

## Comparative Histology of Partial Resistance and the Nonhost Reaction to Leaf Rust Pathogens in Barley and Wheat Seedlings

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### ABSTRACT

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The histological responses involved in partial resistance (PR) and nonhost reaction were compared in three barley and three wheat genotypes inoculated with *Puccinia hordei* and *P. recondita tritici*, the leaf rust pathogens of barley and wheat. Nonhost and PR reactions to the leaf rust pathogens were characterized by a high proportion of colonies that were arrested early (ie, immediately after the formation of the first haustorial mother cells) and were associated with little or no plant cell necrosis. Eight

barley genotypes, four with a low level and four with a high level of PR to *P. hordei*, were inoculated with the leaf rust pathogens *P. hordei*, *P. recondita secalis*, and *P. recondita tritici*. The latter pathogen produced reproductive structures on all four barley genotypes with a low level of PR to *P. hordei*, suggesting that alleles for low PR to *P. hordei* also reduced the effectiveness of the reaction to *P. recondita tritici*.

Apart from the hypersensitive resistance, another and less conspicuous type of resistance is known in the barley/leaf rust (caused by *Puccinia hordei* Oth) relation, which is characterized by a slow rate of epidemic development despite the presence of a susceptible infection type. This resistance has been designated partial resistance (PR) (15). Although the epidemiological and genetical aspects of PR are well documented (6,16), little is known about the underlying mechanisms.

In a histological study of the infection process of the leaf rust fungus in barley seedlings, development of a large proportion of leaf rust colonies in partially resistant genotypes was arrested just after the first infection hyphae and a few haustorial mother cells had formed (11). This "early abortion" resembles the nonhost reactions described by Heath (5).

The present study compares the histological responses involved in PR to barley leaf rust with nonhost reactions to other leaf rust pathogens and investigates whether there is a relationship between level of PR and the nature of the nonhost reaction.

### MATERIALS AND METHODS

In the first experiment, the histology of effects associated with PR and nonhost reaction were compared. The plants were established in two 37 × 39-cm planting boxes, each containing three barley and three wheat genotypes. The plants in one box were inoculated with monospore culture 121A of *P. hordei*, those in the other with a monospore culture of *P. recondita tritici*. The barley genotypes were L94, Vada, and 139-4. L94 is highly infectible to *P. hordei*; Vada and 139-4 have a high level of PR. The wheat cultivars, Saratovskaja 210 (S210), Adonis, and Duri, are fully susceptible to the isolate of *P. recondita tritici* that was used, but their level of PR to this pathogen was unknown. The experiment was carried out in two consecutive series. In each genotype/pathogen combination, six or seven primary leaves were inoculated with urediospores applied in a settling tower. Segments of the central parts of the leaves were collected 200 (in the first series) or 225 hr (in the second series) postinoculation (h.p.i.). They were

cleared and stained for fluorescence microscopy (11). The infection units in the leaves were classified according to phase of development at the day of sampling. The classes were nonpenetrant, aborted substomatal vesicle, early aborted colony, and established colony (13). The proportion of abortion in each phase was calculated by dividing the number of infection units arrested in the given phase by the total number of infection units that entered that phase. The numbers of colonies in the four classes were recorded for each leaf. Also the presence of host cell necrosis, recognized by autofluorescence or cell browning, was noted. Generally, the experimental units for statistical analyses were the averages of the responses per leaf, but sometimes the results from two or three leaf pieces with few infection units were combined to form one larger sample.

In the second experiment, the reactions of eight barley genotypes to the leaf rust pathogens of wheat (*P. recondita tritici*) and of rye (*P. recondita secalis*) were compared with the reactions to *P. hordei*. Particularly, the relationship between the level of PR to *P. hordei* and the nature of the reactions to the two other leaf rust pathogens was studied. Four barley genotypes with a high infectibility (19,20), L94, L92, L98, and Akka, and four with a low infectibility (due to early abortion [11]), Vada, C-118, C-123, and C-92, were grown. Each was inoculated with urediospores of a monospore culture of one of the three leaf rust pathogens. The leaf pieces were collected at 275 h.p.i. for those inoculated with *P. hordei*. Plants inoculated with the other leaf rust pathogens were sampled 2 days later. The experimental process and the nature of the observations were the same as in the first experiment, but in addition the colony lengths of 15 established colonies per leaf were assessed, if available, with an eyepiece micrometer. This experiment was carried out twice in two consecutive series.

### RESULTS

The three leaf rust pathogens could be distinguished by the morphology of their substomatal vesicles (SSV) (Fig. 1). Generally, the SSV of *P. hordei* was cigar-shaped with a transverse septum in the middle. From the SSV, two infection hyphae developed, growing parallel to the long axis of the leaf. The SSV of *P. recondita secalis* was more slender and the septum was either absent or hardly visible. The SSV of *P. recondita tritici* was egg-shaped and developed one infection hypha, which tended to grow transversely to the long axis of the leaf. The thickness of the hyphae

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of *P. hordei* was about half those of the two other leaf rust pathogens (~2.5 and 5.0  $\mu\text{m}$  in diameter, respectively).

In the first experiment, uredia of a low infection type (IT 5 on the scale of McNeal et al [8]) appeared on barley genotype L94 inoculated with *P. recondita tritici*. The colonies had hyphae ~5.0  $\mu\text{m}$  in diameter. Urediospores from these uredia were transferred to a few plants of barley (cultivar L98) and wheat (cultivar Kaspar) by means of a brush. After incubation of these plants, only Kaspar showed a susceptible reaction (IT 9). *P. recondita tritici* did not give visible symptoms in Vada and 139-4, nor did *P. hordei* in the three wheat genotypes.

There were no consistent differences among the plant/rust pathogen species combinations in the proportions of penetrations by appressoria through the stomata (Table 1). There was a tendency towards a reduced stomatal penetration on nonhosts (eg, cultivars Duri and Vada), but the opposite also occurred (S210, series 2). Often, the proportions of nonpenetrating appressoria of either pathogenic or nonpathogenic *Puccinia* species on a plant genotype hardly differed (eg, genotype 139-4). The degree of nonpenetration on the partially resistant genotypes Vada and 139-4 was not higher than on the highly infectible L94.

There was little SSV abortion in either the host or nonhost combinations.

In Table 2, the proportions of early aborted colonies are presented. In the combinations that were considered nonpathogenic, the degree of early abortion was clearly higher than in the pathogenic combinations. In the wheat/*P. hordei* combination, practically all colonies were arrested early. The degree of early abortion of *P. hordei* in the host genotypes Vada and 139-4 was higher than in L94 reflecting differences in level of PR. In the three wheat cultivars, few colonies of *P. recondita tritici* aborted early. The three cultivars did not differ significantly in degree of early abortion (Kruskal-Wallis test,  $P \leq 0.05$ ). In the combinations (host as well as nonhost) with a high degree of early abortion, usually less than 50% of the early aborted colonies were associated with plant cell necrosis, but in wheat the degree of necrosis was not easily reproducible (Table 2).

The leaf segments were sampled before the latent period had elapsed. Consequently, the percentage of colonies that formed uredia could not be assessed. As can be concluded from Table 2, the proportions of established colonies (ie, 1.0 minus the proportion of early aborted colonies) was high in the host/pathogen combinations with L94, S210, Adonis, and Duri and moderate in the partially resistant Vada and 139-4 with *P. hordei*. Of the combinations considered to be nonhost combinations, L94/*P. recondita tritici* gave the highest proportion of established colonies.

In the second experiment, the eight barley genotypes showed no reproducible differences in degree of nonpenetration and SSV abortion in any of the leaf rust pathogens. Therefore, for each leaf rust pathogen the proportions of these types of abortion were averaged over the genotypes (Table 3). The degrees of nonpenetration and SSV abortion of the nonpathogenic *P.*

*recondita tritici* and *P. recondita secalis* were not higher than those of the pathogenic *P. hordei*.

The eight genotypes represented a wide range in level of PR to *P. hordei*, as was manifested by the large differences in proportion of early abortion and the average length of the established colonies of this pathogen (Table 4). In L98, which showed a low level of PR in previous studies (18,20), a high proportion of *P. hordei* colonies aborted early.

All the colonies of *P. recondita secalis* were arrested before they had produced visible symptoms in barley. Arbitrarily, colonies were considered "established" when at least eight haustorial mother cells (HMC) were observed. Such colonies usually were more branched than shown in Fig. 1C; they were much smaller than the established colonies of *P. recondita tritici* in barley and mostly lacked cell necrosis. Colonies with seven or less HMCs were considered early aborted. The proportion of early aborted colonies

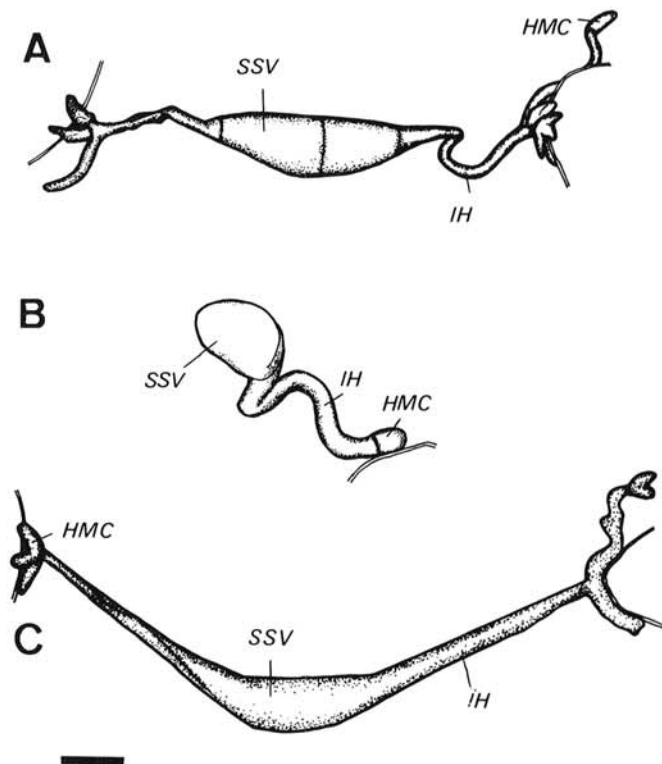


Fig. 1. Typical shape of early aborted colonies of A, *Puccinia hordei*, B, *P. recondita tritici*, and C, *P. recondita secalis* in primary leaves of barley. Indicated are substomatal vesicles (SSV), infection hyphae (IH), and haustorial mother cells (HMC). Part of the walls of barley mesophyll cells are drawn. The bar represents 10  $\mu\text{m}$ .

TABLE 1. Average proportion of nonpenetrating appressoria when the primary leaves of three barley and three wheat genotypes were inoculated with urediospores of *Puccinia hordei* and *P. recondita tritici*

Species inoculated	Genotype	Proportion of nonpenetrating appressoria <sup>a</sup>			
		Series 1		Series 2	
		<i>P. hordei</i>	<i>P. recondita tritici</i>	<i>P. hordei</i>	<i>P. recondita tritici</i>
Barley	L94	0.47	0.46	0.13	0.28
	Vada	0.14	0.24	0.08	0.12
	139-4	0.24	0.26	0.13	0.15
Wheat	S210	<i>P. recondita tritici</i> 0.26	<i>P. hordei</i> 0.31	<i>P. recondita tritici</i> 0.24	<i>P. hordei</i> 0.08
	Adonis	0.64	0.74	0.29	0.27
	Duri	0.26	0.69	0.13	0.15
Average		0.34	0.45	0.17	0.18

<sup>a</sup> Each entry is based on observations of six or seven leaves.

TABLE 2. Average proportions of early aborted colonies of *Puccinia hordei* and *P. recondita tritici* in the primary leaves of three barley and three wheat genotypes and the degree of cell necrosis, associated with early abortion

Species inoculated	Genotype	Proportion of early aborted colonies <sup>a</sup> in Series:				Proportion of early aborted colonies associated with host cell necrosis <sup>b</sup> in Series:			
		<i>P. hordei</i>		<i>P. recondita tritici</i>		<i>P. hordei</i>		<i>P. recondita tritici</i>	
		1	2	1	2	1	2	1	2
Barley	L94	0.08	0.02	0.78	0.83	0.45	0.50	0.18	0.17
	Vada	0.47	0.45	0.90	0.97	0.01	0.04	0.02	0.03
	139-4	0.43	0.46	0.99	0.97	0.00	0.05	0.06	0.03
Wheat	S210	0.06	0.00	1.00	0.99	0.17	...	0.41	0.54
	Adonis	0.08	0.02	1.00	1.00	0.00	0.50	0.16	0.48
	Duri	0.11	0.03	1.00	1.00	0.00	0.33	0.12	0.35

<sup>a</sup> Calculated as proportion to the total number of colonies that formed at least one haustorial mother cell.

<sup>b</sup> Calculated as proportion to the number of early aborted colonies.

TABLE 3. Average proportions of nonpenetrating appressoria and aborted substomatal vesicles (SSV) of three *Puccinia* spp. leaf rust pathogens in seedlings of eight barley genotypes

Rust pathogen	Proportion of non-penetrating appressoria in Series		Proportion of aborted SSVs in Series	
	1	2	1	2
<i>P. hordei</i>	0.10	0.05	0.01	0.03
<i>P. recondita secalis</i>	0.04	0.04	0.01	0.03
<i>P. recondita tritici</i>	0.08	0.05	0.01	0.04

of *P. recondita secalis* was high in all the barley genotypes (Table 4). In the first series, the genotypic differences for early abortion were insignificant, in the second series significant according to the Kruskal-Wallis test ( $P \leq 0.01$ ). The majority of the early aborted colonies of *P. recondita secalis* induced a marked thickening of the mesophyll cell wall at the place of contact with the HMC (Fig. 2A). In the preparations for fluorescence microscopy, these reactions were only visible when white light was used. The deposits probably contained callose, since they showed a bright yellow fluorescence after being stained with aniline blue in 0.07 M  $K_2HPO_4$ , pH 8.9, and exposed to UV light (see reference 4) (Fig. 2B). Such a reaction was absent or much less pronounced than that associated with early abortion of *P. hordei* due to PR. With *P. recondita secalis*, no reproducible differences between the genotypes were found for the average number of HMCs per colony, colony length, or the degree of cell necrosis associated with the colony abortion.

The colonies of *P. recondita tritici* could be classified unambiguously into the two groups, "early aborted" and "established." Also with this fungus, a high proportion of the colonies aborted early, i.e. after the formation of one to four HMCs (Table 4). It could not be established whether a similar deposit of callose-containing material occurred as with *P. recondita secalis*, since the infection hyphae of *P. recondita tritici* grew into the deeper mesophyll layers. In all genotypes, <20% of the early aborted colonies were associated with necrosis of mesophyll cells. Established colonies were more branched than early aborted ones, and usually they were associated with cell browning. In four of the genotypes, one or more established colonies of *P. recondita tritici* produced urediospores. Such uredia were of IT 5 or lower (Table 4) and had thicker hyphae than the few *P. hordei* colonies that appeared due to contamination. On Akka only some uredia were produced in the first series. Particularly on L92, *P. recondita tritici* succeeded in producing uredia: 6 (series 1) and 10% (series 2) of the infection units that developed beyond the SSV stage produced urediospores. The barley genotypes on which *P. recondita tritici* formed uredia were the four that were chosen as representative of genotypes with a low level of PR to *P. hordei*.

## DISCUSSION

There is a remarkable resemblance between the histological responses involved in PR of barley (to *P. hordei*) and the nonhost reactions of wheat (to *P. hordei*) and barley (to *P. recondita secalis* and *P. recondita tritici*). Neither PR of barley nor nonhost reaction of wheat to *P. hordei* result from reduced appressorium formation (10). With both PR and nonhost reactions, the stoma penetration, SSV and infection hyphae formation were not affected (Tables 1 and 3). With cowpea rust, which is induced by *Uromyces phaseoli* var. *vignae*, reduced stoma penetration and SSV formation in some nonhost species was reported (4,5). Rust pathogens of grasses, however, readily formed appressoria and SSVs in graminaceous

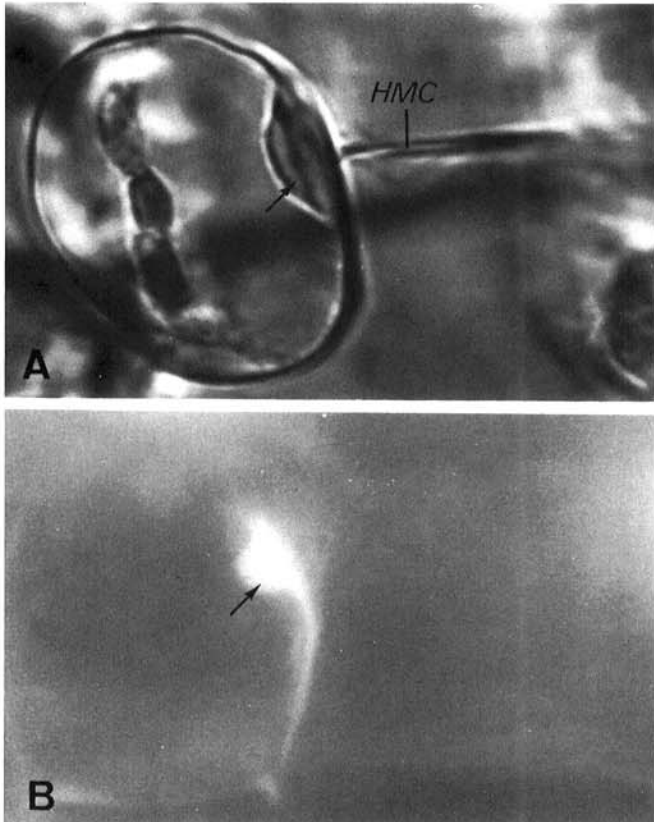


Fig. 2. Deposit of callose containing material (arrow) in barley seedling adjacent to a haustorial mother cell (HMC) of *Puccinia recondita secalis*. A, White light micrograph in a whole-mount preparation prepared for fluorescence microscopy; B, Fluorescence micrograph after staining in 0.005% aniline blue in 0.07 M  $K_2HPO_4$ , pH 8.9. The fluorescence of the deposit is clearly visible. Both figures  $\times 1,560$ .

TABLE 4. Proportion of early aborted colonies, length of established colonies (in micrometers), and infection type measured by the system of McNeal et al (8) of *Puccinia hordei*, *P. recondita tritici*, and *P. recondita secalis* in seedlings of eight barley genotypes

Barley genotype	Proportion of early abortion					Length of established and successful colonies				Infection type		
	<i>P. hordei</i> <sup>w</sup>	<i>P. recondita tritici</i>		<i>P. recondita secalis</i>		<i>P. hordei</i>		<i>P. recondita tritici</i>		<i>P. hordei</i>	<i>P. recondita tritici</i>	<i>P. recondita secalis</i>
		1 <sup>x</sup>	2	1	2	1	2	1	2			
L94	0.09	0.86	0.88	0.96	0.92	1,848 a <sup>y</sup>	1,869 a	254	820	9	1-5	0
L92	0.11	0.83	0.65	0.92	0.96	1,747 a	1,648 b	506	712	9	5	0
Akka	0.16	0.96	0.99	0.94	1.00	1,742 a	1,489 bc	358	603	9	0-5	0
L98	0.48	0.88	0.97	0.99	1.00	1,541 b	1,415 bc	305	95	9	5	0
Vada	0.49	0.98	0.98	0.94	0.98	1,373 c	1,140 d	140	232	9	0	0
C-118	0.56	0.99	1.00	0.99	1.00	1,350 c	1,455 bc	181	0	9	0	0
C-123	0.58	0.98	1.00	0.99	1.00	1,382 c	1,377 c	146	0	9	0	0
C-92	0.48	0.99	1.00	0.97	0.99	1,381 c	1,305 cd	251	0	9	0	0
Significance of genotype effect (Kruskal-Wallis) <sup>z</sup>	**	**	**	-	**							

<sup>w</sup>No significant series effect (Kruskal-Wallis,  $P < 0.01$ ).

<sup>x</sup>The experiment was carried out in two series.

<sup>y</sup>Per column different letters indicate a significant difference ( $P \leq 0.05$ ) according to Duncan's multiple range test.

<sup>z</sup>Asterisks (\*\*) indicate significance ( $P \leq 0.01$ ); - = nonsignificance.

nonhosts (14,21). PR and nonhost reactions to leaf rusts were associated with a high degree of early abortion with little or no cell necrosis (Tables 2 and 4) and a reduced size of the established colonies at the time of sampling (Table 4). In the nonhost reaction, these effects were more extreme than with PR. Not only were the proportions of early abortion higher in nonhost reactions, but the deposition of callose-containing material at the contact points with HMCs of *P. recondita secalis* suggested a more extreme reaction to infection by a nonpathogen. A substantial colony abortion immediately after the formation of the first HMCs was mentioned also for other nonhost combinations (5,9,21). Deposition of callose in association with the lack of haustoria does not seem to be common in nonhost relations (5). The resemblance between PR and nonhost reactions was furthermore expressed by the fact that the early abortion of colonies of *P. hordei* in both nonhost wheat and partially resistant barley Vada was associated with a failure in the formation of haustoria (12).

The results suggested that barley is an intermediate form of host and nonhost to *P. recondita tritici*: five of the nine barley genotypes gave a symptomless reaction to the fungus and in none of the genotypes was sporulation abundant. *P. recondita tritici* was reported to attack *Hordeum* spp. occasionally (1), which leaves it an open question whether barley is to be considered a host or nonhost. The established colonies of *P. recondita tritici* in barley were associated with chlorosis and necrosis of plant tissue (Table 4). Such a hypersensitive type of reaction in hosts inoculated with a poorly adapted forma specialis is routinely found with rusts of Gramineae (2,7). Established colonies of *P. hordei* in partially resistant genotypes of barley are (by definition) of a fully susceptible infection type (Table 4).

The level of PR of the barley genotypes was not obviously related with differences in nature of nonhost reaction to *P. recondita secalis*, but the results suggested a relationship between PR and the nature of reaction to *P. recondita tritici*. The barley genotypes in which *P. recondita tritici* produced urediospores were all highly infectible to *P. hordei* in this study, except for L98, but this genotype had a low level of PR in previous studies (18,20). This suggests that alleles for low PR to *P. hordei* also reduce the effectivity of the reaction to *P. recondita tritici*. There is, however, evidence that slow rusting genes or genes for horizontal resistance are pathogen species-specific (3,17,22). Therefore, more barley genotypes have to be tested with more *P. recondita tritici* isolates to establish whether the relation between level of PR to *P. hordei* and level of nonhost reaction to *P. recondita tritici* suggested by the results of the present study is real or merely coincidental.

#### LITERATURE CITED

- Anikster, Y., and Wahl, I. 1979. Coevolution of the rust fungi on Gramineae and Liliaceae and their hosts. *Annu. Rev. Phytopathol.* 17:367-403.
- Eshed, N., and Dinooor, A. 1980. Genetics of pathogenicity in *Puccinia coronata*: pathogenic specialization at the host genus level. *Phytopathology* 70:1042-1046.
- Gavinlertvatana, S., and Wilcoxson, R. D. 1978. Inheritance of slow rusting of spring wheat by *Puccinia recondita* f. sp. *tritici* and host parasite relationships. *Trans. Br. Mycol. Soc.* 71:413-418.
- Heath, M. C. 1974. Light and electron microscope studies of the interactions of host and nonhost plants with cowpea rust *Uromyces phaseoli* var. *vignae*. *Physiol. Plant Pathol.* 4:403-414.
- Heath, M. C. 1977. A comparative study of non-host interactions with rust fungi. *Physiol. Plant Pathol.* 10:73-88.
- Johnson, D. A., and Wilcoxson, R. D. 1979. Inheritance of slow rusting of barley infected with *Puccinia hordei* and selection of latent period and number of uredia. *Phytopathology* 69:145-151.
- Johnson, T., and Buchannon, K. W. 1954. The reaction of barley varieties to rye stem rust, *Puccinia graminis* var. *secalis*. *Can. J. Agric. Sci.* 34:473-482.
- McNeal, F. H., Konzak, C. F., Smith, E. P., Tate, W. S., and Russell, T. S. 1971. A uniform system for recording and processing cereal research data. U.S. Dep. Agric., ARS 34-121. Washington, DC.
- Mendgen, K. 1978. Attachment of bean rust cell wall material to host and non-host plant tissue. *Arch. Microbiol.* 119:113-117.
- Niks, R. E. 1981. Appressorium formation of *Puccinia hordei* on partially resistant barley and two non-host species. *Neth. J. Plant Pathol.* 87:201-207.
- Niks, R. E. 1982. Early abortion of colonies of leaf rust, *Puccinia hordei*, in partially resistant barley seedlings. *Can. J. Bot.* 60:714-723.
- Niks, R. E. 1983. Haustorium formation by *Puccinia hordei* in leaves of hypersensitive, partially resistant, and nonhost plant genotypes. *Phytopathology* 73:64-66.
- Niks, R. E., and Kuiper, H. J. 1983. Histology of the relation between minor and major genes for resistance of barley to leaf rust. *Phytopathology* 73:55-59.
- Ogle, H. J., and Brown, J. F. 1971. Quantitative studies of the postpenetration phase of infection by *Puccinia graminis tritici*. *Ann. Appl. Biol.* 67:309-319.
- Parlevliet, J. E. 1978. Race-specific aspects of polygenic resistance of barley to leaf rust, *Puccinia hordei*. *Neth. J. Plant Pathol.* 84:121-126.
- Parlevliet, J. E. 1979. Components of resistance that reduce the rate of epidemic development. *Annu. Rev. Phytopathol.* 17:203-222.
- Parlevliet, J. E. 1981. Disease resistance in plants and its consequences for breeding. Pages 309-364 in: *Proc. Plant Breeding Symp. II*. K. J. Frey, ed. Iowa State Univ., Ames.
- Parlevliet, J. E., and Kuiper, H. J. 1977. Partial resistance of barley to leaf rust, *Puccinia hordei*. IV. Effect of cultivar and development stage on infection frequency. *Euphytica* 26:249-255.

19. Parlevliet, J. E., Lindhout, W. H., Van Ommeren, A., and Kuiper, H. J. 1980. Level of partial resistance to leaf rust, *Puccinia hordei*, in West-European barley and how to select for it. *Euphytica* 29:1-8.
20. Parlevliet, J. E., and Van Ommeren, A. 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. II. Relationship between field trials, micro plot tests and latent period. *Euphytica* 24:293-303.
21. Tani, T., Yamamoto, H., Ohasa, Y., and Yamashita, Y. 1978. Non-host response of oat leaves against rust infection. *Ann. Phytopathol. Soc. Jpn.* 44:325-333.
22. Vanderplank, J. E. 1978. *Genetic and Molecular Basis of Plant Pathogenesis*. Springer-Verlag, Berlin, Heidelberg, and New York. 167 pp.