雪腐大粒菌核病抵抗性および雪腐黒色小粒菌核病抵抗性に関与する宿主植物の生理的諸要因

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Factors Involved in the Resistance of Timothy and Perennial Ryegrass to *Sclerotinia borealis* and *Typhula ishikariensis*†

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Summary

Germinating seeds of winter hardy or less hardy varieties of timothy and perennial ryegrass were sown on 15 and 30 Aug. and on 15 Sept. to clarify the effect of growth stage and varietal difference in winter hardiness level on the relationships between physiological factors of plants important for winter hardiness and resistance to two snow mold pathogens, *Sclerotinia borealis* and *Typhula ishikariensis*. Plants were inoculated with the pathogens in December and incubated under snow cover. Freezing tolerance was determined in December. Percent total soluble carbohydrates (TSC) was determined in December and April. Timothy was more disease resistant and freezing tolerant than perennial ryegrass. Winter hardy varieties had higher percent TSC and were more resistant to *S. borealis* and *T. ishikariensis*, but TSC itself was not the criterion determining the level of disease resistance since perennial ryegrass was less resistant yet with higher TSC content than timothy. Resistance to *T. ishikariensis* increased with progressive growth stage. TSC content and plant weight increased with increasing age. Resistance to *S. borealis* was lowest in plants sown on 30 Aug. The youngest plants survived the infection of *S. borealis* despite their lowest freezing tolerance. The unknown resistance factor(s) to *S. borealis* existing in young plants is discussed in terms of their physiological characters during wintering.

Introduction

Many factors are involved in the resistance of winter cereals and forage crops to snow molds, and it appears difficult to estimate each factor separately. Winter hardy species or varieties are generally resistant to snow molds[4], and the principal facotor(s) of resistance may differ from one pathogen to another. OKABE[10] showed that Italian ryegrass (*Lolium multiflorum* Lam.) varieties with high snow endurance level were resistant to *Typhula incarnata* Lasch ex Fr. KIYOMOTO and BRUEHL[5] revealed that winter wheat (*Triticum aestivum* L.) varieties resistant to *T. idahoensis* Remsberg had greater food reserve

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and consumed it more slowly than susceptible varieties. Predisposition of host plants. with low temperature is prerequisite for *Sclerotinia borealis* Bubák & Vleugel to prevail[9,11]. AMANO and OZEKI[1] found a significant positive correlation in winter wheat varieties between freezing tolerance and resistance to *S. borealis*, but not between freezing tolerance and resistance to *T. ishikariensis* S. Imai. They also found that *Typhula*-resistant varieties were severely damaged by *S. borealis*.

We grew two varieties each of timothy (Ti, *Phleum pratense* L.) and perennial ryegrass (PR, *Lolium perenne* L.) which are contrastive in winter hardiness level. They were inoculated with *S. borealis* or *T. ishikariensis* at three different growth stages. The results obtained generally agreed with those of previous studies but imply an unknown factor involved in resistance to *S. borealis*.

Materials and methods

Ti varieties Tammisto and S. 51 and PR varieties Valinge and S. 23 were used. Tammisto and Valinge are winter hardy, and S. 51 and S. 23 are less winter hardy. Germinating seeds were sown in 1/5,000 a Wagner pots containing steam-sterilized soil. Dates of sowing were 15 and 30 Aug. and 15 Sept. Plants were thinned to 5 plants per pot and grown outdoors. Apothecia of *S. borealis* were collected from the field late in fall and stored at −20℃ until used. They were ground in distilled water using a homogenizer, and the suspension was filtered through cheesecloth. Each pot received 4 × 10^5 ascospores on 26 Dec. Inoculum of *T. ishikariensis* biotype A[6] was prepared as previously described[7], and each pot received 15g of inoculum on 18 Dec. Uninoculated controls were applied with fungicides (thiophanate-methyl and copper 8-hydroxy-quinoline). Plants were incubated under snow cover for about 100 days. Resistance to plants was expressed as percent regrowth of tillers on 25 Apr. There were four replications.

On 7 Dec., plants were frozen in a cold chamber by lowering temperature from 0.7 to −10.5℃ at the rate of 1.6℃ per hr to clarify the effect of differences in grass species, varieties, and growth stages on freezing tolerance. Freezing temperature of −10.5℃ was maintained for 16 hr, and plants were thawed at the same rate to 0.7℃. Since there was no visible freezing damage, plants were further subjected to −12℃ for 16 hr three days later. The rate of lowering temperature was 2.6℃ per hr. Temperature was raised to 1℃ at the same rate. Plants were transferred to a heated glasshouse, and tops were trimmed to 3–4cm high. Freezing tolerance of each plant was determined 17 days later and expressed as percent regrowth of tillers. There were two replications.

The tops of ten plants were taken on 11 Dec. and 7 Apr. Dead portions were removed, and remaining green portions were weighed. They were dried in hot air at 130℃ for 30 min and at 70℃ for 10 hr; dry weight and percent dry matter were determined. Dried tillers were milled to pass a 42 mesh sieve, and total soluble carbohydrates (TSC) were extracted in boiling water for 60 min. TSC were subsequently hydrolyzed with 2N H_2SO_4, and reducing groups were determined by the Somogi-Nelson method[8] with fructose as a standard. TSC determination was repeated.

Results

Ti was more resistant to *S. borealis* than PR at every stage of growth although plants sown on 30 Aug. were always severely damaged (Table 1). Less winter hardy varieties, i. e. S. 51 and S. 23, were less resistant to *S. borealis* than hardy varieties, i. e. Tammisto and Valinge (Table 1). Ti was more resistant to *T. ishikariensis* than PR,

Table 1. Response of timothy and perennial ryegrass differing in growth stage to *Sclerotinia borealis* [a]

	Date of sowing 15/ Aug.	30/ Aug.	15/ Sept.	F	LSD (5%)
Tammisto timothy	67.9 [b] (71.8)	48.7 (78.0)	70.3 (76.4)	2.50	
S. 51 timothy	63.1 (84.4)	36.5 (80.1)	64.6 (67.9)	35.24 ***	8.5
Valinge perennial ryegrass	40.6 (83.7)	10.8 (78.3)	48.4 (78.6)	38.85 ***	10.2
S. 23 perennial ryegrass	19.1 (90.0)	9.2 (59.4)	39.8 (71.1)	7.39 *	18.3
F	19.81***	14.22***	7.25***		
LSD (5 %)	15.6	15.9	16.2		

a) Inoculated on 26 Dec.

b) Values indicate percent regrowth of tillers determined on 25 Apr. and are angulary transformed. Values in parentheses indicate percent regrowth of uninoculated controls.

*, ***: Significant at the 0.05 and 0.001 levels, repectively.

and winter hardy varieties were more resistant than less hardy varieties (Table 2). The level of resistance to *T. ishikariensis* tended to increase with age (Table 2). Ti survived the freezing treatment successfully at every stage of growth, and Valinge PR sown on 15 and 30 Aug. survived freezing as well as Ti (Fig. 1). Valinge PR sown on 15 Sept. was slightly damaged, while S. 23 PR was severely damaged. (Fig. 1). Percent dry matter was rather uniform in every grass at every stage (av. 26.7%) except for PR sown on 15 Sept. with a little smaller percent dry matter (22.8%). Older plants weighed more (Fig. 2) and had larger percent TSC (Fig. 3). Plant portions which died during wintering increased with increasing age of plants, and this was much more obvious in PR than in Ti (Fig. 2). Reduction of TSC during wintering, on the contrary, was more remarkable in younger plants (Fig. 3). Correlation coefficients between disease resistance and factors important for winter survival are shown in Table 3. Resistance to *S. dorealis* did not correlate with resistance to *T. ishikariensis*, freezing tolerance, percent TSC before wintering, or TSC reduction during wintering. There was no correlation between resistance to *T. ishikariensis* and freezing tolerance, either. Percent TSC before wintering corre-

Table 2. Response of timothy and perennial ryegrass differing in growth stage to *Typhula ishikariensis* [a)]

	Date of sowing			F	LSD
	15/Aug.	30/Aug.	15/Sept.		(5%)
Tammisto timothy	79.1 [b)] (71.8)	86.9 (78.0)	64.6 (76.4)	0.78	
S. 51 timothy	69.9 (84.4)	86.2 (80.1)	69.2 (67.9)	0.84	
Valinge perennial ryegrass	54.3 (83.7)	36.4 (78.3)	18.4 (78.6)	7.01 *	21.7
S. 23 perennial ryegrass	41.7 (90.0)	40.6 (59.4)	16.2 (71.1)	4.70 *	21.3
F	46.03 ***	24.79 ***	3.50 *		
LSD (5%)	7.5	17.2	47.3		

a) Inoculated on 18 Dec.

b) Values indicate percent regrowth of tillers determined on 25 Apr. and are angulary transformed. Values in parentheses indicate percent regrowth of uninoculated controls.

*, ***: Significant at the 0.05 and 0.001 level, respectively.

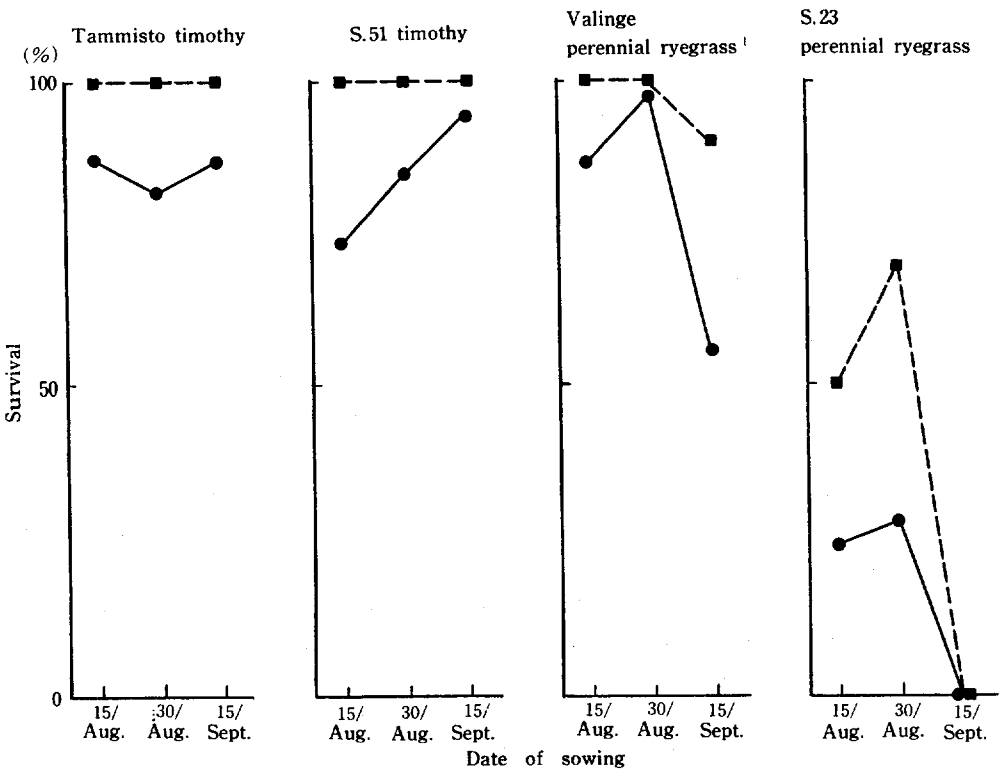

Fig. 1. Freezing tolerance as indicated by percent survival of tillers (●——●) and plants (■ - - ■)

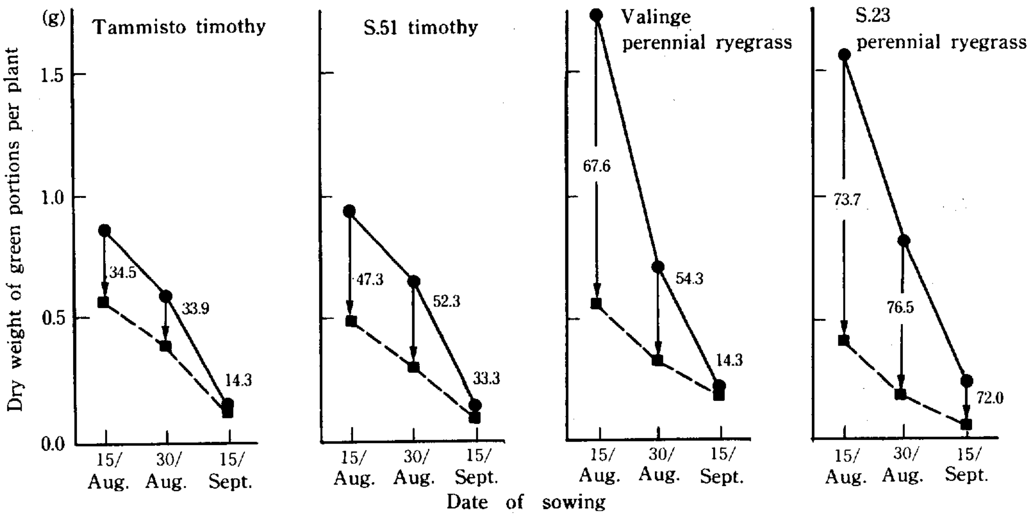

Fig. 2. Dry weight of green portions before (●——●) and after (■ - - ■) wintering. Values in the Fig. indicate percent reduction during wintering.

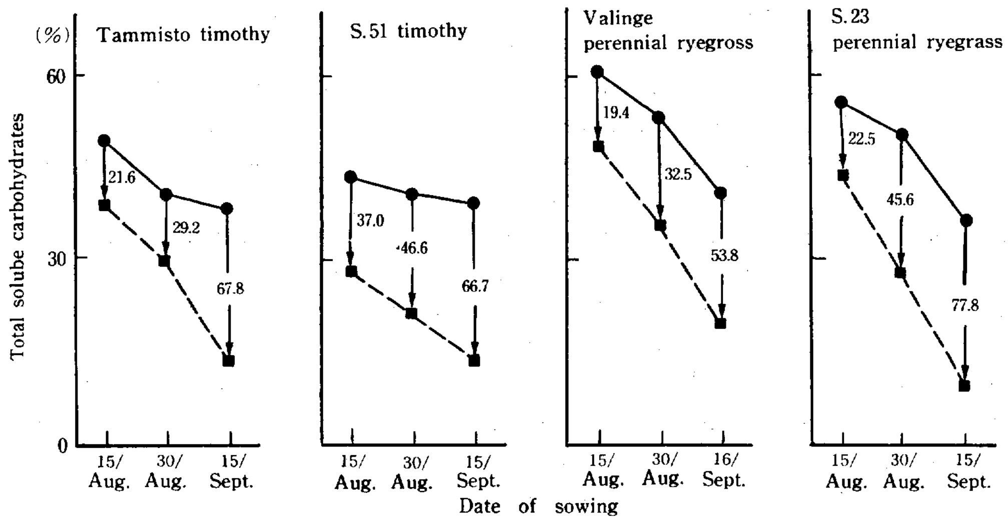

Fig. 3. Percent total soluble carbohydrates of tillers before (●——●) and after (■ - - ■) wintering. Values in the Fig. indicate percent reduction during wintering.

lated well with resistance to *T. ishikariensis* in PR but not in Ti. TSC reduction during wintering had a negative correlation with resistance to *T. ishikariensis* in both Ti and PR.

Table 3. Correlation coefficients between factors important for winter survival of Timothy (Ti) and perennial ryegrass (PR)

		Resistance to *T. ishikariensis*		Freezing tolerance		TSC[a)] before wintering		TSC reduction during wintering	
		Ti	PR	Ti	PR	Ti	PR	Ti	RR
Resistance to *Sclerotinia borealis*	Ti	−.694		.190		.110		.227	
	PR		−.324		−.040		−.444		.365
Resistance to *Typhula ishikariensis*	Ti			−.414		.613		−.853 *	
	PR				.490		.952 **		−.875 *

a) Total soluble carbohydrates.
*, **: Significant at the 0.05 and 0.01 levels, respectively.

Discussion

Winter hardy varieties had higher percent TSC both before and after wintering and were more resistant to *S. borealis* and *T. ishikariensis* than less hardy varieties, but the difference in disease resistance between grass species could not be attributed to the difference in percent TSC since PR generally had higher percent TSC. Freezing treatments were not severe enough to detect the difference between Ti varieties, nor could we find difference in resistance to *S. borealis* between them. Ti plants should have been predisposed with lower temperature to promote the prevalence of *S. borealis*.

Growth stage of plants affects hardiness[12)], freezing tolerance[13)], and resistance to snow molds[2,3)]. Larger and older plants less than one year old generally survived these hindrances in winter better than smaller and younger plants. Resistance to *T. ishikariensis* was associated with percent TSC and dry weight of plants within a variety; i. e, younger plants were less resistant, had lower percent TSC, and weighed less.

The above results agreed with the previous findings except for one : the youngest plants sown on 15 Sept., which were least freezing tolerant, were resistant to *S. borealis* as well as those sown on 15 Aug. Though the results were ommitted because of their too small size, plants sown on 30 Sept. survived the infection of *S. borealis* most successfully despite their most serious freeze damage. This implies the existance of a resistance factor to *S. borealis* other than freezing tolerance. The difference in physiological changes during wintering between old and young plants may be responsible. Older plants lost greater weight of green portions than did younger plants, and they consumed smaller amount of TSC in tillers during wintering. Older plants may preferably utilize food reserve of leaves and consequently, most of their leaves may die from the tip. Younger plants, on the other hand, may keep high respiration to maintain leaves by translocating food reserve from tillers to leaves. Moreover, etiolated sprouts were observed late in winter.

This new finding may be ascribed to milder climate prior to snow cover in Sapporo, but may be masked in colder areas due to the fact that younger plants are less freezing tolerant. Grasses sown in the field either in late Aug. or in mid-Sept. in Kunneppu survived winter much more poorly than those sown in late Jul. or in mid-Aug. (Tsutsui, personal communication). The younger plants must have sustained more severe freezing damage there than in Sapporo, and consequently, *S. borealis* prevailed on them badly. Higher metabolic activity of younger plants throughout winter is considered to be involved in resistance to *S. borealis*, and older plants can be resistant due to their higher freezing tolerance. These two factors are mutually exclusive, and intermediate plants sown on 30 Aug. may have failed to acquire either of the two.

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雪腐大粒菌核病抵抗性および雪腐黒色小粒菌核病抵抗性に関与する宿主植物の生理的諸要因

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摘 要

越冬性の優れた草種であるチモシーと越冬性の劣る草種であるペレニアルライグラスの越冬性の異なるそれぞれ2品種を供試して，雪腐大粒菌核病と雪腐黒色小粒菌核病に対する抵抗性要因を明らかにしようとした．8月15日，8月30日および9月15日にワグネルポットに播種し，戸外で生育させた植物を供試した．接種は12月に行い，積雪下で約100 日間おいた後，被害を評価した．また耐凍性，全可溶性炭水化物（ＴＳＣ）含量など越冬性に関与すると考えられる生理的諸性質についても調査した．チモシーはペレニアルライグラスに比べ両病に対し抵抗性で，また耐凍性も高かった．越冬性の高いとされている品種はＴＳＣ含量が高く，また両病に対しより抵抗性であった．しかし，ＴＳＣ含量はチモシーよりペレニアルライグラスで高く，ＴＳＣそのものは雪腐病抵抗性と直接には関連しないと考えられた．生育段階が進むにつれ雪腐黒色小粒菌核病抵抗性は増加し，同時にＴＳＣ含量や乾物重も増加した．8月15日播種の植物は，いずれの草種，品種においても，雪腐大粒菌核病抵抗性が比較的高く，8月30日播種の植物は最も罹病性であった．9月15日播種の植物は耐凍性が最も低いにもかかわらず，雪腐大粒菌核病抵抗性は8月15日播種の植物と同程度に高かった．9月15日播種の植物にみられるように，耐凍性のない幼植物が雪腐大粒菌核病に対し抵抗性を示したことについて，これらの植物の越冬中の生理的特性との関連で論議した．

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