STUDIES ON PARTIAL RESISTANCE IN WHEAT TO WHEAT LEAF RUST

Authors' abstract

Partial resistance (PR) of wheat to wheat leaf rust acted in two steps. The first step led to a reduced infection frequency (IF), the second step to a longer latency period (LP).
The genes for a prolonged LP inherited in a recessive way and showed additive gene action. The PR-genotype Akabozu carried two and Westphal 12A three LP-prolonging genes. BH 1146 contained one HR gene and two or three LP genes. Transgression was observed in the progenies.
PR, measured as LP, was best expressed in the young flag leaf stage, at low temperatures. LP is a good estimator for PR.
Three epidemiological parameters were used to assess PR in the field: 1) disease severity at the time that susceptible controls are severely rusted, 2) AUDPC, 3) the logistic growth rate. 1) and 2) are reliable estimators for PR, 3) appeared unsuitable. Long LP and low IF were correlated.
Conclusive evidence for or against race specificity of PR has not been obtained. PR was expressed in a wide range of environments, indicating that PR is stable. The ranking orders were similar at all locations and years. The PR studied might be durable. The PR in BH 1146 is effective since 1955.

L.H.M. Broers

Promotoren dr. ir. J.E. Parlevliet, hoogleraar in de plantenveredeling.
dr. J.C. Zadoks, hoogleraar in de fytopathologie.

Th. Jacobs

Promotor dr. ir. J.E. Parlevliet, hoogleraar in de plantenveredeling.

L.H.M. Broers

Th. Jacobs

HISTOLOGICAL, GENETICAL AND EPIDEMIOLOGICAL STUDIES ON PARTIAL RESISTANCE IN WHEAT TO WHEAT LEAF RUST

Proefschrift

ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
in het openbaar te verdedigen
op woensdag 25 januari 1989
des namiddags te kwart voor drie,
respectievelijk te vier uur in de aula
van de Landbouwuniversiteit te Wageningen.

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STELLINGEN

1. De α-tending emulgator glycerollactopalmitaat (GLP) kan beneden de kristallisatietemperatuur een stabiele α-gelfase vormen in de aanwezigheid van water.
   Dit proefschrift.

2. De vergelijking, die Sebba tracht te maken tussen de fysische analogie van de structuur van een waterig schuim en die van levende cellen, is bijzonder hachelijk.
   F. Sebba, Foams and Biliquid Foams-Aphrons. Ed : Wiley and Sons, (1987).

3. Agglomeratie van vetbolletjes in toppings, die een relatief hoog gehalte aan GLP op vetbasis bevatten, hangt nauw samen met de vorming van een α-gelfase op het o/w-grensvlak van de deeltjes.
   Dit proefschrift.

4. Een emulgatormengsel dat beneden de kristallisatietemperatuur van de koolwaterstofketens een α-gelfase kan vormen met water kan dit onafhankelijk doen van het feit of dit mengsel boven de kristallisatietemperatuur mesomorfe fasen vormt met water.
   Dit proefschrift.

5. Hoewel men in de levensmiddelenindustrie graag zou beschikken over vetongevoelige eiwitschuimen, lijkt dit vanuit fundamenteel oogpunt, althans voorlopig, geen haalbare zaak.
   N.a.v. De 23^{e} themadag georganiseerd door de NRLO : luchtige levensmiddelen, hun bereiding en eigenschappen, (1987).

6. De veronderstelling dat amorfe lactose bij bevochtiging met water beneden 93.5°C practisch alleen in α-lactose monohydraat overgaat is aan bedenkingen onderhevig.
   Zie bv. P. Morrissey, In : Developments in Dairy Chemistry-3. , Ed : P.F. Fox, Elsevier Applied Science Publishers, (1985).

7. Het aantreffen van troebelingen in bier kan ondermeer worden veroorzaakt door schuimvorming in de fles.

8. Op grond van resultaten bereikt met pulse NMR aan toppingpoeders mogen Barfod en Krog niet concluderen dat geadsorbeerde eiwitten de kristallisatie van het vet/emulgatormengsel in de gedispergeerde deeltjes onderdrukken.
   N.M. Barfod en N. Krog, J. Am. Oil. Chem. Soc. , 64, 112-119, (1987).

9 Eén en dezelfde emulgator kan zowel schuim stabiliserend als destabiliserend werken.

10. Om de eigenschappen van levensmiddelen, die bestaan uit complexen van biopolymeren, beter te kunnen begrijpen, verdient het de aanbeveling een integratie te bewerkstelligen tussen levensmiddelenfysica en -chemie.

11. Het succes van technologische vernieuwing staat of valt met de betrokkenheid van de werknemers.

12. Golf maakt een goede kans om uit te groeien tot een nationale sport, indien er geen haast gemaakt wordt met het oplossen van onze afvalproblematiek.

13. Uitgerekend op rekencentra lijkt men in de toekomst te zijn uitgerekend.

Stellingen behorende bij het proefschrift " Contribution of the α-gel phase to the stability of whippable emulsions " door J.M.M. Westerbeek, 24 februari 1989 te Wageningen.

1 Het gebruik van de term "vroege abortie"* bij de roest-pathogenen impliceert het voorkomen van "late abortie", aangezien dat laatste in partieel resistente tarwe niet is waargenomen, kan de toevoeging "vroege" vervallen.

* R.E. Niks, 1983. Studies on the histology of partial resistance in barley to leafrust, Puccinia hordei. proefschrift, LH Wageningen.

2 Ondanks een verschillende morfologie van enerzijds de gastheercelwand-toevoegingen in tarwe tegen bruine roest en anderzijds de celwandtoevoegingen in tarwe tegen septoria, in gerst tegen meeldauw en in gerst tegen dwergroest, hebben de celwandtoevoegingen een overeenkomstige funktie.

3 Het werk van een aantal roest-onderzoekers kan niet volledig beoordeeld worden omdat zij het infektietype van de bestudeerde interaktie niet in hun publikaties vermelden.

4 De genen in Westphal 12A en Akabozu die coderen voor een langere latentie-periode moeten opgenomen worden in het systeem van bijna-isogene lijnen.

5 Duurzaamheid van resistenties kan niet tijdens een veredelingsprogramma bepaald worden.

6 Bijna-isogene lijnen zijn bijna altijd niet bijna-isogeen.

Stam, P and A.C. Zeven, 1981. The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing. Euphytica 30:227-238.

Zeven, A.C., and J. Waninge, 1986. The degree of phenotypic resemblance of the near-isogenic lines of the wheat cultivar Thatcher with their recurrent parent. Euphytica 35:665-676.

7 De aanduiding "widely grown for a long period" (Johnson, 1984) moet nader gespecificeerd worden.

R. Johnson, 1984. A critical analysis of durable resistance. Annual Review of Phytopathology 22:309-330.

8 In Zuid en Noord Amerika is geen sprake van een "africanized bee" maar van een "african bee".

Rinderer, T.E., 1986. Bee genetics and breeding. Academic press.

9 De kans dat er zich voor het jaar 2000 een desastreus ongeluk voordoet in de kerncentrale Dodewaard is groter dan de kans dat de centrale stilgelegd en ontmanteld wordt voor dat jaar.

10 Er is geen enkele reden om geen voorstander te zijn van politieke gelijkheid in Zuid Afrika (one person - one vote).

11 Een goede kollega is het halve werk.

Stellingen behorende bij het proefschrift "histological, genetical and epidemiological studies on partial resistance in wheat to wheat leaf rust", publiekelijk te verdedigen door Th. Jacobs, woensdag 25 januari 1989 des namiddags te kwart over vier in de aula van de landbouwuniversiteit te Wageningen.

1 Selectie op resistentie tegen bruine roest in een tarwepopulatie die zeer heterogeen is voor de eigenschap ontwikkelingssnelheid zal in het algemeen tevens leiden tot selectie op laatheid.

2 Het aantal genen dat betrokken is bij de overerving van een resistentie hoeft niets te zeggen over de duurzaamheid van die resistentie.

3 Het bepalen van de logistische groeisnelheid van bruine roest epidemieën in tarwe is een onbetrouwbare, dure en tijdrovende methode om het niveau van partiële resistentie te bepalen.

4 Het selecteren op partiële resistentie te velde met ongedefinieerde isolaten als infectiebron kan leiden tot de selectie van ongewenste overgevoeligheidsresistentie.

5 Het partiële karakter van een resistentie is geen garantie voor duurzaamheid.

6 Selectie op partiële resistentie moet niet zo zeer plaatsvinden in milieu's die optimaal zijn voor de ontwikkeling van het pathogeen maar veel meer in milieu's die optimaal zijn voor de expressie van resistentie tegen dat pathogeen.

7 Het verdient de aanbeveling om de term incomplete resistentie alleen te gebruiken voor onvolledige overgevoeligheidsresistentie.

8 Voor het veredelingsonderzoek is het een goede zaak dat de Vakgroep Plantenveredeling van de Landbouw Universiteit wordt betrokken bij het Centrum voor Plantenveredelingsonderzoek (CPO).

9 Het vervangen van de OCÉ-copieerapparaten door Nashua-copieerapparaten op de Vakgroep Plantenveredeling is een voorbeeld van verkapte arbeidstijdverkorting.

10 Roest Rust Niet.

11 De extrapolatie van de mate van bezuinigingen en het aantal bejaarden doet het ergste vermoeden voor de ouderenzorg in 2025.

12 Al is de sporter nog zo snel de dopingcontrole achterhaald hem wel (als hij niet slim genoeg is).

Stellingen behorende bij het proefschrift "histological, genetical and epidemiological studies on partial resistance in wheat to wheat leaf rust", publiekelijk te verdedigen door L.H.M. Broers, woensdag 25 januari 1889 des namiddags te kwart voor drie in de aula van de landbouwuniversiteit te Wageningen.

ter nagedachtenis aan TONY

'The reason of my liking to keep the marks for cleanness on plants, which have afterwards become rusty, is that I look upon lateness in becoming affected by it, as a measure of resistance to the parasite; and because I consider that individual plants may transmit to their progeny the valuable quality of resisting the parasite until a late stage of their lives, when it has less time for injuring the grain."

W.J. Farrer, 1898. The making and improvement of wheats for Australian conditions. Agr. Gas. N.S. Wales, 9, 131-168.

VOORWOORD

Wij hadden de afgelopen vier jaar het geluk deel te zijn van de *"ROEST-CLUB"* van de vakgroep Plantenveredeling. De sfeer was uitstekend, wij hebben veel kunnen doen. Een groot aantal mensen heeft ons daarbij geholpen. Zonder de medewerking van de kollega's op de vakgroep, de studenten en de partners binnen het EG project hadden we nooit tot het huidige resultaat kunnen komen.

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De Europese Gemeenschap heeft een gedeelte van het projekt gefinancierd. De inbreng van de projectpartners (LUW Fytopathologie: J.C. Zadoks, W. Hoogkamer; IPO: R.W. Stubs, G.H.J. Kema, R. Daamen; SVP: J. Mesdag, J. Scheepers, C.J.M. Denissen, P. van de Putten) en onze halfjaarlijkse bijeenkomsten waren erg waardevol. J.E. Parlevliet was aanvrager van het projekt, verantwoordelijk voor de tussentijdse verslaglegging en het eindverslag. De heren A.M. van Harten en A. Hendriksen hebben het beheer over de financien gevoerd. Zonder deze inspanningen zouden we dit projekt niet hebben kunnen voltooien.

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INTRODUCTION

Th. Jacobs and L.H.M. Broers

There are more than 3000 rust species in the world (Laundon, 1973). Wheat (*Triticum aestivum*) is parasitized by stripe rust (*Puccinia striiformis*), stem rust (*Pucc. graminis* f.sp. *tritici*) and wheat leaf rust (*Pucc. recondita* f.sp. *recondita*, syn. *Pucc. triticina*). Wheat leaf rust is the commonest, most widely distributed of the cereal rusts. Wherever wheat is grown, wheat leaf rust occurs (Chester, 1946).

Growth cycle of the pathogen

Wheat leaf rust is a macrocyclic rust with a sexual cycle (teliospores, basidiospores, aeciospores) and an asexual cycle (urediospores) (Alexopoulos and Mins, 1979). It is the uredial stage which is of great economical importance, causing large yield losses.

Germination of a urediospore on a wheat leaf is initiated by 100 % relative humidity in a dark period of at least six hours. The germ tube grows towards the stoma, over which it forms an appressorium. Inside the leaf a substomatal vesicle is formed from which an infection hypha grows towards a host cell. At the end of a hypha a haustorial mother cell is formed, from which the host cell wall is penetrated. The haustorium inside the host cell extracts nutrients from the cell for the benefit of the colony, which grows intercellularly. In the central part of a mature colony new urediospores are formed which are released after the epidermis has burst.

Resistance

Many modern cultivars are protected against wheat leaf rust by the action of resistance genes which cause hypersensitive host cell collapse. However, the fungal population is able to adapt genetically, resulting in a loss of the effectiveness of resistance. The average life time of wheat leaf rust hypersensitivity resistance (HR) genes is estimated to be about 5 years on global base (Kilpatrick, 1975). In view of this lack of durability of the hypersensitivity resistance genes, research was initiated to investigate other ways to protect the crops against such pathogens. The alternatives can be summarized into two categories.

i) Resistance genes can be managed in such a way that genetic diversity is increased, e.g. the use of multi-lines or cultivar mixtures and/or regional deployment of resistance genes. This management leads to a reduced reproduction of the pathogen population. This decreases the probability of genetical adaptation of the pathogen. This category has been extensively discussed by Mundt and Browning (1985).

ii) More durable forms of resistance have been studied. However, durability of resistance can only be reliably identified in retrospect. A prerequisite for resistance to be considered as durable is that it has been present in a cultivar grown on a large scale and for a long time in environments that are favourable for the pathogen, without loosing its level of resistance (Johnson, 1984). Producing new cultivars is best done by using genotypes with proven durable resistance.

Parlevliet and co-workers studied the barley/barley leaf rust pathosystem. They distinguished two forms of resistance: hypersensitivity resistance (HR) and partial resistance (PR). The HR-genes cause a collapse of the host cell after a haustorium has been formed in the cell. Cells that collapse within a few hours after penetration prevent the supply of nutrients to

the fungus, which leads to a rapid death of the infection structure. This cannot be observed macroscopically. Delayed collapse allows some fungal growth and small yellow, necrotic flecks can be seen by the naked eye. Collapse that is even more delayed allows the formation of urediospores. In all these cases a resistant infection type, ranging from 0 to 6 (scale of McNeal *et al.*, 1971), is observed.

PR can be recognized as a reduced epidemic build-up in the field despite of a susceptible infection type (Parlevliet and van Ommeren, 1975). A susceptible infection type, ranging from 7 to 9, suggests the absence of effective genes for hypersensitive cell collapse. In that case, reduction of the epidemic build-up should be attributed to another resistance mechanism than HR. In partially resistant genotypes the collapse of host cells is probably highly retarded or it does not occur at all.

Research objectives

Based on the definition of PR and the observations made by Parlevliet and co-workers of the barley/barley leaf rust pathosystem, a program was initiated to study PR in wheat to wheat leaf rust. First, a large collection of primitive and advanced spring wheat germplasm was screened for the presence and level of PR. This resulted in a group of genotypes with different levels of PR which were used in the program, which comprised four aspects:

1) histological aspects of PR;
2) epidemiological aspects of PR;
3) genetical aspects of PR;
4) Development of guidelines to recognize PR and to screen for it.

ad 1) Histological comparison of susceptible and partially resistant genotypes could indicate at which phase during the development of a urediospore to a sporulating colony, genes for PR have a major impact.

ad 2) Epidemiological studies should be made of the components of PR measured in the greenhouse, of their relation with PR as measured in the field, the representational value of PR measured in small adjacent plots, the stability of PR in a wide range of environments, the sensitivity of the components of PR to temperature and the race-specific aspects of PR.

ad 3) The genetical aspects of PR were to be evaluated to determine the number of genes present in genotypes with high levels of PR, the dominance relations and the effects of individual genes. The possibility of accumulating genes for PR to obtain higher levels of PR was to be determined as well.

ad 4) Based on the results of the histological, epidemiological and genetical studies, instructions should be generated on screening for PR in wheat to wheat leaf rust: how to recognize it, how to screen for it and how to increase the level of PR.

The following chapters report on these four aspects of PR in wheat to wheat leaf rust.

REFERENCES

Alexopoulos, C.J. and C.W. Mins, 1979. Introductory Mycology. Pp 499-533. John Wiley and Sons.

Chester, K.S., 1946. The nature and prevention of the Cereal Rusts as examplified in the leaf rust of wheat. Chronica Botanica. Waltham, Massachusets, USA.

Johnson, R., 1984. A critical analysis of durable resistance. Annual Review of Phytopathology 22: 309-330.

Kilpatrick, R.A., 1975. New wheat cultivars and longevity of rust resistance 1971-1975. U.S. Agric. Res. Serv. Northeast Reg. ARS-NE NE-64.

Laundon, G.F. 1973. Uredinales. Pp.247-279. In: The Fungi. Vol IVB. Eds: G.C. Ainsworth, F.K. Sparrow and A.S. Sussman. Academic Press, New York.

McNeal, F.M., C.F. Conzak, E.P. Smith, W.S. Tade and T.S. Russel, 1971. A uniform system for recording and processing cereal research data. USDA Agr. Res. Surv. Wash. DC, ARS 34-121.

Mundt, E.C. and J.A. Browning, 1985. Genetic diversity and cereal rust management. In: Cereal Rusts. Vol II. Diseases, Distribution, Epidemiology and Control. Eds: A.P. Roelfs and W.R. Bushnell. Academic Press Inc., London. Pp 527-560.

Parlevliet, J.E., and A.van Ommeren, 1975. Partial resistance of barley to leaf rust *Puccinia hordei*. II. Relationship between field trials, microplot tests and latent period. Euphytica 24: 293-303.

Chapter 1.

GERMINATION AND APPRESSORIUM FORMATION OF WHEAT LEAF RUST ON SUSCEPTIBLE, PARTIALLY RESISTANT AND RESISTANT WHEAT SEEDLINGS AND ON SEEDLINGS OF OTHER GRAMINEAE SPECIES.

Th. Jacobs

SUMMARY

Germination and appressorium formation of wheat leaf rust urediospores were studied in two experiments. No differences could be detected between 11 wheat genotypes (Triticum aestivum), two barley, one Triticum dicoccum, one T. dicoccoides, one T. boeoticum and one Aegilops squarrosa genotype. There were differences in percentage germination and appressorium formation between tips and central portions of the leaves. The results of the study are in line with earlier reports indicating that especially physical signals direct germtube growth and induce appressorium formation. Resistance genes do not influence germination and appressorium formation.

INTRODUCTION

Prepenetration stages in the development of wheat leaf rust (*Puccinia recondita* f.sp. *tritici*, syn. *Pucc. triticina*) urediospores are germination, germ tube growth and formation of an appressorium over a stoma. Chemical and physical stimuli affect growth of the germinating rust spore (Hoch and Staples, 1987; Staples and Macko, 1984). It is believed that germination and germ tube growth require less stimuli than appressorium formation (Goodman *et al.*, 1986). Host resistance is generally not judged an important factor which influences appressorium formation. (Hoch and Staples, 1987). However prepenetration inhibition of a leaf pathogen has been reported (Evans and Pluck, 1978). Hooker (1984) cited Peletz who reported that significantly more corn rust spores germinated on the leaf surface of susceptible than of resistant plants. Poyntz and Hyde (1987) reported a difference in germination but not in appressorium formation between a susceptible and a resistant genotype. Not much is known about the effects of genes for partial resistance on the prepenetration phases.

In this paper germination and appressorium formation of wheat leaf rust have been studied in relation to wheat leaf rust resistance of wheat, barley and species related to ancestors of wheat.

MATERIALS AND METHODS

Two experiments were conducted. In both experiments plants were grown in two rows along the sides of flats (37 x 39 cm). The plants of one genotype occupied part of the row, the location was chosen at random. The seedlings were inoculated at the time that the second leaf was about to emerge. The leaves were flattened on a glass plate placed between the rows. Small iron weights were used to keep the leaves flat with the adaxial sides up. The weights were carefully placed to minimize leaf damage. Urediospores of a monospore culture of the 'Flamingo' race of wheat leaf rust were harvested, dried in an exsiccator during one night, weighed and approximately 1.0 mg spores per flat, mixed with 2.0 mg *Lycopodium* spores, were applied in a settling tower (Eyal *et al.*, 1968). In each corner of a flat a greased glass slide (1.0 x 0.72 cm) was placed for later determination of the number of spores applied. After inoculation, the flats were placed in a greenhouse compartment for incubation during the night. The relative humidity was kept high by means of an electric humidifier. In both experiments three leaves per genotype were not harvested to estimate latency period (Parlevliet, 1975) and infection type on a scale of 0 to 9 (McNeal *et al.*, 1971).

Experiment 1

In the first experiment of five leaves per genotype, leaf segments from the central part of the leaf, each 4.0 cm long, were harvested approximately five and ten hours after the estimated point of darkness (19.00 h), gently boiled in lactophenol:ethanol (1:2) and placed in 0.05% Blancophor for 5 min. (Niks, 1981). Care was taken not to loose germinated urediospores. The glycerol mounted segments were observed under an epi-fluorescence microscope. The total number of germinated urediospores and the total number of appressoria per leaf segment were noted. Urediospores were noted as germinated if the length of the germ tube exceeded the diameter of the spore. The area of the leaf segments was measured. The numbers of germinated urediospores and formed appressoria were converted to numbers per cm^2 for statistical analysis.

Seedlings of twelve genotypes were studied. The barley genotypes L94 and Vada, highly susceptible and partially resistant to barley leaf rust (*Puccinia hordei*), respectively, and non-hosts for wheat leaf rust were compared with six wheat genotypes (nrs 3-8, Table 1-1) and four genotypes related to ancestors of wheat (other *Triticum* species and *Aegilops squarrosa*). The wheat genotypes Saratovskaja 36, CI 9321 and Downy showed a hairy epiderm.

An analysis of variance, four *a priori* tests and one a *posteriori* test (Scheffé) were performed. Assumptions for the *a priori* test were, there is a difference with regard to germination and appressorium formation: i) between barley and wheat, ii) between wheat and *Triticum* species related to ancestors of wheat, iii) between barley and the *Triticum* species and iiii) between hairy and non-hairy wheat genotypes. Calculations were performed with help of the SPSS package on a DEC-10 mainframe of the Agricultural University.

Experiment 2

Experimental procedures were identical to those of Experiment 1. Differences are described below.

A different set of genotypes was used (Table 1-2). Included were the partially resistant genotype Akabozu, the wheat varieties Canthatch, Thatcher and a near-isogenic line of Thatcher incorporating Lr 19 (rl 6040), a gene for hypersensitivity resistance.

The experiment consisted of two series. Seedlings in the first series were sown and inoculated a week earlier and showed longer leaves than seedlings in the second series. Of each genotype five leaves were used. From each leaf two segments, each 4 cm long, were harvested after one night incubation at a relative humidity of 100%. One segment included the top, the other segment was harvested from the central part of the leaf.

A priori assumptions were:

- there is a difference in germination and appressorium formation of urediospores on Thatcher compared to its near isogenic line with Lr 19,
- there is a difference between wheat and the non-wheat genotypes with regard to germination and appressorium formation.

A small experiment was conducted to compare the method used in this paper with a treatment in which the leaves were gently sprayed with a solution of Blancophor. The mean number of germinated urediospores were counted. The experiment showed that non-germinated urediospores, which lacked a germ tube to provide adhesion to the leaf surface, were partly removed by boiling.

RESULTS

Experiment 1

Estimated latency periods ranged from four to eight days (Table 1-1). Most genotypes studied showed a susceptible infection type. The barley genotype Vada and *Triticum boeoticum* did not show any visible response after inoculation with wheat leaf rust. In the barley genotype L94 small colonies with chlorosis developed. None of the *a priori* contrasts was significant. The number of germinated urediospores per genotype was 24.7 per cm^2 five hours after inoculations and 31.7 five hours later. The number of appressoria averaged 12.2 per cm^2 after 5 hours and 13.8 after 10 hours (Table 1-1). In the first experiment there were

no significant differences in numbers of germinated urediospores and of appressoria per cm^2 between the genotypes. The data do not show an association between latency period or infection type and number of germinated urediospores per cm^2.

Experiment 2

The infection types of all genotypes were high (8-9) except for Lr 19 and the barley genotypes L94 and Vada. Relative latency period ranged from 100 for *Aegilops squarosa* to 127 for the partially resistant wheat genotype Akabozu (Table 1-2). The average number of germinated urediospores per cm^2 in series 2 was considerably higher than in series 1. This was probably caused by differences in inoculum density. The two tested *a priori* contrasts were not significant. In the first series no genotypic differences in germinated urediospores per cm^2 or number of appressoria per cm^2 were observed (Table 1-2). In the second series the numbers of ger-

Table 1-1. Estimated latency period in days (LP50), infection type (IT), number of germinated (germ.) urediospores of wheat leaf rust and number of appressoria (appr.) per cm^2 at two times for two barley genotypes, six wheat genotypes and four other gramineae species.

Nr	Genotype	LP50	IT	5 hours		10 hours	
				germ. *	appr. *	germ. *	appr. *
1	L94 (barley)	8	3	23.4	12.2	17.3	6.7
2	Vada (barley)	-	0	18.3	9.0	21.5	8.0
3	Saratovskaja 36	4	9	29.9	14.8	25.0	9.5
4	Melchior	7	9	16.2	7.2	33.4	15.1
5	Bonza Sib	7	9	33.3	16.5	25.1	11.2
6	Kaspar	6	9	11.9	13.6	28.8	15.8
7	CI 9321	6	9	25.5	10.5	42.3	14.6
8	Downy	6	9	24.3	10.8	32.9	6.6
9	*Triticum dicoccum*	8	8	31.0	16.4	28.7	6.1
10	*Triticum dicoccoides*	8	9	35.4	19.3	42.5	23.0
11	*Aegilops squarrosa*	4	9	25.9	9.1	48.3	24.5
12	*Triticum boeoticum*		0	21.5	6.5	34.9	14.7
Average				24.7	12.2	31.7	13.8

*) none of the values within the column differed significantly from each other according to the Scheffé test.

Table 1-2. Latency period relative to *Aegilops squarrosa* (RLP50), number of germinated urediospores (germ.) and appressoria (appr.) per cm^2 of wheat leaf rust on 4 cm long segments from the top and central part of seedling leaves of wheat and other gramineae species.

Nr	Genotype	RLP50	SERIES 1				SERIES 2			
			TOP		MIDDLE		TOP		MIDDLE	
			germ.	appr.	germ.	appr.	germ.	appr.	germ.	appr.
1	Vada	-	65.4^{a}	50.4^{a}	61.8^{a}	43.4^{a}	164.0^{ab}	130.2^{a}	203.5^{c}	161.3^{abc}
2	Melchior	110.5	54.6^{a}	42.4^{a}	64.3^{a}	56.9^{a}	169.2^{ab}	142.2^{a}	171.9^{bc}	138.6^{abc}
3	Kaspar	113.3	63.3^{a}	53.5^{a}	60.6^{a}	49.5^{a}	181.5^{ab}	156.1^{a}	169.7^{ab}	144.9^{abc}
4	CI 9321	107.2	69.8^{a}	54.9^{a}	76.0^{a}	60.6^{a}	163.6^{ab}	131.0^{a}	98.2^{ab}	79.3^{a}
5	Akabozu	126.8	54.2^{a}	43.9^{a}	94.4^{a}	84.9^{a}	147.0^{a}	127.8^{a}	174.8^{ab}	155.9^{abc}
6	Canthatch	106.5	58.4^{a}	47.9^{a}	83.1^{a}	69.4^{a}	159.3^{ab}	131.4^{a}	211.2^{bc}	176.2^{c}
7	Thatcher	107.6	72.2^{a}	60.6^{a}	61.7^{a}	49.1^{a}	175.3^{b}	137.0^{a}	192.6^{ab}	170.2^{c}
8	Lr 19	-	81.5^{a}	66.8^{a}	82.8^{a}	69.1^{a}	165.9^{ab}	133.0^{a}	194.0^{ab}	162.5^{c}
9	*Triticum dicoccum*	109.2	92.4^{a}	75.2^{a}	80.2^{a}	69.2^{a}	125.5^{ab}	101.1^{a}	198.3^{ab}	173.3^{c}
10	*Aegilops squarrosa*	100.0	76.9^{a}	67.1^{a}	119.0^{a}	108.2^{a}	152.4^{a}	136.2^{a}	98.3^{a}	82.3^{ab}

*) per column values with the same letter do not differ significantly at the 0.05 level (Scheffé test).

minated urediospores with or without an appressorium seemed to follow a random pattern. No relation between latency period and number of germinated urediospores appeared from the data. According to the Anova the number of appressoria per cm^2 did not show a genotypic influence on the top segments (series 1, $p = 0.37$, series 2, $p = 0.48$) but on the central parts of the leaves there were differences between the genotypes ($p = 0.01$ and $p = 0.001$ respectively). No counts were made of the density of stomata of the different genotypes. The actual number of stomata per cm^2 probably differed between the genotypes, a correction should then have been made. This probably would not have altered the results to an important extent.

DISCUSSION

Growth of rust urediospores can be interrupted at several phases (Niks, 1982). In principle each of these phases can be affected by the action of resistance genes. From the data presented no clear relation between genes for hypersensitivity resistance or partial resistance and an altered number of appressoria per cm^2 could be detected. If differences were present they seemed to be caused by external influences and showed random variation. The presence of epidermal hairs does not impede appressorium formation. This is not as strange as it may have been anticipated as the hairs are widely spaced and the urediospores are small compared to the hairs. Approximately five hours after the onset of darkness the majority of appressoria were formed. There was a slight increase in germination and appressorium formation after that time.

The Anova indicated that in the central parts of the leaves a genotypic influence on germination and appressorium formation was present. These differences could not be Attributed to specific genotypes (Table 1-2).

Mahindapala (1978) reported higher germination in the mid portion of the young corn leaf, but mature leaves showed more germinated urediospores in the tip portion. On the cultivar Wampum spore germination and appressorium formation was higher at the base than on the tips (Chang and Line,

1983). The differences in % germination and appressorium formation between the top and the central parts of the leaves as observed in this study are probably related to small differences in environmental conditions and do not seem to be influenced by the action of genes for (partial) resistance.

Other workers reported no significant differences in germination and appressorium formation neither between susceptible and slow leaf-rusting wheat genotypes (Chang and Line, 1983; Gavinlertvatana and Wilcoxson, 1978; Lee and Shaner, 1984), or between near-isogenic lines with different hypersensitivity resistance genes to wheat leaf rust (Plotnikova *et al.*, 1985). Effects of a gene for hypersensitive reaction on the early stages of infection could not be detected in stem rust (Gousseau and Deverall, 1983). Niks (1981) studying barley leaf rust concluded that morphological characteristics of the leaf surface of gramineae species provided signals for appressorium formation irrespective of the host or non-host status of the species. A contact sensitive ability of the germ tube was also reported by others (Staples *et al.*, 1983). Wynn (1976) showed that the protruding lip of guard cells induced formation of the appressorium. The present study indicates that the corresponding external leaf characters of the different gramineae studied induce germ tube growth and appressorium formation in a similar way. There was no difference in germination and appressorium formation between wheat, the non-hosts and other species. This study also shows that genes coding for partial resistance or hypersensitive resistance to wheat leaf rust do not influence the pre-penetration phases.

Lewis and Day (1972) stated that the orientation of wax cristals on the epiderm provides tigmotropic clues for germ tube orientation. Germ tube growth thus would become parallel to the short axis of the leaf. This orientation of germlings was reported by others (Johnson, 1934; Staples and Macko, 1984). During this study observations were made that do not fully support that hypothesis. The majority of germ-tubes growed at right angles to veins, but some grew directly to a nearby stoma, not following lines parallel to the short or long axis of the leaf. Other germ tubes first grew transversely but later curved to return to a stoma, suggesting a response of the germ tube to a chemical signal e.g., pH gradient as reported by Edwards and Bowling (1986). The observed patterns of germ tube growth showed a high degree of similarity with those observed by Clifford *et al.* for barley leaf rust (1985, page 51).

REFERENCES

Chang, K.F. and Line R.F., 1983. ABSTRACT. Urediospore germination and growth of *Puccinia recondita* f.sp. *tritici* in leaves of resistant and susceptible wheat cultivars. Phytopathology 73: 816.

Clifford, B.C., T.L.W. Carver and H.W. Roderick, 1985. The implications of general resistance for physiological investigations. In: Genetic and biochemical mechanisms of plant disea-se. Eds: J.V. Groth and W.R. Bushnell. Symposium book nr 4, American Phytopathological Society.

Edwards, M.C. and D.J.F. Bowling, 1986. The growth of rust germ tubes towards stomata in relation to pH gradients. Physiological and Molecular Plant Pathology 29: 185-196.

Evans, R.L. and D.J. Pluck, 1978. Phytoalexins produced in barley in response to the halo spot fungus, *Selenophoma donacis*. Annals of Applied Biology 89: 332-336.

Eyal, Z., B.C. Clifford and R.M. Caldwell, 1968. A settling tower for quantitative inoculation of leaf blades of mature small grain plants with urediospores. Phytopathology 58: 530-531.

Gavinlertvatana, S. and R.D. Wilcoxson, 1978. Inheritance of slow rusting of spring wheat by *Puccinia recondita* f.sp. *tritici* and host parasite relationships. Transactions of the British Mycological Society 71: 413-418.

Goodman, R.N., Z. Kiraly and K.R. Wood, 1986. The biochemistry and physiology of plant disease. Chapter 1. The infection process. University of Missouri Press, Colombia, USA.

Gousseau, H.D.M. and B.J. Deverall, 1985. Effects of the Sr 15 allele for resistance on development of the stem rust fungus and cellular responses in wheat. Canadian Journal of Botany 64: 626-631.

Hoch, R.C. and R.C. Staples, 1987. Structural and chemical changes among the rust fungi during appressorium development. Annual Review of Phytopathology 25: 231-247.

Hooker, A.L., 1984. Corn and Sorghum rusts. In: The Cereal Rusts, volume II, Diseases, Distribution, Epidemiology and Control. Eds: W.R. Bushnell and A.R. Roelfs. Pp 207-236. Academic Press, Inc.

Johnson, T., 1934. A tropic response in germ tubes of urediospores of *Puccinia graminis tritici*. Phytopathology 24: 80-82.

Lee, T.S., and G. Shaner, 1984. Infection processes of *Puccinia recondita* in slow- and fast-rusting wheat cultivars. Phytopathology 12: 1419-1423.

Lewis, B.G. and J.R. Day, 1972. Behaviour of urediospore germ-tubes of *Puccini graminis tritici* in relation to the fine structure of wheat leaf surfaces. Transactions of the British Mycological Society 58: 139-145.

Mahindapala, R., 1978. Host and environmental effects on the infection of maize by *Puccinia sorghi*. I. Penetration development and penetration. Annals of Applied Biology 89: 411-416.

McNeal, F.H., C.F. Konzak, E.P. Smith, W.S. Tate and T.S Russel. 1971. A uniform system for recording and processing cereal research data. USDA, Agricultural Research Service, Washington, D.C. ARS 34-121.

Niks, R.E., 1981. Appressorium formation of *Puccini hordei* on partially resistant barley and two non-host species. Netherlands Journal of Plant Pathology 87: 201-207.

Niks, R.E., 1982 Early abortion of colonies of leaf rust, *Puccinia hordei*, in partially resistant barley seedlings. Canadian Journal of Botany 60: 714-723.

Parlevliet, J.E., 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. I. Effect of cultivar and development on latent period. Euphytica 24: 21-27.

Poyntz, B. and P.M. Hide, 1987. The expression of partial resistance of wheat to *Puccinia recondita*. Journal of Phytopathology 120: 136-142.

Plotnikova, L.Y., B.G. Reiter, L. Yu and L.V. Meshkova, 1985. Race specific resistance and ectophyte stage development of brown rust. Abstract from: Mikologiya i Fitopatologya 19: 334-347.

Staples, R.C., H.J. Grambow, H.C. Hoch and W.K. Wynn, 1983. Contact with membrane grooves induces wheat stem rust urediospore germlings to differentiate appressoria but not vesicles. Phytopathology 73: 1436-1439.

Staples, R.C. and V. Macko, 1980. Formation of infection structures as a recognition response in fungi. Experimental Mycology 4: 2-16.

Staples, R.C. and V. Macko, 1984. Germination of urediospores and differentiation of infection structures. In: The Cereal Rusts. Vol. I. Origins, Specificity, Structure and Physiology. Eds: W.R. Bushnell and A.R. Roelfs. Pp 255-289.

Wynn, W.K., 1976. Appressorium formation over stomates by the bean rust fungus: response to a surface contact stimulus. Phytopathology 66: 136-146.

Chapter 2

ABORTION OF INFECTION STRUCTURES OF WHEAT LEAF RUST IN SUSCEPTIBLE AND PARTIALLY RESISTANT WHEAT GENOTYPES.

Th. Jacobs

SUMMARY

Arrest of the growth of wheat leaf rust infection structures was studied with fluorescence microscopy in seedling leaves and flag leaves of the susceptible spring wheat genotypes Morocco and Kaspar and the partially resistant genotypes Westphal 12A and Akabozu. The percentages non-penetrants and substomatal vesicle abortion were low in all genotypes. In the partially resistant genotypes the percentage abortion of infection structures was higher than in the susceptible genotype Morocco. Aborted infection structures had formed one or two haustorial mother cells. In adult plants differences in the percentage aborted infection structures between susceptible and partially resistant genotypes were more pronounced than in seedlings. The so-called late abortion was not observed.

INTRODUCTION

The infection cycle of a cereal rust can be divided into several phases (Niks, 1982). In principle mycelial growth can be arrested in each of these phases. Zadoks (1972) suggested a component analysis to determine the importance of blockades in the various phases of fungal development in resistant genotypes.

In the barley/*Puccinia hordei* interaction the early abortion of colonies was an important event in primary as well as flag leaves of partially resistant genotypes (Niks, 1982; Parlevliet and Kievit, 1986). According to these authors the so-called late abortion did not show a high correlation with partial resistance in barley to *Puccinia hordei* .

Prepenetration exclusion was reported for wheat leaf rust on leaf sheaths and peduncles of wheat (Romig and Caldwell, 1963). In the wheat/wheat leaf rust interaction differences in urediospore germination and appressorium formation were not related to the presence of resistance genes (Jacobs, 1989).

In this paper the occurrence of arrested infection structures after appressorium formation by wheat leaf rust (*Puccinia recondita* f.sp. *tritici*, syn. *Pucc. triticina*) in susceptible and partially resistant spring wheat genotypes (*Triticum aestivum*) will be described.

MATERIALS AND METHODS

Seedling leaves and flag leaves have been studied of the highly susceptible genotype Morocco, the susceptible genotype Kaspar and the partially resistant genotypes Westphal 12A and Akabozu. Seedlings were grown in 2 rows along the sides of flats (37 x 39 cm). Plants of each genotype covered part of a row. The parts were randomly assigned to genotypes. Temperatures ranged from 14 to 16 °C during the night and from 19 to 21 °C during the day. Four flats with seedlings (growth stage DC 11, Zadoks *et al.*, 1974) were inoculated in the first experiment. Plants for experiments with adult plants were sown weekly in containers for 6 weeks and kept at 10 °C for 5 - 6 weeks to stimulate tillering. The plants were raised in a greenhouse compartment with an average day temperature of 15 °C and a night temperature of 12 °C. Adult plants were selected for inoculation when they had reached the young flag leaf stage (DC 48-59, Zadoks, *et al.*, 1974). In total five sequential series with adult plants were inoculated.

All plants were inoculated in a settling tower with 1 mg urediospores from a monospore culture of the wheat leaf rust race 'Flamingo' mixed with 3 mg *Lycopodium* spores. At each inoculation four greased glass slides were placed between the leaves to asses the number of spores per cm^2 applied. Incubation followed

inoculation, with a relative humidity of 100 % during one night.

Starting the second day after inoculation 3.0 cm long pieces from the central part of four or five leaves per genotype, chosen at random, were harvested daily. The leaf segments were boiled in lactophenol/ethanol (2:1) and stained following the procedure of Rohringer *et al.*, (1977). Instead of Calcofluor a 0.1 % Uvitex 2B solution (Ciba-Geigy) was used. Both chemicals are optical brighteners. Whole mount preparations were made and infection units were observed with a Nikon epifluorescence UV microscope, excitation filter V: 385-425 nm, magnification 100 x, for details 400 x. Per segment 25 - 30 infection structures from the central part of the leaf were observed and classified according to their developmental phase. In this paper, mycelial structures consisting of a germ tube and an appressorium lacking a substomatal vesicle (ssv) are denominated: 'non-penetrants'. If a ssv was present but no infection hypha, the infection structure will be called: 'ssv abortion'. Infection structures, with an appressorium, a ssv and some infection hyphae, which were much smaller than established infection units at the time of observation are called: 'aborted infection structures'. The presence of a substomatal vesicle was confirmed by phase contrast light microscopy observations. The presence of a yellow autofluorescence near infections units was recorded, autofluorescence was confirmed by switching from filter V (385-425 nm) to filter B (420-490 nm) during observations. It was recorded if uredial beds and sporulating areas were present. Not yet sporulating uredial beds in mature colonies were visible as bright red areas under UV light. The presence of a sporulating area in colonies was recorded, at that stage the epidermis was ruptured and round blue spores were visible.

The relative importance of the various components in flag leaves was determined by calculating the number of non-penetrants per cm^2, the number of ssv abortions per cm^2, the number of aborted infection structures per cm^2 and the number of established colonies per cm^2 on day 5, 6 and 7 after inoculation of the partially resistant genotypes and day 4, 5 and 6 for Morocco. The average values of the components were transformed into relative ones, the total over the four components being 100 (Table 2-5).

The infection type on a scale 0 to 9 (McNeal *et al.*, 1971) and the latency period were determined on five leaves per genotype of seedlings grown in the first flat and of adult plants of the first series (according to Jacobs and Broers, 1989).

In a small second experiment with seedlings, the cultivar Chinese Spring and a genotype from Brazil, BH1146, were included. Leaves were harvested on day four and twelve only.

RESULTS

The number of spores applied ranged from 85 to 107 spores per cm^2 and averaged 98 spores per cm^2, indicating near-equal inoculum densities over all series. No data of adult plants of the genotype Kaspar were obtained as flag leaves of Kaspar appeared too late compared to the other three genotypes.

The infection type in seedlings as well as in adult plants was high (8 -9). Differences in latency period were small in seedlings. Akabozu showed a slightly longer latency period (8.9 days) than the highly susceptible genotype Morocco (8.6 days). In adult plants the latency period of Morocco was 11 days, the values for Akabozu and Westphal 12A were 17 and 19 days respectively. The amount of autofluorescence was negligible in Morocco, Westphal 12A and Akabozu. In the genotype Kaspar several aborted infection structures showed yellow autofluores-

Table 2-1. Percentage of non-penetrants, ssv abortion, and aborted infection structures (i.s.) in seedling and flag leaves of four wheat genotypes various days after inoculation with wheat leaf rust.

Seedlings Genotype	% non-pen.[1)]	% ssv abortion[1)]	% aborted i.s.[2)]
Morocco	3.0 a*	1.8 a	0.9 a
Kaspar	4.3 a	2.3 a	19.9 b
Westphal 12A	4.0 a	1.0 a	7.3 a
Akabozu	3.8 a	1.5 a	16.1 b

Flag leaves Genotype	% non-pen.[3)]	% ssv abortion[3)]	% aborted i.s.[4)]
Morocco	5.0 ab	1.7 a	1.5 a
Westphal 12A	9.7 b	4.0 a	14.3 b
Akabozu	4.0 a	1.0 a	29.7 c

* different letters within columns indicate significant differences (P = 0.05, Scheffé test).

1) averages over samples evaluated 2,3,4 and 5 days after inoculation.

2) averages over samples evaluated 4,5,6,7,8,9,10 and 11 days after inoculation.

3) averages over samples evaluated 4,5,6 and 7 days after inoculation.

4) averages over samples evaluated 5,6 and 7 days after inoculation.

cence in the first experiment but hardly so in the second seedling experiment. The variation in percentages of aborted infection structures, percentage of colonies with uredial beds and percentage sporulating colonies between leaves of partially resistant genotypes was high. For instance the percentages aborted infection structures on day four after inoculation in five seedling leaves of Akabozu were 4, 14, 17, 21 and 50 respectively, the percentages of sporulating colonies 15 days after inoculations were 4, 7, 24 and 64 per leaf segment.

Early development

In seedlings the percentages non-penetrants and ssv abortions were low and did not differ between the genotypes (Table 2-1). In adult plants the percentage non-penetrants differed between the genotypes. In both seedlings and adult plants the percentage aborted infection structures showed a clear genotypic effect. In the partially resistant genotypes Westphal 12A and Akabozu more infection structures were aborted than in the susceptible genotype Morocco. Differences between susceptible and partially resistant genotypes were more pronounced in adult plants than in seedlings.

In a second experiment with seedlings the percentages non-penetrants were high and the percentage aborted infection structures in the partially resistant genotype Akabozu was higher than in the susceptible genotype Morocco (Table 2-2).

Aborted infection structures in the genotype Akabozu developed one or two haustorial mother cells (HMC), on rare occasions three HMC were observed (Table 2-3). In the same period as mentioned in table 2-3 66 % of the aborted infection structures in Westphal 12A had developed one HMC and 33 % had developed two haus-

Table 2-2. Percentage of non-penetrants, ssv abortion, aborted infection structures (i.s.) and established colonies in seedlings of wheat genotypes susceptible and partially resistant to wheat leaf rust four days after inoculation and the percentage sporulating colonies twelve days after inoculation, data from experiment 2.

Genotype	day 4				day 12
		aborted			% sporulating
	non-pen	ssv	i.s.	colonies	colonies
Morocco	36	0	1	63	100
Kaspar	35	0	2	63	99
Westphal 12A	26	1	4	69	100
Akabozu	26	2	20	52	95
Chin. Spring	24	2	4	70	-
BH 1146	23	2	13	62	-

torial mother cells. In the genotype Kaspar 66 and 26 percent of the aborted infection structures showed one or two HMC respectively. Considering all three genotypes roughly speaking two out of three aborted infection structures had one HMC and nearly one out of three showed two HMC.

Late development

Seven days after inoculation uredial beds were observed in seedlings, later followed by sporulation (Table 2-4). The percentages of colonies with uredial beds first increased but decreased when the colonies started to sporulate. In adult plants sporulation started ten days after inoculation in the highly susceptible genotype Morocco, and several days later in the partially resistant genotypes. These histological observations correspond with the differences in latency period observed with a 7 X pocket-lens. In seedlings nearly all established colonies had developed a sporulating area at the end of the experiment. All colonies in flag leaves of the highly susceptible genotype Morocco sporulated 19 days after inoculation. In flag

Table 2-3. Number of aborted infection structures of wheat leaf rust with one, two, three or four haustorial mother cells (HMC) on four consecutive days after inoculation in seedling leaves of the partially resistant genotype Akabozu. Data in parenthesis are percentages.

number of HMC	days after inoculation 4	5	7	8
1	23 (66)	16 (60)	18 (86)	10 (50)
2	11 (31)	9 (33)	3 (14)	7 (35)
3	1 (3)	2 (7)	0	3 (15)
4	0	0	0	0

Table 2-4. Percentage of non-sporulating colonies with uredial beds and percentage sporulating colonies in seedlings and flag leaves of wheat genotypes susceptible and partially resistant to wheat leaf rust various days after inoculation.

Seedlings	% colonies with uredial beds						% sporulating colonies				
Genotype	5	7	8	9	10	11 days	7	8	9	10	11 days
Morocco	0	93	61	9	3	0	0	36	90	97	100
Kaspar	0	73	67	6	0	0	2	33	94	100	100
Westphal 12A	0	68	77	49	3	2	2	14	44	97	98
Akabozu	0	16	75	49	18	2	0	11	45	82	98

Flag leaves	% colonies with uredial beds						% sporulating colonies							
Genotype	10	12	13	15	19	22 days	10	12	13	15	19	22	27	29 days
Morocco	94	11	2	1	0	0	1	89	98	99	100	100	100	100
Westphal 12A	0	5	5	12	12	16	0	0	0	3	55	69	98	100
Akabozu	0	5	4	24	5	12	0	0	3	24	80	80	-	-

leaves of Westphal 12A all colonies had developed urediospores 29 days after inoculation. In the partially resistant genotype Akabozu the percentages of sporulating colonies did not increase between day 19 and 22. Probably all colonies in this genotype would have sporulated at a later observation date as several colonies had developed uredial beds at day 22.

The number of established colonies in flag leaves of the partially resistant genotype Akabozu was clearly reduced by the abortion of infection structures (Table 2-5). In the genotype Westphal 12A this was also observed but to a lesser extent. The component ssv abortion was of little importance. The number of non-penetrants varied per genotype and somewhat reduced the number of colonies.

DISCUSSION

There does not seem to be a host genotype effect on percentage non-penetrants and ssv abortion in these experiments. The high percentage non-penetrants in the second experiment could have been caused by

Table 2-5. Relative number of infection structures: non-penetrating, arrested after ssv formation, aborted or successfully having formed a colony after inoculation of flag leaves of three spring wheat genotypes.

Genotype	non-pen.	ssv abortion	aborted i.s.	colonies
Morocco	5	3	2	90
Westphal 12A	9	4	14	73
Akabozu	4	1	25	70

suboptimal incubation conditions, a lowered viability of the urediospores or an unknown other reason. The small differences in percentages non-penetrants and ssv abortions between the genotypes are probably caused by external factors. This contrasts with findings of Romig and Caldwell (1963), but these colleagues studied leaf sheaths and peduncles. Other workers also reported no genotypic effects on wheat leaf rust growth prior to the formation of the first haustorial mother cells (Gavinlertvatana and Wilcoxson, 1978; Lee and Shaner, 1984; Poyntz and Hyde, 1987).

Abortion of infection structures clearly reduces the number of wheat leaf rust colonies in partially resistant wheat genotypes. Up to now this was not reported by other workers. No autofluorescence, indicative for hypersensitive cellcollapse, accompanied aborted infection structures in the partially resistant genotypes Westphal 12A and Akabozu. Possibly growth of fungal structures is arrested prior to cell wall penetration and haustorium formation, or parasitized cells of partially resistant genotypes do not exhibit autofluorescence. In the vicinity of aborted infection structures in seedlings of the genotype Kaspar yellow autofluorescence was present in the first experiment but not in the second experiment. This indicates the action of a gene for hypersensitivity resistance with an expression influenced by environmental conditions.

Partial resistance not only leads to abortion of infection structures, partial resistance also seems to be responsible for a reduction of mycelium growth in partially resistant genotypes compared to the growth in a susceptible genotype. This leads to a delayed formation of sporogenic tissue and postponed sporulation in partially resistant genotypes. In the partially resistant genotypes studied probably all the colonies eventually would have formed a sporulating area. This indicates that in wheat the so-called late abortion (term introduced by Niks, 1982) is an artefact created by the moment of observation. The number of wheat leaf rust colonies not sporulating at the time of observation should be considered as an indication for the growth delaying capacity of partial resistance. However, high levels of partial resistance can reduce the growth of colonies to such an extent that sporulation does not occur even long after the incubation period (Jacobs and Broers, 1989).

Differences between susceptible and partially resistant genotypes were more pronounced in adult plants than in seedlings for all components studied. Selection for high levels of partial resistance should concentrate on adult plants. Parlevliet and Kievit (1986) suggested the name mature plant resistance for these differences between seedlings and adult plants of a genotype. It is not clear if an active resistance mechanism is responsible for these differences. Possibly the smaller size of mesophyl cells and a different physiology of adult plants accounts for the observed differences.

The relative importance of the abortion of infection structures compared to the growth reduction of colonies in epidemics of wheat leaf rust awaits determination. A reduction in latency period and in size of colonies and sporulating area, greatly influences epidemic build up. A reduction in the number colonies reduces the epidemic in the field to a lesser extend. Simulation studies tend to confirm this impression (Shaner, 1983; Zadoks, 1971). Possibly reduction of mycelial growth rate instead of reducing the number of colonies is a more effective resistance mechanism in fungi species with a strong colonizing behaviour (*Pucc. graminis*, *Pucc. striiformis*) and less so in species with small final colony sizes (*Pucc. hordei*) (Parlevliet, pers. comm.). Wheat leaf rust

shows a way of growing intermediate to the above given extremes.

REFERENCES

Gavinlertvatana, S. and R.D. Wilcoxson, 1978. Inheritance of slow rusting of spring wheat by *Puccinia recondita* f.sp. *tritici* and host parasite relationships. Transactions of the British Mycological Society 71: 413-418.

Jacobs, Th., 1989. Germination and appressorium formation of wheat leaf rust on susceptible, partially resistant and resistant wheat seedlings and on seedlings of other Gramineae species. Submitted to the Netherlands Journal of Plant Pathology.

Jacobs, Th. and L.H.M. Broers, 1989. The inheritance of host plant effect on latency period of wheat leaf rust in spring wheat. I: Estimation of gene action and number of effective factors in F1, F2 and backcross generations. Euphytica, in press.

Lee, T.S., and G.Shaner, 1984. Infection processes of *Puccinia recondita* in slow- and fast-rusting wheat cultivars. Phytopathology 74: 1419-1423.

McNeal, F.H., C.F. Konzak, E.P. Smith, W.S. Tate and T.S Russel. 1971. A uniform system for recording and processing cereal research data. USDA, Agricultural Research Service, Washington, D.C. ARS 34-121.

Niks, R.E., 1982 Early abortion of colonies of leaf rust, *Puccinia hordei*, in partially resistant barley seedlings. Canadian Journal of Botany 60: 714-723.

Parlevliet, J.E. and C. Kievit, 1986. Development of barley leaf rust, *Puccinia hordei*, infections in barley. I. Effect of partial resistance and plant stage. Euphytica 35: 953-959.

Poyntz, P. and P.M. Hyde, 1987. The expression of partial resistance of wheat to *Puccinia recondita*. Journal of Phytopathology 120: 136-142.

Rohringer, R., W.K.Kim, D.J. Samborski and N.K. Howes, 1977. Calcofluor: an optical brightener for fluorescence microscopy of fungal plant parasites in leaves. Phytopathology 67: 808-810.

Romig, R.W. and R.M. Caldwell, 1963. Stomatal exclusion of *Puccinia recondita* by wheat peduncles and sheaths. Phytopathology 54: 214-218.

Shaner, G., 1983. Growth of uredinia of *Puccinia recondita* in leaves of slow- and fast- rusting wheat cultivars. Phytopathology 73: 931-935.

Zadoks, J.E., 1971. Systems analysis and the dynamics of epidemics. Phytopathology 61: 600-610.

Zadoks, J.E., 1972. Modern concepts of disease resistance in cereals. In: The way ahead in plant breeding. Eds: F.G.H. Lupton, G. Jenkins and R. Johnson. Proc. 6th Congr. Eucarpia (Cambridge). Pp 89-98.

Zadoks. J.E., T.T. Chang and C.F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Research 14: 415-421.

Chapter 3.

GROWTH OF WHEAT LEAF RUST COLONIES IN SUSCEPTIBLE AND PARTIALLY RESISTANT SPRING WHEATS.

Th. Jacobs and M.B. Buurlage*

* Part of her "doktoraal studie".

SUMMARY

The average size of wheat leaf rust colonies, measured using epifluorescence microscopy was significantly larger in the highly susceptible genotype Morocco than in the susceptible genotype Kaspar and the partially resistant genotypes Westphal 12A, Akabozu and BH 1146. This was already so three days after inoculation. Colony growth in partially resistant genotypes was continuously retarded compared to colonies in the highly susceptible genotype Morocco. No evidence was found for an initial inhibition of the growth of colonies in partially resistant genotypes. In partially resistant genotypes formation of uredial beds and sporulating areas started at a smaller colony size than in susceptible genotypes. Wheat leaf rust colonies in primary leaves of all genotypes studied were much larger than colonies in flag leaves measured at the same number of days after inoculation. Growth and sporulation of not intertwined colonies was not influenced by either a high or a low number of neighbouring colonies.

INTRODUCTION

Partial resistance is defined as a resistance that causes a reduced epidemic build-up of a pathogen despite a susceptible infection type (Parlevliet and van Ommeren, 1975). In a monocyclic infection in the greenhouse, partial resistance can be partitioned into several components (Parlevliet, 1979).

Histological observations of a monocycle of the barley/barley leaf rust (*Puccinia hordei*) interaction, showed that infection structures in partially resistant barley were aborted prior to haustorium formation (Niks, 1982, 1986). Cell wall appositions probably responsible for failure of haustorium development were concentrated around the point of entry into the leaf and not evenly distributed over the whole of the colony (Niks, 1986). This suggests that partial resistance operates especially in the early phases of infection, directly after penetration. Arntzen and Parlevliet (1986), also studying barley/barley leaf rust, concluded that an initial stagnation of growth was the dominant characteristic in partially resistant barley genotypes. At later stages in the monocyclic growth of barley leaf rust fungal growth rates in susceptible and partially resistant genotypes appeared similar Arntzen and Parlevliet, 1986). Abortion of wheat leaf rust (*Puccinia recondita* f.sp. *tritici*, syn. *Pucc. triticina*) infection structures also occurred in partially resistant wheat genotypes (*Triticum aestivum*) (Jacobs, 1989).

This study was initiated to answer the following question:

Do colonies in partially resistant wheat genotypes show an initial retardation of growth, or is the growth of established wheat leaf rust colonies in partially resistant genotypes continuously reduced compared with colonies in susceptible genotypes?

Additional questions were:

Do colonies, irrespective of genotype, have to reach a certain size before sporulation starts? Is the growth of the colony affected by formation of a sporulating area and urediospores? Do adjacent colonies influence each others growth and sporulation?

MATERIALS AND METHODS

Material

Experiments were performed with the highly susceptible genotype Morocco, the susceptible genotype Kaspar and two partially resistant genotypes Westphal 12A and Akabozu. In 1986 the resistant Brazilian genotype BH 1146 was included. In 1985 no adult plants of the genotype Kaspar were

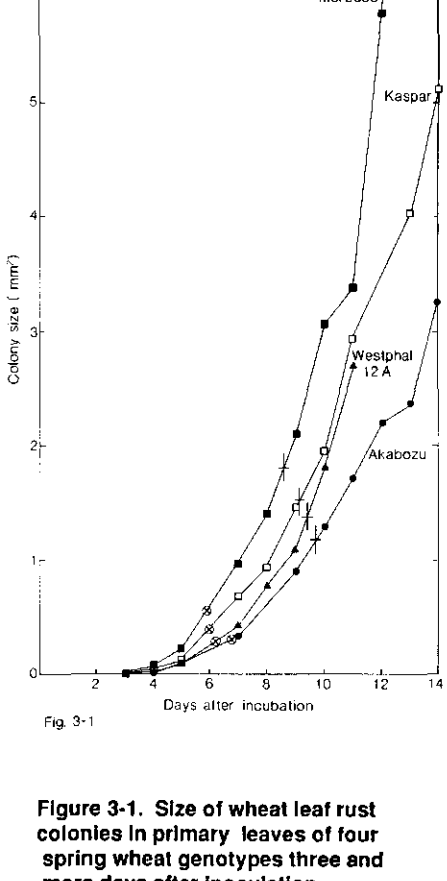

Fig. 3-1

Figure 3-1. Size of wheat leaf rust colonies in primary leaves of four spring wheat genotypes three and more days after inoculation.

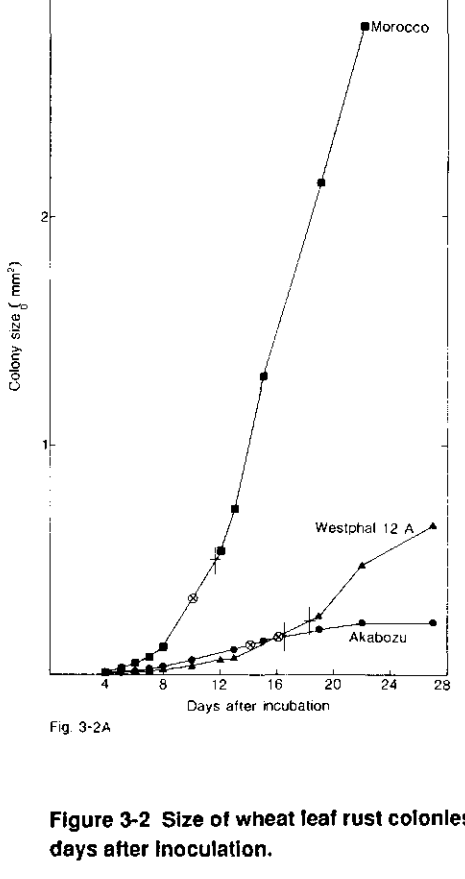

Fig. 3-2A

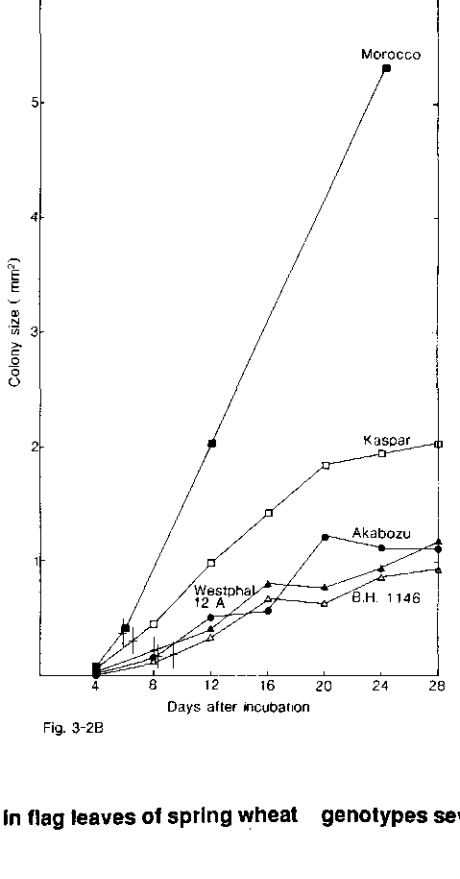

Fig. 3-2B

Figure 3-2 Size of wheat leaf rust colonies in flag leaves of spring wheat genotypes several days after inoculation.

Figure 3-2A: experiment autumn 1985,

Figure 3-2B: experiment spring 1986.

⊗ = first appearance of uredial beds
┼ = time at which 50 % of the final number of colonies sporulated (latency period).

studied. All genotypes studied are spring wheats.

Primary leaves

In july 1985 seedlings were raised in flats and all plants were inoculated simultaneously with a monospore culture of the 'Flamingo' race of wheat leaf rust. Temperatures ranged from 18 to 20 °C during the day and from 14 to 16 °C during the night. Starting at day two, leaf pieces, 3 cm long, were harvested at regular intervals from the central part of the leaves, boiled in lactophenol/ethanol (1:2) and stained with Uvitex 2B (Jacobs, 1989). Each sampling date five leaf pieces were harvested per genotype. Per leaf piece the length and width of 20 to 30 separate colonies, evenly distributed over the leaf, were measured. Intertwined colonies were not included in the observations. Colonies between the leaf margins and the first large vein were discarded. Very small infection structures possessing less then six haustorial mother cells (aborted) were not measured. Just after inoculation some aborted infection structures could have been included, due to the small difference in colony size between aborted and established infection structures. If present, the length and width of uredial beds and sporulating areas in the colonies were measured. The distance between opposite sides of an area was measured with a calibrated eyepiece micrometer using an epifluorescence microscope. The length of an area was obtained by measuring parallel to the long axis of the leaf. Its width was measured perpendicular to the long axis. Occasionally some hyphae extended beyond the front of the majority of fungal

Table 3-1. Number of days of wheat leaf rust colonies to reach a given size in primary leaves and flag leaves of five spring wheat genotypes.

	Colony size in $mm^2 \times 10^{-2}$								
	Primary leaves								
Genotype	25	50	75	100	150	200	400	600	
Morocco	5.1	5.8	6.4	7.1	8.1	8.8	11.3	12.2	
Kaspar	5.7	6.9	7.6	8.1	9.1	10.0	12.9	14.9	
Westphal 12A	5.9	7.2	7.9	8.5	9.5	10.2	-	-	
Akabozu	6.3	7.4	8.0	9.4	10.4	11.5	-	-	
	Flag leaves, 1985								
	10	15	20	25	50	75	100	150	200
Morocco	7.6	8.2	8.7	9.1	10.8	13.0	13.4	15.8	18.3
Westphal 12A	13.7	15.5	17.2	18.9	22.6	-	-	-	-
Akabozu	12.6	15.4	18.9	-	-	-	-	-	-
	Flag leaves, 1986								
	10	15	20	25	50	75	100	150	200
Morocco	-	-	4.4	5.3	6.5	7.5	8.5	10.5	12.0
Kaspar	-	-	4.5	6.0	8.3	9.5	11.5	16.5	25.0
Westphal 12A	-	6.1	7.3	8.7	13.0	17.3	21.3	29.5	34.8
Akabozu	4.3	7.2	8.1	8.7	8.8	11.8	18.9	20.3	-
BH 1146	6.1	8.1	9.1	10.1	14.3	22.0	29.8	35.5	-

Table 3-2. Average number of haustorial mother cells per wheat leaf rust infection structure two and three days after inoculation and their difference (diff), in primary leaves of four spring wheat genotypes.

Genotype	day 2	day 3	diff
Morocco	3.8 a*	13.2 a	9.4
Kaspar	2.1 c	9.7 b	7.6
Westphal 12A	3.1 ab	9.0 b	5.9
Akabozu	2.6 bc	7.4 b	4.8

* Values in each column with different letters differ significantly (P=0.05, according to the Scheffé test).

material, these rare extensions were not included in the measurements. The amount of autofluorescence in colonies was evaluated. After measuring length and width of a colony the number of adjacent colonies was counted in a circle around the exterior of the colony with a radius equal to the length of that colony. If no part of a colony was pre ent within that circle the distance to the nearest colony outside the circle was measured.

On the second and third day after inoculation the number of haustorial mother cells per infection structure was counted. Some aborted infection structures could have been included in these observations.

Flag leaves

Two experiments with adult plants were performed. In autumn 1985 and spring 1986 plants were raised to the flag leaf stage. Just heading plants (stage 48-59, Zadoks *et al.*, 1974) were inoculated, 3 cm long flag leaf pieces were harvested, boiled and stained as described earlier (Jacobs, 1989). Sufficient numbers of just heading plants were inoculated at the same time to allow harvesting of flag leaves at regular intervals. At each sampling date four leaf pieces were harvested per genotype. In 1986 from each flag leaf two adjacent leaf pieces were harvested for mutual comparison, the average of both was calculated. Per leaf piece, the length and width of 30 separate colonies and their sporulating area, if present, were measured. In 1985 day temperatures ranged from 14 - 16 °C and night temperatures varied from 10 to 13 °C. In 1986 the average night temperature was 15 °C, day temperatures were more variable, ranging from 20 to 25 °C, occasionally exceeding 30 °C shortly after noon.

Table 3-3. Colony size at the time that 50 % of the final colonies sporulated (LP50) and estimated maximum colony size of wheat leaf rust in primary leaves and flag leaves of spring wheat genotypes (mm^2).

	colony size at LP50			maximum colony size		
	primary	flag leaves		primary	flag leaves	
Genotype	leaves	1985	1986	leaves	1985	1986
Morocco	1.8	0.45	0.40	11.0	6.0	7.3
Kaspar	1.3	--	0.30	8.6	--	2.1
Westphal 12A	1.2	0.23	0.23	6.6	1.3	1.5
AKabozu	1.1	0.15	0.18	3.0	0.3	1.1
BH 1146	--	--	0.15	--	--	1.0

Table 3-4. Size of the sporulating area, as a percentage of the whole wheat leaf rust colony which is set at 100, in primary leaves and flag leaves of five spring wheat genotypes.

Genotype	primary leaves	flag leaves 1985	flag leaves 1986
Morocco	7.4	14.4	17.6
Kaspar	7.5	--	19.3
Westphal 12A	9.7	15.6	24.6
Akabozu	8.1	17.8	17.7
BH 1146	--	--	21.4

General

The size of the colonies and sporulating area was calculated by multiplying the length and width. Per leaf piece the average size of the 20 to 30 colonies was calculated. These average values were considered the experimental units for analysis. The average size of a colony or sporulating area of a genotype, as presented in Figure 3-1 and Figure 3-2, was calculated as the average of all the leaf pieces harvested at each sampling date. The values of the average colony size per genotype per day were natural log transformed. The number of days to reach a certain colony size was calculated as an interpolation of the natural log transformed data. Maximum colony size was determined through extrapolation as the asymptotically reached value of the hyperbolically shaped natural log transformed growth curves.

In all experiments the latency period (LP) and infection type, on a scale 0 to 9, were determined (Jacobs and Broers, 1989).

RESULTS

The infection type in all genotypes studied was high (8 to 9), except for BH 1146 in 1986 (5 to 6). The colonies of BH 1146 in the 1986 experiment showed yellow autofluorescence which indicates hypersensitive cellcollapse.

Latency period was longer and colony size smaller in partially resistant genotypes compared with those in the highly susceptible genotype Morocco (Figure 3-1 and 3-2). Colony size in primary leaves was considerably larger than the size of colonies in flag leaves when measured at the same number of days after inoculation. The ranking order of the genotypes was the same in primary leaves and flag leaves. In adult plants differences in colony size between the susceptible and partially resistant genotypes were far more clear than in primary leaves (Figure 3-1 and 3-2). At the first sampling date, three days after inoculation, colonies in primary leaves of the partially resistant genotypes Westphal 12A and Akabozu and the susceptible genotype Kaspar were already significantly smaller than colonies in the highly susceptible genotype Morocco. In flag leaves the dif-

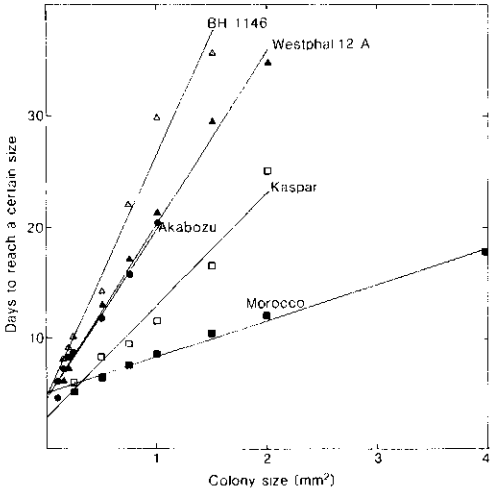

Figure 3-3. Relation between colony size and days to reach a certain size for susceptible and partially resistant spring wheat genotypes. The linear regression lines are given. Data from flag leaves (1986).

Table 3-5. Sizes and sporulating area of wheat leaf rust colonies with the nearest neighbour less than one (<1) or more than one (>1) diameter away in primary leaves of four spring wheat genotypes several days after inoculation (d.a.i.). Eight and nine days after inoculation a subdivision was made between colonies with one and colonies with more than one adjacent colony within a distance of one diameter of the colony perimeter.

COLONY SIZE +

d.a.i.	Morocco			Kaspar			Westphal 12A			Akabozu		
	<1		>1	<1		>1	<1		>1	<1		>1
5	2264		2015	1140		1039	1122		981	796		643
7	9814		8026	5474		4531	5828		4903	3418		3212
	more	one		more	one		more	one		more	one	
8	13057	14033	12862	8879	9351	9603	6994	7657	6690	8272	7295	5975
9	20416	22240	--	15530	13291	--	12464	13219	12017	8916	8188	7216

SPORULATING AREA +

d.a.i.	Morocco			Kaspar			Westphal			Akabozu		
	<1		>1	<1		>1	<1		>1	<1		>1
	more	one	--	more	one	--	more	one		more	one	
9	1404	1473	--	1198	970	--	--	--		--	--	--
10	2325	2136	2553	--	1754	1374	2317	1949	2159	1182	1212	1109
11	--	2842	--	2383	2391	--	2369	2477	2303	1230	1432	1245
12	4976	3783	--	1950	2737	--	--	--		--	1819	2005

--) no colonies in this category.

+) none of the differences in colony size or sporulating area between categories within a genotype are significantly different from each other (Mann-Whitney test (P=0.05)).

ferences in colony size between Morocco and the partially resistant genotypes Westphal 12A and Akabozu were also significant at the first sampling date. This was day 3 in 1985 and day 4 in 1986 (P=0.05, Mann-Whitney test).

The number of days for wheat leaf rust colonies to reach a given size was smaller in primary leaves than in flag leaves. The difference between the highly susceptible genotype Morocco and the partially resistant genotypes Westphal 12A and Akabozu in number of days needed to reach a certain size was small just after inoculation and increased with time (Table 3-1).

The number of haustorial mother cells shortly after inoculation in partially resistant genotypes was smaller than in the susceptible genotype Morocco (Table 3-2).

The average colony size in partially resistant genotypes was smaller than the average colony size in susceptible genotypes at the time that 50% of the final number of colonies sporulated (LP). The values of the maximum colony size in the highly susceptible Morocco greatly exceeded the values of partially resistant genotypes both in primary and flag leaves (Table 3-3). Colony size and size of the sporulating area in flag leaves in spring 1986 were larger then in autumn 1985. This

was probably related with differences in temperature and light between the two experiments.

The size of the sporulating area related to the colony size (set at 100) did not seem to differ between susceptible and partially resistant genotypes. In primary leaves the relative size of the sporulating area was smaller than in flag leaves (Table 3-4).

Colony size increased considerably while uredial beds and sporulating areas were formed (Figure 3-1 and 3-2).

The number of adjacent colonies and their distance from the measured colony did not seem to influence colony size and the size of the sporulating area (Table 3-5).

DISCUSSION

Initial delay or continuous retardation

Colony size in partially resistant genotypes was smaller than in the highly susceptible Morocco. The difference in number of days needed to reach a certain colony size increased with increasing colony size.

According to Arntzen and Parlevliet (1986), studying barley/barley leaf rust, an initial stagnation of growth would lead to large differences between susceptible and partially resistant genotypes in number of days needed to reach a given colony size, already early after inoculation. They reported that these differences did not change substantially with increasing colony sizes. This was not observed in wheat infected with wheat leaf rust (Table 3-1).

The linear regression lines through the data of the respective genotypes cross each other close to the ordinate indicating a common point of initial growth (Figure 3-3).

This leads us to the conclusion that wheat leaf rust colonies in partially resistant genotypes show a continuously reduced growth compared to colonies in susceptible wheat genotypes. This contrasts with the situation in barley/barley leaf rust (Arntzen and Parlevliet, 1986).

A reduced colony growth rate of wheat leaf rust colonies, is not contradicted by the growth measured as the number of haustorial mother cells at two and three days after inoculation (Table 3-2). In primary leaves two days after inoculation the differences in the number of haustorial mother cells between susceptible and partially resistant genotypes are small. Three days after inoculation colonies in partially resistant genotypes have significantly lower numbers of haustorial mother cells than colonies in the highly susceptible genotype Morocco (Table 3-2). However a low number of haustorial mother cells on both days is also expected in partially resistant genotypes under the assumption of an initial inhibition. The data on number of haustorial mother cells (Table 2) do not permit a discrimination between the presence of an initial delay or a continuously retarded growth rate in partially resistant wheat. The reader should be aware of the fact that the presence of a haustorial mother cell not necessarily implies the presence of a haustorium (Niks, 1983).

Smaller colonies in slow leaf rusting spring wheats have been reported by Lee and Shaner (1984) who measured length and width of wheat leaf rust colonies at three moments after inoculation. Gavinlertvatana and Wilcoxson (1978) and Poyntz and Hyde (1987) report smaller wheat leaf rust colonies in flag leaves of wheat measured 120 hours after inoculation. However the number of sequential samples in these studies was three or less. No support for the hypothesis of an initial inhibition or a continuous retardation can be derived from these studies.

Reports from slow stem rusting wheat seedlings (Martin *et al.*, 1977) and slow rusting oat plants (Luke *et al.*, 1984) which were

intensively studied during the first 96 hours after inoculation seem to indicate that a continuous retardation of the growth rate is a general phenomenon in so-called slow-rusting resistant gramineae species. The initial stagnation of leaf rust colonies in partially resistant barley as reported by Artnzen and Parlevliet (1986) clearly deviates from the situation in the above mentioned pathosystems. The nature of the partial resistance in wheat is unknown. Possibly haustorium formation fails and is correlated with the presence of cell wall appositions as reported for partially resistant barley (Niks, 1986).

Critical colony size

It was postulated that fungal colonies sporulated only after reaching a certain, critical size (Clifford and Roderick, 1978; Lee and Shaner, 1984; Wahl *et al.*, 1980). Lee and Shaner working with wheat leaf rust stated that :"although colonies in fast rusting wheat cultivars were consistently larger than those in slow-rusting wheat cultivars, it appeared that uredinium formation began when the colony reached a certain size whether on a fast- or slow- rusting wheat cultivar". They mentioned a critical colony size of 0.12 - 0.14 mm^2 for development of uredial beds in both slow- and fast-rusting wheat genotypes. Uredinal bed formation in flag leaves of the highly susceptible Morocco was observed in the autumn of 1985 when colony size was 0.3 mm^2. In the partially resistant genotypes Westphal 12A and Akabozu uredial beds were first noted in colonies of about 0.1 mm^2. In primary leaves colonies started formation of uredial beds when they were 0.6 mm^2 in Morocco and 0.2 mm^2 in Akabozu. These differences are substantial. The colony size at sporulation (LP) in partially resistant genotypes is considerably smaller than in susceptible genotypes (Table 3-2). If in the autumn of 1985 colonies in flag leaves of the partially resistant genotype Akabozu had started formation of uredial beds and sporulation at the same colony size as colonies in the highly susceptible Morocco (0.3 mm^2), the colonies in Akabozu never even would have sporulated as the final colony size in the 1985 experiment for Akabozu was 0.24 mm^2. The data from the experiments in this paper do not support the hypothesis that "a critical fungal mass" should be reached, regardless the level of resistance, before sporulation starts.

Mature plant resistance

Colonies in primary leaves grow faster, are larger at the time of sporulation and reach larger final sizes than colonies in adult plants. Differences in colony size between susceptible and partially resistant genotypes are smaller in primary leaves than in flag leaves. This phenomenon has been reported before for wheat partially resistant to wheat leaf rust (Broers, 1989; Jacobs, 1989). Parlevliet and Kievit (1986) suggested the name "mature plant resistance" and hypothesized that it might be a third kind of resistance. This was argued against by Jacobs (1989), stating that an altered morphology and physiology of mature plant cells could equally well be responsible. Cells in primary leaves are more widely spaced and larger than cells in flag leaves. The relative sizes of sporulating areas in primary leaves was smaller than those in flag leaves (Table 3-4). It is puzzling that colonies in the so-called "mature plant resistant" flag leaves are more capable of producing spores than colonies of equal size in seedling leaves. The density of haustoria in flag leaves can be different from that in seedlings. Cells in primary leaves can also have a different metabolic activity. Possibly less nutrients are available for the fungus. To

denote these differences as a third kind of resistance seems somewhat premature.

It is difficult to determine the degree in which the formation of uredial beds and spores reduces the colony growth rate. It is especially clear from the data of the highly susceptible Morocco that the fungus is capable of maintaining a high growth rate while producing spores.

Mutual influence of colonies

In this study only separate colonies were measured. Intertwined colonies were not measured. The colonies were classified into one of three categories depending on the distance to the nearest neighbour. Five and seven days after inoculation the average colony size of colonies having an adjacent colony less than one diameter away were slightly larger than colonies having colonies over one diameter away. A similar phenomenon was observed eight and ten days after inoculation (Table 3-5). The values are not significantly different but the trend is showing. If colonies are competing for nutrients the opposite is to be expected. Arntzen and Parlevliet (1986) also report that colonies having an adjacent colony within a circle of one diameter around its exterior are 7% larger than colonies lacking such neighbours. However due to the procedure, measuring the number of adjacent colonies in circles of a given diameter around a random colony, a bias could have occurred. Larger colonies have a wider circle and an increased chance of including a neighbour in that circle. This could explain the observed trend.

Comparing the colony sizes of colonies with only one adjacent colony within a circle of its own diameter around its exterior with colonies having more than one neighbour in a circle of the same size, no differences become evident. Under the assumption of competition for nutrients smaller colonies were expected if more neighbours are present. The sizes of sporulating areas of all four genotypes , susceptible and partially resistant, do not seem to be influenced by the number of adjacent colonies.

There does not seem to be a density dependent growth and sporulation of the wheat leaf rust colonies measured. It is possible that growth of colonies and sporulating area is reduced in intertwined colonies. Data from this study indicate that colonies do not influence each others growth as long as they are separate.

REFERENCES

Arntzen, F.K. and J.E. Parlevliet, 1986. Development of barley leaf rust, *Puccinia hordei*, infections in barley. II: importance of early events at the site of penetration for partial resistance. Euphytica 35: 961-968.

Broers, L.H.M., 1989. Influence of development stage and host genotype on three components of partially resistance to leaf rust in wheat. Submitted to Euphytica.

Clifford, B.C. and H.W. Roderick, 1978. Comparative histology of some barley brown rust interactions. Annals of Applied Biology 89: 295-298.

Gavinlertvatana, S. and R.D Wilcoxson, 1978. Inheritance of slow rusting of spring wheat by *Puccinia recondita* f.sp. *tritici* and host parasite relationships. Transaction of the British mycological Society 71: 413-418.

Jacobs, Th., 1989. Abortion of infection structures of wheat leaf rust in susceptible and partially resistant wheat genotypes. Submitted to Euphytica.

Jacobs, Th. and L.H.M. Broers, 1989. The inheritance of host plant effect on latency period of wheat leaf rust in spring wheat I. Estimation of gene action and number of effective factors in F1, F2 and backcross generations. Euphytica, in press.

Lee, T.S. and G.Shaner, 1984. Infection processes of *Puccinia recondita* in slow- and fast-rusting wheat cultivars. Phytopathology 12: 1419-1423.

Luke, H.H., R.D. Barnett and P.L. Pfahler, 1984. Postpenetration development of *Puccinia coronata avenae* in slow- and fast-rusting cultivars of *Avena byzantina*. Phytopathology 74: 899-903.

Martin, C.D., L.J. Littlefield and J.D. Miller, 1977. Development of *Puccinia graminis* f.sp. *tritici* in seedling plants of slow-rusting wheats. Transactions of the British Mycological Society 68: 161-166.

Niks, R.E., 1982. Early abortion of colonies of leaf rust, *Puccinia hordei*, in partially resistant barley seedlings. Canadian Journal of Botany 60: 714-723.

Niks, R.E., 1983. Haustorium formation of *Puccinia hordei* in leaves of hypersensitive, partially resistant and non-host plant genotypes. Phytopathology 73: 64-66.

Niks, R.E., 1986. Failure of haustorial development as a factor in slow growth and development of *Puccinia hordei* in partially resistant barley seedlings. Physiological and Molecular Plant Pathology 28: 309-322.

Parlevliet, J.E., 1979. Components of resistance that reduce the rate of epidemic development. Annual Review of Phytopathology 17: 203-222.

Parlevliet, J.E. and A. van Ommeren, 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. II. Relationship between field trials, microplot tests and latent period. Euphytica 24: 292-303.

Parlevliet, J.E. and C. Kievit, 1986. Development of barley leaf rust, *Puccinia hordei*, infections in barley. I. Effect of partial resistance and plant stage. Euphytica 35: 953-959.

Poyntz, B. and P.M. Hyde, 1987. The expression of partial resistance of wheat to *Puccinia recondita*. Journal of Phytopathology 120: 136-142.

Wahl, I., R.D. Wilcoxson and J.B. Rowell, 1984. Slow rusting of wheat with stem rust detected in the glasshouse. Plant Disease 64: 54-56.

Zadoks. J.E., T.T. Chang and C.F. Konzak, 1974. A decimal code for the growth stages of cereals. Weed Research 14: 415-421.

Chapter 4.

THE OCCURRENCE OF CELL WALL APPOSITIONS IN FLAG LEAVES OF SPRING WHEATS, SUSCEPTIBLE AND PARTIALLY RESISTANT TO WHEAT LEAF RUST.

Th. Jacobs

SUMMARY

In two experiments, the presence of cell wall appositions in flag leaves of spring wheat genotypes susceptible and partially resistant to wheat leaf rust was studied. More cell wall appositions were observed near aborted infection structures than in established colonies. There was not a marked difference in the number of cell wall appositions per colony between susceptible and partially resistant genotypes. More cell wall appositions per unit area colony were present in partially resistant genotypes. It was concluded that the low number of cell wall appositions could not be responsible for the observed difference in colony size between susceptible and partially resistant genotypes. Partial resistance in wheat to wheat leaf rust can be divided into two phases. The first phase is pre-haustorial and results in a reduction in the number of colonizing infection structures. In the second phase a post-haustorial retardation of fungal growth rate occurs. The latter appears to be the more important phase.

INTRODUCTION

Partially resistant genotypes show a high infection type (Parlevliet and van Ommeren, 1975). Spring wheat genotypes (*Triticum aestivum*) partially resistant to wheat leaf rust (*Puccinia recondita* f.sp. *tritici*, syn. *Pucc. triticina*) have higher numbers of aborted infection structures than susceptible genotypes (Jacobs, 1989). Wheat leaf rust colonies in partially resistant genotypes show a continuous retardation of the growth compared to colonies in susceptible spring wheats. In wheat leaf rust colonies in partially resistant genotypes no autofluorescence, indicative for hypersensitive cell collapse, is present (Jacobs and Buurlage, 1989).

In barley, partially resistant to barley leaf rust, a pre-haustorial exclusion of the fungus was observed (Niks, 1982; 1983; 1986). Aborted barley leaf rust infection structures were associated with host wall alterations at the contact-sites with haustorial mother cells. These cell wall appositions, probably impeding haustorium formation, were more numerous in established colonies in partially resistant barley genotypes than in susceptible barley genotypes (Niks, 1986).

In this paper the presence of host cell wall appositions in wheat leaf rust colonies in flag leaves of susceptible and partially resistant spring wheat genotypes is investigated. In addition, the occurrence of other histological phenomena at early stages of the infection process was studied.

MATERIALS AND METHODS

Two experiments have been conducted, one in spring and one in autumn of 1986. In both experiments plants were raised in the greenhouse and adaxial sides of young flag leaves (growth stage nr 48-59, Zadoks *et al.*, 1974) were inoculated in a settling tower (after a design of Eyal *et al.*, 1968) with 3.0 mg urediospores of a monospore culture of the 'Flamingo' race of wheat leaf rust, diluted with *Lycopodium* spores. The night following the inoculation a relative humidity of 100 % was created with the aid of an electric humidifier. The average day temperature in experiment one was 23 °C and the average night temperature 18 °C. In experiment two the average temperatures were 20 °C and 15 °C respectively. In both experiments 3.0 cm long leaf pieces from central parts of the flag leaves were harvested, fixed during 24 hours in ethanol: dichloro-ethane (3:1 v/v) with 0.15 % trichloro-acetic acid (TCA), boiled in 0.01 % trypan blue for 10 min and more then 24 hours cleared in a nearly saturated solution of chloral hydrate (5:2 w/v). The leaves were then transferred through a series of alcohols from 80 to 100

%, immersed for 5 min in a saturated solution of picric acid in wintergreen oil (methyl salicylate) and airtight mounted in wintergreen oil (modified procedure, Niks, 1986). Observations were made with phase contrast light microscopy (1000 x). Cell wall appositions were visible as luminous structures. An infection structure is defined as an appressorium over a stoma, with at least a substomatal vesicle and one infection hypha.

The spring wheat genotypes used in the first experiment were the highly susceptible genotype Morocco, the susceptible genotype Kaspar and the partially resistant genotypes Westphal 12A and Akabozu.

In the first experiment four leaf pieces per genotype were harvested each sampling time at 96, 144 and 192 hours after inoculation.

The first set of observations were made on the leaf pieces collected 96 hours after inoculation. For 100 infection structures per leaf piece the number of cell wall appositions was recorded. It was determined if the infection structures were aborted or established. Colonies were regarded as established if several branched hyphae and haustoria were present. Aborted infection structures were hardly branched and without haustoria. It was also recorded whether the walls of the guard cells of the penetrated stoma were luminous. The occurrence of luminous cell walls in a zone around the occupied substomatal cavity was evaluated. The presence of one or more dark blue stained, collapsed host cells near an infection hypha was determined.

For the second set of data the number of cell wall appositions per colony of 15 established colonies per leaf piece was noted, 96, 144 and 192 hours after inoculation in experiment 1 and 54, 110 and 250 hours after inoculation in experiment 2. Colonies between the leaf margin and the first vein were not included in the observations. The chosen colonies were evenly distributed over the leaf piece. Each infection structure, the presence of a substomatal vesicle was checked. The infection hypha originating from the substomatal vesicle was followed, often deep into the mesophyll of the leaf. All hyphae of the infection structure were screened and the number of luminous cell wall appositions close to the hyphae was noted.

Colonies in partially resistant genotypes are smaller than colonies in susceptible genotypes. An estimation was made of the number of cell wall appositions per standard colony size. The size of the most susceptible genotype was set at 100. Calculations were based on data published by Jacobs and Buurlage (1989).

In the second experiment the highly susceptible genotype Little Club and the partially resistant genotypes Westphal 12A and Akabozu were studied. Of each genotype five flag leaves were harvested 54, 110 and 250 hours after inoculation. Procedures were as described above. The first set of observations, on 100 infection structures, were made on leaf segments harvested 54 hours after inoculation.

The distribution of cell wall appositions over the colony area was observed 144 and 192 hours after inoculation in experiment one and 110 and 250 hours after inoculation in experiment two.

Four additional flag leaves per genotype were harvested 250 hours after inoculation, boiled and stained with Uvitex 2B (Jacobs, 1989). The length and width of 20 colonies per leaf segment were measured with a calibrated eye-piece micrometer using epifluorescence microscopy. The average of length times width of the colonies of the four leave pieces per genotype was related to the highly susceptible Little Club, being set at 100.

RESULTS

The morphology of the cell wall appositions was quite variable. The majority (70 %) of cell wall appositions consisted of a narrow luminous zone gradually decreasing towards both ends (Figure 4-1 A, B, C, E). The length and width of these cell wall appositions opposite hyphae or haustorial mother cells varied considerably. A minority of cell wall appositions (30%) showed a thick central part opposite a haustorial mother cell and a sharply decreasing thickness on a short distance from the center of the apposition (Figure 4-1 D). Occasionally an encapsulated penetration peg was observed. On rare occasions an enclosed small haustorium was observed or a haustorium in a cell with a cell wall apposition (Figure 4-1 F,G). Thus the majority of cell wall appositions consisted of a narrow luminous zone slightly wider in the central part opposite the haustorial mother cell, the length of the zone greatly exceeding its width.

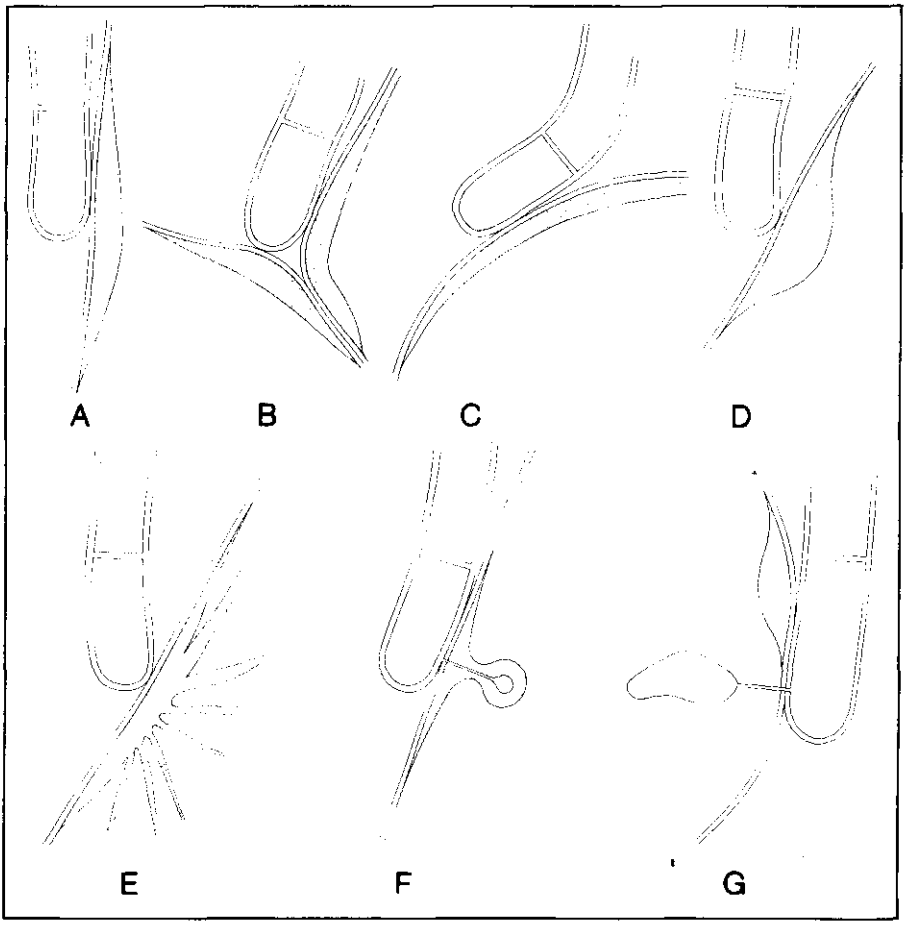

Figure 4-1. Schematic drawings of haustorial mother cells (HMC) and cell wall appositions. A,B,and C are drawings of representative tivetivetivecell wall appositions which are slender and extended over a relatively large area. D,E,F and G are drawings of situations which have been observed on rare occasions. D: Cell wall apposition restricted in length, showing a central thickening. E: Cell wall apposition with fading radial lines. F: Encapsulated haustorial neck and primordial haustorium. G: Cell wall apposition and small haustorium in the same cell.

Aborted infection structures

In all the genotypes studied aborted infection structures were associated with a higher number of cell wall appositions than established colonies (Table 4-1). This association was observed in both susceptible and partially resistant genotypes. The average of the differences in number of cell wall appositions between aborted and establised infection structures was 2.3 in experiment 1 and 1.1 in experiment 2.

Partially resistant genotypes showed a higher percentage of aborted infection structures than susceptible genotypes. The percentage of aborted infection structures in the highly susceptible Little Club was high, possibly due to experimental influences.

Other histological phenomena

Aborted infection structures were more often associated with stoma showing luminous walls of the guards cells than establised colonies. (Table 4-2). The number of infection structures, aborted or not, showing a ring of luminous cell walls around the substomatal cavity were low. Both in susceptible and partially resistant genotypes aborted infection structures were associated with a higher number of collapsed cells than established colonies. (Table 4-2).

Cell wall appositions in established colonies

The number of cell wall appositions per colony between susceptible and partially

Table 4-1. Number of cell wall appositions per established or aborted wheat leaf rust infection structure (i.s.) and percentage aborted infection structures in flag leaves of spring wheat genotypes susceptible and partially resistant to wheat leaf rust in two experiments. In experiment two the colony size 250 hours after inoculation (h.a.i.) was related to that of Little Club (set at 100).

Experiment one	96 hours after inoculation		
	number of cell wall appositions in		% i.s.
Genotype	estab. colonies	aborted i.s.	aborted
Morocco	1.1 a *	3.5 b	9 a +
Kaspar	1.6 a	3.9 a	19 a
Westphal 12A	0.7 a	2.1 b	31 a
Akabozu	1.7 a	4.8 b	53 b

Experiment two	54 hours after inoculation			
	number of cell wall appositions in		% i.s.	250 h.a.i. relative
Genotype	estab. colonies	aborted i.s.	aborted	colony size
Little Club	1.1 a *	2.4 b	35 a +	100 a ++
Westphal 12A	1.3 a	2.3 a	24 a	39 b
Akabozu	1.9 a	2.8 b	55 a	27 b

*) values in a row with equal letters do not differ significantly according to the Mann-Whitney test (P=0.05).

+) values in each column with different letters differ significantly according to the Scheffé test (P=0.05).

++) values in this column with different letters differ significantly according to the Scheffé test (P=0.05), computations on original data.

resistant genotypes hardly differed at a given time after inoculation (Table 4-3). In both experiments the number of cell wall appositions per colony of comparable size, was higher in partially resistant genotypes than in the most susceptible genotype.

The majority of cell wall appositions were observed close to the point of leaf-entry . Even so the cell wall appositions in other parts of the leaf enclosed by the colony, were mostly located near a substomatal cavity. The data are not presented here as distance to a stomatal cavity is difficult to measure.

Haustoria in established colonies were observed but no counts could be made as the hyphae often grew deep into the mesophyll. Granulation of cell contents in less deep cell layers obscured the images of the haustoria and made reliable counts of haustoria in the flag leaves impossible.

DISCUSSION

Aborted infection structures

The difference in the number of cell wall appositions between aborted infection structures and established colonies was small. In an earlier study (Jacobs, 1989), it was shown that the majority (66%) of aborted infection structures showed one haustorial mother cell, some 33 % had formed two haustorial mother cells. Thus it is possible that the presence of one cell wall apposition prevents the formation of a haustorium. Probably most infection structures not able to form the first haustorium, lack the potential to continue their attempts and are aborted. Some infection structures are capable of branching after a failed first attempt and form a second haustorial mother cell. The presence at that moment of

Table 4-2. Percentages of wheat leaf rust infection structures showing a luminous stoma (stoma), a ring of luminous cell walls around the substomatal cavity (ring) or with one or more deep blue collapsed cells in the vicinity of the infection structure (collapse) in established (estab) or aborted (abort) infection structures, in two experiments with susceptible and partially resistant spring wheats.

Experiment one	96 hours after inoculation					
	stoma		ring		collapse	
Genotype	estab	abort	estab	abort	estab	abort
Morocco	10 a*	13 a	2 a	3 a	22 a	39 a
Kaspar	1 a	3 a	1 a	3 a	2 a	14 a
Westphal 12A	6 a	8 a	0 a	2 a	1 a	20 b
Akabozu	12 a	19 a	0 a	6 b	12 a	20 b

Experiment two	54 hours after inoculation					
	stoma		ring		collapse	
Genotype	estab	abort	estab	abort	estab	abort
Little Club	4 a *	14 a	0 a	5 a	0 a	17 a
Westphal 12A	1 a	17 a	0 a	1 a	0 a	7 a
Akabozu	20 a	30 a	0 a	1 a	0 a	7 a

*) values in each row within the categories : stoma, ring or collapse having different letters differ significantly according to the Mann-Whitney pair test (P=0.05).

another cell wall apposition at the site of cell wall penetration could be fatal.

It is worth noticing that the gap between establishment or abortion of an infection structure seems small. The presence of one or two cell wall appositions opposite haustorial mother cells probably determines the fate of an infection structure. For an infection structure it is of utmost importance to form that first haustorium, enabling the established colony to overcome later barriers at cell walls. Figure 4-2 shows the occurrence of cell wall appositions in an aborted and an established hedhedhedhedwheat leaf rust infection structure. It looks as if the balance between abortion and establishment seems to be delicate. This could be the reason why there is such a variation in number of aborted infections structures in partially resistant genotypes between different experiments and between leaves within one experiment (Jacobs, 1989).

Other histological phenomena

For a plant an early detection of a fungal structure is important. With this view the number of luminous stomata and the presence of a ring of luminous cell walls was noted. The differences between established and aborted infection structures were not significant, although a luminous stoma and a ring of luminous ceell walls were more often present near aborted infection structures. Possibly the reaction of the plant to the formation of an appressorium over the guard cells and the formation of a substomatal vesicle enhances the barrier capacity of the plant prior to penetration of the cell wall. These small influences could contribute to the defence of the plant. The presence of cell wall appositions opposite the haustorial mother cells seems more important in determining whether an infection structure is aborted or not.

The above described phenomena can be summarized as pre-haustorium actions of

Table 4-3. Observed and estimated number of cell wall appositions per wheat leaf rust colony in flag leaves of spring wheat genotypes in two experiments at various hours after inoculation (h).

Experiment one

	observed			estimated[1]		
Genotype	96	144	192 h	96	144	192
Morocco	3.0 a+	1.2 a	13.6 a	3.0	1.2	13.6
Kaspar	4.5 a	4.6 a	16.3 a	6.4	7.7	32.6
Westphal 12A	1.4 a	1.0 a	3.7 a	3.1	2.6	18.5
Akabozu	2.3 a	4.2 a	3.2 a	6.2	12.4	21.3

Experiment two

	observed			estimated[1]		
Genotype	54	110	250 h	54	110	250
Little Club	1.5 a	1.1 a	2.3 a	1.5	1.1	2.3
Westphal 12A	1.6 a	1.4 a	2.0 a	4.1	3.1	5.7
Akabozu	2.4 a	1.3 a	8.9 b	8.8	3.5	29.6

+) values in each column followed by a different letter are significantly different (P=0.05) according to the Scheffé test.

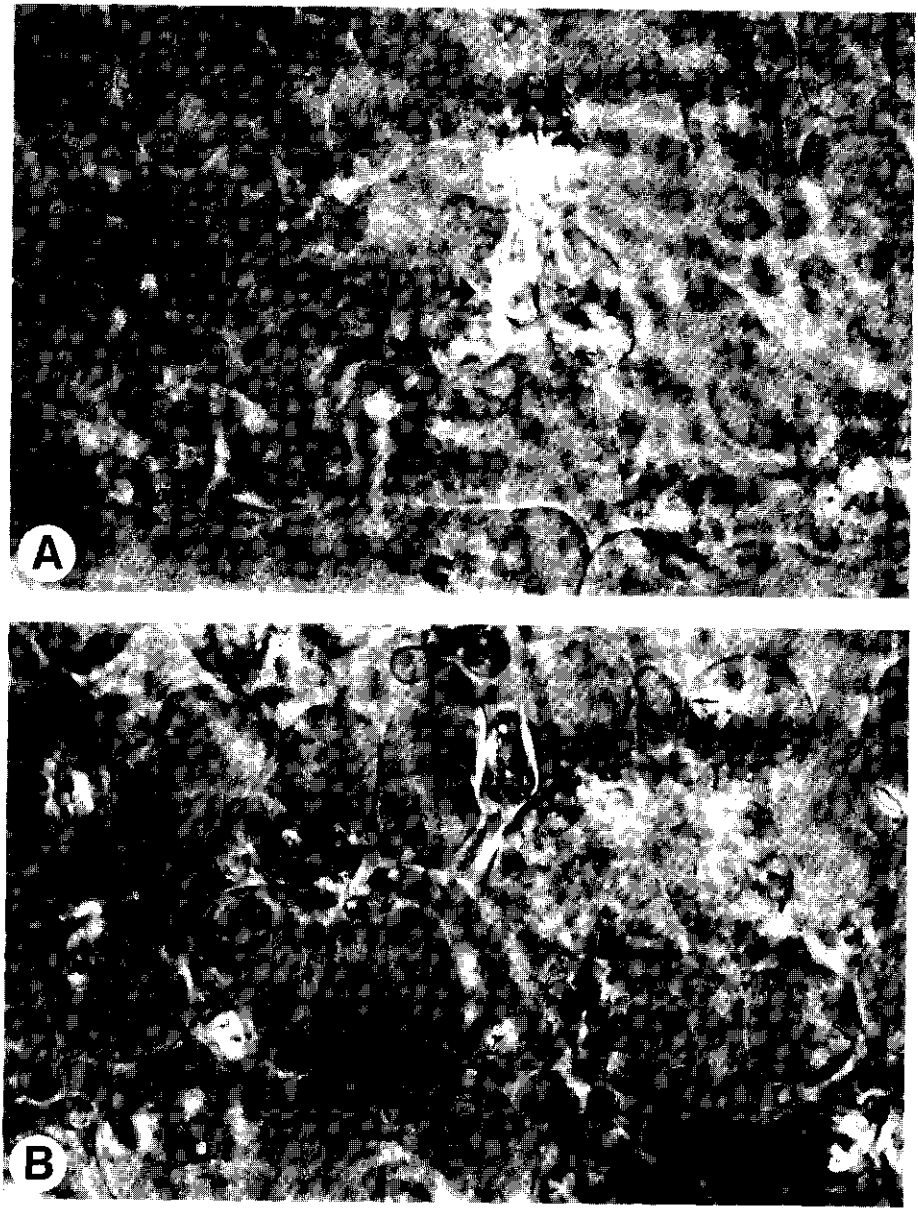

Figure 4-2. Luminous cell wall appositions associated with wheat leaf rust infection structures in flag leaves of the partially resistant genotype Akabozu.
A: Six days old aborted infection structure with several cell wall appositions opposite the haustorial mother cell (arrow).
B: Six days old established colony with two cell wall appositions (large arrow) and intercellular hyphae (small arrows).
Magnification: 1000 X.

resistance (term suggested by Heath, 1974). After penetration of the cell wall and haustorium formation a cell could collapse. In susceptible and partially resistant genotypes aborted infection structures were associated with a higher number of collapsed cells compared to established colonies. This suggests that post-haustorial collapse of cells in some way contributes to the abortion of infection structures. The relative importance of cell collapse appears limited, in most cases less than 20 percent of the aborted infections structures were associated with cell collapse.

Cell wall appositions in established colonies

The influence of cell wall appositions on the retardation of growth of wheat leaf rust colonies in partially resistant wheat is probably negligible. The observed large difference in colony size between susceptible and partially resistant genotypes do not match with the small differences in the number of cell wall appositions. Two hundred and fifty hours after inoculation the number of mesophyll cells surrounded by a wheat leaf rust colony in flag leaves of the highly susceptible Little Club was estimated at 2000. It is difficult to imagine that the observed number of 8,9 cell wall appositions per infection structure (Table 4-3) in the partially resistant genotype Akabozu leads to a relative colony size of less than 30 % of the size of colonies in Little Club (Table 4-2).

Assuming that: i) each mesophyll cell within the area covered by the colony is parasitized only once by a haustorium connected with a haustorial mother cell, and,

ii) the presence of a cell wall apposition impedes haustorium formation, observations learn that at the most 2 % of the attempted penetrations in a nearly sporulating colony in the partially resistant genotype Akabozu would have been frustrated by the presence of a cell wall apposition. Kneale and Farrar (1985) estimated the total number of haustoria per barley leaf rust colony to be 10^4 and showed that more than one haustorium is present in a host cell. If these data also applies to wheat leaf rust, it follows that one in a thousand penetration attempts is frustrated by cell wall appositions.

It is concluded that the observed differences in colony size of wheat leaf rust several days after inoculation between susceptible and partially resistant wheat genotypes are not caused by the formation and presence of cell wall appositions.

In both experiments the number of cell wall appositions per unit area colony in partially resistant genotypes is higher than in the most susceptible genotype. Such a correlation between number of cell wall appositions and level of partial resistance has previously been reported for the barley/barley leaf rust interaction (Niks, 1986).

The majority of cell wall appositions was observed in mesophyll cells in the region near the substomatal cavity. This again is in agreement with the situation in partially resistant barley. Aborted barley leaf rust infection structures and the associated cell wall appositions are located near the substomatal cavity.

Pre- or post-haustorial resistance

Partial resistance in wheat to wheat leaf rust can be divided into two phases.

In the first phase a number of infection structures is aborted (Table 4-1; Jacobs, 1989). Probably, the infection structures are prevented from penetrating the cells to form a haustorium by the presence of cell wall appositions. This happens prior to the formation of a haustorium. The time between apressorium formation and formation of a haustorium mother cell is short. The defence reactions are localized near the sub-

stomatal cavity. The result of the first phase is a lower infection frequency.

The occurrence of post-haustorial cell collapse in aborted infection structures could indicate that partial resistance to wheat leaf rust not only acts prior to the formation of a haustoria.

In the second phase a post-haustorial growth retardation occurs (Jacobs and Buurlage, 1989). This phase probably starts with the formation of the first haustorium and continues during the growth and sporulation of the wheat leaf rust colony. In the interaction between wheat leaf rust and partially resistant wheat this seems to be the most important phase. It determines the size of colonies and the sporulating areas. Sporulation is delayed which leads to longer latency periods. The mechanism responsible for this growth reduction is not known.

The growth retardation is not caused by the formation of cell wall appositions during the extension of established colonies (this study).

An initial retardation as reported on the barley/barley leaf rust interaction (Arntzen and Parlevliet, 1986; Niks, 1986) was not observed (Jacobs and Buurlage, 1989). It is possible that the growth of the wheat leaf rust hyphae is retarded by extra-cellular components on the mesophyll cell walls of partially resistant genotypes. Or possibly the uptake of nutrients through the extra-haustorial matrix into the haustorium is reduced in partially resistant genotypes. This would lead to a slower growth of the colonies compared to the growth in susceptible genotypes.

No autofluorescence indicative for hypersensitive cell collapse was observed in the second phase. The presence of autofluorescence would lead to a low infection type. Only genotypes with a susceptible infection type and a reduced epidemic build-up are called partially resistant (Parlevliet and van Ommeren, 1975).

It is not clear whether the two phases are under a common genetic control or whether they are coded for by different genes which may be closely linked. Most of the genotypes studied showed a longer latency period and a smaller number of infections (Jacobs, 1989; Broers, 1989a). The variation in infection frequency, as exponent of phase 1, and latency period, as exponent of phase 2, within a certain genotype is considerable. This variation hinders detection of genotypes which deviate from the observed association between infection frequency and latency period. However, exceptions have been reported, both in field experiments and in the greenhouse (Broers, 1989b). In partially resistant barley the association between infection frequency and latency period was high (Parlevliet, 1986).

In barley partially resistant to barley leaf rust a pre-haustorial mechanism was reported. Cell wall appositions are formed probably causing the abortion of several infection structures and delaying the initial growth of the established colonies (Niks 1982; 1983; 1986; Artnzen and Parlevliet 1986). In partially resistant wheat the most determining action of resistance to wheat leaf rust takes place after the formation of haustoria.

REFERENCES

Arntzen, F.K. and J.E. Parlevliet, 1986: Development of barley leaf rust, *Puccinia hordei*, infections in barley. II: importance of early events at the site of penetration for resistance. Euphytica 35: 961-968.

Broers, L.H.M., 1989a. Influence of development stage and host genotype on three components of partially resistance to leaf rust in wheat. Submitted to Euphytica.

Broers, L.H.M., 1989b. Partial resistance to wheat leaf rust in 18 spring wheat cultivars. Submitted to Euphytica.

Eyal, Z., B.C. Clifford and R.M. Caldwell, 1968. A settling tower for quantitative inoculation of leaf blades of mature small grain plants with urediospores. Phytopathology 58: 530-531.

Heath, M.C., 1974. Light and electron microscope studies of the interactions of host and non-host plants with cowpea rust *Uromyces phaseoli* var. *vignae*. Physiological Plant Patholology 4: 403-414.

Jacobs, Th., 1989. Abortion of infection structures of wheat leaf rust in susceptible and partially resistant wheat genotypes. Submitted to Euphytica.

Jacobs, Th. and M.B. Buurlage, 1989. Growth of wheat leaf rust colonies in susceptible and partially resistant spring wheats. Submitted to Euphytica.

Kneale, J. and J.F. Farrar, 1985. The localization and frequency of haustoria in colonies of brown rust on barley leaves. New Phytologist 101: 495-505.

Niks, R.E., 1982. Early abortion of colonies of leaf rust, *Puccinia hordei*, in partially resistant barley seedlings. Canadian Journal of Botany 60: 714-723.

Niks, R.E., 1983. Comparative histology of resistance and non-host reaction to leaf rusts in barley and wheat seedlings. Phytopathology 73: 60-64.

Niks, R.E, 1986. Failure of haustorial development as a factor in slow growth and development of *Puccinia hordei* in partially resistant barley seedlings. Physiological and Molecular Plant Pathology 28: 309-322.

Parlevliet, J.E., 1986. Pleiotropic association of infection frequency and latent period of two barley cultivars partially resistant to barley leaf rust. Euphytica 35: 267-272.

Parlevliet, J.E. and A. van Ommeren, 1975. Resistance of barley to leaf rust, *Puccinia hordei*. II. Relationship between field trials, microplot tests and latent period. Euphytica 24: 293-303.

Zadoks. J.E., T.T. Chang and C.F. Konzak., 1974. A decimal code for the growth stages of cereals. Weed Research 14: 415-421.

Chapter 5.

HAUSTORIUM FORMATION AND CELL WALL APPOSITIONS IN SUSCEPTIBLE AND PARTIALLY RESISTANT WHEAT AND BARLEY SEEDLINGS INFECTED WITH WHEAT LEAF RUST.

Th. Jacobs

SUMMARY

Phase contrast light microscopy observations of wheat and barley seedlings infected with wheat leaf rust suggested that cell wall appositions are structural barriers against haustorium formation leading to abortion of infection structures. Near equal numbers of cell wall appositions per infection structure were detected in seedlings of susceptible and partially resistant wheat genotypes. Differences between susceptible and partially resistant genotypes became evident after the first haustorium had been formed. This again indicates the presence of a post-haustorial effect of partial resistance. Some factors influencing nutrient uptake are discussed.

Wheat leaf rust hardly formed haustoria in barley seedlings, the few not aborted infection structures were accompanied with cell collapse. The mechanisms of partial resistance in wheat and barley to their respective leaf rusts seem different, but their non-host reactions appear similar.

INTRODUCTION

The hypersensitive cell collapse in gramineae in response to an attack by biotrophic fungi occurs after penetration of the host cell wall and the formation of a haustorium in the host cell (Heath, 1974, 1976).

In barley partially resistant to barley leaf rust (*Puccinia hordei*), a pre-haustorium exclusion of the fungus was suggested (Niks, 1982, 1986). The author reported an association between failure of haustorium formation and abortion of infection structures without cell collapse. In wheat partially resistant to wheat leaf rust (*Puccinia recondita* f.sp. *recondita*, syn. *Pucc. triticina*) Jacobs (1989b) reported that aborted infection structures were associated with more cell wall appositions than established colonies also suggesting a pre-haustorium exclusion. However a post-haustorium retardation of fungal development was considered to be of more importance in partially resistant genotypes (Jacobs 1989b).

In this paper the occurrence of cell wall appositions in relation to the location of haustorial mother cells and the presence of haustoria is studied. The actual number and size of haustoria in susceptible and partially resistant seedlings was noted as well as the presence of encased haustorial bodies and haustorial necks.

MATERIALS AND METHODS

The wheat genotypes were Morocco (highly susceptible to wheat leaf rust), Kaspar (susceptible), Westphal 12A, Akabozu and BH 1146 (partially resistant). The two barley genotypes were highly susceptible (L94) and partially resistant (Vada) to barley leaf rust. In each of three flats (37 x 39 cm) seedlings of five wheat genotypes and two barley genotypes were grown. Primary leaves of all genotypes were dusted on the adaxial surface with a mixture (6:1) of *Lycopodium* spores and urediospores of a monospore culture of the 'Flamingo' race of wheat leaf rust. A high density of inoculum was applied to facilitate microscopic observation. After incubation, during one night with a relative humidity of 100%, the flats were placed in the greenhouse. Average day temperature was 22 °C and average night temperature was 17 °C.

Per sampling date six leaf pieces of each genotype (two from each flat) were harvested, fixed in ethanol-dichloromethane with 0.15 % trichloric acetic acid (TCA) during 24 hours and boiled in 0.01% trypan blue (Jacobs 1989a; modified procedure A,

Niks 1986). Leaf pieces were mounted and observed with a phase contrast light microscope (400 X) for observation of the substomatal vesicle. Haustoria were observed under emersion oil (1000 X). A suitable combination of filters and very clean lenses, to reduce background disturbance, were necessary.

From each leaf piece 15 infection structures evenly distributed over the leaf were selected. Infection structures between the leaf margins and the first vein and those on double occupied stomata were not included. The location of the infection structures on the leaf was noted (stomata number and row number from a marked leaf edge), to enable observation of the infection structures after the next step in the staining procedure (procedure B). Of each infection structure the direction of growth of each hypha starting from the substomatal vesicle, the location of the haustorial mother cells and the number, size and shape of the haustoria were recorded. The haustoria were classified as small, medium-sized or large. The presence of dark blue collapsed cells was recorded.

After these observations leaf pieces passed a series of dehydrating alcohols and were stained in a saturated solution of picric acid in methyl salicylate, cleared and mounted in methyl salicylate (procedure B, Niks 1986). The same infection structures were observed, the number of haustoria per hypha was checked, missing haustoria were recorded and the number and position of luminous cell wall appositions near the haustorial mother cells were recorded. If haustorial necks or the haustorial body were encased this was also recorded.

In a separate flat seedlings of all genotypes were inoculated on the adaxial side with a low density of urediospores of wheat leaf rust and incubated at a R.H. of 100% during the night. The infection type on a scale of 0 to 9 and the latency period was determined on 3.0 cm long central parts of four leaves per genotype (Jacobs and Broers, 1989). When the first light green halo's were visible on the most susceptible genotype (105 hours after inoculation), five leaf pieces of each genotype were sampled. After the final count for determination of the latency period (250 hours) three leaf pieces per genotype were collected and fixed with Uvitex 2B (Jacobs, 1989a). Observations were made with an epifluorescence microscope, magnification: 100x, excitation: 540 nm. The percentage aborted infection structures was determined (105 hours) by observing at least 100 infection structures per leaf piece. An infection structure was defined as aborted when at the most four haustorial mother cells were present (Jacobs, 1989a). At both harvest times length and width of 20 colonies of three leaf pieces per genotype were measured with a calibrated eye-piece meter. Data were related to the highly susceptible genotype Morocco (set at 100). Of both harvests, the presence of yellow autofluorescence, which indicates hypersensitive cell collapse, near infection structures was recorded.

Several small experiments have been performed to improve the visibility of haustoria, of the surroundings of the haustorium and of the cell wall appositions. None of the chemicals used improved the results obtained by the method used, although using 3,5 dinitro-salicyl acid gave almost equal results as the use of picric acid. The use of xylol instead of methyl salicilate gave visually attractive images of haustoria but made reliable counts difficult. Several tests performed to study the chemical composition of cell wall appositions coloured the whole leaf segment. The tests were judged inappropriate to study the intact fungal-host interface in whole mounts. The tests were: the phloroglucinol-HCl test, the $KMnO_4$ reaction and the chlorine water-sodium sulfite test, all for lignin; the lacmoid test and the aniline blue fluorescence test, for cal-

Table 5-1. Infection type (IT), relative latency period (RLP), percentage aborted infection structures (abortion) and relative colony size 105 and 250 hours (hrs) after inoculation of wheat and barley seedlings infected with wheat leaf rust.

	IT	RLP	Abortion	relative colony size after	
				105 hrs	250 hrs
Wheat genotype					
Morocco	9	100	1^{a*}	100^{a**}	100^{a**}
Kaspar	9	100.3	7^{a}	74^{b}	98^{a}
Westphal 12A	9	105.3	4^{a}	76^{ab}	98^{a}
Akabozu	9	106.2	12^{a}	59^{bc}	58^{b}
BH 1146	9	106.4	6^{a}	45^{c}	81^{ab}
Barley genotype					
L94	4	103	54^{b}	34^{c}	22^{c}
Vada	2	-	92^{c}	4^{d}	2^{c}

*) values in this column followed by the same letter are not significantly different (P<0.05) according to the Scheffé test.

**) as above, calculations on original data.

lose and the JKJ-H_2SO_4 reaction for cellulose.

RESULTS

All wheat seedlings showed high infection types, a common feature in partially resistant wheat (Table 5-1). The latency periods of the partially resistant wheat genotypes Westphal 12A and Akabozu were slightly longer and the percentages aborted infection structures were slightly higher than those of the highly susceptible Morocco. Differences in colony size between susceptible and partially resistant genotypes were prominent 105 hours after inoculation but less so 250 hours after inoculation. The smaller differences between susceptible and partially resistant genotypes at 250 hours compared to 105 hours, were probably caused by the fact that the colonies in the highly susceptible Morocco had reached the margins of the leaves and could not increase in size anymore.

Barley inoculated with wheat leaf rust showed low infection types (Table 5-1). The majority of the wheat leaf rust infection structures were aborted in the two barley genotypes. They had less than four haustorial mother cells and did not show autofluorescence. The wheat leaf rust infection structures (colonies) in barley with more than four haustorial mother cells showed yellow autofluorescence. Most of these colonies were small. In the genotype Vada several colonies did not show more than ten haustorial mother cells. Surprisingly most of the established wheat leaf rust colonies in the barley genotype L94 sporulated.

Visibility of haustoria

After staining procedure A, medium-sized haustoria were visible as balloon-like structures, which were darker than the surround-

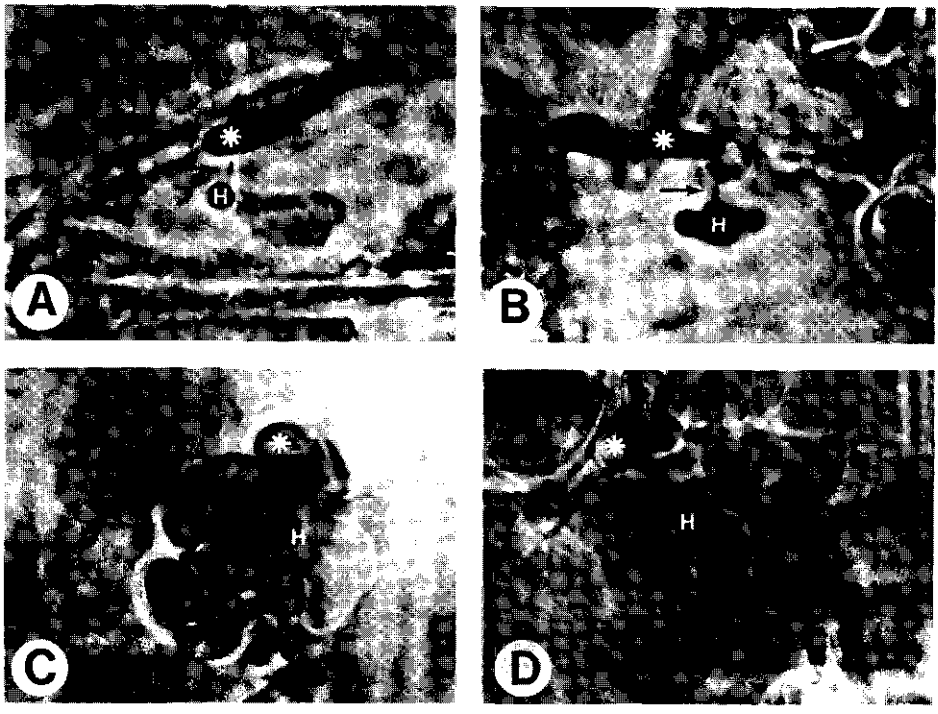

Figure 5-1. Haustoria (H) in wheat seedlings infected with wheat leaf rust. 2A. Small haustorium in a subsidiary cell of the partially resistant genotype Akabozu. 2B. Medium sized haustorium in an epidermal cell of the partially resistant genotype Akabozu. 2C. Large haustorium in an mesophyll cell of the partially resistant genotype Akabozu. 2D. Large haustorium in a mesophyll cell of the highly susceptible genotype Morocco after a xylol treatment.

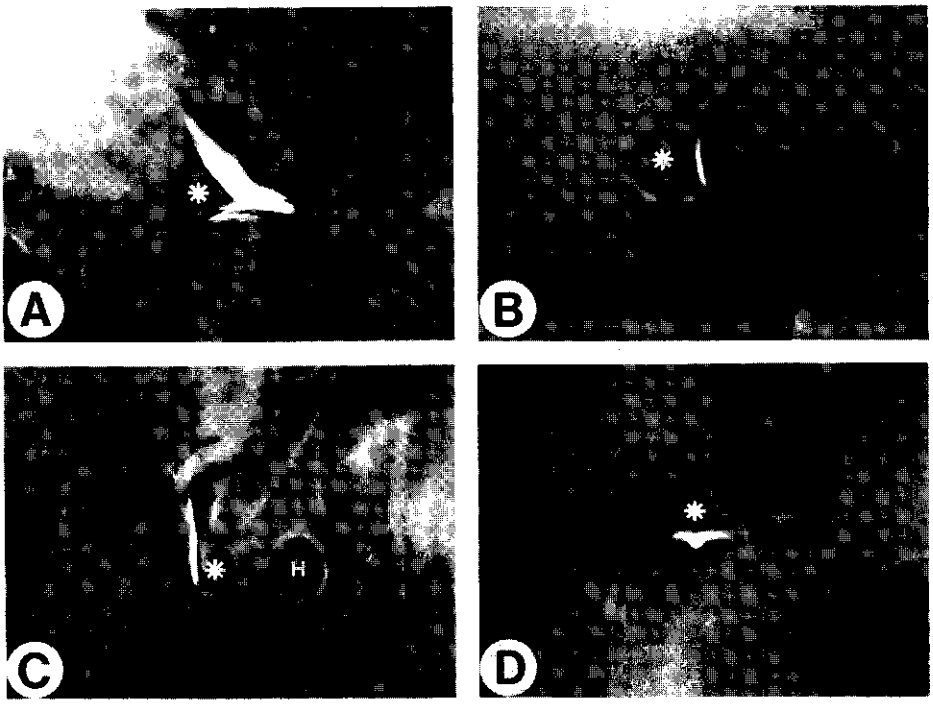

Figure 5-2. Cell wall appositions (arrows) opposite haustorial mother cells (*) in wheat and barley seedlings infected with wheat leaf rust. 1A. Cell wall apposition in the highly susceptible genotype Morocco. 1B. Cell wall apposition in the partially resistant genotype BH 1146. 1C. Haustorial mother cell in BH 1146 in contact with a cell wall apposition on one side and a successful penetration on the other side. 1D. A representative tivetivecell wall apposition in the non-host barley genotype L94, note the absence of granulation in the mesophyl cells.

ing cell contents (Figure 5-1B). Large haustoria were often irregularly shaped and hardly contrasted with their surroundings. They were often slightly lighter than the surrounding cell material and showed a lighter ring around the haustorial walls (Figure 5-1C). The large haustoria are probably mature ones. After procedure B, small darkly stained and circular haustoria were detected (Figure 5-1A). These haustoria clearly showed a neck and sometimes the neck band was visible. On several occasions the nucleus of the host cell was seen near the haustorium. At times the nucleus was located close to the haustorium neck, near the point where the cell wall had been penetrated. Comparison of the observations made after staining with trypan blue (procedure A) and those made after staining with wintergreen oil in methylsalicylate (procedure B) showed that the walls of the substomatal vesicles and hyphae were reasonably well visible after procedure A, but much more obscure after procedure B. The same phenomenon was observed with large haustoria. The location of the large haustoria was exactly known after the staining with trypan blue. Observations made after procedure B, learned that several large haustoria were hardly visible, while others were not seen at all. The opposite was true for small haustoria. Additional counts of small circular haustoria with a clearly visible neck could be made after staining procedure B. The small haustoria were located at positions where previously no haustoria had been recorded.

After procedure B cell contents were more granular and luminous cell wall appositions were visible. In wheat the majority of the cell wall appositions was more drawn out and thinner than the cell wall appositions in barley which often showed a clear central thickening (Figure 5-2). After both procedures leaves of the barley genotypes were much more transparent and showed less granulation than the leaves of the wheat genotypes studied. The data of both procedures have been combined and are presented (Table 5-2). Due to the limited colony

Table 5-2. Average number of haustoria (H) and cell wall appositions (C) per infection structure at 20, 34 or 40 hours after inoculation of wheat and barley genotypes infected with wheat leaf rust.

	20 hours		34 hours		40 hours	
	H	C	H	C	H	C
Wheat genotype +)						
Morocco	0.91^{a}	0.08^{a}	2.59^{a}	0.09^{a}	3.47^{a}	0.09^{a}
Kaspar	0.92^{a}	0.10^{a}	2.21^{a}	0.10^{a}		
Westphal 12A	0.95^{a}	0.16^{a}	2.13^{a}	0.10^{a}		
Akabozu	0.84^{a}	0.30^{a}	1.72^{ab}	0.14^{a}	2.57^{b}	0.23^{a}
BH 1146	1.04^{a}	0.07^{a}	2.08^{a}	0.09^{a}		
Barley genotype						
L94	0.43^{b}	1.23^{b}	0.87^{bc}	5.11^{b}		
Vada	0.05^{c}	3.54^{c}	0.23^{c}	5.83^{b}		

+) values in each column followed by a different letter are significantly different ($P<0.05$) according to the Scheffé test.

size, the data of Table 5-2 include aborted infection structures.

Location of luminous zones

After procedure B the parts of the cell walls of guard cells in contact with the appressorium and the penetration peg were brightly coloured. All wheat and barley genotypes showed these luminous contact-areas.

Several haustorial mother cells in contact with cell walls were associated with cell wall appositions. Luminous cell wall appositions were also recorded at contact-sites with hyphae. Occasionally luminous cell walls were observed in cells not in direct contact with the fungus. Most of these zones resembled cell wall appositions. They were located at sites where two mesophyll cells contacted each other. These cell wall appositions were not in contact with the fungus and are therefore not included in the data.

Association of cell wall appositions and haustoria

In nearly all cases where a haustorial mother cell was in contact with a cell wall and a cell wall apposition was present no haustoria were observed in the mesophyll cells (Figure 5-2 A,B). On rare occasions a haustorium was present in the mesophyll cell with a wall apposition. In such cases the haustorium originated from a haustorial mother cell which extended beyond the cell wall apposition (Figure 4-1 G).

Additional observations were made on the seedling leaves of Akabozu collected 105 hours after inoculation. The exact position of uvitex stained aborted infection structures was noted, the leaf pieces were treated according to procedure A and B and the presence of cell wall appositions and haustoria was observed. In all leaf pieces the aborted infection structures did not show haustoria and cell wall appositions were present near the haustorial mother cells.

Number of haustoria

Wheat leaf rust infection structures had formed, on average, nearly one haustorium 20 hours after inoculation in all wheat genotypes. At that time differences between the wheat genotypes were small (Table 5-2). Significant differences occurred later. The partially resistant genotypes showed infection structures with fewer haustoria than the highly susceptible Morocco.

Twenty hours after inoculation the majority of haustoria in wheat seedlings was small or medium-sized, 34 hours after inoculation the percentage large haustoria had increased to nearly 30 percent and six hours later nearly 40 percent of the haustoria was classified as large (Table 5-3). Differences in percentages of haustoria in the three categories between susceptible and partially resistant genotypes were small. Infection structures in the partially resistant genotype Akabozu had formed less than average large haustoria at 20 and 34 hours after inoculation but not at 40 hours after inoculation.

No morphological differences could be detected between the haustoria in susceptible and partially resistant wheat genotypes. The average number of cell wall appositions per infection structure did not differ between the wheat genotypes. No dark blue collapsed cells were observed near infection structures in any of the wheat seedlings. No encapsulated or partially encapsulated haustoria have been observed in the cells of the wheat and barley genotypes studied.

Table 5-3. Percentage small (S), medium-sized (M) and large (L) haustoria of wheat leaf rust infection structures in seedlings of wheat and barley genotypes 20, 34 or 40 hours after inoculation.

	20 hours			34 hours			40 hours		
	S	M	L	S	M	L	S	M	L
Wheat genotype									
Morocco	39	55	6	27	44	29	28	37	35
Kaspar	27	68	5	26	44	30			
Westphal 12A	24	62	14	25	47	28			
Akabozu	29	71	0	36	44	20	27	30	43
BH 1146	28	69	4	29	42	29			
average	29	65	6	29	44	27	28	34	39
Barley genotype									
L94	30	70	0	33	47	19			
Vada	60	30	0	29	66	5			

Barley

The number of cell wall appositions in the barley seedlings was higher than in the wheat seedlings. The number of cell wall appositions and the percentage abortion of wheat leaf rust infection structures were lower in L94 than in Vada (Table 5-1 and 5-2).

In Vada several haustorial mother cells opposite cell wall appositions were lobed or strangely branched as if several penetration attempts of the haustorial mother cell had failed. This has not been observed in the other genotypes studied. Niks (1983b) reported "unusual lobed haustorial mother cells and hyphal tips" in Vada infected with barley leaf rust.

In barley, established wheat leaf rust infection structures developed few haustoria. The majority of haustoria was small (Table 5-2 and 5-3). In the genotype Vada dark blue collapsed cells were observed.

DISCUSSION

The mechanism for partial resistance in wheat to wheat leaf rust involves two different phases (Jacobs, 1989b). During the first phase a pre-haustorium exclusion of the fungus was postulated, leading to the abortion of infection structures (Jacobs 1989a, 1989b). In the second phase partially resistant genotypes showed smaller colonies than the susceptible genotype (Jacobs and Buurlage, 1989). Data from this study support this dual aspect of partial resistance to wheat leaf rust.

The first phase

Aborted infection structures were associated with a higher number of cell wall appositions than established infection structures (Jacobs, 1989b). In most cases where a haustorial mother cell contacted a cell wall and a cell wall apposition was present no haustorium was observed. This strongly suggests a causal relation between the

processes which lead to the formation of a cell wall apposition and failed haustorium formation. The rare observation of an encapsulated haustorium neck suggests that cell wall appositions are structural barriers.

Cell wall appositions also occured at the contact-site of two cells. It indicates that the luminous wall zones are not only induced by contact with a fungus. It is not impossible that the procedure with picric acid is not a very specific staining method.

In partially resistant wheat seedlings less than fifteen percent of the infection structures were aborted. In earlier reports (Jacobs 1989a, 1989b) the percentage aborted infection structures in seedlings never exceeded 20 percent. In the wheat seedlings studied, the pre-haustorial exclusion seems of limited importance.

The second phase

Wheat leaf rust colonies 105 and 250 hours after inoculation, were smaller in partially resistant wheat genotypes than in the highly susceptible Morocco. Smaller colonies were observed in seedlings and adult plants of partially resistant wheat genotypes (Jacobs and Buurlage, 1989)

In this study the average number of cell wall appositions per infection structure did not differ between the wheat genotypes. Jacobs (1989b) working with flag leaves concluded that the low number of cell wall appositions could not be responsible for the observed difference in colony size between susceptible and partially resistant genotypes.

Colony growth measured by the number of haustoria can be divided into two parts. The first part covers the period between formation of an appressorium and the formation of the first haustorium. This time-span seems on average to be equal for all host genotypes (Table 5-2). Differences between susceptible and partially resistant genotypes become apparent after the formation of the first haustorium. In the second part there is a clear influence of host genotype on the number of haustoria.

Wheat leaf rust colonies in partially resistant genotypes formed fewer haustoria than colonies in susceptible genotypes (Table 5-2). In the average number of haustoria per infection structure, aborted infection structures are included. Assuming 1% abortion for Morocco and 12% for Akabozu (Table 5-1), the number of haustoria per infection structure 34 hours after inoculation would be estimated at 2.62 and 1.93 respectively. Forty hours after inoculation this would be 3.50 and 2.88 respectively. This does not alter the data to a large extent.

Mechanism

Probably the reduction in size of fungal structures in the second phase is of a post-haustorial nature. Carver and Carr (1978) described the occurrence of a haustorial inefficiency in oats resistant to mildew. They mentioned as possible factors influencing the nutrient uptake by haustoria: a physical restriction by the sheath surrounding the haustorium, a semi-permeable sheath membrane, osmotic relations or a lack of suitable nutrients in the host. Light microscopic observations, made during this study, showed that the post-haustorium reduction of colony size is not caused by encasement of haustoria. No statements based on observations from this study can be made on the other factors mentioned above. Carver and Carr also report that "haustoria of equal size may differ in their capacity to extract nutrients from the host cell". If a similar phenomenon operates in wheat, it could explain the near equal percentages of small, medium and large haustoria per colony in susceptible and partially resistant genotypes. It cannot be ruled out that the growth

of hyphae in leaves of partially resistant genotypes is retarded by chemical substances present on the exterior of cell walls or in the intercellular cavities. The growth reduction caused by these substances may be slight and only detectable at later stages of the infection when cumulative effects become clear. It is also possible that the passage through the cell wall in partially resistant genotypes takes more time than in susceptible genotypes. If such is the case, a lower number of medium-sized and large haustoria is expected in partially resistant genotypes. This has not been observed.

The exact mechanism which is responsible for the reduction in size of wheat leaf rust colonies in partially resistant wheat genotypes remains unknown. It is clear that pre-haustorium exclusion by cell wall apposition is of minor importance in wheat partially resistant to wheat leaf rust.

Two pathosystems

An average difference of 0.9 haustorium per infections structure was observed between Morocco and Akabozu 40 hours after inoculation. Niks (1986) reported a difference of 2.9 haustoria 44 hours after inoculation between the highly susceptible L94 and the partially resistant Vada. This difference reflects the difference between the two pathosystems. In partially resistant wheat a continuous reduction of the growth rate was observed (Jacobs and Buurlage, 1989). In wheat a small difference in number of haustoria per infection structure just after inoculation is expected and larger differences longer after inoculation. In partially resistant barley an initial delay was observed (Artnzen and Parlevliet 1986). An early and clear difference in number of haustoria per infection structure between susceptible and partially resistant genotypes is than expected. The difference in number of haustoria per infection structure between the two studies could, in part, reflect differences in experimental procedures (incubation time, temperature).

Non-host reaction

The majority of wheat leaf rust infection structures in barley were aborted (Table 5-1). A minority developed few haustoria. In both cases this was probably caused by a high proportion of failed attempts to form a haustorium, as the number of associated cell wall appositions was high (Table 5-2). The majority of haustoria was small (Table 5-3). In the genotype Vada dark blue collapsed cells were observed. Indicating a post-haustorium reaction of the mesophyll cells.

Niks (1983b) reported that a minority of barley leaf rust infection structures developed a haustorium in mesophyll cells of the wheat genotype Duri. The majority of barley leaf rust infection structures did not develop haustoria in the wheat genotype Duri and were aborted. This was associated with the presence of cell wall appositions. The formation of cell wall appositions is a regularly observed phenomenon in the non-host reaction of cereals (Aist, 1976, Heath, 1980). The similarity between the two non-host reactions is striking. Both barley and wheat show a non-host reaction in which the majority of non-pathogen infection structures are excluded from entering the cell by cell wall appositions. A minority of infection structures becomes arrested after haustoria have been formed in the cell.

Partial resistance and non-host resistance

The number of cell wall appositions and the percentage abortion of wheat leaf rust infection structures were lower in the barley genotype L94 than in the genotype Vada. The barley genotype L94 is highly susceptible

for barley leaf rust and showed sporulating wheat leaf rust colonies. Vada which is partially resistant to barley leaf rust also showed a strong non-host reaction against wheat leaf rust. This corresponds with their reaction to barley leaf rust and supports the view that the non-host reactions of barley bears resemblance with the pre-haustorial acting partial resistance of barley to barley leaf rust (Niks, 1983a). Sporulation of rust pathogens on non-host cereals has also been reported by Helfer (1987).

In barley, a high degree of similarity between non-host reactions of barley to *Puccinia* species and partial resistance to barley leaf rust was reported (Niks 1983a). In wheat, such a similarity between partial resistance and non-host resistance was not observed.

REFERENCES

Arntzen, F.K. and J.E. Parlevliet, 1986. Development of barley leaf rust, *Puccinia hordei*, infections in barley. II: Importance of early events at the site of penetration for partial resistance. Euphytica 35: 961-968.

Aist, J.R., 1976. Papillae and related wound plugs of plant cells. Annual Review of Phytopathology 14: 145-163.

Carver T.L.W. and A.J.H. Carr, 1978. Effects of host resistance on the development of haustoria and colonies of oat mildew. Annals of Applied Biology 88: 171-178.

Heath, M.C., 1974. Light and electron microscope studies of the interactions of host and non-host plants with cowpea rust *Uromyces phaseoli* var. *vignae*. Physiological Plant Pathology 4: 403-414.

Heath, M.C., 1976. Hypersensitivity, the cause or consequence of rust resistance? Phytopathology 66: 935-936.

Heath, M.C., 1980. Reactions of nonsuscepts to fungal pathogens. Annual Review of Phytopathology 18: 211-236.

Helfer, S., 1987. Development of cereal rusts on host and non-host plants. Cereal Rusts Bulletin 15: 44-52.

Jacobs, Th., 1989a. Abortion of infection structures of wheat leaf rust in susceptible and partially resistant spring wheats. Submitted to Euphytica.

Jacobs, Th., 1989b. The occurrence of cell wall appositions in flag leaves of spring wheats susceptible and partially resistant to wheat leaf rust. Submitted to the Journal of Phytopathology.

Jacobs, Th. and L.H.M. Broers, 1989. The inheritance of latency period in susceptible and partially resistant spring wheat genotypes infected with leaf rust. I: Estimation of gene action and number of effective factors in F1, F2, BC1 and BC2 generations. Euphytica, in press.

Jacobs, Th. and M.B. Buurlage, 1989. Growth of wheat leaf rust colonies in susceptible and partially resistant spring wheats. Submitted to Euphytica.

Niks, R.E., 1982. Early abortion of colonies of leaf rust, *Puccinia hordei*, in partially resistant barley seedlings. Canadian Journal of Botany 60: 714-723.

Niks, R.E., 1983a. Comparative histology of resistance and non-host reaction to leaf rusts in barley and wheat seedlings. Phytopathology 73: 60-64.

Niks, R.E., 1983b. Haustorium formation of *Puccinia hordei* in leaves of hypersensitive, partially resistant and non-host plant genotypes. Phytopathology 73: 64-66.

Niks, R.E., 1986. Failure of haustorial development as a factor in slow growth and development of *Puccinia hordei* in partially resistant barley seedlings. Physiological and Molecular Plant Pathology 28: 309-322.

Chapter 6.

THE ULTRASTRUCTURE OF THE INTERFACE BETWEEN SUSCEPTIBLE, PARTIALLY RESISTANT SPRING WHEAT GENOTYPES AND WHEAT LEAF RUST.

Th. Jacobs, F.P. van Hee* and F.M. Engels+.

* Part of his "doktoraal studie".
+ Department of Plant Cytology and Morphology.

SUMMARY

The ultrastructure of host cell wall appositions in the highly susceptible genotype Morocco and in the partially resistant genotype Akabozu was studied shortly after inoculation with wheat leaf rust. The ultrastructure of the haustorium and the host cell of Morocco, Akabozu and another partially resistant genotype Westphal 12A was studied just before sporulation.
The cell wall appositions in Morocco and Akabozu were narrow and long, they extended on both sides beyond the contact zone of the haustorial mother cell and host cell wall. The majority of cell wall appositions in Akabozu consisted of a fibrillar network, in Morocco the majority of cell wall appositions consisted of a layer of dark entities. It is suggested that contrary to the general opinion the cell wall appositions are formed as a coagulation of fungal and host proteins and are not a lignin or callose containing cell wall addition.
Just before sporulation no differences could be detected in extrahaustorial membrane, matrix or haustorial wall between the susceptible and partially resistant genotypes. No collars were observed around the haustorial neck. The cell contents in the highly susceptible Morocco appeared degenerated as if the cells were empty. The amount of cytoplasm was higher and the condition of chloroplasts was better in the partially resistant genotypes. It is suggested that the transfer of nutrients into the haustorium in partially resistant genotypes takes place at a lower speed or requires more energy than in the susceptible genotype. Possibly partially resistant genotypes offer a poor quality nutrients to the fungus.

INTRODUCTION

The majority of electron microscopical studies of rust fungi focus on genotypes which give a susceptible reaction or show hypersensitivity resistance. They have been reviewed by Littlefield and Heath (1979) and Harder and Chong (1984). Up to now we know of no study comparing the ultrastructure of susceptible and partially resistant genotypes.

Partial resistance in wheat (*Triticum aestivum*) to wheat leaf rust (*Puccinia recondita* f.sp. *tritici*, syn. *Pucc. triticina*) was divided into two phases (Jacobs, 1989b). In the first phase infection structures were aborted. This was associated with the presence of cell wall appositions. The aborted infection structures did not form haustoria (Jacobs, 1989c). In the more important second phase a continuous retardation of the growth rate was observed in partially resistant genotypes (Jacobs and Buurlage, 1989) and a post-haustorial inhibition was postulated (Jacobs, 1989c).

In this study the ultrastructure of the cell wall appositions in a susceptible and a partially resistant genotype will be described. The interface between biotrophic fungi and their hosts consists of a highly specialized region termed the extrahaustorial membrane and matrix (Littlefield and Heath, 1979). The ultrastructural properties of this region of nutrient transfer in susceptible and partially resistant genotypes was studied to detect possible differences which could be responsible for their reaction to wheat leaf rust. Other elements of the cell contents of susceptible and partially resistant genotypes were also studied.

MATERIALS AND METHODS

Flag leaves of the highly susceptible genotype Morocco and of the partially resistant genotypes Akabozu and Westphal 12A were used. The partially resistant genotypes used showed a higher percentage aborted infection structures, a longer latency period and smaller colonies than the

highly susceptible genotype Morocco (Jacobs, 1989a; Jacobs and Buurlage, 1989). Plants of all genotypes in stage 48 - 59, (just heading, Zadoks *et al.*, 1974) were selected and inoculated with a monospore culture of the 'Flamingo' race of wheat leaf rust. To ensure a high density of infection structures a fine-liner was used to draw transverse lines on the central part of the flag leaves. On three consecutive days a large number of urediospores was applied on the marked part of the flag leaves with a brush. Each night an incubation was carried out with a relative humidity of 100% (Jacobs, 1989a). Two days after the last incubation period, the marked parts were harvested, the leave margins were removed. The inoculated leaf parts were cut into pieces of 1.0 x 0.5 mm and treated according to the procedure described by Woods and Gay (1987). These are the "A samples" in this paper.

Six days after the last incubation period, central parts of the flag leaves were harvested and treated as described above (the B samples). At that time the flag leaves of the partially resistant genotypes showed halo's and colonies in Morocco nearly sporulated. For comparison, not infected flag leaves of the same stage were harvested. Thin sections of epon embedded material were post-stained for 30 min. at 40 °C with uranylacetate and 2 min. at 20 °C with lead-citrate.

RESULTS

Observations of the A samples

The border between the cell walls of the haustorial mother cells or hyphae and the host cell wall was often obscure. Darkly stained material was present in the corners at these contact-sites (Figure 6-1 and 6-2). Dark material was also present in the host cell wall opposite the haustorial mother cell (Figure 6-2).

Opposite the haustorial mother cells, between mesophyll cell wall and plasmalem-

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Figure 6-1 to 6-6 are taken from samples of the first sampling date (A samples).

Figure 6-1. Contact site of a wheat leaf rust haustorial mother cell and the wheat cell wall, note the presence of dark material in the corner (arrow). In the cell wall apposition two layers can be distinguished (Akabozu). 10.000 X.

Figure 6-2. Darker region in the host cell wall opposite the haustorial mother cell, the corner is filled with dark material (arrow). The cell wall is darker opposite the haustorial mother cell. Between the cell wall and the plasmalemma a cell wall apposition is present. (Akabozu) 13.000 X.

Figure 6-3. Cell wall apposition in the highly susceptible wheat genotype Morocco consisting of a layer of dark entities. 10.000 X.

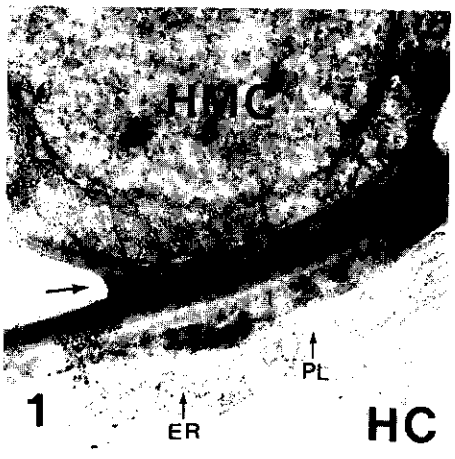
Figure 6-4. The majority of cell wall appositions in the partially resistant wheat genotype Akabozu consisted of a homogeneous layer of fibrillar network. 13.000 X.

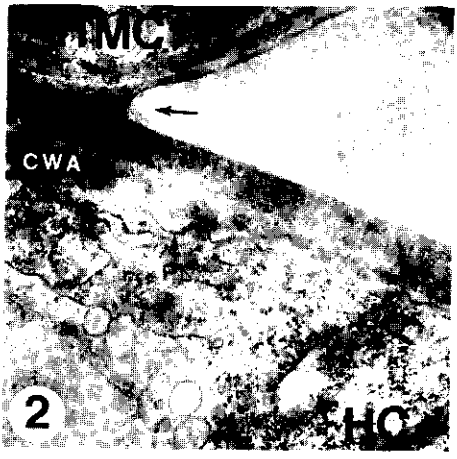
Figure 6-5. Cell wall apposition in the highly susceptible wheat genotype Morocco with central dark entities in a layer of fibrillar network. 13.000 X.

Figure 6-6. A typical cell wall apposition in the partially resistant Akabozu, with several layers of dark entities and an outer layer of fibrillar network. 13.000 X.

Abbreviations:

Chl =	Chloroplast	HC =	Host Cel	N =	Nucleusl
CWA =	Cell wall appositions	HMC =	Haustoriall Mother Cell	PL =	Plasmalemma
ER =	*Endoplasmatic Reticulum*	M =	*Mitichondrium*		


HMC
PL
ER
1
HC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2
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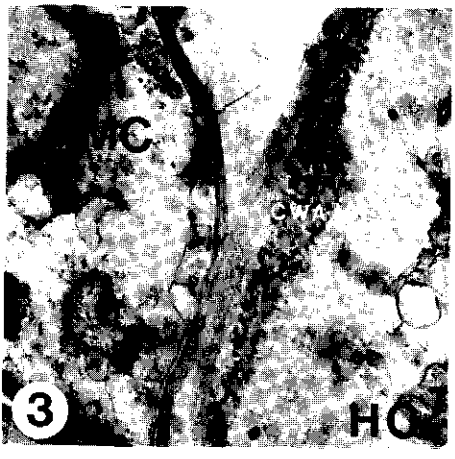
HMC
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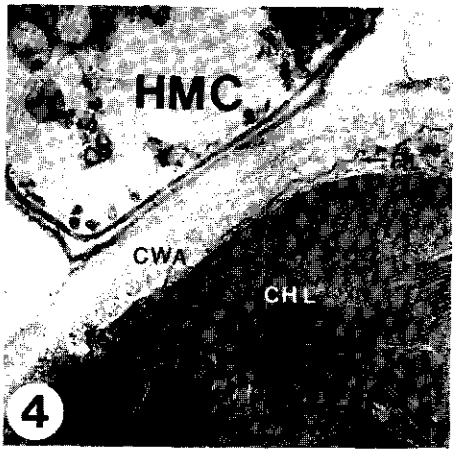
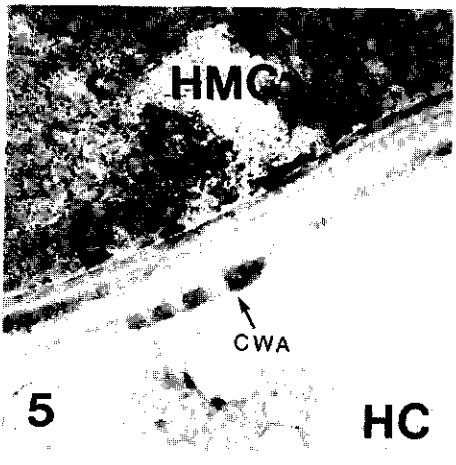
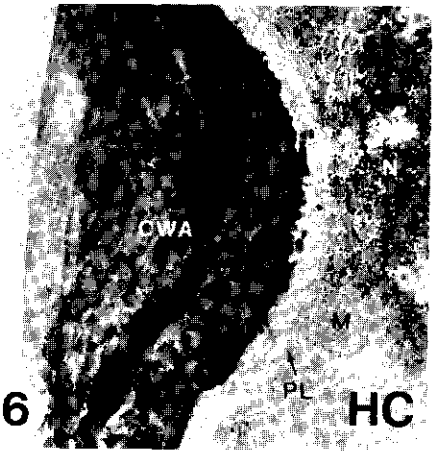

HMC
CWA
CHL
4


HMC
CWA
5
HC


CWA
N
M
PL
6
HC

ma, cell wall appositions were observed in the two genotypes studied (Figure 6-1, 6-2 and 6-4). The cell wall appositions were slender and at the most twice the width of the adjacent cell wall and extended beyond the direct contact-area of haustorial mother cell and cell wall. In several sections a cell wall apposition was present without a haustorial mother cell being observed in that section. In most cases a haustorial mother cell was observed after serial sectioning.

There were marked differences in the structure of cell wall appositions in the highly susceptible genotype Morocco and the partially resistant genotype Akabozu. The majority of cell wall appositions in Morocco consisted of a layer of electron-opaque entities (Figure 6-3). These entities consisted of a dark, often circular center surrounded by a lighter irregular shaped zone. These entities will be called "dark entities". In Akabozu the majority of cell wall appositions consisted of a homogeneous layer of interconnected strings of fibrillar material (Figure 6-4). Both types of cell wall appositions were present in both genotypes studied but dark entities were much more abundant in Morocco and the fibrillar network predominated in Akabozu. In both genotypes intermediate forms have been observed. In Morocco fibrillar network was observed in the central part of the cell wall apposition (Figure 6-5). In Akabozu cell wall appositions have been observed with an electron-opaque center (Figure 6-1 and 6-2). A typical cell wall apposition has been observed once in Akabozu (Figure 6-6). This cell wall apposition was more than five times as thick as the width of the adjacent cell wall. It consisted of layers of dark entities around a central area also filled with dark entities. The most outward layer of the wall apposition opposite the plasmalemma appeared identical to the fibrillar network. In most cells near a cell wall apposition the cytoplasm was parietal and contained numerous cell organelles.

Observations of the B samples

Not infected material of all genotypes showed a normal cytoplasm. The thylakoid membranes in the chloroplasts were regularly distributed and oriented parallel to each other (Figure 6-7).

No cell wall appositions were observed at contact-sites between haustorial mother cells or hyphae and the host cell wall in established colonies of all genotypes studied.

In all genotypes studied haustoria were found. Around the haustoria a layer of cytoplasm was present (Figure 6-8, 6-9 and 6-10). Between the host cell wall and the haustorium several chloroplasts and occasionally a mitochondrium was present. At times the host nucleus was located near the haustorium. The host cell contained a large vacuole. In a few sections a haustorial neck was present, the neck was surrounded by endoplasmatic reticulum orientated parallel to the neck. No collars were observed around the neck in all genotypes studied.

In the highly susceptible genotype Morocco the host cells were vacuolated and contained cytoplasm with a few organelles (Figure 6-8). In several sections the cytoplasm was retracted from the cell wall. In several chloroplasts the thylakoid membranes were irregularly orientated (Figure 6-8, inset). In some cells the chloroplasts contained starch. Only a thin layer of cytoplasm surrounded the haustorium in cells of Morocco. No extensive endoplasmatic reticulum was observed around the haustorium. The extrahaustorial membrane around the haustorium had an undulating appearance. The width of the extrahaustorial matrix, which is the space between the haustorial wall and the extrahaustorial membrane, thus varied. Inside the haustoria large vacuoles and some mitochondria were present. The overall impression of the cells in Morocco was that of empty cells, in a way they looked plundered by the fungus (Figure 6-8). The symptoms

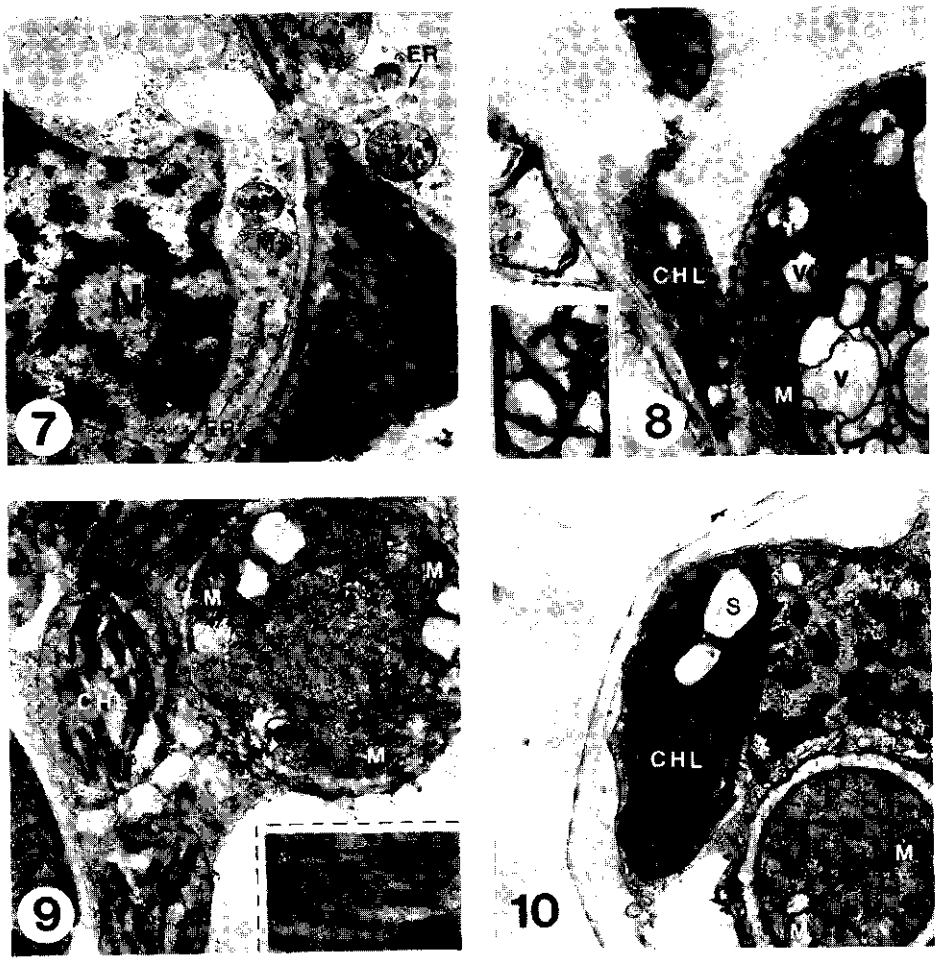


Figure 6-7 to 6-10 are taken from samples of the first sampling date (B samples).

Figure 6-7. Not infected leaf tissue of the partially resistant wheat genotype Westphal 12A with mitochondria and endoplasmatic reticulum. 4200 X.

Figure 6-8. Chloroplasts and a vacuolized haustorium in a cell of the susceptible wheat genotype Morocco. 4200 X. The inset shows irregularly orientated thylakoid membranes in a chloroplast (7200 X).

Figure 6-9. Haustorium, chloroplasts and cell plasma in a cell of the partially resistant wheat genotype Akabozu. 4200 X. The inset shows a chloroplast with normal orientated thylakoid membranes (7200 X).

Figure 6-10. Haustorium, chloroplasts and nucleus in a cell of the partially resistant wheat genotype Westphal 12A. The distance between the haustorial cell wall and the extrahaustorial membrane looks wider than in the pictures of Morocco and Akabozu. The presence of a wider space can be caused by sectioning through the tip of the haustorium and has also been observed in the other genotypes. 4200 X.

Abbreviations:

Chl =	Chloroplast	M =	Mitochondrium	S =	Starch
ER =	Endoplasmatic Reticulum	N =	Nucleus	V =	Vacuole
H =	Haustorium				

observed in cells of the highly susceptible genotype Morocco are similar to symptoms reported for compatible host/rust interactions at later stages of the infection. (Littlefield and Heath, 1979).

Cells of the partially resistant genotype Akabozu contained well organized cytoplasm compared to cells of Morocco (Figure 6-9). Chloroplast in cells of Akabozu hardly showed signs of disorganization (Figure 6-9, inset). Around the haustorium extended layers of endoplasmatic reticulum and occasionally dictyosomes were observed. The haustorium was hardly vacuolated and contained several mitochondria which were localized near the haustorial wall (Figure 6-9). No extensive network of tubules and membranes connected with the extrahaustorial membrane, as reported for compatible interactions (Harder and Chong, 1984), was observed. The host cells and fungus in Akabozu were clearly less disorganized and empty than the cells in the highly susceptible Morocco.

The condition of the cells and haustoria in the partially resistant genotype Westphal 12A appeared intermediate compared that of the highly susceptible genotype Morocco and the partially resistant genotype Akabozu (Figure 6-10).

Mutual comparison of Morocco, Westphal 12A and Akabozu did not show differences in size or orientation of the extrahaustorial membrane, in the width of the extrahaustorial matrix or in thickness of the haustorial wall. No differences were observed in the number of mitochondria in haustoria in Morocco and the partially resistant genotypes. The main difference between Morocco and the partially resistant genotypes consisted of a difference in amount and condition of the cytoplasm of the host cell and of the haustoria.

DISCUSSION

According to Jacobs (1989b) partial resistance in wheat to wheat leaf rust involves two different phases. During the first phase a pre-haustorial exclusion of the fungus was postulated, leading to the abortion of infections structures. The failure of haustorium formation was associated with the presence of cell wall appositions (Jacobs, 1989a, 1989b). In the second phase smaller colonies were observed in partially resistant genotypes (Jacobs and Buurlage, 1989). Possibly the mechanism which leads to a reduced colony size in partially resistant genotypes, is of a post-haustorial nature (Jacobs, 1989c).

The A samples

The events leading to the formation of cell wall appositions in Morocco and Akabozu can be summarized:

i) contact is made between a haustorial mother cell or hypha and the cell wall. Substances can be exchanged between fungus and host.

ii) the host responds with the formation of a network of fibrillar material between cell wall and plasmalemma.

iii) a layer of electron-opaque entities develops between cell wall and plasmalemma.

iiii) additional layers of fibrillar material or dark entities are formed between the plasmalemma and the part of the cell wall apposition already present.

This model includes the majority of phenomena observed and explains the presence of dark entities near the cell wall and the presence of the fibrillar network close to the plasmalemma. It is not clear if the dark entities appear later in time at the location where previously fibrillar material was present, or that the entities are excreted by the fibrillar network towards the cell wall. In the

first case the dark entities would represent a subsequent and more mature stage, in the latter case cell wall appositions would "grow" towards the plasmalemma.

It is interesting that the majority of cell wall appositions in the partially resistant genotype Akabozu were of the fibrillar network type. In the highly susceptible Morocco cell wall appositions with dark entities were more abundant. If the model is correct it follows that the susceptible genotype would be the fastest to complete the formation of cell wall appositions. On the other hand, one would expect the partially resistant genotype to show dark entities in high frequencies. Earlier observations (Jacobs, 1989a, 1989b) indicated that Akabozu was effective in excluding infection structures from the host cell. It must be concluded that the fibrillar network can be as effective a barrier against penetration by the fungus, as the layer with dark entities. The morphology of the cell wall appositions reported here, from the wheat/wheat leaf rust interaction deviates from those reported in the wheat/*Septoria* interaction (Hargreaves and Keon, 1986), in the barley/*Erysiphe graminis* f.sp. *hordei* interaction (Heitefuss and Ebrahim-Nesbat, 1986), and in the partially resistant barley/barley leaf rust interaction (R.E. Niks, pers. comm.). The cell wall appositions found in the latter interaction closely resemble the typical cell wall apposition found in the partially resistant genotype Akabozu (Figure 6-6).

The cell wall appositions are believed to be a product of the host cell in response to fungal attack. The formation is triggered by contact with a haustorial mother cell and will continue until the cell wall appositions are completed. The cell wall apposition are considered to be a structural or possibly a chemical barrier which inhibit the formation of haustoria.

We suggest to look at the formation of cell wall appositions as a direct interaction between fungal cytoplasmic material and excretory products of the host. At the contact site between haustorial mother cell and host cell wall fungal degrading enzymes create a small pore in the host cell wall through which fungal cytoplasm can move toward the host plasmalemma. The host cell excretes substances which react with the fungal cytoplasm, the majority of which are proteins, leading to coagulation and precipitation. The host cell substances could even have an inhibiting and toxic effect on the haustorial mother cell. The area over which cell wall appositions are extended can be explained by diffusion of the reaction products.

The B samples

The reduction of colony size in partially resistant genotypes in the second phase was not caused by the presence of cell wall appositions (Jacobs, 1989b). The mechanism of the postulated post-haustorial retardation is not known.

No collars were observed around the haustorial neck of the fungus in any genotype. It is likely that the retardation of the growth rate of the partially resistant genotypes is not caused by an encapsulation or another kind of physical enclosure of neck and haustoria. Collars have been reported in several rust/host interactions (Littlefield and Heath, 1979; Harder and Chong, 1984) and seem prominently present in oats infected with *Puccinia coronata avenae* (Chong and Harder, 1982). No ultrastructural differences were detected with regard to the extrahaustorial membrane, matrix and haustorial wall between the susceptible and partially resistant genotypes .

We did not find a sheath development around the haustorium as has been reported from several incompatible reactions of hosts against rusts (Manocha, 1975; Heath and Heath, 1971; Coffey and Allen, 1982).

Earlier reports pointed to a post-haustorial nature of partial resistance (Jacobs, 1989b, 1989c). From this study it becomes clear that this resistance is not based on a post-haustorial morphological obstruction of the transport of nutrients through the host/fungus interface. A difference in host cell condition was observed between susceptible and partially resistant genotypes. Most likely this difference in cell condition is a consequence and not the cause of the difference in partial resistance to wheat leaf rust. Cells in the highly susceptible Morocco seem to have lost most of their contents whereas cells in partially resistant genotypes still contain a certain amount of well organized cytoplasm. Cells in Morocco seemed to have released their contents without much hindrance.

It is suggested here that there is a difference between Morocco and Akabozu in the rate of nutrient transport through the extrahaustorial membrane and matrix into the haustorium. In Morocco this rate could be high and in partially resistant genotypes the transfer could be lower. It is difficult to study these differences in rate of transfer in the rust fungi with their intercellular hyphae and intracellular haustoria. The interface of haustoria of powdery mildew whose hyphae extend over the epidermis can be studied (Gay and Manners, 1987). Differences in transfer rate of substances through the haustorial membrane could explain the observations that differences in latency period between susceptible and partially resistant genotypes decrease with increasing temperatures (Broers and Wallenburg, 1989). In such a view the rate of transfer in susceptible genotypes is already high and increases with temperature just as the majority of physiological processes. In partially resistant genotypes the transfer through the extrahaustorial matrix could be low at low temperatures and increase rapidly with temperature when the resistance looses its effectiviness.

The location of mitochondria in the haustoria near the haustorial wall (in accordance with Harder and Chong, 1984), suggests that the uptake of nutrients is an energy-depending process. If the nutrient-intake in partially resistant genotypes requires more energy than in susceptible genotypes fungal growth in the partially resistant genotypes will be reduced.

It has been suggested that the highly specialized haustorium not only extracts basic nutrients from the host cell (Harder and Chong, 1984 p. 470). It is possible that partially resistant genotypes offer a poor quality nutrients to the fungus compared to susceptible genotypes, leading to a reduced growth of the infection structures.

The use of cytochemical methods (e.g. PATCHSP, PACP; Woods and Gay, 1987) or enzyme treatments (Chong and Harder, 1982) could provide valuable information about the interface of fungus and host and help clarify the actual mechanism of growth retardation in partially resistant genotypes.

ACKNOWLEDGEMENTS

The assistance of Juliette Janson is highly appreciated.

REFERENCES

Broers, L.H.M. and S.C. Wallenburg, 1989. Influence of post-infection temperature on three components of partial resistance in wheat to wheat leaf rust. Euphytica in press.

Chong, J. and D.E. Harder, 1982. Ultrastructure of haustorium development in *Puccinia coronata avenae*: some host responses. Phytopathology 72: 1527-1533.

Coffey, M.D. and F.H.E. Allen, 1982. A quantitative histological and ultrastructural analysis of interactions between the flax rust and near-isogenic host lines varying in their degree of incompatibility. Canadian Journal of Botany 61: 1831-1850.

Gay, J.L. and J.M. Manners, 1987. Permeability of the haustorium-host interface in powdery mildews. Physiological and Molecular Plant Pathology 30: 389-399.

Harder, D.E. and J.Chong, 1984. Structure and physiology of haustoria. in: The Cereal Rusts, Vol.I. Origins, Specificity, Structure and Physiology. Eds: W.R. Bushnell and A.P Roelfs. Pp 431-476.

Hargreaves, J.A. and J.P.R. Keon, 1986. Cell wall modifications associated with resistance of cereals to fungal pathogens. in: Biology and molecular biology of plant-pathogen interactions. Ed: J.Bailey. NATO ASI series.

Heath, M.C. and I.B. Heath, 1971. Ultrastructure of an immune and a susceptible reaction of cowpea leaves to rust infection. Physiological Plant Pathology 1: 277-287.

Heitefuss, R. and F. Ebrahim-Nesbat, 1986. Ultrastructural and histochemical studies on mildew of barley (*Erysiphe graminis* f.sp. *hordei*). III. Ultrastructure of different types of papillae in susceptible and adult plant resistant leaves. Journal of Phytopathology 116: 358-373.

Jacobs, Th., 1989a. Abortion of infection structures of wheat leaf rust in susceptible and partially resistant wheat genotypes. Submitted to Euphytica.

Jacobs, Th., 1989b. The occurrence of cell wall appositions in flag leaves of spring wheats susceptible and partially resistant to wheat leaf rust. Submitted to the Journal of Phytopathology.

Jacobs, Th., 1989c. Haustorium formation and cell wall appositions in susceptible and partially resistant wheat and barley seedlings infected with wheat leaf rust. Submitted to the Journal of Phytopathology.

Jacobs, Th. and M.B. Buurlage, 1989. Growth of wheat leaf rust colonies in susceptible and partially resistant spring wheats. Submitted to Euphytica.

Littlefield, L.J. and M.C. Heath, 1979. The ultrastructure of rust fungi. Academic Press, Inc., London.

Manocha, M.S., 1975. Autoradiography and fine structure of host-parasite interface in temperature-sensitive combinations of wheat stem rust. Phytopathologische Zeitschrift 82: 207-215.

Woods, A.M. and J.L. Gay, 1987. The interface between haustoria of *Puccinia poarum* (monokaryon) and *Tussilago farfara*. Physiological and Molecular Plant Pathology 30: 167-185.

Zadoks. J.E., T.T. Chang and C.F. Konzak, 1974. A decimal code for the growth stages of cereals. Weed Research 14: 415-421.

Chapter 7.

THE INHERITANCE OF HOST PLANT EFFECT ON LATENCY PERIOD OF WHEAT LEAF RUST IN SPRING WHEAT. I: ESTIMATION OF GENE ACTION AND NUMBER OF EFFECTIVE FACTORS IN F1, F2 AND BACKCROSS GENERATIONS.

Th. Jacobs and L.H.M. Broers.

SUMMARY

Crosses were made between the highly susceptible Little Club and the partially resistant cultivars Westphal 12A, Akabozu and BH 1146 to obtain F1, F2 and backcross generations. Latency period (LP) was determined in plants inoculated at the young flag leaf stage with a monospore culture of race 'Flamingo' of wheat leaf rust. Broad sense heritability of LP in the F2 averaged 0.8. The genes showed partial to almost complete recessive inheritance. Scaling tests indicated that additive gene action was the most important factor in the inheritance of partial resistance. The tests showed that there were no indications for additive x additive, additive x dominance or dominance x dominance interactions. The number of effective factors was estimated as one or two for Akabozu, three or more for Westphal 12A and two or three for BH 1146. BH 1146 also possessed a (semi-)dominant gene for a lower infection type which was temperature sensitive in its expression. The genes of the various parents had unequal effect on LP.

INTRODUCTION

Partial resistance is characterized by a reduced rate of epidemic development of the pathogen on the condition that a susceptible infection type is present (Parlevliet and van Ommeren, 1975). It can be partitioned into several components, latency period (LP) being the most important one in cereals infected with rusts (Teng *et al.*, 1977; Parlevliet, 1979; Zadoks, 1971).

In the barley/barley leaf rust interaction inheritance of LP is coded for by several (up to 7) genes with small effects, most of them showing additive gene action (Parlevliet, 1976; 1978). In the wheat (*Triticum aestivum*)/wheat leaf rust (*Puccinia recondita* f.-sp. *tritici*, syn. *Pucc. triticina*) interaction two to four partially recessive genes for prolonged LP, with equal effects have been reported (Lee and Shaner, 1985). Other authors reported two to three partially recessive genes present in slow rusting cultivars (Bjarko and Line, 1986). Two partially recessive genes for longer LP were reported in the wheat genotype Suwon 85 (Kuhn *et al.*, 1980). In the above mentioned wheat/wheat leaf rust interactions winter wheat was studied.

This study was initiated to asses the gene action and number of genes in four spring wheat cultivars which differed in LP.

MATERIALS AND METHODS

Crosses were made between the highly susceptible Little Club, and the partially resistant cultivars Westphal 12A, Akabozu and BH 1146. Westphal 12A and Akabozu are lines derived from landraces from Hararge, Ethiopia and Saitama, Japan respectively. BH 1146 is a selection from the cross Ponta Grossa//Fronteira/Mentana made at Bello Horizonte, Brazil in 1946. BH 1146 is grown on a commercial scale in South America since 1955.

Greenhouse raised F1 plants were allowed to self to obtain the F2. F1 plants from crosses between Westphal 12A, Little Club and Akabozu, Little Club were backcrossed to the respective parents as pollen donors. No reciprocal crosses were studied.

The inheritance of LP was studied in the young flag leaf stage because differences in LP between susceptible and partially resistant cultivars were much more pronounced in flag leaves than in seedling leaves (Broers, 1989; Ohm and Shaner, 1976).

Plant culture, inoculation and observations

Plants were raised in square plastic pots of 12 x 12 cm in the greenhouse in Wageningen in the spring of 1987, a smaller num-

ber of plants were raised in late autumn of 1986. Plants from the segregating populations differed in earliness. Since the LP varies considerably with the age of the flag leaf (Parlevliet, 1975; Pretorius *et al.*, 1988) it was necessary to inoculate plants in the same stage. Therefore all plants that reached the required young flag leaf stage (48 - 59 DC, Zadoks *et al.*, 1974) were inoculated together with parental plants in the same stage. To have at each inoculation series parental plants of the right stage available, parents were sown at weekly intervals during a period of six weeks. Each inoculation series four to six plants of the highly susceptible Little Club and of at least one of the partially resistant parents were also inoculated. Spores of a monospore culture of wheat leaf rust race 'Flamingo' were collected a day ahead of the inoculation and dried during one night in an exsiccator. The plants were inoculated by dusting rust spores (1.0 mg per plant) diluted 100 times with *Lycopodium* spores, by means of a cyclone duster. After inoculation, plants were exposed to 100 % R.H. during the night. The next morning the plants were transferred to a greenhouse compartment, day temperatures ranging from 18 - 22 °C, and night temperatures ranging from 15 - 18 °C in 1987. In the autumn of 1986 realized temperatures were lower (day: 15 - 18 °C, night: 12 -15 °C).

When light green flecks, which precede urediosori, became visible, an area of 2-4 cm on the central part of the flag leave, with some 60 -80 flecks, was marked. From the day the first brown ovals were just visible, the number of urediosori were counted every day, using a pocket lens (7 x), until the number did not increase anymore. Per plant three to four flag leaves were observed. The time from inoculation to the time that 50 % of the final number of urediosori were visible was taken as the LP. The infection type (IT) was noted on a scale 0 to 9 (Mcneal *et al.*, 1971) two to three days after the estimated LP.

In total about 35 plants of each parent, 30 plants of each F1, 150 to 200 plants of each F2 cross and 45 to 50 plants of each backcross were studied over seven inoculations in 1987. In 1986 ten plants per F1 were observed and similar numbers of plants as in 1987 in the other generations, over eight series.

Analysis

In each inoculation series the average LP of the flag leaves per plant was related to that of the highly susceptible Little Club, which was given a relative value of 100. Relating the LP to that of Little Club enabled comparison between inoculation series. The relative LPs were transformed to natural log values.

Also calculated were the LP values relative to Little Club (set at 100) and the average of the partially resistant cultivars Westphal 12A and Akabozu, set at 170. Both sets of relative values were analyzed to see which transformation was most satisfactory.

Genetic analysis, gene action

The means of the parents, the F1 and the F2 generations were used to calculate the midparent value (m), the additive (d) and the dominance (h) component of gene action. The degree of dominance was calculated as h/d (Allard, 1960).

Individual scaling tests were performed to test the significance of the deviation from zero of four equations; $a = 2*B1 - P1 - F1$; $b = 2*B2 - P2 - F1$; $c = 4*F2 - 2*F1 - P1 - P2$; $d = 2*F2 - B1 - B2$, with P1, P2, F1, F2, B1 and B2 being the average LP of the six generations. If epistasis is absent, the equa-

tions should be equal to zero (Mather and Jinks, 1982).

For the crosses Westphal 12A x Little Club and Akabozu x Little Club data from backcross generations were available and joint scaling tests were performed (Mather and Jinks, 1982). The first test involved solving simultaneous equations to estimate m, d and h for each cross. A chi-square test gave the probability that the observed values can be explained by the values based on the estimated m, d and h. Low probabilities indicated the presence of interaction (epistasis) between d and h (see Table 7-3, first column).

The joint scaling tests were expanded to include the additive x additive component (i), the additive x dominance component (j) and/or the dominance x dominance component (l). Chi-square tests were performed and the probabilities of the seven expanded models were determined. The joint scaling tests attempt to find the genetic model that provides the best fit of the data for each cross. Chi-square tests indicate how well the data fit a particular model.

Broad sense heritability was calculated according to Allard (1960) and Simmonds (1979).

Genetic analysis, number of genes

Several methods were used to estimate the number of effective factors in the partially resistant cultivars contributing to a longer LP compared with the highly susceptible Little Club. We call the effective factors "genes" for convenience only.

The number of segregating genes has been calculated using several formulas. Formula one (Table 7-4, subscript) is derived from Lande (1981), formulas two to four were published by Wright (1968), formula five was proposed by Kast (1983). The formulas are based on the assumptions that the segregating genes are in one parent only, not linked, have equal effects, have equal degree of dominance, act in the same

Table 7-1. Degree of dominance and broad sense heritability for latency period of wheat leaf rust in six crosses between highly susceptible and partially resistant spring wheat cultivars.

Cross		Degree of dominance[1)]	Broad sense heritability[2)]	
			A	S
Akabozu	xLittleClub	-0.57	0.86	0.83
Westphal 12A	xLittle Club	-0.82	0.61	0.59
BH1146	xLittle Club	-0.82	0.85	0.87
Westphal 12A	xAkabozu	-0.93	0.90	0.85
Westphal 12A	xBH1146	-	0.82	-
Akabozu	xBH1146	-1.14	0.78	0.68

1) Degree of dominance calculated as h/d, h= the departure of the heterozygote (F1) from the midparent value, d= the departure of the parents from the midparent value.

2) Broad sense heritability calculated according to h2 =(VF2-VE)/VF2 with column A: VE = 1/2(VP1 + VP2) (Allard, 1960) and column S: VE = 1/3(VP1 + VP2 + VF1) (Simmonds, 1979).

direction (in this case longer LP) and that interaction components (i,j,l) are not important. If these prerequisites are not met, estimates of numbers of genes can be heavily biased.

The number of genes was also estimated by a method published by Parlevliet (1978). He used the proportion of parental phenotypes in the F2 generation to deduce the number of genes in the parents. The percentage plants showing an LP equal to that of the susceptible parent, or the partially resistant one in the F2 generation is expected to be 25, 6.25 or 1.56 when there are one, two or three genes respectively. This method is effective only if the susceptible parent carries no genes for a longer LP.

Calculations were performed with an Estate P.C. and the VAX mainframe computer of the Agricultural University.

RESULTS

The infection type (IT) in the parents and nearly all the crosses were high, ranging from 7 to 9, with an average of 8 in both years. Only in the F2 of the cross BH 1146 x Little Club in 1987 the IT ranged from one to nine, with equal numbers of plants in each class. The data fit a 3:1 distribution for lower IT (1 to 7) : high IT (7 to 9), and also a 1:2:1 distribution for low IT (0 to 2) : intermediate IT (3 to 6) : high IT (7 to 9). This was not observed in 1986. This indicates the presence of a monogenic hypersensitivity gene with an environmentally sensitive expression.

Results of the analysis performed with the LP related to the highly susceptible Little Club did not differ from those performed with the ln transformed data nor from those calculated with the LP related to Little Club and the partially resistant parents. Therefore only the results of the LP related to Little Club will be presented.

The degree of dominance was negative for all crosses, indicating partial to complete recessive inheritance. LP-prolonging genes in Westphal 12A and BH 1146 showed a more recessive inheritance than those in Akabozu (Table 7-1). Nearly all the means of the F1's between the partially resistant parents and Little Club did not differ significantly from the mean of the highly susceptible Little Club. The F1 of Akabozu x Little Club showed a 20 % longer LP than Little Club in 1987 and 36 % in 1986; the F1 of Westphal 12A x Little Club showed a 9 % increase in 1987; the F1 of BH 1146 x Little Club showed a 9% increase in 1987 and 14 % in 1986 (Table 7-2).

Thus the ability to delay sporulation as measured by LP inherits as a recessive character in crosses between the partially resistant parents and the highly susceptible Little Club.

Expression of gene action measured as degree of dominance (h/d, Table 7-1) in the crosses between the partially resistant cultivars showed incomplete recessive inheritance. This is also shown in Table 7-2 where the F1 values of the crosses between Westphal 12A, Akabozu and BH 1146 resembled the value of the parent with the shortest LP in that cross.

Broad sense heritability estimates ranged from 0.59 to 0.90. High heritability values are indicative for high rates of success in recovering the desired genes in future generations.

Scaling tests

None of the terms of the individual scaling tests differed significantly from zero. For the 1987 cross Akabozu x Little Club, for instance, the terms were a: -7 ± 63; b: 10 ± 45; c: 15 ± 38; d: 6 ± 70;
for Westphal 12A x Little Club a: -12 ± 35; b: 7 ± 26; c: -48 ± 44; d: -22 ± 29;
for BH 1146 x Little Club 1987 c: 95 ± 164; 1986 c: -100 ± 248. This indicates that only

additive and dominance effects contribute to the gene action.

Results of the joint scaling tests also indicated that additive and dominance effects explain the gene action. The additive-dominance model fitted the data from the cross Akabozu x Little Club (P=0.96), as well as from the cross Westphal 12A x Little Club (P=0.50) (Table 7-3 left column). This is a clear indication that additive x additive (i), additive x dominance (j) and dominance x dominance (l) components are of minor importance. None of these components were significantly different from zero. Addition of these components in the expanded models hardly improved the fit of the models (Table 7-3). The value of thenon-allelic interaction components (i, j, l) turned out to be small compared to the other components. In both crosses the additive component (d) appeared to be the most important factor, the dominance component (h) was less important (Table 7-3).

Table 7-3. Values and sign of the midparent value (m), the additive (d), the dominance (h), the additive x additive (i), the additive x dominance (j) and the dominance x dominance (l) components calculated for two crosses between partially resistant and the highly susceptible (Little Club) spring wheat cultivars. Models are based on 3, 4, 5 or 6 components. Chi square values (Chi^2) and significance (P) of the various models are presented.

Akabozu x Little Club								
m	143^{*s}	151^{*s}	144^{*s}	143^{*s}	149^{*s}	149^{*s}	143^{*s}	155^{*s}
d	43^{s}	43^{s}	44^{s}	43^{s}	43^{s}	43^{s}	43^{s}	43^{s}
h	- 24^{s}	- 33	-24	- 13	- 31	- 26	- 18	- 43
i		- 8.5			- 6	- 6		- 11
j			-18		- 16		- 16	- 17
l				- 11		- 4	- 7	8
Chi^2	0.089	0.052	0.017	0.079	0.002	0.073	0.238	0.000
P	0.96	0.99	0.99	0.99	0.99	0.99	0.99	1.00
Westphal 12A x Little Club								
m	128^{*s}	110^{*s}	129^{*s}	129^{*s}	109^{*s}	80^{*s}	130^{*s}	86^{*s}
d	28^{*s}	29^{*s}	29^{*s}	29^{*s}	30^{*s}	29^{*s}	29^{*s}	30^{*s}
h	- 25^{s}	- 4	- 25^{s}	- 46	- 3	75	- 51	57
i		19			21	49		43
j			- 19		- 25		- 26	-20
l				22		-50	27	-38
Chi^2	1.39	0.39	1.67	0.32	0.22	0.30	0.33	0.00
P	0.50	0.94	0.64	0.96	0.97	0.99	0.99	1.00

* = value significant different from zero according to the t-test (P=0.05).

s = value differed more than twice the standard deviation from zero.

Table 7-2. Number of plants per relative latency period class of crosses between six spring wheats, data of parents, F1,F2 for 1987 are presented. For two crosses data of BC1, BC2 were available. Some crosses were also analysed in 1986. The latency period classes are 20 units wide.

Cross, year and generation	Mean latency period	std[1)]	Relative latency period, class means are denoted.															
			75	95	115	135	155	175	195	215	235	255	275	295	315	335	355	375	> 395
			number of plants																
Akabozu x L.Club																			
1987																			
P1 L.Club	100	2.8		29															
BC1	114.5	21.3	1	19	22	8	2	1	1										
F1	118.7	13.8		5	18	9	1												
F2	134.7	29.6		28	56	41	33	17	7	2	2								
BC2	149.3	30.0		2	4	19	9	8	2		1	1							
P2 Akabozu	186.6	15.4			1		9	9	7	5		2							
1986																			
P1 L.Club	100	3.2		26	2														
F1	143.1	12.5			1	8	4	1											
F2	145.0	45.6	2	36	25	21	29	17	13	4	3	4	1	2					
P2 Akabozu	219.5	67.0				2	2	3	2	3	2	1	1		3				
Westphal 12A x L.Club																			
1987																			
P1 L.Club	100	2.8		29															
BC1	106.4	12.4		24	19	3													
F1	105.4	6.8		18	10														
F2	105.4	10.3	1	84	68	11													
BC2	126	16.8		4	21	18	4	2											
P2 Westph.	159.8	8.7				9	14	11	2										
BH1146 x L.Club																			
1987																			
P1 L.Club	100	2.8		29															
F1	113.4	11.6		5	19	2	1												
F2	167.7	40.3			20	21	31	27	16	8	6	6	2	1					
P2 BH1146	248.9	21.8				2		1		8	2	9	7	4	1				

1986																				
P1	L.Club	100	3.2		26	2														
F1		136.6	6.2			1	5													
F2		159.7	61.9		1	28	38	35	20	20	17	10	5	4	3	3	2	1	1	1
P2	BH1146	365.2	12.4															2	1	
Westphal 12A x Akabozu																				
1987																				
P1	Westph.	159.8	8.7				9	14	11	2										
F1		160	18.7				6	15	7	2	1									
F2		181.7	39		1	6	28	42	36	31	27	14	8	3	1	1				
P2	Akabozu	186.6	15.4			1		9	9	7	5		2							
Akabozu x BH1146																				
1987																				
P1	Akabozu	186.6	15.4			1		9	9	7	5		2							
F1		182.3	29.2					4	3	1										
F2		191.8	40.2			1	10	29	37	34	16	9	7	5	3	3				
P2	BH1146	248.9	21.8				2	1	8	2	9	7	4	1						
1986																				
P1	Akabozu	219.5	67.0				2	2	3	2	3	2	1	1		3				
F1		253.2	43.7							1	2	2	2	1	1	2				
F2		256	75.1			2	1	5	9	23	12	14	15	10	8	15	17	14	9	12
P2	BH1146	365.2	12.4															2	1	
Westphal 12A x BH1146																				
1987																				
P1	Westph.	159.8	8.7				9	14	11	2										
F2		154.3	38.9		8	35	52	41	21	16	8	3	1	7						
P2	BH1146	248.9	21.8				2	1	8	2	9	7	4	1						
				75	95	115	135	155	175	195	215	235	255	275	295	315	335	355	375	> 395

1) std = standard deviation.

Table 7-4. Number of independently segregating effective factors (genes) controlling the relative latency period in five spring wheat crosses estimated by five genetic formulas over two years.

Formula[1]		Cross[2]				
		AKAxLC	WESTxLC	BHxLC	WESTxAKA	AKAxBH
Formula 1						
1987	var1	1.4	7.3	1.9	0.1	0.6
	var2	1.3	7.0	1.9	0.1	0.5
	var3	2.4	- 1.9			
	var4	0.9	1.3			
1986	var1	0.9		2.3		0.7
	var2	2.0		2.3		0.7
Formula 2						
1987		1.3	9.3	2.6	0.1	0.8
1986		2.1		1.0		0.9
Formula 3						
1987		0.6	3.2			
Formula 4						
1987		0.2	0.1			
Formula 5						
1987	K1	2.1	19.6			
	K2	1.4	4.8			

1) ***Formula 1:*** $N = (P2 - P1)^2/(8\ varx)$ with x ranging from 1 to 4 (for explanation see below). P2 is the average of the most resistant parent, P1 is average of the least resistant parent, var1 = VF2 - VF1, var2 = VF2 - [1/2VF1 + 1/4VP1 + 1/4VP2], var3 = 2VF2 - VBC1 - VBC2, var4 = VBC1 + VBC2 -[VF1 + 1/2VP1+1/2VP2] VP1 = variance of P1, VP2 = variance of P2, VF1 = variance of F1, VF2 = variance of F2, VBC1 = variance of BC1, VBC2 = variance of BC2.

Formula 2: $N = (P2 - P1)2\ (1.5 - 2h(1 - h))/8(VF2 - VE)$, P2 is the average of the more resistant parent, P1 the average of the more susceptible parent, h = (F1 - P2)/(P2 - P1),
VE = 1/4(VP1 + VP2 + 2VF1).

Formula 3: D = (F1 - P2)2/4(VBC2 - VE), D is the number of genes by which the F1 differs from the resistant parent, VE = 0.5(VF1 + VP2).

Formula 4: D = (F1 - P1)2/4(VBC1 - VE), D is the number of genes by which the F1 differs from the susceptible parent, VE = 0.5(VF1 - VP1).

Formula 5: K1 = (1/2 d2 + 1/2 h2)/ VF2, K2 = (1/2 d2 + 1/2 h2)/ VBC1 + VBC2
with d = 1/2 P1 + 1/2 P2 and h = 6BC1 + 6BC2 - 8F2 - F1- 1.5P1 - 1.5P2.

2) AKA = Akabozu; LC = Little Club; WEST = Westphal 12A; BH = BH1146.

Number of genes

The number of segregating genes estimated with help of the formulas was one or two for the cross Akabozu x Little Club, one to nine for Westphal 12A x Little Club and one to two for BH 1146 x Little Club (Table 7-4). The number of segregating genes in the cross Westphal 12A x Akabozu was estimated zero implying that both cultivars carry the same number of genes for longer LP. Akabozu and BH 1146 appeared to differ for one gene only (Table 7-4).

From the cross Akabozu x Little Club 23 % and 15 % of the F2 plants resembled the susceptible parent in 1986 and 1987 respectively, indicating one or two genes in Akabozu. Fifty one percent of the F2 plants of Westphal 12A x Little Club resembled the highly susceptible Little Club indicating at most one gene. The F2 generation of BH 1146 x Little Club gave one plant resembling Little Club in 1986 and none in 1987, indicating the action of three or more genes. The F2 distributions of all three crosses were skewed towards shorter LP, indicating recessive inheritance of the genes. This leads to high numbers of F2 plants resembling the susceptible parent and an underestimation of the number of genes involved. The number of F2 plants showing the phenotype of the partially resistant parent is not obscured by plants with recessive genes in heterozygous condition, which should in principal lead to better estimates than the previous method. But the large environmental variances of the plants with long LP's and the continuous distributions of the progenies made it difficult to assign plants to distinct classes, giving imprecise estimates. In the cross Akabozu x Little Club about 15 % of the F2 plants resembled the partially resistant parent in 1987 and 28 % in 1986, again indicating the action of one or two genes. Plants of the F2 generation of Westphal 12A x Little Club hardly resembled the partially resistant parent leading to estimates of three or more genes. In 1986 nearly 8 % of the segregating F2 progeny resembled BH 1146 in the cross BH 1146 x Little Club, 17 % in 1987, indicating two and one or two genes respectively. No indications for number of genes could be obtained from the F2 generations between the partially resistant cultivars Westphal 12A, Akabozu and BH 1146 using this approach. Transgression towards shorter or longer LPs was observed in all three crosses. The numbers of segregating plants were low and varied per cross (Table 7-2). The cross Westphal 12A x Akabozu clearly showed F2 plants with a LP longer than that of Akabozu, one F2 plant resembled the highly susceptible Little Club.

DISCUSSION

Gene action

Inheritance of longer LP was expressed as a recessive or partially recessive character in the cultivars studied. Genes in heterozygous condition (F1) showed a LP hardly longer than that of the homozygous susceptible parent. Gene action was to a large extend additive. No epistasis of any importance was observed. Recessive inheritance of genes for prolonged LP or reduced level of disease in slow leaf-rusting wheat cultivars has been reported by other workers (Bjarko and Line, 1986; Kuhn *et al.*, 1980; Lee and Shaner, 1985).

The F2 distribution was continuous and skewed towards shorter LP. A continuous F2 distribution does not necessarily exclude monogenic or digenic inheritance (Lee and Shaner, 1985; Kuhn *et al.*, 1980). Large environmental influences on gene action easily obscure simple Mendelian ratio's. The partially resistant cultivars although homozygous for all loci concerned, showed a high degree of variation for LP.

According to Parlevliet (1978) positive skewness of F2 progenies could be explained by one or more of three reasons. In this study the dominance effect for short LPs probably prevailed. In most crosses the F2 average was closer to the midparent value than to the F1 value. But the data need be looked at with caution, in the cross Akabozu x Little Club there was hardly any difference in average of F1 and F2 in 1986, in 1987 the averages differed considerable. The opposite was true for the cross BH 1146 x Little Club.

Parlevliet (1978) postulated as second reason, a reduced expression of gene action at the short end of the range of LPs, due to physiological restrictions. We believe that the fungus in a highly susceptible cultivar has ample opportunity to grow and sporulate and will do so. Any gene with a small delaying effect on fungal growth will be fully expressed. Parlevliet assumed that the effect of an individual gene was smaller, closer to the physiological barrier (his group 1 crosses). According to our opinion such a smaller effect is the result of a gene which codes for a smaller effect. The expression of the gene does not depend on the nearness of the physiological barrier.

Parlevliet (1978) also postulated a physiological barrier on the upper side of the range. Slow growing colonies would not form a sporulating area, the colonies would not be detected by eye thus leading to an underestimate of the LP. This postulation can be tested. Rust colonies can be made visible with epifluorescence microscopy (Rohringer *et al.*, 1977; Niks, 1983). In this study flag leaves of F2 plants of crosses between Westphal 12A, Akabozu and BH 1146 harvested and stained a long period after the estimated moment of LP showed non-sporulating colonies of different sizes (data not presented). Some colonies showed a central red area, the uredial bed, which later would have developed into a sporulating area. Including these colonies would clearly have led to longer LPs. This means that the LPs of F2 plants with long LPs are underestimated. This upper barrier in the wheat/wheat leaf rust interaction is most likely difficult to determine, but lies well beyond the level of Akabozu or BH 1146, as F2 plants have been found showing a LP far longer than that of BH 1146 (Table 7-2).

In our opinion, the major influence on skewness of the F2 distribution is the (partially) recessive inheritance of the genes involved.

Number of genes

The results of the various methods to calculate the number of genes should be viewed at with caution as several prerequisites are not met and the estimates of the various methods do not match. Despite these pitfalls an estimate was made of the number of genes in each parent by combining the results of the methods.

The number of genes coding for longer LP presumably equals one or two in Akabozu, three or more in Westphal 12A and two or three in BH 1146. It must be concluded that the data from the F1, F2 and backcross generations are insufficient to determine the exact number of genes. Additional information is needed and will be presented in a subsequent paper (Broers and Jacobs, 1989).

The relative increase in LP compared to Little Club was 60 for Westphal 12A, 100 for Akabozu and 200 for BH 1146. This invariably leads to the conclusion that the genes in Westphal 12A had a smaller effect on the increase of LP than the genes in Akabozu. The same conclusion can be derived from comparison of the cultivars Akabozu and BH 1146. The estimates of number of genes in Akabozu and Westphal 12A may be wrong, it is clear however that the number of genes in Westphal 12A is higher than in

Akabozu. Their overall effect is smaller and so the effect of the individual genes.

From the data published by Lee and Shaner (1985) it can be calculated that genes from different parents have unequal effects. The gene effect measured as increase of LP ranged from 1.2 to 3 days depending on the parent. It is very tempting to speculate that in the wheat gene-pool of genes for longer LPs there is a variety of genes differing in their effect on LP, their sensitivity for background genes and environmental influences.

These findings do not exclude the possibility that within a partially resistant cultivar the genes have a different effect on the LP. E.g. the genes in Westphal 12A may have equal effects on LP, each increasing the LP with 20 % (code: 20,20,20), but also unequal effect (30,15,15 or 40,10,10). Due to the large non-genetic variation these gene effects are difficult to detect in crosses with the highly susceptible Little Club or the other resistent cultivars. Parlevliet (1976; 1978) reported unequal gene effects of genes present in one parent. The action of a recessive gene with a fairly large effect and four to five genes with much smaller effects were measured in the partially resistant barley cultivars Vada and Minerva.

The situation in the Brazilian cultivar BH 1146 is less clear. The number of genes involved ranged from one to three and was estimated at two or three. In 1987 the F2 segregated for a lower infection type, indicating one gene with dominant (3:1) or semi-dominant (1:2:1) expression for lower IT. There was a negative correlation between IT and LP in the F2 progeny (r=-0.6). Plants with a lower infection type clearly showed a longer LP. Histological observations of flag leaves of F2 plants showed autofluorescence, which is related to cell collapse. Such a correlation is to be expected as haustoria in collapsed cells are deprived, and colonies will grow slower and sporulate less. The gene for hypersensitive cell collapse and lower IT then acts epistatic over possibly present genes for a prolonged LP. This has been shown for the barley/barley leaf-rust interaction (Niks and Kuiper, 1983). In triticale, hypersensitivity resistance to wheat leaf rust was expressed in the presence of long LP factors (Wilson and Shaner, 1984). Conclusion from several histological experiments over the past few years is that BH 1146 shows varying degrees of autofluorescence in wheat leaf rust colonies. The amount of autofluorescence depended on external factors. BH 1146 clearly possesses a gene coding for lower IT that is sensitive for environmental influences. Expression has been observed in 1987, not in 1986. Temperature sensitive expression of hypersensitivity is common in the wheat/wheat leaf rust interaction (Browder, 1980). How the presence of this gene in BH 1146 influences the estimates of number of genes for longer LP is unknown.

Transgression

In the F2's of Westphal 12A x Little Club and Akabozu x Little Club a few plants showed a LP shorter than Little Club. This was probably due to non-genetic variation. Up to this moment no cultivars have been found by the authors with a LP shorter than that of Little Club. Little Club is as susceptible as the spring wheat Morocco and selections from landraces from Cyprus (Skalavatis numbers) and Nigeria (data not presented).

In the cross Westphal 12A x Akabozu one F2 plant resembled the highly susceptible Little Club. This indicates that the genes in both parents are different. This is not unexpected. The genes differ in their effect on LP and originate from landraces of different parts of the world. Segregation in an F3 and F5 progeny of this cross is described in another paper (Broers and Jacobs, 1989). The cross Akabozu x BH 1146 and Westphal 12A x BH 1146 showed

transgression towards both shorter and longer LP. In the F2 generation of both crosses several plants were observed with a LP nearly equal to the LP of Little Club. This would not have been observed if the cultivars shared one or more genes.

Durability of the genes involved in partial resistance or slow rusting is a highly desired property. Durability should be demonstrated by using the genes in cultivars "grown for a long period over a large area in an environment favoring disease" as Johnson (1984) stated. This not necessarily includes future durability. It makes judgement of durability retrospective, possible only when it is too late for practical breeding purposes. Up to now there does not seem to be a causal relation between durability and the number of genes involved or the mode of inheritance. For the time being the best policy might be to restrain oneself from remarks concerning durability and stimulate the use of genes thought to have that property.

REFERENCES

Allard, R.W., 1960. Principles of Plant Breeding. Wiley, New York and London.

Bjarko, M.E., and R.F. Line, 1986. Inheritance of latent period, infection type and area under disease progress curve in two slow rusting spring wheat cultivars. (Abstract) Phytopathology 76: 841-842.

Broers, L.H.M., 1989 Effect of growth stage and host cultivar on three components of resistance to wheat leaf rust in wheat. Euphytica, in press.

Broers, L.H.M., and Th. Jacobs, 1989. The inheritance of host plant effect on latency period of wheat leaf rust in spring wheat. II: Number of segregating factors and evidence for transgressive segregation in F3 and F5 generations. Euphytica, in press.

Browder, L.E., 1980. A compendium of information about named genes for low reaction to *Puccinia recondita* in wheat. Crop Science 20: 775-779.

Falconer, D.S., 1960. Introduction to Quantitative genetics. Longman Ltd London.

Johnson, R., 1984. A critical analysis of durable resistance. Annual Review of Phytopathology 22: 309-330.

Kast, W.K., 1983. Untersuchungen zur Vererbung der Mehltauresistenz bei Winterroggen. Dissertation University Hohenheim BRD.

Kuhn, R.C., H.W. Ohm and G. Shaner, 1980. Inheritance of slow leaf-rusting resistance in Suwon 85 wheat. Crop Science 20: 655-659.

Lande, R., 1981. The minimum numbers of genes contributing to quantitative variation between and within populations. Genetics 99: 541-553.

Lee, T.S., and G. Shaner, 1985. Oligogenic inheritance of length of latent period in six slow leaf-rusting wheat cultivars. Phytopathology 75: 636-643.

Mather, K., and J.L. Jinks, 1982. Biometrical genetics. The study of continuous variation. Third edition. University Press, Cambridge.

McNeal, F.H., C.F. Konzak, E.P. Smith, W.S. Tate and T.S. Russel, 1971. A uniform system for recording and processing cereal research data. USDA, Agric Res Serv, Washington, D.C. ARS: 34-121.

Niks, R.E., 1983. Early abortion of colonies of leaf rust *Puccinia hordei*, in partially resistant barley seedlings. Canadian Journal of Botany 60: 714-723.

Niks, R.E., and H.J. Kuiper, 1983. Histology of the relation between minor and major genes for resistance of barley to leaf rust. Phytopathology 73: 55-59.

Ohm, H.W. and G.E. Shaner, 1976. Three components of slow leaf-rusting at different growth stages of wheat. Phytopathology 66: 1356-1360.

Parlevliet, J.E., 1975. Partial resistance of barley to leaf rust *Puccinia hordei*. I. Effect of cultivar and development stage on latent period. Euphytica 24: 21-27

Parlevliet, J.E., 1976. Partial resistance of barley to leaf rust, *Puccinia hordei*. III. The inheritance of the host plant effect on latent period in four cultivars. Euphytica 25: 241-248.

Parlevliet, J.E., 1978. Further evidence of polygenic inheritance of partial resistance in barley to leaf rust, *Puccinia hordei*. Euphytica 27: 369-379.

Parlevliet, J.E., 1979. Components of resistance that reduce the rate of epidemic development. Annual Review of Phytopathology 17: 203-222.

Parlevliet, J.E., and A.van Ommeren, 1975. Partial resistance of barley to leaf rust *Puccinia hordei*. II. Relationship between field trials, microplot tests and latent period. Euphytica 24: 293-303.

Pretorius, Z.A., F.H.J. Rijkenberg and R.D. Wilcoxson, 1988. Effect of growth stage, leaf position and temperature on adult-plant resistance of wheat infected by *Puccinia recondita* f.sp. *tritici*. Plant Pathology 37, 36-44.

Rohringer, K., W.K. Kim, D.J. Samborski, and N.K.Howes, 1977. Calcofluor: an optical brightener for fluorescence microscopy of fungal plant parasites in leaves. Phytopathology 67: 808-810.

Simmonds, N.W., 1979. Principles of crop improvement. Longman, London Ltd.

Teng, P.S., M.J. Blackie and R.C. Close, 1977. A simulation analysis of crop yield loss due to rust disease. *Agricultural Systems* 2: 189-198.

Wilson, J., and G. Shaner, 1984. Genetics of leaf rust resistance in triticale. (Abstract) Phytopathology 74: 875.

Wright, S., 1968. Evolution and the genetics of populations. Volume 1 genetic and biometric foundations. The University of Chicago Press, Chicago and London. Pp 469.

Zadoks, J.C., 1971. System analysis and the dynamics of epidemics. Phytopathology 61: 600-610

Zadoks, J.C., T.T. Chang, and C.F. Konzak, 1974. A decimal code for the growth stages of cereals. Weed Research 14: 415-521.

Chapter 8.

THE INHERITANCE OF HOST PLANT EFFECT ON LATENCY PERIOD OF WHEAT LEAF RUST IN SPRING WHEAT. II: NUMBER OF SEGREGATING FACTORS AND EVIDENCE FOR TRANSGRESSIVE SEGREGATION IN F3 AND F5 GENERATIONS.

L.H.M. Broers and Th. Jacobs

SUMMARY

Three partially resistant spring wheat cultivars, with a long latency period were crossed among each other and with the highly susceptible Little Club, showing a very short latency period. Parents, F3 and F5 plants have been inoculated with the wheat leaf rust race 'Flamingo' in the young flag leaf stage to determine the latency period. From the crosses with Little Club, it was concluded that, Westphal 12A carries three, Akabozu two and BH 1146 two or three genes for a longer latency period. BH 1146 also appears to carry one hypersensitivity resistance gene.

Transgressive segregation occurred in crosses between partially resistant cultivars. It was concluded that the genes in Akabozu and Westphal 12A are different, while those in Akabozu and BH 1146 are at least partly different. The possibilities of accumulation of LP-prolonging genes are discussed.

INTRODUCTION

Partial resistance (PR) in wheat (*Triticum aestivum*) to wheat leaf rust (*Puccinia recondita* f.sp. *tritici*, syn. *Pucc. triticina*) retards the disease progress in the field despite a high, susceptible infection type indicating a compatible host-pathogen interaction (Parlevliet and van Ommeren, 1975). It occurs in several cultivars of spring and winter wheat (Broers, 1989; Lee and Shaner, 1985a, 1985b; Poyntz and Hyde, 1987). Latency period (LP) is the most important component of PR (Shaner and Finney, 1980; Teng *et al.*, 1977; Zadoks, 1971).

Jacobs and Broers (1989) concluded from an F1, F2 and backcross analysis that LP in the spring wheat cultivars studied inherited partially recessive and acted in an additive way. Some transgressive segregation was observed. Data indicated that only a few genes governing a longer LP were involved. The exact number of genes could not be estimated from the F1, F2 and backcross generations.

In this study an attempt is made to estimate the number of LP-prolonging genes in four spring wheat cultivars more precisely using F3 and F5 generations. Furthermore, the occurrence of transgressive segregation was studied.

MATERIALS AND METHODS

In 1985 crosses were made with the highly susceptible genotype Little Club and genotypes Westphal 12A, Akabozu and BH 1146, which differed in latency period (LP) and level of PR to wheat leaf rust (Broers, 1989). All genotypes used had susceptible infection types.

The F2 of Westphal 12A x Little Club, Akabozu x Little Club, BH 1146 x Little Club, Westphal 12A x Akabozu and BH 1146 x Akabozu were evaluated for their LP (Jacobs and Broers, 1989). From each cross, 50 F2 plants were selected, 25 with extreme short LP (S1 population) and 25 with an extreme long LP (S2 population).

By single seed descent from randomly chosen F2 plants, an F4 generation was obtained of the crosses Westphal 12A x Little Club, Akabozu x Little Club and Westphal 12A x Akabozu containing 39, 61 and 61 plants respectively.

Eight F3 plants per selected F2 plant and five F5 plants per F4 plant were raised in the greenhouse in 2.2 l. containers with one plant per container. Inoculation was done in sequential series as described by Jacobs and Broers (1989) to neutralize differences in earliness.

LP was determined by estimating the time at which 50% of the finally visible infections had developed into urediosori on the

flag leaves. The actual LP's were converted into relative ones setting the LP of Little Club at 100 and the LP of Akabozu at 180. The F3 evaluations of Akabozu x Little Club, Akabozu x BH 1146 and BH 1146 x Little Club were carried out in the spring of 1987 whereas the F3 of the remaining crosses and the F5 of the crosses Akabozu x Little Club, Westphal 12A x Little Club and Akabozu x Westphal 12A were carried out in the autumn of 1987. The temperatures during the experiments fluctuated between 18 and 25 °C during the day and between 15 and 18 °C during the night.

The crosses Westphal 12A x Little Club, Akabozu x Little Club and BH 1146 x Little Club were evaluated to obtain information about the number of genes (effective factors) governing a longer LP (LP-prolonging genes) in the partially resistant parents. The assumption was made that Little Club was the most susceptible genotype carrying no LP-prolonging genes at all.

The basic concept was that the frequency with which plants occurred having the same genotype for LP as one of the parents, is dependent on the number of genes in the partially resistant parent. In the F2 a fraction of $(1/4)^n$ (n is the number of LP-prolonging genes in the partially resistant parent) will recover the Little Club genotype for LP-prolonging genes(class I), a fraction of $(1/4)^n$ will recover the partially resistant parental genotype for LP-prolonging genes (class II) and a fraction of $1-2*(1/4)^n$ will have a genotype different from both parents (class III). In the F4 generation the fractions are $(7/16)^n$, $(7/16)^n$, $1-2*(7/16)^n$ for class I, II and III respectively.

It was assumed that in the F2 class I and class II genotypes were only to be found in the S1 and S2 population respectively, except in the case that only one LP-gene is present in the resistant parent. In that case the population size of S1 and S2 was too small to contain all expected class I and class II F2 plants.

F3 and F5 lines derived from class I or class II F2 plants should have a mean LP equal to the respective parent and no segregation should occur in these lines. To decide which lines met these prerequisites, two criteria were applied. First, the line mean LP should not differ significantly from the LP of the respective parent according to a t-test at the 5% probability level. Secondly, none of

Table 8-1. Number of F2 and F4 plants recovering the genotype for latency period for wheat leaf rust of the susceptible parent (class I), of the resistant parent (class II) or of a genotype other than those of the parents (class III) and total number of plants observed in the F2 and F4 generation of three spring wheat crosses.

Cross		Number of lines in class			Number of plants	
		I	II	III	F2	F4
F2						
Westphal 12A	x Little Club	4	2	152	158	
Akabozu	x Little Club	7	7	150	164	
BH 1146	x Little Club	0	1	190	191	
F4						
Westphal 12A	x Little Club	6	4	51		61
Akabozu	x Little Club	4	6	29		39

the plants within such a line should deviate more from the respective parent than twice the standard deviation of that parent. F3 and F5 lines that met both criteria were considered to be derived from class I or class II F2 or F4 plants respectively.

Chi-square tests were applied to compare the observed number of F2 and F4 plants in class I, II and III with the expected number of F2 and F4 plants in the respective classes, in models assuming one to four LP-prolonging genes.

Heritabilities were calculated using the formula $R = h^2 * S$ (Mather and Jinks, 1982). R is the response to selection measured as the difference between the F2 mean LP and F3 line mean LP of the S1 respectively the S2 populations. S is the selection differential expressed as the difference between the F2 mean LP and the F2 mean LP of the S1 respectively the S2 population.

RESULTS

The relative LP's of Little Club, Westphal 12A, Akabozu and BH 1146 were 100, 160, 180 and 200, respectively. No F3 or F5 lines were observed with an LP shorter than the LP of Little Club and no transgression towards shorter or longer LP was observed in the crosses involving Little Club as one of the parents. This indicates that Little Club does not carry LP-prolonging genes which are not present in the other genotypes studied. Since Little Club is a very susceptible cultivar, it probably does not carry any LP-prolonging genes at all.

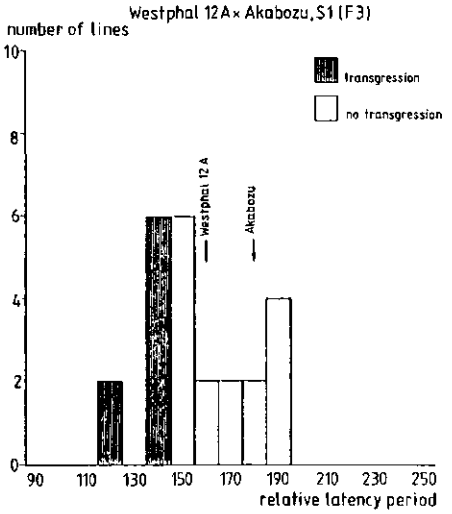
The number of F2 and F4 plants in class I, II and III (Table 8-1) were deduced from the F3 and F5 line means and variation. The observed numbers of plants in the three classes was compared with the expected number derived from models assuming one to four LP-prolonging genes (Table 8-2).

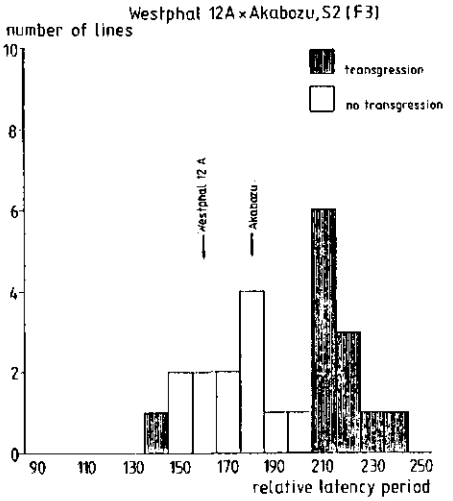
The three gene model fitted the cross Westphal 12A x Little Club best both in the F2 and the F4 generation. In the cross Akabozu x Little Club, the two gene model fitted the data of the F2 generation best. In the F4 generation, both the two and the three gene model could explain the observed number of plants in the three classes. Most likely, Akabozu possesses two LP-prolonging genes. BH 1146 carries probably three or more LP-prolonging

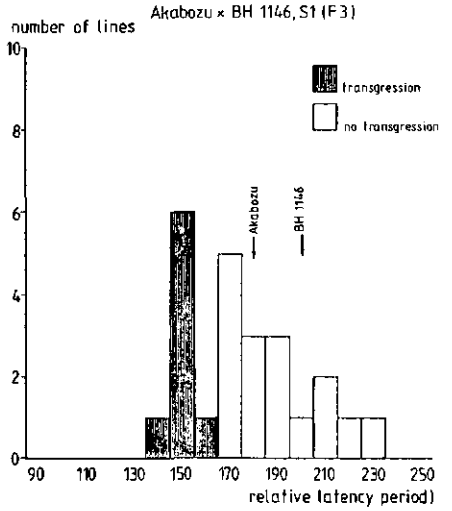
Table 8-2. Chi-square tests to compare models explaining the number of F2 or F4 plants recovering one of the parental genotypes for latency period of wheat leaf rust in three spring wheat crosses for one to four segregating genes with observed numbers.

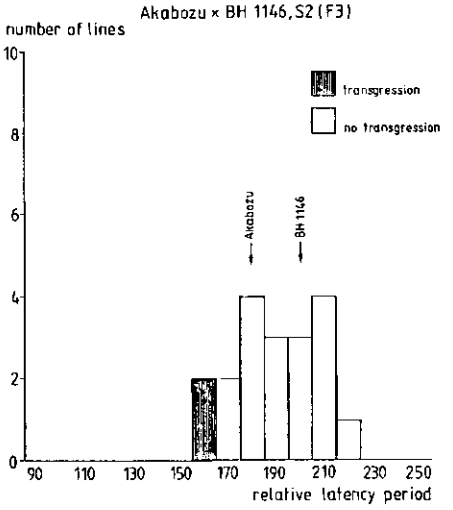
Cross		Chi-square[1]			
		1 gene	2 genes	3 genes	4 genes
F2					
Westphal 12A	x Little Club	140.9*	12.0*	0.9ns	25.0*
Akabozu	x Little Club	117.9*	4.9ns	9.2*	88.1*
BH 1146	x Little Club	187.4*	25.0*	4.5ns	1.2ns
F4					
Westphal 12A	x Little Club	283.2*	12.6*	0.4ns	8.6*
Akabozu	x Little Club	115.2*	2.6ns	3.0ns	21.4*

1) * = model differs significantly from observed situation; ns= model fits the observed situation.









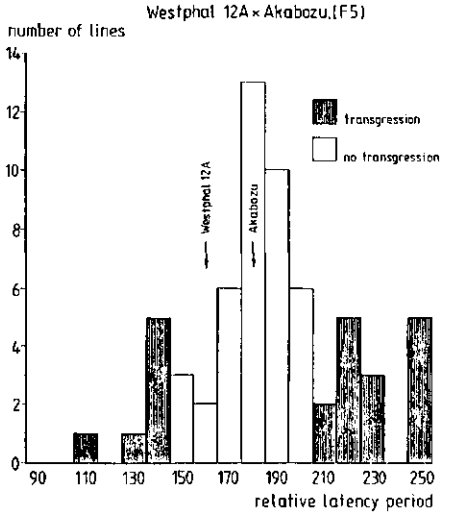


Figure 8-1. Frequency distribution of F3 line means (S1 and S2 population) of Westphal 12A x Akabozu and Akabozu x BH1146 and the F5 line means of Westphal 12A x Akabozu for relative latency periods measured on young flag leaves after inoculation with wheat leaf rust.

genes as both the three and four gene model fitted equally well the observed F2 data of the cross BH 1146 x Little Club. Transgression was observed in Westphal 12A x Akabozu and Akabozu x BH 1146 (Figure 8-1). In Westphal 12A x Akabozu, 8 F3 lines of the S1 population and 7 F5 lines showed a LP-significantly shorter than the LP of Westphal 12A. None of the lines was as susceptible as Little Club. Of the same cross 11 F3 lines (from S2) and 15 F5 lines displayed a longer LP than Akabozu. In the cross Akabozu x BH 1146 8 F3 lines were detected with a LP significantly shorter than Akabozu and significantly longer than Little Club. No lines with a LP longer than that of BH 1146 were found.

Realized heritabilities for the F3 generation are shown in Table 8-3. Heritabilities for the S1 populations ranged from 0.40 to 0.89. Heritabilities calculated from the S2 population data varied from -0.37 to 1.29, which is factually impossible, an explanation will be given below.

DISCUSSION

From the crosses Westphal 12A x Little Club and Akabozu x Little Club it was concluded that Westphal 12A and Akabozu carry three and two genes governing a longer LP respectively. These genes act mainly additively and are partially recessive (Jacobs and Broers, 1989).

If the genes in the respective parents act with equal effects, it is not likely that Akabozu and Westphal 12A have an LP-gene in common (Jacobs and Broers, 1989). This is supported by the data of F3 and F5. Both in the F3 and F5 generation of the cross Westphal 12A x Akabozu clear transgressive segregation occurred. None of the line mean LP's was equal to the LP of Little Club. This could mean that Westphal 12A and Akabozu possess a common LP-gene. In that case neither plants nor lines are expected to show a LP shorter than the LP controlled by the common gene in any generation. However, individual plants from F3 and F5 lines showed LP's equal to Little Club. Thus, the LP-prolonging genes in Westphal 12A and Akabozu are different

The maximum LP expected in the cross Westphal 12A x Akabozu would be 240 (= 100 + 60 + 80) in the case that both parents do not have an LP-gene in common and all five LP-prolonging genes are combined in one genotype. In the case of a common gene, no plants or lines are to be expected with a LP longer than the combined effect of the five LP genes (= 240) minus twice the effect of that common gene. However, F3 and F5 lines were found which showed LP's as long as 240. We conclude therefore that Westphal 12A and Akabozu do not posses a common LP-gene.

In the case of no common LP genes, five genes would segregate in the cross Westphal 12A x Akabozu. The chance to obtain plants without any LP-gene or with all five LP-prolonging genes is one out of 1024 in the F2 and one out of 63 in the F4. Small population sizes in F2 and F4 (160 F2 plants and 61 F4 plants) explain why no plants in the F2 and F4 and no derived F3 and F5 lines lacking LP-prolonging genes al-

Table 8-3. Heritabilities of latency period of wheat leaf rust in five wheat crosses based on the ratio of the selection differential (S) and the response to selection (R) of two selected populations (S1 and S2) per cross.

Wheat cross		Heritability (h^2=R/S)	
		S1	S2
Westphal 12A	x Little Club	0.73	1.29
Akabozu	x Little Club	0.89	0.30
BH 1146	x Little Club	0.74	- 0.37
Westphal 12A	x Akabozu	0.54	0.06
BH 1146	x Akabozu	0.40	- 0.10

together have been found. In the cross Westphal 12A x Akabozu, individual F3 and F5 plants with LP's equal to Little Club have been observed. These plants were probably derived from F2 or F4 plants with one or two LP-prolonging genes in heterozygous condition. One line was found, which might carry all five LP-prolonging genes, as its relative LP-value was 240.

The F2 frequency distributions of the crosses Westphal 12A x Little Club and Akabozu x Little Club were positively skewed. Parlevliet (1978) mentioned three possible causes:

1) dominance effects, genes for a longer LP would inherit in a recessive way.

2) the LP of the most susceptible genotype may represent a fysiological barrier, gene action would not be fully expressed at low LP values.

-geometric cumulative gene action which means that the more genes are present the larger their effect is.

Jacobs and Broers (1989) concluded that the first reason most likely was the most important one and they excluded the second one. Geometric cumulative gene action was not excluded. It causes the F2 mean to be closer to the F1 than to the midparent value. In the previous study, Jacobs and Broers (1989) showed that LP-prolonging genes act mainly additively in Westphal 12A and Akabozu. The longest LP expected in the progeny of the cross Westphal 12A x Akabozu would be 240 provided all five LP-prolonging genes are combined and act additively. As said earlier, this genotype is expected to occur in very low frequencies in F3 and F5 generations. Nevertheless, five out of 61 F5 lines showed mean LP's of about 240 or even longer and several plants in the F3 and F5 generation showed LP's remarkably longer than the expected maximum LP. It is concluded that geometric cumulative gene action might have been of some importance in these cases.

Besides geometric cumulative gene action, circumstances inherent to the experimental design may have caused the extreme long LP's of several plants in the F3 and F5 generation. All plants obtained the same spore density. Latency period and infection frequency are negatively correlated (Broers, 1989; Parlevliet and Kuiper, 1977) and LP is urediosorus-density dependent (Johnson and Taylor, 1976; Metha and Zadoks, 1970). This could mean that the LP of very susceptible plants like Little Club would be somewhat underestimated compared to the LP of very resistant plants due to differences in infection frequency.

Another reason for overestimating the LP of partially resistant genotypes is the influence of temperature. When a period of high temperatures is followed by a period with lower temperatures at the moment that Little Club has just passed its LP, the pustule development on more resistant genotypes will be delayed and the LP will be longer than in the case of constant temperatures during the experiment. The effect will be most pronounced for the very resistant genotypes and may lead to LP's beyond the expected maximum of 240 compared to Little Club.

The inheritance of LP in BH 1146 is not very clear. According to the F3 analysis BH 1146 should possess three or more LP-prolonging genes. In the seedling stage the resistance of BH 1146 behaves like Lr-13 (McIntosh, pers. comm.), which is a hypersensitivity resistance gene present in many South-American wheat cultivars and also present in the ancestors of BH 1146. At high temperatures (25 °C) McIntosh observed low infection types indicating hypersensitivity resistance (HR) whereas at low temperatures (15 °C) he observed high infection types. In a small additional experiment, BH 1146 and a line carrying Lr 13 in a Thatcher-background were tested with our 'Flamingo' race at the 15 °C and 25 °C and all genotype-temperature combinations

gave susceptible infection types. The results indicated that our 'Flamingo' race carries virulence for Lr-13 and for the HR-gene in BH 1146. In 1987, however, the F2 generation of BH 1146 x Little Club showed a 3:1 ratio of susceptible and hypersensitive plants, indicating a monogenic hypersensitivity. This was not observed in the F2 generation in 1986, in F3 and F5 generations or in other crosses involving BH 1146. Probably, the expression of the gene is sensitive to the environment and the genetic background which confirms the observations of Jacobs and Broers (1989).

Assuming that all genes in BH 1146 are LP-prolonging genes different from the LP-prolonging genes of Akabozu, transgressive segregation to both sides would be expected in the cross Akabozu x BH 1446. In the F2 evaluation in 1986, in which the S1 and S2 populations were selected, transgression occurred to both sides. In the F3 analysis reported here, transgression occurred only towards a shorter LP. If the only gene present in BH 1146 would be the HR-gene, 25 % of the F2 plants of the cross Akabozu x BH 1146 would merely segregate for LP-prolonging genes of Akabozu. One out of 64 plants would carry no LP-prolonging genes at all and the derived F3 line would have an LP equal to the LP of Little Club. However, none of the F3 line and none of the individual plants had LP's equal to Little Club, indicating that the HR-gene is not the only gene present in BH 1146. It is concluded that BH 1146 probably possesses a HR-gene (possibly Lr-13) and two or more LP-prolonging genes which are at least partly different from the LP-prolonging genes in Akabozu. The HR-gene possibly acts epistatic over the LP-prolonging genes like in barley/barley leaf rust (Niks, 1983). Besides, the HR-gene may have an increasing effect on LP (Andres *et al.*, 1986).

In the F2 of the crosses studied here, two selections have been made, the S1 and S2 populations. The S1 population was obtained by selection for susceptibility. The heritabilities revealed that selection for susceptibility offers good possibilities (ranged from 0.40 to 0.89). Selection for resistance, the S2 population, did not always give good results. The heritabilities for the S2 populations were low or irrelevant as negative values or values which are larger than one were obtained. As indicated earlier the environment might influence the LP of plants, leading to genotype x environment interactions. These interaction might have occurred leading to negative heritabilities and heritabilities larger than one. Verdoorn (1988) warned against the use of heritabilities based on results from different environments because they give a misleading impression of the genotypic part in the observed variation in segregating generations.

The inheritance of LP in spring and winter wheat showed large similarities. Both in spring and winter wheat, LP inherited partially recessive and oligogenic, LP-prolonging genes in different parents had different effects on LP (major and minor genes) and transgression was observed in crosses between partially resistant cultivars (Lee and Shaner, 1985a, 1985b).

The observed transgression offers possibilities to accumulate LP-prolonging genes like in barley/barley leaf rust (Parlevliet and Kuiper, 1985). Additional new sources of PR will be helpful. An additional approach is the combination of LP-prolonging genes in winter wheat and spring wheat. They represent different gene pools and LP-prolonging genes might therefore be of different origin. Combination of the genes from the two gene pools might lead to very high levels of PR.

REFERENCES

Andres, M.W., R.D. Wilcoxson and A.P. Roelfs, 1986. Effect of pathogen races on the area under the disease progress curve in barley infected with *Puccinia hordei*. Zeitschrift für Pflanzenkrankheit und Pflanzenschutz 93: 632-637.

Broers L.H.M, 1989. Influence of developmental stage and host genotype on three components of partial resistance of wheat to wheat leaf rust. Euphytica, in press.

Jacobs, Th. and L.H.M. Broers, 1989. The inheritance of host plant effect on latency period of wheat leaf rust in spring wheat. I. Estimation of gene action and number of effective factors in F1, F2 and backcross generations. Euphytica, in press.

Johnson, R. and A.J. Taylor, 1976. Spore yield of pathogens in investigations of the race-specificity of host resistance. Annual Review of Phytopathology 14: 97-119.

Lee, T.S. and G. Shaner, 1985a. Oligogenic inheritance of length of latent period in six slow leaf-rusting wheat cultivars. Phytopathology 75: 636-643.

Lee, T.S. and G. Shaner, 1985b. Transgressive segregation of length of latent period in crosses between slow leaf-rusting wheat cultivars. Phytopathology 75: 643-647.

Mather, K., and J.L. Jinks, 1982. Biometrical genetics. The study of continuous variation. Third edition. University Press, Cambridge.

Metha, Y.R. and J.C. Zadoks, 1970. Uredospore production and sporulation period of *Puccinia recondita* on primary leaves of wheat. Netherlands Journal of Plant Pathology 76: 267-276.

Niks, R.E., 1983. Histology of the relation between minor and major genes for resistance of barley to leaf rust. Phytopathology 73. 55-59.

Parlevliet, J.E. 1978. Further evidence of polygenic inheritance of partial resistance in barley to leaf rust, *Puccinia hordei*. Euphytica 27: 369-379.

Parlevliet, J.E. and H.J. Kuiper, 1977. Partial resistance of barley to leaf rust, *Puccinia hordei*. IV. Effect of cultivar and development stage on infection frequency. Euphytica 26: 249-255.

Parlevliet, J.E. and H.J. Kuiper, H.J. 1985. Accumulating polygenes for partial resistance in barley to barley leaf rust, *Puccinia hordei*. I. Selection for increased latent period. Euphytica 34: 7-13.

Parlevliet J.E. and A van Ommeren, 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.

Poyntz, B. and P.M. Hyde, 1987. The expression of partial resistance of wheat to *Puccinia recondita*. Journal of Phytopathology 120: 136-142.

Shaner, G. and R.E. Finney, 1980. New sources of slow leaf rusting resistance in wheat. Phytopathology 70: 1183-1186.

Teng, P.S., M.J. Blackie and R.C. Close, 1977. A simulation of crop yield loss due to rust disease. Agricultural Systems 2: 189-198.

Verdoorn, L.R., 1988. Statistical inference on variance components. Ph.D.thesis, Wageningen Agricultural University, ISN 266302, 221pp.

Zadoks, J.C., 1971. System analysis and the dynamics of epidemics. Phytopathology 61: 600-610.

Chapter 9.

RELEVANCE OF RANDOMIZING HOST GENOTYPES OF WHEAT THAT ARE TESTED FOR LEAF RUST RESISTANCE IN RACE NURSERIES.

L.H.M. Broers

SUMMARY

Ten wheat leaf rust race nurseries were planted in 1984. In five, each with a different race, no randomization of the twenty wheat genotypes was carried out. The remaining five race nurseries, also each with one of the same five races, were completely randomized. Comparison of the wheat genotype assessments was done using three procedures:
i) correlating (Pearson's and Spearman's r) the leaf area affected of the twenty wheat genotypes in the randomized and the non-randomized nurseries;
ii) comparing the best five wheat genotypes in each race nursery;
iii) comparing the W_i-indices of wheat genotypes in the randomized and non-randomized nurseries.
All three procedures showed excellent agreement between the randomized and the non-randomized nurseries. The use of a spreader row was most likely the reason for the large similarity. The consequence is that randomization is not sufficiently relevant to justify the increase in risk of mistakes and the extra administration needed.
The host genotype/pathogen race interaction was significant and rather large. All but two host genotype/pathogen race combinations showed a susceptible infection type, which indicates that partial resistance of wheat to wheat leaf rust caused the major part of this interaction. The results which suggest a gene-for-gene relationship, support the "integrated concept" of Parlevliet and Zadoks.

INTRODUCTION

Zadoks (1961) developed the race nursery as a method to evaluate the resistance under field conditions of crop genotypes to various races of an airborne pathogen. The crop genotypes are sown in clumps of 20 cm diameter, alongside a spreader row which is highly susceptible to all races of the pathogen in the test. A severe epidemic develops after inoculation of the spreader row with the proper race, one race per nursery. At several dates disease is assessed and from the disease readings the relative resistance is calculated.

The race nurseries have been used extensively in the Netherlands to evaluate resistance of winter and spring wheat genotypes to yellow rust (*Puccinia striiformis* f.sp. *tritici*) and wheat leaf rust (*Pucc. recondita* f.sp. *tritici*, syn. *Pucc. triticina*).

The testgenotypes were never randomized in such race nurseries. There is a good practical reason to omit randomization, as many mistakes during planting, managing and analyzing the data can thus be avoided. The assumption was made that the amount of inoculum produced by the spreader row was so much larger than the variable amount of inoculum produced by the adjacent clumps (variable because of differences in resistance) that their neighbour effects were negligible. Randomization was therefore not considered sufficiently relevant to justify the increased risk of mistakes.

The question arises whether the above assumption is actually correct. The aim of this study was to find an answer to this question by comparing randomized and non-randomized experiments.

MATERIALS AND METHODS

Twenty wheat genotypes were used. The susceptible winter wheat cultivar 'Rubis', which served as the control, was obtained from the Department of Phytopathology, Wageningen. The other nineteen genotypes were lines out of a Cyprian landrace, supplied by Dr. A.C. Zeven of the Depart-

ment of Plant Breeding, Wageningen Agricultural University. This landrace is known as Skalavatis (Hadjichristodoulous and Della, 1976) and, according to Bennet (1973), it is susceptible to wheat leaf rust. Morphological, chemical and cytological studies indicate that the nineteen Skalavatis lines are hexaploid, that differ in genotype. The nineteen lines were coded as S48 to S56, S58 to S66 and S68. S70 stood for 'Rubis'.

The five wheat leaf rust races, named 'Felix', 'Flamingo', 'Marksman', 'Clement' and 'Neutral' were obtained from the Department of Phytopathology, Wageningen (Zadoks, 1963). Monospore cultures were maintained and multiplied on seedlings of 'Rubis'.

The basic design was the race nursery as described by Zadoks (1963). Each race nursery consisted of a spreader row (also 'Rubis') with a row of 20 clumps on both sides. Each clump (diameter 20 cm) in a row represented one genotype. The heart to heart distance between clumps was 40 cm. The spreader row was inoculated with the proper wheat leaf rust race. This design offers a test in two replications of 20 genotypes to one isolate, performed at the mature plant stage of the wheat.

The race nurseries were situated in South Flevoland and planted at least 50 m apart in a field of winter swede rape to avoid cross contamination between race nurseries. At the end of February, 1984, the nineteen Skalavatis line were sown in 15 cm jiffy pots at the rate of 15 seeds per pot. After seedling emergence the pots were placed outside to induce good tillering. In the middle of April the pots were transferred to the race nurseries together with those of the susceptible control and of the spreader plants. As 'Rubis' is a winter wheat and thus needs vernalization, these pots had been sown earlier, in November, 1983.

Before transferring the pots to the field, half of the spreader plants were inoculated by dusting with a mixture of spores of the proper race and lycopodium powder. They were incubated for one night at 10 °C and 100% relative humidity in complete darkness.

Ten race nurseries were planted. In five, each with a different race, no randomization was carried out. Row 1 of each race nursery was planted in the same order: S48, S49 etc., with S70 on position 7. Row 2, at the other side of the spreader row, was planted in the reverse order. This was the non-randomized experiment (Exp. I). The remaining five race nurseries, also each with a different race, formed the randomized experiment (Exp. II). Within each row the twenty genotypes were randomized. In Exp. I each entry had always the same neighbours, whereas in Exp. II the neighbours were generally not the same from one row to another.

The level of wheat leaf rust was assessed on 13, 21 and 28 June and 4 July, by estimating the percentage of affected leaf surface (PA) of the upper three leaves, including the flag leaf, using the Peterson scale (0-100%) (Peterson *et al.*, 1948). On 13 June the average PA of the upper three leaves was estimated. On 21 and 28 june and 4 July the PA was assessed for each leaf layer separately. The growth stages were observed on 13 June, the infection types on 21 june.

For statistical analysis of the observations the SPSS-program was used, which was available at the DEC-10 computer of the Agricultural University, Wageningen. Tests were performed for pathogen race effects, wheat genotype effects and wheat genotype-pathogen race interactions assuming a fixed model (Snedecor, 1980) and using transformed PA data (RPA) (per row the PA of each genotype was expressed as a percentage of the highest PA of the row). For comparison of the experiments two correlation coefficients were used: Pearson's linear correlation coefficient and

Spearman's rank correlation coefficient. The W_i-index, developed by Wricke (1962; 1964) was applied to estimate the contribution of individual genotypes to the total host genotype/pathogen race interaction variance:

$$W_i = \sum_{j=1}^{n} (X_{ij} - X_{i.}/n - X_{.j}/m + X_{..}/nm)$$

where

X_{ij} = RPA of genotype i at race j;
$X_{i.}/n$ = Mean RPA of genotype i;
$X_{.j}/m$ = Mean RPA of race j;
$X_{..}/nm$ = overall mean RPA;

If W_i is large, the contribution of genotype i to the interaction variance is said to be large.

Table 9-1. Pearson's linear and Spearman's rank correlations between wheat genotypes from randomized and non-randomized race nurseries, characterized by their relative percentage of leaf area affected.

Wheat leaf rust race	Pearson*	Spearman
'Felix'	0.67	0.70
'Flamingo'	0.94	0.88
'Marksman'	0.90	0.70
'Clement'	0.91	0.77
'Neutral'	0.87	0.82

* All coefficients are significant at P=0.001.

RESULTS

After transformation of PA to RPA it was concluded that the mean RPA of the three leaves on 21 June was the most discriminating variable. The average PA on this day was 34% . These data were used for the comparison of the two experiments.

To compare the randomized and non-randomized experiment three procedures were applied.

i) Statistically by using correlation coefficients. In Table 9-1, Pearson's and Spearman's correlation coefficients are given for five combinations of race nurseries. Both Spearman and Pearson show highly significant correlation coefficients (P

Table 9-2. The ranking of the best five wheat genotypes (best 25%) selected in each of the five wheat leaf rust race nurseries in the non-randomized (N) and the randomized (R) experiment and the percentage of agreement between N and R for each of the five race nurseries.

Ranking	Race									
	Felix		Marksman		Flamingo		Clement		Neutral	
	N	R	N	R	N	R	N	R	N	R
1	S63	S55	S50	S63	S64	S64	S64	S64	S59	S59
2	S54	S50	S63	S50	S65	S59	S65	S65	S52	S63
3	S50	S54	S54	S54	S61	S65	S59	S61	S61	S65
4	S64	S65	S64	S64	S59	S52	S61	S59	S65	S50
5	S55	S64	S60	S60	S52	S61	S60	S52	S63	S61
% of agreement	80%		100%		100%		80%		80%	

< 0.001) indicating good agreement between the two experiments.

ii) The second approach is that of a practical plant breeder. A plant breeder is interested in the results of a selection and its repeatability. Selecting the best 25% genotypes in each race nursery in both experiments gave the results shown in Table 9-2. For two races, the same five genotypes were selected. With the other three races four of five selected genotypes were the same in the two experiments. This corresponds with an average agreement in selection of 88%.

iii) The third approach is again a statistical method. Wricke (1962; 1964) developed an index W_i which estimates the contribution of a genotype to the genotype/environment interaction. The W_i-index can be used here by regarding the five races as five different environments of the host genotypes. From the analysis of variance it was shown that the host genotype/pathogen race interaction was significant ($P < 0.05$). If the two experiments would give similar results, the same genotypes would contribute to the host genotype/pathogen race interaction variance. Table 9-3 shows the W_i-index of the genotypes that contributed most to the interaction variance in both experiments and some that did not contribute to it. The linear correlation coefficient r of the W_i-index between the two experiments was 0.93 ($P < 0.001$). In both experiments the same seven genotypes caused over 75% of the interaction variance.

DISCUSSION

Three different methods were used to compare the randomized and the non-randomized experiments.

i) Both Pearson's and Spearman's correlation coefficients were high and highly significant ($P< 0.001$). On the average the Pearson's correlation coefficient was higher then the Spearman's correlation coefficient. Nine of the 20 wheat genotypes were very susceptible. Their RPA was always over

Table 9-3. The Wricke's indices (Wi) of nine wheat genotypes as a measure for the contribution to the interaction variance, in non-randomized (N) and randomized (R) wheat leaf rust race nurseries based upon the relative percentages of leaf area affected, and the relative contribution of the nine genotypes to the sum of the 20 Wi-indices.

Genotype	N		R	
	Wi	rel Wi	Wi	rel Wi
S50	4820	15	4650	13
S54	3716	12	5418	16
S59	3746	12	2899	8
S61	3427	11	3106	9
S63	4602	15	5003	14
S64	2852	9	3508	10
S65	2050	7	2312	7
S60	73	0.2	243	0.7
S70	114	0.4	413	1.1
Sum of Wi-indices	31300	100.0	34940	100.0

65%. Differences among genotypes in this group were small and seemed mostly due to experimental error. This means that the ranking of these nine genotypes may differ completely from one experiment to another, causing Spearman's correlation coefficient to be lower than Pearson's correlation coefficient. Pearson's correlation coefficient, therefore, is the more suitable one in this study. It can be concluded from the comparison of experiments that there is little difference between the randomized and non-randomized experiments.

ii) Selection of the best five genotypes of each race nursery gave also an excellent agreement between the two experiments. Twentytwo of the possible 25 genotypes were the same in both experiments (88%). The genotypes which were not selected in one experiment but were so in the other, did not differ significantly in most cases from those that were selected. Again, the conclusion is that the two experiments were very much alike.

iii) The last approach used, also shows large similarity between Exp.I and Exp II. The same seven genotypes contribute over 75% to the host genotype/pathogen race interaction variance. Even more important was the high linear correlation coefficient of 0.93 of the W_i-indices of the two experiments.

The use of a highly effective spreader row is most likely the reason for the large similarity between the randomized and non-randomized experiment. The spreader row produces large quantities of spores. Spore dispersal is by wind and the two test rows receive large amounts of spores. Even if there is a predominant wind direction, which might cause differences in inoculum level, as was the actual case, the interplot interference with the spreader row is still so large that interplot interference between adjacent testclumps is overruled.

This way of testing genotypes for partial resistance is a rather 'tough' one (Zadoks and Schein, 1979). Lines with a good level of partial resistance will not contribute to the epidemic. They reflect to a large extend what happens on the susceptible spreader row. Every moment spores can start a new monocyclic process by alloinfection. Autoinfection is negligible (Zadoks and Schein, 1979). Using this design, the level of partial resistance is strongly underestimated (Parlevliet and van Ommeren, 1984). If interplot interference of adjacent clumps has a significant effect on the epidemic development, host genotype/pathogen race interaction can be influenced by systematic planting. A partially resistant line surrounded by two genotypes susceptible to one race but resistant to another race will show a high PA to one race but a low PA to another race. This is an artificial interaction which will not appear in randomized race nurseries. The interaction found here are apparently of the same origin in both experiments, indicating that the systematic planting did not influence the interactions and that the effect of the neighbour plants was negligible.

The consequence of the excellent agreement between the randomized and non-randomized experiments is that systematic planting of the race nurseries, which provide a heavy inoculum pressure through the spreader rows, is allowed. This saves not needed administration, reduces the risk of mistakes, and does not impede statistical analysis.

Since both the randomized and non-randomized experiments are very much alike the data were combined and the analysis of variance was repeated. The host genotype/pathogen race interaction was significant ($P < 0.05$). All but two host geno-type /pathogen race combinations showed a susceptible infection type which indicates that partial resistance for a large part causes the observed interaction. Partial resistance, characterized by a slow epidemic development despite of a susceptible infection type (Parlevliet and van Ommeren, 1975), there-

Table 9-4. Relative percentages of leaf area affected of six wheat genotypes for each of the five races of wheat leaf rust at the second assessment date.

Wheat genotype	Race					Mean
	Felix	Flamingo	Marksman	Clement	Neutral	
S60	61^{b*}	54^{b}	59^{b}	59^{b}	57^{b}	58^{b}
S70	65^{b}	50^{b}	65^{bc}	69^{bc}	65^{bc}	63^{b}
S59	72^{b} ↑	23^{a} ↓	80^{bc} ↑	23^{a} ↓	13^{a}	42^{a}
S63	36^{a}	86^{c}	21^{a}	81^{c}	25^{a}	50^{ab}
S49	79^{b}	84^{c}	79^{bc}	83^{c}	90^{d}	83^{c}
S56	73^{b}	85^{c}	85^{c}	87^{c}	86^{d}	83^{c}

* = entries with the same letter in a column do not differ significantly ($P<0.05$).

fore, seems to act according to a gene-for-gene relationship, allowing for race-specific interactions. These results corroborate the "integrated concept" of Parlevliet and Zadoks (1977) in which both vertical and horizontal resistance are supposed to act on a gene-for-gene base.

The interactions found here are rather large (Table 9-4), indicating that gene effects may be quite large and partial resistance may be of oligogenic rather than of a polygenic nature. This agrees with the results of Lee and Shaner (1985a, 1985b) who found two to four genes for partial resistance to leaf rust in wheat, each with a large impact on the latency period, the most important component of partial resistance.

In field experiments like this one, experimental errors are quite large. Small interactions, which may be present, may remain undetected. The use of microfields (Zadoks, 1972) or the measuring of latency period in the greenhouse might reduce the experimental errors in comparison to the race nursery technique. These alternative techniques could result in finding small interactions, indicative for partial resistance that is polygenically inherited with small gene effects.

REFERENCES

Bennet, E., 1973. Wheats of the Mediterranean Basin. In: "Survey of crop genetic resources in their centers of diversity". Ed: O.H. Frankel, FAO, Rome, Pp1-8.

Hadjichristodoulou, A. and A. Della, 1976. Genetic diversity in Cyprus. Plant genetic resources 32: 8-9.

Lee, T.S., and G. Shaner, 1985a. Oligogenic inheritance of length of latent period in six slow leaf-rusting wheat cultivars. Phytopathology 75: 636-643.

Lee, T.S., and G. Shaner, 1985b. Transgressive segregation of length of latent period in crosses between slow leaf-rusting wheat cultivars. Phytopathology 75: 643-647.

Parlevliet, J.E. and A van Ommeren, 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.

Parlevliet, J.E., and A van Ommeren, 1984. Interplot interference and the assessment of barley cultivars for partial resistance to leaf rust, *Puccinia hordei*. Euphytica 33: 685-697.

Parlevliet, J.E., and J.C. Zadoks, 1977. The integrated concept of disease resistance; a new view including horizontal and vertical resistance in plants. Euphytica 26: 5-26.

Peterson, R.F., A.B. Campbell and A.E. Hannah, 1948. A diagrammatic scale of estimating rust intensity on leaves and stems of cereals. Can J Res C26: 496-500.

Snedecor, G.W., 1980. Statistical methods. The Iowa State Universisty Press, Ames, Iowa, 507pp.

Wricke, G., 1962. Über eine Methode zur Erfassung der Ökologische Streubreite in Feldversuchen. Zeitschrift für Pflanzenzüchtung 47: 9296.

Wricke, G., 1964. Zur Berechnung der Ökovalenz bei Sommerweizen und Hafer. Zeitschrift für Pflanzenzüchtung 52: 127-138.

Zadoks, J.C., 1961. Yellow rust in wheat. Studies in epidemiology and physiological specialization. Tijdschrift voor Planteziekten 67: 69265.

Zadoks, J.C., 1963. The use of race nurseries in cereal resistance breeding. In: "Barley genetics I". Proc 1st Intern Barley Genetics Symp, PUDOC, Wageningen. Pp 242-249.

Zadoks, J.C., 1972. Modern concepts of disease resistance in cereals. In: "The way ahead in plant breeding", Proc 6th Congr Eucarpia. Eds: F.G.H. Lupton, G. Jenkins, R.Johnson, P.B.I., Cambridge, Pp89-98.

Zadoks, J.C., and R.D. Schein, 1979. Epidemiology and plant disease managment. Oxford University Press, New York and Oxford, 427 pp.

Chapter 10.

INFLUENCE OF DEVELOPMENT STAGE AND HOST GENOTYPE ON THREE COMPONENTS OF PARTIAL RESISTANCE TO LEAF RUST IN SPRING WHEAT.

L.H.M. Broers

SUMMARY

Latency period (LP), infection frequency (IF) and urediosorus size (US) of wheat leaf rust were determined on primary leaves and young flag leaves of 18 spring wheat cultivars. A large growth stage and a large cultivar effect on all three components were observed. Partial resistance as measured by the three components was generally better expressed in the adult plant stage than in the seedling stage. Associated variation of the components was observed: long LP, low IF and small US tended to go together. The association was not complete, cultivars with clear deviations from this association for one of the components were found suggesting the existence of at least partly different genetic factors controlling the respective components. LP measured on flag leaves gave the most reliable results and, therefore, could best be used as a selection criterion in breeding programs for partial resistance.

INTRODUCTION

Partial resistance (PR) is a form of resistance that retards the disease progress in the field despite of a high infection type, indicating that no effective hypersensitivity resistance is present. Attention of breeders and scientists has been drawn to PR because it is assumed to be durable in contrast to the transient hypersensitivity resistance.

In barley/barley leaf rust, PR in the field was highly correlated with the latency period of the young flag leaves (Parlevliet and van Ommeren, 1975; Parlevliet *et al.*, 1985). Other components such as infection frequency and spore production were closely associated with latency period but were more difficult to measure (Neervoort and Parlevliet, 1978; Parlevliet and Kuiper, 1977).

Partial resistance to wheat leaf rust (*Puccinia recondita* f.sp. *tritici*, syn. *Pucc. triticina*) has been found in several winter wheat (*Triticum aestivum*) cultivars (Ohm and Shaner, 1976; Shaner *et al.*, 1978). These cultivars always exhibited a longer latency period compared to the highly susceptible checks. It was not clear to what extend the latency period and other components were affected by cultivar and development stage and how the components were associated.

The aim of this study is to investigate the variation of the three components of PR in 18 spring wheat cultivars and to obtain information about the degree of association between the components, the effect of growth stage on the variation of the components and the reliability by which the components can be evaluated.

MATERIALS AND METHODS

Eighteen spring wheat cultivars, obtained from the Department of Plant Breeding, Wageningen Agricultural University, were selected from preliminary field trials because they differed in level of PR. Skalavatis 56 was the most susceptible check and Akabozu the most resistant check. In all experiments a monospore culture of the wheat leaf rust race 'Felix' was used, obtained from the Department of Phytopathology of the Wageningen Agricultural University.

Seedling tests

Plants were grown in square flats (30 x 30 x 5 cm). Each flat contained Skalavatis 56, Akabozu and five or six of the remaining cultivars. One replication consisted of three flats. Per cultivar and per replication at least five plants were used. Three successive experiments (Exp IIa, IIb, IIc) were carried out

with two replications per experiment in a three week period. Primary leaves of 10-days old seedlings (DC 11 on the scale of Zadoks *et al.*, 1974) were inoculated at the upper leaf surface in a settling tower. Per flat 1.3 mg rust spores mixed with 13.0 mg *Lycopodium* spores were used giving ca. 100 urediospores/cm^2.

Adult plant tests

Plants were grown in 2.2 l containers (4 plants per container) in a greenhouse. All plants were inoculated in the young flag leaf stage (DC 48-59 on the scale of Zadoks *et al.*, 1974). As cultivars differed in their developmental rate, it was impossible to inoculate all cultivars together at one and the same time. Therefore, inoculations were carried out in sequential series each including the cultivars that were in the correct growth stage for inoculation, and Skalavatis 56 and Akabozu in the proper stage as checks so that different series could be combined and compared. Per series, four replications were inoculated according to a randomized block design with four flag leaves per cultivar and per replication. Inoculation of the upper surface of the flag leaf was carried out in the settling tower using 1.3 mg spores per replicate giving ca. 100 urediospores/cm^2. In the winter of 1986/1987 (Exp Ia) seven sequential series

Table 10-1. Latency period (LP) of wheat leaf rust race 'Felix' on 18 spring wheat cultivars relative to Skalavatis 56 (set at 100%) in the seedling and adult plant stages.

Cultivar	Seedling	Adult plants		
		Exp Ia	Exp Ib	Mean
Skalavatis 56	100	100	100	100
Little CLub	99	106	96	101
Jufy I	102	100	101	101
Sicco	99	108	101	105
Fr 45/73-2	100	108	103	106
Purple Justin	99	106	107	107
Fundus	102	108	110	109
Minaret	102	105	114	110
Van Hoek	-	115	105	110
Ralle	102	109	112	111
RPB2-297-76E	100	113	111	112
Ze 64-1-14	100	107	120	114
Turano	101	119	119	119
Akabozu	121	167	157	162
Westphal 12A	115	162	159	161
Ponta Grossa 1	-	164	164	164
Kenphad	125	162	181	172
BH 1146	118	184	176	180
LSD 5%	5	14	16	12

- = not included in seedling tests.

were inoculated and in the late spring of 1987 (Exp Ib) six sequential series were realized. Each cultivar was included in at least three series in both winter and spring experiments.

After inoculation, seedlings and adult plants were incubated overnight in a mist chamber where 100 per cent relative humidity in darkness assured germination of and penetration by the rust. Following incubation the plants were transferred to a greenhouse where temperatures ranged from 10 to 15 °C during the night and from 18 to 28 °C during the day.

Latency period (LP) and infection frequency (IF) were measured on all inoculated primary and flag leaves. The LP was determined by counting daily the number of urediosori visible in a marked area on the leaves (using a 7x pocket-lens) until the number of urediosori no longer increased. The time at which 50 per cent of the terminal number of urediosori had appeared was estimated by interpolation. The LP was taken as the time period from the beginning of incubation to the time at which 50 per cent of the urediosori had appeared.

The IF was measured using a metal sheet with a 2 x 0.5 cm window (Parlevliet and Kuiper, 1977). The metal sheet was placed on the leaf so that the window fell within the marked area. The number of urediosori within the window was a direct estimate for IF in urediosori/cm^2.

The urediosorus size (US) was measured using a microscope (magnification 100 x) with a micrometer. Leaves were sampled 15 days (seedlings) and 25 days (adult plants) after inoculation and boiled in a lactophenol:ethanol (1:2, v/v) solution for 3 minutes to fix them. Per leaf the length (L) and width (W) of 15 randomly chosen urediosori were measured. US was obtained using the formula: US =1/4 π*L*W. Seedlings of the experiments IIb and IIc were used for this purpose. Per series four leaves per cultivar were sampled. From the adult plants four leaves per cultivar were used in every series, in both the winter and spring experiments.

The means of the observed values of LP, IF, US were converted into relative values per experiment (seedlings) or per series (adult plants) whereby the observed values of Skalavatis 56 were set at 100%.

RESULTS

Latency period

The LP varied widely among cultivars and significant cultivar differences were detected in both the seedling stage and the adult plant stage (Table 10-1). In the seedling stage the LP ranged from 7.1 to 8.9 days (99 to 125% relative to Skalavatis 56). The adult plant LP ranged from 8.2 to 15.1 days (100 to 184% relative to Skalavatis 56) in Exp Ia and from 8.4 to 15.7 (96 to 181% relative to Skalavatis 56) in Exp Ib.

Latency period in the adult plant stage was always longer than in the seedling stage and the effect of genes governing a longer LP (LP-prolonging genes) was always larger in the adult plant stage than in the seedling stage. For example, LP-prolonging genes of Akabozu lengthened LP with 21% (1.5 days) in the seedling stage and with 62% (5.5 days)in the adult plant stage.

The cultivar effect in relation to LP showed a an irregular distribution over the available range. Therefore, Spearman's rank correlation coefficient was used to describe the relation between the seedling LP and the adult plant LP. The rank correlation coefficients were significant (Table 10-2) but differences in the seedling stage were so small the seedling LP was a poor indicator for adult plant LP.

The cultivars could be divided into two groups according to their LP. Group I con-

Table 10-2. Pearson's linear and Spearman's rank correlation coefficients of three components of partial resistance of wheat to wheat leaf rust as measured on primary leaves and on flag leaves.

	Pearson[1,2]		Spearman		
	IF	US	LP	IF	US
Primary- Flag Exp Ia	0.56^{i}	0.54^{i}	0.60*	0.49^{i}	0.46
Primary- Flag Exp Ib	0.66*	0.51^{i}	0.77**	0.57*	0.49^{i}
Primary- Flag mean	0.68*	0.55^{i}	0.71*	0.57*	0.53^{i}

1) LP = latency period; IF = infection frequency; US = urediosorus size.
2) i = significant at P=0.10; *= significant at P=0.05; **= significant at P=0.01.

Table 10-3. Size of urediosori (US) of wheat leaf rust race 'Felix' on 18 spring wheat cultivars relative to Skalavatis 56 (set at 100%) in the seedling and adult plant stages. Cultivars are ordered according to the mean latency period on the flag leaf (Table 10-1).

Cultivar	Seedling	Adult plants		
		Exp Ia	Exp Ib	Mean
Skalavatis 56	100	100	100	100
Little CLub	108	104	98	101
Jufy I	116	92	75	83
Sicco	84	65	70	68
Fr 45/73-2	111	80	63	72
Purple Justin	61	83	62	73
Fundus	117	70	81	76
Minaret	98	85	70	78
Van Hoek	-	94	68	81
Ralle	130	91	77	84
RPB$_2$-297-76E	116	82	62	72
Ze 64-1-14	106	83	82	83
Turano	103	71	53	62
Akabozu	66	28	40	34
Westphal 12A	88	40	51	46
Ponta Grossa 1	-	45	73	59
Kenphad	46	44	41	43
BH 1146	103	37	36	37
LSD 5%	33	19	21	21

- = not included in seedling tests.

tained 13 cultivars which showed a seedling LP of 99 to 102 and an adult plant LP of 96 to 120. Group II contained five cultivars with a seedling LP in the range of 115 to 125 and an adult plant LP in the range of 157 to 184. The low association between seedling LP and adult plant LP is best exemplified with the group I-cultivars. No significant cultivar differences were detected within this group in the seedling stage and all group I-cultivars would be classified as being as susceptible as Skalavatis 56. In the adult plant stage, however, a cultivar like Turano showed an LP significantly longer than Skalavatis 56, so that Turano could be of interest as a source of LP-prolonging gene(s).

Urediosorus size

In the seedling stage US ranged from 0.23 mm^2 (46% relative to Skalavatis 56) to 0.67 mm^2 (130%). The adult plant US ranged from 0.15 mm^2 (28%) to 0.56 mm^2 (104%) in Exp Ia and from 0.18 mm^2 (36%) to 0.52 mm^2 (100%) in Exp Ib (Table 10-3). In both growth stages, a reduced US was due to a reduction in both pustule length and width.

Significant cultivar effects were found for US in the seedling and the adult plant stages. In the adult plant stage the cultivar effect was larger than in the seedling stage. Only three cultivars showed urediosori smaller than Skalavatis 56 in the seedling

Table 10-4. Infection frequency of wheat leaf rust race 'Felix' on 18 spring wheat cultivars relative to Skalavatis 56 (set at 100%) in the seedling and adult plant stages. Cultivars are ordered according to the mean latency period on the flag leaf (Table 10-1).

Cultivar	Seedling	Adult plants		
		Exp Ia	Exp Ib	Mean
Skalavatis 56	100	100	100	100
Little CLub	100	116	127	122
Jufy I	96	89	85	87
Sicco	101	67	75	71
Fr 45/73-2	86	95	90	93
Purple Justin	83	84	79	82
Fundus	86	68	60	64
Minaret	100	104	81	93
Van Hoek	-	105	108	107
Ralle	97	68	62	65
RPB[2]-297-76E	96	81	80	81
Ze 64-1-14	97	106	72	89
Turano	87	106	72	89
Akabozu	56	63	52	53
Westphal 12A	76	90	78	84
Ponta Grossa 1	-	20	16	18
Kenphad	59	55	33	44
BH 1146	78	56	39	48
LSD 5%	36	25	28	19

- = not included in seedling tests.

stage (Akabozu, Kenphad and Purple Justin) whereas 14 cultivars showed smaller urediosori than Skalavatis 56 in the adult plant stage. Small differences and a large error variance may have caused a lack of discrimination in the seedling stage.

In general, the US relative to Skalavatis 56 was smaller in the adult plant stage than in the seedling stage. Akabozu, for example, showed a US of 0.34 mm^2 (66%) after 15 days in the seedling stage whereas the adult plant US (after 25 days) was 0.15 mm^2 (28%) in Exp Ia and 0.21 mm^2 (40%) in Exp Ib.

The association between the seedling US and the adult plant US was low as expressed by correlation coefficients (Table 10-2). In the seedling stage, cultivar differences for US and cultivar ranking were probably attributed to a non-genetic origin. Therefore, the seedling data for US were an even poorer indicator for adult plant data than those for LP. Only cultivars with extremely small urediosori in the adult plant stage will be recognized in seedling stage. A cultivar like Sicco showed a significantly smaller US than Skalavatis 56 in the adult plant stage but would not be classified as such in the seedling stage (Table 10-3).

Infection frequency

The error variance of IF was large as was the error variance of the spore deposit. Counts of spores on greased slides, placed in the sett-ling tower with the leaves revealed that differences between minimum and maximum spore density could be as much as 30 urediospores/cm^2 at a mean value of 100. Nevertheless, a significant cultivar effect was detected (Table 10-4) in both seedling and the adult plant stage. In the seedling stage the cultivar effect was smaller than in the adult plant stage.

The mean IF in the seedling stage was 38 urediosori/cm^2 (87%). In the adult plant stage the mean IF was 24 urediosori/cm^2 (84%) in Exp Ia and 25 urediosori/cm^2 (74%) in Exp Ib. The results indicate that genetic factors influencing IF were in general better expressed in the adult plant stage than in the seedling stage. IF measured on primary leaves was somewhat correlated with IF measured on young flag leaves (Table 10-2) but as with LP and US, IF in the seedling stage was a poor indicator for IF in the adult plant stage.

Table 10-5. Spearman's rank correlation coefficients between three components of partial resistance of wheat to wheat leaf rust as measured on primary leaves and flag leaves.

Components[1)]			Spearman[2)]
Primary leaves			
LP-	IF		0.49[i]
LP-	US		0.40
IF-	US		0.34
Flag leaves			
LP-	IF		0.64[**]
LP-	US		0.75[***]
IF-	US		0.67[**]

1) LP = latency period; IF = infection frequency; US = urediosorus size.
2) i= significant at P=0.10; **= significant at P=0.01;*** = significant at P=0.001.

Association of components

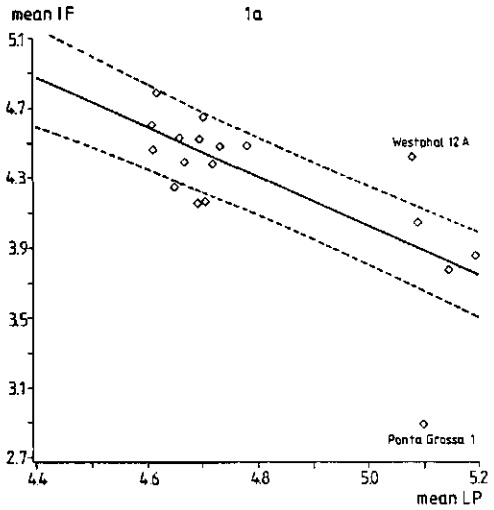
In Table 10-5, Spearman's correlation coefficients between LP, IF and US in the seedling and adult plant stages are presented. The correlation coefficients indicated an associated variation of the components studied. This means that a longer LP, a reduced IF and a smaller US tended to go together. Akabozu, for example, showed in the adult plant stage an LP, an IF and a US which were 160%, 60% and 30% compared to Skalavatis 56, respectively.

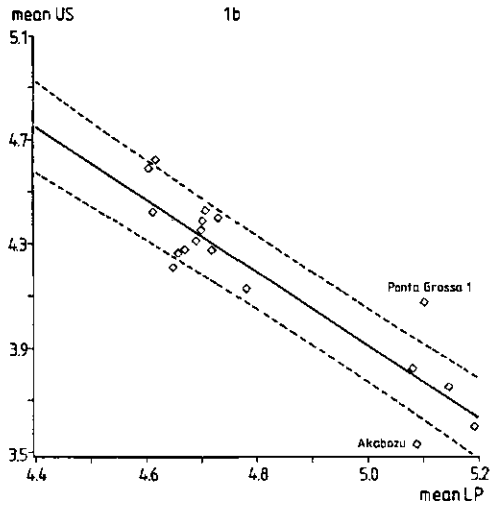
Table 10-6. Classification of 18 cultivars in 7 clusters according to their mean latency period in the flag leaf and the cluster mean latency period (LP_c), infection frequency (IF_c) and urediosorus size (US_c). Entries are means of ln-transformed values (see Table 10-1, 10-3 and 10-4).

Cluster	Cultivars	LP_c	IF_c	US_c
A	Skalavatis 56, Little Club, Sicco, Jufy I	4.62	4.54	4.47
B	FR 45/73-2, Purple Justin, Fundus, Minaret	4.68	4.41	4.31
C	Van Hoek, RPB_2-297-76E, Ralle, Ze 64-1-14	4.72	4.43	4.38
D	Turano	4.78	4.49	4.13
E	Akabozu, Westphal 12A, Ponta Grossa 1	5.09	3.79	3.81
F	Kenphad	5.14	3.78	3.75
G	BH 1146	5.19	3.86	3.60

The correlation of the components, however, was not very high and suggested that different genetic factors coded for the respective components. In the adult plant stage, a cultivar like Purple Justin for example had significantly smaller pustule than Skalavatis 56 but the LP was not significantly different from the LP of Skalavatis 56.

Because the cultivar responses in relation to LP were not regularly distributed over the available range but rather appeared to be clustered, results of linear regression analysis have only limited value as there were effectively far fewer than the apparent 16 (=18-2) degrees of freedom for the error term. Nevertheless, cultivars with interesting deviations from this association between components might be detected with linear analysis. To correct for the bias in the number of degrees of freedom for the error term, the cultivars were divided into clusters. The range for mean LP of the two experiments in adult plant stage (100% to 180%) was divided into classes with a range from 5% starting at 100% and the cultivars were clustered in the classes. This resulted in 7 clusters (Table 10-6). The values for LP, US and IF were logarithmically (ln-) transformed and per cluster averaged over the cultivars giving LP_c, US_c and IF_c. Three linear regression equations were obtained (Figure 10-1). The coefficients of determination were over 90% for all three equations, indicating a strong associated variation of the three components. The individual cultivar responses for LP, US and IF were compared with the three linear equations. Three clear deviating cultivars were found (Figure 10-1). For example, Ponta Grossa 1 had a much lower IF and Westphal 12A a much higher IF than expected according to its LP; Akabozu had smaller urediosori and Ponta Grossa 1 had larger urediosori than expected according to their LP. These deviating cultivars indicated that the association of components is not complete. The result suggests that different components of PR might be governed by different genetic factors, at least in some cultivars.





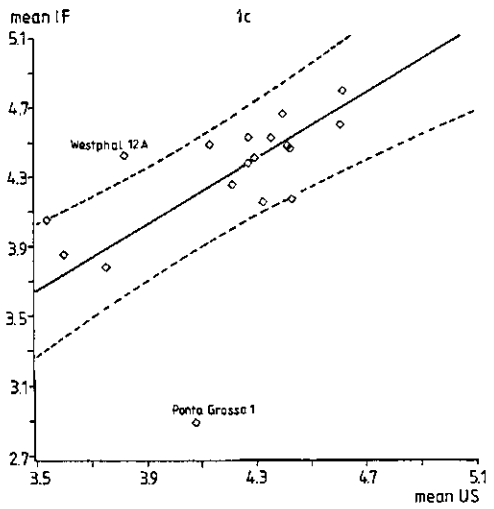


Figure 10-1. Regression of infection frequency and urediosorus size on latency period (1a and 1b) and infection frequency on urediosorus size (1c), with confidence belts (dotted hyperboles) for the regressed variable based on the mean data of the seven classes of cultivars from Table 10-6. The individual cultivar observations are marked as diamonds.

Table 10-7. Pearson's linear and Spearman's rank correlation coefficients between different experiments for three components of partial resistance of wheat to wheat leaf rust as measured on primary leaves and flag leaves.

	Pearson[1,2)]		Spearman		
	IF	US	LP	IF	US
Primary leaves					
Exp IIa- Exp IIb	0.32**	-**	0.73**	0.21*	
Exp IIa- Exp IIc	0.63**	0.69**	0.68**	0.58**	0.51^{i}
Exp IIb- Exp IIc	0.74**	-	0.70**	0.70**	
Flag leaves					
Exp Ia- Exp Ib	0.88***	0.78***	0.77***	0.75**	0.73**

1) LP is latency period; IF infection frequency; US is urediosorus size.

2) i= significant at P=0.10; *= significant at P=0.05;**= significant at P=0.01; *** = significant at P=0.001;

Repeatability

The experiments were compared to check the repeatability of the measurements of the three components. Pearson's linear and Spearman's rank correlation coefficients indicated that the measurement of components in the adult

plant stage is more satisfactory than in the seedling stage (Table 10-7). Akabozu and Kenphad were always among the best five cultivars for the respective components in the different experiments. BH 1146 was in 12 out of 14 experiment-component combinations among the top five, Westphal 12 A in 11 out of 14 and Ponta Grossa 1 (not present in the seedling tests) in all adult plant tests. This result implies that selection on a long LP, a low IF or a small US in repeated experiments will inevitably result in the same cultivars being selected.

DISCUSSION

Latency period (LP), infection frequency (IF) and pustule size (US) are components of partial resistance (PR) of wheat against wheat leaf rust. For all three components clear growth stage effects were detected. Generally, LP was longer, IF was lower and US was smaller on young flag leaves than on primary leaves. Similar results were found in winter wheat/wheat leaf rust (Ohm and Shaner, 1976; Pretorius *et al.*, 1988) and in barley/barley leaf rust (Parlevliet, 1975). The increase in resistance with increasing growth stage can be divided into two categories.

1) Increase in resistance caused by a difference in leaf tissue. Leaf tissue of primary leaves differs from that of flag leaves. Cells are larger and less tightly packed in primary leaves than in flag leaves. The fungus, with its intercellular growth, may therefore be less hindered to grow in primary leaves than in flag leaves. An example of this possible effect is the highly susceptible Skalavatis 56, with an LP on flag leaves 1.5 days longer than on primary leaves and with an US reaching a certain size ten days later in the adult plant stage than in the seedling stage. For IF no tissue effect could be discerned.

2) Increase in resistance because PR-genes are not or not fully expressed in seedling stage. This category can be divided in two sub-categories.

a) Increase in resistance because of the partial resistance genes are not expressed in the seedling stage. In several cultivars, the genetic factors controlling LP, IF or US came to expression only in the adult plant stage. Most of the group I cultivars did not differ from Skalavatis 56 in the seedling stage for either of the three components. In the adult plant stage some had a significantly longer LP, lower IF or smaller US than Skalavatis 56.

b) Increases in resistance because partial resistance genes are better expressed in the adult plant stage than in the seedling stage. This may be exemplified by Akabozu. The seedling values for LP and US were 121% and 60% respectively whereas the adult plant values were 162% and 31% respectively.

The consequence of the relation between partial resistance and growth stage is that breeders should use the young flag leaf stage for selection of PR.

Ranges for LP, IF and US were comparable to ranges observed in winter wheat (Ohm and Shaner, 1976; Shaner *et al.*,1978) and to ranges reported in barley/barley leaf rust (Parlevliet, 1975; Parlevliet and Kuiper, 1977).

Conspicuous was the clustering of the cultivars for LP in both the seedling stage and the adult plant stage (Table 10-1). Two clusters could be recognized, that differed markedly in LP in both seedling and adult plant stage. It is tempting to speculate about the implications of the observed discontinuity. Possibly, LP-prolonging genes exist in low frequencies in the spring wheat gene pool. This idea is supported by preliminary experiments which indicated that more than 90% of over 200 cultivars tested in the seedling stage for LP would be classified in group I (a seedling LP of around 100% relative to Skalavatis 56). In both spring and winter wheat 1 to 3 genes have been reported to govern a prolonged LP. The smallest effect of one LP-prolonging gene in adult plants of winter wheat was estimated to be 1.3 days (Lee and Shaner, 1985; Broers and Jacobs 1989). In the adult plant tests described here, this would mean an effect of about 15% compared to Skalavatis 56. If this is the minimum effect that one LP-prolonging gene can have, group I cultivars posses at most one LP-prolonging gene and group II cultivars two to at most five LP-prolonging genes. This is in agreement with the results of Broers and Jacobs (1989) who reported that Akabozu, Westphal 12A and BH 1146 possess 2, 3 and 3 to 4 genes, respectively, with rather large effects. Group I cultivars carrying one LP-prolonging gene could be of interest as a source of LP-prolonging genes provided they are different from known LP-prolonging genes in Akabozu, Westphal 12A, BH 1146 and some other winter wheat cultivars. As transgressive segregation for LP-prolonging genes has been observed (Lee and Shaner, 1985; Broers and Jacobs, 1989), LP-prolonging genes of group I cultivars might be suitable to create extremely long LP's.

The components tended to vary in an associated way as expressed by the correlation coefficients. Low correlations may be due in part to the large error variances observed with IF and US but also to different genetic factors coding for the respective components. The later explanation is supported by the fact that cultivars occur with deviations from the observed associated pattern found in a linear regression analysis. The results are at variance with observations in winter wheat/wheat leaf rust (Lee and Shaner, 1985) and barley/barley leaf rust (Parlevliet, 1986), where LP and US respectively LP and IF seem to be coded for by the same genes or by closely linked genes. However, Neervoort and Parlevliet (1978) found that the rate of spore production per urediosorus of some barley cultivars did not correspond at all with their LP and infectious period or, in other words, they

also found cultivars with deviations from the associated pattern.

A breeding program must be based on reliable selection criteria. Reliability is partly determined by the repeatability of results. Repeatability was best for latency period in both seedling and adult plant stages and was generally better in the adult plant stage than in the seedling stage. LP measured on young flag leaves should therefore be preferred to other components/growth stage combinations as selection criterion for PR. This is the more so as LP is the most important component of PR (Teng *et al.*, 1977; Zadoks, 1971) and because it is often associated with other components of PR.

The correlation coefficients between seedling and adult plant stages for the three components indicated that components measured in the seedling stages could not explain the adult plant effects in full. This is especially true for the group I cultivars, since almost no cultivar effect was found in this group in the seedling stage whereas several cultivars differed significantly for LP, IF and US from Skalavatis 56 in the adult plant stage. Only cultivars with a very long LP, a very low IF and very small US in adult plants could be recognized in the seedling stage. Therefore, seedling tests are advisable only as rough screening tests for components of partial resistance. The advantage of seedling tests is that they take much less time and far less space than adult plant tests and that no difficulties arise with genotypic differences in development rate. For more precise assessments adult plants tests are recommended.

REFERENCES

Broers, L.H.M. and Th. Jacobs, 1989. The inheritance of host plant effect on latency period of wheat leaf rust in spring wheat. II: Number of segregating factors and evidence for transgressive segregation in F3 and F5 generations. Euphytica, in press.

Lee, T.S. and G.E. Shaner, 1985. Oligogenic inheritance of length of latent period in six slow leaf-rusting wheat cultivars. Phytopathology 75: 636-643.

Neervoort, W.J. and J.E. Parlevliet, 1978. Partial resistance of barley to leaf rust, *Puccinia hordei*. V. Analysis of the components of partial resistance in 8 barley cultivars. Euphytica 27: 33-39.

Ohm, H.W. and G.E. Shaner, 1976. Three components of slow rusting at different growth stages in wheat. Phytopathology 66: 1356-1360.

Parlevliet, J.E., 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. I. Effect of cultivar and development stage on latent period. Euphytica 24: 21-27.

Parlevliet, J.E., 1986. Pleiotropic association of infection frequency and latency period of two barley cultivars partially resistant to barley leaf rust. Euphytica 35: 267-272.

Parlevliet, J.E. and H.J. Kuiper, 1977. Partial resistance of barley to leaf rust, *Puccinia hordei*. IV. Effect of cultivar and development stage on infection frequency. Euphytica 26: 249-255.

Parlevliet, J.E., M. Leyn and A. van Ommeren, 1985. Accumulating polygenes for partial resistance in barley to barley leaf rust, *Puccinia hordei*. II. Field evaluation. Euphytica 34: 15-20.

Parlevliet, J.E. and A. van Ommeren, 1975. Partial resistance of barley to barley leaf rust, *Puccinia hordei*. II. Relationship between field trials, micro-plot tests and latent period. Euphytica 24: 293-303.

Pretorius, Z.A., F.H.J. Rijkenberg and R.D. Wilcoxson, 1988. Effect of growth stage leaf position effects and temperature on adult-plant resistance of wheat infected by *Puccinia recondita* f.sp. *tritici*. Plant Pathology 37: 36-44.

Shaner, G.E., H.W. Ohm and R.E. Finney, 1978. Response of susceptible and slow leaf rusting wheats to infection by *Puccinia recondita*. Phytopathology 68: 471-475.

Teng, P.S., M.J. Blackie and R.C. Close, 1977. A simulation analysis of crop yield loss due to rust disease. Agricultural systems 2: 189-198.

Zadoks, J.C., 1971. System analysis and the dynamics of epidemics. Phytopathology 61: 600-610.

Zadoks, J.C., T. Chang and C. Konzak, 1974. A decimal code for the growth stages of cereals. Weed Research 14: 415-421.

Chapter 11.

PARTIAL RESISTANCE TO WHEAT LEAF RUST IN 18 SPRING WHEAT CULTIVARS

L.H.M. Broers

SUMMARY

Eighteen spring wheat cultivars were tested in microfields and race nurseries for their partial resistance to wheat leaf rust under low and high disease pressure respectively. Large differences existed between the 18 cultivars, Skalavatis 56 being the most susceptible and Ponta Grossa 1 being the most resistant cultivar. Of the three epidemic parameters, disease severity (DS) at the time that the susceptible check was severely diseased, area under the transformed disease severity curve (AUTC) and the logistic growth rate (r), AUTC and DS were highly correlated. Both seemed to be reliable estimators of PR but DS should be preferred for economical reasons. The logistic growth rate seemed to be unsuitable as an estimator of partial resistance.

High and low disease pressure gave similar cultivar ranking. PR can be screened and selected equally well in race nurseries with low space, low time and low cost input as in microfields with high space, time and cost input.

Cultivar differences in development rate had a large impact on the cultivar differences in disease level and can therefore greatly bias the estimation of cultivar resistance. The resistance of early cultivars tended to be underestimated whereas the resistance of late cultivars tended to be overestimated. The effect of differences in developmental rate was most pronounced in the flag leaf. It is advisable to avoid the assessment of disease levels on the flag leaf only and to incorporate in the tests several susceptible and resistant checks that cover the range of development rates in the material to be selected, because otherwise selection for resistance will tend to select also for lateness.

Regression of the epidemiological parameters on three components of partial resistance revealed that latency period (LP) is an important factor in determining the resistance observed in the field explaining on average 67% of the observed variation. Adding infection frequency (IF) and urediosorus size (US) to the linear model increased the proportion of the observed variation in the field explained by the components to 80%. This result supports the idea that the components of PR inherit independently, at least, in part.

INTRODUCTION

The value of resistance in crops depends on its level, its stability (reference to geographical and environmental conditions) and its durability (reference to time). In wheat (*Triticum aestivum*), breeders have achieved high levels of resistance to wheat leaf rust (*Puccinia recondita* f.sp. *tritici*, syn. *Pucc. triticina*) using monogenic, hypersensitivity resistance (HR). HR has frequently been defeated by new races of the rust fungus. Currently, more attention is drawn to other forms of resistance, such as partial resistance (PR), which are hoped to be more durable than HR.

PR is characterized by a slow epidemic build-up despite a high infection type indicating a compatible host-pathogen interaction (Parlevliet and van Ommeren, 1975). In wheat, several studies have been conducted to characterize PR to wheat leaf rust (Ohm and Shaner, 1976; Shaner *et al.*, 1978) but only a few genotypes were used in these studies. The relation between components such as latency period (LP), infection frequency (IF) and urediosorus size (US) on one side, and epidemic parameters on the other is not known. In the barley/barley leaf rust system, LP is the most important component of PR and explains most of the variation for PR in the field (Parlevliet *et al.*, 1985; Parlevliet and van Ommeren, 1975).

The aim of this study was to characterize the PR of 18 spring wheat genotypes under

high and low disease pressures. Comparisons of epidemiological parameters to the three components of PR described in an earlier report (Broers, 1989a) were made to study the relation between the performance of PR in the field and components of PR.

MATERIAL AND METHODS

Eighteen spring wheat cultivars obtained from the Department of Plant Breeding, Wageningen Agricultural University, were selected from preliminary field trials because they differed in level of PR. Skalavatis 56 was the susceptible and Akabozu the partially resistant check. In all experiments the wheat leaf rust race 'Felix' was used (Broers, 1989b) obtained from the Department of Phytopathology, Wageningen Agricultural University.

Two field experiments were conducted. The basic design of Exp I was the race nursery (Broers, 1987; Zadoks, 1963) which consisted of a spreader row (Little Club) with a row of 18 clumps on both sides. Each clump (20 cm diameter) in a row represented one cultivar. The heart to heart distance of adjacent clumps was 40 cm.

Three race nurseries were planted at the university farm in East Flevoland in a large field of winter rye at least 30 meters apart. The cultivars of row 1 of each nursery were planted in the same order. Those of row 2, at the other side of the spreader row were planted in the reverse order. Randomization was not carried out since it seemed to be unnecessary (Broers, 1987). In this way a 'tough' test was obtained for PR to wheat leaf rust because of the high proportion of alloinfection reaching the cultivars from the spreader row ('tough' test; Zadoks and Schein, 1979).

The second experiment (Exp II) consisted of 54 isolated microfields (Zadoks, 1972) arranged according to a randomized block design with three replications in a large field of winter rye. Microfields were separated by at least 10 m of rye. Each microfield measured 2.5 x 2.5 m of which 1.5 x 1.5 m was occupied by spring wheat. Within replications wheat genotypes were assigned randomly to the microfields. The isolation of the individual microfields by distance and by the tall winter rye reduced intermicrofield interference to a minimum. In this way PR was measured with reduced alloinfection ('soft' test; Zadoks and Schein, 1979).

In both experiments the epidemic was started with sporulating spreader plants. On May 10th, 20 plants were placed in each race nursery and two plants in each microfield. Ten days later the spreader plants were removed from the microfields.

The level of wheat leaf rust was assessed on June 30th in microfields, and on July 6th, 10th, 16th and 22th for both experiments. In the race nurseries, disease severity (DS) was assessed by estimating the percentage of affected leaf surface on the upper three leaves, using the Peterson scale (0-100%)(Peterson *et al.*, 1948). DS was subjected to a logistic transformation:
trans(DS) = logit(DS) + 10 =
ln(DS/100-DS) + 10.

Ten was added to obtain positive numbers.

From all microfields 15 tillers were sampled on each assessment date. DS was assessed by counting the number of wheat leaf rust pustules on each of the upper three leaves using a 7x pocket-lens. These numbers were transformed according to the scale of Parlevliet and van Ommeren (1984), which shows a logarithmic progression. After transformation, the data were corrected for differences in leaf area.

Heading date and infection type were recorded for all cultivars.

Analysis of both experiments was performed with the transformed disease severity data. The area under the trans-

Table 11-1. Severity of wheat leaf rust on the upper three leaves on five assessment dates, area under the transformed disease severity curve (AUTC), epidemic growth rate and heading date of 18 spring wheat cultivars. Severity was expressed in number of sori and transformed according to the assessment key of Parlevliet and van Ommeren (1984) for microfields or expressed in percentage of leaf area affected according to the Peterson Scale (Peterson *et al.*, 1948) and transformed to logits and adding 10 for race nurseries. Heading date is expressed in number of days after the heading date of Skalavatis 56 (18-6-1987).

Cultivar	Microfields							Race nurseries						heading
	Assessment date					AUTC	r	Assessment date				AUTC	r	
	30-6	6-7	10-7	16-7	22-7			6-7	10-7	16-7	22-7			
Skalavatis 56	10.7	11.4	12.1	13.4	-	190	0.23	9.2	10.5	13.0	16.9	200	0.48	0
Ralle	8.6	10.3	11.2	12.4	-	171	0.24	6.9	8.0	9.9	12.9	152	0.37	9
Little Club	8.2	10.1	11.4	12.3	-	169	0.26	7.7	9.3	9.7	16.3	168	0.49	14
Sicco	7.6	9.9	10.6	11.8	12.5	161	0.26	6.2	7.3	8.7	9.5	130	0.21	12
Purple Justin	8.2	9.3	10.2	11.4	-	156	0.20	6.3	7.1	8.6	10.1	130	0.24	12
Fr45/73-2	7.5	8.9	10.0	12.3	-	154	0.30	5.7	7.3	9.6	10.9	138	0.33	10
Fundus	7.5	9.3	9.9	10.8	12.1	151	0.20	5.7	6.5	7.5	8.8	116	0.19	11
RPB_2-297-76E	7.0	9.1	9.4	11.1	11.5	147	0.25	6.3	7.2	8.5	9.4	128	0.19	13
Minaret	5.9	8.5	9.2	11.0	11.7	139	0.31	5.0	6.3	8.0	8.9	116	0.25	9
Ze-64-1-14	4.0	6.4	7.7	9.7	10.3	112	0.35	4.8	6.2	8.1	8.9	116	0.26	8
Jufy 1	4.2	6.3	7.2	8.9	9.9	107	0.29	5.1	6.2	8.2	10.0	121	0.31	12
Akabozu	4.1	6.0	6.6	8.2	9.2	100	0.25	2.9	4.5	7.2	8.7	97	0.36	12
Westphal 12A	3.4	5.6	6.1	7.4	9.1	91	0.24	3.5	5.5	7.0	9.1	104	0.34	5
Van Hoek	2.2	4.4	6.0	7.9	9.5	82	0.36	2.7	5.7	8.9	10.1	117	0.46	21
Kenphad	3.7	3.8	5.6	6.2	6.4	77	0.17	2.3	3.0	5.9	8.3	80	0.39	6
BH 1146	1.9	3.1	4.8	5.7	6.2	62	0.25	1.8	3.0	5.2	6.8	70	0.32	4
Turano	2.4	2.7	3.3	4.3	-	50	0.09	1.9	2.5	3.2	4.4	49	0.15	10
Ponta grossa 1	0.8	0.7	1.4	1.4	1.7	17	0.05	1.8	1.8	1.8	1.8	29	0.00	12
mean	5.4	7.0	7.9	9.2	-	119	0.24	4.8	6.0	7.7	9.5	114	0.29	
LSD 5%	1.7	2.0	1.6	1.5	1.8	24	0.10	1.2	1.0	1.1	0.6	16	0.09	

formed DS curve (AUTC) was calculated according to

$$AUTC = \sum_{i=2}^{k} (x_i + x_{i-1})/(t_i - t_{i-1})$$

with k = the total number of observation days, t_i = day i expressed as number of days after sowing, and x_i = the transformed DS on day i. The epidemic growth rate was obtained by linear regression of the transformed DS on time. The regression coefficients were considered to be analogous to the apparent or logistic growth rate described by Van der Plank (1963).

RESULTS

The analysis of variance of the disease severity of the sequential observations revealed that the linear component of the time factor was significant, whereas components of higher order were not significant. This indicates that the used transformations were satisfactory in obtaining additive data.

Infection types indicated that only one cultivar, Turano, showed a clear hypersensitive response to the wheat leaf rust race used in the experiments. On other cultivars, the infection type was high indicating compatible cultivar/race combinations. At later assessment dates infection types tended to

be somewhat lower. This is probably related to the ageing of pustules as evidenced by chlorotic and sometimes even necrotic flecks.

Epidemiological parameters

Analysis of the disease severities revealed large cultivar effects on the wheat leaf rust epidemics in both microfields and race nurseries on all assessment dates. Skalavatis 56 was the most susceptible cultivar and Ponta Grossa 1 was the most resistant cultivar in all cases (Table 11-1).

Generally, the level of wheat leaf rust was higher in the race nurseries than in the microfields ('tough' versus 'soft' testing). On the July 16th, for instance, the average level of wheat leaf rust was 9.2% (logistic transformation and adding 10 gives 7.7; Table 11-1) in the race nurseries whereas in the microfields only about 37 pustules per tiller (9.2 on the scale of Parlevliet and van Ommeren (1984); Table 11-1) were found which is less than 1%. This was to be expected as the cultivars in the race nurseries were continuously subjected to high levels of inoculum produced by the susceptible spreader rows (high alloinfection).

Differences between cultivars were generally larger in the microfields than in the race nurseries. On July 16th, Little Club had 17 times (a difference of 4.1 on the scale of Parlevliet and van Ommeren, 1984) more pustules than Akabozu in the microfields. In the race nurseries, Little Club had only 7.5 times (trans(DS) = 9.7 is equal to 43% for Little Club and trans(DS) = 7.2 is equal to 5.7% for Akabozu) more pustules than Akabozu. This indicates that partial resistance is better expressed in microfields than in race nurseries.

A large cultivar effect was also detected for AUTC. In both experiments Skalavatis 56 had the largest and Ponta Grossa 1 had the smallest AUTC (Table 11-1).

The analysis of variance of the sequential observations revealed a significant cultivar/time interaction, but only the linear component was of importance. This linear component represents the growth rate of the epidemic for the different cultivars. Its significance means that cultivars differed in their epidemic growth rate. Growth rate was less discriminating than disease severity

Table 11-2. Pearson's linear correlation coefficients between three epidemiological parameters in microfields and in race nurseries.

	Microfield[1)]			Race nurseries		
	DS	AUTC	r	DS	AUTC	r
Microfields						
DS						
AUTC	0.98^{***} [2)]					
r	0.58^{*}	0.44				
Race nurseries						
DS	0.92^{***}	0.89^{***}	0.65^{**}			
AUTC	0.93^{***}	0.93^{***}	0.56^{*}	0.98^{***}		
r	0.46	0.40	0.56^{*}	0.67^{**}	0.64^{**}	

1) DS is disease severity on July 16th; AUTC is area under the transformed disease severity curve; r is epidemic growth rate.

2)*** = significant at P<0.001; **= significant at P<0.01; *= significant at P<0.05.

and AUTC, especially in the microfields in which only four cultivars (Ze-64-1-14, Van Hoek, Turano and Ponta Grossa) had a growth rate different from Skalavatis 56 (Table 11-1).

Comparison of epidemiological parameters

The epidemiological parameters disease severity, AUTC and growth rate were compared using Pearson's linear correlation coefficient (Table 11-2). The correlation between disease severity and AUTC was high and significant ($P<0.001$). The relative low correlation coefficients between epidemic growth rate and the other two parameters suggested an interaction between cultivar and epidemiological parameter. In the microfields, for example, Van Hoek had a high growth rate but a low disease severity and a small AUTC whereas Skalavatis 56 had a low growth rate but a high disease severity and a large AUTC (Table 11-1).

The two experimental designs give largely similar results, the correlation coefficients with disease severity and AUTC were high and significant (Table 11-2).

The epidemic growth rate growth should not be used as a measure for PR of wheat to wheat leaf rust as the correlation coefficients related to this parameter were low within and across the experiments.

Effect of development rate on the epidemic

The cultivar differences observed for the epidemic parameters are influenced by differences in development rate. In Figure 11-1 and Figure 11-2, the disease progress curve of Skalavatis 56, Little Club, Van Hoek

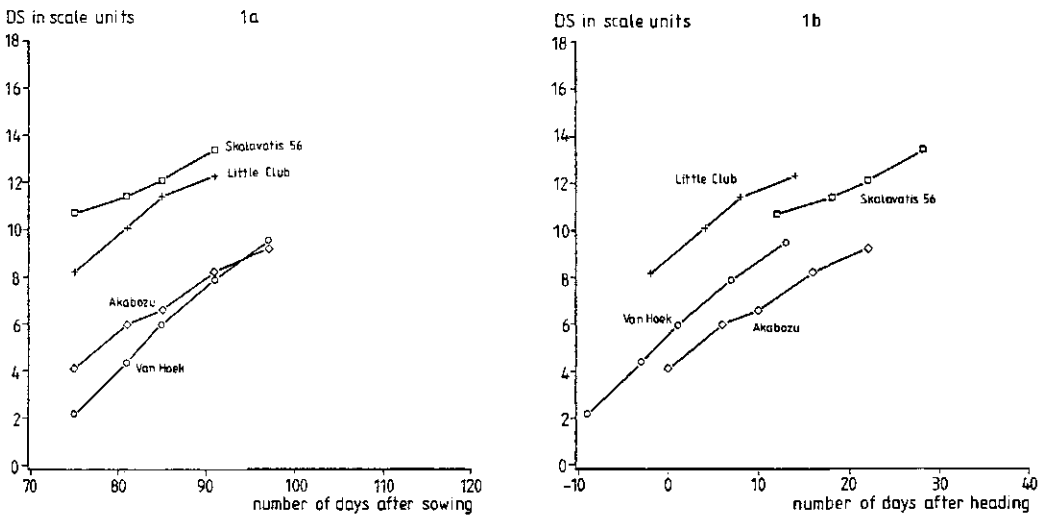


Figure 11-1. Disease progress curves of four spring wheat cultivars in microfields after plotting disease severity (DS) against number of days after sowing (1a) and against the number of days after heading of the respective cultivars (1b). DS is expressed in number of pustules per tiller and transformed according to the scale of Parlevliet and van Ommeren (1984).

and Akabozu are plotted in two different ways for microfields and race nurseries respectively.

1) The x-axis represents the number of days after sowing and the sequential disease assessments accordingly coincide (Figure 11-1a and 11-2a).

2) The x-axis represents the number of days after heading. Assessment dates are related to the heading dates of the respective cultivars (Figure 11-1b and 11-2b).

The effect of the second way of expressing the data is that the disease progress curves were shifted with respect to each other because the four cultivars differed markedly in heading date (Table 11-1). The susceptibility expressed as disease severity on a certain assessment date leads to cultivar differences and a cultivar ranking different from those obtained by susceptibility expressed as disease severity on a certain number of days after heading. In both microfields and race nurseries, Skalavatis 56 was more susceptible than Little Club when disease was assessed on the same number of days after sowing. On the same number of days after heading, however, Little Club was the more susceptible cultivar (Figure 11-1b and 11-2b). A similar observation was made for Akabozu and Van Hoek. The results indicate that neither of both assessment methods are useful to compare the resistance of cultivars that differ largely in heading date.

The correlation between heading date as a measure of development rate and the difference between disease severity on the flag (F) leaf and the F-2 leaf was 0.51 (significant at P=0.05). This indicates that a leaf layer/cultivar interaction exists for disease severity, which is partly depending on the heading date. Statistical analysis showed that the very early-heading Skalavatis 56 and the very late-heading Van Hoek caused

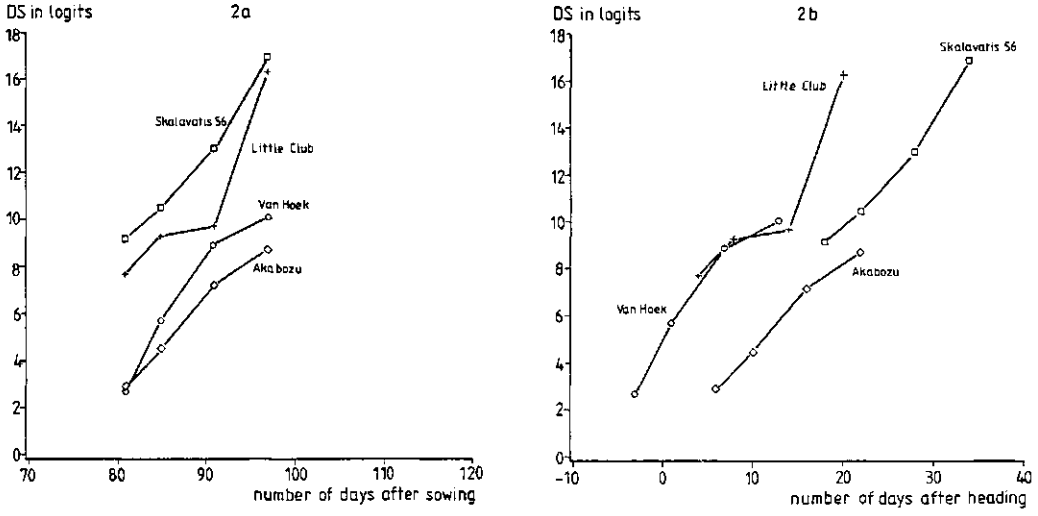


Figure 11-2. Disease progress curves of four spring wheat cultivars in race nurseries after plotting disease severity (DS) against number of days after sowing (2a) and against the number of days after heading of the respective cultivars (2b). DS is expressed in percentage leaf area affected according to the Peterson Scale (Peterson *et al.*, 1948) and transformed to trans(DS) (=logit(DS) + 10).

the largest part of this interaction. Table 11-3 shows that the disease severities of Skalavatis 56 and Van Hoek on the flag leaf differ greatly while at the F-2 leaf the differences are far smaller. The resistance of early cultivars is underestimated and that of late cultivars overestimated in comparison to the other cultivars. The effect of development rate seems to be most pronounced in the flag leaf (Table 11-3).

Differences in development rate were of less importance in the race nurseries than in the microfields. This is seen by the late-heading Van Hoek. In the order from resistant to susceptible on July 16th, Van Hoek ranked 6th in the microfields whereas Van Hoek ranked 14th in the race nurseries. The ranking of Van Hoek in microfields and race nurseries increased with time indicating that the influence of differences in development rate reduced with the progress of the epidemic.

Table 11-3. Severity of wheat leaf rust on the flag leaf (F) and the F-2 leaf, the difference in severity between the F and F-2 leaf (diff) and the heading date expressed as number of days after the heading date of Skalavatis 56 for four spring wheat cultivars. Severity was expressed in number of pustules transformed according to the scale of Parlevliet and van Ommeren (1984).

Genotype	F	F-2	diff	heading
Skalavatis 56	11.3	12.1	0.8	0
Little Club	8.8	12.0	3.2	14
Akabozu	4.7	7.4	2.7	12
Van Hoek	2.8	7.1	4.3	21

Correlation between components of PR and PR measured in the field

The same 18 wheat cultivars were used for a component analysis in seedling stage and adult plant stage (Broers, 1989a). These data have been used to compare three components of PR with the epidemiological parameters of the microfields and the race

Table 11-4. Pearson's linear correlation coefficient between three epidemiological parameters and three components of partial resistance measured in two growth stages in the greenhouse.

	Seedling stage			Adult plant stage		
	LP	IF	US	LP	IF	US
Microfields						
DS1)	- 0.87$^{***2)}$	0.70**	0.44	- 0.84***	0.76***	0.63*
AUTC	- 0.83***	0.65*	0.39	- 0.84***	0.71**	0.65**
r	- 0.43	0.54*	0.47	- 0.48	0.74**	0.33
Race nurseries						
DS	- 0.82***	0.67**	0.47	- 0.78***	0.81***	0.60*
AUTC	- 0.81***	0.69**	0.48	- 0.81***	0.84***	0.70**
r	0.38	- 0.28	- 0.11	- 0.03	0.52*	0.05

1) D.S. is disease severity on July 16th; AUTC is area under the transformed disease severity curve; r is epidemic growth rate; LP is latency period; IF is infection frequency; US is urediosorus size.

2)*= significant at P<0.05; **= significant at P<0.01;*** = significant at P<0.001.

Table 11-5. Explained variation (R^2) of disease severity (DS) and area under transformed disease severity curve (AUTC) of microfields and race nurseries by one, two or three components of partial resistance.

	Explained variation (R^2) by[1)]				
	LP	IF	US	LP+IF	LP+IF+US
Microfield					
DS	0.71	0.59	0.40	0.78	0.83
AUTC	0.70	0.50	0.42	0.71	0.77
Race nurseries					
DS	0.61	0.66	0.36	0.77	0.80
AUTC	0.66	0.70	0.49	0.79	0.81
Mean	0.67	0.61	0.42	0.76	0.80

1) LP is latency period; IF is infection frequency; US is pustule size.

nurseries by means of linear correlation (Table 11-4). Turano was excluded beause of its low infection type, Skalavatis 56 and Van Hoek were excluded because they deviated too much in development from the other cultivars. Latency periods measured on the flag leaf and on the primary leaf gave the best correlations with disease severity and AUTC of both microfields and race nurseries. The epidemic growth rate (*r*) showed low correlations with the components of PR confirming its unsuitability as an estimator of PR of wheat to wheat leaf rust.

The values of the correlation coefficients suggested that individual components could not fully explain the variation found for the epidemiological parameters. On average, LP, IF and US explained 67%, 61% and 42% of the observed variation (Table 11-5). A multiple regression analysis was performed with AUTC and disease severity of both experiments. Linear combinations of the LP and IF gave a 1 to 11 % better explanation of the observed variation for the epidemiological parameters than the most explanatory individual component, whereas linear combinations of LP, US and IF gave a 7 to 14 % better explanation (Table 11-5). The results suggest that the components are independently inherited, at least in part, and that the combined effects of the components determine to a large extent the performance of PR in the field.

DISCUSSION

Epidemiological parameters

Microfield and race nursery designs are two different designs which represent to a certain extent the large field situation of commercial wheat production and the small-plot situation of a breeder's field, respectively. It is important to know whether the results of both designs are comparable and which epidemiological parameter should be used. The results indicated that both designs gave similar results with regard to AUTC and DS assessed on July 16th. This means that selection for PR to wheat leaf rust can be done under high disease pressure. To assess differences in susceptibility it is sufficient to assess DS once during the epidemic. The single disease reading to obtain DS should be carried out at a moment

that the susceptible checks in a breeders plot are severely (75 to 95% on the Peterson Scale) attacked by wheat leaf rust. Assessing DS needs less computation and is less time consuming than assessing AUTC and epidemic growth rate which both need several disease readings throughout the epidemic and more computation. Moreover, the epidemic growth rate was a poor estimator of PR as it was less discriminating than the other parameters and showed low correlations within and across experimental designs. Both the logistic transformation used for race nursery data and the scale of Parlevliet and van Ommeren make the assumptions that (1) the organisms are distributed uniformly in space, (2) the environment is uniform in space and constant in time, (3) all wheat genotypes react in the same way at any given time regardless of development stage and (4) the growth rate of the rust population declines linearly with population size. At least some of these assumptions are violated and, therefore, epidemic growth rate based on these models is of restricted value as parameter for estimating PR. This agrees with the data of Rees *et al.* (1979), which indicate that the ranking according to disease severity and area under the disease progress curve show large similarities but both differed largely from the ranking according to the logistic growth rate.

The level of attack by wheat leaf rust in the microfields was much lower than in the race nurseries. This was expected as the cultivars in the race nurseries were continuously subjected to large spore loads from the spreader rows. It was also expected that cultivar differences would be much larger in the microfields than in the race nurseries as alloinfection and interplot interference in the race nurseries will lead to underestimation of resistance (Zadoks and Schein, 1979). The cultivar differences in the 2.25 m^2 microfields were indeed larger than in the race nurseries, but the effect of different epidemiological conditions was not as clear as with barley and barley leaf rust. The difference between the susceptible barley cultivar L94 and the partially resistant barley cultivar Vada observed in 12 m^2 microfields increased a 100-fold compared to the difference in adjacent small plots (Parlevliet and van Ommeren, 1975). This indicates that either PR of wheat to wheat leaf rust is less sensitive to differences in disease pressure or that the small differences in microfields are due to an artefact or that the effect is a combination if both. The artefact probably has two reasons.

1) The data of the microplots have not been corrected for differences in urediosorus size. The resistance of partially resistant cultivars is underestimated as they have smaller urediosori than susceptible cultivars.

2) In small microplots, the susceptibility of susceptible cultivars is underestimated compared to resistant cultivars. This could be due to the fact that the smaller the plot is, the larger is the proportion of inoculum produced by that plot that will be dispersed beyond the boundaries of the plot, leaving less inoculum for epidemic build-up (Paysour and Fry, 1983). The data of Bowen *et al.* (1984) seem to support this idea as they found higher disease severities in 16 m^2 plots than in 4 m^2 plots.

The combined effect is that the cultivar differences in the microfield are underestimated and that cultivar differences increased only slightly as compared to race nurseries in which differences were underestimated due to alloinfection.

Effect of development rate on the epidemic

The cultivar differences in values of the epidemic parameters, should be interpreted with caution as cultivar differences in development rate may influence the epidemics

of the respective cultivars. The sequential observations described above were related to different development stages according to the earliness or lateness of the cultivars. Therefore, two factors have to be considered to understand the influence of the development rate and the epidemic:

1) only the upper three leaves were included in the observations, and those of early cultivars had been exposed longer to the epidemic at any assessment date than those of late cultivars which had appeared later. This leads to an overestimation of susceptibility.

2) as in early cultivars the upper three leaves appeared earlier than in late cultivars, they were subjected to other infection conditions. Epidemic build-up in early cultivars took place under cooler conditions that increased latency period, and with lower levels of inoculum from the lower leaves, thus leading to underestimation of susceptibility.

The magnitude of both opposing factors is not known, but the results indicate that factor 1 is of greater importance when data obtained at one assessment date are compared. The net result was an overestimation of the resistance of late cultivars and of the susceptibility of early cultivars. When DS values at any one development stage are compared, factor 2 seems to be of greater importance. Susceptibility of early cultivars and resistance of late cultivars were underestimated. According to greenhouse observations (Broers, 1989a), Little Club and Skalavatis are equally susceptible and no differences are to be expected for epidemic parameters in the field. At the same assessment dates, Skalavatis 56 was always more diseased than Little Club. This is due to the influence of factor 1. At similar growth stages, Little Club was more diseased than Skalavatis 56, due to the influence of factor 2 (Figure 11-1, Figure 11-2). Akabozu and Van Hoek differ greatly in resistance according to greenhouse observations but in the field almost no differences were observed at the various observation dates. However, at any development stage van Hoek had much more disease than Akabozu.

Obviously, differences in development rate may cause a misinterpretation of the data obtained in field experiments. It is recommended to include in breeders' plots several susceptible and resistant checks, that cover the range of development rates in these plots so that comparisons and selection can be done within groups of the same development rate.

The results indicated that the effect of development rate on the epidemic is most pronounced on the flag leaf. This was to be expected as the variance of leaf ages across cultivars was larger for flag leafs than for other leafs due to the shorter average leaf age. Assessment of cultivar differences in resistance based on the flag leaf only is, therefore, not recommended.

Correlation between components of PR and PR measured in the field

The correlation coefficients between components of PR and epidemic parameters indicated that LP is a very important factor in determining the epidemic. This is in agreement with the results of Teng *et al.* (1977) and Zadoks (1971), who found in simulated epidemics that small changes in LP had a large impact on the epidemics.

Broers (1989a) suggested that the components LP, IF and US might possibly inherit independently as their correlation coefficients indicated that they were not completely associated. The results obtained here support this idea as individual components explained at most 71% of the observed variation in the field, whereas a combination of components in a multiple regression resulted in a better explanation of the observed resistance in the field. The

independence of the components is largely hidden because selection either under natural conditions or in the breeder's field will tend to select for plants with long LP and low IF and small US. In a sample of cultivars, small exceptions on this rule may exist which will give a less than complete association of the components. A clear example of such an exception is Ponta Grossa 1. Its disease severity was too high in relation to its IF but too low in relation to its LP. After combining LP and IF in a multiple regression model, the disease severity of Ponta Grossa 1 could be explained satisfactory.

The conclusion of this study is that selection of PR can be done under conditions of high disease pressure as in small adjacent plots or race nurseries and also under conditions of low disease pressure as in microfields using either DS or AUTC as an estimator for PR. Small adjacent plots and race nurseries in combination with DS is the least time and space consuming and therefore the least expensive alternative.

REFERENCES

Bowen, K.L., Teng, P.S. and Roelfs, A.P., 1984. Negative plot interference in field experiments with leaf rust of wheat. Phytopathology 74: 1157-1161.

Broers, L.H.M., 1987. Relevance of randomizing host genotypes of wheat that are tested for leaf rust resistance in race nurseries. Euphytica 36: 257-263.

Broers, L.H.M., 1989a. Influence of development stage and host genotype on three components of partial resistance to wheat leaf rust in spring wheat. Euphytica, in press.

Broers, L.H.M., 1989b. Race-specific aspects of partial resistance of wheat to wheat leaf rust. Euphytica, in press.

Ohm, H.W. and G.E. Shaner, 1976. Three components of slow rusting at different growth stages in wheat. Phytopathology 66: 1356-1360.

Parlevliet. J.E., M. Leyn and A van Ommeren, 1985. Accumulating genes for partial resistance in barley to barley leaf rust, *Puccinia hordei*. II. Field evaluation. Euphytica 34: 15-20.

Parlevliet, J.E. and A. van Ommeren, 1975. Partial resistance of barley to barley leaf rust, *Puccinia hordei*. II. Relationship between field trials, micro-plot tests and latent period. Euphytica 24: 293-303.

Parlevliet, J.E. and A. van Ommeren, 1984. Interplot interference and the assessment of barley cultivars for partial resistance to leaf rust, *Puccinia hordei*. Euphytica 33: 685-697.

Paysour, R. E. and Fry, W.E., 1983. Interplot interference: a model for planning field experiments with aerially disseminated pathogens. Phytopathology 73: 1014-1020.

Peterson, R.F., A.B. Campbell, and A.E. Hannah, 1948. A diagrammatic scale of estimating rust intensity on leaves and stems of cereals. Can J Res C26: 496-500.

Rees, R.G., J.P. Thompson and E.A. Goward, 1979. Slow rusting and tolerance to rusts in Wheat. II The progress and effects of epidemics in *Puccinia recondita tritici* in selected wheat cultivars. Australian Journal of Agricultural Research 30: 421-432.

Shaner, G.E., H.W. Ohm and R.E. Finney, 1978. Response of susceptible and slow leaf rusting wheats to infection by *Puccinia recondita*. Phytopathology 68: 471-475.

Teng, P.S., M.J. Blackie and R.C. Close, 1977. A simulation analysis of crop yield loss due to rust disease. Agricultural Systems 2: 189-198.

Van der Plank, J.E., 1963. Plant diseases: Epidemics and Control. Academic Press, New York. 349pp.

Zadoks, J.C., 1963. The use of race nurseries in cereal resistance breeding. Proc 1st Int Barley Genetics Symp, Wageningen. Pp. 242-249.

Zadoks, J.C., 1971. Systems analysis and the dynamics of epidemics. Phytopathology 61: 600-610.

Zadoks, J.C., 1972. Reflections on disease resistance in annual crops. In: Biology of rust resistance in forest trees. Eds. R.T. Bingham, *et al.* US Dept Agr Forest Serv, Misc Publ No. 1221: Pp.43-63.

Zadoks, J.C. and R.D. Schein, 1979. Epidemiology and plant disease management. Oxford University Press, New York and Oxford. 427pp.

Chapter 12.

RACE-SPECIFIC ASPECTS OF PARTIAL RESISTANCE IN WHEAT TO WHEAT LEAF RUST, *PUCCINIA RECONDITA* F.SP. *TRITICI*.

L.H.M. Broers

SUMMARY

Partial resistance (PR) in wheat to wheat leaf rust is characterized by a slow epidemic build-up despite of a susceptible infection type. Two greenhouse tests and two field tests, in which 11 spring wheat cultivars were exposed to five wheat leaf rust races, revealed some indication for race-specificity of PR.

In the greenhouse, the expression of PR was highly dependent on the environment. Significant cultivar-race interactions in the first experiment were not observed in the second experiment probably due to cultivar-environment and cultivar-race-environment interactions.

In the polycyclic field tests several factors played a role in explaining the inconsistency of the cultivar-race interactions, such as differences in initial inoculum, genotypic differences in earliness, interplot interference and environmental conditions.

One cultivar-race combination showed a significant but small interaction towards susceptibility in both field experiments. The interaction was probably too small to be detected in the monocyclic greenhouse tests. The results do not conflict with the idea that a gene-for-gene relationship could exist between PR-genes in the host and genes in the pathogen.

Some problems with regard to the selection of PR in wheat to wheat leaf rust are discussed.

INTRODUCTION

Wheat leaf rust (*Puccinia recondita* f.sp. *tritici*, syn. *Pucc. triticina*) is one of the most important diseases of wheat (*Triticum aestivum*) (Samborski, 1985). Cultivars are often protected by monogenic hypersensitivity resistance. However, the pathogen is able to defeat these genes easily, by producing matching races. Alternative forms of resistance may be more durable. Partial resistance (PR) is such a form. It is characterized by a reduced epidemic build-up despite a high, susceptible infection type (Parlevliet and van Ommeren, 1975). A longer latency period, lower infection frequency, smaller spore production and shorter infectious period are assumed to be associated with PR, latency period being the most important component (Kulkarni *et al.*, 1982; Parlevliet and van Ommeren, 1975; Shaner and Finney, 1980; Teng *et al.*, 1977).

In wheat, PR is found in several spring and winter wheat cultivars and it is oligogenically inherited (Broers and Jacobs, 1989; Lee and Shaner, 1985). Assuming a gene-for-gene relationship with respect to PR, differential interactions between cultivars and races will be less easily detected in the case of PR than in the case of hypersensitivity resistance, due to the quantitative expression of PR. In the case of barley/barley leaf rust, however, Parlevliet (1977) observed a significant and reproducible but small differential interaction for PR. In the data of Kuhn *et al.* (1978) one can identify a small differential interaction for the latency period of wheat leaf rust on wheat seedlings.

The aim of this study is to search for differential interactions in PR of wheat to wheat leaf rust in monocyclic and polycyclic experiments.

MATERIALS AND METHODS

The two greenhouse and the two field experiments described hereafter comprised 11 spring wheat cultivars (Table 12-1). The levels of PR in Table 12-1 refer to adult plants inoculated with the 'Flamingo' race (Broers, 1987; Broers, 1989).

The five Dutch wheat leaf rust isolates used in all experiments were called 'Felix', 'Flamingo', 'Okapi', 'Neutral' and 'Arminda'. The former three isolates were obtained

Table 12-1. Origin and level of partial resistance (PR) of 11 spring wheat cultivars.

Wheat cultivar	Origin	Level of PR
Little Club	Cultivar, USA	very low
Skalavatis 56	Line from a landrace, Cyprus	very low
Skalavatis 59	Line from a landrace, Cyprus	low
Skalavatis 61	Line from a landrace, Cyprus	low
Kaspar	Cultivar, The Netherlands	moderate
Adonis	Cultivar, The Netherlands	moderate
Ze-64-1-14	Homozygous line, The Netherlands	moderate
Van Hoek	Cultivar, The Netherlands	moderate
Westphal 12A	Line from a landrace, Ethiopia	fair
Akabozu	Cultivar, Japan	fairly high
BH 1146	Cultivar, Brazil	high

from the Department of Phytopathology, Wageningen Agricultural University, whereas the latter two were provided by the Institute of Plant Protection (IPO), Wageningen. Virulence tests performed with monospore cultures on seedlings of near-isogeneic lines of Thatcher carrying different wheat leaf rust resistance genes confirmed that the isolates represented five different races (Table 12-2).

Greenhouse experiments

The first greenhouse experiment (Exp. I) comprised the cultivars Skalavatis 56, Little Club, Adonis, Kaspar, Westphal 12A and Akabozu. It was carried out in the autumn of 1985. In the second greenhouse experiment (Exp. II), performed in the late spring of 1986, the cultivars Kaspar and Adonis have been replaced by Skalavatis 59 and BH 1146.

All cultivars were sown at several consecutive sowing dates in order to obtain plants at the same development stage (DC 48-59 on the scale of Zadoks *et al.* (1974) = ears just emerged and young but fully expanded flag leaves) at the time of inoculation. Plants were grown at 15-18 °C and at 16 hours of light per day. Inoculation was done in a settling tower (a design after Eyal *et al.*, 1968) using 1.3 mg of spores mixed with 13.0 mg of *Lycopodium* spores each inoculation. This corresponds with ca. 100 spores/cm^2.

Exp. I comprised four sequential replications whereas Exp. II was carried out in three sequential replications. Per cultivar-race combination two pots with each three or four flag leafs were inoculated. After inoculation, the settling tower was cleaned with 96% ethanol. The ethanol was allowed to evaporate for 45 minutes before the next inoculation with another race. After inoculation, the pots were incubated at 100% relative humidity for one night at 20 °C.

Table 12-2. Virulence factors of five Dutch wheat leaf rust isolates when tested against 27 near-isogeneic Thatcher lines[1)] having different hypersensitivity resistance (Lr-) genes in the seedling stage.

Isolates	Virulence factor to Lr-	No. of virulence factors
Felix	2b, 2c,3ka, 10, 11, 12, 13, 14a, 14b, 15, 16, 18, 20, 21, 22, T, Ech, E, Eg	19
Flamingo	2b, 2c, 10, 12, 13, 14a, 14b, 15, 18, 21, 22, 23, T, B, Eg	15
Okapi	2c, 3, 3ka, 3bg, 10, 11, 12, 13, 14a, 14b, 15, 16, 18, 20, 21, 22, 23, T, Ech, B, Eg	21
Neutral	2b, 2c, 10, 11, 12, 13, 14a, 14b, 15, 16, 18, 21, 22, 23, B, Eg	15
Arminda	2b, 2c, 10, 11, 12, 13, 14a, 14b, 16, 18, 20, 21, 22, B, Eg	15

1) Thatcher lines used carried Lr-1, 2a, 2b, 2c, 3, 3ka, 3bg, 10, 11, 12, 13, 14a, 14b, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, T, Ech, B, Eg

Of each inoculated leaf the latency period (LP) was determined by counting the number of visibly sporulating urediniosori every day from the appearance of the first urediosorus until the number did not increase anymore. The time at which 50% of the urediosori were visible was estimated by linear interpolation. The period between this moment and the start of the incubation was called LP.

Analysis of variance was performed according to a split plot design with races as the main factor and cultivars as the split factor. The statistical unit was the average LP of the two pots per replication.

Field experiments

The first field experiment (Exp. III) included six cultivars: Skalavatis 56, Little club, Kaspar, Ze-64-1-14, BH 1146 and Akabozu. It was carried out in 1985 at the university farm in the Flevopolder near Swifterbant. In a large oat field, 15 plots of 7 x 1 m were kept free of oat. The 15 plots were arranged in five rows of three plots separated by 30 m of oat (between the rows) and 15 m of oats (within each row). Each plot contained six microplots, one cultivar to a microplot, arranged in a row at a heart to heart distance of 1.0 m. Each microplot consisted of eight plants planted in a circle with 10 cm between plants. The plants transplanted into the field plots were raised from seeds in 6 cm-jiffypots. After emergence, the seedlings were placed outside until transplanting took place in the last week of April.

Per isolated plot one wheat leaf rust race was introduced by placing two spreader plants in the isolated plot just before they started to sporulate. Each of the five races were introduced into three isolated plots, each race to one row of three plots. Nine days after the introduction, on May 29th, the spreader plants of the 'Flamingo' race were removed from the plots, six days earlier than for the other races because sporulation on these spreader plants appeared to be much heavier than on the other spreader plants.

On all spreader plants the number of urediosori was counted after removal from the plots.

On June 18th, June 25th and July 9th the levels of atack by of wheat leaf rust were assessed in each microplot. For this purpose ten tillers per microplot were sampled at each date. On the upper three leaves of each tiller, including the flag leaf, the number of urediosori was counted using a 7x pocket-lens. These numbers were transformed according to a logistic scale designed by Parlevliet and van Ommeren (1984) and corrected for leaf area differences. The mean of ten tillers was the statistical unit. Development stage, according to Zadoks *et al.*(1974) and infection type according to McNeal *et al.* (1971) were assessed on June 25th.

The second field experiment (Exp. IV) including the cultivars Skalavatis 56, Skalavatis 59, Skalavatis 61, Little Club, Van Hoek, Ze-64-1-14, Westphal 12A, Akabozu and BH 1146, was carried out in 1986 also at the university farm. In a large field of winter rye 145 plots were set out in five groups of 27 plots. The distance between adjacent groups was 40 m. Each group of 27 plots was formed by three blocks of nine plots. Within a block the plots were arranged in three rows of three plots. The distance between adjacent plots in a group was 6 m. The nine cultivars were randomly assigned to the nine fields in each block. The races were randomly assigned to the five groups of 27 fields. Each plot measured 2 x 2 m of which 1 x 1 m was occupied by wheat. The isolation by distance and by the tall rye crop reduced contamination and interplot interference to a very low level. On April 14th the plots were sown at a seed rate of 120 kg/ha. The inoculation was done on May 21st as in

Table 12-3. The latency period in days in the adult plant stage of five wheat cultivars infected with five wheat leaf rust races in two experiments (Exp).

Exp	Cultivar	Race					Mean[1)]
		Okapi	Felix	Arminda	Flamingo	Neutral	
I	Skalavatis 56	11.9	11.9	12.3	12.1	11.8	10.9[a]
	Little Club	10.7	10.9	11.0	10.7	11.4	12.0[b]
	Ze 64-1-14	13.3	12.6	12.9	13.5	12.9	13.0[c]
	Westphal 12A	19.9	20.7	21.8	21.9	24.3	21.7[d]
	Akabozu	22.2	24.3	22.5	23.3	24.7	23.4[e]
	Mean[2)]	15.6[a]	16.1[a]	16.1[a]	16.3[a]	17.0[a]	
II	Skalavatis 56	9.0	8.8	8.8	8.8	8.8	9.1[a]
	Little Club	9.0	9.1	8.9	9.4	9.1	8.9[a]
	Ze 64-1-14	9.5	9.4	9.2	9.4	9.4	9.4[a]
	Westphal 12A	13.0	13.0	13.6	13.2	14.0	13.4[b]
	Akabozu	12.7	13.1	13.6	13.4	13.8	13.3[b]
	Mean[2)]	10.7[a]	10.7[a]	10.8[a]	10.8[a]	11.0[a]	

1) Cultivar means with the same letters do not differ significantly at P=0.05.

2) Race means with the same letters do not differ significantly at P=0.05.

Exp. III, using two spreader plants per plot. On May 28th, the spreader plants were removed from the plots and the number of sori was counted as a measure for the initial inoculum.

On June 24th, July 4th and July 14th the level of wheat leaf rust was assessed in the plots. From each plot 20 tillers were taken randomly and the number of sori on the upper three leaves was counted as in Exp. III. The amount of rust on the flag leaf was also recorded separately. The data were handled as in Exp. III. The development stage and the infection type were recorded on June 24th.

Both field experiments were analyzed according to a split-plot model for each assessment date separately. Within an experiment assessment dates were combined using a split plot model with repeated measures. In Exp. IV, the initial inoculum was used as covariable and the data were corrected for this variable.

All analyses were performed on the VAX-computer of the Wageningen Agricultural University, Wageningen, using a SAS-program.

RESULTS

Greenhouse experiments

Both experiments were analyzed with untransformed LP-values. Logarithmically transformed LP or LP relative to Skalavatis 56 did not change the results of the analysis. In Table 12-3 the LP's of the 25 common cultivar-race combinations are presented, averaged over four (Exp. I) or three (Exp. II) sequential replicates.

The cultivar-race interaction was significant in Exp. I ($P<0.05$) and indicative in Exp. II ($P<0.10$). Though some cultivar-race interactions could be detected in Exp. I, they did not reappear in Exp. II. Accordingly, no

Table 12-4. The difference between observed latency period in days in the adult plant stage and expected latency period if no differential interaction would occur. Five wheat cultivars were infected with five wheat leaf rust races in two experiments (Exp) (data of Table 12-3).

Exp	Cultivar	Race				
		Okapi	Felix	Arminda	Flamingo	Neutral
I	Skalavatis 56	+0.8	+0.1	+0.6	+0.1	-1.6*
	Little Club	+0.6	-0.1	0.3	-0.5	-0.5
	Ze 64-1-14	+1.3*	-0.5	+0.1	+0.6	-1.5*
	Westphal 12A	-1.9*	-1.3*	+0.2	+0.2	+2.8*
	Akabozu	-0.8	+1.6*	-1.2	-0.3	+0.8
II	Skalavatis 56	+0.5	+0.1	-0.2	-0.1	-0.4
	Little Club	+0.1	+0.2	-0.4	-0.5	-0.4
	Ze 64-1-14	+0.4	+0.2	-0.3	0.0	-0.3
	Westphal 12A	-0.4	-0.4	+0.3	-0.1	+0.6
	Akabozu	-0.6	-0.1	+0.5	-0.2	+0.4

*) Significant at P=0.05.

clear, reproducible and significant cultivar-race interaction could be detected in the greenhouse experiments (Table 12-4).

The LP of the five races did not differ significantly although the LP of 'Neutral' seemed to be somewhat longer than the LP of 'Okapi' (Table 12-3).

Cultivars differed significantly ($P<0.001$) in both experiments. The ranking, however, was not consistent (Table 12-3). Ze-64-1-14 and Little Club had a significantly longer LP than Skalavatis 56 in Exp. I but not so in Exp. II. The same was the case with Akabozu relative to Westphal 12A. In Exp. II the differences between cultivars were smaller. The difference between Skalavatis 56 and Akabozu, for instance, decreased from 11.4 days (95% of Skalavatis 56) in Exp. I to 4.3 days 49% of Skalavatis 56) in Exp. II.

Replication effects and cultivar-replication interaction effects were significant in both experiments ($P<0.001$ and $P<0.05$, respectively). The sequential replications can be seen as different environments. Thus the replication effect can be classified as an environment effect and the cultivar-replication interaction as cultivar-environment interaction.

All these facts indicate that the expression of PR of wheat to wheat leaf rust is, at least to a certain extent, dependent on the environment. This impact of environment on the expression of PR could explain part of the cultivar-race interaction variance.

Adonis and Kaspar, included in Exp. I, showed low, resistant infection types with the races 'Okapi', 'Felix' and 'Neutral'. With the virulent races they had some PR as expressed through their LP. Compared to Skalavatis 56 the LP of Adonis and Kaspar was 1.7 and 1.9 days longer, respectively. Skalavatis 59 and BH 1146 in Exp. II did not lead to different conclusions. No cultivar-race interaction was observed and both cultivars formed compatible combinations with all races. The LP of Skalavatis 59 and BH 1146 were 0.6 days and 6.4 days longer than the LP of Skalavatis 56, respectively.

Field experiments

In the 1985 field experiment, the cultivar-race interaction was highly significant ($P<0.001$) on all three assessment dates. However, most of the cultivar-race interac-

Table 12-5. Leaf area affected expressed in sporulating leaf area transformed according to the key of Parlevliet and van Ommeren (1984) on six wheat cultivars for five wheat leaf rust races (Exp. III, third assessment day).

Cultivar	Race					Mean
	Okapi	Felix	Arminda	Flamingo	Neutral	
Skalavatis 56	12.2	11.4	12.9	12.3	11.9	12.1
Little Club	9.9*	10.6*	9.8	11.7	11.5*	10.7**
Kaspar	5.8*	6.2*	10.6*	12.2*	6.8*	11.3**
Ze-64-1-14	8.9	10.0	5.4*	4.9*	10.7	9.8**
Akabozu	8.7	6.9	6.2	7.5	7.9	7.4
BH 1146	5.0	5.3	4.1	5.9	6.4	5.3
LSD 5%						1.1

*) Low infection type.

**)Excluding low infection type data and corrected for race effects.

Table 12-6. Difference between observed and expected (if no differential interaction would occur) leaf area affected on four wheat cultivars for five wheat leaf rust races (Exp. III, third assessment day, data of Table 12-5).

Cultivar	Race				
	Okapi	Felix	Arminda	Flamingo	Neutral
Skalavatis 56	0.0	-0.6	+2.4*	-0.4	-1.3
Little Club	-1.4	+0.4	-0.4	+1.1	+0.5
Akabozu	+2.0*	-0.3	-1.0	-0.6	-0.1
BH 1146	-0.6	+0.5	-1.0	+0.2	+0.9

*) Significant at P=0.05.

tion variance was caused by Kaspar and Ze-61-1-14 which had low, resistant infection types with some of the races indicating a hypersensitivity resistance (Table 12-5). Therefore, these cultivars were omitted from the analysis. Then, a significant cultivar-race interaction could be detected ($P< 0.05$) only on the third assess-ment date. Two cultivar-race combinations were significantly over-susceptible: Akabozu-'Okapi' (8.7 instead of 6.7 if no interaction

would occur) and Skalavatis 56-'Arminda' (12.9 instead of 10.5 if no interaction would occur) (Table 12-6).

The analysis of variance indicated significant race effects. These, however, do not necessarily represent differences in aggressiveness. The numbers of sori on the spreader plants varied greatly between the races, from some 1700 on 'Flamingo' to about 110 for 'Arminda'. The variance for race effects tended to become smaller during the epidemic development indicating a negative association between initial inoculum and infection rate. This supports the observations of Rouse *et al.*, 1981, who found a negative correlation between epidemic growth rate and the level of the initial inoculum in epidemics of powdery mildew on wheat.

Exp. IV was analyzed in the same way as Exp. III. Results of the analysis appeared comparable to those of Exp. III, though not all cultivars were the same and the experimental design was different. The cultivar-race interaction was significant on all three assessment dates. The main contributor to the cultivar-race interaction variance was Ze-64-1-14 which had lower infection types with some races as in Exp. III. Excluding this cultivar from the analysis resulted in significant cultivar-race interaction on the three assessment dates both for the flag leaf data and the upper three leaves. The data for the upper three leaves of the second assessment date are presented in Table 12-7. The combination Akabozu-'Okapi' was, as in Exp. III, more susceptible than expected (7.9 instead of 6.5 if no interaction would occur) and Akabozu-'Arminda' was more resistant than expected (3.3 instead of 5.4 if no interaction would occur)(Table 12-8).

The analysis of variance indicated highly significant race effects. Here, as in Exp. III, the differences in initial inoculum were probably important in explaining this effect. As in Exp. III, the race effects decreased during the epidemic, suggesting a negative relationship between initial inoculum and infection rate.

Table 12-7. Leaf area affected expressed as sporulating leaf area transformed according to the key of Parlevliet and van Ommeren (1984) of nine wheat cultivars for five wheat leaf rust races on the upper three leaves (Exp. IV, second assessment date).

Cultivar	Race					Mean
	Okapi	Felix	Arminda	Flamingo	Neutral	
Skalavatis 56	13.2	12.7	12.7	12.9	12.8	12.9
Little Club	12.9	12.8	13.1	12.7	12.3	12.8
Skalavatis 59	13.1	12.7	12.1	12.1	11.4	12.2
Skalavatis 61	12.8	12.9	11.1	12.0	12.1	12.3
Van Hoek	10.2	11.2	9.1	8.2	9.3	9.6
Westphal 12A	9.4	9.4	8.0	7.3	7.9	9.2
Ze-64-1-14	9.8	11.9	4.5*	6.3*	7.1	8.4**
Akabozu	7.9	6.4	3.3	4.2	5.0	5.4
BH 1146	4.1	4.6	2.4	2.9	2.2	3.2
LSD 5%						0.5

*) Low infection type

**)Excluding low infection type data and corrected for race effects.

Table 12-8. Difference between observed and expected (if no differential interaction would occur) leaf area affected on the upper three leaves of eight wheat cultivars for five wheat leaf rust races (Exp. IV, second assessment date, data of Table 12-7).

Cultivar	Race				
	Okapi	Felix	Arminda	Flamingo	Neutral
Skalavatis 56	-0.8	-1.3	+0.6	+0.8	+0.6
Little Club	-1.0	-1.0	+1.4	+0.7	0.0
Skalavatis 59	-0.1	-0.5	+0.6	+0.5	-0.6
Skalavatis 61	-0.4	-0.1	-0.7	+0.5	+0.5
Van Hoek	-0.4	+1.2	+0.2	-1.2	+0.2
Westphal 12A	+0.2	+0.3	+0.3	-0.8	-0.1
Akabozu	+2.4*	+0.4	-2.1*	-0.8	+0.1
BH 1146	0.0	+0.9	-0.3	+0.3	-0.8

*) Significant at P=0.05.

DISCUSSION

In the experiments described here, two forms of resistance occurred. Hypersensitivity resistance (HR) as expressed by a low, resistant infection type and partial resistance (PR) as expressed by a slow epidemic build-up despite of a high infection type (Parlevliet and van Ommeren, 1975).

In the greenhouse, HR was easy to recognize. Kaspar and Adonis possessed complete HR, not allowing for any sporulation when infected with 'Okapi', 'Felix' or 'Neutral'. LP could not be determined for these cultivar-race combinations. All other cultivar-race combinations showed high, susceptible infection types and thus, LP could be measured.

The LP was very sensitive to fluctuations of the environment. Apart from the general decrease in LP at higher temperatures, a significant cultivar-environment effect was observed. Environmental conditions (mainly temperature) that shortened the LP reduced cultivar differences markedly, even relative differences became much smaller. When the colonization rate of the pathogen has a higher optimum temperature than the ability of the host to resist this colonization, the ratio of these two, which is the actual observed resistance, will decrease with higher temperatures (Bell, 1982). When different cultivars have different optimum temperatures for the expression of their resistance a cultivar-environment effect may appear. Additionally races may have different optimum temperatures for their colonization rate. These differences might induce race-environment and cultivar-race-environment effects. Cultivar-race-environment effects may have existed in the greenhouse experiments as the cultivar-race interactions in Exp. I were not reproduced in Exp. II. Since clear cultivar-environment effects were observed, decreasing the relative cultivar differences, the cultivar-race interactions may also have decreased and thus may have remained undetected. If so, an experiment at lower temperatures, would increase the chance of finding cultivar-race interactions. According to Browder (1985), the host pathogen interaction is a complex situation in which a specific environment combined with a specific cultivar-race combination would lead to a specific aegricorpus. The disease triangle is probably appropriate for PR in the wheat/wheat leaf rust situation.

In the field, HR and PR were less easy to distinguish. The HR of Kaspar was not complete, allowing for some development of the epidemic, and Ze-64-1-14 showed incomplete HR with some races which was never observed in the greenhouse. Parlevliet and Van Ommeren (1985), working with barley/barley leaf rust used infection types on adult plants in a greenhouse experiment to differentiate between differential interactions caused by HR and PR. In the case of wheat/wheat leaf rust, the approach of Parlevliet and van Ommeren might lead to the misinterpretation that PR observed in the field showed large race-specific interactions, whereas infection types observed in the field indicate otherwise.

All other than above mentioned cultivar-race combinations showed compatible infection types. Only one consistent cultivar-race interaction could be detected in the field experiments. Akabozu-'Okapi' was more susceptible than expected if no interaction would occur. Some cultivar-race interactions that were significant in one experiment were not so in the other. Broers (1987) reported that Skalavatis 59 was less susceptible with 'Flamingo' and more susceptible with 'Neutral'. This is not confirmed in Exp. IV. This leads to the question why cultivar-race interactions are not always consistent in the field. There are at least five reasons.

1) Statistically, there is a good chance that a wrong decision is made if interactions are rare. At 95% probability one out of 20 combinations in Exp. I and two out of 40

Table 12-9. Leaf area affected expressed in sporulating leaf area transformed according to the key of Parlevliet and van Ommeren (1984) of three hypothetical wheat cultivars for three hypothetical wheat leaf rust races on two assessment dates.

Cultivar	Leaf area affected					
	date 1			date 2		
	race 1	race 2	race 3	race 1	race 2	race 3
1	0	0*	0*	4	5	6
2	5	6	7	8	9	10
3	8	9	10	11	12	12*

*) Significant differential interaction

combinations in Exp. II may be falsely classified as interacting significantly interactions by chance only.

2) Initial inoculum may have induced part of the interaction variance. Within races some cultivars may have received more initial inoculum than others. Therefore, with one race, cultivar differences may have increased while with another race cultivar differences may have decreased, resulting in artificial interaction variance. Analysis of the initial inoculum of Exp. IV did not reveal a significant cultivar-race interaction and explained only a small part of the cultivar-race interaction variance observed.

3) Genotypic differences in earliness may influence the ranking of the cultivars on a specific assessment date. The upper three leaves of late-heading cultivars like Van Hoek in Exp. IV, escape from the early stages of

the epidemic resulting in a overestimation of their resistance. Later on they show their real potential. Theoretically, these differences in earliness may cause differential interactions. Assume that three wheat cultivars differing considerably in earliness are tested against three wheat leaf rust races that differ in aggressiveness (real or apparent) (Table 12-9). On the first assessment date, cultivar 1, which is assumed to be very late, does not show any visible infection on the upper three leaves. The other two cultivars react according to their resistance and the aggressiveness of the races. A small differential interaction can be detected. On the second assessment date, cultivar 1 reacts normally. Cultivar 3, an early cultivar, however, is already saturated with rust in combination with race 2 and 3. Here again, an interaction is caused by differences in earliness. The effect of these differences is most pronounced on the flag leaf (Broers, 1989). Flag leaf scoring, therefore, is less representative and, because a smaller leaf area is observed, less accurate than scoring the upper three leaves together.

4) Interplot interference was only important in Exp. III as microplots of susceptible cultivars and resistant cultivars were not separated. Resistant cultivars had more rust in Exp. III than in Exp. IV. Though they were not performed in the same year, the difference between BH 1146 and Skalavatis 56 was 7.5 times larger in Exp. IV than in Exp. III. Thus, interactions in Exp. III may have been less easy to recognize since they remained smaller, as might have been the case with Akabozu-'Arminda', which had the same, but insignificant deviation in Exp. III as in Exp. IV (Table 12-6 and 12-8)

5) As was observed in the greenhouse, differences in environmental conditions

may have influenced the cultivar-race interactions.

PR in wheat to wheat leaf rust is a complex system with an expression that is highly dependent on cultivar, race and environment. For selection purposes, the most complex race can be used to neutralize as many as possible of the known HR-genes. Selection must be carried out in the field and in the greenhouse. Selection only in the field will reveal mixtures of incomplete HR and PR. This was the case with the wheat cultivars selected for PR in the IPHR-programs in Brazil and Zambia. Many of them showed low infection types when tested against Dutch wheat leaf rust races (De Milliano *et al.*, 1986).

The four experiments described here, were performed to find out whether PR of wheat to leaf rust shows race-specific effects. In the field experiments one cultivar-race combination showed a consistent, small, but significant interaction towards susceptibility. Probably, the effect was too small to detect in the LP-assessment in the greenhouse and/or other components than LP may have played a role. In the data of Kuhn *et al.* (1978) a small interaction can be detected. One of the cultivars used was Suwon 85 which possessed digenically inherited PR (Lee and Shaner, 1985). Its level of PR is comparable to that of Akabozu which has two genes for PR (Broers and Jacobs, 1989). For both cultivars, the differential interactions are expected to be rather large since the gene effects are rather large. Small interactions are only to be expected if modifiers in the genetic background react race-specifically. The results presented here do not conflict with the idea that PR in wheat to wheat leaf rust acts according to a gene-for-gene system. Genetical studies can prove this hypothesis.

REFERENCES

Bell, A.A., 1982. Plant pest interaction with environmental stress and breeding for pest resistance: plant diseases In: Breeding for Less Favorable Environments. Eds: H.N. Christiansen and C.F. Lewis. Pp 335-363.

Broers, L.H.M., 1987. Relevance of randomizing host genotypes of wheat that are tested for leaf rust resistance in race nurseries. Euphytica 36: 257-263.

Broers, L.H.M., 1989. Partial resistance to wheat leaf rust in 18 spring wheat cultivars leaf rust. Euphytica, in press.

Broers, L.H.M. and Th. Jacobs, 1989. Inheritance of host plant effect on latency period of wheat leaf rust in spring wheat. II: number of segregating factors and evidence for transgressive segregation in F3 and F5 populations. Euphytica, in press.

Browder. L.E., 1985. Parasite: host: environment specificity in the cereal rusts. Annual Review of Phytopathology 23: 201-222.

De Milliano W.A.J., M.A. Beek and J.C. Zadoks, 1986. International testing of incomplete resistance against brown rust (*Puccinia recondita*) in IPHR wheat lines. Netherlands Journal of Plant Pathology 92: 49-56.

Eyal, Z., B.C. Clifford, and R.M. Caldwell, 1968. A settling tower for quantitative inoculation of leaf blades of mature small grain plants with urediospores. Phytopathology 60: 461-466.

Kuhn, R.C., H.W. Ohm and G.E. Shaner, 1978. Slow leaf rust severity in wheat against twenty two isolates of *Puccinia recondita*. Phytopathology 68: 651-656.

Kulkarni, R.N., V.L. Chopra and D. Sing, 1982. Relative importance of components affecting the leaf rust progress. Theoretical and Applied Genetics 62: 205-207.

Lee, T.S. and G.E. Shaner, 1985. Oligogene inheritance of length of latency period in six slow leaf-rusting wheat cultivars. Phytopathology 75: 636-643.

McNeal, F.H., C.F. Conzak, E.P. Smith, W.S. Tade and T.S. Russel, 1971. A Uniform system for recording and processing cereal research data. USDA Agric Res Surv Washington DC, ARS 34-121, 42pp.

Parlevliet, J.E., 1977. Evidence for differential interaction in the polygene *Hordeum vulgare-Puccinia hordei* relation during epidemic development. Phytopathology 67: 776-778.

Parlevliet, J.E. and A van Ommeren, 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. II. Relationship between field trials, microplot tests and latent period. Euphytica 24: 293-303.

Parlevliet, J.E. and A van Ommeren, 1984. Interplot interference and the assessment of barley cultivars for partial resistance to leaf rust, *Puccinia hordei*. Euphytica 33: 685-697.

Parlevliet, J.E. and A van Ommeren, 1985. Race-specific effects in major genic and polygenic resistance of barley to leaf rust in the field: identification and distinction. Euphytica 34: 689-695.

Rouse, D.I., D.R. MacKenzie and R.R. Nelson, 1981. A relationship between initial inoculum and apparent infection rate in a set of disease progress data for powdery mildew on wheat. Phytopathologische Zeitschrift 100: 143-149.

Samborski, D.J., 1985. Wheat leaf rust In: The Cereal Rusts. Vol. II Diseases, Distribution, Epidemiology and Control. Eds: A.P. Roelfs and W.R. Bushnell. Academic Press, Orlando, Florida. Pp 259-298.

Shaner, G.E. and R.E. Finney, 1980. New sources of slow leaf rusting resistance in wheat. Phytopathology 70: 1183-1186.

Teng, P.S., M.J. Blackie and R.C. Close, 1977. A simulation analysis of crop yield loss due to rust disease. Agricultural Systems 2: 189-198.

Zadoks, J.C., T. Chang and C. Konzak, 1974. A decimal code for the growth stages of cereals. Weed Research 14: 415-421.

Chapter 13.

INFLUENCE OF POST-INFECTION TEMPERATURE ON THREE COMPONENTS OF PARTIAL RESISTANCE IN WHEAT TO WHEAT LEAF RUST.

L.H.M. Broers and S.C. Wallenburg*

*) Part of her "Doktoraal studie".

SUMMARY

Three components of partial resistance (PR) were studied at three post-infection temperatures using seven spring wheat genotypes differing in level of PR and two different wheat leaf rust races. The components were latency period (LP), infection frequency (IF) and urediosorus size (US). The expression of LP was more sensitive to temperature than the expression of the other two components. LP-prolonging genes were better expressed at low temperatures than at high temperatures and cultivar differences tended to increase with decreasing temperature in both seedling and adult plant stages. The reaction of IF to temperature differed from that of LP and US, probably because IF is regulated by another mechanism than LP and US.

It is recommended to perform PR-screening tests at low rather than at high temperatures. If temperatures are maintained at about 8-13 °C (night-day), seedlings can be used to screen for PR instead of the more expensive adult plant tests.

The effectiveness of PR in seedling stage at low temperatures suggests that the seedling stage may have epidemiological significance as the low temperatures (8-13 °C) are relevant for seedlings in the field.

INTRODUCTION

Environment has an important impact on host-pathogen relations. Plant breeders select their breeding material for disease resistance in environments which are suitable for disease development. The environment is considered only when it limits the occurrence of disease. The environment should also be considered as a factor influencing the host-pathogen interaction. In wheat (*Triticum aestivum*), for instance, the expression of several hypersensitivity resistance genes for wheat leaf rust (*Puccinia recondita* f.sp. *tritici* syn. *Pucc. triticina*) is temperature sensitive (Browder, 1980). Therefore, selection for resistance in one environment may result in resistance not effective in another environment.

The effect of environment on the expression of partial resistance (PR) is not yet extensively studied. Ohm and Shaner (1976) and Broers (1989b) observed that cultivar differences were larger in cooler periods than in warmer periods. In barley, the expression of PR to barley leaf rust seems to be relatively temperature insensitive as LP-prolonging genes were fully expressed at all temperatures (Parlevliet, 1975).

In this paper, the influence of temperature on the expression of three components of PR is studied and the results are discussed in relation to selection for PR.

MATERIALS AND METHODS

Seven wheat cultivars were selected which cover the known range of PR to wheat leaf rust in spring wheat. BH 1146, Akabozu and Westphal 12A have high levels of PR, Adonis and Kaspar have intermediate levels of PR, and Skalavatis 56 and Little Club have no PR or hardly at all.

Monospore cultures of the wheat leaf rust races 'Felix' and 'Flamingo' (Zadoks, 1966) have been used as inoculum sources. The two races have different virulence patterns, as evidenced by these monospore cultures (Broers, 1989c). The monospore cultures are seen as representatives of the two races.

Seedling tests

Plants of the cultivars Skalavatis 56, Westphal 12A, Akabozu and BH 1146 were grown in square flats (30 x 30 x 5 cm). Each flat contained two replicates with all four cultivars. Per replicate each cultivar was represented by at least five plants. Three sequential experiments were carried out. An experiment consisted of six flats. Primary leaves of 10-days old seedlings, DC 11 (Zadoks *et al.*, 1974; = second leaf just visible), were inoculated in a settling tower at the upper leaf surface. Per flat 1.3 mg urediospores mixed with 13.0 mg *Lycopodium* spores were used giving a deposit of ca. 100 urediospores/cm^2. Three flats were inoculated with the 'Felix' race and three flats with the 'Flamingo' race. After inoculation, seedlings were placed overnight in a mist chamber where a water saturated atmosphere and complete darkness ensured infection. Following the incubation, flats with the same race were transferred to three different growth chambers with night-day temperatures of 8-13 °C, 15-19 °C and 24-28 °C, respectively. The RH was kept at 70 % and the day length at 16 hours in each growth chamber.

Adult plant tests

Plants were grown in 2.2 liter pots (5 plants per pot) in a greenhouse. All cultivars were sown at several consecutive sowing dates in order to obtain plants at the same development stage, DC 48-59 (Zadoks *et al.*, 1974; = ears just emerged, young but fully expanded flag leaves) at the time of inoculation. The adaxial surface of flag leaves was inoculated in a settling tower using 1.3 mg urediospores mixed with 13.0 mg *Lycopodium* spores.

Two experiments were carried out. In 1986 (Exp I) all seven cultivars were used which were inoculated with the 'Felix'-race only whereas in 1987 (Exp II) only Skalavatis 56, BH 1146, Akabozu and Westphal 12A were used in combination with both the 'Felix' and the 'Flamingo' races.

Exp I was carried out in four sequential series. Each series consisted of six pots per cultivar and two flag leaves per pot were inoculated. After inoculation the plants were incubated in a mist chamber and transferred to the three growth chambers mentioned above. Each cultivar was represented by two pots and four flag leaves at each temperature in each series.

Exp II consisted of five sequential series. Each series contained six pots per cultivar-race combination with two flag leaves inoculated per pot. Per series, each cultivar-race combination was represented by two pots and four flag leaves at each temperature.

Latency period (LP) and infection frequency (IF) were measured on all inoculated primary and flag leaves as described by Broers (1989a) and infection types were recorded on a scale of 0 to 9 (McNeal *et al.*, 1971). Urediosorus size (US) of the seedlings and the flag leaves of Exp II were measured. It was assumed that for each cultivar-temperature combination the US was 0 at its LP. Urediosori of all cultivars at the same temperature and growth stage were allowed to grow for the same time period starting from the time at which 50% of the urediosori was visible (=LP). For seedlings the time periods were 18 days, 8 days and 5 days for 8-13 °C, 15-19 °C and 24-28 °C, respectively. For adult plants the time periods were 25, 11 and 7 days for 8-13 °C, 15-19°C and 24-28°C, respectively. The leaves were harvested and boiled in a lactophenol:ethanol solution (2:1) for 3 minutes to fix them. Per leaf the length (L) and the width (W) of 15 randomly chosen urediosori were measured using a microscope (magnification 100 x) with a micrometer. US was obtained using the formula:

$US = 1/4\pi * L * W$.

Table 13-1. Relative latency period of wheat leaf rust races 'Felix' (Fe) and 'Flamingo' (Fl) on primary leaves of four spring wheat cultivars at three temperatures.

Cultivar	Temperature (night - day)								
	8-13 °C			15-19 °C			24-28 °C		
	Fe	Fl	mean[4]	Fe	Fl	mean	Fe	Fl	mean
Skalavatis 56[1]	101	99	100[a](13.6)	98	102	100[a](7.2)	100	100	100[a](5.0)
Westphal 12A	129	119	124[b]	112	108	110[b]	106	106	106[ab]
Akabozu	147	138	143[c]	112	110	111[b]	112	110	111[b]
BH 1146	143	119	131[b]	113	109	111[b]	104	106	105[ab]
mean[2]	130	118	124	109	108	109	106	106	106
mean[3]	140	125	133	112	109	111	107	107	107

1) absolute latency period in days of Skalavatis 56 in brackets.

2) mean of all cultivars.

3) mean of all cultivars except Skalavatis 56.

4) means with the same letter do not differ significantly (P= 0.05).

Table 13-2. Relative latency period in days of wheat leaf rust race 'Flamingo' on flag leaves of seven spring wheat genotypes at three temperatures.

Cultivar	Temperature (night - day)[4]		
	8-13 °C	15-19 °C	24-28 °C
Skalavatis 56[1]	100[a](12.6)	100[a](6.6)	100[a](5.2)
Little Club	100[a]	100[a]	102[a]
Kaspar	113[b]	106[ab]	102[a]
Adonis	129[c]	126[c]	121[b]
Westphal 12A	126[c]	112[b]	100[a]
Akabozu	160[d]	144[d]	115[b]
BH 1146	231[e]	161[e]	164[c]
mean[2]	137	121	117
mean[3]	152	130	121

1) absolute latency period in days of Skalavatis 56 in brackets.

2) mean of all cultivars.

3) mean of all cultivars except Skalavatis 56 and Little Club.

4) means with same letter do not differ significantly (P=0.05).

Exp I was carried out in winter 1986. Exp II and the seedling tests were carried out in spring 1987.

RESULTS

Infection types of all cultivar-race-temperature combinations were high in both seedling and adult plant stages indicating that the LP, IF and US data were not influenced by undefeated hypersensitivity resistance genes.

Latency period

A large cultivar-temperature interaction on LP was observed in both the seedling and adult plant stages. Per temperature, LP's of all cultivars were expressed in per cent of the mean latency period of Skalavatis 56 at the respective temperatures (Table 13-1, 13-2 and 13-3). Assuming that Skalavatis 56 does not carry any LP-prolonging genes, it represents the reference level at all three temperatures. Values larger than 100 are due to the effect of LP-prolonging genes in the respective cultivar. The average effect of LP-prolonging genes in the seedling stage was 7%, 11% and 33%, at 24-28 °C, 15-19 °C and 8-13 °C, respectively (Table 13-1). In the adult plant stage, these effects were 21%, 30% and 52% for exp I (Table 13-2) and 15%, 49% and 101% for exp II (Table 13-3), respectively. The average effect of LP-prolonging genes increased considerably with decreasing temperature. The effect of LP-prolonging genes is larger in the adult plant stage than in the seedling stage at any temperature. Cultivar differences increased with decreasing temperature in both the seedling and the adult plant stages. In the seedling stage, for instance, no clear cultivar differences could be detected at 24-28 °C whereas at the lowest temperature three significantly different cultivar groups could be detected (Table 13-1).

The temperature effects on the LP in the absence of LP-prolonging genes can be seen from the absolute LP of Skalavatis 56 at the different temperatures. The LP

Table 13-3. Relative latency period of wheat leaf rust races 'Felix' (Fe) and 'Flamingo' (Fl) on flag leaves of four spring wheat cultivars at three temperatures.

Cultivar	Temperature (night - day)								
	8-13 °C			15-19 °C			24-28 °C		
	Fe	Fl	mean[4]	Fe	Fl	mean	Fe	Fl	mean
Skalavatis 56[1]	100	100	100[a](14.4)	101	100	100[a](7.9)	102	98	100[a](6.2)
Westphal 12A	181	182	182[b]	142	135	139[b]	106	102	104[a]
Akabozu	182	178	180[b]	151	146	149[bc]	119	119	119[b]
BH 1146	222	258	240[c]	157	162	160[c]	118	124	121[b]
mean[2]	171	180	176	138	136	137	111	111	111
mean[3]	195	206	201	150	148	149	114	115	115

1) absolute latency period in days of Skalavatis 56 in brackets.

2) mean of all cultivars.

3) mean of all cultivars except Skalavatis 56.

4) means with the same letter do not differ significantly (P=0.05).

Table 13-4. Relative infection frequency of wheat leaf rust races 'Felix' (Fe) and 'Flamingo' (Fl) on primary leaves of four spring wheat cultivars at three temperatures.

Cultivar	Temperature (night - day)								
	8-13 °C			15-19 °C			24-28 °C		
	Fe	Fl	mean[4]	Fe	Fl	mean	Fe	Fl	mean
Skalavatis 56[1]	87	114	100[a](57)	114	86	100[a](65)	96	104	100[a](70)
Westphal 12A	63	91	77[ab]	103	83	93[a]	94	77	86[ab]
Akabozu	54	89	72[b]	100	74	87[a]	54	53	54[b]
BH 1146	49	81	65[b]	86	66	76[a]	80	71	76[ab]
mean[2]	63	93	78	100	77	89	81	76	79
mean[3]	55	87	71	96	74	85	76	69	72

1) absolute infection frequency in urediosori/cm^2 of Skalavatis 56 in brackets.

2) mean of all cultivars.

3) mean of all cultivars except Skalavatis 56.

4) means with the same letter do not differ significantly (P=0.05).

decreased with increasing temperature. The LP at 8-13 °C was on average 2.5 times longer than at 24-28 °C.

A small but significant race-temperature interaction was found in the seedling stage. The LP of Felix was significantly longer than the LP of Flamingo at 8-13 °C, whereas at the other temperatures no significant difference was observed between the two races. No race-temperature interaction in the adult plant stage and no race, cultivar-race and cultivar-race-temperature effects in either growth stage were detected in the analysis of variance. Apparently, the sensitivity in expression of LP is due to the host rather than to the pathogen.

Cultivars present in both adult plant experiments showed different responses in exp I and exp II with regard to LP. The effect of LP-prolonging genes was on average 7% smaller in exp I than in exp II. This result suggests that the pre-infection environment influences the expression of LP-prolonging genes, as the experiments differed only in growing conditions before infection.

Infection frequency

The data of IF are represented in the same way as the LP data. Skalavatis 56 is set at 100% and represents the reference level (no genes that reduce IF). IF values less than 100 represent the effect of genes that reduce IF. The mean effects of IF-reducing genes in the seedling stage were 29%, 15% and 28% for 8-13 °C, 15-19 °C and 24-28 °C respectively (Table 13-4). The mean effects of IF-reducing genes in the adult plant stage were 29%, 37% and 23% in exp I (Table 13-5) and 44%, 16% and 12%

in exp II (Table 13-6) for 8-13 °C, 15-19 °C and 24-28 °C, respectively. Only in Exp II a clear effect of temperature on the expression of

IF-reducing genes could be detected, IF decreasing with increasing temperatures. In seedlings, no cultivar-temperature interaction was observed and in exp I the observed cultivar-temperature interaction was due to a change of rank of some cultivars at 15-19 °C compared to the other temperatures. Thus, no clear, reproducible temperature effect on the expression of IF-reducing genes

Table 13-5. Relative infection frequency of wheat leaf rust race 'Flamingo' on flag leaves of seven spring wheat genotypes at three temperatures.

Cultivar	Temperature (night - day)[4]		
	8-13 °C	15-19 °C	24-28 °C
Skalavatis 56[1]	100[ab](33)	100[a](46)	100[ab](36)
Little Club	109[a]	93[a]	114[a]
Kaspar	93[abc]	60[b]	100[ab]
Adonis	76[bc]	57[b]	86[b]
Westphal 12A	70[c]	80[ab]	75[b]
Akabozu	82[abc]	59[b]	81[b]
BH 1146	36[d]	57[b]	44[c]
mean[2]	81	72	86
mean[3]	71	63	77

1) absolute infection frequency in urediosori/cm^2 of Skalavatis 56 in brackets.

2) mean of all cultivars.

3) mean of all cultivars except Skalavatis 56 and Little Club.

4) means with the same letter do not differ significantly (P=0.05).

Table 13-6. Relative infection frequency of wheat leaf rust races 'Felix' (Fe) and 'Flamingo' (Fl) on flag leaves of four spring wheat cultivars at three temperatures.

Cultivare	Temperature (night - day)								
	8-13 °C			15-19 °C			24-28 °C		
	Fe	Fl	mean[4]	Fe	Fl	mean	Fe	Fl	mean
Skalavatis 56[1]	112	87	100a(39)	95	105	100a(38)	95	105	100a(33)
Westphal 12A	38	49	44c	87	89	88a	126	111	119a
Akabozu	64	77	71b	82	66	74a	117	102	110a
BH 1146	53	51	52bc	84	95	89a	108	108	108a
mean2	67	66	67	87	89	88	112	107	110
mean3	52	59	56	84	83	84	114	107	112

1) absolute infection frequency in urediosori/cm^2 of Skalavatis 56 in brackets.

2) mean of all cultivars.

3) mean of all cultivars except Skalavatis 56.

4) means with the same letter do not differ significantly (P=0.05).

Table 13-7. Relative urediosorus size of wheat leaf rust races 'Felix' (Fe) and 'Flamingo' (Fl) on primary leaves of four spring wheat cultivars at three temperatures.

Cultivar	Temperature (night - day)								
	8-13 °C			15-19 °C			24-28 °C		
	Fe	Fl	mean[4]	Fe	Fl	mean	Fe	Fl	mean
Skalavatis 56[1]	95	105	100[a](0.18)	122	78	100[a](0.14)	112	88	100[a](0.11)
Westphal 12A	80	87	84[ab]	98	93	96[ab]	111	105	108[a]
Akabozu	59	63	61[bc]	88	72	80[b]	97	70	84[a]
BH 1146	78	84	81[b]	86	74	80[b]	90	95	93[a]
mean[2]	78	85	82	99	79	89	102	90	96
mean[3]	72	78	75	91	80	85	99	90	95

1) absolute urediosorus size in mm^2 of Skalavatis 56 in brackets.

2) mean of all cultivars.

3) mean of all cultivars except Skalavatis 56.

4) means with same letter do not differ significantly (P=0.05).

could be detected. A temperature effect on the IF itself was detected in the analysis of variance. However, no consistency could be observed, as can be seen from the absolute IF values of Skalavatis 56 (Table 13-4). In the seedling stage, the IF was highest at the highest temperature (Table 13-4) whereas in exp I of the adult plants the highest IF was observed at the middle temperature and in exp II at the lowest temperature. This inconsistency might have been due to a growth stage-temperature and/or a pre-infection environment-temperature interaction, which, however, could not be demonstrated.

In the seedling stage, a significant race-temperature effect was detected. At the lowest temperature, the 'Felix' race had a lower IF than the 'Flamingo' race whereas at the middle temperature the opposite was true and at the highest temperature no differences existed between the two races. In the adult plant stage, the race-temperature interaction was not significant. As with LP, no cultivar-race and cultivar-race-temperature were detected.

The results indicate that the expression of IF is somewhat sensitive to temperature in both the seedling stage and the adult plant stage but far less so than LP.

Urediosorus size

The data on US are given in Table 13-7 (seedlings) and Table 13-8 (adult plants). Skalavatis 56 was set at 100% and values less than 100 represented the effect of factors that reduced US in the respective cultivars.

Statistical analysis revealed a significant cultivar-temperature interaction. Factors reducing US in partially resistant cultivars were more effective at low than at high temperatures. The effects of US-reducing factors in the seedling stage were 25%, 15% and 5% (Table 13-7) and in

the adult plant stage 61%, 46% and 24% (Table 13-8) at 8-13 °C, 15-19 °C and 24-25 °C, respectively. Both in the seedling and in the adult plant stage, US-reducing factors were sensitive to temperature but to a lesser

Table 13-8. Relative urediosorus size of wheat leaf rust races 'Felix' (Fe) and 'Flamingo' (Fl) on flag leaves of four spring wheat cultivars at three temperatures.

Cultivar	Temperature (night - day)								
	8-13 °C			15-19 °C			24-28 °C		
	Fe	Fl	mean4	Fe	Fl	mean	Fe	Fl	mean
Skalavatis 56[1]	104	96	100[a](0.20)	103	97	100[a](0.17)	107	93	100[a](0.16)
Westphal 12A	38	47	42[b]	74	63	69[b]	92	88	90[ab]
Akabozu	40	41	40[b]	48	32	40[c]	66	53	59[c]
BH 1146	35	36	35[b]	49	57	53[bc]	78	79	78[bc]
mean[2]	54	55	54	68	63	66	86	78	82
mean[3]	38	41	39	57	51	54	79	73	76

1) absolute urediosorus size in mm^2 of Skalavatis 56 in brackets.
2) mean of all cultivars.
3) mean of all cultivars except Skalavatis 56.
4) means with same letter do not differ significantly (P=0.05).

extent than LP. The cultivar differences increased with decreasing temperature, as was the case with LP. However, the cultivar differences between the three partially resistant cultivars did not increase with decreasing temperature.

Contrary to LP, a small but significant race-temperature effect was observed with US. Both in the seedling and in the adult plant stage, the 'Felix' race had smaller urediosori than the 'Flamingo' race at the lowest temperature whereas at the other temperatures the reverse was true. Assuming that urediosorus size is related to spore production and reproductive capacity, this result indicates that the selective advantage of one race compared to the other can vary with temperature.

DISCUSSION

The effect of temperature on LP, IF and US can be divided into three elements. First, there is an effect of temperature on the pathogen, which can be measured in the susceptible check assuming that it does not posses any resistance. Secondly, there is an effect of temperature on factors in the host that prolong LP, reduce IF and reduce US of the pathogen. This effect can be measured by relating the cultivar values for the respective components to the values of the susceptible check (set at 100), to eliminate the first element of temperature sensitivity. Third, an environment genotype (host)-genotype (pathogen) can be postulated, and sometimes confirmed experimentally.

Latency period

A clear temperature effect on LP-prolonging factors was found. A decrease of temperature resulted in a much better expression of LP-prolonging genes and in an increase of cultivar differences in both the seedling stage and the adult plant stage. This result corroborates observations of Ohm and Shaner (1976) and Broers (1989c) who found larger cultivar differences for LP at

cooler periods than at warmer periods. Broers (1989a) recommended to use LP as a selection criterion for PR and to perform tests for LP on young flag leaves and not on primary leaves as cultivar differences were often too small to be detected in the seedling stage. For the temperature used in those experiments (comparable to the middle temperature used here), the present results support this recommendation. At low temperatures, however, the expression of LP-prolonging factors in seedlings improved to a degree that seedlings became suitable to screen for PR. Selection in the seedling stage has the advantage that it can be carried out in winter time so that one selection cycle per year is gained. Selection in the seedling stage is less time and space consuming than selection in the adult plant stage. On the other hand, expensive equipment, such as growth chambers, are needed to keep temperatures sufficiently low. If no such equipment is available and adult plants are used to select for PR, one should still try to keep temperatures as low as possible because cultivar differences decrease rapidly with increasing temperature.

The results have implications for selection for PR in the field. Breeders tend to select in environments which are favourable for disease. It is expected that selection in the field under cooler conditions, which are suboptimal for wheat leaf rust, will give better results than under warmer conditions, due to the temperature sensitivity of LP-prolonging genes. The temperatures in the Netherlands, for instance, are suboptimal for the development of wheat leaf rust for a large part of the season but with artificial infection severe epidemics can be obtained in susceptible cultivars. The differences in disease severity between susceptible and resistant genotypes will be larger at suboptimal than at higher temperatures, making selection more efficient under Dutch conditions than under optimal conditions.

Infection frequency

The effect of temperature on IF-reducing factors was inconsistent in different experiments and much smaller than on LP. IF can be considered as a stochastic variable which expresses the probability that a spore will develop into a sporulating colony. The most crucial period for IF is most likely the 24 hrs after germination of the urediospores. According to Jacobs (1989) two different phases are present in PR of wheat to wheat leaf rust. During first phase cell wall appositions are present at sites where the fungus tries to penetrate a host cell. This probably causes abortion of infection structures leading to a reduction of IF. A large part of this period in which abortion will take place is taken up by the incubation period after inoculation (about 13 hrs). This period was the same for all temperature treatments leaving only short time under different temperature regimes. The second phase starts when a colony has succeeded to infect a host cell; it possibly reduces the effectiveness of the haustoria to take up nutrients from the host cell so that colony growth is reduced and LP is prolonged. The two different phases may explain the different reactions of IF-reducing and LP-prolonging factors to temperature.

Urediosorus size

The effect of temperature on US-reducing factors was similar to but smaller than the effect on LP. The similarity in response of US-reducing factors and LP-prolonging factors reflects the growth potential of wheat leaf rust and, probably, the growth retardation in the second phase (Jacobs, 1989)

Epidemiological implications

A comparison of seedling data with data from adult plants in Exp II shows that at any temperature the seedlings are more susceptible than the adult plants and that differences between cultivars are easier to detect in adult plants than in seedlings. Nevertheless, the seedling stage can have an important impact on the epidemiology of wheat leaf rust. As spring wheat is sown in early spring, the low temperature (8-13 °C) is most relevant to the field situation of seedlings. This implies that cultivar differences for PR in the field are already large just after emergence. During the growing season, the resistance of the plant increases with its development stage (Pretorius *et al.*, 1988) and decreases because the temperature rises at the same time. In the Dutch situation, average temperatures during the adult plant stages of wheat are 15 to 20 °C which means that then the middle temperature is representative of the field situation. The compensatory effects of growth stage and temperature may cause rather constant cultivar differences throughout the growing season. If so, the epidemic will be reduced in PR-cultivars as compared to susceptible cultivars, to a similar extent irrespective of the crop's growth stage.

Durability

In the wheat/wheat leaf rust relationship, PR may function according to a gene-for-gene relationship as some race-specificity has been found (Broers, 1989c; Kuhn *et al.*, 1978). Parlevliet (1983) developed a model by which durability of PR could be explained, assuming a gene-for-gene relationship. Adding temperature sensitivity to the model will increase the durability and stability of PR and decrease the chance of sudden epidemic outbreaks of wheat leaf rust on wheat because the selection pressure of a cultivar on a rust population alters with the environment. The races 'Felix' and 'Flamingo', for example, differ for US in such a way that the 'Felix' race will have a selective disadvantage in cooler periods whereas the 'Flamingo' race will have a selective disadvantage in warmer periods, assuming that US is related to the reproductive capacity of the races. That PR is possibly durable and stable shows the cultivar BH 1146. It has been grown commercially in Brazil for more than 30 years. Its resistance to wheat leaf rust has been recognized as being of the PR-type. This resistance is still effective, though it has been exposed for a long time in an environment conducive to wheat leaf rust (H. van de Vliet, 1985, pers. comm.) Moreover, its resistance is also effective in the Netherlands (Broers, 1989b, 1989c) and in Mexico (Broers and Parlevliet, 1989d), with different wheat leaf rust races and climatic conditions.

REFERENCES

Broers, L.H.M., 1989a. Influence of growth stage and host genotype on three components of partial resistance in wheat to wheat leaf rust. Euphytica, in press.

Broers, L.H.M., 1989b. Partial resistance to wheat leaf rust in 18 spring wheat cultivars. Euphytica, in press.

Broers, L.H.M., 1989c. Race-specific aspects of partial resistance in wheat to wheat leaf rust. Euphytica, in press.

Broers, L.H.M. and J.E. Parlevliet, 1989d. Environmental stability of the expression of partial resistance in spring wheat to wheat leaf rust. Euphytica, in press.

Browder, L.E., 1980. A compendium of information about named genes for low reaction to *Puccinia recondita* in wheat. Crop Science 20: 775-779.

Jacobs, Th., 1989. The occurrence of cell wall appositions in flag leaves of spring wheats susceptible and partially resistance to wheat leaf rust. Submitted to The Journal of Phytopathology.

Kuhn, R.C., H.W. Ohm and G.E. Shaner, 1978. Slow leaf rust severity in wheat against twenty two isolates of *Puccinia recondita*. Phytopathology 68: 651-656.

McNeal, F.M., C.F. Conzak, E.P. Smith, W.S. Tade and T.S. Russel, 1971. A uniform system for recording and processing cereal research data. USDA Agric Res Surv Washington DC, ARS 34-121, 42pp.

Ohm, H.W. and G.E. Shaner, 1976. Three components of slow rusting at different growth stages in wheat. Phytopathology 66:1356-1360.

Parlevliet, J.E., 1975. Partial resistance of barley to leaf rust, *Puccinia hordei* I. Effect of cultivar and development stage on latent period. Euphytica 24: 21-27.

Parlevliet, J.E., 1983. Models explaining the specificity and durability of host resistance derived from the observations on the barley- *Puccinia hordei* system. In: Durable Resistance in Crops. Eds: F. Lamberti, J.M. Waller and N.A. Van der Graaf. Pp 57-78.

Pretorius, Z.A., F.H.J. Rijkenberg and R.D. Wilcoxson, 1988. Effects of growth stage, leaf position and temperature on adult-plant resistance of wheat infected by *Puccinia recondita* f.sp. *tritici*. Plant Pathology 37: 36-44.

Zadoks, J.C., 1966. Field races of leaf rust. Proc. Cereal Rusts Conf., Cambridge, 1964. Pp 92-93.

Zadoks, J.C., T. Chang and C. Konzak (1974). A decimal code for the growth stages of cereals. Weed Research 14: 415-421.

Chapter 14.

ENVIRONMENTAL STABILITY OF PARTIAL RESISTANCE IN SPRING WHEAT TO WHEAT LEAF RUST.

L.H.M. Broers and J.E. Parlevliet

SUMMARY

Five spring wheat cultivars differing in partial resistance (PR) to wheat leaf rust were tested at Wageningen (The Netherlands) on a sandy and a clay site, El Batan (CIMMYT, Mexico) and Ponta Grossa (Brazil) over two years. The cultivars were Skalavatis 56, Little Club (both very susceptible), Westphal 12A, Akabozu and BH 1146 (all three with high levels of PR). The results showed that PR was expressed at all four locations in both years. The level of expression was influenced by the environment but the cultivar ranking was hardly affected. Selection for PR in the field can therefore be carried out over a wide range of environments.

INTRODUCTION

In wheat (*Triticum aestivum*) the emphasis of research into resistance to wheat leaf rust (*Puccinia recondita* f.sp. *tritici*, syn. *Pucc. triticina*) is shifting towards forms that are hopefully more durable than the hypersensitivity resistance (HR). Partial resistance (PR) might be such a form. It is characterized by a slow epidemic built up though its infection type indicates the absence of HR (Parlevliet and van Ommeren, 1975).

Johnson (1984) defines durable resistance as "resistance that remains effective during its prolonged and widespread use in an environment favourable to the disease". This definition contains two elements, time and area, and it refers to the biological stability of the resistance. Experiments to predict durability are, in fact, impossible as they should be performed on large areas, over a long period of time, and in many environments. Abiotic stability is another aspect of resistance, which refers to its expression in a wide range of environments. Experiments performed under a wide range of growing conditions may give some information on the abiotic stability of resistance.

This paper reports about of the expression of PR in wheat to wheat leaf rust over a wide range of growing conditions.

MATERIALS AND METHODS

Five spring wheat cultivars with different levels of PR were used. Akabozu, BH 1146 and Westphal 12A have high levels of PR and Skalavatis 56 and Little Club are very susceptible. All cultivars showed susceptible infection types with a monospore culture of the 'Flamingo' race (Broers, 1989c).

The cultivars were tested at four locations in 1986 and 1987. In Table 14-1, some geographical and physical properties of the four locations are given. In addition to the

Table 14-1. Some geographical and physical properties of four locations to test the partial resistance of five spring wheat cultivars against leaf rust.

Location, country	Latitude	Altitude	Soil type	Growing season
The Netherlands, Wageningen (I)	51°51'N	15 m	clay	April-August
The Netherlands, Wageningen (II)	51°51'N	15 m	sand	April-August
Mexico, El Batan (CIMMYT)	19°32'N	2250 m	loam	May-September
Brazil, Parana, Ponta Grossa	25°05'S	1000 m	clay, low pH	May- October

differences mentioned in Table 14-1 the pathogen populations too differed with the locations. At Wageningen (I and II), a monospore culture of the 'Flamingo' race was used. In Mexico and Brazil, wheat leaf rust developed from natural infection and an unidentified rust population was responsible for the infection.

The basic design for all eight experiments was a randomized block design with three replicates of eight plots. The individual plots consisted of three rows of one meter with a row distance of 25 cm. Adjacent plots were separated by five rows of oats (Wageningen and Brazil) or wheat leaf rust resistant wheat (Mexico) and blocks were separated by a strip of oats or a wheat leaf rust resistant wheat cultivar. The separation of blocks and plots by oats or wheat leaf rust resistant wheat was meant to decrease interplot interference.

In Wageningen, the wheat leaf rust epidemic was started in mid May by placing a sporulating spreader plant in each plot, which were removed after ten days. In Mexico and Brazil, the epidemic started from natural infection.

At weekly intervals the level of wheat leaf rust was assessed. Per plot 15 tillers were harvested. On the upper three leaves of each tiller the number of urediosori was counted. These numbers were transformed according to the scale of Parlevliet and Van Ommeren (1984) and corrected for differences in leaf area. Also the heading dates were recorded.

RESULTS AND DISCUSSION

The terminal disease levels of the five cultivars at four locations in two years are given in Table 14-2. At all locations the disease level on Westphal 12A, Akabozu and BH 1146 was less than on Skalavatis 56 and Little Club, indicating that PR is expressed in all eight the environments. Conspicuous was the difference in disease level between Skalavatis 56 and Little Club. At all locations, Skalavatis 56 showed more wheat leaf rust than Little Club (Table 14-2). This was not expected because a component analysis had revealed that the two cultivars were equally susceptible to wheat leaf rust (Broers, 1989a). The cultivars differed also in earliness. Broers (1989b) mentioned that the susceptibility of late-heading cultivars is underestimated relative to that of early cultivars. Leaves of the late-heading Little Club, especially the flag leaf and the leaf below it,

Table 14-2. Leaf area affected by leaf rust expressed in number of urediosori per tiller transformed according to the scale of Parlevliet and van Ommeren (1984) of five wheat cultivars at four locations in two years.

Cultivar	Location							
	Wageningen I		WageningenII		Mexico		Brazil	
	1986	1987	1986	1987	1986	1987	1986	1987
Skalavatis 56	12.7[a*]	13.5[a]	12.0[a]	13.5[a]	12.7[a]	13.8[a]	12.1[a]	12.0[a]
Little Club	11.2[b]	12.2[b]	11.3[b]	12.7[b]	11.2[b]	12.3[b]	8.7[b]	-
Westphal 12A	5.6[c]	7.5[c]	6.4[c]	6.8[c]	2.1[d]	7.6[c]	4.1[c]	8.4[b]
Akabozu	5.2[c]	7.3[c]	2.0[c]	5.1[d]	1.2[d]	5.8[d]	1.0[e]	6.2[d]
BH 1146	3.7[d]	4.6[d]	5.3[d]	6.7[c]	3.1[c]	8.2[c]	2.3[d]	7.2[c]

* Means in a column with the same letter do not differ significantly (P=0.05)

Table 14-3. Heading dates expressed as number of days relative to the heading date of Skalavatis 56 (set at 0) of five wheat cultivars at four locations in two years.

Cultivar	Location					
	Wageningen I+II		Mexico		Brazil	
	1986	1987	1986	1987	1986	1987
Skalavatis 56	0	0	0	0	0	0
Little Club	12	14	23	25	31	-[1)]
Westphal 12A	4	5	11	12	-19	-25
Akabozu	11	12	26	30	-18	-21
BH 1146	6	7	-4	2	-18	-28

1) did not reach heading stage due to severe rust infection.

were exposed to wheat leaf rust during a shorter time than those of the early Skalavatis 56. Therefore, it is highly probable that the difference in disease level between Skalavatis 56 and Little Club was predominantly, if not solely, associated with the difference in earliness. This gives the possibility to estimate the effect of earliness on the disease level. The average effect of Little Club's later heading, calculated as the ratio between the difference in disease level expressed according to the scale of Parlevliet and Van Ommeren (1984) (scale units) and the difference in heading dates (in days) with Skalavatis 56 averaged over all location-year combinations, was 0.08 scale units per day. The disease levels of Table 14-2 were corrected for differences in heading date using the heading dates in Table 14-3 and the calculated average effect of later heading. For instance, the corrected disease level of Akabozu in Mexico in 1987 was calculated as:
Heading date * 0.08 + uncorrected disease level = (30 * 0.08) + 5.8 = 8.2. In Table 14-4 the corrected disease levels are presented. These results are probably more representative for the real differences in partial resistance at the four locations.

Westphal 12A, Akabozu and BH 1146 still are significantly less affected than Skalavatis 56 and Little Club.

In Mexico and Brazil, the expression of PR, measured as reduced disease severity was better in 1986 than in 1987. The difference between the partially resistant cultivars and the susceptible cultivars was about four scale units larger in 1986 than in 1987. In Wageningen, the expression of PR was the same for both years, but similar changes as in Mexico and Brazil in the effectiveness of PR have been reported for barley/barley leaf rust at sites in The Netherlands between years (Parlevliet and van Ommeren, 1984).

The results indicate that the expression of PR is affected by environmental conditions. This agrees with the greenhouse observations of Broers (1989d), that the expression of PR depends on the post-infection temperature. At lower temperatures, the expression of PR was better than at higher temperatures; cultivar differences tended to decrease with temperature. Differences in temperature between years and/or between locations could easily explain the observed differences in PR. The ranking of the cultivars, though was not affected. However, this temperature sensitivity of PR-genes studied, does not need to

Table 14-4. Leaf area affected by leaf rust expressed in number of urediosori per tiller transformed according to the scale of Parlevliet and van Ommeren (1984) of five wheat cultivars at four locations in two years. Data have been corrected for differences in earliness.

Cultivar	Location							
	Wageningen I		Wageningen II		Mexico		Brazil	
	1986	1987	1986	1987	1986	1987	1986	1987
Skalavatis 56	12.7[a]	13.5[a]	12.0[a]	13.5[a]	12.7[a]	13.8[a]	12.1[a]	12.0[a]
Little Club	12.3[a]	13.5[a]	12.4[a]	14.0[a]	13.3[a]	14.6[a]	11.5[a]	-
Westphal 12A	5.9[b]	7.9[b]	6.7[b]	7.2[b]	3.0[b]	8.6[b]	2.6[b]	6.4[b]
Akabozu	6.1[b]	8.3[b]	2.9[c]	6.1[c]	3.3[b]	8.2[b]	0.0[c]	4.5[c]
BH 1146	4.2[c]	5.2[c]	5.8[b]	7.3[b]	2.8[b]	8.4[b]	0.9[c]	5.0[c]

* *Means in a column with same letter do not differ significantly (P=0.05)*

be the cause of the differences in magnitude in PR reported here, for the PR in barley to barley leaf rust shows similar variations in its magnitude (Parlevliet and van Ommeren, 1984) although the PR-genes in this pathosystem do not show any temperature sensitivity (Parlevliet, 1975).

It is difficult to compare the level of expression of PR across environments as cultivar differences in earliness varied greatly with location. In general, the expression of PR in the partially resistant cultivars studied resulted in a reduction of wheat leaf rust varying from about 50 times (5.8 scale units) to about 1550 times (10.6 scale units) at the time that susceptible cultivars were severely rusted. Sometimes, the effect of PR was reduced due to environmental conditions. An increase in level of PR is useful to avoid drops in effectiveness of PR as observed in Mexico and Brazil. Higher levels of PR can be achieved as transgressive segregation was observed in crosses between Akabozu, Westphal 12A and BH 1146 (Broers and Jacobs, 1989).

Small but significant cultivar-environment interactions were observed. In Wageningen, Akabozu on the clay soil had significantly more wheat leaf rust than BH 1146 whereas on sand the reverse was true. In Mexico, no significant difference in disease severity could be detected between the partially resistant cultivars; in Brazil the cultivar ranking was the same as in Wageningen at clay. The interaction at the Wageningen locations was associated with soil type. The other interactions are not associated with a single factor. Possible causes may be differences in earliness, pathogen population, day length, soil type, and other environmental conditions. The magnitude of these interactions is small in comparison with the difference in disease severity between the group of PR cultivars and the group of susceptible cultivars. The observed interactions do not disturbing the ranking order in essence.

This study clearly revealed that PR is a form of resistance that is expressed in a wide range of environments. Therefore, it is a stable characteristic. The level of expression of PR may vary somewhat with environments. Therefore, the level PR in the cultivars used may sometimes be insufficient to protect satisfactory against wheat leaf rust elsewhere. The ranking of cultivars is not much influenced by the environment. Selection for PR can therefore take place in

a wide range of environments. Broers (1989d) recommended to use environments with suboptimal conditions for wheat leaf rust as resistance is expected to be better expressed and differences between cultivars to be larger than under conditions conducive to the pathogen. Artificial infection will probably be necessary to start the epidemic in such an environment. The experiments at the Wageningen locations (sub-optimal for wheat leaf rust) showed that this way of starting an epidemic gave satisfactory results.

REFERENCES

Broers, L.H.M., 1989a. Influence of development stage and host genotype on three components of partial resistance to leaf rust in spring wheat. Euphytica, in press.

Broers, L.H.M., 1989b. Partial resistance to wheat leaf rust in 18 spring wheat cultivars. Euphytica, in press.

Broers, L.H.M., 1989c. Race-specific aspects of partial resistance in wheat to wheat leaf rust, *Puccinia recondita* f.sp. *tritici*. Euphytica, in press.

Broers, L.H.M., 1989d. Influence of post-infection temperature on three components of partial resistance in wheat to wheat leaf rust. Euphytica, in press.

Broers, L.H.M. and Th. Jacobs, 1989. The inheritance of host plant effect on latency period of wheat leaf rust in spring wheat. II: Number of segregating factors and evidence for transgressive segregation in F3 and F5 generations. Euphytica, in press.

Johnson, R., 1984. A critical analysis of durable resistance. Annual Review of Phytopathology 22: 309-330.

Parlevliet, J.E., 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. I. Effect of cultivar and development stage on latent period. Euphytica 24: 21-27.

Parlevliet, J.E. and A. van Ommeren, 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. II Relationship between field trials, microplot tests and latent period. Euphytica 24: 293-303.

Parlevliet J.E. and A. van Ommeren, 1984. Interplot interference and the assessment of barley cultivars for partial resistance to leaf rust, *Puccinia hordei*. Euphytica 33: 685-697.

Chapter 15.

HOW TO SELECT FOR PARTIAL RESISTANCE IN WHEAT TO WHEAT LEAF RUST.

L.H.M. Broers and Th. Jacobs

How to select for partial resistance

Partial resistance (PR) is defined as the ability of the host to retard the epidemic growth of the pathogen despite of a high infection type (IT). A high IT (7 to 9; scale of McNeal *et al.*, 1971) means that at best chlorotic halos are present around the urediosori. Histologically, little or no autofluorescence should be visible around the infection structures. A high IT indicates the absence of effective hypersensitivity resistance (HR) genes in the genotype studied.

Partially resistant wheat genotypes have a longer latency period (LP), a lower infection frequency, smaller urediosori and lower rates of spore production compared to a highly susceptible genotype. It is hoped that PR to wheat leaf rust is durable in contrast to HR.

Based on the results of the preceding chapters a method is described here to screen for PR in wheat to wheat leaf rust (*Puccinia recondita* f.sp. *tritici*). This method contains three elements:

1) screening against low infection types;
2) assessment of PR in the greenhouse;
3) assessment of PR in the field.

Ad 1) An essential element of PR is the high infection type. Selection against low infection types (IT 7) should be very strict and only truly susceptible genotypes should be considered for further testing.

Testing should be done in the greenhouse. It is our experience that in the field PR and incomplete HR are very difficult to distinguish as both reduce the rate of epidemic development and the IT of PR-genotypes tends to decrease with time due to necrotisation of old urediosori (Chapter 16).

In the greenhouse, the seedling stage is recommended for a first screening. In seedling tests, adult plant resistances (low IT resistances that are only expressed in the adult plant stage) are overlooked. Selected genotypes should, therefore, be tested again in the adult plant stage.

As an inoculum source, a monospore culture of the race with the broadest virulence spectrum available should be used in all experiments (see point 2 and 3) to reduce the chance that interesting PR-genes are obscured by effective HR-genes.

Infection types should be observed 2 to 3 days after the moment that all colonies sporulate.

Ad 2) The most precise estimator for PR is the latency period (LP). This component of PR shows high correlations with PR as measured in the field and it is relatively easy to assess (Chapter 10, 11). LP is defined as the time between the start of infection and the onset of sporulation. There are two ways to measure the onset of sporulation. First, it can be determined by *counting* each day the number of urediosori visible in a marked area on the leaves (using a pocket-lens) until the number of urediosori no longer increases. The time at which 50 per cent of the *final number of urediosori* appeared is estimated by interpolation and considered as the start of sporulation (Chapter 7, 10). Second, the onset of sporulation can be determined by *estimating* the time at which 50 per cent of the *visible infections* (greenish flecks) are sporulating (Chapter 8). In both cases, the LP has to be taken as the time period from the beginning of incubation to the time at which 50 per cent of the final number urediosori respectively of the visible infections appeared. The first method is suitable for precise

Table 15-1. Comparison of seedling and adult plant tests on latency period.

Aspect	Seedlings	Adult plants
sufficient expression	at low temperatures (e.g. 8-13 °C)	at moderate temperatures (e.g. 15-19 °C)
required equipment	growth chamber	greenhouse
experimental size	small	large
time	short	long
differences in development rate	of no importance	of high importance; requires special sowing scheme

measurements of LP on small numbers of plants. The second method is less precise but allows assessment of large numbers of plants e.g. segregating populations.

The expression of LP-prolonging genes is influenced by growth stage and temperature. Young flag leaves (plants just heading, growth stage DC 48-59; Zadoks *et al.*, 1974) show a better expression than seedling leaves (growth stage DC 11) (Chapter 10). The expression is better at low than at high temperatures (Chapter 13). To choose the suitable growth stage some aspects of seedling test and adult plant tests for LP are presented (Table 15-1). Seedling tests need low temperatures (8-13 °C) for a good expression of PR and, therefore, require a growth chamber. Adult plant tests can be carried out in the greenhouse at moderate temperatures (15-19 °C), which are sufficiently low for a good expression of PR. Adult plant tests need more space and take more time than seedling tests.

LP varies with development stage. All plants should be inoculated in the same development stage, to reduce the experimental error. In seedling stage differences in development rate are of no importance. However, adult plants of different genotypes may not reach the young flag leaf stage (growth stage DC 48-59; Zadoks *et al.*, 1974) at one and the same time due to differences in development rate. Therefore, they cannot be inoculated simultaneously in the same young flag leaf stage. To overcome differences in development rate the following procedure can be used. Over a period of several weeks susceptible and partially resistant standard cultivars are sown at weekly intervals. All genotypes to be tested are sown at once about half way this period. Inoculations are carried out in sequential series each including testgenotypes and standards that are, per series, in the same young flag leaf stage (DC 48-59). The standards are added to each series to enable comparison of the different inoculation series.

Irrespective of the growth stage that is used to screen for LP, it should be stressed that always sufficient susceptible and partially resistant standard cultivars should be included in the tests for mutual comparison of different series. Furthermore, IT should be observed again very carefully to be certain that a prolonged LP is not due to a gene that conditions incomplete HR. Thus, entries with low IT should be removed.

ad 3) Several methods are available to assess the level of disease severity of genotypes. The methods are on different epidemiological parameters, different experimental designs and different assessment scales (Chapter 11). In the case of wheat and wheat leaf rust, screening tests with a high disease pressure such as small adjacent plots and race nurseries (using the Peterson Scale (0-100%); Peterson *et al.*,1948) gave results similar to those of screen-

ing tests with a low disease pressure such as isolated plots (using the more precise scale of Parlevliet and van Ommeren, 1984). For economical reasons, small plots are to be preferred. The susceptible and partially resistant controls should be included regularly in field experiments, and comparisons should be made to the nearest check, as the disease pressure may be heterogeneously distributed over the field. If artificial inoculation is necessary to initiate an epidemic, it is recommended to do this with spreader plants early in the growing season (e.g. at tillering). In our experience, this method gives good results.

A satisfactory disease assessment in the field can be obtained from one scoring if it is done at the moment that the susceptible control cultivars are severely rusted (about 75 to 95%, Chapter 11)). Area under the disease progress curve can also be used but needs more assessments. The logistic growth rate is not recommended because in our experiments it was a poor estimator of PR.

The disease assessment should include at least the upper three leaves (including the flag leaf). Assessments on the flag leaf only are more sensitive to differences in development rate. Moreover, assessing on three leaf layers reduces the experimental error because a larger number of leaves is assessed.

Selection of genotypes with a retarded epidemic build-up (low disease level) does not automatically imply that the selected genotypes are partially resistant. Both genotypes with incomplete hypersensitive resistance (HR) and with PR are able to retard the epidemic. To differentiate between HR and PR, infection type data should be used. The problem is that infection type data recorded in the field are rather ambiguous. Incomplete HR will show an IT of 4 to 6. PR should give an IT of 7 to 9. In the field, however, old urediosori tend to become necrotic and thus the infection type will decrease from 7 to 9 to 4 to7 (Chapter 16). It is obvious that PR and incomplete HR are very difficult to separate in field tests. Examination of the IT in the greenhouse is necessary to distinguish between PR and incomplete HR. Genotypes with a very low IT can be removed and do not need an examination in the greenhouse.

A field test also encounters the difficulty of differences in development rate. Selection for PR neglecting the differences in developmental rate, will result in selection for lateness as late genotypes are less attacked at a certain moment in time (Chapter 11). To take differences in development rate into account, the field test should contain susceptible and partially resistant standard cultivars that both cover the range of development rates observed in the test genotypes. The test genotypes and standards should be classified according to development rates. Selection of genotypes with a reduced level of disease can be carried out within groups of the same development rate.

Using the preceding three elements (not necessarily in the order mentioned), a breeder can successfully screen his material for PR. When a breeding program for partial resistance is initiated it is essential that the parents, that will be used in the crosses, are selected very carefully considering all three elements. Otherwise the program may fail to meet its objectives. A practical procedure for a breeder can be as follows. Based on observation of IT and disease levels of many genotypes in artificially infected small plots in the field, genotypes are selected with IT4-6 and a disease level lower than that of the susceptible control. Completely disease-free genotypes should be excluded as they may possess complete HR. In the period following the field observations, seedlings and adult plants of the selected genotypes can be screened for IT and LP in the growth chamber or greenhouse. Genotypes with a high IT

(7 to 9) and a LP longer than that of the susceptible control can be used in a crossing program. This completes the first and perhaps the most important step in breeding for PR: identification of parental genotypes with PR by careful exclusion of hypersensitivity resistance and selection for long LP.

References

McNeal, F.M., C.F. Conzak, E.P. Smith, W.S. Tade and T.S. Russel, 1971. A uniform system for recording and processing cereal research data. USDA Agr. Res. Surv. Washington DC, ARS 34-121. 42Pp.

Parlevliet, J.E. and A. van Ommeren, 1984. Interplot interference and the assessment of barley cultivars for partial resistance to leaf rust, *Puccinia hordei*. Euphytica 33: 685-697.

Peterson, R.F., A.B. Campbell and A.E. Hannah, 1948. A diagrammatic scale of estimating rust intensity on leaves and stems of cereals. Can J Res C26: 496-500.

Zadoks, J.C., T. Chang and C. Konzak, 1974. A decimal code for the growth stages of cereals. Weed Research 14: 415-421.

Chapter 16.

GENERAL DISCUSSION

Th. Jacobs and L.H.M. Broers

Comparison of PR in wheat and barley to their respective leaf rusts

In wheat, the histological data showed that the genes for partial resistance (PR) present in the spring wheat genotypes Westphal 12A and Akabozu operated at two stages during the development of wheat leaf rust infection structures. The first noticeable effect was a reduction of the infection frequency. Several infection structures were probably obstructed by cell wall appositions and did not form haustoria. They were aborted. The second effect of PR genes was on established colonies. After the first haustorium was formed a difference was observed in number of haustoria and growth rate between susceptible and partially resistant wheat genotypes. This difference could not be explained by the presence of cell wall appositions. Possibly, a post-haustorium mechanism operated in the PR genotypes studied. The nature of the mechanism remained unknown. Microscopic observations showed that haustoria in the PR genotypes were not encapsulated.

In barley, the decisive mechanism of PR to barley leaf rust was a pre-haustorium exclusion of the fungus by cell wall appositions. These cell wall appositions were formed immediately after formation of the first haustorium mother cells (HMC), thus leading to early abortion of infection structures. In barley, the cell wall appositions were also formed during a prolonged period after the formation of the first HMC, leading either to late abortion of infection structures or to an initial delay in the growth of established colonies (Arntzen and Parlevliet, 1986; Niks, 1982; 1986).

It is astonishing to see that the mechanism of partial resistance in the barley/barley leaf rust interaction and the wheat/wheat leaf rust interaction differ so much. Both the plant and fungus species are closely related. The partial resistance in both interactions shows a reduced infection frequency, a longer latency period, smaller colony sizes and smaller sporulating areas compared to highly susceptible checks. Despite similarities, the pathosystems tend to show unique characteristics. Therefore, one should be very careful to transfer concepts from one pathosystem to another even if the hosts or the pathogens are closely related.

Induction, generalizing the specific, can be very misleading and requires verification.

Genetics

The prolongation of latency period in the spring wheats studied appeared to be coded by two or three genes. Similar numbers of LP-prolonging genes and genes decreasing the disease severity have been found in wheat leaf rust infected winter wheat (Bjarko and Line, 1988; Lee and Shaner, 1985). This could indicate that the oligogenic inheritance of partial resistance in wheat to wheat leaf rust is a general phenomenon. The number of genes leading to a similar prolongation of LP in partially resistant wheat clearly deviates from the number observed in partially resistant barley (Parlevliet, 1978b).

In our study, the LP-prolonging genes were inherited in a (partially) recessive way. Gene action was mainly additive. In the two winter wheat studies cited above the inheritance of the gene effect also showed a (partially) recessive inheritance and showed mainly additive gene action. The recessive inheritance of PR and additive gene action seems to be a general phenomenon of PR to wheat leaf rust in wheat (Bjarko and Line, 1988; Lee and Shaner, 1985; Chapter 7).

It should be stressed that neither the oligogenic inheritance nor the recessive way of inheritance can be interpreted as evidence for durability of partial resistance.

In our study transgressive segregation for longer latency period was observed. The spring wheat genotypes studied contained partly different LP-prolonging genes. The accumulation of these genes looks rewarding. If the genes for longer latency period in spring and winter wheats also differ, their combined presence could lead to practically disease-free partially resistant genotypes.

Components of PR

Latency period (LP), infection frequency (IF), and urediosorus size (US) are three components of PR in wheat to wheat leaf rust. LP appeared to be a very important factor in determining the disease levels observed in the field (Chapter 10). Data obtained from winter wheat also indicated the importance of LP in the epidemic development of wheat leaf rust (Shaner and Finney, 1980). Simulations of wheat leaf rust epidemics confirm these results (Teng *et al.*, 1977; Zadoks, 1971). A clear growth stage effect on the components was detected (Chapter 10). Generally, LP was longer, IF was lower and US was smaller on young flag leaves than on primary leaves. In winter wheat/wheat leaf rust (Ohm and Shaner, 1976; Pretorius et al., 1988) and barley/barley leaf rust (Parlevliet, 1975; Parlevliet and Kuiper, 1977) similar results were obtained. The ranges for LP, IF and US were of comparable size to the ranges observed in winter wheat (Ohm and Shaner, 1976; Shaner *et al.*, 1978), and to ranges observed in barley/barley leaf rust (Parlevliet, 1975; Parlevliet and Kuiper, 1977).

The components LP, IF and US varied in an associated way. A longer LP, a reduced IF and a smaller US tended to go together. However, some cultivars deviated significantly from this pattern. The presence of deviating genotypes indicates that in some cultivars different genetic factors control the components. This is at variance with observations in winter wheat (Lee and Shaner, 1985) and barley/barley leaf rust (Parlevliet, 1986) where LP and US, and LP and IF, respectively, seemed to be coded for by the same or by closely linked genes. However, i) the different reaction to temperature of LP and IF (Chapter 13), ii) the fact that combining LP and IF in a multiple regression equation resulted in a better explanation of the variation of disease severity than either LP and IF alone and iii) the observation that IF and LP seemed to be governed by different mechanisms (Chapter 4), support the idea that, at least in some genotypes, the genes for a prolonged LP and genes for a decreased IF are not necessarily the same or closely linked.

PR in the field

Disease severity (DS) at the time that the susceptible control is severely infected, was a reliable and economically justifiable epidemiological parameter to estimate PR in the field. The Peterson Scale (Peterson *et al.*, 1948) used to assess DS was satisfactory at the levels of PR now available. When higher levels of PR are obtained, more detailed observations and the use of the scale of Parlevliet and van Ommeren (1984) may be necessary to differentiate between genotypes with high levels of PR. In such cases, experimental designs are required that provide very high disease pressure.

In our study, the logistic growth rate appeared to be unsuitable as estimator for PR in the field. The logistic growth rate showed low correlations with the components LP, IF and US and with the epidemiological parameters, DS and area under the disease progress curve

(AUDPC). Data obtained by Rees *et al.* (1979) also showed that the ranking based on logistic growth rate differed largely from the ranking according to disease severity. A possible explanation for the discrepancy between the logistic growth rate and the other epidemiological parameters, DS and AUDPC, can be that the initial inoculum varied from plot to plot. The variation in initial inoculum was more pronounced in isolated plots. Rouse *et al.* (1981) showed that the epidemic growth rate was negatively associated with the initial inoculum. This negative association could have interfered with the resistance of a particular genotype and could have led to a wrong estimate of the resistance level of that genotype based on the logistic growth rate data.

There may be another reason for the discrepancy between the logistic growth rate and other epidemiological parameters. To obtain the logistic growth rate, data are logistically transformed assuming that: i) the organisms are distributed uniformly in space, ii) the environment is uniform in space and constant in time, iii) all organisms react in the same way at any given time regardless of development stage and iiii) the growth rate of the rust population declines linearly with population size. Clearly, some of the assumptions are incorrect. The environment, for instance, varies with time and the host reaction changes with its development.

PR was studied in the field under low and high disease pressure, representing commercial wheat production and breeder's fields, respectively. The ranking order of the genotypes studied was similar indicating that the best genotypes in breeders' fields (small plots, high inoculum press) also perform best in farmers' fields (large fields, low inoculum pressure). Therefore, selection for PR in the field can be done in small plots.

In the field, differences in development rate of plant genotypes can obscure real differences in PR. Comparison of early and late cultivars that were equally susceptible showed that late cultivars had less disease when assessed at the same time (same number of days after sowing). Late cultivars had more disease when assessed at the same development stage (same number of days after heading). Therefore, susceptible and PR-genotypes that cover the full range of development rates should be used for comparison during screening experiments in breeding programs. Comparison of genotypes should be carried out within groups of genotypes with similar development rate and not between such groups.

Race-specificity

In PR most variation was of the "horizontal type" sensu Van der Plank (1963). However, small significant race-specific interactions have been found in field experiments (Chapter 12). The interactions were of similar size as those found in barley/barley leaf rust (Parlevliet, 1978a). In the data of Kuhn *et al.* (1978), who tested 22 isolates on three winter wheat genotypes in the seedling stage, significant race-specific effects can be found for latency period. These results suggest a gene-for-gene relationship for PR in wheat to wheat leaf rust and support the "integrated concept" of Parlevliet and Zadoks (1977). The presence of race-specific effects does not imply that the resistance is not durable nor the opposite. Parlevliet (1983) developed a model that can explain the durability of PR despite of a gene-for-gene-relationship. This model assumes that if recognition exists between pathogenicity genes in the pathogen and susceptibility genes in the host, the interaction will be compatible. Resistance can only be overcome by a gain mutation, neutralizing the effect of the resistance in the host.

Parlevliet (1983) assumed that gain mutations are difficult to produce and therefore the resistance based on this model is expected to be durable.

The size of the race specific interaction observed in the field experiments was relatively small. If a gene for longer latency period would have been defeated by the pathogen a larger increase of disease severity is expected as LP-prolonging genes have a large effect. This suggests that genes other than LP-prolonging genes influence the epidemic development, for instance IF reducing genes. The component analysis (Chapter 10) showed that it cannot be excluded that separate genes for longer LP and reduced IF are present in PR genotypes. Due to the large variability of IF, the inheritance of IF is difficult to study. Therefore, evidence supporting or contradicting this idea will be hard to obtain.

Comparison of incomplete HR and PR

At the time we started the work reported in this thesis the idea prevailed that there was a clear-cut difference between hypersensitivity resistance (HR) and partial resistance (PR). This was plausible. In barley the two types of resistance can be distinguished fairly easily and are based on different mechanisms (work by Parlevliet, Niks and others).

During this study several similarities between HR and PR in wheat to wheat leaf rust emerged, indicating that HR and PR of wheat to wheat leaf rust were less easy to distinguish than in barley/barley leaf rust. The similarities can be summarized as follows:

1) In wheat, both incomplete HR and PR to wheat leaf rust are of a post-haustorial nature and reduce growth of fungal colonies. Cells of a host carrying incomplete HR collapse after the fungus has formed a haustorium (Heath, 1974). The cell collapse leads to autofluoresence (AF) and is accompanied by colony growth retardation (Tomerlin *et al.*, 1984). The growth retardation of fungal colonies in partially resistant genotypes, most likely, starts after the formation of the first haustorium. In partially resistant genotypes the extensive host cell collapse and AF do not occur.

2) A small number of genes is reported to be involved in the inheritance of HR and PR. HR is mostly monogenic or digenic and most HR-genes inherit in a dominant way. In PR-genotypes, one to three genes governed a prolonged LP, but they inherit in a recessive way (Chapter 7 and 8; Lee and Shaner, 1985)

3) The expression of HR and PR is temperature sensitive. Several H.R.-genes lead to incompatibility at low temperatures and to compatibility at high temperatures, other HR-genes show a reversed temperature sensitivity (Browder, 1980). The effect of LP-prolonging genes is much more pronounced at low temperatures.

4) Incomplete HR and PR have the same epidemiological effect. They reduce the epidemic build-up, prolong LP and reduce IF (Chapter 10 and 11; Eversmeyer *et al.*, 1980).

In addition to the similarities between HR and PR, difficulties were encountered to distinguish incomplete HR and PR based on the definition of PR. Genotypes showing an intermediate or high IT were hard to classify as HR or PR as factors other than resistance influenced the infection type. Several adult plant resistance gene do not lead to a low IT in seedlings (Browder, 1980). On the other hand not all HR seedling genes are expressed in adult plants (Overlaet, 1963). In the greenhouse the IT in flag leaves of a genotype can be high at low temperatures and lower at high temperatures (BH 1146, Table 16-1). The IT of adult plants can be high in the greenhouse but lower in the field. This has been observed in adult plants

Table 16-1. Changes in infection type in some wheat-wheat leaf rust combinations due to various factors.

Genotype	Race	Infection type
		0 1 2 3 4 5 6 7 8 9
		>30 °C <30 °C*
BH 1146	All	←——→
		F G**
Ze-64-1-14	Flamingo	←——→
		G F
Kaspar	Felix	←——→
		o y***
Akabozu	All	←——→

*temperature in °C; **G=greenhouse; F=Field; *** o=old urediosori; y= young urediosori.

of Ze-64-1-14 with the 'Flamingo' race. The opposite effect, high IT in the field, low in the greenhouse was observed in Kaspar with the 'Felix' race. This discrepancy between greenhouse and field data can probably be attributed to environmental dependent expression of HR genes (e.g. temperature, day-length, light intensity). The IT of colonies sporulating for a while tends to be lower than the IT of just sporulating colonies. This has been observed in leaves of partially resistant genotypes in the greenhouse and in the field (Akabozu, Table 16-1). In some genotypes (BH 1146, Ponta Grossa 1), a high infection type was observed in adult plants in the greenhouse. Microscopical observations of these genotypes showed a large amount of aborted infection structures with autofluorescence. In both genotypes a HR gene (presumably Lr 13) is present.

It is clear that under certain conditions an intermediate IT can be observed on leaves of partially resistant genotypes. On the other hand a high IT not necessarily excludes HR. The distinction between PR and HR cannot be made by a single determination of IT but requires a careful investigation. Otherwise undesired HR may be selected.

Stability and durability

Stability and durability are two different characteristics of PR. Stability refers to expression under a range of environments. Resistance is stable if it is expressed in a wide range of environments despite of differences in temperature, day length or other environmental variables. Durability refers to the biological expression of resistance. A cultivar possesses durable resistance if the cultivar has been grown commercially at a large scale for a long time in an environment conducive to the disease, without a major loss of the effectiveness of the resistance due to adaptation of the pathogen.

PR in wheat to wheat leaf rust can be considered as a stable form of resistance as it was expressed in a wide range of environments (Brazil, Mexico, The Netherlands) and the ranking of the cultivars studied was hardly affected by different environments. The level of ex-

pression of PR was affected by environmental conditions. This agrees with the greenhouse observations (Chapter 13) that the expression of LP depends on the post-infection temperature. Differences in temperature between years and/or between locations may easily explain the observed differences in the level of PR at the various locations. The temperature sensitivity of LP-genes in wheat does not need to be the cause for the differences in level of PR between years and between locations. PR in barley to barley leaf rust showed similar variations in its expression (Parlevliet and van Ommeren, 1984) although the PR-genes in this pathosystem do not show any temperature sensitivity (Parlevliet, 1975).

Stability is not necessarily an indicator of durability. CIMMYT triticales proved to be resistant to stem rust when tested in widely different areas over several years. However, shortly after their introduction in Australia the resistance was defeated by a new race of the stem rust pathogen. The resistance appeared to be governed by a single gene (Sr27) (McIntosh, 1988)..

In the case of wheat leaf rust, evidence is present that PR in wheat is durable. BH 1146, has been grown commercially since 1955 in Brazil under conditions that are favourable to wheat leaf rust. Its resistance, recognized as being of the PR-type, is still effective in 1988 and it can be said to be durable. This is a retrospective conclusion. It is not correct to relate the durability of a resistance to the occurrence of stability.

References

Arntzen, F.K., and J.E. Parlevliet, 1986. Development of barley leaf rust, *Puccinia hordei*, infections in barley. II: importance of early events at the site of penetration for resistance. Euphytica 35: 961-968.

Bjarko, M.E., and R.F. Line, 1988. Quantitative determination of the gene action of leaf rust resistance in four cultivars of wheat, *Triticum aestivum*. Phytopathology 78: 451-456.

Browder, L.E., 1980. A compendium of information about genes for low reaction to *Puccinia recondita* in wheat. Crop Science 20: 775-779.

Eversmeyer, M.G., C.L. Kramer and L.E. Browder, 1980. Effect of temperature and host:parasite combination on the latent period of *Puccinia recondita* in seedling of wheat plants. Phytopathology 70: 938-941.

Heath, M.C., 1974. Light and electron microscope studies of the interactions of host and non-host plants with cowpea rust-*Uromyces phaseoli* var. *vignae*. Physiolological and Plant Patholology 4: 403-414.

Kuhn, R.C., H.W. Ohm and G.E. Shaner, 1978. Slow leaf rust severity in wheat against twenty two isolates of *Puccinia recondita*. Phytopathology 68: 651-656.

Lee, T.S. and G. Shaner, 1985. Oligogenic inheritance of length of latent period in six slow leaf-rusting wheat cultivars. Phytopathology 75: 636-643.

McIntosh, R.I, 1988. The role of specific genes in breeding for durable stem rust resistance in wheat and triticale. In: Breeding strategies for resistance to the rusts of wheat. Eds: N.W. Simmonds and S. Rajaram. Mexico, D.F., CIMMYT. Pp 1-9.

Niks, R.E., 1982. Early abortion of colonies of leaf rust, *Puccinia hordei*, in partially resistant barley seedlings. Canadian Journal of Botany 60: 714-723.

Niks, R.E., 1986. Failure of haustorial development as a factor in slow growth and development of *Puccinia hordei* in partially resistant barley seedlings. Physiolgical and Molecular Plant Pathology 28: 309-322.

Ohm, H.W. and G.E. Shaner, 1976. Three components of slow rusting at different growth stages in wheat. Phytopathology 66: 1356-1360.

Overlaet, J., 1963. De roestziekten van tarwe in de kultuurvoorwaarden van Belgie: opzoekingen in fysiologische specialisatie, resistentie en epidemiologie. Dissertatie Leuven. 217 Pp.

Parlevliet, J.E., 1975. Partial resistance of barley to leaf rust, *Puccinia hordei*. I. effect of cultivar and development stage on latent period. Euphytica 24: 21-27.

Parlevliet, J.E., 1978a. Race-specific aspects of polygenic resistance of barley to leaf rust, *Puccinia hordei*. Netherlands Journal of Plant Pathology 84: 121-126.

Parlevliet, J.E., 1978b. Further evidence of polygenic inheritance of partial resistance in barley to leaf rust, *Puccinia hordei*. Euphytica 27: 369-379.

Parlevliet, J.E., 1983. Models explaining the specificity and durability of host resistance derived from the observations on the barley-*Puccinia hordei* system. In: Durable Resistance in Crops. Eds: F. Lamberti, J.M. Waller and N.A. van der Graaf. Plenum Publishing Corporation. Pp 57-78

Parlevliet, J.E., 1986. Pleiotropic association of infection frequency and latency period of two barley cultivars partially resistant to barley leaf rust. Euphytica 35: 267-272.

Parlevliet, J.E. and H.J. Kuiper, 1977. Partial resistance of barley to leaf rust, *Puccinia hordei*. IV. effect of cultivar and development stage on infection frequency. Euphytica 26: 249-255.

Parlevliet, J.E. and A. van Ommeren, 1984. Interplot interference and the assessment of barley cultivars for partial resistance to leaf rust, *Puccinia hordei*. Euphytica 33: 685-697.

Parlevliet, J.E., and J.C. Zadoks, 1977. The integrated concept of disease resistance; a new view including horizontal and vertical resistance in plants. Euphytica 26: 5-26.

Peterson, R.F., A.B. Campbell, and A.E. Hannah, 1948. A diagrammatic scale of estimating rust intensity on leaves and stems of cereals. Can J Res C26: 496-500.

Pretorius, Z.A., F.H.J. Rijkenberg and R.D. Wilcoxson, 1988. Effect of growth stage leaf position effects and temperature on adult-plant resistance of wheat infected by *Puccinia recondita* f.sp. *tritici*. Plant Pathology 37: 36-44.

Rees, R.G., J.P. Thompson and E.A. Goward, 1979. Slow rusting and tolerance to rusts in Wheat. II The progress and effects of epidemics in *Puccinia recondita tritici* in selected wheat cultivars. Australian Journal of Agricultural Research 30: 421-432.

Rouse, D.I., D.R. Mackenzie and R.R. Nelson, 1981. A relationship between initial inoculum and apparent infection rate in a set of disease progress data for powdery mildew on wheat. Phytopathologische Zeitschrift 100: 143-149.

Shaner, G.E. and R.E. Finney, 1980. New sources of slow leaf rusting resistance in wheat. Phytopathology 70: 1183-1186.

Shaner, G.E., H.W. Ohm and R.E. Finney, 1978. Response of susceptible and slow-leaf rusting wheats to infection of *Puccinia recondita*. Phytopathology 68: 471-475.

Teng, P.S. and M.J. Blackie and R.C. Close, 1977. A simulation of crop yield loss due to rust disease. Agricultural Systems 2: 189-198.

Tomerlin, J.R., M.G. Eversmeyer, C.L. Kramer and L.E. Browder, 1984. Environmental and host effects on colony development of *Puccinia recondita* f.sp. *tritici*. Phytopathology 74: 225-229.

Van der Plank, J.E., 1963. Plant diseases: Epidemics and Control. Academic Press, New York and London. 349 Pp.

Zadoks, J.C., 1971. Systems analysis and the dynamics of epidemics. Phytopathology 61: 600-610.

SUMMARY

Histology

Microscopical observations showed that there was no difference between susceptible and partially resistant genotypes in percentage germination, appressorium formation, substomatal vesicle formation nor in the percentage of infection structures that formed a first infection hyphae.

Partial resistance of wheat to wheat leaf rust appeared to act in two steps. In the first step, several infection structures were aborted. Probably due to the presence of cell wall appositions they were unable to form haustoria. This pre-haustorium exclusion leads to a decreased infection frequency (IF). In the second step, a reduction in size of established colonies was observed. The reduction was not caused by the presence of cell wall appositions nor by the encapsulation of haustoria. The mechanism is unknown but it is presumably of a post-haustorial nature. The growth reduction leads to longer latency periods (LP).

Genetics

Three partially resistant spring wheat genotypes with a long LP were crossed among each other and with the highly susceptible Little CLub. From the segregating generations it was concluded that Akabozu carries two and Westphal 12A three LP-prolonging genes. BH 1146 seemed to contain one gene for hypersensitive cell collapse and two or three genes for a prolonged LP. The LP-prolonging genes in Akabozu and Westphal 12A differed from each other, as transgression was observed in their progenies. At least some LP-prolonging genes of BH 1146 were different from those present in Akabozu.

The genes for a prolonged LP were inherited in a recessive or partially recessive way and showed additive gene action.

Epidemiology

PR measured as latency period (LP), infection frequency (IF) and urediosorus size (US) was better expressed in the young flag leaf stage than in the seedling stage. The LP measured in the flag leaf appeared to be relatively easy and accurate to measure. LP is a satisfactory estimator for PR as LP was well correlated with PR measured in the field (see below). To determine LP, relatively low temperatures should be used as differences in LP between genotypes increased with decreasing temperatures.

Three epidemiological parameters were used to assess PR in the field: 1) disease severity at the time that susceptible controls are severely rusted (75 to 95 per cent), 2) the area under the disease progress curve and 3) the logistic growth rate. The former two parameters are reliable estimators for PR. The latter, however, appeared to be unsuitable as it showed low correlations with the other two parameters and with the three components of PR mentioned above.

The components LP, IF and US showed an associated variation. Long LP, low IF and small US tended to go together. Some genotypes deviated from the observed pattern, Ponta Grossa 1, for instance, had an IF which was to low relative to its LP and the disease severity of

Ponta Grossa 1 was too low compared to its LP. The results indicate that the components of PR are at least in some cases controlled by different genes.

Race-specificity

Conclusive evidence for or against race-specificity of PR has not been obtained. In the field, one significant and reproducible cultivar-race interaction was observed. In the greenhouse, measuring LP, also significant cultivar-race interactions have been detected. However, these interactions were not reproducible. Therefore, it is not clear whether cultivar-race interactions do occur regularly and what their significance is with respect to durability.

Stability and durability

Irrespective of the temperature sensitivity of the expression of LP, PR was expressed in a wide range of environments (Brazil, Mexico, the Netherlands) indicating that PR is stable. The ranking orders were similar at all locations and years. Differences in the level of PR were observed among years and locations. The results indicate that the levels of PR in the genotypes studied, might be insufficient under conditions very conducive to wheat leaf rust. The PR studied might be durable. The PR in one of the cultivars studied (BH 1146) is still effective notwithstanding the commercial use of that cultivar since 1955 on a large scale in environments conducive to wheat leaf rust.

SAMENVATTING

Histologie

Uit mikroskopische waarnemingen bleek dat er geen duidelijke verschil bestond in de percentages kieming, vorming van het appressorium, van het substomatale blaasje en van de eerste infektiehyphe tussen vatbare en partiëel resistente genotypen.

Partiële resistentie in tarwe tegen bruine roest leek uit 2 fasen te bestaan. In de eerste fase trad abortie van infektiestrukturen op. Een aantal infektiestrukturen vormde geen haustorium. Dit werd waarschijnlijk verhinderd door materiaal dat aan de celwand werd toegevoegd. Het verschijnsel kan bestempeld worden als een pre-haustoriale werking en leidt tot verlaging van de infektiefrequentie (IF).

In de tweede fase werd de groei van gevestigde kolonies vertraagd. De groeivertraging werd niet veroorzaakt door het verhinderen van penetratie en evenmin door inkapseling van haustoria. Het mechanisme is onbekend, maar waarschijnlijk post-haustoriaal van aard. De groeivertraging had een verlenging van de latentieperiode (LP) tot gevolg.

Genetika

Drie partiëel resistente zomertarwerassen met een lange latentieperiode (LP) werden onderling en met het zeer vatbare ras Little Club gekruist. Uit de LP gegevens van de splitsende generaties werd afgeleid dat Akabozu twee en Westphal 12A drie genen voor een langere latentieperiode bevatten. In BH 1146 lijkt een gen voor over-gevoeligheidsresistentie aanwezig te zijn naast twee of drie genen die de LP verlengen. De genen in Akabozu en Westphal 12A verschillen van elkaar, in de nakomelingschappen trad transgressie op. BH 1146 en Akabozu hebben een of meer genen die van elkaar verschillen. De genen voor langere LP erven (gedeeltelijk) recessief over en vertonen duidelijk additieve effekten.

Epidemiologie

Partiële resistentie gemeten aan de hand van latentieperiode (LP), infektiefrequentie (IF) of uredosorusgrootte (UG), kwam beter tot expressie in jonge vlagbladeren dan in kiembladeren. De LP gemeten aan het vlagblad bleek een gemakkelijk en nauwkeurig te meten schatter te zijn om het niveau van PR te bepalen. LP gemeten in het vlagblad vertoonde een hoge korrelatie met PR gemeten in het veld. LP kan het best bij lage temperaturen bepaald worden omdat verschillen tussen genotypen toenemen met afnemende temperaturen.

Partiële resistentie in het veld is bepaald aan de hand van drie epidemiologische parameters: 1) de mate van aantasting op het moment dat de vatbare kontrole voor 75 tot 95 procent was aangetast, 2) de oppervlakte onder de kurve van het ziekteverloop en 3) de logistische groeisnelheid van de epidemie. De eerste twee parameters zijn goede schatters van PR gebleken. De logistische groeisnelheid bleek onbruikbaar als schatter voor PR vanwege een slechte korrelatie met de andere twee parameters en met de drie komponenten van PR.

De komponenten LP, IF en UG vertoonden een hoge onderlinge korrelatie. Een lange LP, een lage IF en een kleine UG bleken veelal samen te gaan. Enkele genotypen weken echter af van dit patroon. Ponta Grossa 1, bijvoorbeeld, had een te lage IF ten opzichte van diens LP. Ponta Grossa had tevens een aantasting die lager was dan verwacht werd op grond van diens LP. Deze resultaten duiden er op dat de komponenten van PR tenminste soms op verschillende genen berusten.

Fysiospecificiteit

De vraag of fysiospecificiteit een rol speelt by partiële resistentie, kan niet eenduidig worden beantwoord. In het veld, is een signifikante en herhaalbare ras-fysio interaktie gevonden. In de kas, waar LP gemeten werd, zijn ook signifikante ras-fysio interakties gevonden, maar deze waren niet herhaalbaar. De vraag blijft dus open hoe frequent ras-fysio interakties optreden en of deze interakties betekenis hebben voor de duurzaamheid van PR.

Stabiliteit en duurzaamheid

Ondanks het feit dat de expressie van LP genen temperatuur afhankelijk is, bleek dat PR in het veld effektief is in zeer verschillende milieu's (Brazilië, Mexico en Nederland). De rangordes van de rassen in de verschillende milieu's waren nagenoeg gelijk aan elkaar. Wel werden er verschillen in de mate van PR waargenomen tussen jaren en lokaties. Dit duidt er op dat de niveaus van PR die wij bestudeerd hebben soms ontoereikend zouden kunnen zijn om tarwe voldoende tegen bruine roest te beschermen. Over de duurzaamheid van PR kan alleen gemeld worden dat de vooruitzichten goed zijn omdat de PR van één van de gebruikte rassen (BH 1146) nog steeds effektief is ondanks dat het sinds 1955 op grote schaal wordt verbouwd in een milieu dat zeer geschikt is voor de ontwikkeling van bruine roest.

CURRICULUM VITAE

Theo Jacobs *(Theodorus Martin Gerardus Maria) werd op 16 oktober 1955 geboren in Meerlo, Noord-Limburg. Hij behaalde zijn Atheneum-B diploma in mei 1974. Van september 1974 tot januari 1983 was hij ingeschreven als Biologie-student aan de Landbouwhogeschool te Wageningen. Het verzwaarde hoofvak Populatie-genetika heeft hij in Liverpool Engeland uitgevoerd. Zijn keuzevakken waren Vegetatiekunde en Bodemkunde. Tijdens de praktijktijd was hij verbonden aan het Smithsonian Tropical Research Institute te Panama. Gedurende zijn studie heeft hij de onderwijsbevoegdheid behaald. Na enkele tijdelijke banen heeft hij een extra hoofvak Plantenveredeling voltooid. Van november 1984 tot november 1987 werkte hij als promotie-assistent bij de vakgroep Plantenveredeling, in Wageningen. Daarna schreef hij in samenwerking met Léon Broers dit proefschrift. Vanaf februari 1989 werkt hij als koördinator van het DGIS programma Duurzame Resistentie.*

CURRICULUM VITAE

Léon Broers *(Leonardus Hendricus Maria) werd op 12 december 1960 geboren in Kerkrade, Zuid-Limburg. Na 6 jaar Atheneum-B te hebben gevolgd aan het Antonius Doctor College in Kerkrade, behaalde hij zijn VWO-diploma in mei 1979. Van september 1979 tot september 1985 volgde hij aan de toenmalige Landbouwhogeschool de studie Plantenveredeling, met als doctoraalvakken Plantenveredeling, Fytopathologie en Erfelijkheidsleer. Hij bracht zijn praktijktijd door op het 'Centro Internacional de Mejoramiento de Mais y Trigo' (CIMMYT) in Mexico. Tijdens zijn studie is hij begonnen als promotie-assistent bij de vakgroep Plantenveredeling, waar hij van november 1984 tot november 1987 werkzaam was. Daarna heeft hij dit proefschrift geschreven in samenwerking met Theo Jacobs. Vanaf februari 1989 werkt hij als onderzoeksmedewerker van de Vakgroep Plantenveredeling aan een projekt in het kader van het DGIS programma Duurzame Resistentie met als standplaats Mexico.*