Interactive Effects of Freezing and Common Root Rot Fungi on Winter Wheat

J. A. Fernandez, D. S. Wofford, and J. L. Horton

Associate professor of Plant Pathology, assistant professor of Crop Science, and research technician, respectively, University of Wyoming, Laramie 82071.

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ABSTRACT

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Wheat plants inoculated with *Bipolaris sorokiniana* and *Fusarium acuminatum* were subjected to freezing (−12 C) under controlled conditions. Plant tops were clipped and the effects of fungi, freezing, and the interactions of these factors on plant regrowth (top dry weight and percent reattainment of top dry weight measured prior to temperature treatments) and plant health (number of healthy adventitious coronal roots) were studied. If plants were not frozen, neither fungus significantly affected reattainment of top weight and only *B. sorokiniana* reduced top dry weights and the number of healthy coronal roots compared to those of uninoculated plants. After the plants were subjected to freezing, however, both fungi significantly reduced top dry weight, percent reattainment of top weight and the number of healthy coronal roots. These results and field observations suggest that freezing may predispose wheat to damage by fungi normally considered to be weak pathogens and exacerbate the damaging effects of fungi known to be vigorous pathogens.

Common root rot is a well-known major disease of wheat (*Triticum aestivum* L.). It has a complex etiology that appears to vary regionally. The major components of the common root rot complex most often named are *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem., *Fusarium culmorum* W. G. Smith, and *F. graminearum* Schwabe (3,5,7,10–14,18,21). In a study of common root rot in the dryland winter wheat-producing areas of Colorado and Wyoming (8), however, the fungi most frequently associated with the disease were *B. sorokiniana* and *F. acuminatum* Ell. & Ev.

Foot and root diseases of cereals may be exacerbated by environmental stresses. Moisture stress is an important factor in Fusarium foot rot of wheat (4). Freeze stress predisposes barley to infections by *F. avenaceum* (Corda ex Fr.) Sacc. (15). Cold, dry winters and low soil moisture in summer are thought to be associated with severe outbreaks of common root rot of winter wheat (5).

In the early spring of 1984, numerous fall-sown wheat fields in southeastern Wyoming exhibited stand decline. Winter temperatures were as low as −30 C in this area and, undoubtedly, were involved in stand decline. However, later in the spring, many surviving fields had patchy areas of plants that exhibited typical symptoms of common root rot. Routine isolations from diseased roots and crowns almost invariably produced cultures of *F. acuminatum* (J. A. Fernandez, *unpublished personal observations*).

From these field observations and reports in the literature, an association of freezing damage and common root rot of wheat was suspected. The purpose of this study was to investigate the interaction of freezing and infections of wheat by the common root rot fungi under controlled conditions.

MATERIALS AND METHODS

The experimental design employed in this study was a completely randomized 2 × 3 factorial with six replications. The factors were temperature (+2 C and −12 C) and fungi (none, *F. acuminatum*, and *B. sorokiniana*).

Isolates of *F. acuminatum* and *B. sorokiniana* were obtained from diseased wheat in a previous study (8) and maintained at 5 C in a mixture of soil, peat moss, and perlite (20). One isolate of each was selected for this study. Inoculum was prepared by growing fungi for 10 days on a sand-cornmeal-inorganic salts medium in 125 ml widemouth jars according to the methods of Ludwig et al (9).

A 1:1 mixture (v:v) of sand and a commercial potting medium (Metro-Mix 200; W. R. Grace & Co., Cambridge, MA) was infested with inocula at a rate of 1% by weight. Sterile sand-cornmeal-inorganic salts medium (9) was added to the sand-potting medium to serve as controls.

Wheat cultivar Hail was planted 25 mm deep, four seeds per 8 × 8 × 7-cm pot. Plants were grown for 3 wk in a greenhouse at 16–21 C, then "hardened" for 3 wk in a growth chamber at 2 ± 2 C) with 18 hr of light (~150 $\mu E/m^2/sec$) per day.

After being hardened, plant tops were clipped to 10–12 mm and the potting medium was watered to saturation. Plant tops were dried at 95 C and weighed. Plants that did not receive the freeze treatment were placed in an incubator set at 2 ± 2 C. Plants that received the freeze treatment were placed in an incubator at 2 ± 1.5 C) and subjected to a regime similar to that described by Gullord et al (6). Thermocouple probes (type T, copper-constantan, range −30 to + 40 C) were placed in three pots to a depth of 5 mm to monitor temperatures near the wheat crowns. Incubator temperature was lowered 1 C/hr until thermocouple temperatures of −2 C were obtained. Plants were held at −2 C for 20 hr to ensure freezing of all free water in the pots and plants. Temperature was lowered 2 C/hr until thermocouple temperature readings of −12 C were obtained. Plants were held at −12 C for 1 hr then warmed to +3 C at 5 C/hr and allowed to thaw overnight. All plants were placed in the greenhouse at 16–21 C and allowed to recover for 2 wk.

Following recovery, plant tops were clipped, dried at 95 C, and weighed. Plants were removed from pots and roots were washed. The number of healthy (nondiscolored) adventitious coronal roots per pot was counted. If present, one diseased adventitious coronal root per pot was excised and placed on carnation leaf agar (20) for culture and identification of any fungi present.

Data were analyzed by using analysis of variance for a completely randomized 2 × 3 factorial with six replications and, for top weights prior to freezing, as a completely randomized design with twelve replications. Means were compared utilizing the Student-Newman-Keuls test (19). The relation between variables was examined by using simple linear regression analysis techniques.

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RESULTS

Prior to freezing, wheat plants grown in the uninfested substrate yielded the highest mean top dry weight (331 mg), followed in order by those grown in the presence of *F. acuminatum* (288 mg) and *B. sorokiniana* (213 mg). All means differed significantly ($P = 0.05$) according to the Student-Newman-Keuls test.

Freezing (−12 C) significantly reduced mean top dry weights within each inoculation regime compared to their respective unfrozen (+2 C) controls (Table 1). Within the group of plants not subjected to freezing, those grown in the uninfested substrate and the substrate infested with *F. acuminatum* yielded significantly higher top dry weights than those grown in the presence of *B. sorokiniana*. However, in the group of plants subjected to the freeze treatment, plants grown in substrate infested with either *F. acuminatum* or *B. sorokiniana* yielded dramatically less than the uninfested control.

Growth measured as percent reattainment of top dry weight (dry weight after recovery per dry weight prior to temperature treatments × 100) was similarly affected by freezing (Table 2). Within each inoculation regime, plants subjected to freezing reattained a significantly lower percentage of their prior weights than those not subjected to freezing. Within the group of plants not subjected to freezing, no statistically significant differences in percent reattainment were observed among the inoculation regimes. Within the group subjected to freezing, however, plants grown in substrate infested with either *F. acuminatum* or *B. sorokiniana* reattained a significantly lower percentage of weight than did the control.

Freezing did not significantly reduce the number of healthy adventitious coronal roots of plants grown in uninfested substrate (Table 3). Plants grown in substrate infested with either *F. acuminatum* or *B. sorokiniana* and subjected to freezing, however, produced significantly fewer adventitious coronal roots than the unfrozen controls. Within the group of plants not subjected to freezing, only those grown in the presence of *B. sorokiniana* produced significantly fewer healthy coronal roots than the controls. Within the group subjected to freezing, plants grown in substrate infested with either *F. acuminatum* or *B. sorokiniana* produced significantly fewer healthy coronal roots than those of the controls.

Linear regressions described a significant ($P = 0.01$) positive relationship between number of healthy adventitious coronal roots and top dry weight for plants subjected to freezing and also for the nonfrozen controls (Fig. 1). In both cases, nearly 80% of the variation in top dry weight was explained by the regression. Other regressions were computed, but none approached statistical significance.

Samples of discolored coronal roots were assayed from all inoculated plants that were frozen. Three pots in the uninfested control contained plants with discolored roots and produced one isolate each of *B. sorokiniana, F. acuminatum*, and an unidentified

TABLE 1. Top dry weights of wheat plants grown in soil uninfested or infested with root-rotting fungi and exposed to two temperatures

Temperature (C)	Top dry weight (mg)[z] None	*Fusarium acuminatum*	*Bipolaris sorokiniana*
2	577 aAB	635 aA	452 aC
−12	223 bA	50 bB	27 bB

[z]Values are means for six replicate pots, four plants per pot. Means followed by the same letter (lower case for comparing columns, upper case for rows) are not significantly different ($P \leqslant 0.05$) according to the Student-Newman-Keuls procedure.

TABLE 2. Percent reattainment of top dry weights of wheat plants grown in soil uninfested or infested with root-rotting fungi and exposed to two temperatures

Temperature (C)	Reattainment[z] (%) None	*Fusarium acuminatum*	*Bipolaris sorokiniana*
2	194 aA	227 aA	221 aA
−12	59 bA	17 bB	12 bB

[z]Values are means for six replicate pots, four plants per pot. Means followed by the same letter (lower case for comparing columns, upper case for rows) are not significantly different ($P \leqslant 0.05$) according to the Student-Newman-Keuls procedure.

TABLE 3. Numbers of healthy adventitious coronal roots of wheat plants grown in soil uninfested or infested with root-rotting fungi and exposed to two temperatures

Temperature (C)	Healthy adventitious coronal roots[y,z] (no.) None	*Fusarium acuminatum*	*Bipolaris sorokiniana*
2	21 aA	20 aAB	16 aB
−12	20 aA	6 bB	3 bB

[y]Values are means for six replicate pots, four plants per pot. Means followed by the same letter (lower case for comparing columns, upper case for rows) are not significantly different ($P \leqslant 0.05$) according to the Student-Newman-Keuls procedure.

[z]Healthy coronal roots were those showing no discoloration. Means were rounded to nearest whole number following analysis.

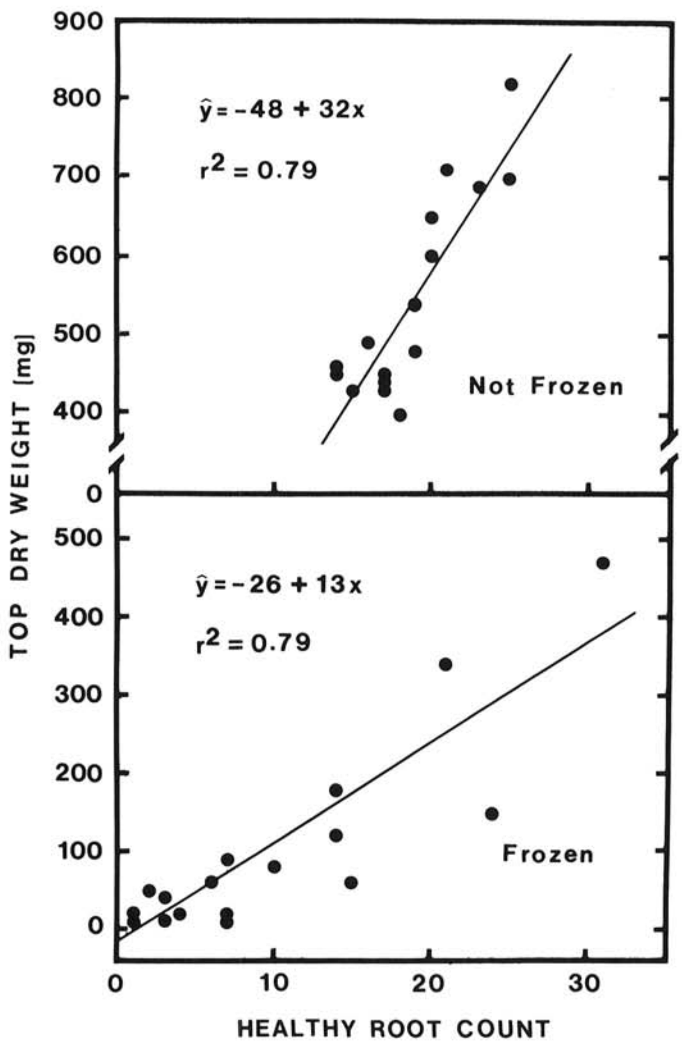

Fig. 1. Regression of the number of healthy adventitious coronal wheat roots on top dry weight of nonfrozen plants (top) and following recovery from being frozen for 1 hr at −12 C (bottom).

Fusarium sp. Four such pots were present in the plants infested with *F. acuminatum* and these produced four isolates of *F. acuminatum*. Six such pots of plants infested with *B. sorokiniana* produced six isolates of *B. sorokiniana*. Of the group of plants that had not received the freeze treatment, none exhibited discolored coronal roots.

DISCUSSION

The differences observed among top dry weights prior to initiation of the temperature treatments seem a valid indication of the relative pathogenicity of the fungi under nonstressful conditions. Under warm conditions, *B. sorokiniana* is a vigorous pathogen of seedling wheat, and in this test it produced the most detrimental effects prior to the cold tests. *F. acuminatum*, on the other hand, is generally considered a weak pathogen of cereals (2,16,17); thus, the intermediate effect of *F. acuminatum* on top dry weights prior to initiation of temperature treatments was not surprising.

The highly significant detrimental effects of the freeze treatment, fungal inoculations, and the significant interactions of these factors is important. The obvious differences in top dry weight between the freeze-treated, uninoculated plants and those grown in the presence of either *F. acuminatum* or *B. sorokiniana* point out the synergistic detrimental effects of these two factors. Equally important is the increased relative effect of *F. acuminatum*, i.e., its effect is as pronounced as that of *B. sorokiniana* following exposure to freezing. These effects appear even more pronounced when regrowth is measured as percent reattainment of the original top dry weight. Significant differences were observed between inoculation regimes only in the group that had received the freeze treatment. That this was possibly a function of the detrimental effects of the fungi prior to the freeze treatment is unlikely since a nonsignificant regression ($r^2 = 0.003$) was obtained between prior top dry weights and percent reattainment within this group.

The effect of the treatments on the number of healthy adventitious coronal roots follows a similar pattern. The dramatically increased detrimental effects of both *F. acuminatum* and *B. sorokiniana* under the low temperature treatment regime should be noted.

That the results obtained using top dry weights are a function of root health is strongly supported by the highly significant regressions obtained when the number of healthy adventitious coronal roots was regressed on final top dry weights (Fig. 1). Equally supportive of this was the consistent isolation of root-rotting fungi from discolored coronal roots.

The freezing temperature (−12 C) used in this study was chosen since it was near the LT_{50} value (−11.6 C) for high-intensity freezing reported for winter wheat cultivars with low winter hardiness (6). In addition, winter temperatures almost invariably reach −12 C and even lower in the dryland winter wheat-producing areas of southeastern Wyoming (1).

In a previous study (8) it was speculated that the more frequent isolation of *F. acuminatum* from diseased wheat during the spring and summer was due to predisposition of wheat to infection by *F. acuminatum* by fall infections with *B. sorokiniana*. It now appears logical that freezing injury during the winter predisposes plants to such infection and allows *F. acuminatum* to become as aggressive a pathogen of wheat as *B. sorokiniana*. It is entirely possible that other fungi known to colonize wheat roots and normally considered to be weak pathogens may produce similar effects following freezing injury.

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