Reaction of Winter Wheat to Pythium Snow Rot

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ABSTRACT

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In laboratory tests with *Pythium iwayamai*, small wheat plants (three to four leaves, no tillers) were more resistant to snow rot than were larger plants (seven to nine tillers per plant). In a search for sources of resistance, only six of 77 wheats inoculated with *P. iwayamai* gave 50% or greater survival after incubation for 63–77 days under flooded conditions at 0.5 C. The most resistant line, Sel. 7439, averaged 66% survival. In early seeded field trials with natural inoculum, no wheat tested had sufficient resistance in the field to provide >50% survival. Resistance to snow rot is not related to resistance to snow mold and is different from winterhardiness. Large plants obtained by early seeding survive snow mold better than small plants from late seedings, and small plants obtained by late seeding survive snow rot better than large plants. Although the breeding of one cultivar with resistance to both diseases may be possible if a wheat such as CI 14106 is used as a resistant parent, the contrasting response to seeding dates makes cultural control more difficult.

Diseases of winter wheat (*Triticum aestivum* L.) frequently occur beneath snow in north central and eastern Washington, but actual losses vary markedly depending on the location and the weather (5). Snow mold caused by *Typhula idahoensis* Remsberg, *T. ishikariensis* Imai, *T. incarnata* Lasch ex Fr., and *Fusarium nivale* (Fr.) Ces., as well as snow rot caused by *Pythium iwayamai* Ito and possibly *P. okanoganense* Lipps (11), occur in these areas. In Washington, snow mold is more important than snow rot because it can affect entire fields, but snow rot is confined to natural drainage ways and low areas where water collects.

Field observations and tests under controlled conditions indicate that resistance to snow molds is nonspecific, polygenic, and strongly influenced by plant size and vigor (1,2,4,10). The leaves of large, well-tillered plants are susceptible, but crowns are not destroyed except under severe attack. Late seeded or very small plants with no tillers frequently survive in the field with no appreciable damage from snow mold and, presumably, have escaped infection (3,8,16).

Commercial use of Sprague (CI 15376), a snow mold resistant wheat, has reduced losses, and losses from snow rot have been easily recognized in areas where water from snow melt accumulated. Growers in severe snow mold areas were concerned that Sprague had lost its resistance, but on examination, it was evident that snow rot was mistaken for snow mold. Survival of winter wheat in limited areas of Washington requires winterhardiness and resistance to snow mold and to snow rot.

The purpose of this study was to determine if resistance to snow rot is related either to snow mold resistance or to winterhardiness. The relationship of plant age and size to susceptibility was examined, and winter wheats with varying levels of mold resistance and winterhardiness were tested under artificial and field conditions for resistance to Pythium snow rot.

MATERIALS AND METHODS

Winter wheats from Europe, Asia, and North America were tested for resistance to Pythium snow rot in the laboratory, field, or both. The wheats represented hard white, soft white, hard red, soft red, and club market classes. Some were very winter-hardy and others were used as sources of resistance to snow molds.

Inoculum. Inoculum containing zoospores and sporangia was produced by growing two single zoospore isolates of *P. iwayamai* (IMI 209669 and N/Y.z.1) on a corn meal/sand mixture (15 g of corn meal, 485 g of sand, and 120 ml of deionized water autoclaved 45 min at 121 C). The inoculated medium in glass jars was incubated at 10 C for 3 wk and shaken periodically to insure complete colonization of the corn meal/sand mixture.

Cultures of both isolates were mixed

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in a 1:1 ratio and then adjusted with autoclaved sand to an inoculum concentration of 140 propagules per gram of sand as determined by a dilution plate technique (14). An autoclaved corn meal/sand culture of isolate IMI 209669 was similarly diluted with sand and used as the control.

Zoospore inoculum was prepared by transferring 2- to 3-mo-old Difco corn meal agar cultures of *P. iwayamai* (IMI 209669) to Difco lima bean agar and incubating at 10 C until the colony diameter reached 5 cm. Four or five 1-cm disks were cut from the periphery of the colonies and placed into each glass petri dish with 20 ml of deionized water. After 24-hr incubation at 5 C, the deionized water in each dish was decanted and replaced with fresh 5 C deionized water. Forty-eight hours later dishes with motile zoospores were transferred to 0.5 C, and after 2–3 hr, zoospores in suspension were decanted into 250-ml beakers and adjusted to the proper concentration by dilution with deionized water precooled to 0.5 C.

Effect of plant size. Seeds of three winter wheats—CI 14106, Peck (CI 17298), and Nugaines (CI 13968)—were planted at 2-wk intervals from 2 September to 14 October 1977 in 15-cm-diameter plastic pots containing a Palouse silt loam/sand/vermiculite mix (6:4:3, v,v,v). Emerged plants were thinned to four per pot, with four pots for each treatment. The plants were hardened outdoors and were fertilized with ammonium nitrate and potassium nitrate until 2 December 1977.

Plant growth ranged from seven to nine tillers per plant in the earliest planting (plant size 4) to the three to four leaf stage with no tillers in the last planting date (plant size 1 by December 2). Pots were then inverted, soil and plants were removed intact, each pot was lined with a plastic bag, and the soil and plants were then put back into the bag-lined pot. The soil in each pot was flooded with tap water to a depth of 1 cm, and 30 g of the corn meal/sand inoculum was applied evenly to the water covering the soil surface. The plants were then covered with wet absorbent cotton and incubated at 0.5 C in the dark for 60–77 days. The pots were then drained, the cotton cover removed, and the plants placed on greenhouse bench (8–15 C) for 2 wk. At the end of this recovery period, the number of surviving plants per pot after each incubation time was recorded.

Inoculations with zoospores. Seeds of five wheats—Sel. 7439, CI 14106, Sprague, Nugaines, and Luke (CI 14586)—were planted in sand in 237-ml Styrofoam cups (one seed per cup) 15 September 1978 and placed outdoors. Plants were fertilized with 10% Hoagland's solution no. 2 (7). On 5 December 1978 plants in cups were brought into the greenhouse, flooded with tap water to a depth of 1 cm above the sand surface, covered with wet pads of absorbent cotton, and incubated at 0.5 C in the dark.

After 0, 13, and 27 days, 40 plants of each wheat were taken from the low-temperature chamber and washed from the sand in cold tap water. Their roots were wrapped in moist paper and the tops of four plants at a time of each wheat were submerged in fresh 200-ml zoospore suspensions of 5,000 *P. iwayamai* spores per milliliter. A total of 20 plants of each wheat were submerged in zoospore suspensions and 20 were submerged in deionized water for 24 hr at 0.5 C.

The plants were subsequently transplanted into sand in Styrofoam cups (one plant per cup), flooded with tap water, covered with cotton, and placed in the 0.5 C chamber. After 85 days all cups were placed on the greenhouse bench (8–15 C) and drained; the cotton cover was removed, and the plants watered with 10% Hoagland's solution to facilitate recovery. The number of surviving shoots per plant was recorded after a 10-day recovery period.

Sources of resistance. Seeds of 77 wheats and one winter barley (*Hordeum vulgare* L.) (Table 1) were planted in soil in pots on 18 September 1977 and thinned to four plants per pot after emergence. Plants were maintained outdoors until 20 November 1977. At this time plants averaged from four to seven tillers, depending on the wheat. These plants were transferred to pots lined with plastic bags and inoculated with corn meal/sand cultures of *P. iwayami* as described.

Four pots of each wheat were incubated at 0.5 C in the dark for 63 and 77 days. The number of surviving plants per pot was recorded after a 2-wk recovery period on the greenhouse bench. Controls were uninoculated Sprague and Nugaines maintained under identical conditions.

Ninety-nine wheats and three barleys were tested in the field. These included the wheats and the barley previously tested in the low-temperature chamber. Each line was planted in a single four-row plot 3.05 m long, and every fifth plot was a control of either Peck, Nugaines, Sprague, or CI 14106. The seeds were planted with a deep-furrow drill with 40.6-cm row spacing on 25 August 1977 near LaFleur, Okanogan County, WA. The plot was in a low area where water could collect from melted snow. Estimates of the percent of surviving plants were recorded on 19 April 1978.

RESULTS

Effect of plant size. After 60 or 77 days at 0.5 C under flooded conditions, more plants of CI 14106, Peck, and Nugaines survived attack by *P. iwayamai* if plants were small (three to four leaf stage, plant size 1), than if large (seven to nine tillers per plant, plant size 4, Fig. 1). Some of the smallest plants were killed, but those that survived had watersoaked lesions restricted to the leaf tips. Survival of CI 14106 was greater than that of either Peck or Nugaines, but only in plants with five to six and seven to nine tillers per plant (plant sizes 3 and 4 respectively) was this

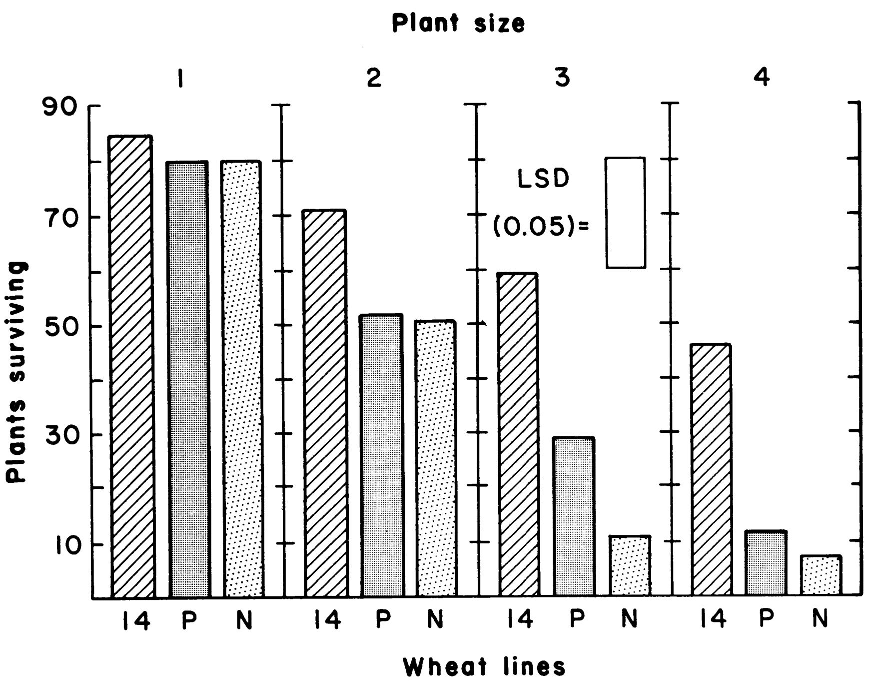

Fig. 1. The effect of plant size on survival of CI 14106 (14), Peck (P), and Nugaines (N) winter wheat inoculated with corn meal/sand culture of *Pythium iwayamai* (140 propagules per gram of sand). Data are the percent of inoculated plants surviving compared with controls and are means of four pots incubated at 0.5 C for 60 days and four pots for 77 days with four plants per pot. Plant sizes: 1 = three to four leaf stage, no tillers; 2 = two to four tillers per plant; 3 = five to six tillers per plant; 4 = seven to nine tillers per plant.

difference statistically significant.

Effect of zoospore inoculum. The mean number of tillers per plant was 6.6, 6.3, 5.0, 3.2, and 3.0 for CI 14106, Sel. 7439, Sprague, Nugaines, and Luke, respectively. Therefore, not all plants were in the same stage of growth when inoculated. After 85 days at 0.5 C under flooded conditions, inoculated plants had typical symptoms of snow rot. Many plants were killed, yet a few plants had some living tillers. More tillers survived of CI 14106 than of Sprague, Nugaines, or Sel. 7439. Luke was the most susceptible. The results were too variable, probably because of differences in growth stages, to warrant detailed presentation.

Sources of resistance. The mean number of surviving plants per pot (of a possible 4.0) for the 77 wheats tested in the low-temperature chamber, ranged for 2.62 for Sel. 7439 to 0.0 for Itana and Froid (Table 1). Only six wheats (Sel. 7439, PI 173440, CI 14106, Linne, Jo 3057, and HJA-B215) had 50% or more surviving. Froid and Itana, both winter-hardy wheats, were the most susceptible wheats tested. All wheats had fewer plants surviving after 77 days than after 63 days of incubation (data not presented).

Snow rot in the field was limited to areas of standing or running water, which resulted in irregular areas of severe disease surrounded by plants that escaped the disease. In the diseased areas the highest survival was 30% (CI 14106, PI 167822, Centurk, Bezostaja 2/B, Jo 3077, Id 00072, Sel. 7342) and the lowest was 0% (Arrow, Baca, Burt, Cappelle, Cardon, CO 725052, HJA 01481, Jo 3057, Jo 3067, Jyvâ, Nugaines, OK-66V2629, Peck, Sprague, and PI nos. 155229, 166797, 178383, 190490, 191423, 282905, and 372014). The dead plants had typical symptoms of snow rot, with crown and leaf tissues filled with oospores of *P. okanoganense* (11). As in the laboratory trial with *P. iwayamai*, there was no relationship between snow rot reaction and winterhardiness. Some wheats, such as CI 14106, with resistance to snow mold had some resistance to snow rot, but all plants of some wheats resistant to snow mold were eliminated by snow rot (Sprague and PI nos. 166797, 178383, and 181268). The field test was more severe than the laboratory test.

DISCUSSION

Selection of snow rot resistant lines in the field may be complicated by possible variations in the distribution of natural inoculum in drainage water from melting snow. Even slight depressions in the soil surface where water collects could have higher concentrations of inoculum and greater incidence and severity of snow rot than other areas. Predisposition could also be influenced by running, standing, or frozen water. The necessity of placing small plots in limited and irregular areas where water is likely to collect during snow melt adds to the difficulty in selecting resistant wheats in the field.

Most wheat plants colonized by *P. okanoganense* in the field did not recover after the water from snow melt drained away. Plants that survived either had only a few infected tillers or escaped infection. Koch's postules have been completed with *P. iwayamai* (13) (Table 1). Based on isolation from dead plants, we believe that *P. okanoganense* is also a pathogen at low temperatures. Although no proof is presented, resistance to *P. iwayamai* may be similar to resistance to *P. okanoganense*, since the infection processes by these species are similar (12).

Bruehl (3) and colleagues (4) demonstrated that the snow mold fungi, *T. idahoensis* and *F. nivale*, readily killed plants in the three to four leaf stage, but larger plants with several tillers were more resistant. The response to *P. iwayamai* was the reverse of that to the snow mold fungi. Results of the present study (Fig. 1) and observations by Iwayama (9) and Hirane (6) in Japan indicate that larger plants are more susceptible than smaller plants to snow rot. The lack of resistance in larger plants with greater carbohydrate reserves, which contributes to resistance to snow molds (3,10), indicates that carbohydrate reserves are not important in resistance to snow rot.

Sprague and Nugaines reacted equally to snow rot in the laboratory (37% survival) and in the field (0% survival), yet Sprague is moderately resistant and Nugaines is susceptible to snow mold. Some of the wheats with resistance to snow mold (Sel. 7439, CI 14106) were apparently also resistant to snow rot (Table 1). However, other lines used as parents in breeding for snow mold resistance (PI 173467, PI 181268) were highly susceptible to snow rot. Resistance to the two diseases is apparently unrelated and independent.

The resistance of Sel. 7439 and CI 14106 to snow rot is not great. Papavizas and Ayers (15) noted that, although pea cultivars with tolerance to *Aphanomyces euteiches* Drechs have been selected under artificial conditions, no resistant

Table 1. Survival of wheats inoculated with *Pythium iwayami* under flooded conditions at 0.5 C in the dark

Wheat	Survival[a] (%)	Wheat	Survival[a] (%)
Sel 7439**[b]	65 a[c]	UT 755204, H	22 defghij
PI 173440***	59 ab	Arrow	22 defghij
CI 14106***	50 abc	HPG 245	22 defghij
Linne*, H[d]	50 abc	Caribo	22 defghij
Jo 3057, H	50 abc	Wanser	22 defghij
HJA-B215*, H	50 abc	Centurk	22 defghij
PI 166797	47 abcd	Franklin	22 defghij
Sel A65257w579***	43 abcde	Sel 101	19 efghij
PI 178383***	37 bcdef	Roughrider	19 efghij
Bezostaja 2/B*, H	37 bcdef	Frederick	19 efghij
Sprague**	37 bcdef	Luke*	19 efghij
Nugaines	37 bcdef	Consul	19 efghij
Yorkstar	34 cdefg	PI 284553	19 efghij
AN 57143*, H	34 cdefg	Burt	19 efghij
CI 9342**	34 cdefg	Stepnaja, H	15 fghij
Peck	34 cdefg	Ark	15 fghij
Id 71043	34 cdefg	Cardon	15 fghij
Kavkaz, H	34 cdefg	McCall, H	15 fghij
Varma, H	34 cdefg	PI 191423	15 fghij
Elo, H	34 cdefg	Baca, H	15 fghij
Moro*	31 cdefgh	Mt 6930	13 fghij
Ticonderoga	31 cdefgh	PI 181268***	13 fghij
Ionia	31 cdefgh	Ks 70H179	9 ghij
Daws	31 cdefgh	Hyslop	9 ghij
Omar	31 cdefgh	Winoka, H	9 ghij
Paha	31 cdefgh	Golils Ns738	9 ghij
McDermid	31 cdefgh	Champlein	6 hij
OK 66v2629	31 cdefgh	Lancota	6 hij
Lindon, H	28 cdefghi	Coulee	6 hij
Rubigus	28 cdefghi	Frank Nord	6 hij
WA 007003	28 cdefghi	Cappelle	6 hij
Vakka*, H	28 cdefghi	Cerco	3 ij
Co 725061, H	28 cdefghi	Norsdaat	3 ij
Genessee	28 cdefghi	N98-35C	3 ij
Nisū	25 cdefghij	Winovka, H	3 ij
1567	25 cdefghij	TP-107	3 ij
Virgo, H	25 cdefghij	Froid, H	0 j
Svalof	25 cdefghij	Itana, H	0 j
Jyvâ	25 cdefghi	Kamiak barley	0 j

[a] Based on four plants per pot, four pots at 0.5 C for 63 days plus four pots for 77 days.
[b] Relative resistance to snow mold: * = some, ** = moderate, *** = resistance source.
[c] Means followed by the same letter are not significantly different at $P = 0.05$ using Duncan's multiple range test.
[d] H indicates wheat lines known to be winter-hardy.

cultivars of peas have been developed for commercial use. It is probable that, as in Aphanomyces root rot of peas, the level of resistance to snow rot is too weak to be useful in the field.

Winterhardiness does not provide protection against the snow rot *Pythium* spp. because Baca, Froid, Itana, and Karkov were susceptible.

For a snow rot resistant cultivar to be commercially acceptible, it must also have resistance to snow mold. The low amount of resistance to snow rot detected in several snow mold resistant wheats and the inverse relationship to plant size, and thus to seeding date, indicate that breeding one cultivar with resistance to both diseases may be difficult. Wheats with greater resistance to snow rot should be identified before a breeding program is started.

An early seeded winter wheat nursery containing 140 wheats in replicated plots was destroyed by what must have been Pythium snow rot during the 1968-1969 season at Pullman, WA. The plot was on very gently sloping to nearly level land between hills. About 60 cm of settled snow covered the wheat from December into March. In February water was trickling down the drill rows beneath the snow and the wheat was dead by April. There was no evidence of *Typhula* spp. and, except for some plants on the better drained periphery of the plot that were attacked by *Fusarium nivale*, the rotted crowns and leaves were filled with oospores. Pythium snow rot was suspected, but the disease could not be produced with Pythium isolates inoculated by standard methods (5).

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