Greenhouse Evaluation of Adult-Plant Resistance Conferred by the Gene *Lr*34 to Leaf Rust of Wheat

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ABSTRACT

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The latent period of *Puccinia recondita* f. sp. *tritici* in flag leaves of line RL6058 (with gene *Lr*34) was most extended at greenhouse temperatures of 13–17 C compared with that in the leaf-rust-susceptible wheat cultivar Thatcher. A day/night regime of 25–29/13–15 C also increased latent period in RL6058, but no such differences between RL6058 and Thatcher were evident at continuous 26–30 C. Uredinia per square centimeter of flag leaf surface were fewer on RL6058 than on Thatcher. Temperature did not influence the expression of this character in RL6058, but more uredinia developed on young flag leaves than on older ones. The size of uredinia on flag leaves depended on the ambient greenhouse temperature and was more restricted at 25–29/13–15 C and continuous 13–17 C than at 26–30 C. Because of the assumed durability of *Lr*34 and its ability to interact with other genes to enhance the level of resistance, more deliberate exploitation of *Lr*34 could be justified. This study indicated that selection for *Lr*34 can be done in greenhouses with adequate temperature control.

Additional keywords: resistance components

The gene *Lr*34 for resistance to leaf rust, caused by *Puccinia recondita* Rob. ex Desm. f. sp. *tritici*, occurs in many wheat (*Triticum aestivum* L.) genotypes of diverse origin (5,6,8,19). The original source of *Lr*34 may have been Alfredo Chaves, a land cultivar found in Brazil in about 1921, or Americano 44D, a selection from a land cultivar in Uruguay in 1918 (17). *Lr*34 is also present in the cultivar Frontana, which has often been used in wheat breeding programs (7). *Lr*34 is located on chromosome 7D (13) and was inherited as a partially dominant gene in a cross between Chinese wheat accession PI 58548 and the wheat cultivar Thatcher (5). Initially, Dyck (5) referred to *Lr*34 as a gene characterized by a 2+ seedling infection type. Subsequently, the temporary gene symbol *Lr*T2 was assigned (7), and more recently, gene *Lr*T2 has been designated *Lr*34 (6).

In wheat seedlings, the resistance conferred by *Lr*34 is expressed by infection type 2+, but without accompanying chlorosis (5,7). Furthermore, and most significantly, *Lr*34 conditions high levels of adult-plant resistance in the field (6,7,14). *Lr*34 also interacts with other genes for resistance such as *Lr*T3 and *Lr*33 to enhance levels of resistance to leaf rust (6,18). The potential of *Lr*34 is further substantiated by its apparent durability. Roelfs (17) surmised that within *T. aestivum*, durable resistance to leaf rust is most probably associated with the gene combinations *Lr*13 + *Lr*34 and *Lr*12 + *Lr*34. In wheat seedlings, however, expression of *Lr*34 has been reported as specific sensu Browder (2) to temperature, light, and the genetic constitution of both host and pathogen (7). Knowledge regarding such specificity is extremely important in establishing selection criteria of genes for resistance in breeding programs.

Exactly how long the adult-plant resistance of *Lr*34 has been effective or whether races virulent to this gene exist has not been established. However, the gene's diverse occurrence (19) suggests it might be durable sensu Johnson (10). Within bread wheat, few effective *Lr* genes remain for exploitation in resistance breeding programs (19), and a more detailed description of *Lr*34 resistance in a greenhouse environment may enhance its future management. This paper describes the components of adult-plant resistance due to *Lr*34 in different temperature environments.

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MATERIALS AND METHODS

Germinated seeds of line RL6058 (Thatcher*6/PI58548 [*Lr*34]) and Thatcher (susceptible control) were planted in 18-cm plastic pots (two plants per pot), containing 4 kg of soil, in a greenhouse set at 19–23 C. Daylight was supplemented with light from cool-white fluorescent tubes at 900 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 12 hr each day. Three plantings, 21 days apart, were made. Fourteen days after each planting, plants were fertilized with a soil drench of 0.5 g of urea (46% N) and 0.8 g of superphosphate (10.5% P) per pot.

First inoculation. Seventy-one days after the first planting, and 49 days after the second, the adaxial surfaces of flag leaves were quantitatively inoculated with a suspension of 0.4 mg of fresh urediniospores of isolate 3SA132 of *P. r.* f. sp. *tritici* per milliliter of lightweight mineral oil (Soltrol 130), using an Andres inoculation device (1). Isolate 3SA132 is representative of the most common race of *P. r.* f. sp. *tritici* in South Africa and is avirulent to the genes *Lr*3a, 3bg, 3ka, 11, 16, 20, 26, and 30 and virulent to *Lr*1, 2a, 2b, 2c, 10, 14a, 15, 17, and 24. Three sections of adhesive tape were inoculated in a similar manner to determine the number of spores deposited per square centimeter. The 71-day-old RL6058 and Thatcher plants were at Zadoks growth stages 59 (emergence of ear completed) and 71 (kernel water ripe), respectively (22). The 49-day-old RL6058 and Thatcher plants were at growth stages 45 (boots swollen) and 59, respectively.

After incubation in a dew chamber for 16 hr (15), a set of plants representative

of the described genotypes and growth stages was placed in each of three greenhouse compartments at 26–30 C, 13–17 C, and a 12-hr 25–29/13–15 C day/night regime. In each compartment, cool-white fluorescent tubes arranged above the plants emitted 900 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 12 hr daily.

Second inoculation. Flag leaves of 57-day-old RL6058 and Thatcher plants from the third planting were inoculated according to the procedures described above. At the time of inoculation, RL6058 and Thatcher plants were at growth stages 55 (half of ear emerged) and 61 (beginning of flowering). After the incubation period, plants were maintained in a greenhouse at 13–17 C with supplementary daylight illumination as mentioned above.

Latent period, number of uredinia per square centimeter of flag leaf surface, and uredinium size were determined as described elsewhere (15). Latent period and number of uredinia were determined on the flag leaf of the main tiller of 10 plants per wheat genotype at a specified growth stage per temperature. Uredinium size was calculated by measuring the diameters of 10 randomly selected uredinia on each of four inoculated flag leaves per treatment.

Both experiments were arranged as completely randomized designs, and data were analyzed for variance accordingly. Tukey's HSD procedure for comparison of means was applied where analyses of variance showed significant variation.

Table 1. Effect of temperature and two adult-plant growth stages on latent period on flag leaves of wheat line RL6058 (with gene *Lr* 34) and wheat cultivar Thatcher (susceptible control) infected with isolate 3SA132 of *Puccinia recondita* f. sp. *tritici*

	Latent period (hr)[y]					
	RL6058			Thatcher		
Temperature regime (C)	GS 59[z]	GS 45	Mean	GS 71	GS 59	Mean
26–30	213 efg	195 efg	204 C	192 fg	191 fg	192 C
25–29/13–15	243 cd	222 de	233 B	216 def	187 g	202 C
13–17	304 a	271 b	288 A	264 bc	205 efg	235 B
Mean	253	229		224	194	

[y] Values followed by different lowercase letters and means followed by different uppercase letters differ significantly at $P < 0.05$ according to Tukey's HSD procedure.
[z] GS = growth stages according to Zadoks scale (22): 45, boots swollen; 59, emergence of ear completed; 71, kernel water ripe.

Table 2. Effect of temperature and two adult-plant growth stages on number of uredinia of isolate 3SA132 of *Puccinia recondita* f. sp. *tritici* per square centimeter of flag leaf surface of wheat line RL6058 (with gene *Lr* 34) and wheat cultivar Thatcher (susceptible control)

	Number of uredinia/cm^2 of flag leaf surface[y]					
	RL6058			Thatcher		
Temperature regime (C)	GS 59[z]	GS 45	Mean	GS 71	GS 59	Mean
26–30	3.2 c	6.9 bc	5.1	11.6 a	10.4 ab	11.0
25–29/13–15	4.7 c	6.0 c	5.4	13.8 a	12.4 a	13.1
13–17	4.3 c	11.9 a	8.1	12.4 a	13.3 a	12.9
Mean	4.1 C	8.3 B		12.6 A	12.0 A	

[y] Values followed by different lowercase letters and means followed by different uppercase letters differ significantly at $P < 0.05$ according to Tukey's HSD procedures.
[z] GS = growth stages according to Zadoks scale (22): 45, boots swollen; 59, emergence of ear completed; 71, kernel water ripe.

Table 3. Effect of temperature and two adult-plant growth stages on size of uredinia produced by isolate 3SA132 of *Puccinia recondita* f. sp. *tritici* on flag leaves of wheat line RL6058 (with gene *Lr*34) and wheat cultivar Thatcher (susceptible control)

	Uredinium size (mm^2)[y]					
	RL6058			Thatcher		
Temperature regime (C)	GS 59[z]	GS 45	Mean	GS 71	GS 59	Mean
26–30	0.367 abcd	0.325 abcd	0.346 A	0.564 d	0.249 ab	0.407 AB
25–29/13–15	0.274 ab	0.278 ab	0.276 A	0.484 abcd	0.239 a	0.362 A
13–17	0.279 ab	0.299 abc	0.289 A	0.558 cd	0.506 bcd	0.532 B
Mean	0.307 A	0.301 A		0.535 B	0.331 A	

[y] Values followed by different lowercase letters and means followed by different uppercase letters differ significantly at $P < 0.05$ according to Tukey's HSD procedures.
[z] GS = growth stages according to Zadoks scale (22): 45, boots swollen; 59, emergence of ear completed; 71, kernel water ripe.

RESULTS

First inoculation. Latent period varied significantly because of host genotype, growth stage, and temperature and their interactions, except for the interaction between cultivar and growth stage (Table 1). Latent periods in RL6058 at growth stages 45 and 59 and in Thatcher at growth stage 71 was most extended at 13–17 C. These latent periods were significantly longer than that in Thatcher plants (growth stage 59) at 13–17 C. At the day/night regime of 25–29/13–15 C, no significant differences between latent period in RL6058 at both growth stages and in Thatcher at stage 71 were indicated. The latent period in Thatcher at growth stage 59, however, was significantly shorter. At 26–30 C, the latent periods determined in all treatments were statistically similar.

The effects of temperature and adult-plant growth stage on the number of uredinia per square centimeter of flag leaf surface of RL6058 and Thatcher are presented in Table 2. Microscopic evaluation of 12 units of 1 × 1 cm on the three sections of adhesive tape showed that the inoculation device deposited 25.7 ± 7.2 spores per square centimeter. The mean area studied per leaf was 9.83 cm^2. Uredinium density varied significantly because of host genotype, growth stage, temperature, and all interactions except for temperature × cultivar and temperature × growth stage × host genotype. On RL6058 plants at growth stage 45 at 13–17 C, 11.9 uredinia developed per square centimeter, compared with 3.2–6.9 uredinia per square centimeter for the other growth stage × temperature interactions. No significant differences were evident for numbers of uredinia on Thatcher leaves. Means indicated that more uredinia developed on Thatcher than on RL6058.

Except for temperature and its interaction with growth stage, all other factors were significant sources of variation in uredinium size (Table 3). According to the means for growth stages, uredinia on leaves of RL6058 at both developmental stages were similar in magnitude to those on leaves of Thatcher at stage 59 but were smaller than those on Thatcher at stage 71. Temperature did not significantly affect the size of uredinia on RL6058 leaves. At the time of evaluation, higher temperature restricted development of uredinia on leaves of Thatcher plants inoculated at growth stage 59. Uredinia on Thatcher plants at 13–17 C were larger than those on Thatcher plants kept at the other temperatures and also larger than those on RL6058 at 13–17 C.

Second inoculation. Significant differences for latent period (hours), number of uredinia per square centimeter of flag leaf area, and uredinium size (square millimeter) between the *Lr*34

genotype and Thatcher were evident (Table 4). In RL6058, latent period was more prolonged and uredinia were fewer and smaller than in Thatcher.

Table 4. Latent period, number of uredinia per square centimeter. and uredinium size determined in the second inoculation of flag leaves of wheat line RL6058 (with gene *Lr*34) and wheat cultivar Thatcher (susceptible control) with isolate 3SA132 of *Puccinia recondita* f. sp. *tritici* at 13–17 C

Cultivar or line	Growth stage[y]	Latent period (hr)	Number of uredinia/cm^2	Uredinium size (mm^2)
RL6058	55	297 a[z]	3.4 a	0.197 a
Thatcher	61	233 b	10.7 b	0.344 b

[y] According to Zadoks scale (22): 55, half of ear emerged; 61, beginning of flowering.
[z] Column values followed by different letters differ significantly at $P < 0.05$ according to Tukey's HSD procedure.

DISCUSSION

In cereal rust host-pathogen systems, an increase in temperature generally shortens latent period (9,11,20,21). Although the latent period of the *P. graminis* f. sp. *avenae*/oat system was also decreased at higher temperatures, that of the *P. coronata*/oat system was shorter at 20–25 C than at 30–35 C (12). In the *P. r.* f. sp. *tritici*/wheat system, a prolonged latent period, mediated by low temperature, has been associated specifically with genes *Lr*18 (21) and *Lr*22a (15). Furthermore, Browder and Eversmeyer (4) demonstrated that the slow-rusting characteristic of the cultivar Suwon 85 could be qualitatively identified in seedlings at 5 or 12 C but not at 19 or 26 C. Exposure to different postinoculation temperature regimes has also been shown to be important in the expression of resistance conditioned by *Lr*1, *Lr*16, and *Lr*17 (3).

Our study showed that the latent period in RL6058 was significantly extended at temperatures maintained in the range of 13–17 C. At temperatures in the range of 26–30 C, no significant differences in latent period between RL6058 and Thatcher were evident. In the greenhouse, a diurnal 25–29/13–15 C temperature regime allowed some distinction of long latent period mediated by *Lr*34. It is apparent, therefore, that under natural diurnal temperature fluctuations, latent period effects induced by low temperatures, *Lr*34, and a corresponding gene for avirulence may largely be negated by higher temperatures.

The number of uredinia that developed on flag leaves was lower on RL6058 than on Thatcher and did not seem as temperature-dependent as the other rust-reducing components. The expression of this character in RL6058 was greater in young leaves than in older ones, however, and caution should be exercised when selecting for *Lr*34 on a basis of reduced uredinium density on young, recently emerged flag leaves. The age of leaf tissue has previously been reported to influence the expression of adult-plant resistance to leaf rust (15,16).

The size of uredinia on flag leaves is an important component of adult-plant resistance in wheat to *P. r.* f. sp. *tritici* (15). Moreover, the study by Pretorius et al (15) showed that the size of uredinia on RL6044 was markedly reduced by maintaining inoculated plants at 15 C rather than 21 C. Our data, especially from the second inoculation, showed that uredinium size can be effectively used as a criterion of *Lr*34 resistance. Optimum expression of this character is enhanced by low temperatures. In the greenhouse, uredinia on flag leaves of RL6058 were not associated with chlorosis or necrosis, and, phenotypically, the low reaction of *Lr*34 did not resemble hypersensitivity.

The effect of inoculum density on *Lr*34 resistance should be investigated. At 26–30 C, the number of uredinia was the only component that identified *Lr*34. It can be concluded, therefore, that if high inoculum concentrations are applied in qualitative evaluations in greenhouses without sufficient temperature control, the expression of *Lr*34 would most probably be completely masked. Conversely, less precise inoculation methods should allow identification of *Lr*34, provided facilities with adequate temperature control are available. The delayed appearance of pustules of small dimensions should clearly distinguish a *Lr*34 genotype from a susceptible genotype at one point in time.

Because *Lr*34 allows the expression of other genes more fully, Dyck (5) suggested that the widespread occurrence of *Lr34* may be partly ascribed to inadvertent selection. In addition to its complementary abilities, *Lr*34 alone confers high levels of resistance to leaf rust in the field (6,14; Drijepondt, *unpublished*). More deliberate exploitation of *Lr*34 in wheat cultivar development appears to be warranted. Our data support the hypothesis of Browder (2) that parasite-host genotypes are specific to the environment and also extend the information currently available on the adult-plant expression of a potentially useful leaf rust resistance gene.

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