Histology of the Relation Between Minor and Major Genes for Resistance of Barley to Leaf Rust

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ABSTRACT

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*Pa*7 and *Pa*3, two major genes that confer hypersensitive resistance to leaf rust (caused by *Puccinia hordei*) in barley were introduced into three genetic backgrounds with different levels of partial resistance (PR) (the genetic complement controlling PR will be referred to collectively as "PR genes") to study the interaction between both types of resistance. The growth rate and the degree of abortion of the colonies in the genotypes were determined by fluorescence microscopy. The degree of host cell necrosis was recorded. The PR genes affected the success of colony establishment in the host and reduced the growth and development rate of colonies after establishment. This effect was also apparent in the presence of *Pa*7, the effects of which were seen relatively late in the infection process. Apparently, PR genes and *Pa*7 acted independently and consecutively. *Pa*3 acted shortly after the establishment of the colonies and largely obscured the effect of the PR genes. Nevertheless, the level of PR can be assessed in the presence of *Pa*3 by determining the proportion of early aborted colonies not associated with host cell necrosis.

Additional key words: *Hordeum vulgare*, vertical resistance, horizontal resistance.

Since Vanderplank (24) introduced the concept of vertical and horizontal resistance (VR and HR, respectively), these two types of resistance have been compared in many publications. One host genotype may possess alleles for both HR and VR, but many authors surmise that the expression of HR is obscured if the host also possesses effective alleles for VR (eg, reference 20). As a consequence, selection for HR, which is desirable because of its assumed durability, would be possible only in the absence of VR.

In the barley/leaf rust (caused by *Puccinia hordei* Otth) relationship, the major genes conferring a hypersensitive reaction with avirulent races are considered to be VR genes. These genes are designated *Pa*-genes (13). The minor genes conferring partial resistance (PR) are presumed to belong to the type of HR genes (8.14). PR is characterized by a reduced epidemic buildup, despite a susceptible infection type (IT) (15). Both types of resistance can occur in one genotype (17).

In this paper, we present the results of a histological study of the relation between both types of resistance.

MATERIALS AND METHODS

A backcrossing procedure was used to introduce the dominant alleles *Pa*7 of Cebada Capa and *Pa*3 of Rika × (Rika × Baladi) (see reference 13) into three recipient cultivars of barley: L94, Zephyr, and Vada, which have undetectable, moderate, and high PR, respectively (19). From each of the six major gene/recipient combinations one hypersensitive plant was singled out after the fourth backcross (B4). After selfing, each B4 plant produced a population that segregated for the major gene. These populations were screened for IT with isolate 121A, which is avirulent to *Pa*7 and *Pa*3. The seedlings with the lowest IT were assumed to be homozygous for the dominant allele of the major gene (*Pa7 Pa7* or *Pa3Pa3*, respectively). The progenies from two selfed very hypersensitive and two selfed fully susceptible plants (*pa7pa7* or *pa3pa3*, respectively) (ie, the second generation after B4) for each major gene/recipient combination were used in this study. The two donor cultivars, indicated as CC and Rika, respectively, also were studied. The lines are designated according to the presence (P, which stands for *Pa*) or absence (p, which stands for *pa*) of the dominant allele of the *Pa* gene (*Pa* 7 or 3). The genetic background of the line, which resembles that of the recipients (L94, Zephyr, or Vada), is indicated by L, Ze, or Va (see also Table 1). The 24 lines were grown in twelve 37 × 39-cm planting boxes, or flats. Each flat contained one line derived from a hypersensitive plant and one fully susceptible sister line. Each line was represented by 16 seedlings. CC and Rika were grown in an additional flat. The primary leaves were inoculated with isolate 121A by using a settling tower. Between 80 and 190 spores per square centimeter were applied, of which 50% formed an appressorium over a stoma. Central segments of the leaves were sampled 2.5, 4.5, 6.5, and 14.5 days postinoculation (d.p.i.), but for the lines with a Vada background the final sampling was at 16.5 d.p.i. because of the reduced rate of uredial development. Four segments per line were collected per sampling day. The leaf pieces were stained (22) with Blancophor BA 267% (Bayer, Leverkusen, West Germany) replacing the Calcofluor. The observations were carried out with Zeiss model NXL epifluorescence equipment. Details of the foregoing procedures are given elsewhere (10).

Infection units (each unit representing the thallus developing from a single urediospore [25]) were classified according to developmental stage and by the measurement of colony lengths. Here, germ tubes were not observed, nor were germination and appressorium formation studied. The developmental phases of the infection process and the designations of the corresponding infection units are given in Table 2 (see also reference 10). Each leaf was screened for as many infection units as were necessary to find 20 measurable established colonies that either had aborted late or successfully produced urediospores. The size of the colonies was assessed with an eyepiece micrometer by measuring the length of the projection of the colony on the long axis of the leaf. Intertwined colonies were considered immeasurable. They were, however, recorded to determine the proportions of infection units per developmental phase.

Host cells were classified as necrotic if they showed one or more of three qualities: browning of the cell contents, collapse of cell walls, and autofluorescence. The latter criterion has been used also by Samborski et al (23). The number of autofluorescent host cells per infection unit was counted or assessed. With large colonies the proportion of the colony area with cell browning was estimated.

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In the statistical analyses, the experimental units were (the means of) the responses per leaf.

RESULTS

One of the P3-Va lines segregated for hypersensitive resistance. The observations on this line were discarded for the leaves collected at 4.5 d.p.i., since the results suggested that the leaves sampled on that day belonged to susceptible segregants. No segregation for hypersensitivity occurred in the other lines, indicating that these lines descended from parents that were homozygous for this character.

Colony growth. The average lengths of the established colonies at the four times of sampling are presented in Table 1. The effect of *Pa3* on the colony growth was pronounced: a significant lag in colony length was apparent as early as 2.5 d.p.i. The growth retarding effect of *Pa7* was less serious. At 2.5 d.p.i. the colonies in the P7 lines did not prove to be smaller than in the p7 lines. The effect of *Pa7* only became detectable when the colonies in the susceptible counterparts averaged at least 300 μm.

Four susceptible lines per recipient genotype were available to compare the effect of the minor genes for PR on colony growth. No systematic differences between p3 and p7 lines were found within a recipient genotype. In accordance with the level of PR of the recipients, the colonies in the susceptible L-lines had a higher rate of growth than those in the susceptible Va-lines. The colonies in the Ze-lines grew at an intermediate rate.

The effect of the PR genes on colony growth in the lines with *Pa7* was evident. At 6.5 d.p.i. there was a significant interaction effect between the *Pa7* allele and the PR-background (ANOVA, critical level $P < 0.01$), indicating that the larger the average colony length in the susceptible line, the larger the relative arrears in the hypersensitive counterpart. In the lines carrying the *Pa3* allele, the colonies in the L-lines reached the largest size, as expected. The colony lengths in Ze- and Va-lines showed no significant differences; in both types of lines the average colony length hardly increased with time. The growth-retarding effect of PR genes was obscured by the strong expression of *Pa3*.

Colony abortion. The proportion of infection units blocked as nonpenetrants and as aborted substomatal vesicles (SSV) were calculated from the data obtained from all 16 leaves per line. The mean proportion of nonpenetrants was 6%, without conspicuous differences between the lines (Kruskal-Wallis nonparametric test, $P = 0.14$). The differences in SSV-abortion were not significant either (Kruskal-Wallis, $P = 0.10$) and too low to be of interest (average proportion 0.9%).

For the degree of early abortion, pronounced differences between the lines were found (Table 3, Fig. 1). In all the Va-lines

TABLE 1. Average colony length (μm) of *Puccinia hordei* in 26 barley lines at four measuring times

Host genotype	Time of sampling[u] 2.5	4.5	6.5	14.5	16.5
P7-L[v]-1	174[w]g[x]	330 f	429 fg	973 f	···
P7-L-2	174 g	326 f	447 g	940 f	···
p7-L-1	178 g	419 gh	768 j	1,640 j	···
p7-L-2	172 g	385 g	773 j	1,436 i	···
P3-L-1	*134* def[y]	174 bc	230 cd	446 c	···
P3-L-2	*133* de	187 bcd	262 d	490 c	···
p3-L-1	171 g	430 h	816 j	1,490 i	···
p3-L-2	178 g	400 gh	761 j	1,433 i	···
P7-Ze-1	141 ef	257 e	379 ef	780 e	···
P7-Ze-2	142 ef	255 e	330 e	772 e	···
p7-Ze-1	143 ef	328 f	572 h	1,188 gh	···
p7-Ze-2	141 ef	314 f	585 h	1,194 gh	···
P3-Ze-1	*94* a	*123* a	*126* a	220 ab	···
P3-Ze-2	*117* bc	*125* a	*133* ab	169 a	···
p3-Ze-1	151 f	310 f	651 i	1,279 h	···
p3-Ze-2	144 ef	273 e	553 h	1,290 h	···
P7-Va-1	143 ef	206 bcd	241 d	...	656 d
P7-Va-2	137 ef	200 bcd	255 d	...	740 de
p7-Va-1	128 cde	217 d	379 ef	...	1,118 g
p7-Va-2	135 def	209 bcd	341 e	...	1,229 gh
P3-Va-1	*109* b	···	*116* a	...	299 b
P3-Va-2	*120* bcd	*116* a	*128* a	...	168 a
p3-Va-1	141 ef	211 cd	401 fg	...	1,210 gh
p3-Va-2	135 ef	209 bcd	405 fg	...	1,185 gh
Cebeda Capa[z]	135 def	194 bcd	182 bc	287 b	···
Rika[z]	*105* ab	*113* a	*112* a	211 ab	···

[u] Expressed in days after inoculation.
[v] P indicates the presence and p the absence of the dominant allele of the major gene; 7 and 3 refer to *Pa7* and *Pa3*, respectively; L, Ze, and Va indicate the recipient genotypes of cultivars L94, Zephyr, and Vada, respectively.
[w] Each entry is the mean length of 80 established colonies in four primary leaves unless indicated otherwise.
[x] Per column, entries with a common letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.
[y] Entries in italics are averages of the lengths of early aborted and of established colonies, since the latter could not be recognized unambiguously.
[z] Cebada Capa is the donor of the *Pa7* allele; Rika ((= Rika × (Rika ×Baladi))) is the donor of the *Pa3* allele.

TABLE 2. Designation and definition of the infection units of *Puccinia hordei* in barley, according to their stage of development

Developmental phase	Designation of the infection unit	Definition
Prepenetration	Nonpenetrant[z]	Appressorium over stoma without formation of a substomatal vesicle
Substomatal vesicle (SSV) formation	Aborted SSV[z]	SSV without hyphae
Establishment	Early aborted colony[z]	SSV with primary infection hyphae and up to six haustorial mother cells
Colonization	Established or late-aborted colony[z]	Branched hyphae, six or more haustorial mother cells; sporogenic tissue may be present
Reproducion	Successful colony	At least some uredio-spores are formed

[z] Arrested in the indicated developmental phase.

TABLE 3. Percentage of early aborted colonies of *Puccinia hordei* in barley seedlings with different genotypic backgrounds and different alleles of major genes for resistance

Genotypic background	Line	Alleles of the major genes[y] *Pa7*	*pa7*	*Pa3*	*pa3*
L94	1	10 bc[z]	4 ab	32 de	4 ab
	2	2 a	3 a	33 ef	2 a
Zephyr	1	7 abc	3 a	50 gh	5 ab
	2	12 c	4 ab	22 d	3 a
Vada	1	52 h	55 hi	54 hi	42 fg
	2	37 ef	42 fg	33 def	43 fg
Cebada Capa		31 de			
Rika				63 i	

[y] *Pa7* and *Pa3* cause a hypersensitive reaction, *pa7* and *pa3* a susceptible reaction.
[z] Entries with a common letter are not significantly different ($P \leq 0.05$) based on Duncan's multiple range test.

and in the P3-L and P3-Ze lines, a high proportion of colonies was found that fitted the (morphological) definition of early abortion as given in Table 2 (Table 3). Many other colonies in the *Pa*3-carrying lines were blocked little beyond the establishment stage: these possessed more branched hyphae suggesting a successful establishment, but had only five to eight haustorial mother cells. As a consequence, a distinction between established and early aborted colonies often was impossible with these lines. The presence of the *Pa*3 allele did not raise the level of early abortion in the Vada background. In contrast with *Pa*3, the *Pa*7 allele had scarcely any effect on the level of early abortion.

The degree of late abortion was assessed from the leaf segments of the final sampling date. The colonies that had not reached the reproduction phase at this date were classified as late aborted

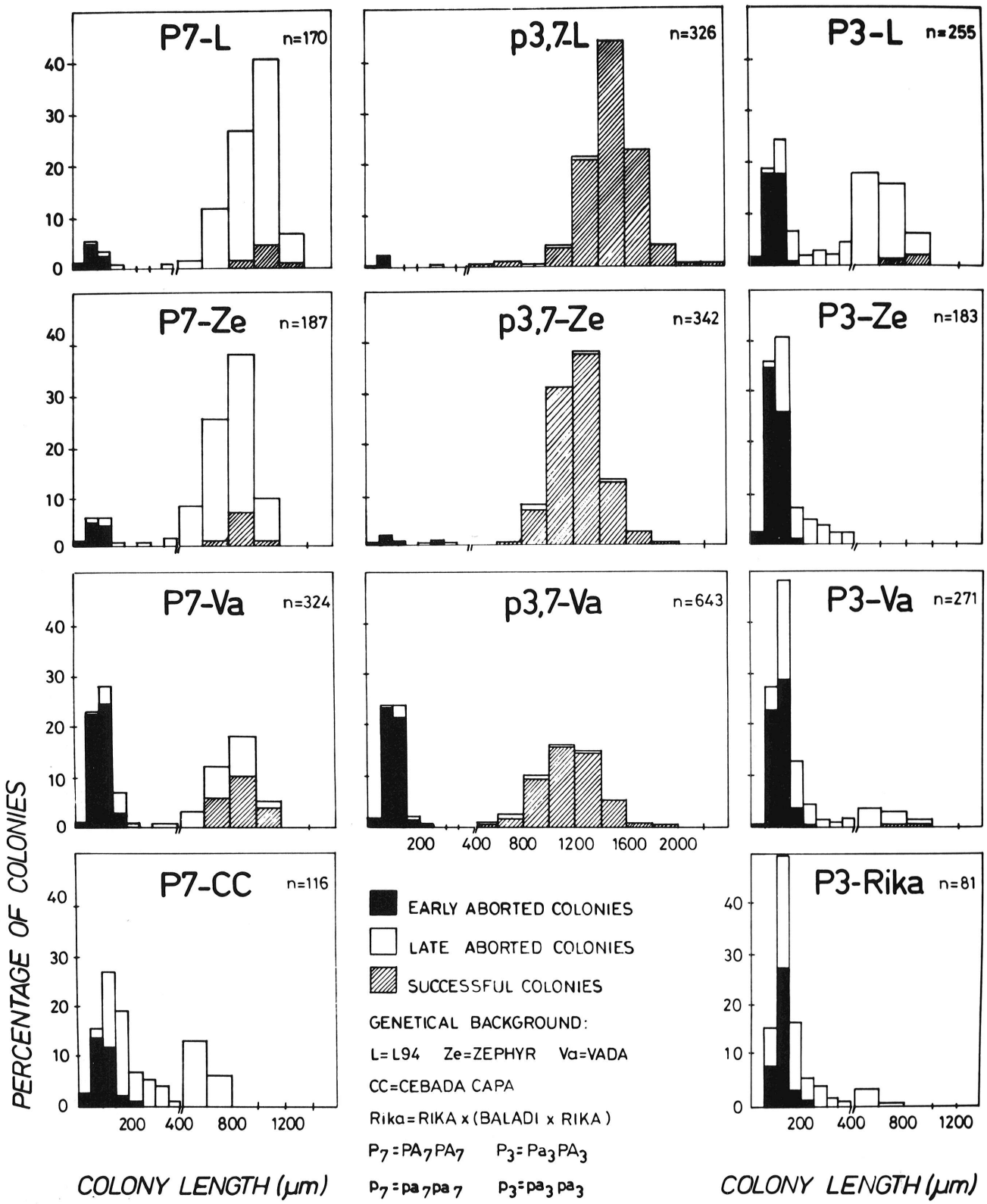

Fig. 1. Frequency distribution of the colony length of *Puccinia hordei* in seedlings of barley genotypes differing in genetic background and in the presence (P) or absence (p) of a major gene (*Pa*7 or *Pa*3) for resistance. The leaves were collected 16.5 (Vada background) or 14.5 (the others) days after inoculation. Each graph is based on the measurements of *n* colonies. The three classes of colonies are defined in Table 2. Note the change of scale at 400 μm.

colonies. It must be borne in mind that the level of late abortion may depend on the date of sampling. Almost all established colonies in the genotypes without a dominant allele for hypersensitivity had reached the reproduction phase at the final sampling date (Table 4, Fig. 1). Those that had not were not likely to have the potential to become reproductive. The susceptible Va-lines showed a barely higher level of late abortion than the L- and Ze-lines.

Of the *Pa7*-containing genotypes the level of late abortion was highest in Cebada Capa. No reproduction of the pathogen was observed in this cultivar (Table 4) and the colony growth ceased at a rather small colony size (Table 1, Fig. 1). In the other genotypic backgrounds the *Pa7* gene did not prevent reproduction completely. Especially in the P7-Va lines a substantial proportion of the established colonies succeeded in the formation of at least a few urediospores (Fig. 1).

With the genotypes carrying *Pa3*, late abortion generally occurred at smaller colony sizes than with *Pa7*. Except for the P3-L lines, the majority of the late-aborted colonies were arrested just beyond the establishing phase (see above). Of the colonies that continued growth, only a few had reached the reproduction phase at the final sampling date. In the P3-L lines, relatively numerous colonies reached lengths of over 400 μm (Table 1, Fig. 1).

Host cell necrosis. Generally, small colonies were associated with the necrosis associated with autofluorescence, large colonies with browned and collapsed cells that were not autofluorescent.

In the genotypes with a high level of early abortion due to PR (Va-lines and CC) (Table 3) the early aborted colonies were rarely associated with host cell necrosis (Table 5). The early abortion due to *Pa3* (Table 3), on the contrary, was almost always associated with necrotic host cells (Table 5).

TABLE 4. Percentage of late-aborted colonies of *Puccinia hordei* in barley seedlings with different genotypic backgrounds and different alleles of major genes for resistance

Genotypic background	Line	Alleles of the major genes[x]			
		Pa7	*pa7*	*Pa3*	*pa3*
L94	1	88[y]de[z]	0 a	99 e	1 a
	2	99 e	2 a	91 de	2 a
Zephyr	1	97 e	2 a	100 e	2 a
	2	86 d	3 ab	100 e	2 a
Vada	1	64 c	5 ab	96 de	14 b
	2	56 c	8 ab	100 e	10 ab
Cebada Capa		100 e			
Rika				100 e	

[x] *Pa7* and *Pa3* cause a hypersensitive reaction, *pa7* and *pa3* a susceptible reaction.

[y] Entries are the average percentages of established colonies that failed to form urediospores. The data are obtained from leaf segments collected 16.5 (Vada-background) or 14.5 (the others) days after inoculation.

[z] Entries with a common letter are not significantly different ($P \leq 0.05$) based on Duncan's multiple range test.

TABLE 5. Percentage of early aborted colonies of *Puccinia hordei* associated with at least one autofluorescent host cell in barley seedlings with different genotypic backgrounds and different alleles of major genes for resistance

Genotypic background	Alleles of the major genes[z]			
	Pa7	*pa7*	*Pa3*	*pa3*
L94	32	23	88	16
Zephyr	10	14	92	10
Vada	5	3	29	3
Cebada Capa	11	...	...	...
Rika	...	...	88	...

[a] *Pa7* and *pa3* cause a hypersensitive reaction, *pa7* and *pa3* a susceptible reaction.

Pa7 and *Pa3* differed in the degree to which host cells became necrotic in established colonies. At 2.5 d.p.i. the established colonies in *Pa7* carrying lines were associated with cell necrosis as much as or little more than the susceptible lines (Table 6). From 4.5 d.p.i. on, the *Pa7* gene induced a browning of host cells that progressed until at the final sampling date in the larger colonies cell browning occurred over 25–50% and in CC up to 100% of the colony area. These colonies were macroscopically visible as small dark spots on the leaves.

Pa3 induced cell necrosis around the majority of the colonies in the P3-L, P3-Ze, and Rika genotypes as early as 2.5 d.p.i. (Table 6). In P3-Va, the degree of necrosis appeared to be less, probably owing to the early abortion caused by PR genes, which seldom goes with host cell necrosis. The colonies seemed to be "knocked down" by *Pa3* either just at or shortly after establishment. The cell necrosis mostly concerned the cells that were associated with the haustorial mother cells of the young colony.

In all genotypes carrying *Pa3*, especially in the P3-L lines, part of the colonies seemed to recover from the early inhibitory effects of *Pa3*. Their mycelium passed by the necrotic host cells and continued growth without provoking further cell necrosis. These larger colonies were associated with only a few browned host cells in the center of the colony. The browning seldom occupied more than 25% of the colony area. Since *Pa3* causes a macroscopically visible chlorosis and necrosis of the host tissue around the infection sites, it appears that the larger colonies also were associated with host cell alterations that were not detectable by the methods used here.

In the susceptible lines, particularly the L- and Ze-lines, many established colonies were associated with autofluorescent cells (Table 6), but the number of these cells per colony was small and the amount of necrosis was negligible relative to the total colony area.

DISCUSSION

As is known from macroscopical investigations, the minor genes for PR to leaf rust in barley cause a reduced rate of development of the colonies (8,12). In the present experiment, the uredia on the susceptible lines with the partially resistant Vada background appeared a few days later than on the lines with L94 and Zephyr background. In connection with the reduced rate of development, the growth rate of the colonies is affected by the PR genes (Table 1), as has been reported before (2,4,5). In addition, minor genes for PR can reduce the number of uredia per square centimeter leaf area (18) by causing a substantial early abortion of colonies, which seldom is associated with host cell necrosis (10) (see data collected from the p7-Va and p3-Va lines in Tables 2 and 5).

TABLE 6. Average percentage of established colonies of *Puccinia hordei* associated with at least one necrotic (= autofluorescent, browned, or collapsed) host cell 2.5 days after inoculation

Genotypic background	Line	Alleles of the major genes[x]			
		Pa7	*pa7*	*Pa3*[y]	*pa3*
L94	1	48 (1.4)[z]	49 (1.9)	84 (1.6)	40 (1.3)
	2	55 (1.3)	21 (1.2)	95 (2.1)	51 (1.5)
Zephyr	1	33 (1.5)	21 (1.4)	88 (2.4)	20 (1.1)
	2	36 (1.4)	35 (1.3)	98 (2.2)	20 (1.6)
Vada	1	28 (2.0)	6 (1.3)	25 (1.3)	10 (1.3)
	2	29 (1.4)	20 (1.2)	35 (1.4)	5 (1.5)
Cebada Capa		15 (1.2)			
Rika				75 (1.7)	

[x] *Pa7* and *Pa3* cause a hypersensitive reaction, *pa7* and *pa3* a susceptible reaction.

[y] In lines carrying *Pa3*, the established colonies could not be recognized unambiguously from the early aborted colonies. The data concern all colonies that formed at least one haustorial mother cell.

[z] Within parentheses the average number of necrotic cells associated with the colonies concerned are given.

The dominant alleles of the two major genes for resistance to leaf rust considered (*Pa*7 and *Pa*3) clearly differed in their mode of action, irrespective of the genetic background. The action of *Pa*7 became manifest several days after the establishment of the fungus by a gradual growth retardation associated with extensive cell browning. In contrast, *Pa*3 caused an early cessation of the fungal growth associated with host cell necrosis. Part of the colonies continued growth without provoking further necrosis of host cells. Similar differences in action between major genes for resistance were reported, eg, for flax and flax rust (9), wheat and stem rust (21), and wheat and powdery mildew (7). The genetic background may modify the mode of action of the hypersensitivity alleles although the general characteristics of the resistance reaction are not lost (6,21). In the present study, the genetic background also influenced the expression of the major genes. With *Pa*7, relatively many established colonies reached the reproductive phase in the Vada background, whereas in donor cultivar Cebada Capa no successful colonies were found (Fig. 1). Since both Cebada Capa and Vada possess a high gene dose for PR (17), it is likely that the mitigation of the action of the *Pa*7 allele in the Vada background is attributable to other than PR genes. Apparently, the PR genes and *Pa*7 act independently and consecutively: only the colonies that are not arrested by early abortion due to PR have to cope with the barrier raised by *Pa*7. These results confirmed the findings of Parlevliet (16) who demonstrated that the more minor genes for low infectibility are present, the fewer necrotic flecks appear in the presence of *Pa*7. Clifford (3) also found a good correlation between the level of PR in the genetic background and the number of necrotic spots due to hypersensitivity. He investigated cultivars that were recessive for *Pa*7, but dominant for at least *Pa*2. The growth retarding effect of PR genes remained unimpeded in the presence of *Pa*7 (Table 1), in accordance with the slower appearance of necrotic flecks found by Parlevliet (16) in lines with *Pa*7 in a high-PR background.

The effects of the PR genes were less easily recognized in the presence of *Pa*3, since this major gene acts about as early as the PR genes. Differences in growth rate due to PR genes were largely obscured by the substantial abortion of colonies due to *Pa*3-either at or shortly after establishment. The results suggest that *Pa*3 acts even more intensely in the Zephyr background than in the donor cultivar Rika. Despite the low degree of reproduction in the P3-lines at the final sampling date, it cannot be excluded that more colonies would have reached the reproductive stage if the sampling of the leaves had been postponed some days. A fair degree of sporulation has been observed in the three *Pa*3-carrying recipients (J. E. Parlevliet, *unpublished*).

It could be demonstrated that the early abortion by *Pa*3 occurs shortly after the formation of the first haustoria (11). The cells in which these haustoria are formed become necrotic. This explains the high association of early abortion due to *Pa*3 with host cell necrosis in genotypes with little PR (Table 5). Considering the formation of the first haustoria as successful establishment, it is more appropriate to speak of early hypersensitivity. Early abortion by PR genes occurs before the formation of haustoria as a failure of establishment (11). This means that the PR genes and *Pa*3 again act consecutively, but in a very short time interval. Only the colonies not arrested by PR genes (without necrosis) may be arrested by *Pa*3 (with necrosis). The level of PR in genotypes with an early acting allele for hypersensitivity can be assessed by the degree of host cell necrosis. This implicates that Rika does not have an appreciable level of PR. A similar difference in the degree of host cell necrosis between race-specific and race-nonspecific resistance was reported for oats and powdery mildew (1).

In the presence of a late-acting major gene for resistance, the level of PR in the genetic background can be estimated much more easily than in the presence of an early acting major gene. The results suggest that minor and major genes for resistance acted independently and constituted different defense systems. Plants combining hypersensitive resistance with a high proportion of small aborted colonies lacking host cell necrosis should be promising parental material, because they may carry a high level of possibly durable resistance in their genetic background.

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