Combined Resistance in Sugar Beet to *Rhizoctonia solani*, *Phoma betae*, and *Botrytis cinerea*

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ABSTRACT

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Germ plasms FC 701/4 and FC 712 of sugar beet with resistance to crown and root rot caused by *Rhizoctonia solani* were developed at Fort Collins, Colorado, and germ plasms with resistance to storage rot caused by *Phoma betae* and *Botrytis cinerea* were developed at Fargo, North Dakota. In three greenhouse experiments and a field test, germ plasms F1002 and F1004, selected for resistance to *P. betae* and *B. cinerea*, also possessed a moderate level of resistance to *R. solani*. F1002 was selected originally from FC 701/4, an early root rot-resistant release. F1004 was selected originally from a USSR germ plasm that had been developed for resistance to storage rot caused by *B. cinerea*. In a storage rot evaluation experiment, germ plasm FC 712 was as resistant to *P. betae* and *B. cinerea* as were germ plasms developed specifically for resistance to storage rot. Hybrid cultivars ACH 139, with resistance to *R. solani*, and ACH 146, with resistance to *Aphanomyces cochlioides*, were moderately resistant to *P. betae* and *B. cinerea* and should store better than root rot-susceptible cultivars.

Additional keywords: multiple disease resistance

Yield losses from crown and root rot of sugar beet (*Beta vulgaris* L.) caused by *Rhizoctonia solani* Kühn (teleomorph = *Thanatephorus cucumeris* (Frank) Donk) occur annually. Control measures include cultural practices, such as crop rotation and prevention of hilling-up of soil around sugar beet crowns during cultivation (18). Genetic resistance is the most effective control, and germ plasms resistant to *R. solani* have been developed (9–12).

Sucrose losses from storage rot caused primarily by *Phoma betae* Frank (teleomorph = *Pleospora bjoerlingii* Byford) can amount to 1–2% by weight of stored roots (5). Several sugar beet cultivars and germ plasms were evaluated in 1979 for resistance to storage rot caused by *P. betae* and *Botrytis cinerea* Pers. ex Fr. as part of the breeding program for resistance to storage rot at Fargo, North Dakota. One of the germ plasms, FC 701/4, was developed by the Agricultural Research Service, U.S. Department of Agriculture, at Fort Collins, Colorado, for resistance to crown and root rot caused by *R. solani* (9). Germ plasm FC 701/4 also expressed resistance to the storage rot phase of *P. betae* and moderate resistance to *B. cinerea* (4). This combined resistance was speculated to be a chance incident.

Several germ plasms with enhanced levels of resistance to *R. solani* have been released from the program at Fort Collins (9–12), and several germ plasms with enhanced resistance to *P. betae* and *B. cinerea* have been released from the program at Fargo (2,6,7). Some of these germ plasms (Table 1), developed independently at each location, were tested at the Northern Crop Science Laboratory, Fargo, to determine if they possess combined resistance to the three pathogens and if the original observation (4) was a chance incident. A commercial hybrid with resistance to Rhizoctonia root rot derived from Fort Collins germ plasm also was examined.

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MATERIALS AND METHODS

Inoculum. The isolate of *R. solani* used was from a rotted sugar beet root and belongs to anastomosis group 2-2. Inoculum for *R. solani* was produced on sterile barley kernels. Barley was soaked overnight in 1% potato-dextrose broth, then drained, and 250 ml was placed in 1-L Erlenmeyer flasks. The barley was sterilized by autoclaving twice for 1 hr at a 1-day interval. Barley in each flask was inoculated with one-fourth of a young culture of *R. solani* that had grown to about 9 cm in diameter on potato-dextrose agar (PDA) in a petri dish. The fungus was grown on the sterile barley at 25 C for 2 wk. Colonized barley was dried at room temperature and used intact for experiments in the greenhouse. For the field experiment, the barley inoculum was ground in a hammer mill to pass a screen with 3-mm-diameter holes.

Inoculum of *P. betae* was produced by growing the fungus on green bean agar (19) under continuous fluorescent light of 10 $\mu E \cdot m^{-2} \cdot s^{-1}$. Conidia were suspended by flooding cultures with sterile distilled water. The conidial suspension was used to uniformly inoculate PDA contained in culture dishes. After 4 days, cultures were used for inoculations to evaluate roots for resistance.

The inoculum of *B. cinerea* was increased by growing the fungus on PDA in continuous fluorescent light of 10 $\mu E \cdot m^{-2} \cdot s^{-1}$. The agar cultures were homogenized briefly and portions were used to inoculate PDA plates that were used for storage rot evaluations.

Greenhouse experiments. In the first greenhouse experiment, four germ plasms resistant to storage rot and one commercial hybrid susceptible to root rot were compared with root rot-resistant FC 712 for reaction to *R. solani*. Two-month-old sugar beets, one per 36-cm-diameter pot, were inoculated by placing 20 colonized barley grains on opposite sides of each root (40 per root) about 5 cm below the soil line. Sterile barley was used in the control treatment. Roots were harvested 4 wk after inoculation, and fresh root weights were recorded. Rotted portions of the roots were excised, and the amount of root rot was expressed as the percent (w/w) rotted tissue of the whole root. This test was repeated. In a third test, additional germ plasms were used representing a wider known range in reaction to *R. solani* than those in the first two tests. The germ plasms were arranged in a completely randomized block design with five replicates in the first test and nine in the second and third tests. Analyses of vari-

ance were performed on arcsin-transformed data.

Field test. Four replicates of eight germ plasms and three cultivars were planted 16 May 1988 in a split-plot randomized complete block design. Main plots were either inoculated with the fungus or amended with sterile barley (controls). Subplots (genotypes) were two rows wide and 9.3 m long. Ground barley inoculum of *R. solani* was prepared as described above. Inoculum was applied with a two-row granule applicator adjusted to deliver 85–120 cfu/cm of row. Inoculum density of ground barley, expressed as colony-forming units per gram, was determined by plating a weighed amount on the selective agar medium of Ko and Hora (13). Sugar beets were inoculated on 6 July 1988 after the stand had been thinned to one plant per 25 cm. Extremely hot, dry weather followed this inoculation, so a second inoculation was done on 18 July. After inoculation, plots were cultivated in a manner that placed soil in and around the crowns to favor infection (18). The plots then were sprinkle-irrigated for 24 hr.

Stand counts were recorded at inoculation and at harvest. Prior to harvest, leaves and petioles were removed with a mechanical defoliator. Roots were loosened in the soil with a two-row lifter. Each root in the inoculated plots was examined and given a disease index (DI) rating on a scale of 0 = healthy to 7 = dead, based on the evaluation scheme used by Ruppel et al (17) at Fort Collins. Roots were then washed, weighed, and placed in perforated plastic bags and stored at 4–6 C and 95% relative humidity to await analysis for sucrose content and juice impurities.

Storage rot evaluations. Roots from the control plots were harvested, washed, weighed, and stored in perforated plastic bags for at least 80 days at 4–6 C and 95% relative humidity. Blocks (1 cm) of individual root tissue were evaluated for response to infection by *P. betae* and *B. cinerea* with a standardized method in which blocks (one per root) were placed on petri dishes containing agar cultures of the test fungi (3). Fifteen roots were evaluated per plot. Sucrose content (16) and extractable sucrose (8) were obtained using standard analytical methods.

RESULTS

Greenhouse experiments. Germ plasm FC 712 was more resistant than the susceptible cultivar Ultramono based on the amount of rotted tissue in the first two greenhouse tests (Table 2). The storage rot-resistant germ plasms F1004P and F1006 did not differ from FC 712 in both tests. The responses of F1005 and F1004G were inconsistent. In the first test, F1005 developed a resistant response and F1004G was susceptible, whereas in the second test, F1004G was resistant and F1005 susceptible. In the third greenhouse test, FC 712, ACH 139, and F1004G expressed resistant reactions to *R. solani* and F1004P expressed an intermediate reaction; the remaining entries were similar to one or both of the susceptible controls.

Field test. The germ plasm FC 712 was the most resistant to *R. solani* (Table 3). The next most resistant group consisted of the storage rot germ plasms F1002, F1004G, and F1004P and the commercial cultivar ACH 139. The germ plasm F1005 was intermediate between the most resistant and the most susceptible germ plasms. The germ plasms F1006 and F1009 and the cultivar ACH 146 were as susceptible as the susceptible controls, C16 and Monohikari (Table 3). The germ plasms F1002 and F1004P and the cultivar ACH 139 had stand losses similar to those of FC 712 (Table 4). Stand losses by F1004G and F1005 were intermediate and less than those of the susceptible controls. The germ plasms and the cultivar ACH 139 that had stand losses lower than those of the susceptible controls had the highest root yields and amounts of extractable sucrose per hectare (ESPH).

Storage test. Six storage rot-resistant germ plasms (labeled "F") responded similarly to *P. betae* (Table 3). The root rot-resistant FC 712 expressed resistance to *P. betae* and *B. cinerea* that was comparable to the resistance of the storage rot-resistant cultivars. The two commercial cultivars, ACH 139 and ACH 146, had intermediate ratings that were significantly better than that of Monohikari, the more susceptible of the two control cultivars.

DISCUSSION

Resistance in FC 712 to *R. solani* and *P. betae* was evident in these experiments, as it was with FC 701/4 in 1979. FC 712 also expressed resistance to *B. cinerea*, which FC 701/4 did not in 1979. Certain storage rot-resistant germ plasms possessed a moderate level of resistance to *R. solani*. Three storage germ plasms (F1002, F1004G, and F1004P) and the commercial cultivar ACH 139 had more root rot resistance than the susceptible controls in the field but not as much as FC 712.

Except for F1006, F1009, and ACH 146, effects of root rot resistance were observable in stand loss, total root yield, and ESPH. The moderate resistance of ACH 139 to *P. betae* and *B. cinerea* and the resistance of ACH 146 to *B. cinerea* indicated that these commercial cultivars will store better than highly susceptible cultivars such as Monohikari. The level of storage rot resistance in FC 712 was equal to that in the germ plasms developed for resistance to storage rot, suggesting that storage rot-resistant genotypes can be developed concomitantly in a *Rhizoctonia* breeding nursery. Such development requires procedures that are less labor-intensive than the storage rot selection method.

Although FC 712 is a germ plasm resistant to *R. solani*, ESPH and root yield in this test were reduced 38–39%, with only a 10% reduction in stand and a DI of only 1. This loss was comparable to that of other cultivars with two to three times greater stand loss. The fungus

Table 1. Sugar beet germ plasms and cultivars tested for combined resistance to *Rhizoctonia solani, Phoma betae*, and *Botrytis cinerea*

Germ plasm or cultivar	Description and source
FC 712	Germ plasm developed at Fort Collins, Colorado, for resistance to *R. solani*
F1002	Resistant to *P. betae* and *B. cinerea*; selected from FC 701/4, germ plasm developed for resistance to *R. solani*
F1004G	Resistant to *P. betae*, *B. cinerea*, and *Penicillium claviforme*; selected from germ plasm introduced from USSR with resistance to *B. cinerea*
F1004P	Same as F1004G; F1004 segregates for green and pink hypocotyl
F1005	Same as F1004 but selected from a different Soviet introduction
F1006	Same as F1004 and F1005; mass selection for resistance from the world collection of *Beta vulgaris*
F1009	Resistant to *P. betae*, *B. cinerea*, and *P. claviforme* and with a low respiration rate; selected from a broad range of germ plasms
ACH 139	Hybrid with resistance to *R. solani*
ACH 146	Hybrid with resistance to *Aphanomyces cochlioides*
C16	Inbred used as susceptible check
Monohikari	Hybrid used as susceptible check

Table 2. Amount of root rot on sugar beet germ plasms and cultivars after inoculation with *Rhizoctonia solani* in two greenhouse tests

Germ plasm or cultivar	Percent (w/w) rot of whole root	
	Test 1	Test 2
FC 712	21 a[z]	24 a
F1004P	19 a	21 a
F1006	43 ab	29 ab
F1005	33 ab	43 bc
F1004G	67 c	22 a
Ultramono	86 c	53 c

[z] Means followed by the same letter within a column are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 3. Response of sugar beet germ plasms and cultivars to *Rhizoctonia solani* in greenhouse and field tests and to *Phoma betae* and *Botrytis cinerea* in a storage test

Germ plasm or cultivar	*R. solani*		Storage rot rating[x]	
	Greenhouse	Field		
	% Rot (w/w)	DI[y]	*P. betae*	*B. cinerea*
FC 712	21 a	1.0 a[z]	2.1 a	1.1 ab
ACH 139	38 ab	3.5 a	3.1 b	3.0 d
F1004G	38 ab	3.4 a	2.0 a	0.8 a
F1004P	60 bc	2.8 a	1.7 a	1.1 ab
F1002	65 cd	2.7 a	2.2 a	1.4 abc
F1006	69 cd	5.4 bc	1.6 a	1.6 bc
F1005	82 cde	4.6 b	2.3 a	0.9 a
ACH 146	83 cde	5.6 c	3.1 b	2.0 c
F1009	89 de	5.7 c	2.2 a	1.4 abc
C16	92 de	6.8 d	3.4 b	3.3 d
Monohikari	97 e	6.3 cd	4.2 c	4.6 e

[x] The distance rot progressed through a 1-cm block of root tissue in 2 wk at 22 C, with 0 = 0 mm, 1 = not over 2 mm, 2 = over 2 and less than 4 mm, 3 = over 4 and less than 6 mm, 4 = over 6 and less than 8 mm, 5 = over 8 mm or entire block rotted. Roots had been stored 80–90 days at 4–6 C.

[y] Disease index of root rot: 0 = healthy to 7 = completely rotted (Ruppel et al [17]).

[z] Means followed by the same letter within each column are not significantly different (P = 0.05) according to Duncan's multiple range test.

Table 4. Stand, root yield, and extractable sucrose per hectare (ESPH) of sugar beet germ plasms and cultivars in response to infection by *Rhizoctonia solani* in a field test

Germ plasm or cultivar	Percent loss compared with healthy control		
	Stand	Yield (t)	ESPH
FC 712	10 a[z]	39 ab	38 a
F1004P	22 ab	44 abc	61 abc
F1002	23 ab	40 abc	49 a
ACH 139	32 a	32 a	44 a
F1004G	37 bc	40 abc	52 ab
F1005	52 cd	54 abc	62 abc
ACH 146	61 de	69 cd	78 cd
F1006	62 de	66 bcd	74 bcd
F1009	71 de	64 bcd	82 cd
Monohikari	81 e	87 d	90 d
C16	94 f	92 d	98 d

[z] Means followed by the same letter within each column are not significantly different (P = 0.05) according to Duncan's multiple range test.

probably caused undetected pruning of secondary feeder roots, resulting in the lowered yields.

A common biochemical mechanism of resistance may be functioning in those germ plasms that express resistance to *R. solani, P. betae*, and *B. cinerea*. All three fungi produce pectolytic enzymes (1,14,15). When *P. betae* and *B. cinerea* were cultured identically to *R. solani*, pectin lyase was detected in the culture filtrate of *P. betae* but not of *B. cinerea* (W. M. Bugbee, *unpublished*). Partially purified proteins from root extracts of FC 712 inhibited pectin lyase from both *R. solani* and *P. betae* in vitro. A root extract from the root rot-susceptible Ultramono was not inhibitory (W. M. Bugbee, *unpublished*).

The recognition of combined resistance reported here will benefit further genetic enhancement efforts and places added value on the germ plasms and root rot-resistant cultivars already released.

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