Specificity of the Puccinia recondita f. sp. tritici: Triticum aestivum 'Bulgaria 88' Relationship

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ABSTRACT

Infection-type (IT) data obtained by inoculating *Triticum aestivum* 'Bulgaria 88' (B88) and an experimental line, 'Purdue selection 45' (PD45) with 25 cultures of *Puccinia recondita* f. sp. *tritici* indicated specificity in the relationship of B88 and *P. recondita* f. sp. *tritici*. All cultures except one produced a low IT on B88 compared to the IT produced on PD45 by the same cultures; two distinctly different low ITs were observed within those produced by the 24 cultures. The other culture produced a higher IT on B88 than on PD45. These data indicated an hypothesis that B88 has two genes for low reaction to *P. recondita* f. sp. *tritici*. A comparison study of B88 with host-lines having *Lr1, Lr2A, Lr2B, Lr2C, Lr2D, Lr3, Lr9*, and *Lr11* indicated that one of the hypothesized genes for low reaction in B88 is *Lr11*; these comparisons provided no evidence that the other hypothesized gene is any of the known named genes for low reaction. A further study comparing B88 with the host-line having *Lr11* [LR11 (Wichita)] with other cultures and in three environments supported the hypothesis that B88 has *Lr11*. Infection types produced on B88 and LR11 (WI) were identical in a 15-20 C growth chamber environment and were similar, although different, in two greenhouse environments. These results imply that the resistance of B88 is specific rather than general.

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Van der Plank (10) and Caldwell (3) have recently emphasized host-plant resistance to disease which is not specific to parasite pathogenicity. This category of host-plant resistance offers numerous advantages in plant disease control when and where it is available and can be placed into the genotype of commercial cultivars. Van der Plank (10) has listed these advantages and discussed them in detail. He showed, on a theoretical basis, that using nonspecific (horizontal) resistance flattens the slope of disease development curves whereas using specific (vertical) resistance delays epidemics in proportion to the frequency of the parasite population having avirulence to the specific resistance of the host. Caldwell et al. (4) have reported that the cultivar 'Bulgaria 88' (B88) has a general resistance to wheat leaf rust; they termed this resistance as "slow rusting" compared with the experimental line, 'Purdue Selection 45' (PD45). They based their conclusions on disease development curves plotted from counts of pustule numbers on flag leaves made at predetermined times and stations in field plots after they had inoculated plot centers with *Puccinia recondita* f. sp. *tritici*.

In seeking a rapid method of screening for the resistance in B88, tests were made to determine if any consistent, visually measurable difference could be found between infection types (ITs) on B88 and PD45 when inoculated with uredial cultures of *P. recondita* f. sp. *tritici* having a wide range of specific pathogenicity. Differences were observed which suggested specificity in the *P. recondita* f. sp. *tritici*: B88 relationship and a study of these differences was conducted.

MATERIALS AND METHODS.—Seeds of *Triticum aestivum* L. em. Thell. Bulgaria 88, P.I.94407, and an experimental line Purdue Selection 45, Purdue 45-1834-1, were obtained from J. J. Roberts, USDA-ARS, Department of Agronomy, Purdue University, Lafayette, Indiana. Seeds of near-isogenic lines having single known genes for low reaction to *P. recondita* f. sp. *tritici* in a Wichita (WI) background were obtained from E. G. Heyne, Agronomy Department, Kansas State University, Manhattan.

In this paper, corresponding gene pairs in host:parasite interactions are symbolized as the symbols of genes for parasite pathogenicity and the symbols for genes for host reaction conjugated by a slash (/) character according to the method used by Loegering (7). This results in symbolization such as *PLr11/Lr11* indicating corresponding gene pair 11 in *P. recondita* f. sp. *tritici:Triticum* spp. (9).

In two experiments, PD45 and B88 were grown in the greenhouse at approximately 20 C with 12-hr day-length, including supplemental lighting. Ten days after seeding, the plants were inoculated by the "brushing" technique (2) with uredial cultures of *P. recondita* f. sp. *tritici* having a wide range of known combinations for specific pathogenicity. Inoculated plants were exposed to an overnight moist period at 15-20 C. Observations of ITs produced by each host-line:culture interaction were made 12 days after inoculation and were coded in the IT coding system recently proposed by Browder (1). In this system, the IT on plants in a host-line:parasite-culture:environment test judged by the observer to be most important was coded with three characters. The first and second character represent visual estimates of the relative amount of sporulation and size of typical lesions, coded on a 0-9 scale. A third character was used to describe certain characteristics of the IT

according to a predetermined code:description key where 1 indicated chlorosis surrounding sporulation, 3 indicated necrosis surrounding the sporulation, 5 indicated a mosaic pattern of variably sized lesions on individual leaves, and 8 indicated increasing sized lesions from the leaf base to leaf apex. Other codes are used for other descriptions (1). A fourth character was used to code fractional tenths of plants in a test having ITs other than that described by the first three characters of the coding system. Thus, three-character codes are used in discussion to describe particular ITs although four-character codes are listed in the table in this paper to depict the total host-line: parasite-culture:environment phenotypes observed.

In another experiment, eight near-isogenic host-lines, each having single known genes for low reaction to *P. recondita* f. sp. *tritici*, *Lr1*, *Lr2A*, *Lr2B*, *Lr2C*, *Lr2D*, *Lr3*, *Lr9*, and *Lr11* (5, 9), were inoculated along with B88 and PD45 with 12 cultures of *P. recondita* f. sp. *tritici*. The seeding, inoculating, and coding methods described above were used, except that greenhouse temperature rose to a 24 C maximum on some days during the experiment. Comparison analyses of IT data obtained from these experiments were made, using the concepts described by Loegering et al. (8). Under these concepts, the production of a higher IT on a line of unknown reaction genotype than on a near-isogenic line having a known gene for low reaction, by the same culture, shows that the unknown does not have the known gene for low reaction in the near-isogenic line. Conversely, positive association of low and high ITs on a line of unknown reaction genotype and a near-isogenic line having a known gene for low reaction inoculated with the same cultures provides some evidence that the unknown host-line may have the gene for low reaction in the near-isogenic line. Similarity of low ITs on the known and unknown under different environments provides additional evidence of similarity of reaction genotype.

In two final experiments, direct comparisons were made between ITs produced on B88, PD45, and a host-line having *Lr11*, Wichita*6/Hussar, KS7110704 [LR11 (WI)]. A wider range of cultures was used in one of these experiments, although some of the cultures used in the earlier experiment were included. Some of the materials were grown in three environments during postinoculation development in the other experiment. A growth chamber environment of 20-C day, 15-C night, 75% relative humidity (RH), 12-hr day at 1,500 ft-c, and a greenhouse environment of 20-24 C, 12-hr day were used to test for similarity of the low ITs produced on B88 and LR11 (WI). A greenhouse environment of 30 C, 12-hr day, was used to test for high temperature lability of *PLr11/Lr11* and one of the low ITs produced on B88.

Some of the cultures of *P. recondita* f. sp. *tritici* used in this study were research cultures deposited in the American Type Culture Collection's Plant Rust (PR) collection. These were: UN09-66A, PR66; UN17-68A, PR62; UN01-68B, PR51; 66-763, PR60; UN01-68A, PR67; UN2-70-22, PR77; 6B-NA65-9, PR76; UN02-64A, PR3; and 66-36-03, PR61. The other cultures were selected from the 1971 pathogenicity survey of *P. recondita* f. sp. *tritici* in the United States, because of some unusual combination of specific pathogenicity. Some of these cultures are available from the author.

RESULTS.—The results obtained from inoculation of B88 and PD45 with 25 cultures of *P. recondita* f. sp. *tritici* showed no distinct variation in ITs on PD45 (Fig. 1-B, D, F). The IT produced on B88 by 24 of the 25 cultures was lower than that produced on PD45 by the same culture. Two cultures produced 031 ITs on B88 (Fig. 1-A) and 22 cultures produced 235 ITs on B88 (Fig. 1-C). One culture, 6B-NA65-9, produced a higher IT on B88 than on PD45 (Fig. 1-E, F). This experiment was repeated once with similar results. A 10-20 percent mixture of off-type plants having IT 881 or higher was found in the B88 seed source used in these experiments.

Comparison analyses (8) of IT data obtained from the inoculation of B88, PD45, and eight near-isogenic host-lines showed that B88 does not have any of the genes for low reaction: *Lr1*, *Lr2A*, *Lr2B*, *Lr2C*, *Lr2D*, *Lr3*, or *Lr9*. However, these analyses indicated that the gene for low reaction involved in conditioning the 235 IT on B88 may be *Lr11*. The intermediate ITs were variable on B88 and LR11 (WI) both between the two host-lines inoculated to the same culture and between tests of the same line inoculated with different cultures. Variation of the intermediate ITs ranged from 235 to 671 on B88 and from 235 to 565 on LR11 (WI). Eleven of 12 cultures were avirulent to both B88 and LR11 (WI); one culture was virulent to both lines. The interaction *PLr11/Lr11* is known to be high-temperature labile (11). Thus, further experiments were coducted to determine if ITs produced on B88 were similar to those produced on LR11 (WI) when inoculated with a wider range of cultures and subjected to different environments.

Figure 2 shows a summary of results obtained in an experiment designed to further compare the ITs produced on B88 with those on LR11 (WI) with other cultures of *P. recondita* f. sp. *tritici*. This summary is shown as a paired comparison analysis suggested by J. F. Schafer (*personal communication*). One additional culture, 66-36-03, was found to produce a high IT on B88; this culture also produced a high IT on LR11(WI). Although ITs were again variable in this experiment, pathogenicity to LR11(WI) and to B88 was positively associated; this further supports the hypothesis that B88 carries *Lr11*. The 031 ITs observed on B88 when inoculated to some of the cultures were apparently due to epistasis of the *PLr11/Lr11* interaction by another corresponding gene pair.

IT data obtained from the experiment conducted to compare ITs produced by the *PLr11/Lr11* interaction and the ITs produced on B88 are shown in Table 1. When grown under the growth chamber environment, ITs produced on LR11(WI) and B88 were identical for each culture and pathogenicity to the two cultivars was positively associated. However, when grown in the 20-24 C greenhouse environment,

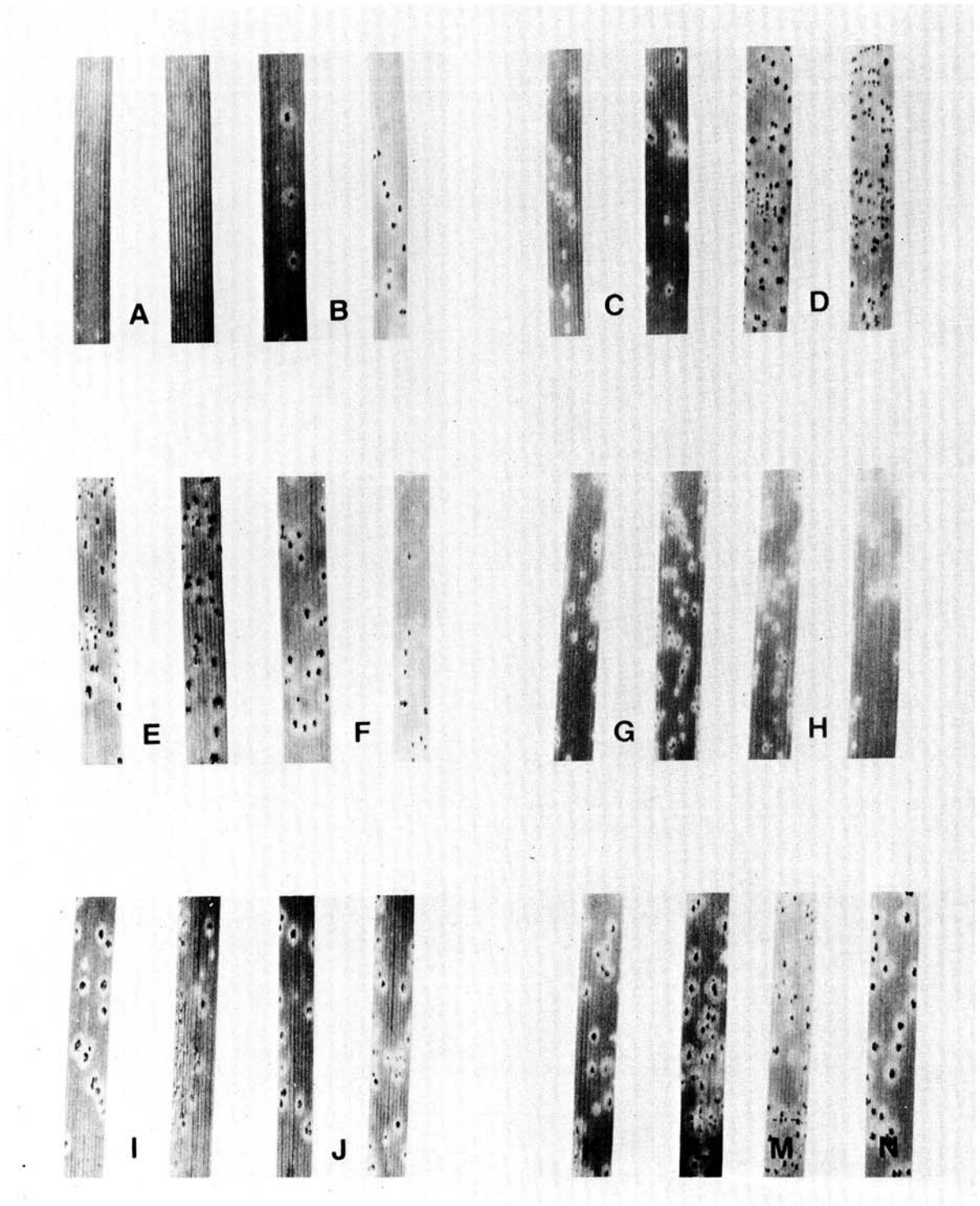

Fig. 1. Infection types produced by different *Triticum aestivum* line:*Puccinia recondita* f. sp. *tritici* culture:environment interactions. Interactions are labeled by host-line abbreviations, literal culture numbers, and environment where GH = greenhouse and GC = growth chamber. **A)** B88:0953-1:20 C GH; **B)** PD45:0953-1:20 C GH; **C)** B88:65284-1:20 C GH; **D)** PD45:65284-1:20 C GH; **E)** B88:6B-NA65-9:20 C GH; **F)** PD45:6B-NA65-9:20 C GH; **G)** LR11(WI):UN02-64A:20 C GC; **H)** B88:UN02-64A:20 C GC; **I)** LR11(WI):UN02-64A:30 C GH; **J)** B88:UN02-64A:30 C GH; **K)** LR11(WI):UN01-68A:30 C GH; **L)** B88:UN01-68A:30 C GH; **M)** WI:UN01-68A:30 C GH; **N)** PD45:UN01-68A:30 C GH.

there were differences in the low ITs produced by the avirulent cultures, although pathogenicity to B88 and LR11(WI) was positively associated in these tests also. ITs observed on LR11(WI) were slightly lower than on B88 inoculated with the same culture. In the 30-C greenhouse test, no low ITs were observed on B88, but on LR11(WI) four cultures produced an IT characterized by only slightly reduced sporulation from that of a typical "high" IT. Two cultures, UN01-68A and 66-763, produced even lower ITs, coded as 678 and 561, on LR11(WI). Only one culture, UN17-68A, produced a high IT on LR11(WI). Observation of these ITs during development indicated they were indeed different from the other ITs on LR11(WI) at the high temperature environment. Figure 1, G-J, shows comparisons of the ITs produced on LR11(WI) and B88 by one culture at two temperatures. Figure 1, G and H, compare the low ITs produced by culture UN02-64A when grown at 20 C. Figure 1, I and J, compares ITs produced by UN02-64A when grown at 30 C. These photographs show that the interactions of both the lines with culture UN02-64A were temperature-labile. Figure 1, K-N, shows ITs produced by culture UN01-68A on: (K)-LR11(WI); (L)-B88; (M)-Wichita; and (N)-PD45 under the high temperature environment.

DISCUSSION.—These results clearly indicate, although they do not prove by conventional genetic methods, that there is genetic specificity in the relationship of *P. recondita* f. sp. *tritici* and the cultivar B88. Using methods based on the gene-for-gene relationship (6, 7, 8), two genes for low reaction can by hypothesized in B88; one of these, interacting with its corresponding gene for low pathogenicity, which occurred in a few of the cultures used in this study, conditioned the 031 IT (Fig. 1-A). The other hypothesized gene for low reaction, interacting with its corresponding gene for low pathogenicity, which occurred in most of the cultures used in these experiments, conditioned the 235 IT (Fig. 1-C). All of the evidence available from this study indicated that the second hypothesized gene for low reaction in B88 is *Lr11*.

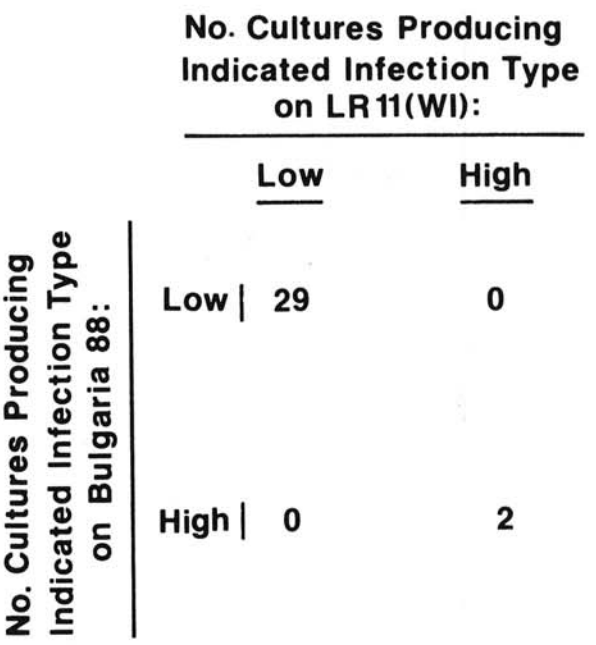

Fig. 2. Numbers of cultures of *Puccinia recondita* f. sp. *tritici* producing indicated infection-type combinations on LR11 (Wichita) and Bulgaria 88 wheats.

The lower ITs observed on LR11(WI) than on B88 at high temperature does not refute the hypothesis that B88 has *Lr11* but suggests that Lr11(WI) has a gene(s) for low reaction to *P. recondita* f. sp. *tritici* other than *Lr11*. Johnston & Heyne (5) previously noted that a Wichita backcross derivative having *Lr11* resulted in lower ITs than did the donor cultivar 'Hussar' when inoculated with avirulent cultures. Figure 1, K-N, shows this difference is due to the effect of the Wichita background on IT.

TABLE 1. Infection types produced on 'Purdue Selection 45', LR11 ('Wichita'), and 'Bulgaria 88' wheats by seven cultures of *Puccinia recondita* f. sp. *tritici* when grown in three environments

Cultivar	Environment[a]	Infection type produced by culture: UN02-64A	6B-NA65-9	0967-1	UN17-68A	66-36-03	UN01-68A	66-763
Purdue Selection 45	1	8810	8810	8810	7810	8810	8810	8810
LR11(WI)[b]	1	6780	8810	6780	6750	8810	6780	6780
Bulgaria 88	1	6780	8910	6781	6751	8810	6780	6780
Purdue Selection 45	2	8810	8810	8811	8810	8810	8810	8910
LR11(WI)	2	3410	8810	5610	6710	8810	2310	2310
Bulgaria 88	2	5610	8810	6752	6751	8810	6781	6781
Purdue Selection 45	3	8910	9910	9910	8910	8810	8910	8810
LR11(WI)	3	7810	7810	7810	8910	7810	6710	5610
Bulgaria 88	3	8810	8810	8810	8910	8810	8810	8810

[a] Environment 1 = growth chamber 20-C day, 15-C night, 12-hr day, 1,500 ft-c, 75% RH. Environment 2 = greenhouse 20-24 C, 12-hr day. Environment 3 = greenhouse 30 C, 12-hr day.

[b] LR11(WI) is Wichita*6/Hussar, KS7110704.

Although these data and analyses allow only the formulation of hypotheses rather than proof of the hypotheses, one of the values of present knowledge of the gene-for-gene relationship is the formulation of usable working hypotheses concerning reaction genotype of host-lines and pathogenicity genotype of parasite cultures from IT data and application of these hypotheses without their formal proof.

The higher IT produced on B88 by culture 6B-NA65-9 than on PD45 (Fig. 1-E, F) may be particularly significant in regard to the hypothesis of general resistance in B88 by Caldwell et al. (4). It appears that when the specific genes for low reaction in B88 are overcome by corresponding genes for high pathogenicity in *P. recondita* f. sp. *tritici*, as in culture 6B-NA65-9, that greater amounts of sporulation occur in that host:parasite relationship than in the PD45:6B-NA65-9 relationship. Thus, some parasite cultures having the specific pathogenicity of culture 6B-NA65-9 may have greater disease-producing potential on B88 than on PD45, which is generally regarded as a very susceptible line (4). The range of cultures used in this study did not represent a random sample of any *P. recondita* f. sp. *tritici* population (2), particularly that experimental population used by Caldwell et al. (4). However, only two of 41 cultures were virulent to B88. Pathogenicity genotypes conditioning the 235 IT on B88 were most frequent. High pathogenicity at the *PLr11* locus occurs at a low frequency (1-5 percent) in U.S. population of *P. recondita* f. sp. *tritici* (*unpublished data*).

Based on these considerations, it is suggested that the apparent general resistance, termed "slow rusting" by Caldwell et al. (4) may have been due to a specific resistance in the cultivar brought about by a gene or genes for low reaction which is easily measurable through ITs produced by different parasite cultures and which can be overcome by genes for high pathogenicity, as evidenced by the pathogenicity of culture 6B-NA65-9. It is further suggested that when these genes for low reaction are overcome by genes for high pathogenicity in *P. recondita* f. sp. *tritici*, the cultivar may, in fact, be a "faster rusting" cultivar than even PD45. If so, this would be an example of the "Vertifolia effect" described by Van der Plank (10).

It is also suggested that the interaction of specific genes for low reaction and low pathogenicity may condition ITs having reduced sporulation, rather than zero sporulation, and these may effect a flatter disease development curve than the curve effected by host:parasite materials with the high IT and increased sporulation. The distinction between specific resistance brought about by these kinds of genes for low reaction in a cultivar and a general resistance in field studies where genes for low pathogenicity prevail may be difficult. Van der Plank's distinction of horizontal resistance as having a slope-flattening effect compared to epidemic delay caused by vertical resistance may be only applicable where host:parasite incompatability inhibiting sporulation is conditioned by host genes for low reaction interacting with prevailing parasite genes for low pathogenicity. Specific corresponding gene pairs conditioning a visually measurable low IT may also have a slope-flattening effect on disease epidemic development.

Because the cereal rust diseases are complex interactions of host, parasite, and environment, it is often difficult to separate specific and nonspecific relationships of hosts and parasites involved in the development of these diseases.

LITERATURE CITED

1. BROWDER, L. E. 1971. A proposed system for coding infection types of the cereal rusts. Plant Dis. Rep. 55:319-322.
2. BROWDER, L. E. 1971. Pathogenic specialization of the cereal rust fungi, especially Puccinia recondita f. sp. tritici: Concepts, methods of study, and application. U.S. Dep. Agr. Tech. Bull. 1432. 51 p.
3. CALDWELL, R. M. 1968. Breeding for general and/or specific plant disease resistance. p. 263-272. *In* K. W. Finlay & K. W. Shepherd [ed.]. Proc. Third Int. Wheat Genet. Symp., Canberra, Australia, Aug. 1968. Plenum Press, New York. 479 p.
4. CALDWELL, R. M., J. J. ROBERTS, & Z. EYAL. 1970. General resistance ("slow rusting") in Puccinia recondita f. sp. tritici in winter and spring wheats. Phytopathology 60:1287 (Abstr.).
5. JOHNSTON, C. O., & E. G. HEYNE. 1964. Wichita wheat back-cross lines for differential hosts in identifying physiologic races of Puccinia recondita. Phytopathology 54:385-388.
6. LOEGERING, W. Q. 1968. A second gene for resistance to Puccinia graminis f. sp. tritici in the Red Egyptian 2D wheat substitution line. Phytopathology 58:584-586.
7. LOEGERING, W. Q. 1972. Specificity in plant disease. p. 29-31. *In* R. T. Bingham, R. J. Hoff, & G. I. McDonald [ed.]. Biology of rust resistance in forest trees. Proc. NATO-IUFRO Advanced Study Inst., Aug. 1969, U.S. Dep. Agr. (Forest Service) Misc. Publ. 1221. 681 p.
8. LOEGERING, W. Q., R. A. MC INTOSH, & C. H. BURTON. 1971. Computer analysis of disease data to derive hypothetical genotypes for reaction of host varieties to pathogens. Can. J. Genet. Cytol. 13:742-748.
9. SOLIMAN, A., E. G. HEYNE, & C. O. JOHNSTON. 1964. Genetic analysis for leaf rust resistance in the eight differential varieties of wheat. Crop Sci. 4:246-248.
10. VAN DER PLANK, J. E. 1968. Disease resistance in plants. Academic Press, New York and London. 206 p. Lib. Congress Cat. No. 68-23505.
11. WILLIAMS, E., JR., & C. O. JOHNSTON. 1965. Effect of certain temperatures on identification of physiologic races of Puccinia recondita f. sp. tritici. Phytopathology 55:1317-1319.