Evaluation of the Concept of Horizontal Resistance in the Barley/Puccinia hordei Host-Pathogen Relationship

J. E. Parlevliet

Institute of Plant Breeding, Agricultural University, Lawickse Allee 166, Wageningen, The Netherlands.
Accepted for publication 24 October 1975.

ABSTRACT

PARLEVLIET, J. E. 1976. Evaluation of the concept of horizontal resistance in the barley/Puccinia hordei host-pathogen relationship. Phytopathology 66: 494-497

The partial resistance (slow rusting) effect and the cultivar effect on latent period (LP) in the barley-leaf rust relationship have been shown to be highly correlated. Because of this the LP, which can be measured accurately, was used to investigate the nature of this relationship in more detail. The LP's of five isolates were measured on the primary and flag leaves of six barley cultivars. Three types of interaction between cultivar and isolate effects on the LP were observed. (i). The isolate effect on LP could depend on the stage of crop development. On the flag leaves, isolates 11-1 and 18 gave longer LP's than the other three isolates. In the seedling stage, isolate 11-1 resulted in a LP shorter than, and isolate 18 in a LP equal to, those caused by the other three isolates. (ii). The deviation in LP of isolates 11-1 and 18 was far more pronounced in the partially resistant than in the susceptible cultivars. (iii). Differential interactions also occurred. Compared with isolates 1-2, 12, and 15, isolate 18 resulted in a longer LP on all cultivars (except Julia) in the flag leaf stage. Since there are no signs that slow rusting or partial resistance to leaf rust in barley is unstable, the results suggest that either partial resistance comprises elements of vertical, race-specific, resistance, or the tests used to differentiate horizontal and vertical resistance are unsatisfactory.

During inventory of barley for resistance to leaf rust caused by *Puccinia hordei* Otth. several types of resistance were encountered, which could be discerned as *seedling resistance* with low infection types (0 to 1) in all growth stages, *intermediate resistance,* with infection types of 2 to 3 in all growth stages, *adult plant resistance* characterized by a susceptible infection type (4^- to 4) in the seedling stage and a resistant reaction in the adult plant stage (0 to 2) and *partial resistance,* having a susceptible infection type in all growth stages (4 in seedlings, 3 to 4 in adult plants).

Partial resistance may vary as a result of differences in its components, infection frequency, latent period, sporulation rate, and infectious period (2, 11, 12) and is expressed in differences in the proportion of disease-affected leaf area. This type of resistance has been studied in more detail because it has the characteristics of horizontal resistance as described by van der Plank (11, 12). An investigation of 16 cultivars showed that the partial resistance differs greatly among cultivars (Table 3) and that it is highly correlated ($r = -0.92$) with the latent period (LP) in the flag leaves (7, 9). The LP is defined as the period between infection (inoculation) and the formation of new uredospores (new pustules becoming visible).

According to van der Plank (12) horizontal resistance is characterized by the absence of interaction between cultivars of the host and isolates of the pathogen. The absence or presence of interaction can be studied either by means of an analysis of variance or by ranking the isolates in order of their pathogenicity on the various cultivars. The two tests, however, are not identical; the former registers all deviations from additivity, the latter only the differential interactions. Ou et al. (6) tested 24 rice cultivars with 50 isolates of *Xanthomonas oryzae*. The statistical method showed cultivar-isolate interactions, whereas ranking the isolates gave no distinct interactions. This happened because the 50 isolates displayed a much wider range of pathogenicity on some cultivars than on others, although the order of pathogenicity was approximately maintained.

In the barley-leaf rust relationship the LP (one of the components of partial resistance, and highly correlated with it) can be measured quite accurately. Therefore, this host-pathogen system seemed to be suitable for investigation of the nature of partial resistance by van der Plank's tests.

MATERIALS AND METHODS

Five spring barley cultivars, which distinctly differ in partial resistance (7, 9) and five leaf rust fungus isolates were used. The isolates 1-2, 11, 12, 15, and 18 were collected, respectively, from volunteer barley plants at Wageningen in September 1971; near Süd-Lohn, 150 km east of Wageningen in October 1972; near Epping, north-east of London, England, in October 1973; near Aalten, 50 km east of Wageningen in November 1973; and near Dwingelo, 200 km northeast of Wageningen in January 1974. A monospore culture of each isolate was maintained by repeated subculturing on barley seedlings. Monospore cultures were made and multiplied again before the experiments started.

Plants were grown in the greenhouse in black 12×12-cm square plastic pots. By using different sowing dates the cultivars could all be inoculated in the same stage, when the flag leaves were young, but fully expanded at, or just

TABLE 1. Latent periods for pustule formation in the primary leaves of seedlings of six barley cultivars inoculated with five isolates of Puccinia hordei

Isolate	Latent period for indicated cultivar (days)[a]						
	L94	L98	Zephyr	Berac	Julia	Vada	Mean
1-2	7.3	8.1	7.8	7.9	8.0	9.2	8.03
12	7.2	8.0	7.9	7.9	7.9	9.4	8.05
15	7.3	8.0	7.8	8.0	8.0	9.1	8.03
Mean of the above	7.3	8.0	7.8	7.9	8.0	9.2	8.04
11-1	7.1	7.8	7.6	7.8	7.7	8.4	7.73
18	7.3	8.0	7.8	7.9	7.9	9.2	8.02
SE[b]	0.09	0.09	0.09	0.16	0.09	0.12	

[a]The latent period was determined by measuring the period between inoculation and the moment when 50% of the pustules were barely visible.

[b]SE = standard error of the difference between two effects.

TABLE 2. Latent periods in the young flag leaves of six barley cultivars inoculated with five isolates of *Puccinia hordei*

Isolate	Latent period for indicated cultivar (days)[a]						
	L94	L98	Zephyr	Berac	Julia	Vada	Mean
1-2	7.6	9.1	11.0	12.3	12.7	14.5	11.2
12	7.9	9.4	11.0	11.9	12.5	14.9	11.3
15	7.9	9.6	11.4	12.6	12.8	15.1	11.6
Mean of the above	7.8	9.4	11.1	12.3	12.7	14.8	11.3
11.1	8.1	9.4	11.7	14.0	15.1	17.2	12.6
18	8.3	9.9	11.7	13.9	12.8	18.6	12.5
SE[b]	0.14	0.16	0.34	0.68	0.35	0.56	

[a] The latent period was determined by measuring the period between inoculation and the moment when 50% of the pustules were barely visible.

[b]SE = standard error of the difference between two effects.

after, ear emergence. At this stage the cultivar effect on the LP is greatest (7). Plants were grown in flats for the seedling tests.

Four adult plants and 8-10 seedlings were inoculated per isolate by spraying a mixture of uredospores and lycopodium spores over the plants. The dilution with lycopodium spores gives a far more uniform distribution of the uredospores.

The plants were then placed at a relative humidity of 100% for about 16 hours. The plants were kept in a greenhouse with night temperatures of about 10 C and day temperatures of 20-25 C.

The LP was measured on two flag leaves of four adult plants each and on the primary leaves of six seedlings in each treatment by counting the numbers of visible pustules (first browning in the center of the light green halo) on marked areas of the leaves every day until no more pustules appeared. From these data the time between inoculation and that when 50% of the pustules were barely visible was estimated. When selecting leaf areas to be marked, the basal and top ends of the leaves were avoided as well as areas with a high density of pustules. The density was considered sufficiently low, when a large proportion of the halos (being visible two to three days before sporulation) did not touch each other before rupturing. This was done since the LP appears to be shorter at the extremes of the leaves and at high pustule densities.

The infection types were assessed on a 0-4 scale (1) one or two days after most uredosori had ruptured the epidermis. This was 9 to 20 days after inoculation, depending on the LP of the host-pathogen combination. This scale is easy to use with seedlings, but less so with adult plants. In the latter case, a susceptible infection type (type 4) is characterized by a pustule surrounded by a light green halo. This halo can become chlorotic varying from slight (type 4^-) to substantial (3^+ or 3) or may even tend towards some necrosis (a 3^-). The types 2 and 1 have small uredia surrounded by chlorotic and necrotic tissue which is in general irregularly shaped and clearly different from the 3-types.

The experiment was repeated three times, twice in the spring of 1974 and once in the spring of 1975. In the third series a sixth cultivar, Berac, was included.

The normal analysis of variance could not be applied to the adult plant tests due to lack of homogeneity of the variance. In order to assess possible isolate effects and isolate-cultivar interactions the standard error of the difference between isolate effects within each cultivar was estimated.

RESULTS

The LP not only differed among cultivars, but also varied with the stage of development of the plant (7). The rows "mean of above" in the Tables 1 and 2 show this clearly. In the seedling stage, L94 causes a slightly shorter, and Vada a somewhat longer, LP than the other four cultivars. In the young flag leaf stage the differences are far greater and cultivars with similar LP in the seedling stage differed greatly like L98, Zephyr, and Julia.

The variation between isolates was much smaller, which is not surprising. The cultivars were chosen to represent a wide range of variation and the isolates were taken more or less at random.

The isolates 1-2, 12, 15, and 18 were remarkably similar in the seedling stage (Table 1). The slightly shorter LP caused by 11-1, taken over all cultivars, is highly significant. At the flag leaf stage the situation is quite different. Isolates 1-2, 12, and 15 were still similar but 11-1 and 18 appeared distinct from them and from each other (Table 2). Isolates 11-1 and 18 gave a longer LP than the other three, the difference being small for the susceptible cultivars, but tending to increase with the more resistant cultivars.

Three types of statistically significant ($P = 0.01$) cultivar-isolate interactions could be discerned:

(i) An interaction between isolates and stage of development. The LP caused by isolate 11-1 was shorter in the seedling stage and longer in the flag leaf stage compared to those of 1-2, 12, and 15. Isolate 18 was quite similar to 1-2, 12, and 15 in the seedling stage, but clearly different (longer LP) in the flag leaf stage.

(ii) The deviation from the mean LP (the means are shown in the rows "mean of above" in the Tables 1 and 2) caused by the isolates 11-1 and 18 was far more pronounced in cultivars effectuating a long LP than in those giving a short one. This is statistically an interaction although the ranking order remains the same.

(iii) The third type of interaction is a real differential one. Compared to the average LP effected by 1-2, 12, and 15, isolate 11-1 gave a markedly longer LP with the cultivars Berac, Julia, and Vada. Isolate 18 caused a similar increase in LP as 11-1 with the cultivars Berac and Vada, but not so with cultivar Julia, where the LP equalled those of 1-2, 12, and 15. This interaction could not be discerned in the seedling stage.

As far as the infection types are concerned, all cultivar-isolate combinations were given a type 4 in the seedling stage. In the flag leaf stage there was a clear tendency toward increased chlorosis of the pustule-surrounding halo with increasing LP, from a type 4 in the cultivars L94, L98, and Zephyr to 3⁻-4⁻ in Vada inoculated with 11-1.

DISCUSSION

The partial resistance of barley to leaf rust has all the characteristics of horizontal resistance as defined by van der Plank (12). Differences between cultivars exist, not only in latent period, but also in infection frequency, sporulation rate, and infectious period (2). These differences combined, result in remarkably large differences in level of disease at the grain filling stage in Western Europe as can be seen in Table 3 from data derived from Parlevliet (9).

Various levels of partial resistance are common among the West-European cultivars (9), although no conscious selection has been done apart from removing the very susceptible lines. This suggests a more stable situation, where erosion of partial resistance either does not occur or proceeds slowly. On the other hand seedling resistances exist and are clearly of the vertical type. The most common races in Western Europe appear to be virulent on nearly all or most differential cultivars (3, 8, 10), indicating a low stability of this type of resistance. Seedling resistance has been used very little in Western Europe.

The statistical interaction and the ranking method test were unsatisfactory in determining whether or not the partial resistance studied was horizontal. The latter test, however, seems more satisfactory as it measures only differential interactions. Van der Plank also preferred ranking over the analysis of variance approach (12). We conclude either that partial resistance comprises elements of vertical resistance or that the tests were not valid. The latter is quite possible. Both tests in fact try to determine whether or not the resistance in the host population varies independently with the degree of pathogenicity. In case of independent variation no interactions between cultivars and isolates are expected. A change in resistance is not met by a change in pathogenicity; the resistance is stable and said to be horizontal. This, however, implies that resistance is a fitness-neutral characteristic for the pathogen, an improbable situation. In host-pathogen systems, where both the resistance and the degree of pathogenicity vary in a quantitative way, like the one studied here, the resistance of the host and the level of pathogenicity of the pathogen must be important factors in the fitness complex of the pathogen. In such a case, dependency (and thus interaction) between resistance and level of pathogenicity are to be expected. This, however, does not mean that such isolate-specific effects inevitably lead to erosion of the resistance. Whether the resistance of the host erodes depends on the genetic homeostasis or tendency of the pathogen population to resist genetic change (4, 5, 12). Stability of resistance due to genetic homeostasis in the pathogen population may, therefore, go together with some host-pathogen or differential interactions as our data suggest. This would mean that as far as the stability of resistance is indicated concepts like horizontal, uniform, or race-nonspecific resistance versus vertical, differential, or race-specific resistance may in fact be less unambiguous than generally is indicated.

TABLE 3. Epidemic build-up of leaf rust, *Puccinia hordei*, in isolated field plots of six barley cultivars seven weeks after inoculation at stage 6-7 of the Feekes' scale

	Number of uredosori on ten tillers per cultivar					
Year	L94	L98	Zephyr	Berac	Julia	Vada
1973	28,000	10,000	5,100	. . .	170	11
1974	7,500	7,000	400	19	15	5

LITERATURE CITED

1. CHAMBERLAIN, N. H., J. K. DOODSON, and M. H. MEADWAY. 1972. A technique for the evaluation of the resistance of barley varieties to infection with brown rust (Puccinia hordei Otth.). J. Nat. Inst. Agric. Bot. 12:440-446.
2. CLIFFORD, B. C. 1972. The histology of race non-specific resistance to Puccinia hordei Otth. in barley. Proc. Eur. and Mediterr. Cereal Rusts Conf., Prague, 1:75-78.
3. CLIFFORD, B. C. 1974. The choice of barley genotypes to differentiate races of Puccinia hordei Otth. Cereal Rust Bull. 2(1):5-6.
4. FALCONER, D. S. 1961. Introduction to quantitative genetics. Oliver and Boyd, Edinburgh and London. 365 p.
5. LERNER, I. M. 1954. Genetic homeostasis. Oliver and Boyd, Edinburgh. 134 p.
6. OU, S. H., F. L. NUQUE, and J. P. SILVA. 1971. Pathogenic variation among isolates of Xanthomonas oryzae of the Philippines. Plant Dis. Rep. 55:22-26.
7. PARLEVLIET, J. E. 1975. Partial resistance of barley to leaf rust, Puccinia hordei I. Effect of cultivar and development stage on latent period. Euphytica 24:21-27.
8. PARLEVLIET, J. E. 1976. The genetics of seedling resistance to leaf rust, Puccinia hordei Otth., in some spring barley cultivars. Euphytica 25: (In press).
9. PARLEVLIET, J. E., and A. VAN OMMEREN. 1975. Partial resistance of barley to leaf rust, Puccinia hordei II. Relationship between field trials, microplot tests and latent period. Euphytica 24:293-303.
10. RINTELEN, J. 1975. Physiologic specialization of Puccinia

hordei Otth. and reactions of varieties and cultivars to physiological races. Proc. Third Barley Genetics Congress, München, Germany. July 1975 (In press).
11. VAN DER PLANK, J. E. 1963. Plant diseases: epidemics and control. Academic Press, New York and London. 349 p.
12. VAN DER PLANK, J. E. 1968. Disease resistance in plants. Academic Press, New York and London. 206 p.