryons				6299-B Monokaryons				69-3M Monokaryons			
			1	2	4	3	13	15	16	14	9	10	11	12	5	6	7	8
			Incompatibility loci and alleles															
			A_1 B_1	A_1 B_2	A_2 B_1	A_2 B_2	A_1 B_1	A_1 B_3	A_2 B_1	A_2 B_3	A_3 B_1	A_4 B_1	A_3 B_2	A_4 B_2	A_4 B_4	A_5 B_4	A_4 B_2	A_5 B_2
5999-5	1	A_1 B_1[a]	0	0	0	+[b]												
	2	A_1 B_2	0	0	+	0												
	4	A_2 B_1	0	+	0	0												
	3	A_2 B_2	+	0	0	0												
6274	13	A_1 B_1	0	0	0	+	0	0	0	+								
	15	A_1 B_3	0	0	+	+	0	0	+	0								
	16	A_2 B_1	0	+	0	0	0	+	0	0								
	14	A_2 B_3	+	+	0	0	+	0	0	0								
6299-B	9	A_3 B_1	0	+	0	+	0	+	0	+	0	0	0	+				
	10	A_4 B_1	0	+	0	+	0	+	0	+	0	0	+	0				
	11	A_3 B_2	+	0	+	0	+	+	+	+	0	+	0	0				
	12	A_4 B_2	+	0	+	0	+	+	+	+	+	0	0	0				
69-3M	5	A_4 B_4	+	+	+	+	+	+	+	+	+	0	+	0	0	0	0	+
	6	A_5 B_4	+	+	+	+	+	+	+	+	+	+	+	+	0	0	+	0
	7	A_4 B_2	+	0	+	0	+	+	+	+	+	0	0	0	0	+	0	0
	8	A_5 B_2	+	0	+	0	+	+	+	+	+	+	0	0	+	0	0	0

[a]A,B, denote mating-type loci; 1,2, etc. subscripts denote incompatibility alleles.
[b]+ = compatible mating, 0 = incompatible mating.

TABLE 4. Survival of Nugaines, Moro, C. I. 9342, and C. I. 14106 wheats following inoculation and incubation with six field dikaryons of *Typhula idahoensis*

Wheat	Plant survival (%)[a] after inoculation with *T. idahoensis* field dikaryons						
	6274	6258	69-3M	5999-5	Goldmark	6299-B	Non-inoculated
Nugaines	0	5	12	22	44	91	95
Moro	32	21	73	72	93	96	97
C.I. 9342	53	70	94	98		98	100
C.I. 14106	98	100	91	98		98	100

[a]Survival determined in 10 inoculated pots (replicates) containing approximately seven plants per pot.

showed the same trend on wheats of differing resistance levels, with no differential virulence to host cultivars.

Virulence of synthesized 5999-5 F_1 dikaryons was determined by both parental monokaryons (Table 5). The data are presented in terms of percentages of surviving plants. The means (8-10 replications) for each dikaryon are different at the 0.01 level of significance with a standard error of treatment means (s_x) of 9.2% ($LSD_{0.05}$ = 25%).

The parent dikaryon killed 78% of the test plants. Fifty-three (37%) F_1 dikaryons were more virulent (85-100% kill), 16 (11%) F_1 dikaryons were equal in virulence (70-84% kill) to the parent dikaryon, 47 (32%) were moderately to weakly virulent (20-69% kill), and 27 (19%) F_1 dikaryons were essentially avirulent (less than 20% kill).

Virulence of monokaryons was not related to radial growth on either CMYEA or PDA (Fig. 1), but a slight relationship between the rate of radial growth of dikaryons on CMYEA and PDA and virulence was indicated (Fig. 2).

Progeny obtained from the F_1 dikaryon formed between 5999-5 monokaryons 27 and 71 further illustrate (Table 6) that virulence is influenced by particular combinations of monokaryons, and not by genes carried by a single monokaryon. Only one dikaryon (8 × 11) in this small F_2 sample was as virulent as the parent from which it was derived. Genes for virulence were still segregating in the F_2.

DISCUSSION

Each of the original field dikaryons was a homogeneous dikaryon, for only four incompatibility alleles were found in each (Table 2). Matings among the tester monokaryons from the field dikaryons revealed five A alleles and four B alleles (Table 3). These results confirm earlier findings that a relatively small sample of dikaryons from a limited geographical area may possess both common and different incompatibility alleles (7). There is wide variability in ease of recovering monokaryons. Field dikaryon 5999-5 was successfully used for genetic studies of virulence, but it proved to be a poor subject for studying the sexual stage. Field dikaryon 6274 produced a very active sexual stage and vigorous monokaryons.

Field dikaryon 5999-5 appears to have a number of loci determining virulence, and virulence is not governed by a single dominant gene in most monokaryons at least, since most monokaryons produced both virulent and avirulent dikaryons (Table 5). Monokaryons 9, 73, 76, and 77 (Table 5) produced only virulent dikaryons in

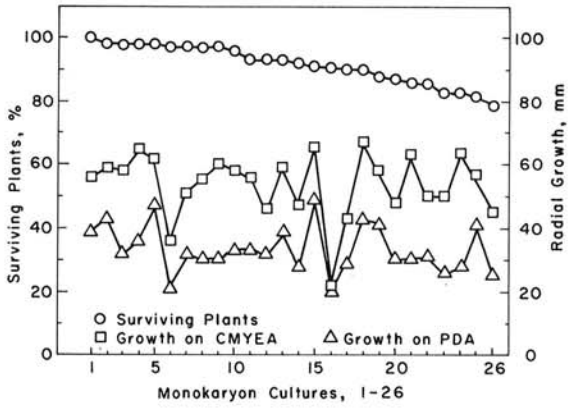

Fig. 1. Growth of 26 different 5999-5 monokaryons of *Typhula idahoensis* after 21 days incubation in darkness at 10 C on potato-dextrose agar (PDA) and corn meal agar yeast extract (CMYEA), and virulence of the monokaryons on the winter wheat Nugaines. Radial growth (colony diameter) data represent the average growth on two dishes of each medium for each monokaryon culture. Virulence was determined on plants in six pots (replicates) containing seven to ten plants per pot.

TABLE 5. Segregation of virulence on the winter wheat cultivar Nugaines among F_1 dikaryotic progeny of *Typhula idahoensis* field isolate 5999-5[a]

	Source of dikaryons												
	A_1B_1 Monokaryons												
	1	12	16	22	27	29	72	73	75	76	77	78	79
A_2B_2 Monokaryons	Virulence, surviving plants (%)												
3	9	2	45	13	3	23	2						
5	0	95	33	92	21	97	83						
8	8	98	42	97	37	88	79						
9	5	30	3	15	2	5	0						
10	3	32	32	12	37	12	2						
19	56	98	3	90	22	83	10						
21	25	93	53	95	36	96	63						
24	63	45	19	45	7	15	3						
25	72	83	42	86	18	100	74						
26	31	25	50	32	58	47	7						
28	32	88	5	85	7	81	66						
30	33	86	68	56	25	70	68						
70	37	38	49	71	47	85	55	9	21	3	7	52	68
71	25	75	13	80	12	85	30	7	32	3	16	85	63
74			12	43	8	22	9	3	18	0	4	7	5
80			7	84	0	85	61	12	12	0	23	75	83
81			0	0	4	80	15	0	5	0	2	18	31

[a]Virulence of parental isolate 5999-5 is 79%.

TABLE 6. Virulence of field dikaryon 5999-5, of a synthesized F_1 dikaryon in the $(A_1B_1)/(A_2B_2)$ incompatibility configuration, and of F_2 $(A_1B_1)/(A_2B_2)$ dikaryons on Nugaines wheat

Inoculum	Plants killed (%)
5999-5 (field isolate)	78[a]
Synthesized F_1 dikaryon	79
Synthesized F_2 dikaryons	
3×2[b]	12
3×11	17
5×2	50
5×11	0
8×2	29
8×11	73
9×2	0
9×11	9
No inoculum	1

[a]Based on a total of 40-80 wheat plants per dikaryon.

[b]The numbers designate the monokaryons from the F_1 dikaryon used to synthesize the F_2 dikaryons. The F_1 dikaryon was derived by pairing monokaryons 27×71. (Table 5).

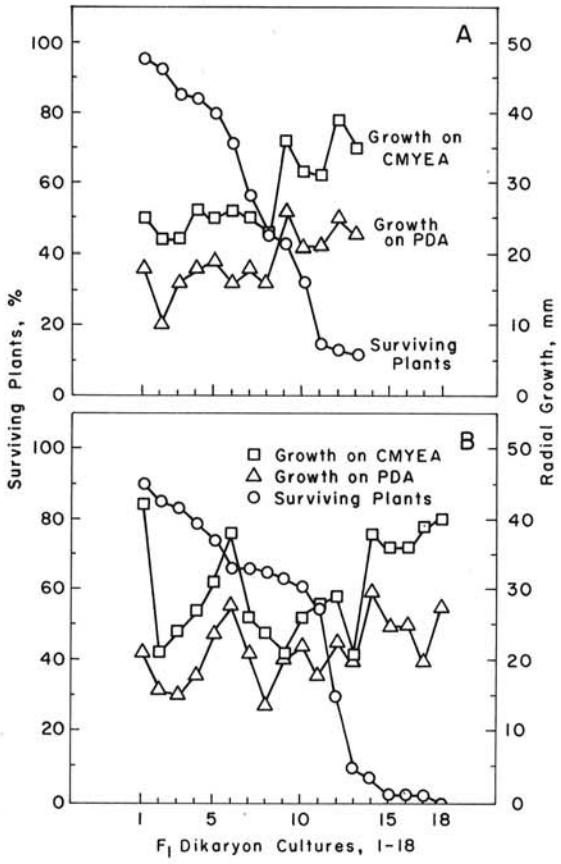

Fig. 2-(A, B). Growth of F_1 dikaryons synthesized from monokaryons of *Typhula idahoensis* field dikaryon 5999-5 after 13 days in darkness at 10 C on potato-dextrose agar (PDA) or on corn meal agar with yeast extract (CMYEA), and virulence of the dikaryons on the winter wheat Nugaines. **A)** Monokaryon 22 served as one parent in all crosses. **B)** Monokaryon 72 served as one parent in all crosses. Radial growth (colony diameter) and virulence determined in two culture plate- and six pot (each containing seven to ten plants) -replicates, respectively.

combinations with A_1B_1 monokaryons. Monokaryon 8 would have few genes for virulence because it forms only one virulent dikaryon when paired with A_1B_1 monokaryons.

There is no evidence for gene-for-gene interactions. The complete spectrum of virulence obtained from a field isolate is consistent with the idea that virulence is quantitatively inherited. Segregation of virulence in F_2 dikaryons of a synthesized F_1 dikaryon of field dikaryon 5999-5 proved further segregation of virulence (Table 6), and further supported the existence of several genes for virulence.

Virulence of F_1 dikaryons of the highly virulent field dikaryon 6274 also depends upon specific pairs of monokaryons (9). In 6274, 10 F_1 dikaryons were synthesized. One synthesized dikaryon was more virulent than the parent dikaryon and nine were avirulent. This is surprising because one might suspect that all genes for virulence in this highly virulent isolate would be homozygous. Segregation and recombination should have produced dikaryons of high virulence or intermediate reaction. Although the sample size was small, the data indicate that virulence is due to specific combinations of genes that may be allelic or additive in function.

The absence of pathogenicity in many dikaryons (Fig. 1 and Table 5) supports the hypothesis that genes for virulence are not linked to chromosomes marked by incompatibility genes. Virulence and rate of dikaryon growth data show that these two factors are not linked, but probably indicate that a minimum growth rate is needed for dikaryons to express a certain degree of virulence (Fig. 2).

No significant virulence has been observed in monokaryons (Fig. 1) (9) or in incompatible rairings (9). Only nine of 26 5999-5 monokaryons killed as many as 10-22% of their host plants (Fig. 1). These results differ from earlier studies which showed that a monokaryon of *T. idahoensis* could kill 50% of its host plants after 48 days of incubation (6). The finding of no correlation between monokaryon virulence and growth rate on culture media (Fig. 1) supports previous studies of Cunfer (6).

The evidence for multiple genes for virulence is logical if resistance to snow mold is conferred by multiple incompletely dominant genes (5, 9). These conclusions are in general agreement with genetic studies on several plant pathogenic fungi reviewed by Day (8) and Webster (12). Genes for resistance usually are dominant and genes for virulence usually are recessive (12). Bolkan and Butler (1) demonstrated that genes for virulence in *Rhizoctonia solani* Kuehn [= *Thanatephorus cucumeris* (Frank) Donk] are multiple and recessive. In *R. solani* avirulence is determined by the cumulative effect of dominant genes. *Typhula idahoensis,* unlike *R. solani,* lacks virulent monokaryons.

LITERATURE CITED

1. BOLKAN, H. A., and E. E. BUTLER. 1974. Studies on heterokaryosis and virulence of Rhizoctonia solani. Phytopathology 64:513-522.
2. BRUEHL, G. W. 1967. Lack of significant pathogenic specialization within Fusarium nivale, Typhula idahoensis, and T. incarnata and correlation of resistance

in winter wheat to these fungi. Plant Dis. Rep. 51:810-814.
3. BRUEHL, G. W. 1967. Correlation of resistance to Typhula idahoensis, T. incarnata, and Fusarium nivale in certain varieties of winter wheat. Phytopathology 57:308-310.
4. BRUEHL, G. W., R. MACHTMES, and R. KIYOMOTO. 1975. Taxonomic relationships among Typhula species as revealed by mating experiments. Phytopathology 65:1108-1114.
5. 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.
6. CUNFER, B. M. 1974. Sexual incompatibility and aspects of mono- and dikaryotic phases of Typhula idahoensis. Phytopathology 64:123-127.
7. CUNFER, B. M., and G. W. BRUEHL. 1973. Role of basidiospores as propagules and observations on sporophores of Typhula idahoensis. Phytopathology 63:115-120.
8. DAY, P. R. 1974. Genetics of host-parasite interaction. Freeman, San Francisco. 238 p.
9. KIYOMOTO, R. K. 1975. Role of soluble reducing sugars in winter wheat resistance to Typhula idahoensis. Part 1. Ph.D. Thesis, Washington State University, Pullman. 83 p.
10. REMSBERG, R. E. 1940. Studies in the genus Typhula. Mycologia 32:52-96.
11. TOMIYAMA, K. 1955. Studies on the snow blight disease of winter cereals [in Japanese, English summary]. Hokkaido Nat. Agric. Exp. Stn. Rep. 47. 234 p.
12. WEBSTER, R. K. 1974. Recent advances in the genetics of plant pathogenic fungi. Annu. Rev. Phytopathol. 12:331-353.