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Author(s)	YUMOTO, Setsuzo; SHIMAMOTO, Yoshiya; TSUDA, Chikahiro
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STUDIES ON ECOTYPIC VARIATIONS AMONG  
NATURAL POPULATIONS OF TIMOTHY  
(*PHLEUM PRATENSE* L.)

VIII. Growth potential at low temperature  
and cold tolerance

Setsuzo YUMOTO\*, Yoshiya SHIMAMOTO  
and Chikahiro TSUDA

Laboratory of Industrial Crops, Faculty of Agriculture,  
Hokkaido University, Sapporo, Japan, 060

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Introduction

Previous experiments in this series have revealed that ecotypic differentiation of timothy (*Phleum pratense* L.) in Hokkaido is apparent for the growth habit in early spring<sup>9,11,12</sup>. When the northeastern population is compared with the southwestern one, the former is characterized by the low growing activity under low temperature conditions in early spring.

It is well known in forage plants that growth potential at low temperature is inversely related to cold tolerance; poor growth potential at low temperature usually combines with high cold tolerance<sup>1,2,4,6,7</sup>. Then, the reason why the northeastern population shows the low growing activity in early spring must lie in the preservation of high level of cold tolerance, by which it would resist the erratic late frost injury. Besides, it would be necessary for the northeastern population to have high cold tolerance for surviving the severe winter. These circumstances stimulate further study of inter-population variation of cold tolerance to demonstrate the adaptive significance in timothy in Hokkaido.

The present study describes the variations of relative growth rate at 10°C and survival rate after exposure to -5°C. Furthermore, the pattern of variation of survival rate is examined in relation to climatic conditions at habitat in winter.

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\* Present address: Hokkaido Prefectural Tenpoku Agricultural Experiment Station, Hamatonbetsu, Hokkaido, 098-57

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### Materials and Methods

Thirty-two timothy populations were studied in Sapporo, of which 13 and 19 populations were collected from the northeastern and southwestern areas of Hokkaido, respectively. Seeds gathered at original habitat in the summer of 1976 were not available because of their low viability. Accordingly, open-pollinated seeds harvested in 1978 from ten plants of each representative population were used. The locations of habitat are shown in Fig. 1.

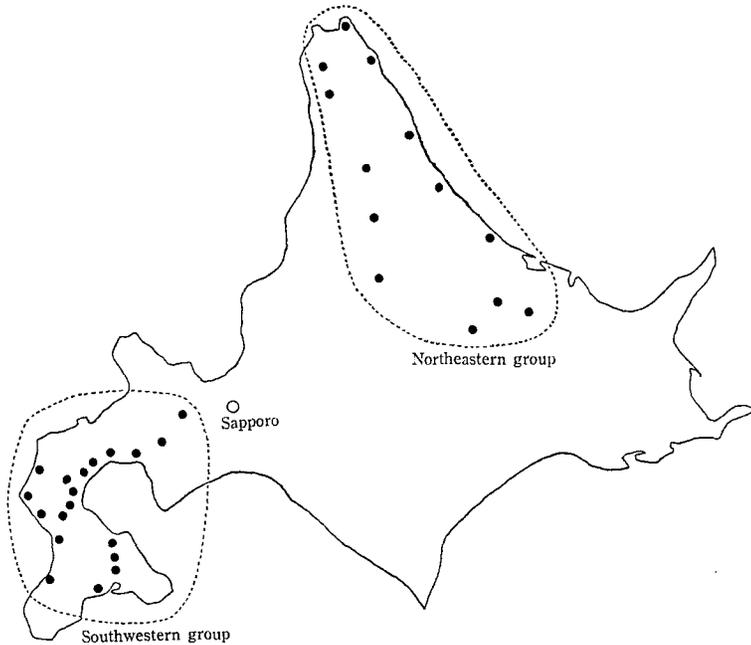


Fig. 1. Map of Hokkaido showing collection sites of population.

Seeds were sown on agar medium in petri-dish on August 25, 1981 and germinated seeds were planted in a plastic box filled with fertile soil. Afterward, boxes were put into a glasshouse and they were kept in it until experimental usage.

The following two experiments were designed as a randomized block with two replications. Each replication was represented by each box and each population within replication consisted of 15 seedlings. Day length in a growth cabinet was eight hours, provided by two fluorescent tubes (40 watt) 30 cm above the box.

### **Relative growth rate at 10°C**

Dry weight of shoot was measured on five seedlings per replication on October 15 and then other seedlings were put into the growth cabinet. Thereafter, they were grown at a constant temperature of 10°C till November 15, when dry weight of shoot was measured on ten seedlings per replication. Relative growth rate during the period was calculated from the dry weight data.

### **Survival rate after exposure to -5°C**

The seedlings grown in the glasshouse till November 17 were transferred into the growth cabinet. After they were subjected to 5°C for two days and subsequently exposed to -5°C for five days in it, they were returned to the heated glasshouse for recovering. On December 24, number of living seedlings was recorded on ten seedlings per replication. Seedling with any green tissue was considered to be alive. Two populations from the northeastern area were not available, since many of their seedlings displayed poor growth before freezing treatment.

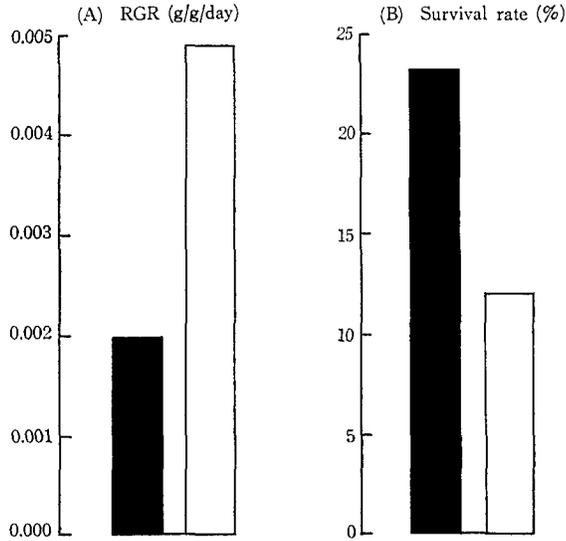
## **Results**

Mean values of relative growth rate at 10°C and survival rate after exposure to -5°C in the northeastern and southwestern populations are graphically presented in Fig. 2. Analyses of variance for these characters showed that relative growth rate of the southwestern population was significantly larger than that of the northeastern one, but inverse was true for survival rate (Table 1). On the other hand, there was not a significant difference among populations within each local group.

In order to clarify the relationship of cold tolerance of population with climatic conditions at the habitat in winter, two simple correlation coefficients for survival rate versus monthly mean of daily minimum temperature and monthly total precipitation from December to March were calculated (Table 2). These climatological data were taken from the meteorological station nearest the original habitat. Significant negative correlation was detected between them in each month; population from the habitat with colder temperature and lower snow fall in winter was more tolerant to cold. As a result of multiple regression analysis of survival rate with these climatic elements, it was found that multiple correlation coefficient was the highest in December and the lowest in March.

The relationship of survival rate with the two climatic elements in December was illustrated in Fig. 3. It is worth noting that population from

the habitat where both temperature and precipitation are the lowest showed much higher survival rate than the other populations.



**Fig. 2.** Relative growth rate (RGR) at 10°C and survival rate at -5°C.  
 Closed bar: northeastern group  
 Open bar: southwestern group

**TABLE 1.** Analyses of variance for relative growth rate (RGR) of shoot at 10°C and survival rate ( $\sin^{-1}$ ) at -5°C.

Source of variation	df	Mean squares	
		RGR ( $\times 10^6$ )	Survival rate
Population	31 (29)	29.5	179.77
NG vs. SG	1	129.6*	934.86*
Northeastern group (NG)	12 (10)	34.3	350.84
Southwestern group (SG)	18	20.7	42.78
Block	1	57.1	655.39
Error	31 (29)	26.2	193.60

\*: Significant at 5% level.

Numerals in parentheses represent degrees of freedom for survival rate

TABLE 2. Multiple regression analyses of survival rate ( $\sin^{-1}$ ) on monthly mean of daily minimum temperature (T) and monthly total precipitation (P) in winter.

Month	Simple correlation coefficient		Multiple correlation coefficient	Standard partial regression coefficient	
	T	P		T	P
December	-0.434*	-0.531**	0.626**	-0.339	-0.416
January	-0.424*	-0.506**	0.542**	-0.225	-0.392
February	-0.426*	-0.522**	0.554**	-0.216	-0.412
March	-0.377*	-0.369*	0.446*	-0.273	-0.260

\*, \*\*: Significant at 5% and 1% levels, respectively.

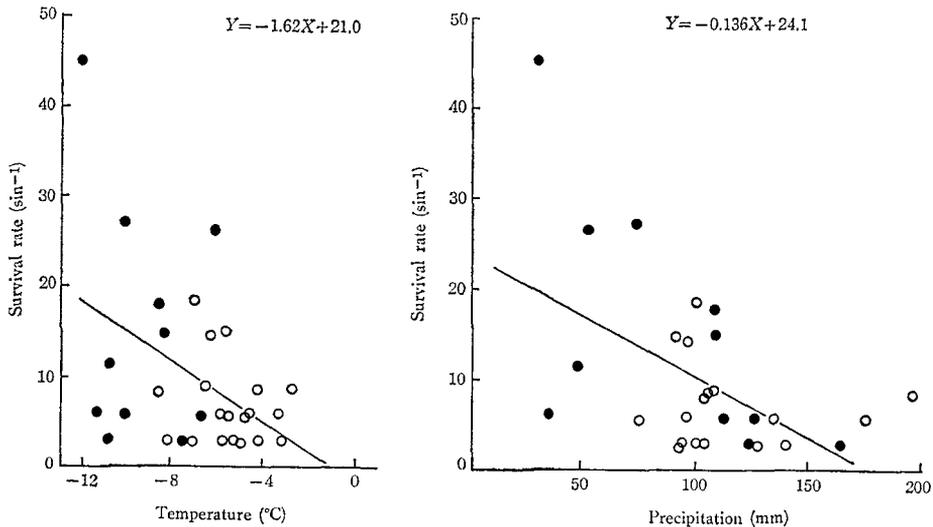


Fig. 3. Relationship of survival rate at  $-5^{\circ}\text{C}$  with mean of daily minimum temperatures and total precipitation of December in northeastern (●) and southwestern (○) populations.

### Discussion

In the present study, the northeastern and southwestern populations of timothy in Hokkaido were different in relative growth rate at  $10^{\circ}\text{C}$  and survival rate after exposure to  $-5^{\circ}\text{C}$ . As expected from previous experiments<sup>9-11)</sup>, the northeastern population showed lower growth rate and higher survival rate than the southwestern one. On the other hand, survival rate was related to both monthly mean of daily minimum temperature and month-

ly total precipitation in winter. These results suggest that population differentiation of cold tolerance in Hokkaido has evolved through the adaptive response to winter conditions, and depth of snow cover as well as cold temperature is an important selective factor.

It has been reported in forage plants that inverse relationship exists between growth potential at low temperature and cold tolerance<sup>1,2,4,6,7</sup>. The above results agree with this. In respect to the inverse relationship of alfalfa, DADAY<sup>8</sup> elucidated by genetical analysis that it was not due to either pleiotropic effect or close linkage of genes controlling the two characters, but arose from independent response to natural selection. Moreover, it was demonstrated in Italian ryegrass<sup>9</sup> and orchard grass<sup>9</sup> that combining a high level of cold tolerance with good growth potential at low temperature was genetically possible. Thus, it seems probable that inverse relationship observed in the present study is also simply the results of independent genetic response of the two characters to natural selection. Because poor growth potential and therewith high level of cold tolerance induced by hardening in autumn are essential metabolic processes to encourage winter hardiness and, therefore, they have much selective advantage in the area where winter conditions are more severe.

It would be concluded from the present study that, through the adaptive response to the severe winter conditions, the northeastern population of timothy in Hokkaido has possessed a physiological trait to induce higher level of cold tolerance by reducing growth potential in response to cold temperature. This physiological trait must underlie the differential growth habit in early spring.

### Summary

In order to investigate the variations of growth potential at low temperature and cold tolerance in natural populations of timothy in Hokkaido, relative growth rate at 10°C and survival rate after exposure to -5°C were examined using seedling material of 32 populations.

Relative growth rate was higher in the southwestern population than in the northeastern one, but survival rate was greater in the northeastern population.

Survival rate was negatively correlated to monthly mean of daily minimum temperature and monthly total precipitation in winter.

Thus, it was indicated that ecotypic differentiation of cold tolerance has evolved through the adaptive response to prevailing winter conditions at habitat.

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