Mechanisms Associated with Wheat Leaf Rust Resistance Derived from *Triticum monococcum*

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Accepted for publication 22 January 1996.

ABSTRACT

Jacobs, A. S., Pretorius, Z. A., Kloppers, F. J., and Cox, T. S. 1996. Mechanisms associated with wheat leaf rust resistance derived from *Triticum monococcum*. Phytopathology 86:588-595.

The infection of wheat (*Triticum aestivum*) line KS93U9 (Karl*3//PI 266844/PI 355520) with *Puccinia recondita* f. sp. *tritici* was studied to determine histological mechanisms of resistance. The development of fungal structures was studied at two host growth stages in KS93U9, its leaf rust-resistant *T. monococcum* parent lines PI 266844 and PI 355520, and its susceptible recurrent parent 'Karl'. *T. monococcum* accession Tm2126/5, previously reported to exhibit prehaustorial resistance to *P. recondita* f. sp. *tritici*, was included in the study. Using fluorescence microscopy, infection sites on leaf sections infected with pathotype UVPrt9 and stained with Uvitex 2B were examined for, respectively, the percentage of prestomatal exclusion of the fungus (germ tubes not forming appressoria and appressoria not forming over stomata), aborted penetration (nonpenetrating appressoria and aborted substomatal vesicles), early abortion (less than six haustorium mother cells per infection site), and successfully established colonies. In general, the resistant lines responded similarly for prestomatal exclusion and aborted penetration, but differences in early abortion and colony formation were observed. In seedlings, prestomatal exclusion could be attributed to the inability of fungal germ tubes to produce appressoria, whereas the formation of nonstomatal appressoria occurred more commonly in adult plants. At both growth stages, most aborted penetration attempts were accounted for by arrested substomatal vesicles rather than nonpenetrating appressoria. All infection sites displaying early abortion in KS93U9 were associated with host cell necrosis, whereas no hypersensitivity was observed in primary leaves of Tm2126/5. In adult Tm2126/5 plants, however, a large proportion of the infection sites exhibited hypersensitivity. Similarly, infection sites in PI 266844 and PI 355520 were frequently accompanied by necrotic leaf tissue. Staining of the leaves with trypan blue and a saturated solution of picric acid in methyl salicylate, and viewing with phase contrast microscopy, showed that papillae commonly occurred at infection sites in the *T. monococcum* lines, but not in KS93U9. Expression of *T. monococcum*-derived resistance in KS93U9 was not different from hypersensitive reactions typically associated with existing sources of major gene resistance to wheat leaf rust. Furthermore, components of resistance indicated, that at a histological level, mechanisms of resistance in the hexaploid KS93U9 background were altered when compared with parent lines.

Additional keywords: histology, infection structures.

Germ plasm sources of diverse genetic origins are important in the improvement of crop plants through breeding. Since cultivated plant species often lack sufficient variation, related species are frequently used for the introgression of desired traits (1,10). Named genes for resistance to leaf rust, caused by *Puccinia recondita* Rob. ex Desm. f. sp. *tritici*, have been transferred to wheat from *Triticum umbellulatum* (Zhuk.) Bowden (*Lr9*), *T. ventricosum* Ces., Pass., and Gib. (*Lr37*), *Thinopyrum ponticum* (Podp.) Barkworth & Dewey (*Lr19*, *Lr24*, and *Lr29*), *Thinopyrum intermedium* (Host.) Barkworth & Dewey (*Lr38*), *T. speltoides* (Tausch) Gren. ex Richter (*Lr28*, *Lr35*, and *Lr36*), and *T. tauschii* (Coss.) Schmal. (*Lr21*, *Lr22a*, *Lr32*, *Lr39*, *Lr40*, and *Lr41*) (18). Although none of the currently listed (18) genes originated from *T. monococcum* L., this species has been reported as highly resistant to wheat leaf rust (4,17,20,22). Furthermore, a histological study of the infection process of *P. recondita* f. sp. *tritici* has indicated the onset of nonhypersensitive resistance mechanisms prior to haustorium formation (prehaustorial resistance) in certain *T. monococcum* accessions (17). Despite the apparent potential of *T. monococcum* as a source of resistance to wheat leaf rust, Johnson and Lupton (9) warned that an alien origin of resistance does not necessarily imply durability.

The Wheat Genetics Resource Center at Kansas State University has recently transferred leaf rust resistance from *T. monococcum* to bread wheat (*T. aestivum* L.). To ascertain whether the germ plasm developed in this program contains leaf rust resistance mechanisms similar to the ephemeral posthaustorial types, histological studies were conducted. Resistance expression in a hexaploid line derived from *T. monococcum* was compared with the diploid resistant and hexaploid susceptible parents, and with a *T. monococcum* accession previously shown to have prehaustorial resistance to leaf rust.

MATERIALS AND METHODS

Host genotypes. The expression of leaf rust resistance was studied in the bread wheat line KS93U9 (Karl*3//PI 266844/PI 355520) and the *T. monococcum* accessions PI 266844, PI 355520, and Tm2126/5. The hard red winter wheat cultivar Karl, used as the recurrent parent in KS93U9, was included as the leaf rust-susceptible control. Seeds of PI 266844 and PI 355520 were obtained from the U.S. Department of Agriculture, Agriculture Research Service, Small Grains Collection, Aberdeen, Idaho; and Tm2126/5 was provided by R. E. Niks, Wageningen Agricultural University, the Netherlands. Production of BC_1 seed from the crosses Karl*2//PI 266844/PI 355520 was described by Cox et al.

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Publication no. P-1996-0416-01R

(2). Briefly, F_1 plants between PI 355520 (which produces fertile hybrids with hexaploid wheat) and PI 266844 were used to pollinate emasculated spikes of 'Karl'. F_1 embryos were rescued (5) and 15 of 19 resulting F_1 plants were female-fertile. These were pollinated using 'Karl'. Resulting BC_1 plants were crossed as female plants to 'Karl', and selfed progeny of BC_2 plants were evaluated for leaf rust reaction in the field at Manhattan, Kansas, under heavy natural infection in 1991. Leaf rust-resistant F_3 progeny of one F_2 plant was selected and inoculated in the greenhouse with pathotype PRTUS25 of *P. recondita* f. sp. *tritici* to confirm seedling resistance. Resistant BC_2F_2-derived F_4 progenies were produced in the field in 1992. Seed from progenies of Karl*3//PI 266844/PI 355520 were bulked to form the germ plasm release KS92WGRC23 (3). In 1993, 50 individual progenies that had been bulked in KS92WGRC23 were evaluated in the field, and one was selected as KS93U9 on the basis of agronomic similarity to 'Karl'.

Inoculation and incubation. Approximately 12 seedlings of each of the experimental lines were grown in a steam-sterilized mixture of soil and peat moss in 10-cm-diameter plastic pots. Three replicate pots per treatment were included. Ten days after planting, primary leaves were taped to a vertically positioned board and inoculated by spraying exposed adaxial surfaces with freshly collected urediniospores of pathotype UVPrt9 of *P. recondita* f. sp. *tritici* suspended in light mineral oil (1 mg of spores/ml of oil). Seedlings were dried for 45 min in fan-circulated air before placement in the dark in a dew-simulation chamber at 21 to 22°C. Immediately after a 16-h dew period, plants were transferred to a controlled-environment cabinet in which 20°C was maintained. Cool-white fluorescent tubes and incandescent bulbs, arranged 50 cm above leaves, provided 200 $\mu mol/m^2/s$ of photosynthetic active radiation for 14 h each day. The experiment was repeated in a second, independent study, as well as with plants at a more advanced growth stage. In the latter study, the terminal leaves of 8-week-old plants, grown in a steam-sterilized soil/peat moss mixture in 1-liter-capacity pots, were inoculated with UVPrt9. Inoculation procedures and the length of the dew-chamber cycle were similar to that described previously. At the time of inoculation, KS93U9, 'Karl', PI 266844, and PI 355520 were between the decimal growth stages 30 to 33, and Tm2126/5 was at growth stage 41 (21). Upon termination of the dew-chamber period, plants were transferred to a greenhouse in which 18 to 25°C was maintained. Cool-white fluorescent tubes supplemented natural daylight with 14 h of 120 $\mu mol/m^2/s$ of photosynthetic active radiation each day.

Fluorescence microscopy. In the seedling experiment, three primary leaves of each genotype were randomly sampled from each of the three replicates 2 and 7 days postinoculation (dpi). Similarly, nine leaves were sampled from adult plants 7 dpi. Two-centimeter primary leaf sections and 1-cm adult-plant leaf sections were prepared for fluorescence microscopy (12,19). Uvitex 2B (Ciba-Geigy Corp., Basel, Switzerland) (17) was used as the fluorescent stain. Observations were carried out at ×100 or ×400 with a Nikon Labophot epifluorescence microscope (Nikon Corp., Tokyo, Japan), using the filter combinations UV-1A (330- to 380-nm excitation filter and 420-nm barrier filter) for fungal structures and B-2A (450- to 490-nm excitation filter and 520-nm barrier filter) for observations of plant cell necrosis. In the seedling investigations, 20 infection sites, defined by the formation of an appressorium over a stoma, on each of three leaf segments per line were studied. The only exception was for the percentage of prestomatal exclusion, calculated as the proportion of germ tubes not producing any appressoria and appressoria not forming over stomatal openings. For this parameter, all germinated spores on the entire surface of each leaf segment were scanned. Aborted penetration was determined as the percentage of infection sites in which appressoria did not penetrate a stomatal opening or infection structure development failed to proceed beyond the formation of substomatal vesicles (16). To obtain information on the mechanism of aborted penetration, the relative proportions of non-penetrating appressoria and aborted substomatal vesicles were noted. The number of infection sites displaying early abortion of infection structures was counted. An early-aborted infection site was designated by the observation of at least one, but not more than six, haustorium mother cells (14). The number of early-aborted infection sites with or without host cell necrosis was also recorded. Infection sites culminating in colonies (more than six haustorium mother cells) were quantified either with or without host cell necrosis. Colonies were then differentiated as either sporulating or nonsporulating. The number of haustorium mother cells per infection site was counted at ×100 magnification and confirmed at ×400 when necessary. When more than 30 haustorium mother cells were encountered, no further counts were made because of lack of accuracy. Dimensions of colonies, and of host cell necrosis associated with colonies, were measured with a calibrated eyepiece micrometer and corresponding areas (mm^2) calculated according to the formula: $\pi \times$ length $\times$ width/4. Coalescing colonies and those near the leaf edge were excluded from all measurements. A hypersensitivity index (11) was calculated by dividing the necrotic area associated with each infection site with the corresponding area of fungal colonization. Leaves sampled 2 dpi were evaluated only for the number of haustorium mother cells and colony size.

Phase contrast microscopy. Haustoria and cell wall appositions were studied in primary leaves only. The number of leaves and sampling procedures were similar to the preparation of materials for fluorescence microscopy. Leaf segments (2-cm long) collected 2 dpi were stained with trypan blue and a saturated so-

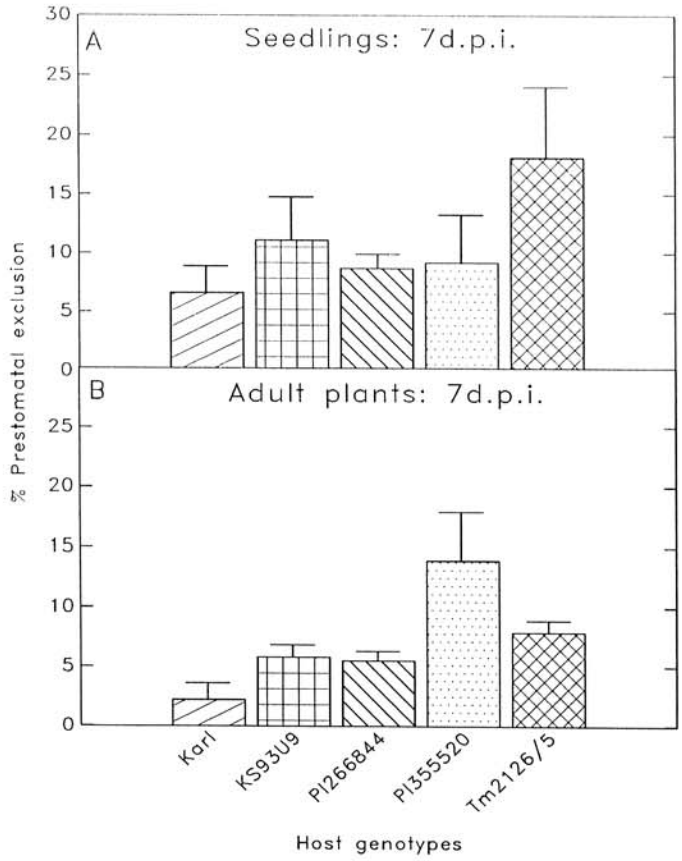

Fig. 1. Percentage of prestomatal exclusion of pathotype UVPrt9 of *Puccinia recondita* f. sp. *tritici* determined 7 days postinoculation (dpi) on adaxial leaf surfaces of **A,** seedlings and **B,** adult plants of the bread wheats 'Karl' (leaf rust-susceptible) and KS93U9 (leaf rust-resistant), and the *Triticum monococcum* lines PI 266844, PI 355520, and Tm2126/5 (leaf rust-resistant). Error bars represent standard deviations.

lution of picric acid in methyl salicylate to elucidate haustoria and cell wall appositions (15). Screenings of leaf sections were done using a Nikon Optiphot microscope (Nikon Inc.) at ×100, whereas detailed observations were made at ×1,000 (oil immersion). Ten randomly selected infection sites, but not close to the edges of leaves, were screened on each of nine leaf segments. The numbers of papillae and haustoria occurring at each infection site were recorded.

Experimental design and data analyses. The statistical program SOLO (BMDP Statistical Software, Inc., Los Angeles) was

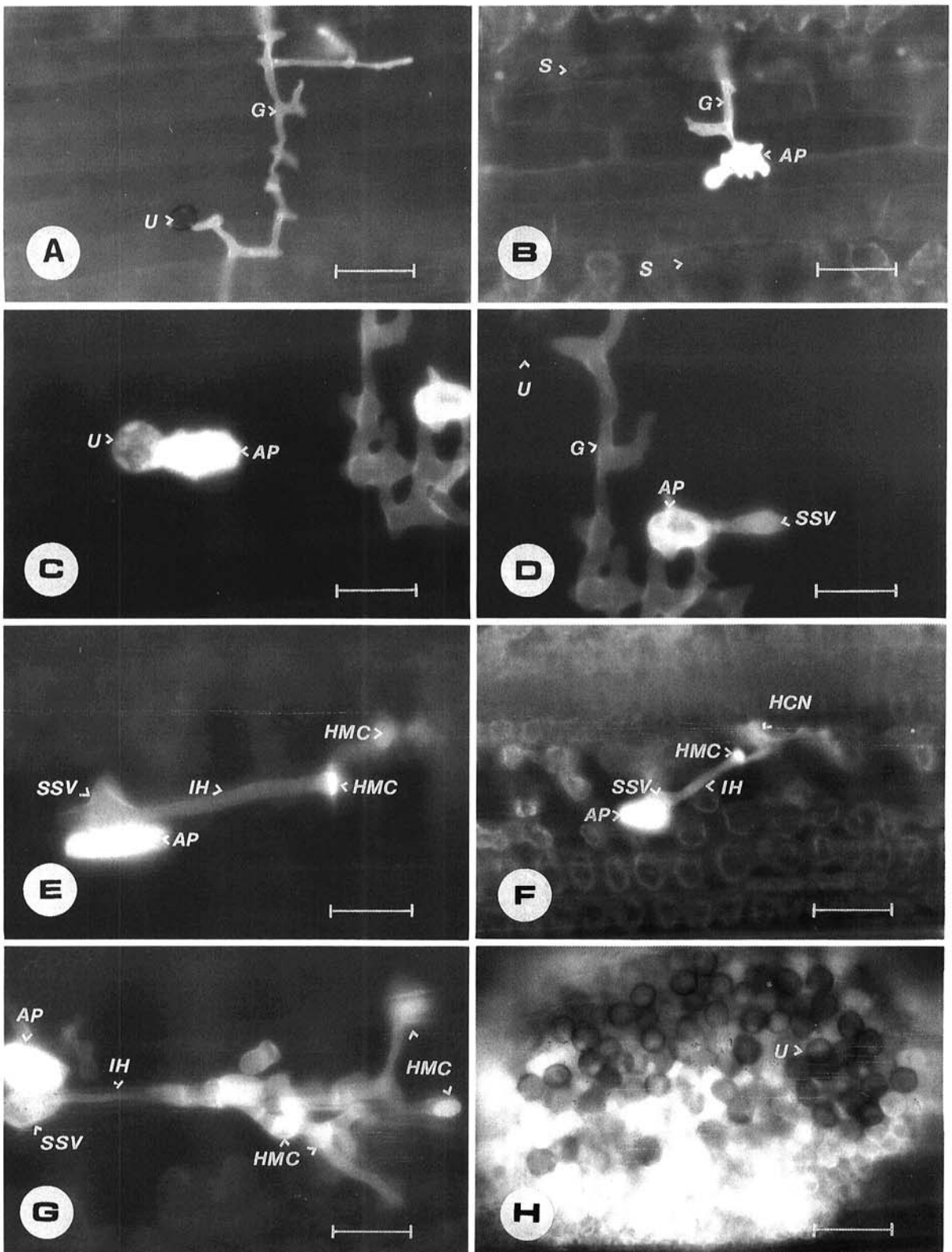

Fig. 2. Components of resistance studied, using fluorescence microscopy, in *Triticum aestivum* and *T. monococcum* to pathotype UVPrt9 of *Puccinia recondita* f. sp. *tritici*. **A,** Germ tube without an appressorium on 'Karl' 2 days postinoculation (dpi). **B,** Germ tube with nonstomatal appressorium on KS93U9 7 dpi. **C,** Aborted substomatal vesicle on PI 266844 2 dpi. **D,** Germ tube with nonpenetrating appressorium on PI 266844 2 dpi. **E,** Early abortion on KS93U9 2 dpi. **F,** Early abortion with host cell necrosis on KS93U9 7 dpi. **G,** Colony formation on 'Karl' 2 dpi. **H,** Sporulating colony on 'Karl' 7 dpi. All fungal structures were from pathotype UVPrt9: U = urediniospore; G = germ tube; AP = appressorium; SSV = substomatal vesicle; IH = infection hyphae; HMC = haustorium mother cell; HCN = host cell necrosis; and S = stoma. Scalebar in **A**, **B**, **F**, and **H** represents 50 μm; scalebar in **C**, **D**, **E**, and **G** represents 25 μm.

used for analyses of variance and for calculating standard deviations of treatment means. Seedling and postseedling data were analyzed separately.

RESULTS

Prestomatal exclusion. On primary leaf surfaces, prestomatal exclusion of the fungus ranged between 6.6% of the infection sites for 'Karl' and 18.1% on the resistant line Tm2126/5 (Fig. 1A), and between 2.2% on adult leaves of 'Karl' and 13.9% on PI 355520 (Fig. 1B). On seedlings, the failure of germ tubes to produce appressoria (Fig. 2A) was more common than nonstomatal appressoria (Fig. 2B). On adult leaves of most lines, the opposite was true (Table 1). Approximately equal proportions of absent or nonstomatal appressoria constituted prestomatal exclusion on adult leaves of Tm2126/5 (Table 1).

Aborted penetration. Very little variation occurred in the percentage of aborted penetrations on seedlings of the resistant lines, which were all significantly different from the leaf rust-susceptible 'Karl' (Fig. 3A). However, on adult leaves, aborted penetration was similar in 'Karl' and KS93U9, but less frequent than on PI 266844, PI 355520, or Tm2126/5 (Fig. 3B). Considering the components of aborted penetration, nonpenetrating appressoria were not as conspicuous as was the abortion of substomatal vesicles (Fig. 2C and D) at both growth stages (Table 1). In adult leaves, aborted penetration could be attributed almost exclusively to the failure of substomatal vesicles to develop further (Table 1).

Early abortion. Early abortion of fungal structures occurred frequently on many lines (Fig. 4). However, variation in genotype response was noted for this parameter. At both seedling and adult-plant growth stages, very few infection sites aborted prematurely in 'Karl' (Fig. 4A and B). Early abortion (Fig. 2E) did not appear an important component of resistance in PI 355520 seedlings (Fig. 4A), but was prominent at the more mature growth stage (Fig. 4B). None of the early abortion sites in seedlings of 'Karl' and Tm2126/5 exhibited host cell necrosis (Table 1). In contrast, all early abortions in KS93U9 were associated with a hypersensitive response (Fig. 2F). Compared with seedlings, the occurrence of host cell necrosis was much more pronounced in adult plants of PI 266844 and Tm2126/5, in which 81.7 and 76.9% of all early abortions were associated with hypersensitive cell death (Table 1).

Number of haustorium mother cells. Haustorium mother cell counts for seedlings 2 and 7 dpi, and for adult plants 7 dpi, are presented in Figure 5A to C. At the first sampling time, 'Karl', PI 266844, and PI 355520 contained more haustorium mother cells than KS93U9 and Tm2126/5 (Fig. 5A). The number of haustorium mother cells in the latter two lines remained approximately the same, 7 dpi, but increased in the other lines (Fig. 5B). In the adult stage, the leaf rust fungus produced very few haustorium mother cells in the resistant lines (Fig. 5C).

Papillae and haustorium counts. The numbers of haustoria (Fig. 6A) and brightly fluorescing papillae (Fig. 6B) observed with phase contrast microscopy at primary leaf infection sites are shown in Figure 7. Papillae were frequently observed in the three *T. monococcum* lines PI 266844, PI 355520, and Tm2126/5, but none occurred in KS93U9. Using the trypan blue staining procedure, necrosis in KS93U9 was commonly detected as densely stained cells in association with haustoria (Fig. 6C). Haustoria were only occasionally observed in Tm2126/5.

Formation and size of colonies and uredinia. Infection sites with more than six haustorium mother cells, set as the criterion for successful establishment of a colony, were not observed in KS93U9 and Tm2126/5 (Fig. 5B). The percentages of infection sites classified as colonies (Fig. 2G) in 'Karl', PI 266844, and PI 355520 are shown in Figure 8. The production of urediniospores was evident in many of those sites culminating in colonies in

TABLE 1. Histological components of resistance to *Puccinia recondita* f. sp. *tritici* in a bread wheat line deriving resistance from *Triticum monococcum* and in parental and reference *T. aestivum* and *T. monococcum* lines

Histological component[a]	Growth stage	Sampling time[b]	Lines				
			'Karl'[c]	KS93U9[d]	PI 266844[e]	PI 355520[e]	Tm2126/5[e,f]
Prestomatal exclusion (%)							
No appressorium formed	Seedling	7	54.8 ± 8.1	58.9 ± 8.1	63.8 ± 10.3	44.0 ± 11.9	61.6 ± 6.3
	Adult plant	7	0	0	27.8 ± 4.8	26.2 ± 4.2	51.7 ± 20.2
Nonstomatal appressorium	Seedling	7	45.2 ± 8.1	41.1 ± 13.3	36.2 ± 10.3	56.0 ± 11.9	38.4 ± 4.3
	Adult plant	7	100	100	72.2 ± 4.8	73.8 ± 4.2	48.3 ± 20.2
Aborted penetration (%)							
Nonpenetrating appressorium	Seedling	7	40.7 ± 12.6	35.5 ± 9.2	31.1 ± 7.5	32.1 ± 4.3	35.7 ± 9.2
	Adult plant	7	0	0	10.7 ± 3.8	5.2 ± 0.4	16.8 ± 3.9
Aborted substomatal vesicle	Seedling	7	59.3 ± 12.6	64.5 ± 9.1	68.9 ± 7.5	67.9 ± 4.3	64.3 ± 9.2
	Adult plant	7	100	100	89.3 ± 3.8	94.8 ± 0.4	83.2 ± 3.9
Early abortion (%)							
Host cell necrosis	Seedling	7	0	100	30.6 ± 2.5	42.9 ± 53.5	0
	Adult plant	7	20.2 ± 11.5	100	81.7 ± 2.7	41.8 ± 13.2	76.9 ± 11.1
Colony formation							
Colony area (mm^2)	Seedling	2	0.0047 ± 0.0008	–[g]	0.0032 ± 0.0004	0.0031 ± 0.0003	–
	Seedling	7	0.6333 ± 0.1363	–	0.0265 ± 0.0156	0.1659 ± 0.0827	–
	Adult plant	7	0.1217 ± 0.0043	–	–	–	–
Sporulating colonies (%)	Seedling	7	82.2 ± 7.2	0	0	26.7 ± 33.3	0
	Adult plant	7	47.1 ± 3.3	0	0	0	0
Uredinium area (mm^2)	Seedling	7	0.625 ± 0.0131	–	–	0.0415 ± 0.0270	–
	Adult plant	7	0.0192 ± 0.0049	–	–	–	–

[a] All infection sites examined were classified as either prestomatal exclusion, aborted penetration, early abortion, or colony formed (Fig. 10). The relative proportions of subcomponents within prestomatal exclusion and aborted penetration are shown. For sites in which the fungus aborted early, the percentage exhibiting host cell necrosis is given. In addition to the sizes of colonies and uredinia, the percentage of infection sites culmination in sporulating pustules is also shown.

[b] Sampling time is given in days postinoculation.

[c] Leaf rust-susceptible control.

[d] Bread wheat line (Karl*3//PI 266844/PI 355520).

[e] *T. monococcum* accessions.

[f] *T. monococcum* control previously described to exhibit prehaustorial resistance to leaf rust.

[g] – = no colonies developed.

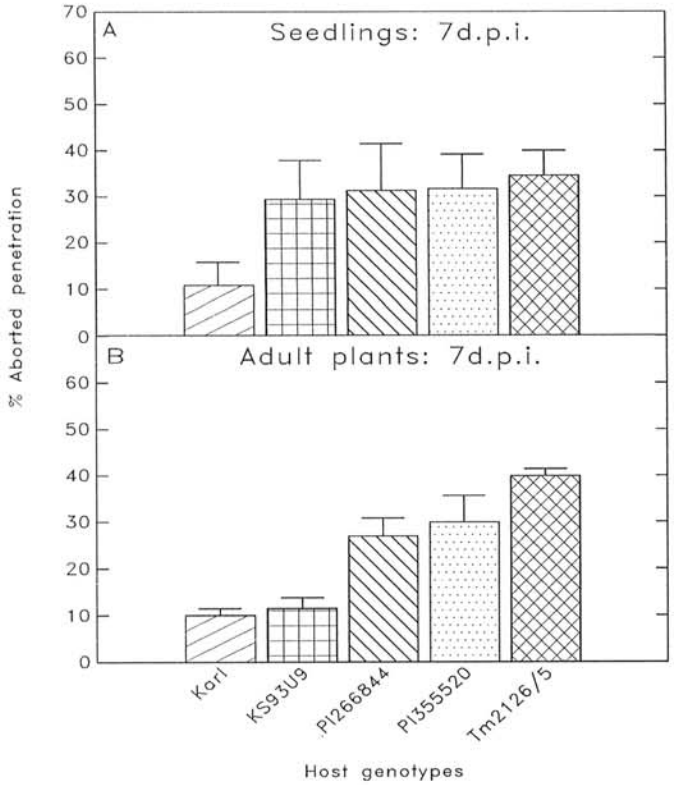

Fig. 3. Percentage of abortive penetration of pathotype UVPrt9 of *Puccinia recondita* f. sp. *tritici* determined 7 days postinoculation (dpi) on adaxial leaf surfaces of **A,** seedlings and **B,** adult plants of the bread wheats 'Karl' (leaf rust-susceptible) and KS93U9 (leaf rust-resistant), and the *Triticum monococcum* lines PI 266844, PI 355520, and Tm2126/5 (leaf rust-resistant). Error bars represent standard deviations.

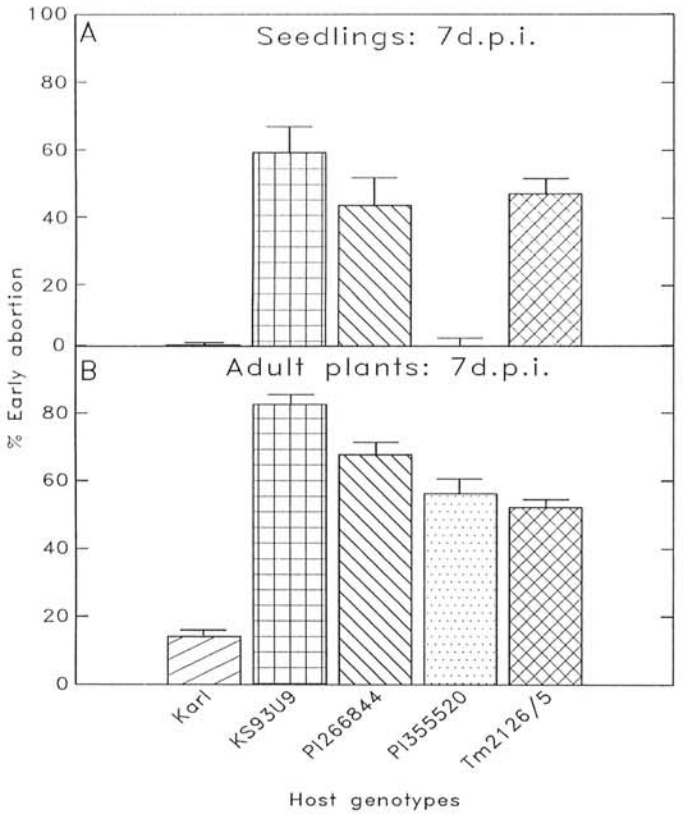

Fig. 4. Percentage of early abortion of pathotype UVPrt9 of *Puccinia recondita* f. sp. *tritici* determined 7 days postinoculation (dpi) in **A,** seedlings and **B,** adult plants of the bread wheats 'Karl' (leaf rust-susceptible) and KS93U9 (leaf rust-resistant), and the *Triticum monococcum* lines PI 266844, PI 355520, and Tm2126/5 (leaf rust-resistant). Error bars represent standard deviations.

'Karl' (Table 1) (Fig. 2H). Although 26.7% of the infection sites in PI 355520 sporulated in seedlings, no sporulation was observed in adult leaf tissue. At 2 dpi, colonies in 'Karl' measured 0.0047 mm^2; those in PI 266844 and PI 355520 measured 0.0032 mm^2 and 0.0031 mm^2, respectively (Table 1). In adult plants, colonies in 'Karl' were 0.1217 mm^2 in size. Measurements of colonies in the resistant lines were not possible, since all infection sites in adult leaves were quantified to have five or less haustorium mother cells. Uredinium sizes measured on 'Karl' and PI 355520 are given in Table 1.

Hypersensitivity index. The relationship between the amount of necrotic leaf tissue and extent of fungal colonization as determined for primary leaf infections is given in Figure 9. Index values larger than one, as observed for seedlings of PI 266844 and PI 355520, indicated that the area of necrotic host tissue exceeded the actual colony dimensions.

DISCUSSION

The relative proportions of prestomatal exclusion, aborted penetration, and early abortion of infection structures, as well as the percentage of infection sites classified as colonies, are shown in Figure 10. According to these parameters, and specifically the inability of the fungus to successfully establish colonies, resistance in KS93U9 seedlings appeared similar to the *T. monococcum* line Tm2126/5. The absence of hypersensitivity in Tm2125/6 (Table 1) indicated, however, a different host response than in the *T. aestivum* line KS93U9. The frequent occurrence of host cell necrosis at infection sites in both seedling and adult KS93U9 leaves suggested that the resistance transferred from *T.*

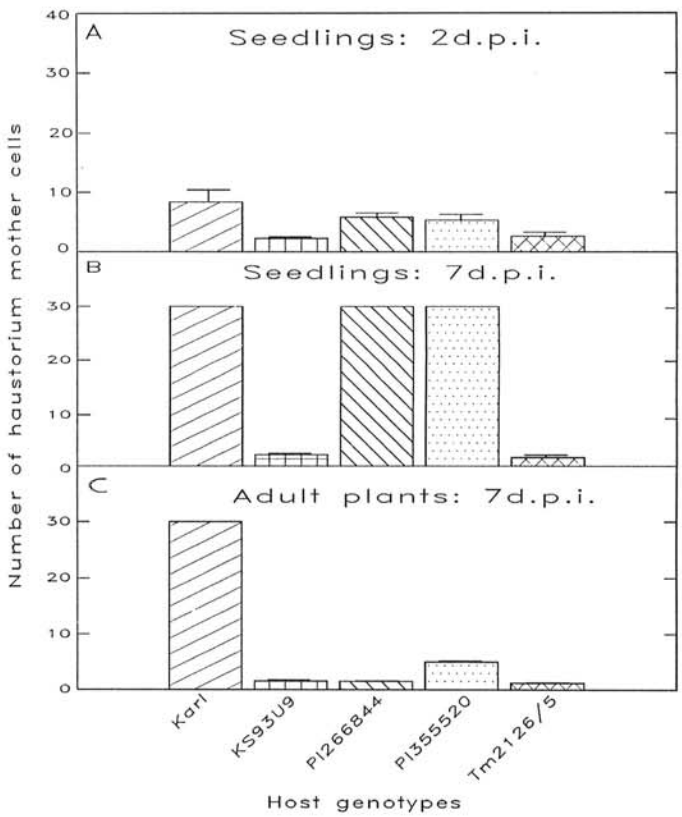

Fig. 5. Number of haustorium mother cells of pathotype UVPrt9 of *Puccinia recondita* f. sp. *tritici* detected in **A,** seedlings 2 days postinoculation (dpi); **B,** seedlings 7 dpi; and **C,** adult plants 7 dpi of the bread wheats 'Karl' (leaf rust-susceptible) and KS93U9 (leaf rust-resistant), and the *Triticum monococcum* lines PI 266844, PI 355520, and Tm2126/5 (leaf rust-resistant). Error bars represent standard deviations. Because of possible counting errors in large colonies, the number of haustorium mother cells therein was estimated at 30.

monococcum is not different from other existing sources conferring hypersensitive resistance to *P. recondita* f. sp. *tritici*. Using the South African pathotype UVPrt9, results were in agreement with a previous report of prehaustorial, nonhypersensitive resistance to leaf rust in Tm2126/5 (17).

In contrast to the seedling data, however, histological investigations in leaves from adult Tm2126/5 plants clearly showed that most (76.9%) early abortion sites were associated with host cell necrosis (Table 1). Differences between seedlings and adult plants in expression of resistance are not uncommon in the wheat leaf rust system (18). Evidently, similar growth stage-related mechanisms exist for leaf rust resistance in *T. monococcum*. From the present detailed histological observations, especially during the prepenetration and penetration phases, expression of resistance varied between primary and older leaves. The advantage of Tm2126/5 seedlings in excluding the fungus prior to stomatal penetration was not observed to the same degree in adult plants. According to prestomatal exclusion and early abortion, adult PI

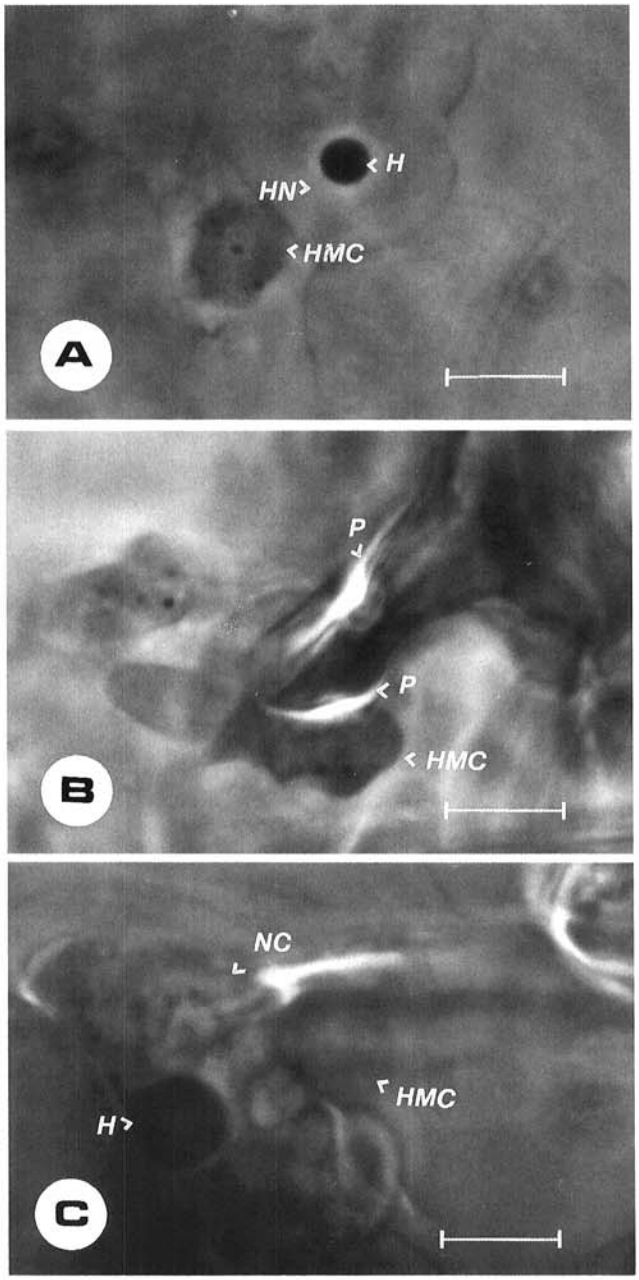

Fig. 6. Components of resistance studied, using phase contrast microscopy, in *Triticum aestivum* and *T. monococcum* to pathotype UVPrt9 of *Puccinia recondita* f. sp. *tritici*. **A,** Haustorium of pathotype UVPrt9 of *P. recondita* f. sp. *tritici* in a mesophyll cell of 'Karl' 2 days postinoculation (dpi); **B,** papillae in Tm2126/5 2 dpi; and **C,** haustorium in necrotic host cell in KS93U9 2 dpi. Abbreviations used are HMC = haustorium mother cell; HN = haustorium neck; H = haustorium; P = papilla; and NC = necrotic cell. Scalebar represents 5 μm.

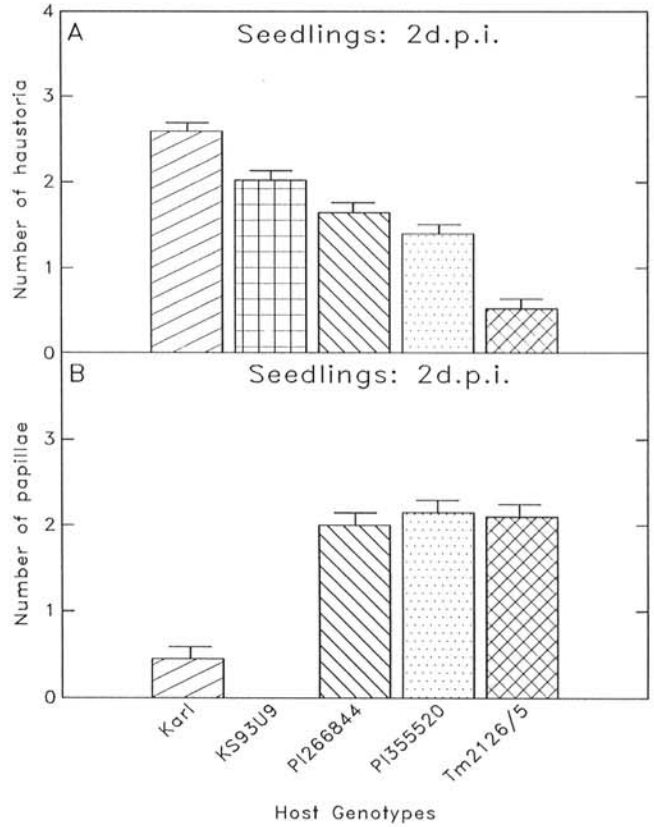

Fig. 7. Numbers of **A,** haustoria per infection site of pathotype UVPrt9 of *Puccinia recondita* f. sp. *tritici* and **B,** papillae per infection site observed 2 days postinoculation (dpi) in primary leaves of the bread wheats 'Karl' (leaf rust-susceptible) and KS93U9 (leaf rust-resistant), and the *Triticum monococcum* lines PI 266844, PI 355520, and Tm2126/5 (leaf rust-resistant). No papillae were observed in KS93U9. Error bars represent standard deviations.

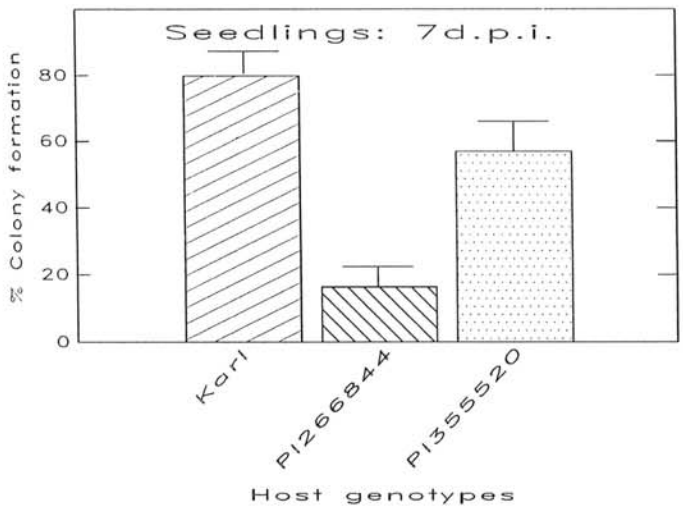

Fig. 8. Percentage of infection sites of pathotype UVPrt9 of *Puccinia recondita* f. sp. *tritici* successfully culminating in colonies in primary leaves of the bread wheat 'Karl' (leaf rust-susceptible) and the *Triticum monococcum* lines PI 266844 and PI 355520 (leaf rust-resistant). Less than six haustorium mother cells per infection site, set as criterion for establishment of a colony, formed in the resistant lines KS93U9 and Tm2126/5. Error bars represent standard deviations.

355520 plants also appeared much more resistant to leaf rust than seedlings. Based on adult-plant evaluation, our data suggested that Tm2126/5 resistance might not be completely different from hypersensitivity typically associated with ephemeral major rust resistance genes. A further implication was that histological characterization of resistance components should preferably be conducted at more than one growth stage. The differences between seedlings and adult plants, based on quantification of the respective histological parameters, are exemplified by Figure 10.

According to the variation in resistance mechanisms from prehaustorial without necrosis to posthaustorial with necrosis, Niks and Dekens (17) concluded that generalization about mechanisms in diploid wheats is problematic. Results from our study, specifically histological data supporting statistical differences between the resistant lines and 'Karl', indicated a continuum of resistance expression. This continuum was discussed by Heath (6), who recognized inhibition of germination, leaf topographical effects, penetration barriers, inhibition of infection structure formation or development, and reduced mycelium growth as plant defense mechanisms. The high proportion of sites in which infection structures aborted early, as indicated by the observation of between one and six haustorium mother cells and haustorium mother cell counts similar to those in Tm2126/5, initially suggested that KS93U9 displayed prehaustorial resistance to leaf rust. Limitation of fungal growth in KS93U9 was further emphasized by statistically equal counts of haustorium mother cells at 2 and 7 dpi. However, quantification of haustoria in the resistant lines indicated that they occurred more readily in KS93U9, PI 355520, and PI 266844 than in Tm2126/5. Early abortion of fungal structures in KS93U9 thus appears to have resulted from haustorium-induced hypersensitive cell death inhibiting fungal development, rather than papillae formation. Necrotic cells in KS93U9 were clearly visible with both staining techniques as either yellow or excessively stained cells, providing evidence that the line actually exhibited posthaustorial resistance with necrosis.

An interesting observation was that no cell wall appositions were detected in KS93U9, whereas, on average, two of these luminous structures were visible at each infection site in PI 266844, PI 355520, and Tm2126/5 (Fig. 7). Papillae have previously been shown to be an important mechanism of resistance to leaf rust in certain *T. aestivum* accessions. Jacobs (7,8) provided evidence that failed haustorium formation was related to the occurrence of cell wall appositions in wheat seedlings and adult plants. Since callose deposition usually succeeds other detrimental effects on the haustorium, Littlefield and Heath (13) did not consider encapsulation by callose an important mechanism of haustorium failure. The absence of papillae in KS93U9 served to emphasize that when resistance is transferred, the actual mechanisms in the progeny are not necessarily the same as in the parent lines. This phenomenon was also evident from the fact that the relative proportions of prestomatal exclusion, aborted penetration, early abortion,

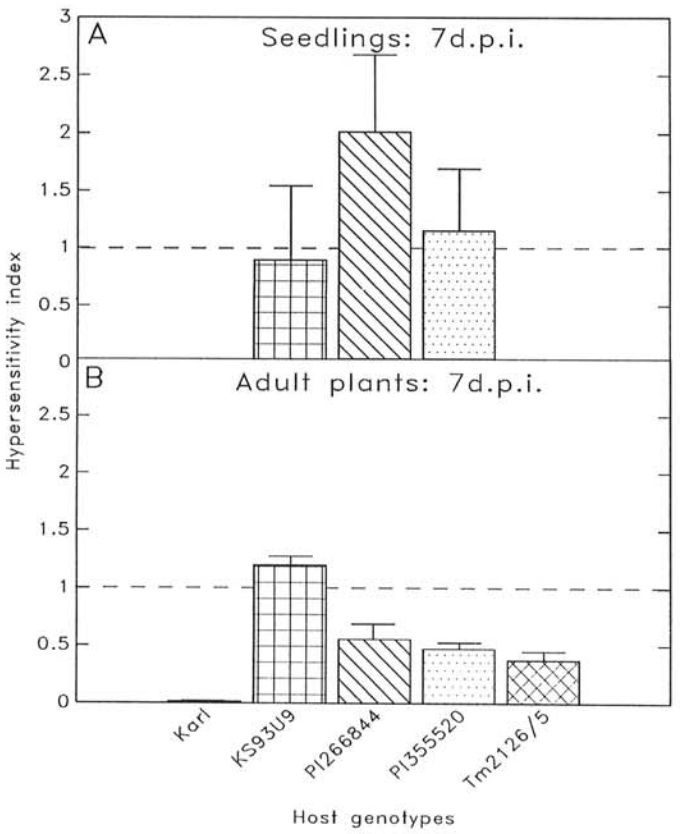

Fig. 9. Hypersensitivity index calculated by expressing the necrotic leaf area as a fraction of the area of fungal growth in **A,** primary leaves and **B,** adult-plant leaves of the bread wheats 'Karl' (leaf rust-susceptible) and KS93U9 (leaf rust-resistant), and the *Triticum monococcum* lines PI 266844, PI 355520, and Tm2126/5 (leaf rust-resistant). Values exceeding 1.0 indicated extensive hypersensitivity in relation to the colonized leaf area. No host cell necrosis was observed in 'Karl' or Tm2126/5 seedlings. Error bars represent standard deviations.

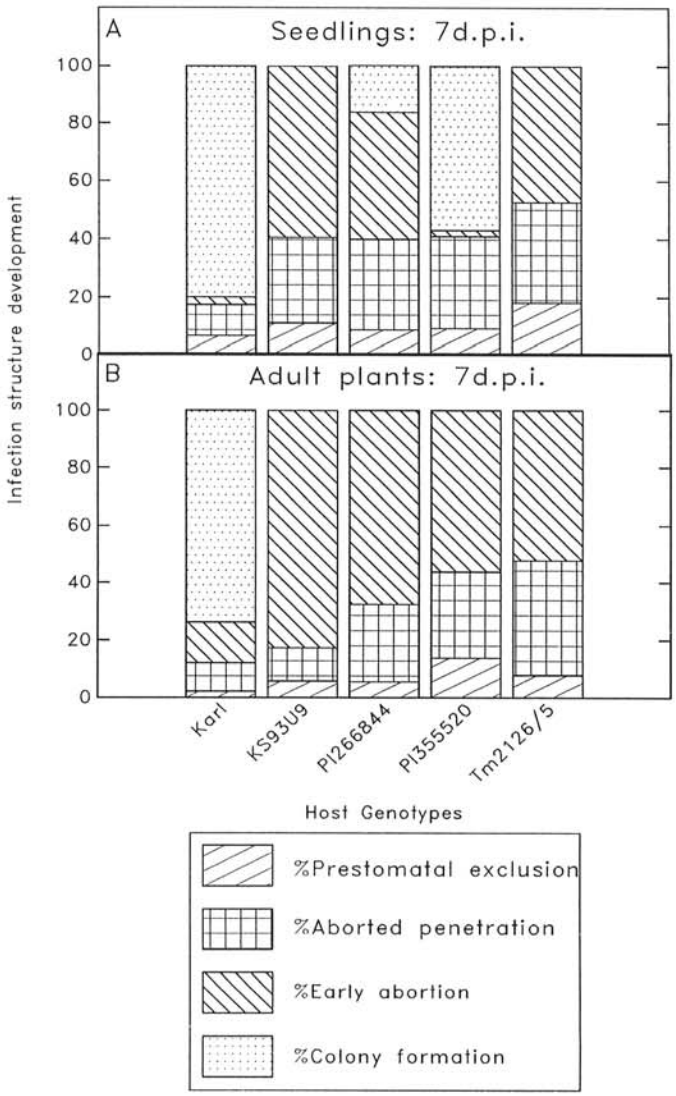

Fig. 10. Percentage of infection sites of pathotype UVPrt9 of *Puccinia recondita* f. sp. *tritici* classified as, respectively, prestomatal exclusion, aborted penetration, early abortion, and successfully established colonies in the bread wheats 'Karl' (leaf rust-susceptible) and KS93U9 (leaf rust-resistant), and the *Triticum monococcum* lines PI 266844, PI 355520, and Tm2126/5 (leaf rust-resistant).

and colony formation varied considerably between seedlings of KS93U9 and its two resistant parents (Fig. 10). More colonies of larger dimensions formed in seedlings of the lines PI 355520 and PI 266844 than in KS93U9. According to this parameter, expression of *T. monococcum* resistance was enhanced in the hexaploid background. The high frequency of early abortion in KS93U9 seedlings seemed attributable to PI 266844, since very few infection sites were classified as such in PI 355520. Differences between the *T. monococcum* and *T. aestivum* lines could be attributed to species differentiation in reaction to leaf rust and the functioning of resistance mechanisms at two levels of ploidy, as well as to possible effects of the susceptible recurrent parent 'Karl' in KS93U9. Although no information is currently available on the genetics of resistance in KS93U9 and whether the line actually contains genes from both *T. monococcum* sources, the seedling infection type of KS93U9 to pathotype UVPrt9 suggested a strong resemblance to the PI 266844 phenotype (infection type 0). In adult plants, differences in the histological components between KS93U9, PI 266844, and PI 355520 were not as pronounced. KS93U9 differed from its resistant parents by expressing a lower percentage of aborted penetration, but more frequent early abortion.

No conclusive evidence suggesting a novel resistance type was obtained for KS93U9. Our study, however, provided information on the expression of *T. monococcum* resistance in a hexaploid background, as well as the importance of resistance expression across the full spectrum of defense mechanisms against *P. recondita* f. sp. *tritici*.

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