Effect of Quantitative Resistance in Wheat on the Development of *Puccinia striiformis* During Early Stages of Infection

L. H. M. Broers and **R. M. López-Atilano,** International Maize and Wheat Improvement Center, Lisboa 27, Apdo Postal 6-641, 06600 Mexico DF, Mexico

ABSTRACT

Broers, L. H. M., and López-Atilano, R. M. 1996. Effect of quantitative resistance in wheat on the development of *Puccinia striiformis* during early stages of infection. Plant Dis. 80:1265-1268.

Flag leaves of three quantitatively resistant wheat cultivars and one highly susceptible wheat cultivar were inoculated with urediospores of *Puccinia striiformis* to study the effect of quantitative resistance on development of the fungus during the first 6 days of the infection cycle. The results indicated that the most important mechanism of resistance is reduction in the frequency of formation of appressoria. On resistant cultivars, twice as many germ tubes failed to produce appressoria and grew over stomata than on the susceptible cultivar. Of the infection structures that oriented themselves on stomata, most were able to form substomatal vesicles. In resistant cultivars, the number of infection sites that developed substomatal vesicles decreased over time. This result suggests a resistance mechanism that disintegrates substomatal vesicles. In addition, the formation of primary infection hyphae is delayed considerably in resistant cultivars. Although large cultivar differences for quantitative resistance have been observed at the cellular level, these cannot fully explain differences at the plant level or field level. Therefore, events that happen after the first 6 days of fungal development, like reduced growth and late abortion, may further explain differences in level of resistance among the quantitatively resistant wheat genotypes.

Durable resistance in wheat (*Triticum aestivum* L.) to *Puccinia striiformis* West-end. f. sp. *tritici*, causing yellow or stripe rust, is a form of quantitative resistance (1, 5,9). In the field, reduced epidemic rates are observed that result from prolonged latency periods, reduced infection frequencies, and slower growth rates of the fungus (2). Cultivars with quantitative resistance have a susceptible infection type in the seedling stage but in the adult plant stage the infection type decreases with increasing levels of resistance (11). The infection cycle of *P. striiformis* can be divided into several steps: germination, formation of an appressorium, penetration of the stoma, formation of a substomatal vesicle, formation of the first infection hypha, formation of a haustorium, ramification of hyphae, and sporulation. Theoretically, resistance can have an effect on each of these steps. A comparison of resistant and susceptible cultivars can determine the important components of resistance. Few studies have been carried out to characterize quantitative resistance in wheat to yellow rust at the cellular level (9,14,18).

Mares and Cousen (9) studied the development of the fungus on four winter wheat cultivars ranging from very resistant to very susceptible. They observed that quantitative resistance was associated with reduced fungal growth rates and appearance of scattered necrotic cells. This necrosis was more pronounced with increasing levels of resistance. Similar studies on quantitative resistance in barley to leaf rust (*P. hordei*) showed that cell wall appositions explained the lower infection frequency and the longer latency period of cultivars with different levels of quantitative resistance (10). Unlike the case of wheat yellow rust, necrosis was not observed. A reduced fungal development was also observed for quantitative resistance in wheat to *P. recondita* (6,8). However, the observed cell wall appositions could not explain the reduced size of infections in resistant cultivars. As for leaf rust in barley, no necrosis was observed and the reduced fungal development is so far unexplained (6).

We initiated a study to investigate the effects of quantitative resistance in spring bread wheat to *P. striiformis* in the early stages of fungus development, to increase our understanding of the mechanism of quantitative resistance.

MATERIALS AND METHODS

Four bread wheat cultivars with different levels of quantitative resistance were used: Morocco (highly susceptible), Opata 85 (intermediately resistant), Pavon 76 (intermediately to highly resistant), and Parula (highly resistant) (3). All four cultivars are characterized by a susceptible infection type in the seedling stage (2) with isolate 89009 of race 14E14 (7), which is virulent toward the resistance genes *Yr2*, *Yr3*, *Yr6*, *Yr7*, and *YrA*. In addition, the cultivars differ considerably in latency period and infection frequency when inoculated in the adult plant stage (2).

Eight plants of each cultivar were grown in individual pots in the greenhouse. Seeds were planted at weekly intervals over a period of 6 weeks to assure for inoculation plants of all cultivars in the same young-flag-leaf stage (DC 49-59) (19).

Two experiments were carried out. For each experiment, six pots per cultivar were used. Eight flag leaves per pot were inoculated by applying a 1:2 (wt/wt) urediospore (isolate 89009)/*Lycopodium* mixture with a paintbrush. Plants were incubated at 15°C at 100% relative humidity for 18 h in a dark room. After incubation, pots were transferred to a greenhouse and arranged according to a randomized complete block design with six replications. Plants were given approximately 14 h of light per day. Day and night temperatures were 19 to 22°C and 15 to 18°C, respectively.

At 48, 96, and 144 h after inoculation, one randomly chosen inoculated flag leaf was collected from each pot. The central part of each leaf was boiled in a 2:1 (vol/vol) lactophenol/ethanol solution and stained according to the procedure of Rohringer et al. (12). Instead of Calcofluor, Uvitex 2B (0.1%, wt/vol) (CIBA-Geigy, Basel, Switzerland) was used as an optical brightener. Whole mount preparations were made and infection structures were observed with a Zeiss epifluorescence UV microscope equipped with a HBO-50 mercury lamp, a 395 to 440 nm excitation filter, a FT560 chromatic divider, and a 470 nm suppresser filter.

Germ tubes were very long and often fragmented, probably as a result of the boiling treatment. Therefore, assessments of infections based on individual germinated spores could not be made. Instead, stomata that were touched by a germ tube were considered as potential infection sites. Stomata were grouped into five classes depending on the development of the observed infection structures: (i) class 1, germ tube grows across the stoma; (ii)

Corresponding author: L. H. M. Broers.

Present address of first author: Lochow Petkus France, Route Nationale 154, 28150 Allonnes, France.

Accepted for publication 18 July 1996.

Publication no. D-1996-0819-04R

class 2, germ tube forms an appressorium on a stoma but does not penetrate; (iii) class 3, germ tube forms an appressorium on a stoma, penetrates the stoma, and forms a substomatal vesicle—no primary hyphae with haustorium mother cells are visible; (iv) class 4, same as class 3 except primary hyphae with five or fewer haustorium mother cells are visible; and class 5, same as class 4 except more than five haustorium mother cells are visible—the infection is considered established.

At 48 h after inoculation, as many stomata were assessed as required to find five class 4 infections. No class 5 infections were observed. At 96 and 144 h after inoculation, as many stomata were assessed as required to find five class 5 infections.

Based on these observations, the following totals were calculated: sum of class 1 to class 5, T = total number of observed stomata with germ tube contact; sum of class 2 to class 5, A = total number of stomata with appressoria; sum of class 3 to class 5, S = total number of stomata with substomatal vesicles; and sum of class 4 to class 5, H = total number of stomata with infection hypha and haustorium mother cells; and E = total number of established infections (class 5).

The totals were used to calculate survival ratios (20) of infection structures. In this case, survival ratios (R) give the proportion of infection structures that continued to develop from one step to the next step in the infection cycle of the fungus. In total, four survival ratios were calculated for each step in fungus development with the above sums: $ATR = A/T$; $SAR = S/A$; $HSR = H/S$; and $EHR = E/H$. For example, the fraction of infection structures with a substomatal vesicle that successfully formed a primary hyphae with a haustorium mother cell can be calculated as H/S. A low survival ratio means that few infection structures succeeded to the next step in the infection cycle.

For statistical analysis, fractions were subjected to an angular sine transformation ($\arcsin\sqrt{(\text{fraction})}$) (16). Untransformed data are presented in the tables.

Analysis of variance was applied to each assessment time separately according to a randomized block design with six replicates. For significant main effects, multiple comparisons were made with least significant difference tests at $P = 0.05$. Assessment time was added to the model as shown in Table 1 to test for an assessment time effect and for an assessment time × cultivar interaction.

Two leaves from each pot were used to assess the latency period (period from inoculation until first pustule appeared) and the average percentage of infected leaves. For both variables, pot means were calculated and analyzed according to a randomized complete block design with six replications.

RESULTS

Formation of appressoria. Of those stomata that were touched by a germ tube, only 26% in experiment 1 and 18% in experiment 2 had an appressorium over them (Table 2). For all other cases in which stomata were touched by a germ tube, the germ tube overgrew it without forming an appressorium. Cultivars differed significantly in frequency of stomata with an appressorium (Table 3). The fungus formed appressoria on Morocco at more than twice the frequency it did on resistant cultivars. No statistically significant difference was found among resistant cultivars although Parula had in five out of six cases the lowest percentage of stomata with an appressorium. In a combined analysis of the two experiments, Parula had a significantly lower percentage of stomata with an appressorium than the other cultivars. An effect of assessment time was not clear. In experiment 1, significantly fewer stomata with an appressorium were observed at 48 h after inoculation than at 96 or 144 h after inoculation (Table 2). In experiment 2, no statistically significant difference was detected among the three assessment dates, although the tendency was the same as for experiment 1: fewer stomata with an appressorium at 48 h after inoculation than at 96 or 144 h after inoculation.

Formation of substomatal vesicles. Within 48 h after inoculation 88 (experiment 2) to 90% (experiment 1) of the infection sites with an appressorium were able to penetrate the stomata and form a substomatal vesicle. No significant cultivar effect was detected at this assessment time (Table 4). At 144 h after inoculation, the percentage of infection sites with a substomatal vesicle was reduced to 70 (experiment 1) and 73% (experiment 2), averaged over cultivars, and a significant cultivar effect was detected: a higher percentage of infection structures formed a substomatal vesicle on Morocco than on resistant cultivars (Table 4). Between 48 and 144 h after inoculation there was a reduction in the number of substomatal vesicles in resistant cultivars. On resistant cultivars, 26 (experiment 2) to 28% (experiment 1) fewer infection structures with substomatal vesicles were found at 144 than at 48 h after inoculation. On Morocco no or a much smaller reduction was observed.

Formation of haustorium mother cells. The proportion of infection structures that formed haustorium mother cells increased with time (Tables 1, 2, and 5). The cultivar effect (Table 1) was similar to that on other development stages of the infection cycle: a larger proportion of infection structures with haustorium mother cells was found on Morocco than on resistant cultivars (Table 5). The time effect on formation of haustorium mother cells was not cultivar dependent as the cultivar × time interaction was not significant (Table 1). These data suggest that on resistant cultivars the formation of the first infection hyphae with a haustorium mother cell is delayed compared with Morocco.

Establishment of infections. The time effect on the development of established infection structures was significant in both experiments (Table 1). None of the cultivars had infection structures with more than five haustorium mother cells at 48 h after inoculation (Table 2). At 144 h after inoculation, 85 (experiment 1) to 91% (experiment 2) of the infections had more than five haustorium mother cells. In ex-

Table 1. Analysis of variance of survival ratios of four stages of development of stripe rust on four bread wheat cultivars at three assessment times after inoculation[x]

		Mean square							
		app[y]		ssv		hmc		est	
Source	df	exp 1	exp 2	exp 1	exp 2	exp 1	exp 2	exp 1	exp 2
Replication	5	0.035	0.016	0.066	0.028	0.058	0.067	0.221**	0.022
Cultivar	3	0.336***[z]	0.290***	0.206**	0.466***	0.389***	0.459***	0.234**	0.026
Time (T)	2	0.109***	0.011	0.337***	0.398***	4.752***	3.538***	9.148***	13.260***
G × T	6	0.013	0.101***	0.040	0.132*	0.075	0.026	0.091	0.013
Error	55	0.013	0.014	0.036	0.036	0.048	0.049	0.047	0.018

[x] Survival ratios are calculated as the number of stomata with infection structures in a certain development stage divided by the number of stomata with infection structures in the preceding development stage.

[y] app = stomata with infection structures that formed an appressorium; ssv = stomata with infection structures that penetrated and formed a substomatal vesicle; hmc = stomata with infection structures having haustorium mother cells; est = stomata with infection structures with at least five haustorium mother cells.

[z] * = significant at $P = 0.05$; * *= significant at $P = 0.01$; *** = significant at $P = 0.001$.

periment 1, there were significant differences among cultivars at 144 h after inoculation (Table 6). In experiment 2, the cultivar effect was significant both at 96 and 144 h after inoculation. The same cultivar ranking was observed as for experiment 1. (Table 6).

Components of resistance. Latency period and average percentage of infected leaves showed a significant cultivar effect in both experiments (Table 7). Morocco had the shortest latency period and the highest average percentage of infected leaves. The differences among the resistant cultivars were similar to those found in other studies (2; L. Broers, *unpublished data*).

DISCUSSION

The low infectibility of *P. striiformis* on quantitatively resistant wheat cultivars as observed in earlier studies (2) can be attributed at least partially to a mechanism that prevents the recognition of stomata by germ tubes and the subsequent formation of appressoria. On resistant cultivars, the formation of appressorium was reduced by more than 50% compared with appressorium formation on a highly susceptible cultivar. Similar avoidance has been observed on *Hordeum chilense* inoculated with *Puccinia hordei* (13). Low appressorium formation could be caused by two mechanisms: (i) growth of germ tubes is disoriented and germ tubes do not find stomata or (ii) germ tubes are able to find stomata but they are not triggered to form appressoria and penetrate; hence, they grow over the stomata. The cultivar effect described here reflects only the second mechanism.

Differentiation of an appressorium may be triggered by morphological characteristics of the stoma (17). The lack of differentiation observed here suggests that these characteristics are less well developed on resistant cultivars, resulting in a reduced formation of appressoria. We can also conclude that this mechanism is not unique to the quantitative resistance gene *Yr18,* as only two of the cultivars (Parula and Opata 85) carry it (15).

The three resistant cultivars had similar levels of this avoidance mechanism. The differences in other components of resistance among them are therefore likely to be caused by mechanisms that act at later stages of the infection cycle. Those mechanisms may be governed by resistance genes different from the gene(s) causing avoidance.

The success rate of forming a substomatal vesicle was also influenced by resistance. The reduction in frequency in formation of substomatal vesicles on resistant cultivars with time suggests that some of the substomatal vesicles observed at 48 h after inoculation disintegrate later. A possible explanation might be that resistant cultivars form or contain higher levels of pathogenicity-related (PR) proteins such as glucanase at infection sites. These PR-proteins might be able to digest the cell wall of the substomatal vesicle that contains β-glucan. Collapse of substomatal vesicles was also observed in the durably resistant winter wheat cultivar Little Joss after infection with *P. striiformis* (4). This collapse was attributed to the presence of phytoalexins.

Both experiments demonstrated a delay or retardation in formation of the first infection hyphae on resistant cultivars. This is the first step in prolonging the latency period of *P. striiformis* on these cultivars. Differences were small among resistant cultivars in the frequency of appressoria, yet there were large differences among these cultivars for latency period and infection frequency. This suggests that differences among resistant cultivars are determined at later stages of fungus development.

As a consequence of the delay observed in the formation of the first infection hy-

Table 2. Survival ratios of stripe rust infections structures at five development stages during the first 6 days of the infection cycle on adult wheat plants of wheat[x]

	Experiment 1				Experiment 2			
Development stage[y]	48 h	96 h	144 h	Mean	48 h	96 h	144 h	Mean
sto	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
app	0.19 a[z]	0.31 b	0.27 b	0.26	0.17 a	0.18 a	0.20 a	0.18
ssv	0.90 a	0.81 b	0.70 b	0.81	0.88 a	0.70 b	0.73 b	0.77
hmc	0.07 a	0.48 b	0.72 c	0.42	0.12 a	0.42 b	0.72 b	0.42
est	0.00 a	0.71 b	0.85 c	0.52	0.00 a	0.82 b	0.91 c	0.58

[x] Survival ratios are calculated as the number of stomata with infection structures in a certain development stage divided by the number of stomata with infection structures in the preceding development stage.

[y] sto = stomata with germ tube contact; app = stomata with infection structures that formed an appressorium; ssv = stomata with infection structures that penetrated and formed a substomatal vesicle; hmc = stomata with infection structures having haustorium mother cells; est = stomata with infection structures with at least five haustorium mother cells.

[z] Means in a row within an experiment with the same letter do not differ significantly ($P = 0.05$); means are the average of four genotypes and six replications.

Table 3. Proportion of stomata touched by a germ tube that had an appressorium for flag leaves of four wheat cultivars at 48, 96, and 144 h after inoculation with urediospores of isolate 89009 (race 14E14) of *Puccinia striiformis*

	Experiment 1				Experiment 2			
Cultivar	48 h	96 h	144 h	Mean	48 h	96 h	144 h	Mean
Morocco	0.39 a[z]	0.50 a	0.40 a	0.43 a	0.39 a	0.43 a	0.22 ab	0.35 a
Opata 85	0.13 b	0.23 b	0.27 a	0.21 bc	0.07 c	0.11 b	0.29 a	0.16 b
Pavon 76	0.12 b	0.31 b	0.27 a	0.25 b	0.07 c	0.11 b	0.11 b	0.10 b
Parula	0.11 b	0.20 b	0.14 b	0.15 c	0.09 bc	0.08 b	0.18 ab	0.12 b

[z] Means in a column followed by the same letter do not differ significantly ($P = 0.05$).

Table 4. Proportion of infection structures with an appressoirum that formed a substomal vesicle on flag leaves of four wheat cultivars at 48, 96, and 144 h after inoculation with urediospores of isolate 89009 (race 14E14) of *Puccinia striiformis*

	Experiment 1			Experiment 2		
Cultivar	48 h	96 h	144 h	48 h	96 h	144 h
Morocco	0.91 a[z]	0.89 a	0.87 a	0.91 a	0.84 a	1.00 a
Opata 85	0.93 a	0.82 a	0.68 ab	0.86 a	0.61 b	0.57 b
Pavon 76	0.88 a	0.78 a	0.70 ab	0.91 a	0.68 b	0.65 b
Parula	0.89 a	0.76 a	0.55 b	0.85 a	0.65 b	0.69 b

[z] Means in a column followed by the same letter do not differ significantly ($P = 0.05$).

Table 5. Proportion of infection structures with a substomatal vesicle that formed haustorium mother cells on flag leaves of four wheat cultivars at 48, 96, and 144 h after inoculation with urediospores of isolate 89009 (race 14E14) of *Puccinia striiformis*.

	Experiment 1			Experiment 2		
Cultivar	48 h	96 h	144 h	48 h	96 h	144 h
Morocco	0.17 a[z]	0.58 a	0.91 a	0.29 a	0.64 a	0.81 a
Opata 85	0.01 b	0.50 ab	0.75 a	0.05 b	0.26 b	0.72 a
Pavon 76	0.06 b	0.46 ab	0.73 a	0.12 b	0.43 b	0.63 a
Parula	0.03 b	0.36 b	0.48 b	0.02 b	0.34 b	0.68 a

[z] Means in a column followed by the same letter do not differ significantly ($P = 0.05$).

Table 6. Proportion of infection structures with at least one haustorium mother cell that formed at least five haustorium mother cells on flag leaves of four wheat cultivars at 48, 96, and 144 h after inoculation with urediospores of isolate 89009 (race 14E14) of *Puccinia striiformis*

	Experiment 1			Experiment 2		
Cultivar	**48 h**	**96 h**	**144 h**	**48 h**	**96 h**	**144 h**
Morocco	0.00	0.78 a[z]	1.00 a	0.00 a	0.89 a	1.00 a
Opata 85	0.00	0.81 a	0.91 ab	0.00 a	0.92 a	0.96 ab
Pavon 76	0.00	0.61 a	0.79 bc	0.00 a	0.79 ab	0.83 b
Parula	0.00	0.65 a	0.71 c	0.00 a	0.70 b	0.83 b

[z] Means in a column followed by the same letter do not differ significantly ($P = 0.05$).

Table 7. Latency period and average percentage of infected leaves on flag leaves of four wheat cultivars after inoculation with urediospores of isolate 89009 (race 14E14) of *Puccinia striiformis*

	Latency period (days)			Average percentage of infected leaves		
Cultivar	**Experiment 1**	**Experiment 2**	**Mean**	**Experiment 1**	**Experiment 2**	**Mean**
Morocco	8.9 a[z]	9.1 a	9.0 a	92 a	100 a	96 a
Opata 85	13.6 b	12.8 b	13.2 b	81 ab	72 b	77 b
Pavon 76	17.6 c	17.9 c	17.8 c	66 b	50 b	58 c
Parula	18.4 c	21.4 d	19.9 d	6 c	10 c	5 c

[z] Means in a column followed by the same letter do not differ significantly ($P = 0.05$).

pha, the proportion of established infection structures (>5 haustorium mother cells) was lower on the resistant cultivars than on Morocco. Small differences among resistant cultivars were apparent. Opata 85, with a low level of resistance, had a higher survival ratio of established infection structures than Pavon 76 and Parula.

The histological data presented here partially explain the difference between the highly susceptible Morocco and the three resistant cultivars. However, our results do not account for differences among the resistant cultivars. In a study in which components of resistance were assessed, Opata 85, Pavon 76, and Parula had infection frequencies of 45, 9, and 0.6%, respectively, relative to that of Morocco (2). These relative infection frequencies are likely to be an overestimation because the way infection frequency was assessed gives rise to an underestimation of the absolute infection frequency of Morocco. Assuming an underestimation by a factor two, the relative infection frequencies of the resistant cultivars would be 22.5, 4.5, and 0.3%.

The infection frequency for each cultivar in the study presented here was estimated by multiplying the four survival ratios. This resulted in relative infection frequencies of 27, 21, and 9%. Only for Opata 85 was a reasonable estimate obtained. The infection frequency of the other two resistant varieties was largely overestimated. Therefore, the proportion of failed infection attempts detected in the first 6 days of the infection cycle cannot fully explain the low infectibility of Pavon 76 and Parula. Phenomena that were not studied here, such as germ tube orientation and events taking place at later stages during the fungus development, may further determine the cultivar differences in infection frequencies among the resistant cultivars.

The objective of this study was to reveal information about the mechanism of quantitative resistance to *P. striiformis* in wheat. Our data have shown that resistance acts at most stages of fungal development. The major effect of quantitative resistance on the fungus was an avoidance mechanism that reduced the formation of appressoria. This was followed by a possible disintegration of substomatal vesicles and a delayed development of infection hyphae and subsequent establishment. We speculate that the avoidance mechanism might be durable as it is probably a (morphological) barrier acting before intimate contact between host and parasite. The durable nature of the stripe rust resistance in Pavon 76 supports our hypothesis.

ACKNOWLEDGMENTS

The research was financed by the Dutch Minister for Development Cooperation (DIGS/DST/SO) as part of a collaborative project between the International Maize and Wheat Improvement Center (CIMMYT), Mexico, and the Department of Plant Breeding of the Wageningen Agricultural University (WAU), The Netherlands. We thank J. E. Parlevliet (WAU), R. Niks (WAU), and E. Duveiller (CIMMYT) for critically reading the manuscript.

LITERATURE CITED

1. Broers, L. H. M. 1993. Breeding for partial resistance in wheat to stripe rust. Pages 179-183 in: Durability of Disease Resistance. T. Jacobs and J. E. Parlevliet, eds. Wageningen, The Netherlands.
2. Broers, L. H. M. Components of quantitative resistance a in spring bread wheat to yellow rust and their relations with field assessments. Euphytica. (In press.)
3. Broers, L. H. M., Cuesta Subía, X., and López-Atilano, R. M. 1996. Field assessment of quantitative resistance to yellow rust in ten spring bread wheat genotypes. Euphytica 90: 9-16.
4. Cartwright, D. W., and Russell, G. E. 1981. Possible involvement of phytoalexins in durable resistance of winter wheat to yellow rust. Trans. Br. Mycol. Soc. 76:323-325.
5. Danial, D. L. 1993. Is partial resistance a suitable approach against stripe rust in wheat? Pages 185-189 in: Durability of Disease Resistance. T. Jacobs and J. E. Parlevliet, eds. Wageningen, The Netherlands.
6. Jacobs, T. 1989. The occurrence of cell wall appositions in flag leaves of spring wheats, susceptible and partially resistant to wheat leaf rust. J. Phytopathol. 127:239-249.
7. Johnson, R., Stubbs, R. W., Fuchs, E., and Chamberlain, N. H. 1972. Nomenclature for physiological races of *Puccinia striiformis* infecting wheat. Trans. Br. Mycol. Soc. 58:475-480.
8. Lee, T. S., and Shaner, G. 1984. Infection processes of *Puccinia recondita* in slow-and fast-rusting wheat cultivars. Phytopathology 74:1419-1423.
9. Mares, D. J., and Cousen, S. 1977. The interaction of yellow rust (*Puccinia striiformis*) with winter wheat cultivars showing adult plant resistance: Macroscopic and microscopic events associated with the resistant reaction. Physiol. Plant Pathol. 10:257-274.
10. Niks, R. E. 1983. Haustorium formation by *Puccinia hordei* in leaves of hypersensitive, partially resistant and nonhost plant genotypes. Phytopathology 73:64-66
11. Qayoum, A., and Line, R. F. 1985. High-temperature, adult-plant resistance to stripe rust of wheat. Phytopathology. 75:1121-1125.
12. Rohringer, R., Kim, W. K., Samborski, D. J., and Howes, N. K. 1977. Calcofluor: An optical brightener for fluorescence microscopy of fungal plant parasites in leaves. Phytopathology 67:808-810.
13. Rubiales, D., and Niks, R. E. 1992. Low appressorium formation by rust fungi on *Hordeum chilense* lines. Phytopathology 82: 1007-1012.
14. Russell, G. E. 1976. Germination of *Puccinia striiformis* uredospores on leaves of adult winter wheat plants. Ann. Appl. Biol. 82:71-78.
15. Singh, R. P. 1992. Genetic association of leaf rust resistance gene *Lr34* with adult plant resistance to stripe rust in bread wheat. Phytopathology 82:835-838.
16. Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics. A Biometrical Approach. McGraw-Hill, Inc., Singapore.
17. Terhune, B. T., Allen, E. A., Hoch, H. C., Wergin, W. P., and Erbe, E. F. 1991. Stomatal ontogeny and morphology in *Phaseolus vulgaris* in relation to infection structure initiation by *Uromyces appediculatus*. Can. J. Bot. 69:477-484.
18. Warren, M., and Keane, P. J. 1993. Mechanisms of adult plant resistance to wheat stripe rust. (Abstr. 12.3.14). Page 227 in: Int. Congr. Plant Pathol., 6th.
19. Zadoks, J. C., Chang, T., and Konzak, C. 1974. A decimal code for the growth stages of cereals. Weed Res. 14:415-421.
20. Zadoks, J. C., and Schein, R. D. 1979. Epidemiology and Plant Disease Management. Oxford University Press, New York.