Physiologic and Environmental Factors that Affect the Severity of Snow Mold of Wheat

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ABSTRACT

Recovery from attack by *Typhula idahoensis* was strongest from plants with several tillers and weakest from plants with two to four leaves. Whereas early seeding results in the largest plants with the greatest ability to recover, it also results in the production of more sclerotia. Deep seeding did not lessen the effects of mold.

Varieties with some resistance enter winter with greater carbohydrate reserves and utilize carbohydrates at a slower rate than fully susceptible wheats. Fully susceptible wheats remain greener longer in the dark at +1 or 0 C than do more resistant wheats. *Typhula idahoensis* reproduces as prolifically on resistant as on susceptible wheats.

Typhula idahoensis is the most virulent species at 1.5, 0, and −1.5 C. Poorest wheat recovery occurs after attack at 0 C. *Fusarium nivale* and *T. incarnata* are second and third in virulence, respectively, but neither causes mold at −1.5 C. *Sclerotinia borealis* did not attack the wheat in our experiments.

Sclerotinia borealis grew about twice as fast on agar media when sucrose was used to lower the water potential as when KCl was used, and its growth was fastest at water potentials between about −10 to −30 bars. *Typhula idahoensis* and *T. incarnata* were favored by higher water potentials. They were more equally tolerant of salts and sucrose. *Fusarium nivale* grew best at higher water potentials, but some isolates tended to be quite sensitive to KCl and relatively tolerant to sucrose.
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Severe snow mold of winter wheat in Washington depends upon prolonged, deep snow cover. Deep snow (50 cm or more) maintains darkness (22) at the soil surface, prevents photosynthesis, stabilizes humidity, and favors vegetative development of the snow mold fungi. It protects aerial and perthophytic hyphae from sunlight, and insulates the soil surface. If deep snow falls on lightly frozen or unfrozen soil, the temperature reaches 0.0 to 0.5 C and remains constant for prolonged periods (4, 22).

The wheat plant beneath deep snow on lightly frozen or unfrozen soil is subjected to gradual starvation. It respires at a significant rate at or near 0 C (13), and exhaustion of food reserves through lack of photosynthesis predisposes it to snow mold. Wheat utilizes carbohydrates first, then proteins as sources of energy. Old leaves (senescent) are subject to food loss by translocation, and they become susceptible first (22).

Selection of wheats with a measurable degree of resistance to snow molds provided an opportunity to test the predisposition by starvation hypothesis.

Field observations (4, 8, 14, 26) and experiments in snow mold chambers (2) proved that resistance to snow mold involves plant size. Very small plants with one or two leaves and no tillers frequently survive (escape) with no mold. Very large plants from very early seedings lose all their leaves, but the crowns usually survive. Greatest disease losses occur with intermediate-sized plants. Farm practices in Washington stress the importance of large plants to survival.

But more than size is involved in resistance. In field tests, at least 12,000 wheats were screened for snow mold resistance; only a few were resistant. All were seeded at the same time, and all were large when exposed. When hardened wheats were held (noninoculated) at 1 C in darkness, susceptible wheats remained green longer than resistant wheats (2). The greener susceptible wheats held in the dark indicated a rate of metabolism sufficient to maintain the leaf. These observations suggested that at least three mechanisms acted in resistance: plant size, a carbohydrate conserving mechanism, and unknown physiologic resistance factors (2).

Sclerotinia borealis Bubak & Vleugel develops best when the soil remains frozen (5, 12, 21, 22). *Typhula idahoensis* Remsberg competes best at very close to 0 C. *Typhula incarnata* Lasch ex Fr. can develop in the crowns of wheat in the absence of snow in the cool, wet late winter and early spring (3, 20), particularly in lighter soils. *Fusarium nivale* (Fr.) Cesati is serious on winter wheat only under snow, even though it produces disease on susceptible turf grasses in the absence of true snow mold conditions (7, 20). In culture, *F. nivale* has the highest temperature opt, followed in order by *T. incarnata, T. idahoensis,* and *S. borealis*. These general temperature responses undoubtedly are reflected in their distribution (5, 12, 21).

Ekstrand (6) and Tomiyama (22) proved that several snow mold pathogens can grow in culture at −5 to −7 C, if freezing of the medium is avoided. When Tomiyama permitted media to freeze, *Sclerotinia graminearum* Elen. continued to grow but *T. incarnata* did not. Laboratory and field observations led him to conclude that *S. graminearum* withstood greater osmotic forces than did *T. incarnata*. Volk (24) stated that *T. graminum* Karst. grew best on 0.5% agar and almost as well on 1.0% agar, and that 4% agar greatly reduced growth of this fungus.

The present study compares the aggressiveness of *Fusarium nivale, Typhula idahoensis, T. incarnata,* and *Sclerotinia borealis* at 1.5, 0, and −1.5 C; the influences of these temperatures on carbohydrate metabolism of

wheats differing in snow mold resistance; and the abilities of the four pathogens to tolerate diverse water potentials.

MATERIALS AND METHODS.—*Inoculation and incubation in snow mold chambers.*—Inoculum of two isolates of each pathogen was produced by transferring mycelial fragments suspended in a water blank to a moist sand-bran-dextrose medium (4) with incubation at 10 C for *Typhula* spp. and *F. nivale,* and 0-5 C for *S. borealis.* The medium was placed in quart canning jars to a depth of about one-third the capacity of the jar, to permit mixing by shaking. The inocula were used fresh (undried) after 4-8 weeks. The shorter period is preferred for *F. nivale.*

Wheat was seeded at a uniform depth in early September in a 1:1 mixture of Palouse silt loam and river sand in 6-inch clay pots. The planted pots were watered and placed in sand beds outdoors for growth and hardening under natural light and temperature fluctuations. When young, the plants were thinned to three/pot. In late November or early December, the pots were removed from the sand beds, and brought into the greenhouse. About 35-40 cc of inoculum were added to the plant and soil surface, and a pad of wetted absorbent cotton was placed firmly over the plants so that all leaves were covered. The inoculated plants were then placed in dark chambers ("snow mold" chambers) maintained at selected low temperatures. The pots were placed in water 1.5-2.5 cm deep for subirrigation. Water was sprayed on the cotton if it became somewhat dry. Control plants were neither inoculated nor covered with cotton. Natural (nonsterile) soil was used and slight snow mold developed in the controls.

After incubation in the snow mold chambers, the plants were transferred to greenhouse benches under natural daylight at 13-20 C. The cotton was removed and mold observed, and the plants were watered if survival and growth of the molded plants was to be determined. Recovery is usually rapid under these conditions. Etiolated control plants become green in 2-3 days.

Weights of all aboveground growth were taken after 11-12 days in the greenhouse to compare recovery.

When important departures from the above procedures occurred, they will be given where appropriate.

Carbohydrate and chlorophyll determinations.—Plants were held in the snow mold chambers in the dark without inoculum and without wet cotton. Elimination of inoculum and the cotton covering resulted in leaves free of mold.

Leaf and crown plus root samples were taken after 0 to 8 weeks in the dark, depending upon the experiment. In carbohydrate determinations, the soil was washed from the roots and the plants were cut into two portions: leaves; and crowns plus roots. Plant tissue was autoclaved at 105 C for 5 min to halt all enzyme activity, dried at 20 C, ground in a Wiley mill to pass a 20 mesh screen, and stored dry until analyzed. Total available carbohydrates were determined by the method of Weinmann (25), utilizing hydrolysis by commercial takadiastase (K & K Laboratories, Inc., Plainview, N.Y., USA) of 0.2-g samples for 48 hr at 37 C to digest carbohydrate assumed to be readily available to the plant. Enzyme hydrolysis was followed by a mild acid hydrolysis with HCl to convert available carbohydrates to "glucose equivalents" and glucose determined by titration with cerium sulfate.

Chlorophyll was measured with a Spectronic 20 colorimeter at 665 and 645 nm for chlorophyll A and B, and the chlorophyll conversions were combined. The first extractions were made by grinding leaves with sand in a mortar and pestle, using hexane as the solvent. Later extractions used a more efficient and quicker extraction, boiling 15 leaf discs (cut at 2 cm intervals along leaves with a No. 1 cork-borer) per sample in 80% ethanol (16).

Water activity media.—Water activity media were made using the osmotic forces of solutes as calculated by Scott (19). Modifications of his system, employing both sugar and salt solutions at 0, 1, 5, and 10 C, were used. All media were molal solutions. Water activity (a_w) data for sucrose, NaCl, and KCl were taken from the tables of Robinson & Stokes (18). Activity (a_w) in solution is equivalent to relative humidity, and related by a constant and logarithmic relation to water potential. Plastic petri dishes (100 × 15 mm) containing 25 ml of medium were enclosed in plastic bags during incubation to minimize water loss. Inoculum consisted of mycelial blocks about 3 × 3 mm cut from near the advancing margin of vigorous cultures incubated at either 1 or 5 C, depending upon the experiment. Growth was estimated by colony diam minus the inoculum block.

RESULTS.—*Size of plants.*—Early seeding not only produced plants that recovered strongly (Fig. 1, top); it also produced the most sclerotia per plant (Fig. 1, bottom).

Depth of seeding.—*Typhula idahoensis,* our most aggressive snow mold pathogen, typically destroys all expanded leaves of both resistant and susceptible wheats. The difference in survival and recovery of the plants resides in the crowns. This led to the supposition that deeper seeding might increase the amount of crown tissue, and increase the ability of wheat to survive and recover from snow mold. In 1966-67, wheats differing in resistance were seeded at depths varying from 2-8 cm at a single date. Deeper-than-normal seeding did not increase the resistance of the wheats. The extreme depths resulted in poor emergence and weaker plants.

The experiment was repeated in 1967-68 with seeding depths of 1.2, 2.4, and 5 cm. Two susceptible and two resistant wheats were seeded 12 September. The 5-cm depth retarded emergence and delayed development of the wheat. In this trial, *F. nivale* and *T. idahoensis* were used. Again, varying the depth of seeding had so little influence on survival and recovery (Table 1) that further work with depth of seeding was abandoned.

Effect of −1.5, 0, and 1.5 C on F. nivale, T. idahoensis, and T. incarnata snow molds of winter wheat.—Five resistant (C.I. 9342, C.I. 14106, P.I. 167822, P.I. 172582, and P.I. 173438) and five commercial (Burt, McCall, Moro, Nugaines, and Wanser) wheats

were seeded 5 and 6 September 1967. They were inoculated 29 November with two isolates of each pathogen, individually, in three replicates. One group of plants was removed 15 January 1968; group two, 26 January; group three of *T. idahoensis* was removed 9 February, and the remainder of group three on 14 February. Incubation varied from 45 to 73 days.

We intended to maintain the highest temperature at 2 C, but this was too warm as the wheats raised the cotton and became etiolated. It was lowered to 1 C, and the earlier 2 and later 1 C are reported as 1.5 C.

Two isolates of *Sclerotinia borealis* were included,

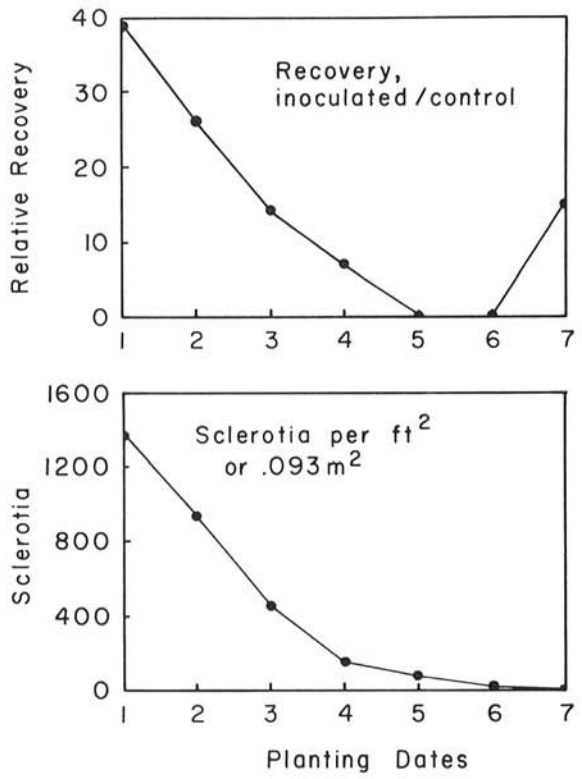

Fig. 1. Influence of planting dates (= plants of differing stages of development) on recovery of wheat from snow mold as judged by per cent green wt inoculated/control (top), and upon production of sclerotia of *Typhula idahoensis* per surface area of a field with wheat comparably attacked (bottom). Wheat at dates 1-7, respectively, had 6-8, 8-10, 5-7, 2-3, 0-1, 0, and 0 tillers/plant, and wheat of date 5 had 2-3 leaves; of date 6, two leaves, and of date 7, 0-1 leaf emerged when inoculated. Seven resistant, three susceptible, and four intermediate wheats were seeded in four replicates on 28 August; 7, 18, 28 September; and 9, 19, 28 October 1967, to obtain plants in different stages of development. Incubation at 1 C began 27-28 November 1967. One replicate was removed on each date: 9, 12, 18, 21 January 1968. Incubation in the chamber varied from 43-55 days.

Recovery was assessed by obtaining the green wt of green shoot tissues after 9-12 days in the greenhouse. Data are expressed in terms of green wt, inoculated plants/noninoculated control plants. Each point on the graph represents data from plants in 56 inoculated/56 control pots. Emergence was not complete in the last seeding.

TABLE 1. Influence of depth of seeding on the recovery of four wheats from speckled (*Typhula idahoensis*) and pink (*Fusarium nivale*) snow molds as determined by green wt of regrowth

	Seeding depth (cm)						
	1.2	2.4	5.0	1.2	2.4	5.0	
Wheat	*T. idahoensis*			*F. nivale*			Avg
McCall	0[a]	0	0	10	28	20	10
Nugaines	3	2	3	26	28	36	16
C.I. 14106	26	30	24	108	80	52	53
P.I. 173438	25	22	28	103	56	83	53
Avg	13	13	14	62	48	48	

[a] Data represent percentages, total green wt of green tissues, inoculated/noninoculated (controls), four replicates/treatment.

but no disease resulted. The plants of the *S. borealis* series were thus used as additional controls.

Typhula idahoensis destroyed the wheat most rapidly at all three temperatures, and was most destructive at 0 C (Table 2). *Fusarium nivale* was second, but it caused no disease at −1.5 C. *Typhula incarnata* was third in virulence, and it too caused no disease at −1.5 C. The destruction recorded (Table 2) under *S. borealis* (control) at 1.5 C is due to "volunteer" snow mold (*F. nivale* and *T. incarnata*) that came from the soil.

Sclerotia of *T. idahoensis* were produced abundantly, at 1.5 and 0 C, on both resistant and susceptible wheats (Table 3), but only a few immature sclerotia were observed on plants incubated at −1.5 C. The longest incubation period (73 days) was probably too short to enable this fungus to reproduce on the host at a sustained −1.5 C.

Carbohydrate reserves.—Carbohydrate reserves were determined for the noninoculated controls of the temperature experiment after 6.5, 8, and 10 weeks in the dark at 1.5, 0, and −1.5 C. In this experiment, only one sample was taken for each variety at each temperature at each date. In total mg glucose per 0.2-g sample, C.I. 14106 was highest with 120 mg; C.I. 9342 and P.I. 167822 had 98 mg; Nugaines, 81 mg; Wanser, 70; and Burt, 68 mg of glucose. The glucose content of 0.2 g of root and crown varied from 0.64 mg in C.I. 14106 to 0.30 mg in Burt. The paucity of data does

TABLE 2. Relative recovery made by five resistant and five susceptible wheats after incubation with two isolates each of *Fusarium nivale, Typhula idahoensis,* and *T. incarnata* at 1.5, 0, and −1.5 C

	Temp, C		
Pathogen	1.5	0	−1.5
Control	81[a]	97	100
F. nivale	47	70	101
T. idahoensis	18	9	37
T. incarnata	52	60	94

[a] Each datum contains the wt of 90 plants. The reduction in controls at 1.5 C is caused by *F. nivale* and *T. incarnata* originating from the soil. All figures are relative to the control at −1.5 C. Data represent percentages, total green wt of green tissues, inoculated/noninoculated (controls).

TABLE 3. Numbers of sclerotia of two isolates of *Typhula idahoensis* per plant of two resistant (C.I. 9342, C.I. 14106) and two susceptible (Burt, Wanser) wheats at 1.5 and 0 C

	Isolate 5999-5		Isolate 6258	
Wheat	1.5	0	1.5	0
C.I. 9342	124[a]	121	208	180
C.I. 14106	145	78	119	
Burt	89	82	104	70
Wanser	74	73	126	48

[a] Each datum represents the average number of sclerotia per plant on samples varying from 10 to 30 plants.

not warrant further exposition, but high carbohydrate content was correlated with resistance.

In 1968-69, a more extensive determination of carbohydrates was made using five resistant and five susceptible wheats with samples taken after incubation in the dark at 1 C for 0, 4, and 8 weeks. Three determinations were made for each variety at each date. The average total mg glucose equivalents per 0.2-g sample/wheat for all three dates was as follows: C.I. 14106, 94; P.I. 173438, 81; C.I. 9342, 79; P.I. 166797, 71; P.I. 167822, 67; Omar, 61; Nugaines, 58; Burt, 57; and Itana and Wanser, 54. The resistant wheats all had greater total carbohydrate than the commercial varieties, and the correlation of carbohydrate reserve with known field reactions to snow mold (4) is good.

The resistant wheats at 0 weeks (before incubation in the dark) averaged 95 mg glucose equivalents; the susceptible wheats averaged 79 mg, or 83% of that of the resistant plants. After 8 weeks in the dark, the resistant wheats averaged 58 mg, the susceptible wheats 32; or only 55% of that of the resistant plants. The resistant wheats entered the dark with more carbohydrate, and they utilized it more slowly. The difference in favor of the snow mold-resistant varieties increased with incubation (glucose equivalents, susceptible/resistant at 0 weeks, 83%; 4 weeks, 72%; 8 weeks, 55%) (Fig. 2).

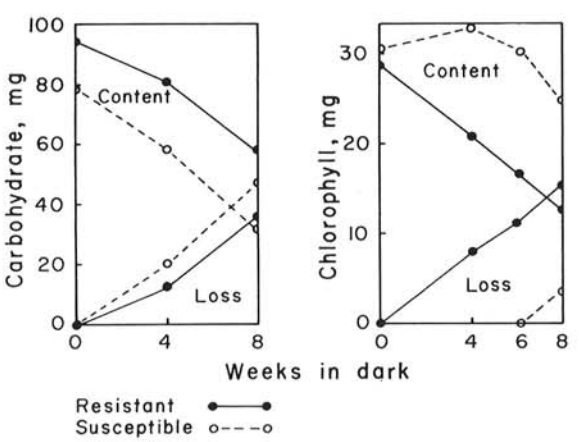

Fig. 2. Loss of total carbohydrate (left) (mg of glucose equivalents per 0.2 g dry leaves and crowns + roots) and chlorophyll (right) by resistant and susceptible wheats incubated in the dark at 1 C for 0 to 8 weeks.

TABLE 4. Total available carbohydrates in leaves and crowns + roots of wheat, resistant and susceptible to snow molds, after incubation at 1 C in the dark for 0, 4, and 8 weeks

	Resistant			Susceptible		
	Incubation period, weeks					
Plant part	0	4	8	0	4	8
	mg	*mg*	*mg*	*mg*	*mg*	*mg*
Leaves	62[a]	43	38	53	33	24
Crown + roots	33	37	21	26	25	8

[a] Each datum is the average of three samples/variety per date. Five resistant and five susceptible wheats = 15 samples/datum; mg of glucose equivalents per 0.2 g of dried tissue.

The data also indicated that, during the 0-4 week period of darkness, carbohydrates were translocated from leaves to the roots and crowns by both resistant and susceptible wheats (Table 4). During the 0-4 week period of darkness, carbohydrates in the leaves decreased but remained constant in roots and crowns.

Chlorophyll determinations.—Leaf samples for chlorophyll determinations were taken from wheats in the temperature series after 6.5, 8, and 10 weeks in the dark at 1.5, 0, and −1.5 C. Wheats of the same series of plants that had been left outdoors were sampled 24 January 1968. Plants at 1.5 C became chlorotic quickly, and the chlorophyll content of resistant (C.I. 9342, C.I. 14106) wheats was 98% that of susceptible (Burt, Wanser) wheats when data of the three sampling dates were averaged. The chlorophyll content of the resistant wheats averaged only 69% of that of the susceptible wheats at 0 C, and 95% at −1.5 C. Thus, at either warm (1.5) or cold (−1.5 C) conditions, the wheats were indistinguishable, but at 0 C, chlorosis was more severe in resistant wheats.

In 1968-69, chlorophyll content of resistant wheats after 0, 4, 6, and 8 weeks in the dark at 1 C decreased more rapidly than it did in susceptible wheats. Thus, while the resistant wheats were richer in carbohydrate (Fig. 2, left), they became more chlorotic (Fig. 2, right) than susceptible wheats.

Water activity media.—Trials with osmotic forces produced by KCl, NaCl, sucrose, and dextrose in various media at 0, 1, 5, and 10 C indicate that there is no precise drought limit for a given species. Rather, "drought tolerance" will vary with the pH of the medium, its composition, temperature, isolate of the species, and most important in some cases, with the source of the osmotic forces.

Sclerotinia borealis grew slowly on "standard" potato-dextrose, cornmeal, or cornmeal dextrose agar media. These media have relatively high water potentials, probably above −2 bars (a_w 0.9985 at 20 C). When sucrose reduced the water potential to about −15 to −25 bars, *S. borealis* grew about five times as fast as it did in "standard" media at 1 C, and at that temperature it equaled or exceeded the growth rate of the other pathogens at their water potential opt (Fig. 3). When KCl or NaCl was used to induce water stress,

the growth of *S. borealis* was much less than when sucrose was used.

Typhula idahoensis and *T. incarnata* responded similarly to water stress, both species tolerated osmotic forces from sucrose, and they were somewhat tolerant to KCl and NaCl. The *Typhula* spp. and *F. nivale* grew best with high water potential, and their growth rate diminished markedly with stresses greater than about −6 bars. These fungi, thus, contrast sharply to *S. borealis* in their responses to water potential.

When three isolates of *F. nivale* were grown on Difco cornmeal agar to which KCl, sucrose, KCl + sucrose, or dextrose were added to increase osmotic forces, one isolate tolerated KCl fairly well at 10 C; the growth of two other isolates was severely limited by KCl in contrast to an equal osmotic force derived

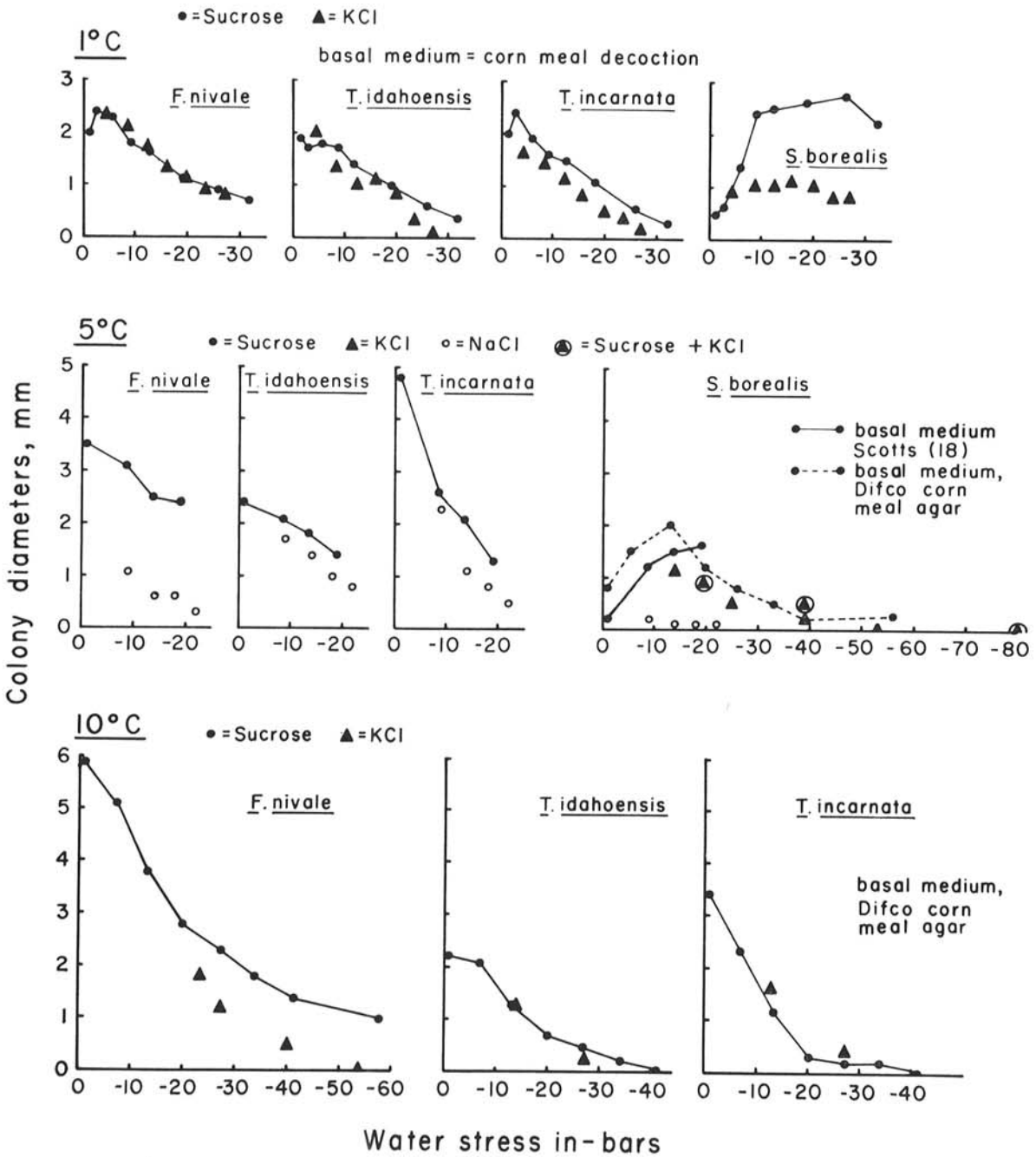

Fig. 3. Growth of *Fusarium nivale, Sclerotinia borealis, Typhula idahoensis,* and *T. incarnata* on agar media differing in water potential induced by different osmotic solutions. Incubation was at 1, 5, and 10 C, growth based on colony diam.

from either sucrose or dextrose. At a water potential of −13 bars after 13 days, the diam of three isolates were 6, 14, and 51 mm when KCl lowered the water potential. When sucrose was used, diam of the same three isolates were 53, 48, and 47 mm, respectively, or more nearly alike.

When standard Difco potato-dextrose agar (PDA) medium was used in varying concentrations (13, 19.5, 26, 39, and 52 g/liter of distilled water) to provide increasing levels of solutes and agar (the latter at 0.5, 0.75, 1, 1.5, and 2%), the growth of the various fungi was influenced little at 1 C. The diam of colonies of *F. nivale* averaged 44 mm at 13 g and 32 mm at the 52 g PDA concentration. The diam of *S. borealis* colonies averaged 20 mm at the 13-g, and 30 mm at the 52-g concentration. *Typhula incarnata* and *T. idahoensis* grew equally at all five concentrations. When PDA medium was made at 13 g/liter (0.5% agar) and agar alone added in increments to a total of 4% agar, the effect on rate of growth of all four fungi was small. Increased agar did not adequately increase "matric" forces.

DISCUSSION.—*Size of the wheat plant.*—The size of the wheat plant, as in previous field observations (8, 14, 26) and snow mold chamber experiments (2), influenced survival and the ability of survivors to recover. The largest plants in our snow mold chamber trials (6-10 tillers/plant) are small compared to those obtained from early seeding and low seeding rates in the field. McCall wheat, seeded at 40 lb./acre 13 August 1969, averaged about 20 tillers/plant. Farmers in the chronic snow mold areas of Washington prefer to seed in the first half of August. The large, robust crowns usually survive and produce more wheat, even though the leaves are totally destroyed, than the small wheat from late seedlings that may escape attack altogether. Sclerotia production is as great on resistant as on susceptible wheats. When and if sufficiently resistant wheats become available, planting dates could be retarded, and the smaller plants would reduce the numbers of sclerotia of *T. idahoensis,* leading to a steady diminution of the inoculum level of this pathogen.

The long-time danger from early seeding, so far as *T. idahoensis* is concerned, is illustrated by our sclerotia production data. If each large, early-seeded, diseased wheat plant provides 250 sclerotia, and there are 400,000 such plants/acre, 10^8 sclerotia are produced/acre; a smaller plant from a medium seeding date with 50 sclerotia each with 500,000 plants/acre provides a potential 2.5×10^7 sclerotia/acre, or one-fourth the number from early seeding. Consistent late seeding with small plants would not maintain an effective inoculum level of *T. idahoensis*. The disease cycles of *T. incarnata* and *F. nivale* are sufficiently different so that inoculum levels of these pathogens would not be so directly influenced by plant size.

Physiologic aspects of resistance.—Earlier studies in the USSR (23) and particularly in Japan (22) stressed the role of nutrition in survival under snow mold conditions. The fact that our more resistant wheats entered the winter with a greater carbohydrate reserve and used it at a slower rate near 0 C supports the hypothesis that starvation plays a significant role in predisposition. While we stress the role of carbohydrates in resistance, starvation alone does not kill the wheat. This is proven by the success of fungicides in controlling snow molds (9).

The third, unknown component of resistance (something in addition to plant size and food reserves) probably depends upon available food reserves and upon maintenance of a certain min level of metabolism. C.I. 9342 was selected in Washington at 3,000 feet elevation (915 m), where snow cover in mold years persists from 100 to 120 days. C.I. 14106 was selected in Idaho at 6,000 feet (1,830 m), where snow cover in the sites used often persists 150 days. In Washington, C.I. 9342 recovered more quickly than C.I. 14106 in 2 years out of 3, when meaningful field observations were possible. In Idaho, C.I. 14106 has consistently survived better than C.I. 9342. Possibly, C.I. 9342 has a greater amount of the unknown resistance factor, but its lower carbohydrate reserve fails to maintain the required metabolic level under more prolonged darkness.

As predisposition progresses, the wheat plant apparently has difficulty in regulating carbohydrate utilization. If the mg of reserve carbohydrate per unit of leaf plus crown and root are averaged after 0, 4, and 8 weeks in the dark at 1 C, the figures are 48, 41, and 29 for resistant, 39, 29, and 16 for susceptible wheats, respectively. Conversion to per cent of original reserves transforms the figures to 100, 85, and 60 for resistant, 100, 74, and 41 for susceptible wheats. The rate of expenditure was thus greater during the second 4-week period than during the first 4 weeks.

Chlorophyll content of leaves of resistant wheats decreased more rapidly at 0 C in the dark than in susceptible wheats. The carbohydrate data indicate that translocation from leaves to crowns occurs during the first 4 weeks. The susceptible wheats apparently maintain the leaves in a higher metabolic state than the resistant wheats. The resistant wheats may have evolved in an environment of deep snows, and "sacrificing" the leaves to strengthen the crowns would be a survival mechanism. Leaves in the dark perform no useful function.

The resistance of certain wheats, not only to different isolates of a species, but to such diverse species as *F. nivale, T. idahoensis,* and *T. incarnata* (1) supports the role of some common factor(s) in resistance.

Winter hardiness vs. snow mold resistance.—In latitudes and elevations where snow molds are significant, winter wheats must have a degree of winter hardiness. Cold may come when no protective snow cover exists. Winter hardiness (11) and snow mold resistance are both enhanced by carbohydrate reserves, but hardiness and mold resistance are not the same. Many of the most hardy wheats of the world have been grown in Washington, and all commercial varieties of the past were susceptible (8, 14). Yet some of the observations on hardiness may aid in understanding resistance to snow molds. Newton & Anderson (15) reported in 1931 that the respiration rate of Minhardi decreased when tissue samples were taken from outdoor plants during the period September through November. As hardiness

and dormancy increased, respiration at 0 C decreased. Respiration per unit of tissue decreased by half between 10-20 September and 17-30 November. Early snowfall increases losses from mold, particularly when it occurs before the soil is frozen. Early snow may do more than merely extend the period of exposure. Early snow may prevent completion of the hardening process and allows wheats to go under snow with a higher rate of basal metabolism than normal. If the higher metabolic rate of incompletely hardened wheat persists, the rate of carbohydrate loss and predisposition would be increased, and contribute to seasonal fluctuations of disease severity.

Winter hardiness is usually greatest in wheat in the 4-6 leaf stage (early tillering) (17). Such wheat is highly vulnerable to mold. Early seeding in Washington results in wheat with at least 10 tillers/plant and reduced potential winter hardiness.

In winter hardiness, survival depends upon crown, not on leaves or roots. Janssen (10) removed roots and leaves from the crown, and survival (and subsequent yield) was not affected.

Low temperature hardening is associated with sugar accumulation and light intensity. A general, but not always varietal, relationship exists between high sugar content and hardiness (11). Sucrose was the main sugar that accumulated during hardening. Emphasis of the crown in hardiness parallels its emphasis in survival under snow mold.

Temperature and moisture relations.—Snow mold of winter wheat probably develops in the most uniform environment of any disease of terrestrial plants, yet the small differences in temperature and moisture beneath deep snow govern the type of disease that develops. In Hokkaido, Japan (22), as in Douglas County, Washington (4), two, three, or four snow mold fungi may infest the same fields. In one season, one fungus may predominate, or one fungus may predominate in one portion of a field and another in the remainder. Most of these differences do not reflect inoculum levels so much as they do the environment.

Sclerotinia graminearum dominated when the snow cover was inadequate to prevent the soil from freezing (22). Tomiyama (22) found that *S. graminearum* grew in a frozen agar medium that did not support growth of *T. incarnata*. Both fungi grew on the same medium at the same temperature if freezing was avoided. *S. graminearum* grew better on concentrated media; *T. incarnata* grew better on more dilute media. Tomiyama concluded that *S. graminearum* did best when the soil was frozen because it tolerated higher osmotic forces. The present study fully supports his conclusion. *Sclerotinia borealis* (= *S. graminearum* ?, 21) in our study grew most rapidly when water potential was reduced (at −10 to −25 bars). Osmotic forces of this magnitude reduced the growth of *F. nivale* and *T.* spp. Ice crystals remove water, and the snow mold *Sclerotinia* spp. apparently are favored by lowered water potentials.

Fusarium nivale and *T. incarnata* were not pathogenic at −1.5 C, but *T. idahoensis* was. As these pathogens all grow on super-cooled media at −5 to −7 C (6, 22), the different responses to temperature observed near freezing may be influenced more strongly by water relations than by temperature directly, but our data failed to support such a supposition.

Sclerotinia borealis failed to attack wheat in our experiments. *Sclerotinia graminearum* did not attack wheat in Tomiyama's (22) trials. We both used sclerotial and mycelial inoculum. Possibly, unlike *Typhula* spp., the sclerotia do not serve directly as a source of inoculum, but function only through the production of ascospores (22).

LITERATURE CITED

1. 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. Reptr. 51: 810-814.
2. Bruehl, G. W. 1967. Effect of plant size on resistance to snow mold of winter wheat. Plant Dis. Reptr. 51: 815-819.
3. Bruehl, G. W. 1967. Diseases other than rust, smut, and viruses, p. 375-410. *In* K. S. Quisenberry & L. P. Reitz [ed.]. Wheat and wheat improvement. Amer. Soc. Agron., Inc., Madison, Wisc.
4. Bruehl, G. W., R. Sprague, W. R. Fischer, M. Nagamitsu, W. L. Nelson, & O. A. Vogel. 1966. Snow molds of winter wheat in Washington. Washington Agr. Exp. Sta. Bull. 677. 21 p.
5. Cormack, M. W., & J. B. Lebeau. 1959. Snow mold infection of alfalfa, grasses, and winter wheat by several fungi under artificial conditions. Can. J. Bot. 37:685-693.
6. Ekstrand, H. 1955. Overwintering of winter cereals and forage grasses. Summary of results and program for continual investigations. Medd. Växtskyddsanst, Stockholm, Sweden 67:1-125.
7. Gould, C. J. 1957. Turf diseases in western Washington in 1955 and 1956. Plant Dis. Reptr. 41:344-347.
8. Holton, C. S. 1953. Observations and experiments on snow mold of winter wheat in Washington State. Plant Dis. Reptr. 37:354-359.
9. Jamalainen, E. A. 1964. Control of low temperature parasitic fungi in winter cereals by fungicidal treatment of stands. Ann. Agr. Fenniae 3:1-54.
10. Janssen, G. 1929. Effect of date of seeding of winter wheat on plant development and its relationship to hardiness. Amer. Soc. Agron. J. 21:444-466.
11. Kneen, E., & M. J. Blish. 1941. Carbohydrate metabolism and winter hardiness of wheat. J. Agr. Res. 62:1-26.
12. Lebeau, J. B. 1964. Control of snow mold by regulating winter soil temperature. Phytopathology 54:693-696.
13. Martin, J. H. 1927. Comparative studies of winter hardiness in wheat. J. Agr. Res. 35:493-535.
14. McKay, H. C., & J. M. Raeder. 1953. Snow mold damage in Idaho's winter wheat. Idaho Agr. Exp. Sta. Bull. 200. 5 p.
15. Newton, R., & J. A. Anderson. 1931. Respiration of winter wheat plants at low temperatures. Can. J. Res. 5:337-354.
16. Osborne, Daphne, & D. R. McCalla. 1961. Rapid bioassay for kinetin and kinens using senescing leaf tissue. Plant Physiol. 36:219-221.
17. Roberts, D. W. A., & M. N. Grant. 1968. Changes in cold hardiness accompanying development in winter wheat. Can. J. Plant Sci. 48:369-376.
18. Robinson, R. A., & R. H. Stokes. 1955. Electrolyte solutions. Academic Press, Inc. N. Y. 512 p.
19. Scott, W. J. 1953. Water relations of Staphylococcus aureus at 30°C. Australian J. Biol. Sci. 6:549-564.
20. Sprague, R. 1950. Diseases of cereals and grasses in

North America (fungi, except smuts and rusts). Ronald Press Co., N. Y. 538 p.
21. SPRAGUE, R., W. R. FISCHER, AND PEGGYBETH FIGARO. 1961. Another sclerotial disease of winter wheat in Washington. Phytopathology 51:334-336.
22. TOMIYAMA, K. 1955. Studies on the snow blight disease of winter cereals. Rep. No. 47, Hokkaido Nat. Agr. Exp. Sta., Sapporo, Japan. 234 p.
23. TUMANOV, I. I., J. N. BORODINA, & T. V. OLEINIKOVA. 1935. The role of snow cover in the wintering of crops. Bull. Appl. Bot. Gen. Plant Breed. Ser. 3. 6:3-57.
24. VOLK, A. 1937. Untersuchungen über Typhula graminum Karst. Z. f. Pflanzenkr. 47:338-365.
25. WEINMANN, H. 1947. Determination of total available carbohydrates in plant material. Plant Physiol. 22: 279-290.
26. YOUNG, P. A. 1937. Sclerotium blight of wheat. Phytopathology 27:1113-1118.