Characterization of *Lr34*, a Major Gene Conferring Nonhypersensitive Resistance to Wheat Leaf Rust

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ABSTRACT

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Wheat leaf rust resistance gene *Lr34* is claimed to contribute to durability of resistance in wheat cultivars in combination with other genes for resistance. We compared the effect of *Lr34* with that of *Lr12* and *Lr13* (all in Thatcher background) and with the partial resistance of Akabozu and BH1146. Seedlings of all lines displayed a compatible infection type. *Lr34* increased latency period and decreased infection frequency, especially at low temperature. The gene caused a small but significant increase in early abortion of sporelings. The number of haustoria per sporeling 42 h after inoculation was reduced significantly, but this was not associated with papilla formation. In adult plants the effect of *Lr34* was much clearer. In the flag leaf *Lr34* decreased infection frequency and increased latency period. Many infection units did not develop further than the stage in which they caused pale (nonhypersensitive) flecks. Also at the microscopic level we found no increased hypersensitivity due to *Lr34*. *Lr34* shared features both with *Lr13* and with the genes for partial resistance in Akabozu and BH1146. The main difference with *Lr13* was the association of the latter with chlorosis at the macroscopic level and cell necrosis at the microscopic level. *Lr34* and the partial resistance in Akabozu and BH1146 are based on reduced rates of haustorium formation in the early stages of infection, in association with no or relatively little plant cell necrosis. However, the reduction of haustorium formation in Thatcher-*Lr34* appeared to be due to a low rate of intercellular hyphal development and not to papilla formation as in Akabozu and BH1146. We argue that *Lr34* should be considered a major gene conferring partial resistance *sensu* Parlevliet.

Additional keywords: *Puccinia recondita* f. sp. *tritici, Triticum aestivum*

In wheat (*Triticum aestivum* L.) 45 major genes for resistance to the leaf rust fungus *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* Eriks. & E. Henn. have been identified at 36 loci (32). Almost all these genes cause resistance that is associated with chlorosis or necrosis or with immunity.

Resistance gene *Lr34* (formerly designated *LrT2*) has been reported to cause resistance that is not clearly associated with chlorosis or necrosis (6,9,17). *Lr34* has received much attention in recent years, since this gene is present in many wheat cultivars throughout the world that have shown durable resistance to leaf rust. It enhances the effect of other resistance genes (6,17,34). The durability of the resistance is ascribed to interactions with adult plant resistance genes *Lr12* and *Lr13* in particular (31).

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Lr34 is associated with leaf tip necrosis (36), with adult plant resistance to stem rust (11), with adult plant resistance gene *Yr18* to stripe rust (37), and with tolerance to barley yellow dwarf virus (38). *Lr34* is located on chromosome 7D although it may change to another position due to translocation (13).

In wheat cultivars that lack other effective *Lr* genes, *Lr34* expresses resistance in a quantitative way (6,17,39). It causes an increased latency period and decreased infection frequency and uredium size (6).

In this respect the effect of *Lr34* is very similar to the partial resistance of wheat to *P. r.* f. sp. *tritici* (20) and of barley to *P. hordei* G. Otth (29,30). In addition, both *Lr34* and genes for partial resistance are expressed more clearly in the adult plant than in the seedling stage.

Histological observations demonstrated that partial resistance in barley and wheat is based on poor haustorium development and slow growth rates of the leaf rust fungi (18,21,25,26). Early aborted sporelings were found, which were associated with cell wall appositions (papillae) that may impede haustorium formation (18,26). The aborted sporelings were not associated with plant cell necrosis. Lee and Shaner (21), however, did not find such early aborted sporelings in their slow-rusting wheat cultivars.

The present study was performed to determine the effect of *Lr34* on the leaf rust fungus at the histological level in the seedling and adult plant stage and to compare it with the effect of *Lr12* and *Lr13* (all in Thatcher background) and the partial resistance of Akabozu and BH1146 (1, 3,4,18,19,30).

MATERIALS AND METHODS

Plant material. The studies were performed on Thatcher (susceptible) and its isolines Thatcher-*Lr12* (RL6011 = Tc*6/ Exchange), Thatcher-*Lr13* (RL4031 = Tc*7/Frontana) and Thatcher-*Lr34* (RL-6058 = Tc*6/PI58548). These lines were developed by Dyck and co-workers (9, 15). Seed of the lines was kindly provided by J. A. Kolmer, Agriculture Canada, Winnipeg. We also included the extremely susceptible line Morocco (18,19,21) and the partially resistant wheat lines Akabozu and BH1146 (1–4,18,19). *Lr12* and *Lr13* both confer hypersensitive resistance in the adult plant stage (31). Akabozu and BH1146 were reported by Broers and Jacobs (3) to have a high level of partial resistance. According to them, BH1146 may possess a hypersensitive gene (probably *Lr13*) and two or more latency period prolonging genes, at least some of which are different from the latency period prolonging genes in Akabozu.

Pathogen. In all experiments the *P. r.* f. sp. *tritici* isolate Flamingo was used. The virulence/avirulence factors were published by Broers (2), and the isolate should be race DBB according to the designation of Long and Kolmer (22). The isolate was multiplied on adult plants of cv. Little Club and kept in a desiccator till used.

Seedling tests. Plants were grown in soil in plant boxes (37 × 39 × 5 cm). Each replication consisted of one box with eight seedlings of the seven lines studied. Eleven days after sowing, the primary leaves of the seedlings were fixed in a horizontal position with the adaxial side upward. Per plant box, 2 mg of urediospores was mixed with *Lycopodium* spores (1:9, vol/vol), and applied using a settling tower. The inoculum density was about 130 spores/cm^2.

After inoculation the plant boxes were incubated overnight in a mist chamber at 100% relative humidity and darkness and then transferred to a greenhouse compartment at 20 to 25°C (25°C) or climate room (8 and 16°C).

Six successive experimental runs (series) were performed at 25°C, three at 16°C and two at 8°C for macroscopical observations (see below). For five of the six series at 25°C leaves were sampled for microscopical observations. Segments of three leaves per line were collected 5 days after inoculation. The macroscopic observations were carried out on the remaining five primary leaves.

The experimental units for the statistical analyses (analysis of variance [ANOVA], least significant difference [LSD] test, STATISTIX SX 4.1 [Analytical Software, Tallahassee, FL]) were the averages over all leaves of a line within a series.

Adult plant tests. Plants were grown individually in 12 × 12 cm pots in a greenhouse. All lines were sown at several dates in order to obtain plants at the same development stage, DC (Decimal Code) 48-59 (ears just emerged, young but fully expanded flag leaves) (40) at the time of inoculation. Five series were performed. Five pots per line were used per series. Inoculation was performed by dusting urediospores mixed with *Lycopodium* spores over the plants. One milligram of urediospores was used per pot. Incubation was as described before. Following incubation, plants were transferred to a greenhouse at about 20 to 25°C. Three flag leaves per line in four consecutive series were sampled for microscopical observations 5 days after inoculation. The macroscopic observations were carried out on at least five flag leaves per line per series.

The experimental units for the statistical analyses (ANOVA, LSD test) were the averages over all leaves of a line within a series.

Macroscopic observations. Infection type (IT), latency period, and infection frequency were determined. IT was recorded 14 days after inoculation according to a 0 to 9 scale (23). IT1 indicates small chlorotic or necrotic spots without uredia; IT7, IT8, and IT9 indicate well-sporulating uredia surrounded by some, little, or no chlorosis, respectively. Latency period was determined by counting daily the number of uredia visible in a marked area on the leaves till the number of uredia no longer increased. This area was taken as large as needed to contain about 20 to 50 pale flecks before the uredia matured. The latency period was taken as the time period from the beginning of incubation to the time at which 50% of the uredia had appeared (27). Infection frequency was determined on the marked areas of the leaves. The final number of uredia was used to calculate the number of uredia per cm^2.

Microscopic observations. Middle segments of 1 to 3 cm^2 of the collected leaves were prepared as whole mounts for fluorescence microscopy as described by Rohringer et al. (33), but instead of Calcofluor we used Uvitex 2B (Ciba-Geigy, Arnhem, the Netherlands). The preparations were examined at 200× with a Nikon epifluorescence equipment (Fluophot, V-excitation, 380 to 425 nm wave length transmission). The sporelings were scored and classified according to their stage of development (24). Sporelings that developed a germ tube and not an appressorium over a stoma were ignored. We defined early aborted sporelings as individuals that formed a primary infection hypha and not more than six haustorial mother cells (24). Sporelings that had developed more haustorial mother cells were classified as established. Filters with 420 to 490 nm transmission were used to observe necrosis of host cells, which display a golden yellow autofluorescence. The length (L) and width (W) of ten arbitrarily chosen established colonies per leaf was measured with an eyepiece micrometer. Colony size (CS) was calculated as the geometric mean of L and W, $CS = SQRT(¼ \pi \cdot L \cdot W)$. The statistical analysis for percentage of sporelings aborted or associated with necrosis or sporogenic tissue was performed on arcsin-transformed data. The percentage of established colonies with sporogenic tissue was calculated as the percentage of all established colonies (not including the early aborted sporelings).

Fluorescence microscopy using Uvitex 2B or Calcofluor does not permit direct observations on haustoria. In order to check whether sporelings were arrested before haustorium formation, and whether resistance was associated with formation of papillae, we used chloral hydrate–trypan blue clearing and staining, followed by a wintergreen oil clearing (26). Two series (one at 20 to 25°C and one at 16°C) of the seedling test were sampled. Three leaf segments per line were collected 42 h after inoculation. The segments were fixed in ethanol/dichloro-ethane (1:3 vol/vol) with 0.15% trichloro-acetic acid for 24 h, boiled in 0.03% trypan blue in lactophenol/ethanol (1:2, vol/vol) for 10 min, and cleared in a nearly saturated aqueous solution of chloral hydrate (5:2, vol/vol) to remove trypan blue from the chloroplast membranes. Leaf segments were embedded in glycerol and systematically screened for infections. The position of each infection in relation to stoma and row number was recorded so that it was possible to re-examine it after further processing the leaf segments. The number of hyphal tips per colony, number of haustoria per colony, and relative maturity of each haustorium (young, medium, mature) were scored. Small spherical and dark staining haustoria were scored as young, large oblong and vacuolated haustoria were scored as mature. Twenty sporelings that had formed at least one haustorial mother cell were observed per line per series for their haustoria and possible presence of necrosis.

After haustoria were observed, the leaf segments were washed in chloral hydrate and then transferred through a series of alcohol solutions ranging from 85 to 100%. After immersion for 5 min in a

Table 1. Infection type (IT), latency period (LP), and infection frequency (IF) of wheat line Thatcher, its isolines Thatcher-*Lr12*, Thatcher-*Lr13*, and Thatcher-*Lr34*, the susceptible wheat line Morocco, and the partially resistant wheats Akabozu and BH1146 to *Puccinia recondita* f.sp. *tritici* race Flamingo at the seedling and adult plant stage[v]

	Seedling									Adult plant		
	25°C			16°C			8°C			25°		
Lines	IT	LP[w]	IF[w]	IT	LP	IF	IT	LP	IF	IT	LP	IF
Morocco	9	103 cd[x]	95 ab	9	106 b	85 abc	9	103 b	82 ab	9	74 c	288 a
Thatcher	9	100 d (*137*)[w]	100ab (*32*)[w]	9	100 b (*208*)[w]	100 ab (*85*)[w]	9	100 b (*278*)[w]	100 a (*28*)[w]	8	100 b (*221*)[w]	100 cd (*11*)[w]
Thatcher-*Lr12*	9	99 d	90 ab	9	99 b	102 ab	9	94 b	83 ab	7 to 8	101 b	100 cd
Thatcher-*Lr13*	7 to 8	100 d	103 a	7 to 8	101 b	105 a	7 to 8	99 b	98 a	1(9)/8[y]	106 ab	4 e
Thatcher-*Lr34*	9	109 bc	84 ab	9	136 a	67 bcd	9	140 a	61 bc	1(9) to 9/9[z]	123 a	21 de
Akabozu	9	121 a	84 ab	9	142 a	52 cd	9	154 a	42 c	9	112 ab	186 b
BH1146	9	112 b	66 b	9	148 a	42 d	9	150 a	34 c	9	115 ab	134 bc

[v] On the basis of six, three, two, and four series respectively

[w] LP and IF are expressed as values relative to Thatcher (= 100%). The actual average values for Thatcher are presented in parentheses: for LP in hours, for IF in number of pustules per cm^2.

[x] Numbers within a column followed by a letter in common are not significantly different (least significant difference, $P = 0.05$).

[y] Chlorotic flecks and few susceptible-type pustules in the flag leaf, but showing susceptible-type pustules in lower leaves.

[z] Pale flecks and few susceptible-type pustules in the flag leaf, but showing susceptible-type pustules in lower leaves.

saturated solution of picric acid in wintergreen oil (methyl salicylate) the segments were cleared and mounted in plain wintergreen oil (26). The number of haustoria per sporeling as determined after the trypan blue staining was checked on the same sporelings. Per sporeling the number of bright cell wall appositions in contact with hyphal tips or haustorial mother cells were counted. All observations were carried out using a phase contrast microscope (Nikon, Optiphot) at 1,000× magnification.

RESULTS

Infection type. All lines studied showed a high IT in the seedling stage (Table 1). On Thatcher-*Lr13* the IT was slightly lower than on the other lines. There was no effect of temperature on the IT.

Lr13 and *Lr34* decreased the infection type in adult plants (Table 1). On Thatcher-*Lr13* the flag leaf displayed chlorotic flecks and very few pustules that were not associated with necrosis (IT1(9)), while in the lower leaves no flecks and more pustules of high IT were present. The reaction of Thatcher-*Lr34* was similar (IT1(9)-9), but the flecks were pale rather than yellow as is the case in susceptible lines before the maturation of the uredia.

Latency period. The absolute latency period was longer at low temperatures than at high temperatures and longer on adult plants than on seedlings (Table 1). In both plant stages and at all temperatures the latency period on Thatcher-*Lr34* was significantly longer than on Thatcher, and similar to that on the partially resistant Akabozu and BH1146 (Table 1). At the adult plant stage the latency period on flag leaves of Thatcher-*Lr34* was difficult but not impossible to assess due to low infection frequency. The effect of *Lr34* and partial resistance on latency period was especially large at lower temperature and at the adult plant stage. We found no significant differences between Thatcher, Thatcher-*Lr12,* and Thatcher-*Lr13* for latency period in any of the plant stages or temperature regimes (Table 1). In the seedling stage, Thatcher showed at least as short a latency period as the susceptible Morocco. In the adult plant stage, however, the latency period on Thatcher, Thatcher-*Lr12,* and Thatcher-*Lr13* was significantly longer than on Morocco.

Infection frequency. The absolute infection frequency was higher on the seedlings at 16°C than on those at 25 and 8°C. We presume that this was the effect of inadvertent differences in inoculum quality or quantity applied. There were no significant differences for infection frequency (Table 1) among lines in the seedling stage at 25°C. At 8°C the infection frequency on seedlings of Thatcher-*Lr34* was significantly lower than on Thatcher and similar to that on the partially resistant lines.

In the adult plant stage Thatcher-*Lr34* and Thatcher-*Lr13* had a lower infection frequency than Thatcher, Akabozu, and BH1146. Thatcher and the Thatcher isolines had a lower infection frequency than Morocco.

Analysis of fungus development and host response. Apart from an insignificant proportion (less than 0.1%) of sporelings failing to penetrate stomata after appressorium formation, the sporelings could be classified as early aborted or established. The early aborted sporelings of *P. r.* f. sp. *tritici* had usually only one or two haustorial mother cells. At the time of sampling, the established colonies (usually with dozens of haustorial mother cells) in susceptible plants would often have started to form sporogenic tissue and urediospores (Table 2). Necrotic plant cells could be associated with both early aborted and established colonies.

Seedlings. In seedlings of Thatcher-*Lr34* we found a significantly higher percentage of early aborted sporelings than in Thatcher, and the established colonies were smaller than those in Thatcher. Necrosis was not frequently observed in Thatcher-*Lr34*. Thatcher-*Lr34* did not differ significantly from the partially resistant Akabozu and BH1146 in any of the aspects. In the seedling stage there was no significant difference between Thatcher and Morocco for any of the developmental stages that were measured. In Thatcher-*Lr13* the incidence of necrosis associated with established colonies was higher than in Thatcher.

Adult plants. Differences among the wheat lines in early abortion were more pronounced in adult plants than in seedlings. There was more early abortion in Thatcher-*Lr34* than in Thatcher. Thatcher-*Lr34* differed from Thatcher-*Lr13* by the lower rate of necrosis associated with early aborted and established sporelings. Early abortion in Thatcher, Thatcher-*Lr12,* and Thatcher-*Lr13* was significantly higher than in the susceptible check Morocco. Especially in Thatcher, Thatcher-*Lr12,* and Thatcher-*Lr13*, the early aborted sporelings were frequently (37 to 71%) associated with plant cell necrosis. In Thatcher-*Lr34*, Akabozu, BH1146, and Morocco necrosis appeared to be less common.

The average colony size 5 days after inoculation in Thatcher-*Lr34* was significantly smaller than in Thatcher, Thatcher-*Lr12,* and Morocco. The colony size was similar to that in the partially resistant Akabozu and BH1146. Compared with Morocco, established colonies in the other lines were less advanced in their development (formation of sporogenic tissue) and growth (colony size). Thatcher-*Lr13* gave an average colony size intermediate between that in Thatcher and the partially resistant lines.

Haustorium formation. Data on haustorium and papilla formation are presented in Table 3. The results obtained at 20 to 25°C and at 16°C showed similar differences among the lines. We therefore present the averages for the two series. At the time of sampling, 42 h after inoculation, sporelings in Thatcher-*Lr34,* Akabozu, and BH1146 had developed fewer haustoria and fewer hyphal tips per sporeling than sporelings in Thatcher and in Mo-

Table 2. Analysis of development of *Puccinia recondita* f. sp. *tritici* in Thatcher wheat isolines and susceptible and partially resistant wheat lines at 5 days after inoculation

	Seedling[v]					Adult plant[w]				
	Early abortion[x]		Established colonies			Early abortion[x]		Established colonies		
Lines	(%)	With necrosis (%)	With necrosis (%)[y]	With sporogenic tissue (%)[y]	Colony size (µm)	(%)	With necrosis (%)	With necrosis (%)[y]	With sporogenic tissue (%)[y]	Colony size (µm)
Morocco	5 bc[z]	19 a	6 ab	55 a	593 a	3 c	1 c	4 e	46 a	431 a
Thatcher	3 c	9 ab	1 b	65 a	591 a	22 b	37 abc	68 bc	15 ab	247 b
Thatcher-*Lr12*	3 c	0 b	7 ab	54 a	589 a	23 b	46 ab	92 ab	7 b	240 b
Thatcher-*Lr13*	5 abc	9 ab	17 a	58 a	625 a	31 ab	71 a	98 a	2 b	200 bc
Thatcher-*Lr34*	9 ab	5 ab	1 b	47 a	445 b	48 a	2 bc	42 cd	0 b	146 c
Akabozu	11 a	7 ab	2 b	29 a	447 b	32 ab	25 bc	27 de	11 b	158 c
BH1146	11 a	1 b	4 b	30 a	430 b	39 ab	29 abc	27 cde	1 b	195 bc

[v] Average of five series, three primary seedling leaves per series.
[w] Average of four series, three flag leaves per series.
[x] Sporelings that formed a primary infection hypha and not more than six haustorial mother cells.
[y] Calculated as the percentage of all established colonies (not including the early aborted sporelings).
[z] Numbers within a column followed by a letter in common are not significantly different (least significant difference, $P = 0.05$).

rocco. In Thatcher-*Lr12* and Thatcher-*Lr13* haustorium formation was similar to that in Thatcher.

The relatively poor haustorium formation in Akabozu and BH1146 was associated with frequent papilla formation (in 43 to 48 % of the sporelings). In Thatcher-*Lr34* papilla formation was not more frequent than in Thatcher.

At 42 h after inoculation a lower proportion of mature haustoria was found in Akabozu and BH1146 than in the other lines. Thatcher-*Lr34* was similar to Thatcher. Necrosis of cells was rarely observed in any of the lines.

DISCUSSION

All studies on the effect of *Lr34* are performed by comparing Thatcher-*Lr34* with the recurrent parent Thatcher. In the seedling stage Thatcher was at least as susceptible as Morocco, but in the adult plant stage Thatcher was significantly more resistant in most respects (Tables 1, 2). This indicates that Thatcher has a certain level of adult plant resistance. Although the reduction in IT was slight (from 9 to 8), the adult plant resistance of Thatcher seems to be based on hypersensitivity, since sporelings were frequently associated with necrosis (Table 2). Thatcher has been reported to possess the gene *Lr22b* for adult plant resistance (10), which should confer low infection type (10,32) to avirulent races. Our wheat leaf rust isolate Flamingo produced IT8. It may be that Flamingo carries virulence to *Lr22b* (2). In that case the moderate resistance observed in Thatcher in the present experiment may be a residual effect of the *Lr22b* gene or the effect of additional minor gene(s) for adult plant resistance. This implies that probably the *Lr34* in Thatcher-*Lr34* is not in a completely susceptible genetic background.

Reported infection types caused by *Lr34* vary widely. The infection types are expressed on a 0 to 4 scale. The gene has been reported to cause IT 2+ to 3+ in the seedling stage (8,11,12). Singh and Gupta (39) reported that, in the seedling stage, IT varied among tests from ; to 3+ depending on leaf age, temperature, and other environmental factors. On adult plants of Thatcher-*Lr34* the following infection types have been reported: 0;12– (17), 1++ to 3++ depending on temperature (7); 0 to 3+ depending on the race (possibly due to other genes than *Lr34)* (35). Low temperature and low light intensity resulted in lower IT (7,14,36,37).

Drijepondt and Pretorius (6) stated that "*Lr34* is expressed by infection type 2+, but without accompanying chlorosis" and they observed that the low reaction type caused by *Lr34* in adult plants "did not resemble hypersensitivity." Also, Dyck (9) observed that in the seedlings with IT2+ there was no real chlorosis. These remarks on the absence of chlorosis are in agreement with our observation. We have the strong impression, supported by the data in Table 2, that the pale flecks are caused by retarded, or even arrested, small, established colonies that are not frequently associated with necrosis, and hence should not be considered to indicate hypersensitivity. It may be that at least part of the reports on low infection type caused by *Lr34* is based on interpretation of pale flecks as being chlorotic. We recommend that in descriptions of infection types possible pale flecks should be mentioned separately rather than lumped with chlorotic flecks.

Our results indicate that the effect of *Lr34* shares features both with *Lr13* and with the genes for partial resistance in Akabozu and BH1146. Both *Lr34* and *Lr13* were more effective in the flag leaf than in the leaves under the flag leaf, which agrees with the observations by Drijepondt et al. (8) on *Lr34*. Both genes increased the adult plant resistance in Thatcher (Table 1) (11,15). For *Lr34*, however, there was also a modest but significant effect in the seedling stage on latency period (at all three temperatures) (Table 1), on infection frequency (at 8°C) (Table 1), and on colony size (Table 2). The main difference, however, is, that *Lr13* was associated with a hypersensitive reaction, whereas *Lr34* was not (Table 2). Broers (2) reported isolate Flamingo to be virulent to *Lr13*, but since he determined the virulence factors on seedlings, he may not have been able to judge the effectiveness of *Lr13*. Our results indicate that isolate Flamingo is avirulent to *Lr13*.

Also *Lr12* has been reported to cause adult plant resistance (31). However, in none of the aspects the differences between Thatcher-*Lr12* and Thatcher were statistically significant. This indicates that the wheat leaf rust isolate was virulent to *Lr12*.

We also compared *Lr34* with the partial resistance in Akabozu and BH1146. Partial resistance was defined by Parlevliet (28) as a type of resistance that retards epidemic development in the field, although plants show a compatible (nonhypersensitive) infection type. The slow epidemic build-up by the fungus on partially resistant cereals can be attributed to the combined effect of several components, including a long latency period and a low infection frequency (29,30).

The effect of *Lr34* resembled that of the partial resistance genes in Akabozu and BH1146 in the increased effectiveness at low temperatures and in the adult plant stage (Tables 1, 2), the absence of hypersensitivity (Table 2), the reduction of haustorium formation (Table 3), the long latency period and low infection frequency (Table 1), high early abortion in the adult plants, and small colony size (Table 2). Such effects were reported for partial resistance by Broers, Jacobs, and co-workers (1–4,18,19), and by Lee and Shaner (21), although the latter did not find early aborted colonies. The small colonies in their slow leaf-rusting cultivars were interpreted as not aborted, but still continuing their development (21). Such an explanation does not apply to our material. We collected the leaf segments 5 days after inoculation, and the early aborted sporelings had formed only one or two haustorial mother cells (HMCs) by then.

In the present study, the infection frequency on flag leaves of the partially resistant lines Akabozu and BH1146 was significantly lower than on those of Morocco, but not lower than on those of Thatcher (Table 1). The lack of significant difference with Thatcher may be ascribed to the adult plant resistance in Thatcher, discussed above.

The effect of *Lr34* on the leaf rust fungus differed from that of the combined minor genes in Akabozu and BH1146 by the stronger reduction of the infection frequency in the flag leaf (Table 1). It caused a much higher proportion of pale flecks than found in the partially resistant lines Akabozu and BH1146. We argue that, since these flecks do not differ fundamen-

Table 3. Haustorium formation by *Puccinia recondita* f.sp. *tritici* on seedlings of Thatcher wheat isolines and susceptible and partially resistant lines[u]

Lines	Haustoria per sporeling[v]	Maturity of haustoria[w]	Sporelings without haustoria (%)	Hyphal tips per sporeling[x]	Sporelings with ≥1 papilla (%)[y]
Morocco	1.31 a[z]	52:37:11	18	4.7 ab	4
Thatcher	1.61 a	46:31:23	5	5.2 a	12
Thatcher-*Lr12*	1.36 a	53:32:15	11	4.4 abc	5
Thatcher-*Lr13*	1.37 a	52:21:27	14	4.1 bc	18
Thatcher-*Lr34*	0.96 b	56:34:10	27	3.6 cd	8
Akabozu	0.89 bc	33:46:21	29	3.5 cd	48
BH1146	0.55 c	35:60:5	51	2.7 d	43

[u] Average of two series harvested 42 h after inoculation; series 1 incubated at 20 to 25°C and series 2 at 16°C.
[v] For haustoria/sporeling: no significant series effect; no series × line effect.
[w] Percentage of the total number of haustoria mature:medium:young.
[x] For hyphal tips/sporeling: significant series effect and series × line effect.
[y] Data available only for the second series (16°C).
[z] Numbers within a column followed by a letter in common are not significantly different (least significant difference, $P = 0.05$).

tally from the flecks that are found on susceptible lines before the end of the latency period, such flecks do not indicate incompatibility. A further difference with partial resistance was that the reduction of the haustorium formation in Thatcher-*Lr34* appeared to be due to a lower intercellular hyphal development and not to papillae formation as in Akabozu and BH1146 (Table 3) (18).

Broers and Jacobs (3) suggested that the resistance of BH1146 may be due to *Lr13* and several additional genes that prolong the latency period. Roelfs et al. (32) assumed a combination of *Lr13* and *Lr34* in this line. Our data seem at variance with these suggestions. In the Thatcher isolines *Lr13* and *Lr34* both decrease infection frequency in adult plants much more strongly on the flag leaf than on the lower leaves. This phenomenon was not observed in BH1146, which had a rather high infection frequency on the flag leaf (Table 1). In addition, *Lr13* caused substantial plant cell necrosis (Table 2), which was not observed in BH1146. Although we acknowledge that modifying genes in the genetic background may play a role, we conclude that the differences between the Thatcher isolines and BH1146 make it questionable whether *Lr13* (and probably also *Lr34*) are present in BH1146.

In our opinion, the resistance caused by *Lr34* fits Parlevliet's definition of partial resistance (28). As a consequence, *Lr34* demonstrates that partial resistance in wheat to leaf rust may be the consequence of a single major gene (9,11). It has often been suggested that durability of resistance can be increased by combining genes that govern different mechanisms of defense (5,16). We suggest that in the wheat cultivars that contain both *Lr12* or *Lr13* and *Lr34* (31) two different resistance mechanisms have been brought together, viz., a hypersensitive and a nonhypersensitive. This may contribute to the claimed durability of the resistance in these cultivars.

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