Biology and Control of Ergot on Male Sterile Wheat and Barley

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ABSTRACT

A technique of inoculation of male sterile barley and wheat with *Claviceps purpurea* was developed, involving the removal of the upper 2-4 mm of the glumes prior to inoculation with a conidial suspension. Nearly 100% head and floret infection resulted. Maximum infection occurred with 10^5 or more conidia/ml. Inoculation during and shortly after anthesis resulted in high levels of floret infection. With unfertilized florets, susceptibility declined at 10 days and was lost completely 15 days after the initiation of anthesis. Fertilized ovaries were susceptible right after fertilization. Four days after fertilization, susceptibility decreased until no infection occurred 9 days after fertilization. Under field conditions using male sterile barley, 2,400 μg/ml benomyl applied 3 times just prior to and during anthesis gave some control of ergot. Floret infection was reduced from 83 to 5% when 1,000 μg/ml benomyl was applied to florets with the upper portion of their glumes removed. This indicated that benomyl must reach the surface of the ovary at or before infection to be effective, as benomyl did not act as an eradicant against this pathogen. In male sterile wheat, Chris had some degree of resistance in comparison to other varieties tested. Phytopathology 61:1075-1080.

Additional key words: *Claviceps purpurea*, *Hordeum vulgare*, *Triticum* sp., fungicides, benomyl.

The substantial increase in yields of corn (*Zea mays* L.), sorghum (*Sorghum vulgare* Pers.), and other crops by use of hybrid vigor has stimulated many scientists to propose schemes for production of hybrid barley (*Hordeum vulgare* L.) and wheat (*Triticum* spp. L.) (16, 18). Hybrid seed is produced by forcing cross-pollination between the two different parent lines. The prevention of self pollination is accomplished by hand emasculation or by use of male sterility. Male sterility may be either genetic or cytoplasmic, but to date only genetic male sterility is known in barley (10, 11). Cytoplasmic male sterility and, more recently, genetic male sterility in wheat are being developed for hybrid seed production (7).

Since the florets of male sterile lines of self-pollinated cereals are open for a long time, they are more susceptible to floral diseases (17). The seriousness of ergot caused by *Claviceps purpurea* (Fr.) Tul. in male sterile cereals is illustrated by the observation that, in 1970, 76% of the heads and 36% of the florets of male sterile barley were infected under natural conditions in field plots at Bozeman, Mont. Similar levels of infection have also been observed in other areas of Montana, particularly in fields under irrigation. These levels of infection would cause not only loss in yield, but grain produced for hybrid seed would have to be cleaned to remove the numerous ergot sclerotia.

While several control measures can be used for control of ergot, none is completely effective. Use of ergot-free seed could reduce infection, but there may be some sclerotia from a previously infected crop that could serve as inoculum. Inoculum could also come from infected grasses bordering the field (2). Another control measure would be to grow the crop in an area where development of ergot is restricted by environmental conditions. For instance, very little ergot is observed in Arizona on male sterile barley. However, transportation of hybrid seed from areas such as Arizona to Montana would be economically undesirable. Therefore, if hybrid wheat and barley are to be grown in the northern states, the seed will also have to be produced in these areas. In the absence of completely resistant germ plasm, the prevention of infection might offer some control. While protective fungicides have been ineffective in controlling ergot, the use of an effective systemic fungicide that would reach the site of infection, i.e., the base of the ovary (1), could offer some hope for chemical control.

Because of the seriousness of ergot in male sterile barley and wheat, further study of the biology of this pathogen was undertaken. Since previous work did not involve male sterile wheat and barley as hosts, studies were conducted on inoculation technique, infection period, resistance, and chemical control.

Materials and Methods.—*Plant materials*.—Seed of the 2-row male sterile balanced tertiary trisomic barley 67-c-383 BTT 2^7 ms was supplied by R. F. Eslick (Montana State Univ., Bozeman). Seed of male sterile wheat varieties and elite lines was supplied by W. W. Roath (Dekalb Agricultural Assoc., Fargo, N.D.).

In the greenhouse, seeds were planted in sandy loam in 17.5 cm plastic pots. Supplemental illumination with incandescent lights was used when necessary to induce heading. Field tests were conducted on the Montana State University Agronomy Farm near Bozeman. Rows 3.5 m long spaced 30 cm apart were used. Four rows of male sterile barley were alternately planted between four guard rows of Hypana fertile barley. Similarly, four rows of the wheat male sterile varieties were planted between four rows of fertile wheat or barley.

Cultures.—The culture of *C. purpurea* used in these studies was isolated from honey dew of naturally infected male sterile barley. It was further maintained on an ammonium citrate agar medium (ACM) slightly

modified from that described by Kybal et al. (13), and consisted of the following: $Ca(NO_3)_2 \cdot 4\ H_2O$, 1.44 g; $MgSO_4 \cdot 7\ H_2O$, 0.51 g; KCl, 0.125 g; dibasic ammonium citrate, 20 g; sucrose, 200 g; KH_2PO_4 monobasic, 0.1 g; agar, 12 g; distilled water to 1 liter. In some studies, a calcium nitrate agar medium (CNM) described by McCrea (14) was used, containing the following: $Ca(NO_3)_2 \cdot 4\ H_2O$, 1 g; KH_2PO_4 monobasic, 1.25 g; $MgSO_4 \cdot 7\ H_2O$, 0.625 g; maltose, 30 g; agar, 12 g; water, 1 liter. The cultures were grown at a room temperature of 24-26 C. Unless otherwise indicated, the conidia from 10- to 12-day-old cultures were used.

Conidia were harvested by shaking the cultures with water. The conidia-mycelium suspension was then filtered through six layers of cheesecloth. The conidial concentration was determined with a haemocytometer, and further diluted with water to the desired conidial concentration.

Inoculation techniques.—The florets of male sterile barley open 4-5 days after they emerge from the boot. Generally, heads with florets open were selected for inoculation. The glumes were cut 2-4 mm from the top with scissors. An aqueous conidial suspension of the desired concentration was either dropped into the open florets with a capillary tube or was sprayed onto the florets with an atomizer or a sure-shot sprayer, Model A (Milwaukee Sprayer Mfg. Co., Milwaukee, Wis.). Inoculated heads were usually covered with glassine bags clipped tightly at the base of the head to maintain high humidity and also to avoid any outside source of inoculum or contamination.

Fungicides.—The systemic fungicide, benomyl, was supplied as technical material or as a 50% wettable powder by E. I. du Pont de Nemours & Co., Inc., Wilmington, Del. Nabam and Dithane M-45 (manganese++, 16%, zinc++, 2%; and ethylene bisdithiocarbamate ion, 62%) were supplied by Rohm & Haas Co., Philadelphia, Pa. The other chemicals used were: F 849 (2-amino-4-methyl-5-thiazolecarboxanilide) and G 696 (2,4-dimethyl-5-thiazolecarboxanilide) of Uni-Royal Chemical Co., Bethany, Conn.; Bayer 33172 [2-(2-Furyl)-benzimidazole] 97% active ingredient, Chemagro Corp., Kansas City, Mo.; captan, Stauffer Chem. Co., San Francisco, Calif.; Thiabendazole [2-(4-Thiazolyl)-benzimidazole] 60% active ingredient, Merck Chemical Div., Rahway, N.J.

The emulsifiable mineral oils LS-1194, LS-0925, LS-1183, and LS-1197 were supplied by American Oil Co., Whiting, Ind. Orchex 792 and Orchex 796 were supplied by Esso Research and Engineering Co., Agricultural Products Lab., Linden, N.J. The perfume oils, Bouquet No. 22, Bouquet No. 821, Palma Bouquet, and Blue Stone Bouquet with trademark MM&R, were supplied by Magnus, MaBee & Reynard Division of BFM Corp., Paramus, N.J.

The insecticide Diazinon AG 500 [*o-o*-diethyl-*o*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothiale 48%, xylene 36%] was supplied by Geigy Chemical Co., Ardsley, N.Y.

Fungitoxicity tests.—Benomyl was suspended in 1 ml acetone, to which water was added to make the final concentration of benomyl 20 times that required in the growth medium. Nabam and Dithane M-45 were suspended in water. One ml of each concentration of the fungicide was then pipetted into petri dishes. After solidification, they were inoculated with a 0.9-mm mycelial disc of a 10- or 12-day-old culture of *C. purpurea* growing on ACM agar. Controls received the appropriate amount of water or acetone. The cultures were maintained at room temperature for 15-20 days, and the growth was measured from the center of the inoculum disc. The toxicity of the fungicides, perfume oils, and mineral oils was also tested as follows: A conidial suspension of *C. purpurea* was sprayed on solidified ACM agar or CNM agar. Then a sterilized filter paper disc (Difco Laboratories), 7-mm diam, saturated with various concentrations of the test material suspended in water or acetone, was placed on the inoculated media. Observations on the inhibition of growth around the disc were taken after 25-30 days' incubation at room temperature.

The effect of benomyl and Dithane M-45 on growth of *C. purpurea* was also determined by measuring their effect on mycelial dry weight. When the temperature of sterilized ACM medium without agar was about 40-43 C, 2.5 ml of the appropriate concentration of test material plus 2 ml of a thick conidial suspension were added to 25 ml of media. These flasks were kept stationary at room temperature for 14-20 days. The mycelial mat was filtered on Whatman No. 4 paper, dried overnight at 100 C, and weighed.

Bioassay of benomyl.—Residues of benomyl or its breakdown product were bioassayed in plant parts using *Penicillium expansum* Lk. ex Thom. in the manner described by Erwin et al. (5).

RESULTS.—*Inoculation technique.*—In greenhouse tests, a low infection percentage resulted when male sterile barley plants (with florets kept open by various methods, such as placing the plants in humidity chambers) were inoculated by spraying a conidial suspension on the heads. If the upper 2-4 mm of the glumes were removed, 80 and 93% of the florets were infected when inoculated with 10^6 conidia/ml using a capillary tube or an atomizer, respectively. If only the awns were removed, 81 and 83% floret infection occurred using the same inoculation techniques, respectively.

Inoculum density.—Florets of greenhouse-grown male sterile barley and Mexican Sterile No. 2 wheat with glumes clipped back were inoculated using a capillary tube. About 20-30 μliters of a conidial suspension were placed in each floret. Infection took place with as few as 10 conidia/ml on barley and 1,000 conidia/ml on wheat, but maximum infection occurred when 10^5 or more conidia/ml were used (Fig. 1). Since an average of 25 μliters of inoculum was applied to each barley floret, it appears that one conidium can probably initiate infection, with maximum infection occurring when 2,500 or more conidia/floret are used. Suspension of the conidia in 2% sucrose rather than in water did not increase the in-

fection percentage in barley. Occasionally, infection occurred with no honey dew or sclerotial development. Such ovaries were observed microscopically, and many more conidia were found than had been used for inoculation. Moreover, such ovaries were shrunken and discolored as compared to healthy ones. Some sclerotia were so minute that they were not visible until florets were dissected. Therefore, all the floret infection percentages include these infected shrunken ovaries and minute sclerotia.

Infection period.—Field-grown unfertilized male sterile barley florets were inoculated from 5 days prior to anthesis to 25 days after anthesis. An aqueous conidial suspension (10^6 conidia/ml) was atomized onto florets with glumes clipped back which had been previously bagged to prevent infection and fertilization. Unfertilized ovaries were most susceptible from 5 days prior to anthesis to 5 days after anthesis. Susceptibility declined at 10 days and was lost completely after 15 days.

Some investigators have suggested that fertilized ovaries are resistant to infection (6). Were this true, control of ergot in male sterile barley and wheat could be accomplished if rapid pollination occurred. To determine if fertilization of the ovary confers resistance to infection by *C. purpurea,* florets of field-grown, male sterile barley were inoculated at various times after they had been hand-pollinated. Heads just emerging from the boot were bagged. Four days later, the glumes were clipped back, and a mature stamen from Hypana fertile barley was placed in each floret and the heads bagged. Daily, up to 10 days after pollination, the florets were inoculated as described for unfertilized ovaries. At the time of inoculation, the percentage of the ovaries that had been fertilized was determined when possible. However, prior to the 5th day after fertilization, it was impossible to tell whether an ovary had been fertilized. Therefore, the percentage of fertilized ovaries for the first 4 days was calculated on the basis of the minimum percentage of fertilized ovaries observed later. From the results shown in Fig. 2, it appears that fertilized ovaries were very susceptible right after fertilization. Following the 4th day, susceptibility decreased until no infection occurred 9 days after fertilization.

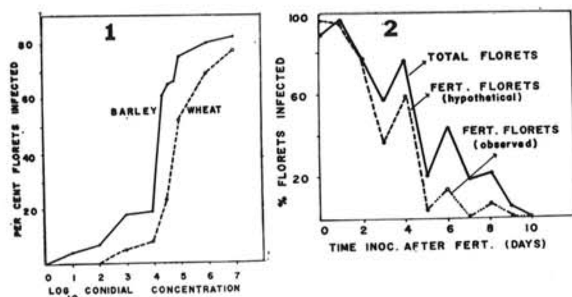

Fig. 1-2. 1) The effect of inoculum density of *Claviceps purpurea* on infection of nonfertilized florets of male sterile barley and wheat. **2)** The effect of fertilization of male sterile barley ovaries on susceptibility to *Claviceps purpurea.*

Varietal reaction.—Plants of wheat male, sterile, elite lines and varieties with unclipped glumes were inoculated with a conidial suspension (10^6 conidia/ml) applied with a capillary tube. The elite line A3032 was slightly more susceptible than A3048, with 43 and 30% of the florets being infected, respectively. With the varietal lines, when the per cent of heads containing at least one ergot sclerotium was determined, the variety Chris appeared to be the least susceptible (Table 1). However, there was little difference between the varieties when the per cent of florets infected was determined on the basis of heads containing at least one sclerotium. To determine if the difference in per cent heads infected was due to somatic resistance, individual florets with glumes clipped back were inoculated using a capillary tube. Per cent floret infection ranged from 50% for Chris up to 78% for Mexican Sterile No. 2, thus substantiating the field observations that Chris was the least susceptible of the varieties tested.

Fungicide studies.—Fungitoxicity tests indicated that the following materials did not inhibit growth of *C. purpurea:* all mineral oils at 8,000 μg/ml; all perfume oils at 2,000 μg/ml; Pentachloronitrobenzene (PCNB) at 5,000 μg/ml; and Thiabendazole at 12,500 μg/ml. Benomyl inhibited growth more than 50% at levels of 2 μg/ml and above. Dithane M-45 and nabam did not inhibit growth to any extent at 10 μg/ml. When mycelial discs in contact with 400 μg/ml benomyl for 17 days were removed and replanted on fresh ACM agar without benomyl, growth resumed, indicating that benomyl is fungistatic in its action. In studies on the effect of various concentrations of benomyl on mycelial dry weight, 100 and 10 μg/ml of benomyl reduced the dry weight by 98 and 74%, respectively. Dithane M-45 was moderately inhibitory at concentrations down to 9 μg/ml.

In field experiments in 1968, male sterile barley plants left for natural infection were sprayed once during anthesis with several fungicides, perfume oils, or mineral oils. Those fungicides, reported to be sys-

Table 1. Degree of susceptibility of male sterile wheat varieties to ergot

Var	Heads infected[c]	Florets infected in infected heads	Total florets infected	Florets with grain
	%	%	%	%
Mexican Sterile 1[a]	71	18	13	11
Pembina[a]	47	23	11	7
Chris[a]	33	17	6	8
Mexican Sterile 2[b]	86	22	19	1
Selkirk[b]	63	22	14	3
Justin[b]	51	19	10	3

[a] Grown between fertile Lemhi wheat.
[b] Grown between fertile Hypana barley.
[c] Inoculated twice with a conidial suspension (10^6 conidia/ml) with a back pack sprayer. Glumes were not clipped. Based on examination of a minimum of 155 heads and 900 florets.

temic in plants, were sprayed prior to or during anthesis. Benomyl sprayed at the rate of 500 µg/ml reduced the per cent of heads with at least one sclerotium from 94 to 77%. An application of captan at the rate of 1,600 µg/ml during anthesis reduced infection by 24%, whereas the other materials reduced infection only slightly or not at all.

To determine if soil application of benomyl would allow enough material to be translocated to the heads and thus control infection, a field trial was set up in 1969 using 30, 90, 150, and 210 lb./acre of active ingredient. The fungicide was applied to the furrows at the time of seeding in 30-cm rows. In addition, some of the plants also received a foliar and head application of fungicide at various times. The plants were inoculated during anthesis with a conidial suspension (10^5 conidia/ml) using a back pack sprayer. No control was obtained by the sole use of any of the soil applications. Some control did result when 500 and 1,000 µg/ml benomyl were applied to foliage prior to or during anthesis. For example, 4 applications of 500 µg/ml benomyl during and following anthesis at 4-day intervals reduced infection of heads from 85 to 78%, whereas 1,000 µg/ml benomyl applied on the same schedule resulted in 66% infection. Dithane M-45 (1,000 µg/ml), applied 3 times at 4-day intervals, also reduced infection to 59%.

In 1970, male sterile barley plants were inoculated 3 times at 4- to 7-day intervals during anthesis with a conidial suspension (10^6 conidia/ml) using a back pack sprayer. Technical benomyl was applied at the rate of 1 and 2 lb./acre in 100 gal of water (about 1,200 and 2,400 µg/ml, respectively). Since insects are believed to be the main vectors of secondary inoculum, a broad spectrum insecticide, Diazinon, was also applied to see if insect control would also result in ergot control. On the basis of heads infected, Diazinon at 2 pints/acre and benomyl at 1 and 2 lb./acre provided some control (Table 2). When the per cent of total florets infected was considered, all the treatments provided control, particularly benomyl at 2 lb./acre.

Since under natural conditions the maximum reduction in percentage of heads infected by application of benomyl was only 29%, the possibility existed that the fungicide was not always reaching the ovary surface. Therefore, a test was set up in which the glumes of male sterile barley florets were clipped back prior to application of the fungicide, thus allowing entry of the fungicide into the floret. Under these conditions benomyl provided excellent control, reducing the floret infection from 83% to 5%. Nabam also reduced infection to 30%, while Dithane M-45 provided little control.

To determine if benomyl can act as an eradicant as reported by Delp & Klopping (4) for several fungi, the florets of greenhouse-grown male sterile barley were inoculated as described above. Benomyl was then atomized onto the florets either on the day of inoculation or 2 and 4 days after inoculation. When the fungicide was applied 4 and 2 days after inoculation, 97 and 63% of the florets were infected, as compared to 11% infection when the fungicide was applied at the time of inoculation.

To determine if residues of benomyl or its breakdown product (3) remain on florets of field-grown, male sterile barley, noninfected florets were removed from plants that had been previously sprayed 3 times with benomyl at the rate of 1 or 2 lb./acre. The last spray was applied 22 days prior to the analysis of the florets. The ovaries were removed intact and placed on solidified potato-dextrose agar in which conidia of *Penicillium expansum* (10^6 conidia/ml) had been incorporated. This particular isolate of *P. expansum* was known to be sensitive to benomyl at concentrations down to 0.4 µg/ml. Twenty per cent and 75% of ovaries of florets sprayed with 1 and 2 lb./acre, respectively, showed inhibition zones, indicating a residual effect for at least 22 days. These data also indicate that benomyl did not reach all of the florets. This may be the reason only partial control of ergot is achieved with use of this material.

TABLE 2. Foliar and head application of benomyl or diazinon for control of ergot in field-grown male sterile barley

Treatment	Rate	Heads[d] infected	Florets infected in infected heads	Total florets infected
		%	%	%
Control		100	74	74
Diazinon[a]	1 pint/acre	99	60	69
Diazinon[b]	2 pints/acre	82	59	48
Benomyl[c]	1 lb./acre	82	46	38
Benomyl[c]	2 lb./acre	71	26	18

[a] Applied once prior to anthesis.
[b] Applied thrice during anthesis.
[c] Applied thrice prior to and during anthesis at 4- to 7-day intervals.
[d] Based on examination of a minimum of 1,115 heads and 1,025 florets.

DISCUSSION.—The technique reported here, of clipping back glumes and inoculating once with an aqueous conidial suspension of 10^6 conidia/ml, resulted in infection of 100% of the heads of male sterile wheat and barley. This is in contrast to infection levels of 20-50% reported in earlier studies for fertile grain (9). If only the awns are clipped, the percentage of florets infected varies a great deal, probably due to the variation in the number of florets open at the time of inoculation. The observation that the infection percentage for florets of male sterile wheat is lower (80%) than that for male sterile barley (95-98%) is probably related to the fact that wheat florets hold a smaller volume of inoculum than do barley florets. The close fit of the wheat glumes, palea, and lemma are responsible for this decreased volume.

Inoculation of florets with an atomizer resulted in slightly higher levels of infection than were observed when the inoculum was dropped into the floret with a capillary tube. The forcible discharge of the conidia into the floret when an atomizer is used may result in more conidia reaching the base of the ovary reported

to be the site of infection (1). In addition, more inoculum tends to run off the side of the florets when applied with a capillary. However, the method of inoculation by use of a capillary may be desirable if controlled levels of inoculum are needed in studies of the relative susceptibility of different genetic lines of wheat or barley. The disadvantage of the capillary inoculation technique is its time consumption.

Apparently, very few conidia are needed to infect one ovary, probably no more than from 1 to 10 (Fig. 1). However, maximum percentage infection was obtained when 2,000 or more conidia/ovary were used. This number of conidia is probably not too different from that occurring on the body and legs of an insect that had previously visited a honey-dewed floret.

The duration of the period of susceptibility of fertilized and unfertilized barley ovaries was 9 and 15 days after anthesis, respectively, thus confirming the earlier work of Kirchhoff (12) with fertile rye. Fertilized ovaries of male sterile barley were highly susceptible during the first 5 days following fertilization, with resistance appearing to develop after the 5th day, until no infection occurred past the 10th day (Fig. 2). This suggests that if protective chemicals could be placed on the ovary during the 10- to 15-day period following anthesis, the plant would be protected from infection until the resistance mechanisms in the fertilized ovary became effective. Under field conditions, however, plants produce tillers over a considerable length of time, resulting in heads in various stages of development. This would necessitate the multiple application of protective chemicals to make sure that all ovaries receive protection as they develop.

The possibility of using soil applications of a systemic fungicide for control of ergot was reported by Hardison (8). Preliminary tests by us indicated that with male sterile barley, seed germination was adversely affected by soil applications of benomyl at rates of 60 lb./acre or higher. Moreover, when benomyl was applied to the furrow at rates up to 210 lb./acre, no control of ergot was observed. However, control of ergot in male sterile barley was obtained when the heads were sprayed with benomyl at the rate of 1 or 2 lb./acre in 100 gal of water (Table 2). In this case, the fungicide was applied 3 times over a period of 18 days and decreased the infection of heads and florets by 36 and 56%, respectively.

One problem in the use of benomyl for ergot control appears to involve the penetration of the chemical to the ovary surface. When unclipped glumes were sprayed, there was about a 56% reduction in infection, but if the glumes were clipped prior to spraying of benomyl, a 95% reduction in infection resulted. This indicates that benomyl reached the ovary surface in much greater amounts when plant tissue (i.e., the upper parts of the glumes) was removed. Since the fungicide must reach the ovary surface to be effective, the percentage of florets in anthesis at the time of fungicide application would affect the degree of ergot control. In addition, the fact that benomyl does not appear to have an eradicant effect on ovary infection would necessitate the application of benomyl prior to infection, probably soon after the florets open. Thus, the longer the duration of tillering of the plants, the longer would be the over-all period for open florets. Such a condition would necessitate multiple applications of fungicide unless the amount of tillering is controlled. Application of the insecticide, Diazinon, did reduce infection, but not to the extent to make its use worthwhile. For this aspect of ergot control to be effective, insect control over a wide area would have to be accomplished.

Under most conditions, fertile barley and particularly fertile wheat are rarely attacked by *C. purpurea*. This resistance is believed to be functional in nature, due to the fact that these cereals are largely self-pollinated and highly fertile. Hence, the flowers are not open to receive inoculum of *C. purpurea*. Recently, partial resistance to ergot was reported for several lines of spring wheat (15). Among the male sterile wheat varieties tested, there appear to be varying degrees of susceptibility. The variety Chris not only had fewer heads infected than the other varieties, but within an infected head, fewer florets were infected. Therefore, use of this variety as a source of resistance to ergot warrants further investigation.

While none of the control measures tested was effective by itself, the possibility exists that a combination of one or more of them might prove to be quite effective. This would include: the use of resistant or tolerant germplasm; the use of a variety that produced tillers over a very short time period; the use of a good pollinator to insure rapid and complete fertilization; and the use of a systemic fungicide to provide protection during the 10- to 15-day period after anthesis when the host is most susceptible. The lack of the necessary germplasm at present hinders the testing of all of these possibilities.

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