# Carbohydrate Accumulation and Depletion by Winter Cereals Differing in Resistance to Typhula idahoensis

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ABSTRACT

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The wheat selection C. I. 14106, which is resistant to *Typhula idahoensis,* accumulated available carbohydrates in its crown tissues early in the cold-hardening process; but resistant C. I. 9342, moderately resistant Moro, and susceptible Nugaines accumulated less carbohydrates and at a slower rate. Early acquisition of carbohydrate in the crown of C. I. 14106 was correlated with early acquisition of resistance. Moro and C. I. 9342 required longer periods of cold hardening to acquire carbohydrates and resistance. During incubation at 0.5 C in the dark, carbohydrate concentration declined in Nugaines at a faster rate than in C. I. 14106, C. I. 9342, or Moro. The $F_1$ progeny of reciprocal crosses were intermediate between their respective resistant parents and Nugaines in carbohydrate accumulation during cold hardening, carbohydrate use under snow mold conditions, and in resistance. This indicated no cytoplasmic inheritance either of resistance or of patterns of carbohydrate metabolism. Resistance in $F_1$s was partially dominant. Only $F_1$ progeny of C. I. 14106 accumulated carbohydrates rapidly. Under snow mold conditions, progenies of C. I. 14106 and C. I. 9342 used carbohydrate at a slower rate after 30 days than did Nugaines. There was no correlation between snow mold resistance and crown carbohydrate content of 15 wheats sampled in the field in early autumn, but there was a high correlation if they were sampled in the spring after snow melt.

Winter wheats (*Triticum aestivum* L.) with useful resistance to snow mold fungi (*Typhula idahoensis* Remsb., *Typhula ishikariensis* Imai, *Typhula incarnata* Lasch ex Fr., *Fusarium nivale* (Fr.) Ces., and *Sclerotinia borealis* Bubak & Vleugel) exist (7, 8) and they have been the subject of a recent review (10). Genetic studies of resistance in winter wheat to *T. idahoensis* indicate quantitative inheritance involving several genes for resistance (8). Cultivars resistant to *T. idahoensis* also are resistant to *T. incarnata* and *F. nivale* (3, 4). There is also a correlation of resistance in cereals to *T. ishikariensis, T. incarnata, F. nivale,* and *S. borealis* in Scandinavia (10). The wide host ranges of these fungi, in some cases not restricted to the Gramineae, is also evidence for a lack of high degrees of pathogenic specialization (4, 10). No physiologic races have been observed in *T. idahoensis, T. incarnata,* or *F. nivale* (3, 4, 8, 12, 15). The resistance of winter wheat to snow mold is nonspecific and polygenic.

Snow mold resistance in winter wheat is influenced by plant size (5, 7, 9, 10, 14, 21). Larger and older plants of winter wheats C. I. 14106, C. I. 9342, Moro, and Nugaines survive infections and recover better than smaller plants when inoculated with *T. idahoensis* and incubated in controlled environment trials, even though their leaves are destroyed. However, plant size alone, is relatively inefficient in increasing resistance in the susceptible Nugaines wheat (5).

Resistant wheats use their reserves at a slower rate in darkness near 0 C than do susceptible wheats (6). It was suggested that leaves of susceptible wheats use carbohydrates more readily than leaves of resistant plants, thus depriving crowns of a translocatable source of carbohydrate (6). Tomiyama (19) showed that susceptibility to *T. incarnata* increased as carbohydrates were exhausted and proteolysis in leaves increased; resistance thus terminated when leaf tissues became senescent. Similar results have been reported for *T. incarnata* on winter barley (12). On the other hand, field observations of C. I. 9342 and C. I. 14106 suggested that resistance in C. I. 9342 is in part due to something other than to carbohydrate relationships alone (6).

In the present study, accumulation and use of available sugars were followed in diseased and disease-free winter wheats that possessed different levels of snow mold resistance, to detect any nutritional response to pathogen attack. The inheritance of resistance, and the accumulation and use of carbohydrates by winter wheat $F_1$ progeny were observed, to determine whether resistance is dominant or recessive and whether cytoplasmic inheritance of resistance is involved.

## MATERIALS AND METHODS

**Inoculum.**—*Typhula idahoensis* isolate 5999-5 was transferred as mycelial fragments onto sterilized wheat

kernels and incubated in darkness at 10 C for 30 to 45 days. Wheat kernel medium was prepared in 0.95-liter (1-quart) jars by mixing 225 cc of dry wheat grain with 150 cc hot water and autoclaving the mixture at 121 C for 50 minutes. Jars were shaken after 2 weeks to permit even distribution and development of mycelium. Mature sclerotia are developed in approximately 1 month. Nondried, fresh inoculum was used to inoculate plants.

**Hosts.**—Hudson and Luther winter barley (*Hordeum vulgare* L.) and Nugaines, Moro, Sprague, C. I. 9342, and C. I. 14106 winter wheats were seeded in mid-September at about 3-4 cm deep and eight to 10 seeds per clay pot (15.3 cm diameter) in a mixture (1:1, v/v) of Palouse silt loam and sand. The seeded pots were placed outdoors in sand beds to allow plants to cold-harden under natural conditions. Water and fertilizer were applied as needed. In November or early December plants were removed from the sand beds, inoculated uniformly over plant and soil surface with 40-50 cc of inoculum (wheat kernels and sclerotia), and covered with a pad of wet absorbent cotton. Control plants were neither inoculated nor covered with cotton.

In the autumn of 1972 and 1973 wheats were cold-hardened for varying lengths of time in an attempt to vary plant size and periods of carbohydrate accumulation, and hence to attain the same degree of resistance in inherently different wheats. Thus, although seeded at the same time, resistant wheat 14106 was inoculated and placed in snow mold chambers on 29 October 1972 and 23 October 1973: Moro and C. I. 9342 on approximately 10 November 1972 and 1973; and Nugaines on 19 November 1972 and 15 November 1973. At each date, six inoculated and six noninoculated pots of each wheat were placed into snow mold chambers and incubated for 50 to 60 days. In contrast, in 1974 all plants were placed in snow mold chambers on 23 to 25 November with no attempt to adjust for degree of resistance.

The $F_1$ progeny of reciprocal crosses and their parents were studied in autumn, 1974. In recording pedigrees (16), the female parent in a cross is placed first and is separated from the male parent by a single slash which designates a cross.

**Incubation and recovery.**—Snow mold chambers were maintained at 0.2 to 0.8 C with pots watered by subirrigation. During incubation the cotton covering the leaves was kept moist. After incubation, the cotton was removed, and plants were placed in the greenhouse to recover under natural light at 10-15 C. Recovery from snow mold was evaluated by weight of new leaf growth (5) and by the number of surviving plants after 25 to 40 days in the greenhouse. Recovery was evaluated in five to eight pots (each pot was considered a replicate), providing 35 to 64 plants for each treatment. In the study of reciprocal cross progeny, only three inoculated pots (approximately 24 plants) and two noninoculated pots of each $F_1$ progeny were evaluated for recovery at a given sampling date.

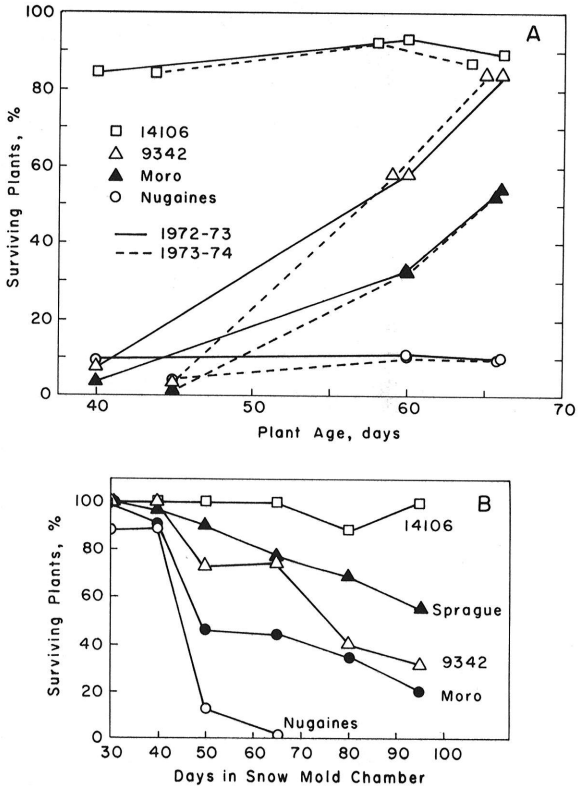


**Fig. 1-(A, B).** Survival of winter wheats (C. I. 14106, C. I. 9342, Sprague, Moro, and Nugaines) after incubation at 0.5 C in the dark with *Typhula idahoensis* isolate 5999-5. **A)** Plants of different ages incubated 50-60 days. **B)** Plants the same age (84-86 days) incubated for 30-95 days.

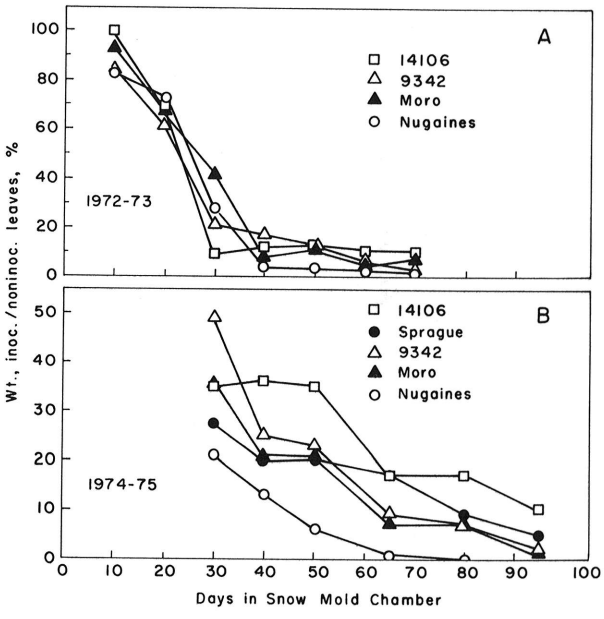


**Fig. 2-(A, B).** Recovery of wheat after attack by *Typhula idahoensis* as evaluated by growth of new leaf tissue expressed as the proportion of the weight of new leaves produced by diseased plants to the leaves present on healthy plants. **A)** Age of plants varied at start of incubation to compensate for differences in susceptibility (C. I. 14106 about 46 days old, C. I. 9342 and Moro about 58 days old, and Nugaines about 67 days old when incubation began. **B)** All wheats 84-86 days old when incubation began.

**Carbohydrate analyses.**—Plants were analyzed for total available carbohydrates in roots, crowns, and leaves during exposure outdoors and during incubation in the snow mold chambers. Plants from two or three pots of each treatment at each sampling date were combined, washed, divided into roots, crowns, and leaves, and immediately steamed under low pressure at 105 C for 5 minutes to halt enzyme activity. Heated samples were air-dried at room temperature, ground in a Wiley mill to pass a 0.97-mm (20-mesh) screen, and stored in a freezer until analyzed. Available carbohydrates were determined according to methods outlined by Smith (18) and Weinmann (20). Total nonstructural carbohydrates rather than individual sugars and starches were determined.

Finely-ground, air-dried plant materials were extracted first in 10 ml of distilled water for 40 minutes over boiling water. Carbohydrates in the cooled mixture were hydrolyzed with commercial takadiastase (K & K Laboratories, Plainview, New York) (18). Enzyme hydrolysis was followed by mild acid hydrolysis in 0.1 N HCl to expose all reducing groups; these were measured on aliquots by copper reduction-iodine titration (20). The results are expressed as glucose equivalents per 0.2 g dry tissue, and will be referred to as total available carbohydrates.

The samples of air-dried plant material in almost all cases weighed 0.1 to 0.2 g and sometimes 0.05 g. A linear relationship between sample size and total available

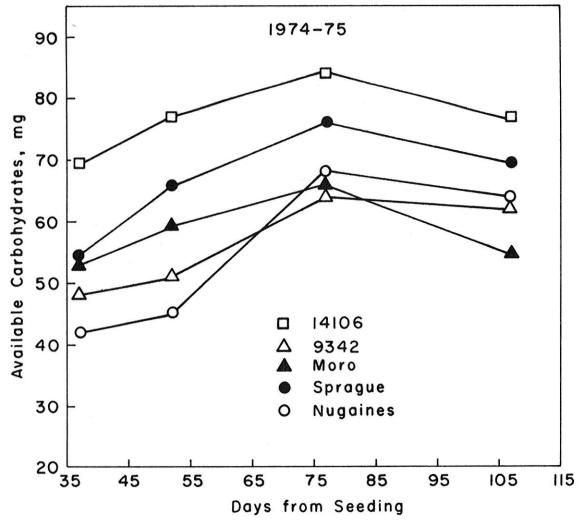


**Fig. 3.** Available carbohydrates (mg per 0.2 g dry tissue) in crowns of winter wheats during cold hardening outdoors in autumn, 1974.

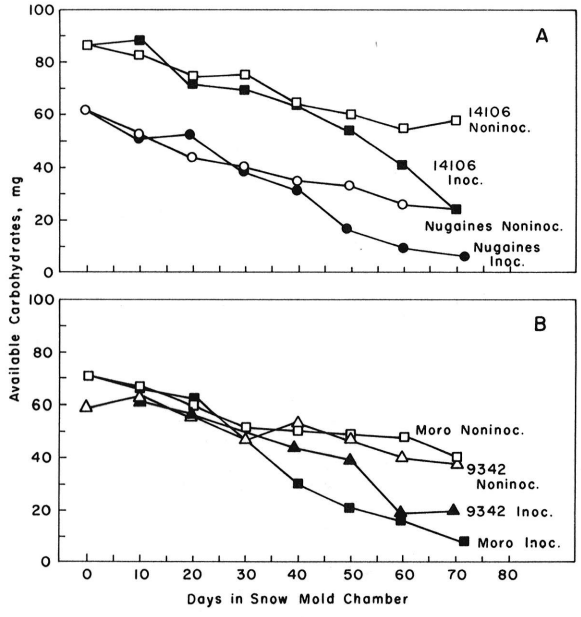


**Fig. 4-(A, B).** Effect of *Typhula idahoensis* attack upon loss of available carbohydrates (mg/0.2 g dry tissue) from crowns of winter wheat. **A)** C. I. 14106 and Nugaines, healthy and diseased. **B)** Moro and C.I. 9342, healthy and diseased.

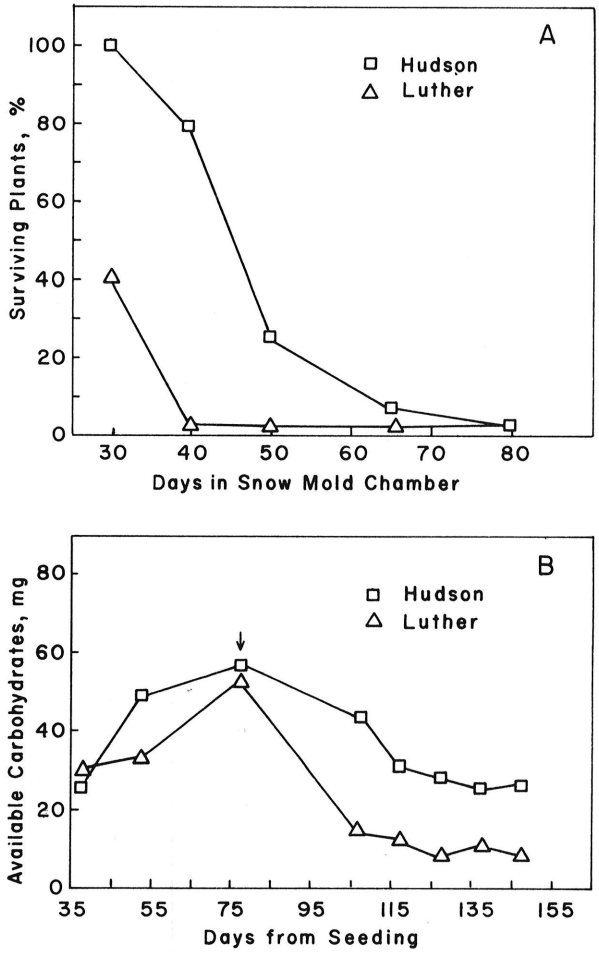


**Fig. 5-(A, B).** Comparison of two winter barleys, Hudson and Luther. **A)** Survival after incubation with *Typhula idahoensis*. **B)** Carbohydrate content (mg/0.2 g dry weight) in crowns during cold hardening outdoors (to left of arrow) and during incubation in darkness at 0.5 C (to right of arrow).

carbohydrates was obtained with sample sizes over the range, 0.05-0.3 g of dried plant material (11). The data of at least two independent samples are presented as averages.

**Plant material from field samplings.**—Samples were taken of 15 winter wheats from Douglas County, Washington, on 10 October 1973 and 3 May 1974 of the 1973-74 season. All samples were dug and transported to the laboratory within 24 hours at ambient temperature in open plastic bags and analyzed as above.

## RESULTS

**Resistance in snow mold chamber trials.**—The resistance of the wheats to *T. idahoensis* was strongly influenced by their age (degree of development when exposed to snow mold conditions, Fig. 1-A). C. I. 14106 was already resistant at 40 days, 9342 increased in resistance with age, and Nugaines did not increase in resistance. When all plants were 84-86 days old when placed under snow mold conditions (Fig. 1-B), C. I. 14106 was resistant for at least 100 days, the duration of the test, and Sprague was moderately resistant after 90-100 days.

The effect of plant age on resistance also was evident in the 1972-73 experiment in which C. I. 14106 was grown 49 days, Moro and C. I. 9342 were grown 55 days, and Nugaines 65 days prior to incubation with *T. idahoensis*. These wheats were equally damaged as determined by leaf growth following the attack (Fig. 2-A). The differences between wheats were less distinct when recovery or leaf growth was the criterion of damage (Fig. 2-B) than when plant survival was the criterion (Fig. 1-B).

**Available carbohydrates in winter wheat.**—Although carbohydrates in roots, leaves and crowns were determined separately (11), only crown data are presented. Crowns have the highest concentrations of available carbohydrates and they are the part most critical to survival and recovery after attack. C. I. 14106 accumulated carbohydrates at a rapid rate, Sprague was quite efficient, and the other wheats accumulated carbohydrates at the rate of average winter wheats (Fig. 3). Carbohydrate accumulation peaked in late November and then gradually declined (1,2).

Carbohydrates declined linearly in the crowns of hardened healthy wheats held at 0.5 C in the dark and in the crowns of inoculated wheats for the first 30 days of incubation. After 30 days, carbohydrates declined more rapidly in the diseased plants, falling to less than 75% of that in healthy plants at 40 days in Moro, at 50 days in Nugaines, and at 60 days in C. I. 9342 and C. I. 14106 (Fig. 4).

**Cytoplasmic effects in reciprocally-produce $F_1$ progeny of resistant × susceptible wheats.**—The $F_1$ offspring of crosses of Nugaines (susceptible) × C. I. 9342 and C. I. 14106 were intermediate between the parents in both resistance and carbohydrate accumulation, regardless of which wheat was the female. Neither carbohydrate accumulation nor resistance, therefore, gave evidence of maternal or cytoplasmic effects.

**Carbohydrate metabolism and resistance to Typhula idahoensis in two winter barley cultivars.**—Hudson barley was more resistant than Luther (Fig. 5-A). Hudson accumulated more carbohydrate and conserved it in darkness at 0.5 C better than Luther (Fig. 5-B). These limited data suggest that a correlation exists in barley between carbohydrate conservation and snow mold resistance.

**Carbohydrates in winter wheats from field samples.**—Fifteen winter wheats were sampled 10 October 1973 and after snow melt, 3 May 1974, and analyzed for available carbohydrates in roots, leaves, and

TABLE 1. Available carbohydrates in crowns, leaves, and roots of 15 winter wheats sampled from the Douglas County snow mold nursery in autumn, 1973 and spring, 1974, following a 150-day period of snow cover. The wheats are listed in descending order according to carbohydrates in the crowns on the first sampling date

| Winter wheat | Relative resistance to snow mold | Available carbohydrates (mg)[b] | | | | |
|---|---|---|---|---|---|---|
| | | Sample from 10 October 1973[c] | | | Sample from 3 May 1974[d] | |
| | | Crowns | Leaves | Roots | Crowns | Roots |
| C. I. 173440 | 1 | 76.7 | 50.8 | 31.3 | 16.9 | 14.7 |
| C. I. 178383 | 3 | 73.8 | 63.1 | 33.4 | 7.0 | 7.3 |
| C. I. 181268 | 2 | 70.1 | 48.1 | 20.6 | 10.0 | 14.7 |
| C. I. 14106 | 1 | 69.5 | 58.1 | 37.7 | 19.0 | 16.2 |
| Gaines | 5 | 66.1 | 47.4 | 15.5 | 1.1 | N.D.[e] |
| C. I. 173438 | 1 | 65.1 | 58.1 | 37.7 | 19.4 | 20.7 |
| C. I. 172582 | 2 | 64.5 | 58.8 | 33.0 | 13.0 | 7.0 |
| Sprague | 3 | 63.5 | 44.2 | 31.3 | 6.3 | 9.3 |
| C. I. 166797 | 3 | 61.5 | 44.6 | 28.4 | 4.7 | 2.4 |
| Wanser | 5 | 59.5 | 52.1 | 12.8 | 0.8 | 1.6 |
| C. I. 9342 | 3 | 58.8 | 52.1 | 18.9 | 5.1 | 2.5 |
| C. I. 173467 | 2 | 57.4 | 50.8 | 31.1 | | |
| C. I. 167822 | 2 | 56.6 | 50.8 | 27.5 | 9.4 | 10.4 |
| Moro | 4 | 56.1 | 57.4 | 34.1 | 1.7 | N.D. |
| Burt | 5 | 55.1 | 45.4 | 24.7 | 3.0 | N.D. |

[a]Most resistant wheats, 1; most susceptible, 5, in the resistance ranking.
[b]Soluble reducing sugars (glucose equivalents) per 0.2 g dry tissue. Values represent average of two determinations.
[c]Five to 12 plants per sample of each winter wheat.
[d]Leaves destroyed by mold, so there are no data for leaves after snow melt.
[e]N.D. signifies concentration too low to detect.

crowns. Ranking them by their carbohydrate content in the fall did not place them in the order of their resistance. The carbohydrates remaining in the crowns after a severe disease attack, however, was correlated with resistance (Table 1).

## DISCUSSION

Wheat C. I. 14106 accumulated carbohydrates more rapidly in the fall than the other wheats that were studied, and it was more resistant in early stages of development than the others. The carbohydrate reserve must be adequate to sustain a wheat under prolonged snow cover, and it appears that all wheats, if seeded early so that they have several tillers when winter comes, will have an adequate carbohydrate reserve. The resistant wheats differ primarily, not in how much food they store, but in how much remains after the mold attack. Resistant wheats must maintain a relatively low rate of respiration throughout the incubation period. In this way they maintain life processes needed to restrain the advance of the pathogens into the crown at a minimum, effective rate, and yet have sufficient energy remaining to replace the rotted leaves in the spring.

Earlier data (6) suggested that some carbohydrates were translocated during darkness at 0.5 C from leaves to the crowns of resistant plants, thus contributing to crown survival by early sacrifice of the leaves. The data of the first 30 days in darkness (Fig. 4) do not substantiate this hypothesis. Carbohydrates in the crowns of resistant and susceptible plants, inoculated and controlled, declined in a linear manner, providing no evidence of translocation.

After 30 days, the crowns of diseased plants lost carbohydrates more rapidly than did crowns of healthy plants. By 30 days the leaves of all plants were in advanced stages of rotting and translocation from them to crowns would be unlikely. The accelerated loss of crown reserves is evidence of accelerated metabolism in response to attack when the crown is threatened. Increased respiration is characteristic of early infections in most plant diseases, except that in snow mold the increased respiration continues for a protracted period. If the above conjectures are correct, resistance involves active physiological activity, and the carbohydrate reserves are essential to sustain this activity (19).

The central role of carbohydrate conservation suggested that cytoplasmic factors could be significant. The only cytoplasmic (maternal) effects observed among the $F_1$ progeny of reciprocal crosses were that, when Nugaines was the maternal parent, seedling emergence was slower than when the resistant parent was the mother plant (11). This effect was transitory, and by the third leaf stage, reciprocal offspring were equal. These plants were exposed to *T. idahoensis* 80-90 days after seeding, and no difference among reciprocal progeny either in carbohydrate concentration or in resistance was detectable by that time. In these trials, as well in some earlier observations (8), it made no difference which plant was used as the female and which was used as the pollen parent in making crosses. McDaniel (13) and Sarkissian and Srivastana (17) reported that plant hybrids heterotic in their early growth rate had higher respiration rates at early stages of germination as well as mitochondria with higher efficiency of energy conservation. No significant heterotic or cytoplasmic effects were observed here for snow mold resistance.

It may be significant for breeders to note that the total carbohydrate per gram of host crowns and roots after prolonged disease attack (Table 1) was greatest in P. I. 173438, C. I. 14106, P. I. 173440, and in P. I. 181268.

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