Race-Specific Resistance in Soybean cv. Davis to *Phytophthora megasperma* f. sp. *glycinea*

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ABSTRACT

Ryley, M. J., and Obst, N. R. 1992. Race-specific resistance in soybean cv. Davis to *Phytophthora megasperma* f. sp. *glycinea*. Plant Dis. 76:665-668.

In Queensland, Australia, the soybean (*Glycine max*) cultivar Davis has been used extensively in the public breeding program as a source of resistance to *Phytophthora megasperma* f. sp. *glycinea*. This type of resistance, associated with roots and conditioned by the *Rps2* gene, provided a high level of protection against many races until recently. Davis and other cultivars derived from it are grown throughout the state and have rarely been affected by Phytophthora root and stem rot. Since 1988, *P. m. glycinea* has been isolated from affected plants of these cultivars at several localities. Every isolate was classified as race 15, which comprised less than 10% of all isolates collected in Australia between 1979 and 1988. Race 1 was dominant during this period. Two root inoculation experiments showed that plants of Davis, Manark, and Centaur were resistant to races 1 and 4 but highly susceptible to race 15. Bragg and Dragon were moderately susceptible to all three races, whereas Semstar was highly susceptible to races 1 and 15 and moderately resistant to race 4. There is evidence that the long-term cultivation of cultivars with high levels of resistance to race 1 at Hermitage Research Station via Warwick, Queensland, has resulted in an increase in the proportion of race 15 isolates relative to race 1 isolates. It is apparent that field resistance in Davis is race-specific and that the continued use of genotypes with resistance derived from Davis will result in the selection of races virulent to such genotypes.

Since the first Australian record of Phytophthora root and stem rot of soybean, *Glycine max* (L.) Merr., caused by *Phytophthora megasperma* Drechs. f. sp. *glycinea* T. Kuan & D. C. Erwin (9), the disease has been found in most of the soybean growing areas of eastern Australia (1,13). Until 1989, only two races, 1 and 15, had been identified in Australia, with race 1 accounting for more than 90% of all isolates (1,13). In early 1990, race 4 was found at one location in central New South Wales (14) and, more recently, an isolate that cannot be classified into any described race has been found (M. J. Ryley, *unpublished*).

Two types of resistance to *P. m. glycinea* have been identified in soybeans—immunity and field resistance. Immunity confers complete resistance to one or more races (race-specific), is controlled by a single gene, and is easy to identify in breeding lines. However, its major disadvantage is that it promotes the appearance and buildup of new races by selection pressure (15). At least 25 races of *P. m. glycinea* have been identified in North America (6) where immunity had been the main type of resistance used in breeding programs (23). In soybeans, field resistance is a term used to describe cultivars that are susceptible to hypocotyl inoculation and that rarely show symptoms of infection by *P. m. glycinea* under field conditions (12). Cultivars with high levels of field resistance suffer little, if any, loss in yield when grown in soils infested with *Phytophthora* (8,12,24). In glasshouse evaluations, seedlings of highly field resistant genotypes such as Davis have low or no mortality and little height reduction (3).

Other terms have been used to describe this type of resistance. Jimenez and Lockwood (4) assessed the "field tolerance" of such cultivars in a laboratory test. Schmitthenner (15,16) and Walker and Schmitthenner (23) define "tolerance" in a manner very similar to our definition of field resistance as given above; the tolerance index of Walker and Schmitthenner (23) is a product of seedling survival and reduction in height of inoculated seedlings. Tooley and Grau (19–22) claim that tolerance is attributable to the ability of plants to restrict and localize the activity of the pathogen in the taproot and lower stem, resulting in reduced incidence and severity. They used the term "rate reducing resistance" and assessed it by measuring area under the disease progress curve (AUDPC), apparent infection rate, disease incidence at growth stages V7 and R5, and cotyledon inoculation (19–22). The term "root resistance" was used to describe the reaction of cv. CNS to two races of *P. m. glycinea* (17). We believe that these terms are synonymous and that the resistances of many genotypes variously described as having field resistance, tolerance, rate-reducing resistance, or root resistance, once believed to be race nonspecific (15,16,19), are in fact race-specific.

In the first few seasons after Phytophthora root and stem rot was found in Queensland, plant losses of up to 90% were recorded (10). Trials conducted on a site naturally infested with race 1 of *P. m. glycinea* showed that commercial Queensland soybean cultivars and breeding lines possessed a wide range of resistance (10). Genotypes such as Hill, Davis, and Nessen (HS 1115, Hinn × Semmes) were resistant; Bragg, Forrest, and Dragon (DB 1583, Davis × Bragg) had moderate levels of resistance; and Fitzroy, Semstar, and Ross were susceptible. Hypocotyl inoculation provided evidence that only Nessen had immunity to race 1 (10). Glasshouse trials using a root inoculation technique confirmed the high levels of field resistance of Hill and Davis to this race (3). In Queensland, a widespread change of cultivars to Davis and cultivars with a Davis background, such as Centaur, Triton, and Manark, resulted in a significant reduction in the incidence and severity of Phytophthora root and stem rot. Kilen et al (5) reported that the resistance of CNS (from which Davis is derived) was different from genotypes that were immune to one or more races of *P. m. glycinea* and that its resistance was controlled by a single dominant gene, which they named *Rps2*.

Until the 1988–1989 season (November–May), plants of genotypes with resistance derived from Davis were rarely affected by Phytophthora root and stem rot in Queensland and then only after a long period of waterlogging (5–7 days). These observations were reinforced by experimental evidence that showed that Davis did not suffer significant yield losses in two trials conducted on a site infested with race 1 of *P. m. glycinea* (12). During the 1988–1989 and 1989–1990 seasons, there were several outbreaks of Phytophthora root and stem rot on Davis-derived genotypes in southern Queensland. Every isolate collected from infected plants was classified as race 15. This association prompted an investigation into the responses of several Queensland cultivars to inoculation with isolates of the three Australian races of *P. m. glycinea*.

MATERIALS AND METHODS

Isolates. Six isolates (Table 1) were used in two experiments. The isolates had previously been classified into races by inserting a weft of mycelium grown in

Accepted for publication 23 January 1992.

sterile V8 juice broth (V8B) (200 ml of Campbells V8 juice and 800 ml of distilled water, adjusted to pH 6.5 with calcium carbonate) into 5-day-old seedlings of a modified North American differential set consisting of the cultivars Harosoy, Harosoy 63, Sanga, Wells II, Altona, PI 103091, and PI 86972-1. The seedlings were incubated in a growth cabinet at 26 ± 1 C under a 14-hr light (380–400 $\mu E \cdot m^{-2} \cdot s^{-1}$), 10-hr dark regime and rated as resistant (hypocotyl collapsed) or susceptible (no lesion or restricted nonkilling lesion on hypocotyl) 5 days after inoculation (14). Isolate BRIP 17341 killed 100% of Harosoy seedlings giving a reaction typical of race 1; BRIP 17344 killed 100% of Harosoy and small proportions (<10%) of Harosoy 63 and Sanga (atypical race 1); and BRIP 17342, 17343, and 17345 killed 100% of Harosoy and PI 86972-1 (race 15). The New South Wales isolate DAR 63062 killed 100% of Harosoy, Harosoy 63, and Wells II (race 4).

Genotypes. Five cultivars, Davis, Manark (DB 1597, Davis × Bragg), Centaur (DB 1668), Dragon (DB 1583), and Semstar, were tested in experiment 1, and cv. Bragg, in addition to these five, was included in experiment 2. Previous glasshouse and field trials using race 1 of *P. m. glycinea* had shown that Manark and Centaur had resistance equal to that of Davis, Bragg and Dragon were moderately resistant, and Semstar was susceptible (3,10) (M. J. Ryley, *unpublished*).

Procedure. A modified version of a root inoculation technique (3) was used in both experiments. Each isolate was grown separately as a still culture in V8B. After 7 days, the mycelial mats were harvested, washed, and pressed between paper towels to approximately 75% moisture content. The weight of wet mycelium necessary to give an inoculum level of 0.072 g dry weight of mycelium kg^{-1} potting mix was added to 400 ml of distilled water and macerated in a Waring blender for 20 sec. Sterile U.C. potting mix (mix C, fertilizer V) (7) was used in both experiments. Distilled water was added to the mycelium suspension to bring the final moisture content of the potting mix up to 30%. The inoculum then was incorporated into the mix by hand. In both experiments, there was a control treatment consisting of sterile, uninfested potting mix.

The experiments were conducted in 900-ml watertight plastic containers into which 800 g of infested or sterile potting mix was placed. Twelve seeds (germination 90%) of each cultivar were distributed uniformly over the surface of the mix and covered with 100 g of mix. A plastic lid was fitted to effect an airtight seal, and the containers were incubated in a growth cabinet at 26 ± 1 C under 14 hr of daylight (380–400 $\mu E \cdot m^{-2} \cdot s^{-1}$) and 10 hr of darkness. In both experiments, the pots were completely randomized in the growth cabinet and there were three replicates of each genotype × isolate combination. After 3 days, the lids were removed and the mix was saturated for 7 days by flooding to approximately 10 mm above the surface of the potting mix. Five days after flooding ceased, counts were made of the total number of emerged seedlings and the number of healthy seedlings in each container. Each seedling was removed from the mix, and the hypocotyl length (from uppermost lateral root to apical meristem) was measured.

For each genotype × isolate combination, a field resistance index (FRI) was calculated by the following formula: [(number of healthy seedlings)/(number of emerged seedlings)] × [(mean height healthy inoculated seedlings)/(mean height uninoculated seedlings)] × 100.

An analysis of variance was performed on the mean values of the FRI and differences were tested using a protected least significant difference test (Genstat 4.04B, Lawes Agriculture Trust, Harpenden, England). Data were arcsine-transformed before analysis as the residuals from the analysis on the raw data indicated heterogeneous error. In each experiment, FRI data were analyzed for each isolate within races and for pooled values of isolates of the same race.

Table 1. Source and race classification of isolates of *Phytophthora megasperma* f. sp. *glycinea* used in experiments 1 and 2

Isolate[a]	Race	Cultivar	Date	Locality
BRIP 17341	1	Dragon	8 January	Hermitage Research Station via Warwick, Qld. (28°12′S 152°06′E)
BRIP 17342	15	Manark	8 January	Hermitage Research Station via Warwick, Qld.
BRIP 17343	15	?[b]	21 February	Norwin, Qld. (27°34′S, 150°20′E)
BRIP 17344	1 AT[c]	Semstar	14 March	Brookstead, Qld. (27°42′S, 151°20′E)
BRIP 17345	15	Buchanan	20 March	Millaroo Research Station, Qld. (approx. 20°03′S, 147°16′E)
DAR 63062	4	Ridley	April	Forbes, NSW (approx. 33°23′S, 148°01′E)

[a] Isolates were deposited in BRIP (Plant Pathology Herbarium, Queensland Department of Primary Industries [QDPI], Indooroopilly, Queensland, Australia) as dried cultures and in DAR (NSW Department of Agriculture and Fisheries Plant Pathology Herbarium, Rydalmere, New South Wales, Australia) as a living culture. Duplicates of all isolates have been stored at Plant Pathology Branch, QDPI, Toowoomba, Queensland, Australia, in liquid nitrogen and distilled water.

[b] Unknown cultivar.

[c] Atypical race 1 reaction. BRIP 17344 killed 100% Harosoy seedlings and <10% of Harosoy 63 and Sanga seedlings.

RESULTS

In both experiments, Manark had the highest level of resistance to race 1 isolates, followed by Davis or Centaur and then Dragon and Semstar (Figs. 1 and 2). In experiment 2, the FRI value for Bragg was slightly higher than that for Dragon (Fig. 2). Although the order of Davis and Centaur were reversed in experiment 1 and experiment 2, the differences in FRI between the two with respect to race 1 were significantly different ($P = 0.05$) only in experiment 1 (Fig. 1). In experiment 1, Dragon was the most resistant to race 15, followed by Manark, Centaur, Semstar, and Davis (Fig. 1), whereas in experiment 2, the order (most resistant to least resistant) was Dragon, Bragg, Manark, Semstar, Centaur, and Davis (Fig. 2). The reactions of the cultivars to the race 4 isolate were similar to those for race 1 except that Semstar was more resistant than Bragg and Dragon (Fig. 2).

The FRI values of three cultivars (Manark, Davis, and Centaur) after inoculation with race 15 isolates were significantly lower ($P = 0.05$) than FRI values for these cultivars after inoculation with races 1 or 4 (Figs. 1 and 2). For Bragg, differences in pooled FRI values between the three races were not significant at $P \leq 0.05$ (Fig.2). The FRI for Dragon was significantly lower for race 1 than for races 4 and 15 in experiment 2 (Fig. 2), whereas in the first experiment there was no significant difference ($P \leq 0.05$) between races 1 and 15 (Fig. 1). The other cultivar, Semstar, was significantly more resistant ($P = 0.05$) to race 4 than to both of the other races (Fig. 2).

Although there were differences in FRI values between isolates of the same race for individual cultivars, the isolate × cultivar interaction was not significant at $P \leq 0.05$ in either experiment.

DISCUSSION

Cultivars that lack immunity react differently to races of *P. m. glycinea*. Davis, Centaur, and Manark are resistant to races 1 and 4 but highly suscep-

tible to race 15. Others, such as Bragg and Dragon, are susceptible to all three races, whereas Semstar is highly susceptible to races 1 and 15 but moderately resistant to race 4. The differential responses of cultivars that lack immune genes to races of *P. m. glycinea* also has been reported by Thomison et al (17,18) in the United States. After root inoculation, the "tolerant" cultivars York and Ware were resistant to races 1 and 5 but highly susceptible to race 10, whereas Williams was susceptible to all isolates except a race 1 isolate from Illinois (18). Coincidentally, the first two cultivars have CNS (a parent of Davis) in their breeding background (2). In a later study, Thomison et al (17) confirmed the reactions of York, Ware, and Williams to races 1 and 10 and also found that other cultivars such as Celeste, Toano, and Ripley reacted in a manner similar to York and Ware. Others, such as CNS and Emerald, were resistant to both races. Thomison et al (17) called the resistance of CNS "*Rps2* root resistance" and those of York, Ware, and others "tolerance." However, they considered that the reactions of these latter cultivars were not tolerance sensu Schmitthenner which had been characterized as race nonspecific (15,16). However, neither Schmitthenner (15,16) nor Tooley and Grau (19–22) dismissed the possibility of race specificity in tolerant genotypes.

In our studies, cultivars with CNS-type resistance reacted no differently to races of *P. m. glycinea* than did the tolerant genotypes studied by Thomison (17,18); they were resistant to some races and susceptible to others. There seems little reason to separate the two groups based on reaction type. The differences between genotypes with *Rps2* resistance and those with other types of nonimmune race specific resistance may be in the genetics and/or mechanisms of resistance. Race specificity to *P. m. glycinea* in soybean genotypes may be found to be widespread as more genotype × race combinations are tested. Further detailed research on these aspects is needed to gain a better understanding of the diversity of resistance in soybeans to the pathogen.

Most of the cases of field infection of Davis, Manark, and Centaur by *P. m. glycinea* in Australia have been from the Darling Downs region of southern Queensland. Only two isolates of race 15 (comprising only 3% of all isolates) had been collected up until the 1988–1989 season in that region (11) and both from plants of Davis which had been waterlogged. Since then, eight isolates of race 15 have been found on the Darling Downs, all of which have been isolated from Davis, Manark, or Centaur. It seems likely that selection pressure by genotypes with Davis-type resistance has led to an increase in importance of race 15 relative to race 1.

This concept is further reinforced by the history of soybean production and the race composition of *P. m. glycinea* at Hermitage Research Station via Warwick, Queensland (southern Darling Downs). The Queensland Department of Primary Industries (QDPI) soybean breeding program is conducted at this center, and there is a long history of continuous soybean cropping in fields

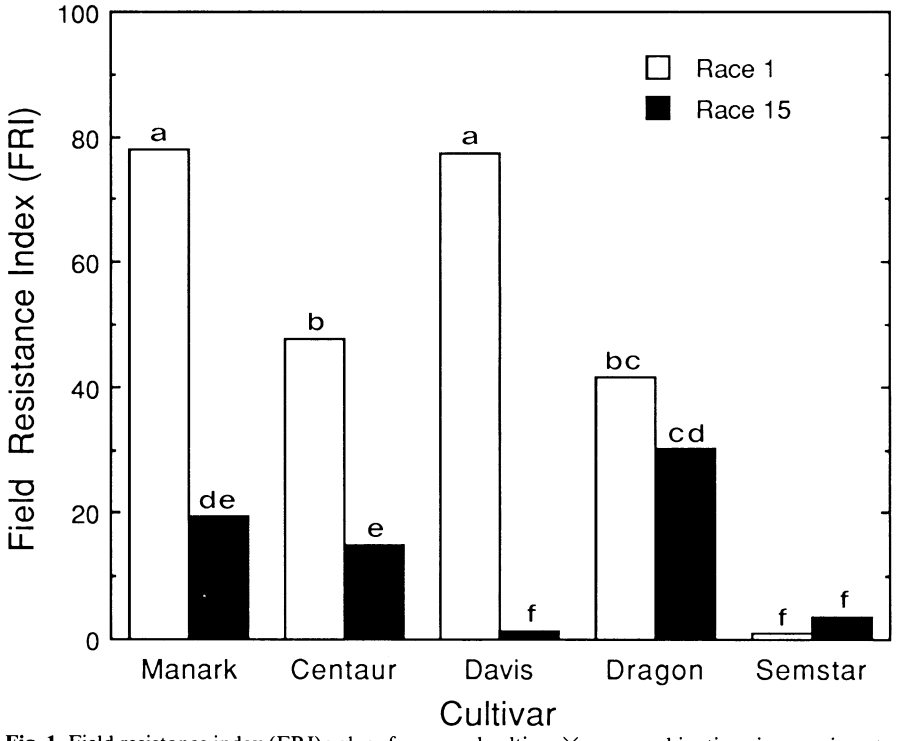

Fig. 1. Field resistance index (FRI) values for several cultivar × race combinations in experiment 1. Data are pooled values of two race 1 isolates and three race 15 isolates. Bars headed by the same letter do not differ significantly ($P \leq 0.05$). FRI = [(number of healthy seedlings)/(number of emerged seedlings)] × [(mean height healthy inoculated seedlings)/(mean height uninoculated seedlings)] × 100.

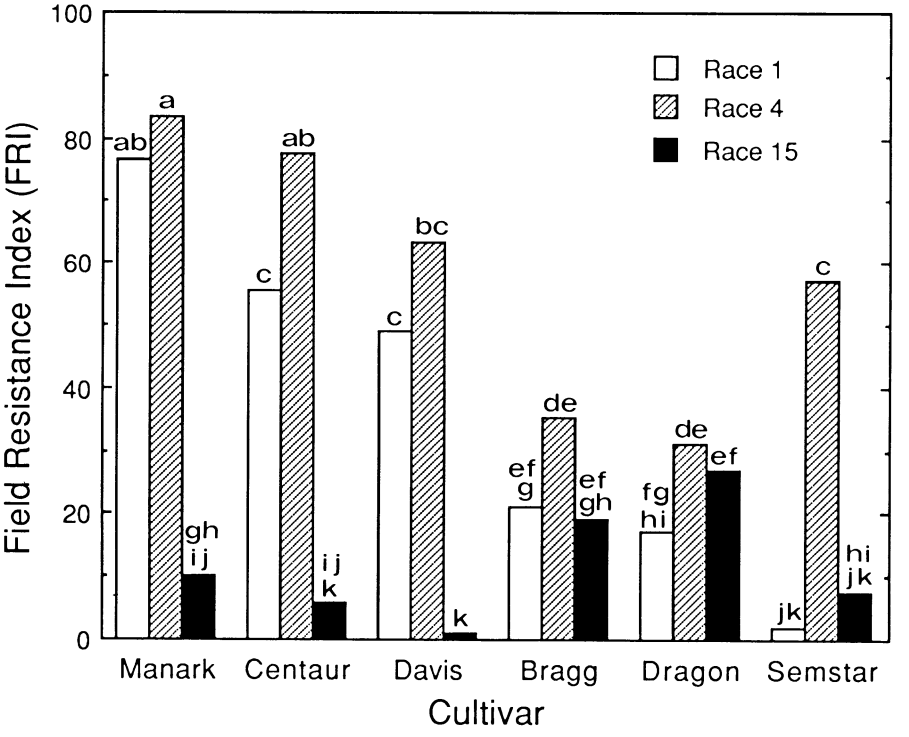

Fig. 2. Field resistance index (FRI) values for several cultivar × race combinations in experiment 2. Data are pooled values of two race 1 isolates, two race 15 isolates, and one race 4 isolate. Bars headed by the same letter do not differ significantly ($P \leq 0.05$). FRI = [(number of healthy seedlings)/(number of emerged seedlings)] × [(mean height healthy inoculated seedlings)/(mean height uninoculated seedlings)] × 100.

naturally infested with the pathogen. Trials conducted in these fields during 1981–1982 and 1982–1983 showed that Davis was highly resistant (10) (M. J. Ryley and J. L. Rose, *unpublished*) and did not suffer significant yield losses (12). A large proportion of the breeding lines grown at the Station had Davis in their background. All 58 of the isolates collected from affected soybean plants or soil at Hermitage Research Station between 1980 and 1988 were race 1 (M. J. Ryley and J. A. G. Irwin, *unpublished*). Race 15 was first isolated at Hermitage Research Station from soil in December 1988 and from affected plants of Davis in December 1989. Of the 16 isolates collected from soil and from plants infected with *Phytophthora* (including cultivars that lack the *Rps2* gene) at the station since 1988, nine were classified as race 15 and the rest as race 1 (M. J. Ryley, *unpublished*). Some workers (15,16,23) have stated that tolerance should not favor the buildup of one race relative to others. Our experience with Davis and Davis-derived lines at Hermitage Research Station does not support this view.

Another explanation for the susceptibility of cultivars with CNS-type resistance to race 15 is that new strains of the race have developed as a result of selection pressure by such cultivars. However, there is no evidence to support this hypothesis. In our experiments, one of the race 15 isolates (BRIP 17345) was obtained from cv. Buchanan growing in a CSIRO variety trial at Millaroo Research Station, northern Queensland, more than 1,000 km from the Darling Downs region. Movement of seed and personnel involved in these trials between the two areas was minimal, so there was very little opportunity for the introduction of race 15 from southern Queensland. The isolate obtained from Manark in southern Queensland (BRIP 17342) was no more pathogenic on Davis, Manark, and Centaur than the North Queensland one, BRIP 17345.

These findings have important implications in soybean breeding programs that use field resistance. In the QDPI breeding program, sources of field resistance other than that of Davis need to be incorporated to ensure that there is a wide genetic base to minimize the selection of virulent races. In addition, field resistant genotypes should be screened against a number of isolates of each race (in glasshouse trials) and at a number of sites (in field evaluations) to avoid creating false impressions of the relative resistances of genotypes.

ACKNOWLEDGMENTS

We thank J. L. Rose, Hermitage Research Station via Warwick, Queensland, for seed of the cultivars used in this study and A. Kelly for statistical analyses. Funding support by the Australian Oilseeds Research Council is gratefully acknowledged.

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