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Variation within isolates of *Typhula incarnata* from localities differing in winter climate

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Isolates of *Typhula incarnata*, a snow mold fungus, were collected from four localities with different winter climates. Their ecological traits such as mycelial growth rate, sclerotium size, carpogenic germination of sclerotia, and aggressiveness were compared between populations in order to reveal infraspecific differentiation associated with climatic differences. Population variability was evident only in sclerotium germination: isolates from more snowy localities germinated faster than those from less snowy localities. *T. incarnata* is regarded as a versatile pathogen with no specialized forms in contrast with *T. ishikariensis*. The germination rate of sclerotia is considered very critical in the life history of *T. incarnata*.

Key Words——sclerotium germination; specialization; *Typhula incarnata*; variation.

Typhula incarnata Lasch ex Fr. is a ubiquitous snow mold fungus (Bruehl et al., 1966; Matsumoto and Sato, 1983) and was regarded as synonymous with *T. graminum* Karst. and *T. itoana* S. Imai (McDonald, 1961). The former two species were interfertile (Røed, 1969), but Cavelier and Auquier (1980) recognized differences in mycelial growth, host range, and suitable climatic conditions between isolates of *T. incarnata* from Belgium and Switzerland. Many ecological traits of *T. incarnata* suggest that this fungus is versatile and not specialized in contrast with *T. ishikariensis* S. Imai, which shows infraspecific differentiation into specialists adapted to diverse winter climates (Matsumoto, 1992).

Typhula incarnata is not strongly virulent and, unlike *T. ishikariensis*, can colonize plant debris saprophytically (Matsumoto and Sato, 1982; Jacobs and Bruehl, 1986). *Typhula incarnata* produces sclerotia on subterranean plant parts as well as leaves (Bruehl et al., 1966; Matsumoto, 1989). Snow cover is not prerequiste for the fungus to prevail on plant tops (Jackson and Fenstermacker, 1969; Matsumoto, unpublished), and its basidiospores can infect plants (Hindorf, 1980; Matsumoto and Tajimi, 1985). This fungus has broader optimum growth temperature range than *T. ishikariensis* (Matsumoto, 1989). Thus, *T. incarnata*, as a species, can be regarded as a generalist; however, no one has ever examined infraspecific differentiation in this species to reveal the existance of specialized forms adapted to different winter climates.

This paper reports a comparative study of *T. incarnata* isolates collected from different climatic regions and concludes that sclerotial germination is more critically associated with different winter climates than other ecological traits in *T. incarnata*.

Materials and Methods

***Typhula incarnata* isolates used** Six isolates of *T. incarnata* were collected each from Nayoro, Sapporo, Toyama, and Yamaguchi. These localities differed in the number of days with snow cover per annum, i.e., Nayoro, more than 150 days; Sapporo, 120–150 days; Toyama, 60–90 days; and Yamaguchi, 10–20 days.

Mycelial growth Mycelial discs (5 mm diam) were removed with a cork borer from marginal growth of fresh PDA (potato-dextrose agar) cultures and placed with mycelial side down in the center of 9-cm diam PDA plates. Plates were incubated at 0, 7 and 12°C for 21 days or at −3°C for 40 days in duplicate, and mycelial growth rate per day was determined.

Mycelial growth in antagonism with soil microorganisms was determined as described previously (Matsumoto and Tajimi, 1988) with a slight modification. Unsterile field soil was passed through a 1-mm screen, adjusted to 30% water holding capacity, and stored in a polyethylene bag at room temperature until use. The edge of each PDA plate (9 cm diam) was inoculated with a 5-mm diam mycelial disc, and the surface was entirely covered with soil and incubated at 0, 7, or 12°C in duplicate. Cultures to be incubated at 0°C were pregrown at 5°C for 2 days before soil treatmet. After incubation for 21 days soil cover was removed, and radial mycelial growth was determined under the microscope after staining with cotton blue. The reaction of isolates to soil microbial antagonism was expressed as percent suppres-

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sion compared to untreated controls.

PDA cultures were grown at 12°C for 14 days in a CO_2 incubator adjusted at 2% (v/v) CO_2 concentration, which simulated CO_2 concentration under snow (Mariko et al., 1994). Results were presented as relative growth to controls, which were incubated in an ordinary incubator. Measurements were made in duplicate.

Sclerotium size One hundred sclerotia produced on pot-grown perennial ryegrass (*Lolium perenne* L.) plants, which had been inoculated with wheat bran-vermiculite culture and incubated under snow throughout winter, were collected in spring and photographed against a white background. Diameter of each sclerotium was randomly determined along the horizontal axis on a photograph.

Carpogenic germination of sclerotia Sclerotia produced on perennial ryegrass plants were passed through a 2-mm screen, and those remaining on a 1-mm screen were collected. Five sclerotia were placed in each well of a Nunc Multidish containing humid, unsterile soil. There were two germination regimes. One consisted of a 10-h photoperiod produced by fluorescent lights at 8°C and 14 h of darkness at 6°C (Matsumoto and Tajimi, 1990); the other was incubation outdoors in the shade at temperatures ranging from −4 to 14°C. Germination was observed daily under a dissecting microscope, and the emergence of a stipe was taken as the criterion of germination. Twenty-five sclerotia were used for each isolate, and each isolate was tested in duplicate.

Aggressiveness Aggressiveness was evaluated by three different methods. Winter wheat (*Triticum aestivum* L., cv Chihokukomugi) plants were grown in a glasshouse to the three-leaf stage, and leaf blades in the middle were datached and wound-inoculated with a 5-mm diam mycelial disc and incubated in a moist container at 0 and 4°C for 31 and 19 days, respectively. The radius of the water-soaked lesion was determined for each isolate with five repetitions.

Seeds of Akimidori orchardgrass (*Dactylis glomerata* L.) were sown in plastic flats (45×39×7 cm) containing steam-sterilized soil in early September (Matsumoto and Tajimi, 1990). Seedlings were thinned to 30 plants per flat and grown in a glasshouse for a month. They were subsequently grown outdoors for about 2 months prior to inoculation. Sixty-five grams of wheat-bran vermiculite culture were sprinckled over each flat. Plants were incubated under snow cover for 52 days, then transferred to an unheated glasshouse. The estimate of aggressiveness was based on plant regrowth, which was visually rated 7 days after transfer, using a scale of 0 (no damage) to 6 (plants killed). There were two replications.

Plastic flats were evenly divided into six blocks with three strips of plastic board, and each block was sown with 10 seeds of Kitakamikomugi winter wheat in early October. There were two regimes of inoculation. In one regime, plants were grown outdoors till inoculation with 16 g of wheat-bran vermiculite culture for each block. They were then incubated under snow cover for up to 97 days in duplicate. In the other inoculation regime, plants were grown in a glasshouse for 10 weeks, and the high temperature inoculation method (Nakajima and Abe, 1990) was employed at 12°C in triplicate. Aggressiveness of each isolate was expressed as LI_{50} (Nakajima and Abe, 1990), which was similar to LD_{50} and indicated the number of incubation days required to kill 50% of plants.

Results

Mycelial growth There was no population difference in mycelial growth under all the conditions examined (Table 1). Isolate variability in mycelial growth in axenic culture was significant in populations from Nayoro and Toyama at 0, 7, and 12°C. Mycelial growth was suppressed by soil microbial antagonism by ca. 10, 60, and 80% at 0, 7, and 12°C, respectively. Yamaguchi population was more resistant to antagonism at 0°C, but this was not significantly different from other populations. Mycelial growth at 2% CO_2 was about 80% of that in the atmosphere. Relative growth of each isolate ranged from 46 to 106%, but population variability was again not significant.

Sclerotium size Sclerotium size varied considerably within and between isolates with an overall mean of 1.27 mm. Nayoro population had larger sclerotia than others,

Table 1. Mycelial growth of *Typhula incarnata* populations on PDA under different conditions.

Population[a]	Mycelial growth in axenic culture (mm/day)				Percent suppression[b]			Growth at 2% CO_2 (%)[c]
	Incubation temperature (°C)				Incubation temperature (°C)			
	−3	0	7	12	0	7	12	
Nayoro	0.71±0.10	1.28±0.13	1.60±0.36	1.90±0.27	9.4±17.2	68.2±8.5	76.9±5.5	81.3±8.4
Sapporo	0.73±0.07	1.33±0.07	1.94±0.09	1.36±0.07	13.5±11.1	63.8±9.7	80.2±3.2	81.0±19.0
Toyama	0.70±0.09	1.23±0.16	1.71±0.21	1.14±0.22	8.9±12.6	59.4±7.1	83.2±10.1	79.3±14.2
Yamaguchi	0.70±0.05	1.23±0.09	1.92±0.19	1.29±0.11	1.9±14.5	68.2±8.4	76.9±5.0	86.3±13.8
F	0.1782ns	0.9394ns	2.8668ns	2.6375ns	0.533ns	1.0045ns	0.8552ns	0.5201ns

Each result represents the average ± standard deviation.
[a] Six isolates each were used.
[b] Cultures were covered with unsterile soil. Figures are relative values to controls (100).
[c] Cultures were grown at 12°C for 14 days. Figures are relative values to controls.
ns: Not significant.

Table 2. Sclerotium size of *Typhula incarnata* populations.

Population	Size (mm)
Nayoro	1.37±0.12
Sapporo	1.29±0.17
Toyama	1.21±0.07
Yamaguchi	1.21±0.12
F	2.2985[ns]

One hundred sclerotia produced on inoculated plants were determined for each isolate. Each result represents the average ± standard deviation.
ns: Not significant.

but the difference was not statistically significant (Table 2).

Sclerotium germination Sclerotia in an incubator were found to germinate carpogenically in about two weeks, and a half of them germinated about 23 days after incubation in every population (Fig. 1). There was, however, no population difference. On the contrary, sclerotia kept outdoors in the shade showed significant population differences in germination rate (Fig. 2). Populations from Nayoro and Sapporo germinated more frequently than those from Toyama and Yamaguchi between days 23 and 30; and from days 31 to 36 the Nayoro population showed a higher percentage germination than populations from Toyama and Yamaguchi, and the Sapporo population showed higher germination than the Yamaguchi population. From day 37 to the end of experiment (day 45), the Yamaguchi population showed lower germination than those from Nayoro, Sapporo, and Toyama. Isolates with germination rates of more than 50% at the end of the experiment numbered 4, 3, 1, and 0 out of 6 for populations from Nayoro, Sapporo, Toyama, and Yamaguchi, respectively.

Aggressiveness No population difference in aggressiveness was evident as determined by the three methods (Table 3). Lesion extension on winter wheat leaves averaged more than 0.5 mm/day at 0°C and tended to be faster in populations from Toyama and Yamaguchi at 4°C. However, there was no statistically significant difference between populations. Disease severity of orchardgrass plants ranged from 3.73 to 4.06, again with no statistically significant difference. The Yamaguchi

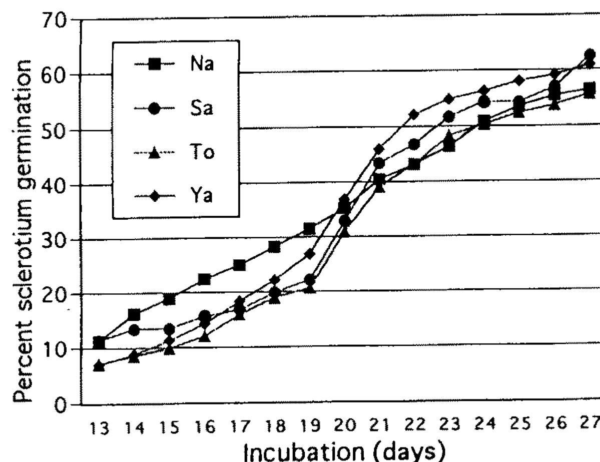

Fig. 1. Carpogenic germination of *Typhula incarnata* sclerotia incubated at 8°C in the light for 10 h and 6°C in the darkness for 14 h. Na: Nayoro; Sa: Sapporo; To: Toyama; and Ya: Yamaguchi.

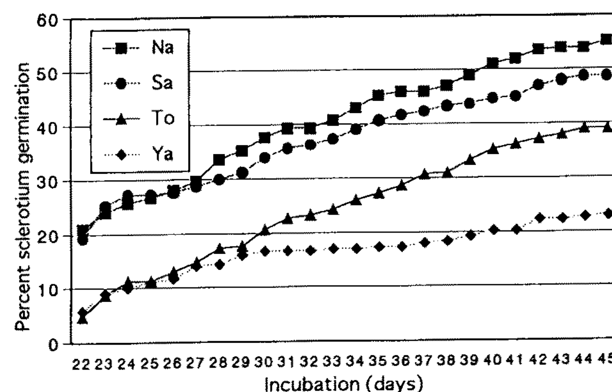

Fig. 2. Carpogenic germination of *Typhula incarnata* sclerotia incubated outdoors in the shade at temperatures ranging from −4 to 14°C. Na: Nayoro; Sa: Sapporo; To: Toyama; and Ya: Yamaguchi.

population was less aggressive under snow than others as determined by LI_{50}, but the difference was not significant. Population variability was evident in LI_{50} determined at 12°C.

Table 3. Comparison of aggressiveness among *Typhula incarnata* populations.

Population	Lesion extension[a] (mm/day)		Disease severity[b]	LI_{50}[c]	
	0°C	4°C		under snow	12°C
Nayoro	0.53±0.10	0.62±0.26	4.03±0.39	81.2±7.3	18.0±0.5
Sapporo	0.53±0.13	0.53±0.14	3.73±0.43	85.3±8.0	16.5±0.9
Toyama	0.57±0.16	0.77±0.23	4.06±0.57	85.8±11.6	17.1±0.5
Yamaguchi	0.55±0.06	0.77±0.22	3.93±0.48	97.9±20.8	17.4±0.6
F	0.1521[ns]	1.7142[ns]	0.6234[ns]	0.4268[ns]	5.8523**

Each result represents the average ± standard deviation.
[a] Wheat leaves were wound-inoculated with mycelial discs.
[b] Disease rating of inoculated orchardgrass from 0 (no damage) to 6 (plants killed).
[c] Number of days required to kill 50% of inoculated wheat plants.
ns: Not significant; **: Significant at the 0.01 level.

Discussion

Typhula incarnata populations did not show any differentiation associated with climatic differences except in sclerotium germination. Maraite et al. (1981) reported considerable varitation in the speed of sclerotium germination by isolates from Belgium, Germany, Norway, Switzerland, and U.S.A. but did not correlate it with climatic differences. Our results indicated that, when incubated outdoors, populations from less snowy localities did not germinate as readily as those from snowy localities. This is the only adaptative variation that isolates of *T. incarnata* exhibited as a population, and may be explained as follows. In less snowy areas, suitable climatic conditions for the pathogen are highly unpredictable due to unstable weather in late fall and winter, i.e., frequent high temperature and low humidity occur, and to considerable annual fluctuations (Matsumoto and Tajimi, 1990). Genotypes whose sclerotia germinate readily may not survive in such a changeable habitat.

However, there was no population variability when sclerotia were incubated under controlled conditions. They germinated more frequently. Possibly, the germination regime was too optimal to detect population variability. The size factor should also not be overlooked. Matsumoto (1992) regarded a wider range in sclerotium size within single isolates of *T. incarnata* as an adaptation to survive diverse climates: small sclerotia and large sclerotia were considered to have different roles. Tränkner and Hindorf (1982) found that sclerotia more than 0.7 mm in diam germinated carpogenically and small ones myceliogenically. The effect of size, dormancy, and environmental factors on sclerotium germination should be further investigated in detail.

We were previously unable to detect even isolate variability in aggressiveness of *T. incarnata* due to poor disease incidence by the methods which were effective for *T. ishikariensis* (Matsumoto and Abe, unpublished), and consequently, more severe conditions with less resistant cultivars and longer incubation periods were adopted here. Still, isolate variability was obvious only in the results of the high temperature incubation method, which revealed a population difference. However, the population difference observed should not be accepted as such since the percent of killed plants often fluctuated among replicates within a treatment, due presumably to the lack of hardening treatment. Tasugi (1930) also recognized the lack of isolate variability in aggressiveness, in contrast with *T. ishikariensis*, which contained different subgroups within a species (Matsumoto, 1992).

In conclusion, *T. incarnata* is a weak, undifferentiated but ubiquitous pathogen and does not show population variability in aggressiveness or mycelial growth. The wide range of distribution of this fungus is ascribed to its ecological versatility. Each of its different abilities is considered available in different habitats in this fungus.

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