Reactions of Sugar Beet to Powdery Mildew: Genetic Variation, Association Among Testing Procedures, and Resistance Breeding

E. D. Whitney, R. T. Lewellen, and I. O. Skoyen

Research plant pathologist, research geneticist, and agronomist, respectively, U.S. Agricultural Research Station, U.S. Department of Agriculture, Agricultural Research Service, Salinas, CA 93915.
We acknowledge the statistical service of Bruce Mackey.
Accepted for publication 13 July 1982.

ABSTRACT

Whitney, E. D., Lewellen, R. T., and Skoyen, I. O. 1983. Reactions of sugar beet to powdery mildew: Genetic variation, association among testing procedures, and resistance breeding. Phytopathology 73:182-185.

Studies were done on sugar beet (*Beta vulgaris*) cultivars to obtain an estimate of the genetic variation and heritability for disease reaction to powdery mildew, incited by *Erysiphe polygoni*, and to determine the association among field, greenhouse, and laboratory reactions. S_1 and full-sib progeny families were grown in the field to estimate variance and heritability of mildew reactions. Genetic variances were significant, and broad-sense heritability estimates for disease reaction were 54–75%. Consistently severe disease developed on susceptible genotypes in the greenhouse following inoculation of plants 6–10 wk old. Mildew ratings from greenhouse evaluations were significantly correlated with field ratings, indicating that greenhouse tests on young plants can be used to predict field reactions. In greenhouse tests, significant interaction for mildew ratings occurred between cultivars and the age (6, 8, or 10 wk) at which inoculation occurred. However, this interaction would cause little difficulty in correctly differentiating cultivars unless their reactions were similar. The evaluation of young seedlings (4 wk at inoculation) in flats in the greenhouse only discriminated lines with wide differences in reaction. Ratings from a laboratory in vitro test with inoculated leaf strips did not correspond with ratings obtained from greenhouse or field evaluations. The occurrence of genetic variation for disease resistance and the association between field and greenhouse ratings suggested that a breeding program based on individual plant or line reaction in greenhouse tests should improve resistance to *E. polygoni*. From a susceptible, open-pollinated source, two cycles of mass selection for resistance in the greenhouse reduced the mean reaction from 8.0 for the parent to 5.3 for the second cycle synthetic in a greenhouse test and from 7.0 to 4.6 in a field test.

Additional key words: *Erysiphe betae*, general resistance, horizontal resistance, slow mildewing.

For the first time in the United States, powdery mildew, incited by *Erysiphe polygoni* D.C. "type" (3) (= *E. betae* Weltzier), reached epiphytotic levels on sugar beet (*Beta vulgaris* L.) in 1974. After first being identified in the Imperial Valley of California (3), the fungus spread into most other sugar beet growing areas of the United States (6). The fungus overwinters in sugar beet fields in the southwestern United States, causing recurring epiphytotics as the conidia blow north and eastward each spring and summer (6,7). The disease can be controlled easily by sulfur and other fungicides (1,9). Even so, economic and environmental advantages would result from growing resistant cultivars. Most cultivars and breeding lines grown in the Unites States are more or less susceptible, but variations within and among these are evident. Most breeding lines resistant to curly top are highly susceptible (9).

The purposes of these studies were to determine the variability for reactions to the fungus within a broad-base, random-mating population adapted to California; to determine the association between field and greenhouse disease reactions; to evaluate the feasibility of initiating a mildew-resistance breeding program within locally adapted germ plasm; to identify conditions that provide the best differentiation of disease reactions in greenhouse testing; and to determine whether an in vitro laboratory testing procedure identifies resistant genotypes.

MATERIALS AND METHODS

Genetic variability. Randomly derived S_1 and full-sib (FS) families (progenies) were obtained from a random-mating, self-fertile population designated 791, which was developed at Salinas, CA. In 1974, 80 S_1 and FS families were grown in adjacent field tests at Salinas. Each entry was replicated four times. In addition, a common check was included every eighth plot. The check for the test of S_1 progenies was a composite of selfed seed from population 791. The check for the FS progeny test was composited seed obtained from genetic male-sterile plants randomly open-pollinated by the fertile plants in the same population. Single-row plots were 71 cm wide and 6.1 m long. Separate analyses of variance were performed on the families and checks.

Plants in these tests were not treated with sulfur for mildew control. When powdery mildew became epiphytotic in the Salinas Valley (9) in July and August, these plants were uniformly exposed to inoculum. By late July, the disease had reached a moderate level of severity. On 5 August, the S_1 and FS families were scored on a plot basis. A linear scale of 0–4 was used, in which 0, 1, 2, 3, and 4 approximated 0, 25, 50, 75, and 100%, respectively, of the matured leaf area covered by mildew. The plots were rated again on 15 August, using an expanded linear scale of 0–9, in which each increment equaled an approximate 10% increase in leaf area covered with mildew.

Determinations of inheritance of resistance were based on the means and ranges of the powdery mildew reactions and the estimated components of variance. Estimates of genetic variances were obtained by equating mean squares to their expectations for both the S_1 and FS tests. Broad-sense heritabilities (H) were estimated as $H = V_g/(V_g + V_e)$ where V_g = total genetic variance and $V_g + V_e$ = phenotypic variance.

Association between field and greenhouse disease reactions. For studies involving associations among field and greenhouse reactions, disease ratings for the S_1 families used in the greenhouse tests were obtained from the 1974 field tests. Disease reactions for the other breeding lines and hybrids used in the greenhouse tests were obtained from adjacent field tests with similar cultural treatments.

In 1974 greenhouse tests 1 and 2, nine randomly selected S_1 families and four open-pollinated cultivars were used. In tests 3 and 4, 17 S_1 families were selected on the basis of field scores and tested with the same open-pollinated cultivars as in tests 1 and 2. Each cultivar (10 plants per test, replications completely randomized)

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

was inoculated by shaking infected plants over the test plants. Usually one infected plant was used to inoculate six to eight plants; however, the number depended on the amount of fungal sporulation on the source plant. Seven hours before inoculation, plants were placed in a dark room 4.9 × 4.0 × 2.7 m and the humidity was increased with a fan circulating air over a 0.6 × 1.8-m sheet of water (5). The relative humidity varied from 60 to 80% and the temperature from 20 to 26 C (Abbeon relative humidity and temperature indicator). The plants were removed after 48 hr and placed on benches in a talc-shaded greenhouse maintained at 18–26 C. Each plant was visually scored for mildew at 2, 3, 4, and 5 wk after the inoculation on the scale of 0–9. To aid in evaluating each plant, a series of infected plants representing each score were used as a standard. About 2 mo after inoculation, the number of dead and living leaves of each plant were counted. These greenhouse and laboratory data were analyzed statistically and correlations between variables calculated. The data from the four common cultivars were extracted from the four greenhouse tests and analyzed over tests.

Resistance breeding. Field and greenhouse tests similar to those conducted in 1974 were made in 1978. Twenty-nine half-sib (HS) families from the second cycle of mass selection for mildew resistance based upon greenhouse evaluations, the susceptible parental source, and a resistant check were included in these tests. The plants were inoculated in the greenhouse instead of being placed in an inoculation room. The greenhouse was shaded with an application of talc, and evaporative coolers were used to control the temperature and increase the humidity. Natural infection occurred in the field. In the field test, each family was confined to one plot, whereas the resistant check and the parental source were repeated five and six times, respectively. Each family and check had about 30 plants per plot. Two greenhouse tests were conducted, with families, parent, and check replicated 15 times per test. Individual plant readings were made 2, 3, 4, and 5 wk after inoculation. After individual plant readings were made in the greenhouse, selections were aligned, and two readers estimated the mildew severity on the group of 15 plants comprising each HS family and check.

Differentiation of disease reactions in greenhouse. To determine the best age to inoculate plants to maximize differences between a resistant (S72-315) and a susceptible cultivar (417H21), seed of these experimental cultivars was planted on three dates. The test was repeated, with each cultivar replicated 10 times at each age. When test plants were 6, 8, and 10 wk old (approximately six, 10, and 14 true leaves, respectively), they were inoculated by shaking infected plants over them. Each plant was visually scored for mildew at 2, 3, 4, and 5 wk after inoculation on the scale of 0–9. The data were analyzed statistically by a multivariate analysis of variance and by linear contrast (4).

To evaluate differential mildew reactions on seedling plants, seed of four cultivars (S72-315, 417H21, 791B, and 3204) used in the four 1974 greenhouse tests were sown in rows (37 cm) in flats of soil. Plants were inoculated when about 1 mo old and evaluated 1 mo later.

In vitro tests. Before inoculation of plants in tests 1 and 2, the fourth true leaf from five plants of each cultivar was removed and tested for mildew reaction in the laboratory following the procedure of Jones and Hayes (2). Two strips 2.5 cm wide were cut perpendicular to the midvein from each leaf, placed in two petri dishes that contained 20 ml of a benzimidazole solution (40 mg/ml), and inoculated. The date at which each piece was completely dead was used as an indication of reaction.

RESULTS

Genetic variability. Reaction to powdery mildew ranged from moderately resistant to highly susceptible among the S_1 and FS progenies (Table 1). High levels of resistance were not observed within the 791 population. Frequency distributions for both progeny types were skewed toward susceptibility, with ratings between 3 and 4 (on the 0–4 scale) being most common on 5 August. Transformations improved the symmetry of the distributions but did not change either the relative values of the variance components or, therefore, the heritabilities. The correlation between field ratings made 5 and 15 August on the S_1 families was highly significant ($r = 0.89$).

For both the S_1 and FS families, genetic variances were highly significant (Table 1). Experimental errors estimated from S_1 families and checks were similar. Broad-sense heritabilities were high.

Association between field and greenhouse disease reactions. Of the family and line measurements used to evaluate reaction to powdery mildew in the greenhouse, values based upon mean individual plant scores were the most consistent predictors of resistance in the field ($r = 0.72$, $P = 0.01$). Ratings based upon overall family or line scores were less precise predictors of field reactions ($r = 0.49$, $P = 0.01$). No association was obtained between field ratings and greenhouse ratings based upon number or percent of dead leaves per plant, nor were the in vitro scores correlated with field reactions.

The highest consistent correlations between field and greenhouse data when all tests were considered were for greenhouse readings 5 wk after inoculation. For tests 1 and 2, the highest correlation was for the reading 2 wk after inoculation ($r = 0.82$). In tests 3 and 4, the highest correlation was for the reading at 5 wk ($r = 0.74$). All correlations between greenhouse readings and field readings were significant. All correlations were highly significant between field readings on the 0–9 scale for 15 August and the four greenhouse readings taken 2, 3, 4, and 5 wk after inoculation. The coefficient of variation decreased from approximately 20 to 14% between readings at 2 and 5 wk after inoculation, respectively, for ratings from tests 1, 2, 3, and 4 and for the means of aligned plants.

The correlations between field readings (0–9) and means of individual plant readings when summed over greenhouse tests for data taken 2, 3, 4, and 5 wk after inoculation were 0.57, 0.58, 0.63, and 0.66, respectively, and highly significant. When the composite readings (aligned plants) for the four 1974 tests were averaged over tests and compared to field readings on the scales of 0–4 and 0–9, the correlations were highly significant, 0.45 and 0.46, respectively. The correlations for tests 1 and 2 were not significant; however, for tests 3 and 4, the correlations were highly significant between the field scores and the greenhouse scores.

Although mildew ratings in the greenhouse increased between 2 and 5 wk after inoculation, the difference between the most resistant and the most susceptible entries remained fairly constant (Table 2). However, mildew increased somewhat more slowly on cultivar 3204 than on the susceptible cultivars 791B and 417H21.

Test results for 1978 confirmed those obtained in 1974. Field and greenhouse readings were highly correlated except for mean values of individual plant readings (Table 3). The correlation between the two greenhouse tests was highly significant. The r values for disease reactions read by two different scorers for composited plants in two greenhouse tests (1 and 2) and one field test were 0.85, 0.90, and 0.79, respectively, and were highly significant.

Resistance breeding. Mean powdery mildew values for

TABLE 1. Means, ranges, and estimates of variance components and broad-sense heritabilities (H) made from S_1 and full-sib (FS) families for reaction to powdery mildew from the 791 sugar beet populations in 1974

Family	Rating date	Disease reaction[a]		Variance		H
		Mean	Range	Genetic[b]	Error	(%)
S_1	Aug. 5	3.1	1.3–4.0	0.45	0.17	72.5
Check	Aug. 5	3.2	3.0–3.5		0.20	
S_1	Aug. 15	7.0	4.3–9.0	1.39	0.47	74.7
Check	Aug. 15	6.9	6.5–7.5		0.52	
FS	Aug. 5	3.6	2.5–4.0	0.15	0.13	53.6
Check	Aug. 5	3.5	3.3–4.0		0.25	

[a] Families rated on 5 August were on a scale of 0–4, in which 0, 1, 2, 3, and 4 represent approximately 0, 25, 50, 75, and 100%, respectively, of the matured leaf area covered with mildew; those rated 15 August were on a linear scale of 0–9, in which each increment equals an approximate 10% increase in leaf area covered with mildew and 9 = 90–100%.

[b] Genetic variances were statistically significant, $P = 0.01$.

individual and composite readings for greenhouse and field tests are given in Table 4 for the second cycle HS families, the parental source, and resistant check. Large differences within HS families and within parent and check plots (30 plants per plot) were observed. On the scale of 0–9, individual greenhouse scores from HS families ranged from 1 to 8 after two cycles of resistance selection and from 5 to 8 for the parental source when scored 4 wk after 6-wk-old plants were inoculated. The results from the companion field test were similar (Table 4).

TABLE 2. Comparison of mean field scores and greenhouse scores for powdery mildew of sugar beet cultivars common to all four greenhouse tests in 1974

Cultivar	Field scores	Greenhouse scores[a] at week after inoculation			
		2	3	4	5
S72-315	2.0[b]	1.93 a[c,d]	3.03 a	3.05 a	3.48 a
3204	4.0	3.93 b	4.90 b	5.50 b	6.08 b
791B	7.5	5.03 c	5.30 bc	6.00 bc	6.13 b
417H21	7.6	5.33 c	5.70 c	6.48 c	6.63 c

[a] Scored on a 0–9 scale, with each scale unit equivalent to 10% leaf area affected.
[b] Each datum is the mean of four replications.
[c] Each datum is the mean of four tests with 10 plants each.
[d] Means within columns followed by the same letter are not significantly different at $P = 0.05$, according to Duncan's multiple range test.

TABLE 3. Correlations between field and greenhouse scores[a] for powdery mildew on 29 sugar beet half-sib progenies, parents, and resistant checks in 1978

Treatment[b] number	Greenhouse data				Field data	
	Mean[c]		Composite[d]		Mean[c]	Composite[d]
	Test 1	Test 2	Test 1	Test 2		
	1	2	3	4	5	6
1	...	0.86**[e]	0.73**	0.67**	0.27	0.42**
2	...	...	0.70**	0.78**	0.37*	0.59**
3	...	...	...	0.80**	0.38*	0.68**
4	...	...	...	...	0.50**	0.80**
5	...	...	...	...	...	0.71**

[a] Scored on a 0–9 scale, with each scale unit equivalent to 10% of leaf area affected.
[b] Vertical and horizontal treatment numbers are synonymous.
[c] Each plant was individually scored and the mean of the selection calculated.
[d] All plants of a selection were given one score on a plot basis.
[e] Asterisks * and ** are significant r values at $P = 0.05$ and 0.01, respectively.

TABLE 4. Powdery mildew ratings[a] on 29 sugar beet half-sib (HS) progenies, parents, and resistant checks for individual and composite readings of the greenhouse and field tests in 1978

Treatments	Disease reaction				Coefficient of variation	Least significant difference	
	HS progenies						
	Range	Mean	Parent	Check	(%)	0.05	0.01
Greenhouse							
Mean[b]	2.6–4.3	3.5	5.2	1.0	20.8	0.4	0.5
Composite[c]	3.2–7.8	5.3	8.0	1.5	28.9	1.0	1.3
Field							
Mean[d]	3.7–6.2	5.2	6.0	4.0	13.3	0.4	0.5
Composite[e]	2.0–6.0	4.6	7.0	2.5	23.9	0.6	0.8

[a] Scored on a 0–9 scale, with each scale unit equivalent to 10% of leaf area affected.
[b] Each datum is the mean rating of 15 plants taken 2, 3, 4, and 5 wk after inoculation in each of two tests.
[c] Mean disease rating on a whole line basis of greenhouse tests 1 and 2.
[d] Mean of individual plant ratings from the field test (about 30, 180, and 150 plants per HS progeny, parent, and check, respectively).
[e] Mean (of two readers) disease rating on a plot basis.

Differentiation of disease reactions in greenhouse. Cultivar 417H21 had significantly more mildew than cultivar S72-315 at all ages. A cultivar-plant age interaction resulted from a greater difference between cultivars when 6 or 10 wk old than when 8 wk old. Severity on the resistant cultivar was a linear function of plant age, but on the susceptible one, it was a quadratic function of plant age. Mildew readings taken 2, 3, 4, and 5 wk after inoculation showed the same linear vs quadratic response pattern.

Seedling tests conducted in flats of soil indicated that only large differences between entries can be differentiated when 1-mo-old seedlings are inoculated and read 1 mo later.

In vitro tests. Very low negative correlations were observed between the in vitro laboratory readings (days until death) and either greenhouse or field readings.

DISCUSSION

The results of the field test indicate that most of the variation for powdery mildew reaction within random-mating population 791 is due to genetic effects (Table 1). However, these data were obtained for a single test, and the estimates for genetic variances and heritabilities might tend to be biased upward if interactions between genotype and environment occur. These estimates also were obtained from a single population and may not be indicative of sugar beet in general. The highest resistance observed in this population was moderate. Higher levels of resistance are known to occur in sources not adapted to California, eg, S72-315 (Table 2).

High positive correlations between field and greenhouse tests in both 1974 and 1978 show that results from greenhouse evaluations can be used with confidence to predict the reaction of cultivars under natural field infection. The similarity of correlations between greenhouse and field tests in 1974 and 1978 support the reliability of the techniques used, and therefore these techniques should provide guidelines to other sugar beet powdery mildew investigations. Although an interaction between cultivar and plant age at inoculation suggested that resistant lines react differently than susceptible lines, the difference was not large enough to cause confusion when scoring resistant and susceptible cultivars if the differences in reaction were large. If the differences were small, age at inoculation could decrease the precision of differentiating genotypes. This also would be true for length of time between inoculation and scoring if slow mildewing were associated with cultivars being tested. This greater difference in reaction between lines shortly after inoculation suggests that early readings (2 wk after inoculation) may best identify slow-mildewing genotypes. When all greenhouse tests over all cultivars are considered with respect to field reactions, the optimum mildew ratings were those taken 5 wk after the inoculation of 6-wk-old plants. With experience, individual scorers of mildew incidence become sufficiently skilled to make readings with high reliability.

Although in vitro test inoculations appeared to establish adequate levels of infection, the amount of mildew per leaf piece was difficult to use as a criterion of reaction, as was done by Jones and Hayes (2) for oats. Therefore, length of time for leaf tissues to become senescent was used. The lack of uniform inoculation may be the cause of low correlations between in vitro tests and field test data. Although this in vitro test was not conclusive, further evaluations should be done to develop a reliable laboratory test.

The testing of very young seedlings proved to be a reliable method of evaluating cultivars, but small differences were not discriminated. Seedling tests could be used to identify and eliminate highly susceptible lines.

Selection for powdery mildew resistance was effective. The improvement in resistance of the population was consistent with heritability estimates. Selection based on assessment of individual plant reaction or on assessment of overall family reaction both effectively upgraded resistance within a variable population (Table 4).

E. polygoni is xerophytic, but spore germination increases with increasing relative humidity (10). Placing of plants to be inoculated into a humidity-conditioned room or greenhouse should provide an environment conducive to mildew development if temperatures

do not become excessive (above 30 C) (10). In these studies, we found that susceptible plants inoculated and maintained in environmental chambers and greenhouses at 60–80% RH and 20–30 C consistently had severe mildew development.

Over several years of testing (*unpublished data*), we have found the greatest differences among lines evaluated in the field at the time when susceptible lines first reach maximum severity. After that time, differences are less distinct. All of the lines developed fungal colonies and sporulation but differed in severity (proportion of leaf area affected). The individual fungal colonies did not differ greatly in appearance among genotypes. The reaction expressed by some resistant lines appeared to be similar to what others have termed "slow mildewing" (8) (Table 2) and may be a type of general or horizontal resistance. Even though a high level of resistance was not observed in most lines with curly top resistance, the moderate levels of resistance exhibited by some families and lines should reduce less direct losses and also slow the development of epiphytotics. In field experiments (*unpublished data*), yield losses due to disease were proportional to their rating for mildew reaction. If selection within adapted populations does not produce sufficiently high levels of resistance, then obtaining additional factors for resistance from nonadapted sources may be necessary.

LITERATURE CITED

1. Hills, F. J., Hall, D. H. and Kontaxis, D. G. 1975. Effect of powdery mildew on sugarbeet production. Plant Dis. Rep. 59:513-515.
2. Jones, I. T., and Hayes, J. D. 1971. The effect of sowing date on adult plant resistance to *Erysiphe graminis* f. sp. *avenae* in oats. Ann. Appl. Biol. 68:31-39.
3. Kontaxis, D. G., Meister, H., and Sharma, R. K. 1974. Powdery mildew epiphytotic on sugarbeets. Plant Dis. Rep. 58:904-905.
4. Kramer, C. 1972. A First Course in Methods of Multivariate Analysis. Virginia Polytechnic Institute, Blacksburg, VA. 351 pp.
5. Magyarosy, A. C., Schurmann, P., and Buchanan, B. B. 1976. Effect of powdery mildew on photosynthesis by leaves and chloroplasts of sugar beets. Plant Physiol. 57:486-489.
6. Ruppel, E. G., Hills, F. J., and Mumford, D. L. 1975. Epidemiological observations on the sugarbeet powdery mildew epiphytotic in western U.S.A. in 1974. Plant Dis. Rep. 59:283-286.
7. Ruppel, E. G., and Tomasovic, B. J. 1977. Epidemiological factors of sugar beet powdery mildew. Phytopathology 67:619-621.
8. Shaner, G. 1973. Evaluation of slow-mildewing resistance of Knox wheat in the field. Phytopathology 63:867-872.
9. Skoyen, I. O., Lewellen, R. T., and McFarlane, J. S. 1975. Effect of powdery mildew on sugarbeet production in the Salinas Valley of California. Plant Dis. Rep. 59:506-510.
10. Spencer, D. M. 1978. The Powdery Mildews. Academic Press, San Francisco. 565 pp.