Multiple Inoculation Technique for Evaluating Resistance of Single Barley Seedlings to Three Fungi

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ABSTRACT

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A multiple inoculation technique was developed for evaluating resistance of barley seedlings to *Rhynchosporium secalis, Puccinia hordei,* and *Erysiphe graminis hordei.* Differences in disease reaction were highly significant among cultivars inoculated with the three fungi. Disease reaction in the multiple inoculation test correlated well with disease reaction in concurrent single inoculation tests. The technique should prove valuable for evaluating barley seedlings where seed supplies or space are limited and results are needed rapidly.

Disease evaluation of barley (*Hordeum vulgare* L.) seedlings is of great value to plant breeders in the development and release of cultivars. Early generation detection of disease reaction permits more rapid incorporation of resistant germ plasm into breeding programs. Frequently, a shortage of seed of certain types (introductions, crosses, etc.) prevents detection of the interaction between host and pathogen.

To conserve valuable seed, a method of inoculating different leaves of individual plants with two separate fungi was used successfully in genetic studies (1,6). We used similar techniques in developing a multiple (three) inoculation technique for evaluating disease reaction of individual plants.

MATERIALS AND METHODS

We used the three pathogens that cause scald, leaf rust, and powdery mildew, namely *Rhynchosporium secalis* (Oud.) J. J. Davis, *Puccinia hordei* Otth, and *Erysiphe graminis* DC F. sp. *hordei* Marchal. Seeds of 50 commercial barley cultivars were planted in peat moss cubes, divided into four groups, each replicated three times. Plants were grown in the greenhouse before inoculation. Group 1 was not inoculated (control). Group 2 was inoculated with all three fungi. Group 3 was inoculated only with the leaf rust pathogen and group 4 only with the powdery mildew pathogen.

Cultures of *R. secalis* were increased on lima bean agar (7,8). A spore suspension was streaked on agar and incubated 12 days (18 C). Dishes of the agar plus the fungus (one dish/200 ml of water) were comminuted in a Waring blender about 3 min. Plants were inoculated with a Schrader spray gun (3) and incubated 48 hr in a moist chamber at 20 C. Inoculated plants were moved to a growth chamber where the temperature was maintained at 16–19 C under 175 μEinsteins sec^{-1} m^{-2} of cool white fluorescent light for 12 hr/day for 12 days, then readings were made.

Leaf rust spores of culture 57-19 (*P. hordei*) were increased on the susceptible cv. Husky. Spores were collected on paper twice weekly, placed in a 5-mm glass vial (2), sealed, and then stored in liquid nitrogen (4) until used. Seedlings were placed in a moist chamber (20 C) and inoculated by dusting with a spore-talc mixture. Plants remained in a moist chamber 24 hr before they were placed in the growth chamber. Readings were made 12 days later.

Culture CR-3 of *E. graminis hordei* was increased on susceptible seedlings (one to two leaf stage) of cv. Manchuria incubated at 20 C. Plants to be tested were inoculated in the growth chamber by dusting conidiospores of the fungus from infected plants (6). Readings were made 8–10 days after inoculation.

The sequence for multiple inoculation began 10 days after planting the seeds. Seedlings were usually in the one or two leaf stage at the time of inoculation. After *R. secalis* was inoculated on group 2 plants, they were placed in the moist chamber. The following day (day 11) plants were removed and inoculated with spores of *P. hordei* and then returned to the moist chamber for an additional 24 hr. Concurrently, group 3 plants were inoculated only with spores of *P. hordei.* Twenty-four hours after inoculation with *P. hordei,* both the multiple and single inoculation groups of plants were transferred to the growth chamber (day 12). One week later (day 19), the group 2 plants were inoculated with *E. graminis hordei.* Concurrently, the group 4 plants were inoculated only with *E. graminis hordei.* Seedlings were usually in the three leaf stage at the time of this inoculation. Five days later (day 24) readings were made on scald and leaf rust. Three days later (day 27) readings were made on powdery mildew. The inoculation technique required 27 days

from seeding to final readings on all three diseases. At 27 days, plants were usually in the fourth leaf stage.

We used the standard system of rating cereal seedling disease on a 0–9 scale (0 = no symptoms and 9 = maximum severity) for all diseases (5). The multiple and concurrent single inoculation tests were each considered as randomized complete block experiments having three replications for analysis of variance. Correlation coefficients between the multiple and single inoculation tests for *P. hordei* and *E. graminis hordei* were determined by using cultivar means. *R. secalis* was inoculated singly and therefore no correlation could be made.

RESULTS AND DISCUSSION

Highly significant differences were detected in the multiple inoculation test among the cultivars for their reaction to scald, leaf rust, and powdery mildew (Table 1). Similarly, in the single inoculation tests (Table 1), highly significant differences were detected among entries for their reaction to leaf rust and powdery mildew.

Inoculum of *R. secalis* was first applied. Therefore, scald infection was established before the cultivars were inoculated with the other pathogens in the multiple inoculation test. The disease reactions averaged slightly higher in the single inoculation test than in the multiple inoculation test for both leaf rust (5.7 and 5.3, respectively) and powdery mildew (5.5 and 5.3, respectively).

In general, the multiple inoculation test underestimated the cultivar disease reaction compared with the single inoculation test when the disease reactions had low values (values less than 4.5) for both leaf rust and powdery mildew. When the cultivar disease reaction had a high value (values greater than 4.5), the multiple inoculation test had smaller underestimations of the cultivar disease reaction in the single inoculation test for both leaf rust and powdery mildew.

Overall, the entry results were extremely similar. The cultivar leaf rust reaction in the multiple inoculation test was highly significantly correlated with the cultivar reaction in the single inoculation test ($r = 0.94$, $P < 0.01$). The cultivar reaction for powdery mildew in the multiple inoculation test was also highly significantly correlated with the cultivar reaction in the single inoculation test ($r = 0.93$, $P = < 0.01$). Hence, the results of the multiple inoculation test and the single inoculation tests were similar.

In addition to illustrating similarities between the multiple and single inoculation results, Table 1 provides valuable information on the cultivar reaction to the three pathogens.

Our purpose in developing a multiple inoculation test was to obtain maximum information from a small quantity of seed. Another way of saving seed would be to reduce the number of replications. In this experiment, we used three replications. Of the total variation in the analysis of variance in the multiple inoculation test, 95, 93, and 95% of the variation for scald, leaf rust, and powdery mildew, respectively, was due to differences among the cultivars. The remaining variation was due to the differences between replications or in the residual error term. Because the differences in cultivars account for so much of the variation and the differences in replications and the residual error account for so little, we believe that replication is not necessary for evaluation of limited seed and numerous entries. Susceptible checks should be used in such screening tests, however, to determine uniformity of infection.

Table 1. Average disease ratings [a] for seedlings of 41 commercial barley cultivars inoculated by single or multiple inoculation technique with spores of *Rhynchosporium secalis, Puccinia hordei,* and *Erysiphe graminis hordei*

		Inoculation				
		Multiple			Single	
Barley cultivar	CI No.	Scald	Leaf rust	Powdery mildew	Leaf rust	Powdery mildew
Austral	6483	9.0	4.0	7.7	3.7	7.3
Barsoy	11904	8.7	7.3	4.3	8.7	4.3
Beecher	6566	2.3	7.0	7.7	8.0	7.7
Bolivia	1257	9.0	1.3	3.3	2.0	5.0
Boone	15494	2.0	0.7	2.0	3.0	5.7
Cebada Capa	6193	8.7	0.7	4.0	0.0	4.3
Chilean D	1433	9.0	4.7	2.0	4.0	3.7
Colonial 2	8062	8.7	7.7	8.3	8.7	7.7
Decatur	10546	5.0	6.7	8.0	7.0	7.7
Dover	10435	8.3	6.3	3.0	6.7	2.3
Egypt 4	6481	9.0	6.0	6.7	7.3	7.0
Estate	3410	8.7	1.7	6.3	1.3	6.7
Franger	8811	7.7	5.0	2.0	5.0	1.7
Gold	1145	8.7	2.7	7.7	2.7	7.3
Gold Foil	1866	8.7	1.0	1.0	2.3	2.7
Harrison	10667	6.3	5.0	3.3	4.3	3.7
Hudson	8067	3.3	7.7	4.3	8.0	4.0
Huron	15534	5.3	6.7	2.7	7.0	2.3
Husky	9537	7.7	4.3	6.7	4.7	8.0
Ishtar	1615	7.7	7.0	3.3	7.0	3.3
Lakeland	13734	3.7	4.7	3.3	4.3	4.0
Lechtaler	6488	8.7	3.0	6.0	3.0	5.3
Long Glumes	6168	8.3	1.7	3.7	2.0	2.0
Manchuria	16138	9.0	6.7	2.3	8.0	2.3
Marocaine	8333	7.0	6.0	7.0	5.3	7.3
Monte Cristo	1017	9.0	6.3	1.7	6.7	2.7
Morocco	6311	9.0	4.0	6.0	5.0	6.0
Oderbrucker	940	8.3	1.7	8.0	3.3	7.3
Park	15768	9.0	7.0	2.7	7.7	4.0
Perry	15731	8.0	5.3	3.0	7.0	4.3
Post	15695	8.7	6.7	2.0	6.3	1.7
Quinn	1024	3.0	4.3	7.0	5.7	7.7
Rabat	4979	8.7	3.7	2.0	3.7	1.7
Rapidan	14006	9.0	7.3	3.7	7.0	3.7
Ricardo	6306	9.0	3.3	2.3	4.0	3.0
Rojo	5401	7.0	6.0	8.3	7.3	8.0
Sudan	11507	9.0	5.3	9.0	4.3	8.7
Swiss	3430	2.3	7.7	4.7	8.7	4.0
Unitan	10421	3.7	7.0	8.0	7.3	8.0
Volbar	15557	3.0	6.3	6.3	7.0	6.3
Weider	1021	8.7	3.7	3.0	3.7	3.0
$\bar{X}$ [b]		7.5	5.3	5.3	5.7	5.5
LSD 0.05		1.0	1.1	1.3	1.5	1.1
CV		7.9	13.2	15.3	16.3	12.0

[a] Average of three replications, based on a rating scale of 0 = no symptoms to 9 = maximum severity.
[b] Mean, LSD 0.05, and CV were derived using all 50 cultivars.

LITERATURE CITED

1. Hiura, U. 1960. Studies on the disease-resistance in barley. IV. Genetics of the resistance to powdery mildew. Ber. Ohara Inst. Landwirtsch. Biol., Okayama Univ. 11:235-300.
2. Kilpatrick, R. A., Harmon, D. L., Loegering, W. Q., and Clark, W. A. 1971. Viability of urediospores of *Puccinia graminis* f. sp. *tritici* stored in liquid nitrogen, 1960-1970. Plant Dis. Rep. 55:871-873.
3. Kilpatrick, R. A., and Johnson, H. W. 1956. Spray gun for greenhouse inoculations. Phytopathology 46:345-346.
4. Loegering, W. Q., McKinney, H. H., Harmon, D. L., and Clark, W. A. 1961. A long term experiment for preservation of urediospores of *Puccinia graminis tritici* in liquid nitrogen. Plant

Dis. Rep. 45:384-385.
5. McNeal, F. H., Konzak, C. F., Smith, E. P., Tate, W. S., and Russell, T. S. 1971. A uniform system for recording and processing cereal research data. U.S. Dep. Agric. ARS 34-121.
6. Moseman, J. G., and Greeley, L. W. 1966. Reactions of one barley plant to several cultures of *Erysiphe graminis* f. sp. *hordei*. Phytopathology 56:1428-1429.
7. Schein, R. D. 1958. Pathogenic specialization in *Rhynchosporium secalis*. Phytopathology 48:477-480.
8. Schein, R. D., and Kerelo, J. W. 1956. Culturing *Rhynochosporium secalis*. Plant Dis. Rep. 40:814-815.