Factors Related to Partial Resistance of Barley to Leaf Rust

G. D. Statler and J. E. Parlevliet

Department of Plant Pathology, North Dakota State University, Fargo 58105, and Department of Plant Breeding (I.V.P.), Lawickse Allee, 166, Wageningen, The Netherlands.

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ABSTRACT

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Several components of partial resistance were studied in three barley (*Hordeum vulgare*) cultivars known to be widely divergent in their reactions of partial resistance to *Puccinia hordei*. Uredinia were counted daily to determine infection density and latent period. Inoculated leaf samples taken daily were cleared and stained for fluorescent microscopy. The factors studied histologically included proportion of successful infection units, penetration, number of haustorial mother cells (HMC), early abortion, late abortion, colony size, and uredinium sporulating area. Latent period (LP_{50}) was longer for the cultivars with partial resistance. Infection density and proportion of successful units was lower for cultivars with partial resistance. The average percentage of germinated urediniospores that had not penetrated was higher for the cultivar with the highest level of partial resistance (17-5-16) than for the very susceptible cultivar (L94). The percentage of early-aborted colonies was much higher for cultivars with partial resistance than for the susceptible cultivar. The percentage of late-aborted colonies was significantly greater for cultivar 17-5-16 than for the other cultivars. The average area of *P. hordei* colonies and the number of HMC was greater for the susceptible cultivar than for those with partial resistance. The size of the sporulating uredinium was not significantly different for any of the cultivars. The most definitive histological difference between susceptible and partially resistant barley cultivars to *P. hordei* was the amount of early abortion in the cultivars with partial resistance. The most important factor of partial resistance was the slower development of fewer sporulating colonies.

Partial resistance in barley (*Hordeum vulgare*) to barley leaf rust, incited by *Puccinia hordei* Otth., has been characterized by a reduced rate of epidemic development in spite of a susceptible infection type (9). Partial resistance in barley has been related to reduced infection frequencies, longer latent periods, and reduced rates of sporulation (12). The latent period has been the most reproducible and easiest component to measure (15). Latent period has been well correlated with partial resistance in the field (15,16). The differences in latent period between cultivars have been more pronounced in flag leaves than in seedling leaves (9).

Partial resistance has been shown to be polygenically inherited and generally race-nonspecific (11). This resistance has been considered relatively durable compared with race-specific resistance characterized by a hypersensitivelike infection type (4,16).

Niks (7,8) investigated the histology of low infectibility attributable to partial resistance of barley seedlings to barley leaf rust. He found early abortion to be the most important factor of low infectibility.

The current study was undertaken to histologically study the components of partial resistance in barley seedlings inoculated with *P. hordei*. The factors evaluated were colony growth, latent period, infection density, penetration, early abortion, and late abortion.

MATERIALS AND METHODS

Three barley cultivars known to be widely divergent in their reactions to partial resistance were chosen for this study. Cultivar L94 was susceptible to *P. hordei*. Cultivars 17-5-16 and Vada were known to be partially resistant, with 17-5-16 having the highest level of partial resistance. Parlevliet et al (15) found about 5,000 uredinia per tiller on Akka (susceptibility comparable to that of L94), 100 uredinia for Vada, and only 0.5 for 17-5-16 in a field experiment.

Seedlings of the cultivars were grown in flats $40 \times 40 \times 10$ cm in the glasshouse during late September and early October 1985 at the Department of Plant Breeding, Wageningen, The Netherlands. There were 60 seedlings per flat and one flat per cultivar. There were four replicates. A completely randomized design was subjected to analysis of variance and Duncan's multiple range test.

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The daytime temperature was about 22 ± 3 C, and the nighttime temperature was about 12 ± 2 C.

Seedlings were prepared for inoculation by pinning primary leaves to the soil in flats in a horizontal position with their adaxial sides up. Four Vaseline-coated slides were placed in the corners of each flat to determine inoculum density and distribution. Inoculum consisted of 1.5 mg of *P. hordei* urediniospores, isolate 1-2-1A, mixed with *Lycopodium* spores. Isolate 1-2-1A was a monospore culture derived from isolate 1-2 (11). This isolate represents the race dominating the barley leaf rust population in Western Europe. The inoculum was applied in a settling tower (3). The pins were removed after inoculation. Plants were incubated for 16 hr under natural darkness in a glasshouse equipped with a hygrostat and time switch connected to a humidifier to simulate dew formation. Plants were returned to the glasshouse bench after inoculation.

Latent period (LP_{50}) was determined using the method of Parlevliet (9) by counting the visible uredinia on a 2-cm leaf section of six leaves until no more uredinia developed. The time between inoculation and 50% of the uredinia just visible (LP_{50}) was calculated from these data (10). Infection density was determined by dividing the total number of colonies by the number of urediniospores applied per square centimeter.

A sample consisted of a 3-cm section from the central portion of four leaves collected about 16 hr after the onset of the infection process. Thereafter, samples were taken daily until sporulation, then on alternate days to day 14 after inoculation.

The leaf segments were prepared as whole mounts for fluorescent microscopy, using a modified method of Rohringer et al (17). A 0.1% solution of Uvitex 2B (Ciba-Geigy Ltd., Manchester, UK) was used in place of Calcofluor White MZR. The preparations were examined with a Nikon epifluorescence microscope equipped with an absorption filter of 460 nm.

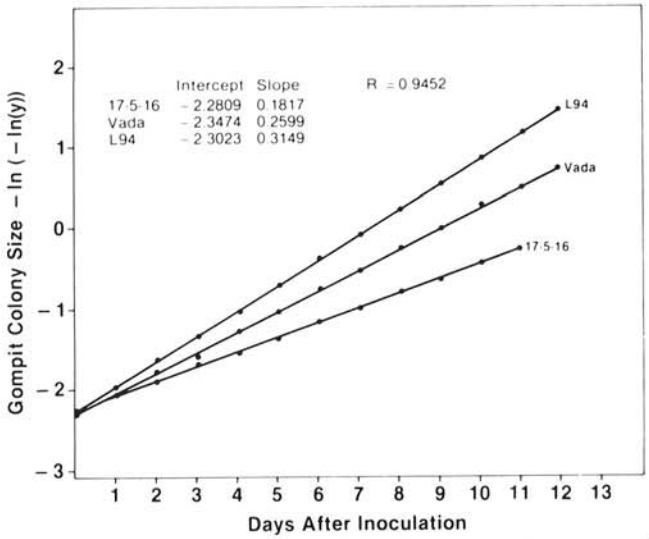

Fig. 1. Area of *Puccinia hordei* colonies after transforming to gompit values (Gompertz model) in three barley cultivars during a 14-day period after inoculation.

Twenty-five penetrated stomata were used as the experimental unit (subsample) for statistical analyses. After day 2, the experimental unit consisted of 25 colonies that had penetrated the stoma but had not aborted early. The proportion of successful infection units was determined by dividing the number of established colonies with urediniospores by those that had at least formed appressoria. The length and width of 25 colonies was measured each sampling day. The rate of increase in colony size in the three barley cultivars was analyzed by the Gompertz model (1,6). The length and width of the sporulating area was also recorded on alternate days to day 14. The numbers of penetration units containing only appressoria, penetration peg, or substomatal vesicles (SSV) were also recorded. Haustorial mother cells (HMC) were counted on days 1, 2, and 3. Early-aborted colonies were determined from day 3 onward as described by Niks (8) as those containing fewer than six HMC and hyphae with little or no branching. Late-aborted colonies were recorded as those colonies that had not sporulated by the last observation date, day 14. Uredinial eruption was not observed after day 14.

RESULTS

The LP_{50} was longer for the cultivars with partial resistance. The average LP_{50} was 10.2 days for 17-5-16, 8.1 days for Vada, and 6.8 days for L94 (Table 1).

The infection density was lower for the cultivars with partial resistance, especially for 17-5-16 (Table 1). The average infection density was 7.7% for 17-5-16, 15.5% for Vada, and 23.5% for L94. These differences were all significant at $P = 0.01$.

The proportion of successful infection units was 46.6% for 17-5-16, 58.5% for Vada, and 89.6% for L94. The differences for successful infection units were highly significant ($P = 0.01$) for all cultivars (Table 1).

The average percentage of uredeniospores that had formed an appressorium but had not penetrated was 8.6 for 17-5-16, 4.4 for Vada, and 2.2 for L94. The value for cultivar 17-5-16 was significantly greater than that for L94 ($P = 0.05$). The average percentage of infection units with SSV only was 8.0, 4.0, and 3.7 for 17-5-16, Vada, and L94 respectively; differences were not significant ($P = 0.05$).

The average percentage of early-aborted colonies was much higher for cultivars with partial resistance than for the susceptible cultivar (Table 1). The average percentages of early abortion were 47.5% for 17-5-16, 37.5% for Vada, and 4.4% for L94. These values were all significantly different at $P = 0.01$. The average percentage of late-aborted colonies was significantly greater ($P = 0.01$) for 17-5-16 than for the other cultivars (Table 1).

The average number of HMCs was greater for the susceptible cultivar L94 than for the partially resistant cultivars at days 2 and

TABLE 1. LP_{50}, infection density, proportion of successful and aborted infection units, and average number of haustorial mother cells for *Puccinia hordei* in three barley cultivars

Cultivar	LP_{50}[u] (%)	Infection density[v] (%)	Proportion successful units[w] (%)	Early abortion[x] (%)	Late abortion[y] (%)	Haustorial mother cells (no. at day 3)
17-5-16	10.2	7.7 a[z]	44.6 a	47.5 c	26.0 b	7.4 a
Vada	8.1	15.5 b	58.8 b	37.2 b	2.0 a	10.4 b
L94	6.8	23.5 c	89.6 c	4.4 a	0.0 a	13.4 c

[u] LP_{50} = time (days) between inoculation and 50% or uredinia just visible.
[v] Infection density = number of colonies per square centimeter divided by number of spores per square centimeter.
[w] Proportion successful units = number of established colonies divided by number of units with appressoria (average over sampling days (3–6).
[x] Early abortion = number of colonies with six or fewer haustorial mother cells and little branching divided by total number of infection units that passed the abortive penetration stage (average over sampling days 3–6).
[y] Late abortion = number of colonies with no sporulation on day 14 divided by total number of colonies beyond early abortion.
[z] Values with the same letter within each column are not significantly different according to Duncan's multiple range test ($P = 0.01$ for infection density, early abortion, and late abortion; $P = 0.05$ for proportion successful units and haustorial mother cells).

3, but differences were not significant on day 1. Analysis by the Gompertz transformation indicated differences in colony size between cultivars and days after inoculation ($P > 0.01$). The average colony area was greater for the *P. hordei*-susceptible cultivar L94 than for the partially resistant cultivars ($P > 0.01$) (Fig. 1). The average colony size for cultivar 17-5-16 was smaller than for Vada. The slopes of the regression lines for cultivars L94 and Vada were significantly different from that for 17-5-16 ($P > 0.01$) (Fig. 1). The average sporulating area of *P. hordei* pustules was not significantly different for the three cultivars.

DISCUSSION

The LP_{50} of the barley cultivars 17-5-16 and Vada was 3 and 1 days longer, respectively, than that of susceptible cultivar L94. This longer LP_{50} for the cultivars with partial resistance agrees with previous reports (12,16). A longer LP_{50} could reduce the number of disease cycles in the field and appeared to be highly correlated with partial resistance (5,15,16).

The infection density was lower for the two partially resistant cultivars than for the susceptible cultivar L94 in this study. This agrees with the findings of Parlevliet and Kuiper (14), who found a high correlation between infection density in the seedling stage and partial resistance in the field.

The proportion of successful penetration units was much lower for the two cultivars with partial resistance than that for the susceptible cultivar L94. The proportion of successful units in Vada was comparable to infection density, about 65% of that of cultivar L94. The proportion of successful penetration units for 17-5-16 was about 50% of that of cultivar L94, whereas the infection density was only about 33% of that of cultivar L94. Even with this variation, the number of successful infection units was much lower for the cultivars with partial resistance. Niks (8) also reported a reduced proportion of successful penetration units for barley cultivars with partial resistance.

The percentage of penetration units that contained an appressorium but had not penetrated was significantly greater in the partially resistant cultivar 17-5-16 than in the susceptible cultivar L94. The percentage of penetration units that had an SSV but no further development were not significantly greater for the cultivars with partial resistance. Clifford (2) speculated that the low infectibility of Vada to barley leaf rust could be due to SSV abortion. However, Clifford (2) found no evidence for cultivar differences in resistance attributable to appressorium formation, penetration, or SSV formation. Parlevliet and Kievit (13), however, found differences between partially resistant and susceptible cultivars in the adult but not the seedling stage when nonpenetration and SSV abortion were combined in an abortive penetration category. Although the percentage of nonpenetrants was significantly higher for cultivar 17-5-16 in our study, it may be premature to cite this as an important aspect of partial resistance in the seedling stage.

The average colony size of *P. hordei* was greater for the susceptible cultivar L94 than for the cultivars with partial resistance (Fig. 1). These differences could have been even larger if the early-aborted colonies had been used in the averages for colony size. Colonies of *P. hordei* were smaller and took longer to sporulate in the cultivars displaying partial resistance. We hypothesized that veins could limit colony width. However, the area and length of the colonies of all three cultivars were significantly correlated ($r = 0.98$). This indicated that either colony length or area could be used to determine growth. The fact that there was a greater number of HMC for L94 may simply be due to a faster growth of L94 at all stages. Lee and Shaner (5) also found smaller colonies and fewer HMC in slow- than in fast-rusting wheat cultivars.

Late abortion was more pronounced for cultivar 17-5-16 than for Vada or L94. In fact, 26% of the colonies of cultivar 17-5-16 had failed to sporulate by day 14 after inoculation. Differences were not found between Vada and L94. Niks (8) found no differences in late abortion, but he used Vada and L94 in his studies and not the more resistant cultivar 17-5-16.

It is apparent from these studies that the most definitive histological differences between susceptible and partially resistant barley cultivars in the seedling stage were differences in colony size and the amount of early and late abortion detected in the cultivars with partial resistance. This resulted in fewer sporulating colonies that took longer to develop in cultivars showing partial resistance. These results partially agree with findings of Niks (8) and Clifford (2), who reported that the final expression of partial resistance was the slower development of fewer and smaller sporulating colonies.

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