Promotor : dr.ir. J. Sneep, emeritus hoogleraar in de leer van de plantenveredeling.

Co-promotor : dr. J.C. Zadoks, hoogleraar in de fytopathologie.

Dit proefschrift is bewerkt onder leiding van dr.ir. J.E. Parlevliet, wetenschappelijk hoofdmedewerker aan de vakgroep Plantenveredeling.

Studies on the histology of partial resistance in barley to leaf rust, *Puccinia hordei*

R.E. Niks

Studies on the histology of partial resistance in barley to leafrust, *Puccinia hordei*

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C.C. Oosterlee,
hoogleraar in de veeteeltwetenschap,
in het openbaar te verdedigen
op vrijdag 18 maart 1983
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen.

Groot zijn de werken des HEREN,
na te speuren door allen die er behagen in hebben.
Majesteit en luister is zijn doen,
en zijn gerechtigheid houdt eeuwig stand.

Psalm 111 : 2,3

STELLINGEN

1. Het feit dat compatibiliteit uitzondering is in plant-schimmel combinaties wordt door Ward en Stoessl ten onrechte gebruikt als argument dat niet de overgevoeligheidsreactie, maar de compatibele reactie van een waardplant op een herkenningsreactie berust.

   Ward, E.W.B. & A. Stoessl (1976), Phytopathology 66: 940-941.

2. Parlevliet en Zadoks baseren hun bewering dat waard-pathogeen systemen die berusten op een gen-om-gen basis in de natuur uiteindelijk een stabiel evenwicht bereiken ten onrechte op het model van Mode.

   Mode, C.J. (1958), Evolution 12: 158-165.
   Parlevliet, J.E. & J.C. Zadoks (1977), Euphytica 26: 5-21.

3. Schaarste aan gegevens in de Middeleeuwse literatuur over desastreuze epidemieën in gewassen kan wijzen op een gunstig evenwicht tussen de toen geteelde landrassen en de pathogeen populaties.

   Orlob, G.B. (1971). Ann. Rev. Phytopath. 9: 7-20.

4. Het ontbreken van een statistisch significante cultivar x isolaat interactie in grootheden die gebruikt worden om de mate van resistentie te schatten, impliceert geen duurzaamheid van de resistentievorm.

   Kuhn, R.C., H.W. Ohm & G.E. Shaner (1978), Phytopathology 68: 651-656.

5. De opvatting dat avirulentie bij pathogenen als meeldauw en roestschimmels een "altruïstische eigenschap" is, die de coexistentie van het pathogeen met de waard regelt, is waarschijnlijk onjuist als de waardsoort een autogaam gewas is.

   Eshel, I. (1977), Theor. Popul. Biol. 11: 410-424.
   Parlevliet, J.E. (1981). Proc. Plant Breeding Symp. II, Iowa State Univ.: 309-364.

6. Bij taxonomisch onderzoek in Puccinia en Uromyces spp. dient ook de morfologie van het mycelium, zoals de vorm van het substomatale blaasje, betrokken te worden.

   Dit proefschrift.

7. Baker's conclusie dat panmixie in de F_2 generatie van een zelfbevruchtend gewas de selectiemogelijkheden in de F_3 duidelijk vergroot, is onjuist. De resultaten van het werk waarop hij zijn conclusie baseert, tonen aan dat panmixie op korte termijn weinig voordeel biedt.

   Baker, R.J. (1968), Crop Sci. 8: 547-550.

8. De bewering van Lewis, dat de fylogenie van het segmentatiepatroon van de vlieg redelijk goed begrepen is, is niet te verifiëren.

   Lewis, E.B. (1978), Nature 276: 565-570.

9. In ieder psychiatrisch ziekenhuis behoort een psychogeriatrisch circuit te bestaan, waarvan, naast een kliniek, ook een polikliniek deel uitmaakt.

10. Trimmen is een hondebaan.

Stellingen bij het proefschrift van R.E. Niks, getiteld "Studies on the histology of partial resistance in barley to leafrust (Puccinia hordei)", te verdedigen op 18 maart 1983 in de Aula van de Landbouwhogeschool, Wageningen.

Woord vooraf

Bij de voltooiing van dit proefschrift wil ik graag mijn dankbaarheid tot uitdrukking brengen aan allen die hebben bijgedragen aan de totstandkoming ervan.

In de eerste plaats ben ik dank verschuldigd aan mijn ouders, die mij op zo royale wijze in de gelegenheid hebben gesteld een studie aan de Landbouwhogeschool te volgen.

Mijn promotor, prof.dr.ir. J. Sneep, ben ik erkentelijk voor de mij geboden gelegenheid dit proefschrift aan het IvP te bewerken en voor zijn belangstelling voor mijn onderzoek.

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Mijn begeleider, dr.ir. J.E. Parlevliet, dank ik voor de vrijheid die hij me liet het onderzoek naar eigen inzicht uit te voeren. Zijn kennis maakte hem tot een waardevolle vraagbaak en ik ben hem erkentelijk voor zijn hulp bij het gereedmaken van dit manuscript voor de offset.

Zonder de hulp van Henk Kuiper zou dit werk niet alleen niet mogelijk, maar ook veel minder plezierig geweest zijn. Ik heb het erg gewaardeerd dat hij met enthousiasme een grote verscheidenheid aan werkzaamheden, waaronder honderden uren microscopie, heeft verricht.

In het kader van hun doctoraalstudie hebben Philippine Goettsch en Erik Jongedijk aan het onderzoek meegewerkt. Hun enthousiasme voor het werk deed me deugd.

Dr. M.S. Ramanna dank ik voor zijn stimulerende belangstelling en nuttige wenken m.b.t. de tekst van enkele van de manuscripten. Ik mocht profiteren van zijn rijke ervaring in het cytologische werk. Ir. Ies Bos gaf waardevolle hulp bij de statistische verwerking van de gegevens.

Wout Hoogkamer en Corrie Geerdts dank ik voor het beheren van de isolaten. Tony van Ommeren verschafte gegevens over de gebruikte isolaten. Luuk Suurs en Jan Molenveld voorzagen me van de benodigde chemicaliën, waarvoor mijn oprechte dank. De heren A. Florissen en

B. Navest ben ik erkentelijk voor de technische verwerkelijking van ideeën die tot doel hadden de gebruikte methoden te perfectioneren.

Annie Marchal ben ik dankbaar voor het uitstekend typen, niet alleen van de manuscripten, maar ook van de tussentijdse rapporten. Ook naar Rinia Baldew van de afdeling Tekstverwerking, die de definitieve versie van het proefschrift heeft getypt, gaat mijn dank uit.

De heer J.S. de Block corrigeerde de manuscripten op het Engels. De heer H.W. Sengers heeft op uitstekende wijze een eindeloze reeks foto's afgedrukt. De grafieken werden getekend door de heer W.C.T. Middelplaats (p. 23) en door de heer W.D.J. van der Vliet (p. 35). De heer A.M. Vroegop en collega's van de offsetdrukkerij van de Landbouwhogeschool verzorgden de produktie van "de boekjes".

Ook de overige, niet met name genoemde collega's van het IvP wil ik in mijn dank betrekken, daar ze een sfeer creëerden waarin het prettig werken was.

Tenslotte wil ik mijn vrouw, Marlien, hartelijk bedanken voor haar levendige belangstelling in het verloop van het onderzoek en voor haar waardevolle adviezen. Hiervoor, en voor nog veel meer, ben ik haar zeer dankbaar.

Contents

1. Appressorium formation of *Puccinia hordei* on partially resistant barley and two non-host species

by

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Accepted 30 July 1981

ABSTRACT

One of the components of partial resistance of barley to leaf rust, *Puccinia hordei*, is a reduced infectibility. It was investigated whether this low infectibility may rest on a hampered appressorium formation of the leaf rust fungus. The appressorium formation on the primary leaves of 11 barley genotypes with an intermediate-to-low infectibility was compared with that on the highly infectible L94. The number of stomata per cm^2 leaf area occupied by appressoria of *P. hordei* was determined per genotype by means of fluorescence microscopy. No consistent differences could be detected, indicating that the mechanisms causing a low infectibility of partially resistant barley seedlings act at a phase later than the formation of the appressoria. On the non-host wheat not fewer appressoria were formed than on L94, but no appressoria were found on a lettuce genotype. The latter probably lacks the stimuli that enable the fungus to find stomata.

Additional keywords: Hordeum vulgare, leaf rust, horizontal resistance.

INTRODUCTION

Little is known about the mechanisms that give plants horizontal resistance sensu Van der Plank (1968) to fungal pathogens, but it is supposed that various mechanisms, both active and passive, may underlie horizontal resistance (Robinson, 1976).

Partial resistance (PR) of barley (*Hordeum vulgare* L.) to leaf rust (*Puccinia hordei* Otth) can be regarded as a case of horizontal resistance: it is largely race non-specific and is controlled by polygenes (Parlevliet, 1977, 1978a). PR is manifested in a reduced rate of epidemic build-up in spite of a susceptible infection type (Parlevliet, 1978b). One of the factors responsible for the reduction of the epidemic build-up is the low infectibility of partially resistant genotypes (Parlevliet and Kuiper, 1977).

The aim of this study was to investigate whether low infectibility is caused by a hampered appressorium formation of the leaf rust fungus.

MATERIALS AND METHODS

Plant material. The pure lines from barley composite cross XXI (Suneson and Wiebe, 1962) show a large variation in infectibility to leaf rust (Niks and Parlevliet, 1979). Nine lines of low infectibility were used to investigate appressorium formation. These lines are designated C-lines. The barley cultivar Julia, which has an intermediate level of infectibility was also used. L94 and Vada, barley cultivars having a very high and very low infectibility respectively, were used as references. The genotypes were divided into two groups, since two flats (37 × 39 cm) were required to grow six seedlings per genotype. Three consecutive series with the twelve genotypes were grown.

In a second experiment the appressorium formation on non-hosts was studied. The wheat (*Triticum aestivum* L.) cultivars Duri, Adonis and Saratovskaja 210 (S210), representatives of the graminaceous non-hosts, and a genotype of lettuce (*Lactuca sativa* L.), a dicotyledonous non-host species, were compared with barley cultivar L94. In the first two replications each genotype was represented by six plants, grown in a flat. In the third and fourth replications the

lettuce was omitted, and the number of plants of the wheat cultivars and L94 was increased to ca. 12 per entry.

Inoculation. The primary leaves of the barley and wheat seedlings and the first true leaves of lettuce were inoculated when they had reached their final size. The lettuce was sown approximately two weeks before the barley and wheat. The leaves were pinned to the soil in the flats into a horizontal position with their adaxial sides up. Per flat, eight greased slides were added to check the density and distribution of the inoculum. This inoculum, urediospores of leaf rust isolate 121A, was applied in a settling tower (Eyal et al., 1968). After inoculation the flats were transferred to a greenhouse compartment. The urediospores were allowed to germinate and to form appressoria under natural darkness, while the relative humidity was kept at saturation point by means of an electric humidifier. As free water affects the regularity of the appressorium distribution on the leaves, a properly adjusted hygrostat and time-switch were connected to the humidifier. In the morning the leaves were covered with droplets as if with dew.

Sampling, staining and observation. Approximately 36 h after the onset of the infection process, a segment from the central part of each of the leaves was collected. The leaf segments were processed as described by Rohringer et al. (1977), but Blancophor BA 267% (Bayer, Leverkusen) was used instead of Calcofluor. Per leaf segment the occupied stomata per 25 random microscope fields (1.54 mm^2 each) were counted at × 125 magnification, using the Zeiss epifluorescence equipment NXL (Rohringer et al., 1977). The numbers of occupied stomata per 25 microscope fields were the experimental units for statistical analysis.

Stomatal densities were determined by screening five microscope fields (2.75 mm^2 each) per leaf at × 60 magnification, using a normal white light microscope.

RESULTS

Appressorium formation on host genotypes. The number of occupied stomata per 25 microscope fields was averaged over the six leaf segments per genotype and converted into densities per cm^2. About 2-5% of the occupied stomata carried two appressoria and one stoma was

found to have three. The distribution of the inoculum was even: the coefficient of variation was less than 10%. The inoculum density averaged 250 to 450 urediospores per cm^2.

Table 1. Relative number of stomata per cm^2 leaf area occupied by appressoria of Puccinia hordei on the primary leaves of 12 barley genotypes. Per series the grand mean has been set at 100%.

Barley genotype	Series I	II	III	$\bar{x}$	Barley genotype	Series I	II	III	$\bar{x}$
C-29	145	82	99	109	C-70	111	108	95	105
L94	127	100	98	108	Vada	102	114	95	104
C-92	81	116	107	101	L94	104	106	94	101
Vada	89	106	102	99	C-118	107	81	114	101
C-17	93	98	99	97	C-120	95	106	97	99
C-41	78	104	101	94	C-197	111	86	99	99
C-123	87	97	94	93	Julia	72	99	111	94
Mean number of occupied stomata per cm^2 (=100%)	164	129	283			194	104	287	
Genotype effect ($P \leq 0.05$, ANOVA)	yes	no	no			yes	no	no	

The percentages of urediospores giving rise to an appressorium were approximately 55, 40 and 63% for the respective replications.

The average numbers of occupied stomata are presented as relative values to the grand means in Table 1. The genotype effect was significant in the first series only (ANOVA, $P < 0.05$). This effect might have been caused by fortuitous factors, since in the second and third series not even a tendency towards a ranking of the genotypes similar to that in the first series was found. To investigate whether the genotypic differences in occupation of the stomata in the first series was attributable to differences in stomatal density, the number of stomata per mm^2 leaf area was determined. The stomatal densities in this series differed significantly between the genotypes indeed (ANOVA, $P < 0.01$), ranging from 25.3 (C-70) up

to 32.0 (C-118) stomata per mm² leaf area. The correlation with the number of occupied stomata per cm², however, was not significant ($r = -0.15$).

Appressorium formation on non-host genotypes. In the four replications with the non-host genotypes, approximately 17, 25, 28 and 33% of the urediospores produced an appressorium on the graminaceous genotypes.

Tabel 2. Relative number of stomata per cm² leaf area occupied by appressoria of Puccinia hordei on the primary leaves of one barley and three wheat cultivars and on the first true leaves of a lettuce genotype.
Per series the mean number of occupied stomata per cm² on L94 has been set at 100%.

Species	Cultivar	Series I	Series II	Series III	Series IV
Barley (host)	L94	100[a][1]	100[c]	100[a]	100[b]
Wheat (non-host)	Duri	132[a]	64[b]	127[a]	130[c]
	Adonis	112[a]	45[a]	138[a]	100[b]
	S210	102[a]	38[a]	118[a]	72[a]
Lettuce (non-host)		0	0	-	-
Mean number of occupied stomata per cm² on L94 (=100%)		84	88	112	127

[1] Per column different letters indicate a significant difference ($P \leq 0.05$) according to Duncan's multiple range test.

The appressorium formation on the three non-host wheat cultivars was not consistently less successful than on the highly infectible barley cultivar L94 (Table 2). On lettuce, however, no appressoria were found. On the typically jigsaw-puzzle-like epidermis of the dicotyledonous lettuce, the germ tubes grew randomly over the leaf surface, without finding stomata. On wheat, the germ tubes grew directionally towards the stomata as on barley. The graminaceous species equal each other in their structure of the leaf surface. Wheat cultivar S210, however, has a densely haired epidermis. In spite of

this, the appressorium formation on this cultivar was hardly lower than on the other genotypes. The wheat and barley genotypes differed little in the number of stomata per mm^2 leaf area. In the fourth series, the densities ranged from 28.4 (Adonis) to 31.6 (L94) stomata per mm^2.

DISCUSSION

In the search for possibly durable types of resistance to rust fungi, it is useful to investigate the different phases of the infection process in order to elucidate the resistance mechanisms that are operative (Zadoks, 1972). Here, the role of possible barriers during the germination and appressorium formation by *P. hordei* were studied.

There may already be a biochemical interaction between the plant and the germinating rust fungus (Ehrlich and Ehrlich, 1971). Grambow and Riedel (1977) demonstrated that *in vitro* differentiation of appressoria of *P. graminis* f.sp. *tritici* can be stimulated with certain substances extracted from wheat leaves. It is conceivable that cultivar differences in the quality or quantity of such compounds may result in host genotypic differences in the degree of appressorium formation by rusts. There are few accounts, indeed, that report on differences in appressorium formation by cereal rusts that are not based on apparent morphological differences. The reported differences, however, are small and erratic (Brown, 1968; Russell, 1976) or (Stubbs and Plotnikova, 1972) not reproducible (H.D. Frinking, pers.comm.).

Other studies indicate that the success of the germination and appressorium formation by rust fungi merely depends on morphological stimuli (Dickinson, 1970; Wynn, 1976). As a consequence, a normal germination and appressorium formation should occur on non-host species with a leaf surface structure similar to that of the host and little appressorium formation when the epidermal structure is different. This was actually reported (Heath, 1974; Wynn, 1976; Tani et al., 1978). Within a host species a reduced appressorium formation due to an aberrant epidermal structure (Mlodzianowski et al., 1978) or a reduced leaf wettability (Cook, 1980) may occur.

The present results show an irregular host genotype effect on the appressorium formation by *P. hordei* (Table 1). The data suggest that the host effects were rather caused by chance than by genetic differences for characters that influence the urediospore germination and appressorium formation. Therefore, the reduced infectibility of partially resistant barley is most likely caused by a resistance mechanism that is effective after the appressoria have been formed. Even the density of available stomata was not correlated with the degree of appressorium formation. This is understandable, since the number of available stomata was at least five times as high as the number of deposited urediospores and thus did not form a limiting factor.

Neither wheat nor lettuce show symptoms after inoculation with *P. hordei*. From the results (Table 2) it is clear that the 'resistance' of both species arises from different mechanisms, as a normal appressorium formation was found on wheat and no appressoria were found on lettuce. The non-infectibility of wheat to *P. hordei* apparently rests on a mechanism that is effective after the appressorium formation. These results agree with the findings of Tani et al. (1978). They reported that most of the rusts pathogenic on Gramineae are able to form appressoria on graminaceous non-host species, but fail to do so on dicotyldonous non-hosts. Apparently, the graminaceous hosts and non-hosts provide stimuli that enable a good appressorium formation, whereas on the dicotyledonous non-hosts appressorium formation is prevented, probably because the stimuli are lacking. The similarity of the epidermal structure of graminaceous leaves, which differs from the structure of dicotyledonous leaves, and the reported sensibility of rust germ tubes to morphological stimuli suggest that the success of appressorium formation of barley leaf rust on different species depends on the structure of the leaf surface. Less radical morphological differences such as hairiness (wheat cultivar S210) hardly affect the formation of appressoria.

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2. Early abortion of colonies of leaf rust, *Puccinia hordei*, in partially resistant barley seedlings

by

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ABSTRACT

Partial resistance (PR) in barley to leaf rust is assumedly a case of durable resistance. PR is characterized by a reduced rate of epidemic development in spite of a susceptible infection type. One of the components of PR, low infectibility, was studied histologically by means of fluorescence microscopy.

Quantitative analyses of the phases of the infection process beyond appressorium formation showed that the reduced infectibility of partially resistant barley seedlings rests on a significant 'early abortion' of colonies. This type of abortion occurs at about the moment of the formation of the first haustoria, when the young colonies have formed up to five or six haustorial mother cells. Early abortion is only incidentally associated with the collapse of host cells. Not only the variation in infectibility among barley genotypes but also the variation in infectivity among leaf rust isolates is based mainly on differences in the degree of early abortion. The occurrence of a high degree of early abortion in several unrelated barley genotypes indicates that the PR genes are part of a generally occurring system.

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INTRODUCTION

Hypersensitive resistance to biotrophic pathogens has been used widely by breeders because, owing to its simple inheritance and ease with which it is recognized, the character can be manipulated very well in breeding programmes. The important disadvantage of this type of resistance is that it is usually short lived. It is not surprising therefore that the attention is shifting from hypersensitive resistance towards other, more durable types of resistance. This shift, however, has not yet had a significant impact in the actual breeding programmes. Genotypes with non-hypersensitive types of resistance are less easy to select in the field and little is known about gene action and inheritance of the pertinent genes of the host/parasite system. A deeper insight into the principles underlying durable types of resistance may stimulate and facilitate the use of them in resistance breeding.

It is the aim of the present study to help enlarge insight into the nature of non-hypersensitive or partial resistance. The barley/leaf rust (*Puccinia hordei* Otth) relationship has been chosen as a model. The term partial resistance (PR) is used in the sense of Parlevliet (1978): "PR is characterized by a reduced rate of epidemic development in spite of a susceptible infection type": 7-9 on the scale of McNeal et al. (1971). The reduced rate of epidemic buildup results from the combined effect of several components: low infectibility, long latent period, low sporulation rate, and short infectious period. PR appears to be a durable type of resistance since many West-European barley cultivars have good PR to leaf rust although the genes have not been consciously introduced or selected for in recent times (Parlevliet et al. 1980; Parlevliet 1981).

The present study is confined to one component of PR: reduced infectibility. This aspect has been studied by Parlevliet and Kuiper (1977) at a macroscopic level. They measured the infectibility of several cultivars by determining relative infection densities on the leaves. They used the term 'infection frequency' for infectibility. Here the term infectibility is used, as it indicates the ability of a plant to become infected by a pathogen. Infectibility says that a character of the plant is at issue, while in-

fection frequency is a result of infectibility and density and quality of the inoculum.

Little is known about the histology of low infectibility. It has been suggested that there may exist several mechanisms which can block the infection process in different phases (Hayes 1973; Russell 1976; Zadoks and Schein 1979). If these mechanisms have a genetic basis, it should be possible to lower the level of infectibility by genetic recombination (Zadoks and Schein 1979). To determine in what stage of the infection process the defence system of the host becomes effective, a detailed histological analysis is required. Zadoks (1972) proposed to resolve the resistance into components by a quantitative analysis of phases in the infection cycle. So far, comprehensive and successful studies with such an approach have rarely (Zadoks and Schein 1979), if ever, been reported for cereal-rust relationships.

This paper reports when and in what proportions the colonies are arrested by PR before they reach the reproductive phase. The study has been carried out using the components analysis approach suggested by Zadoks (1972).

MATERIALS AND METHODS

Host genotypes

To be able to generalize on the histology of the low infectibility component of PR, it is essential to study a number of unrelated barley lines with a low infectibility but with a susceptible infection type. Former studies (Parlevliet 1976a; Niks and Parlevliet 1979) showed that the barley composite XXI contains such lines. The composite originates from the 6200 spring barleys in the American World Collection, gathered from all parts of the world (Suneson and Wiebe 1962). Since the composite has been multiplied in isolation for more than ten years, it can be considered a mixture of fairly homozygous lines. No artificial selection has been applied. A number of lines that were assumed to have an infectibility about as low as the partially resistant control cultivar (cv.) 'Vada' (Niks and Parlevliet 1979) were used in the present experiments. They are designated as C-lines. Their genetics regarding hypersensitivity genes and PR genes is unknown, but all lines used had a susceptible

infection type with isolate 121A, unless stated otherwise. The cultivars 'L94', 'Julia' and 'Vada', which show respectively a very high, intermediate, and low level of infectibility (Parlevliet and Kuiper 1977), were also included. All experiments concerned seedlings.

Pathogen isolates

In all experiments the isolate 121A was used, which is a monospore culture derived from isolate 1-2 (Parlevliet 1976b). In experiment 3, eight isolates were tested; their countries of origin and their putative alleles of avirulence are listed in Table 1.

Table 1. The assumed virulence/avirulence factors of the eight leaf rust isolates in experiment 3 regarding the known Pa resistance genes of barley.

Isolate	Country of orgin	Virulence/avirulence factors*
18	Holland	1,4/2,3,5,6,7,8,9
A	United Kingdom	1,4/2,3,5,6,7,8,9
201	Israel	2,4,6,8/1,3,5,7,9
22	France	2,4,6,8/1,3,5,7,9
9	Kenya	2,3,4,5,8/1,6,7,9
121A	Holland	1,2,4,5,6,8/3,7,9
F	United Kingdom	1,2,4,5,6,8/3,7,9
5	Israel	1,2,3,4,5,6,7,8/9

* Parlevliet and Van Ommeren, unpublished.

Isolate 5 is a monospore culture from the Israelian isolate T-46, which carries virulence towards almost all known hypersensitive resistance genes (Golan et al. 1978). The isolates were multiplied on adult plants of cv. 'L98'. The vials with inoculum were stored in liquid nitrogen at approximately -160°C.

Inoculation and incubation

The plants were sown in 37 x 39 cm flats in two or three parallel rows. After the complete unfolding of the primary leaves (about 14 days after sowing), the leaves were pinned to the soil in the flats, to bring them into a horizontal position with the adaxial

side facing upward so as to obtain an equal spore distribution over the leaves. Eight greased slides were placed among the leaves of each flat. With these the inoculum density and spread were determined by means of a microscope. A weighed quantity of urediospores was applied to each flat, using a settling tower, constructed according to the device of Eyal et al. (1968). The inoculum was applied by means of a 'Kabierske' powder blower mounted on a tube inserted 50 cm under the top of the cylinder of the settling tower. The other end of the tube with its funnel shaped opening directed down just reached the axis of the tower. The inoculum, mixed with Lycopodium spores, was gently blown down in the cylinder. After about 10 min the tower was opened and the flat was removed.

After inoculation the flats were transferred to a greenhouse compartment. The first night the relative humidity (RH) was kept at saturation point by means of an electric humidifier. A hygrostat and a time-switch were adjusted so as to prevent an excessive condensation which preliminary tests had shown to give erratic germination. After the first natural dark period (at least 10 h) the pins were removed from the leaves, and the plants were kept in the greenhouse at temperatures not exceeding 30°C. The RH was kept far below saturation point, so no germination of urediospores could occur after the first night.

Staining, preparation, and observation

Middle segments generally of 1 to 3 cm^2 of inoculated leaves were collected 2 to 4 days after the emergence of uredia and prepared as whole mounts for fluorescence microscopy (Rohringer et al. 1977). Instead of Calcofluor, Blancophor BA 267% (Bayer, Leverkusen) was used. Both are optical brighteners that stain the cell wall rather than the cytoplasm of fungi. The preparations were examined with a Zeiss epifluorescence equipment NXL (Rohringer et al. 1977) under x200 magnification, sometimes at x500 for observation of details. The infection units in the segments were scored and classified according to their stage of development. An infection unit is defined as the mycelial structure that originates from a urediospore (Zadoks and Schein 1979), but germ tubes that failed to form

an appressorium are not included here. Some structures became more visible when the regular white transmitted light of the same microscope was switched on. Replacement of filter BP 390-440 by H436 facilitated observation on necrosis of host cells, which display a golden yellow autofluorescence.

Macroscopically and microscopically determined infectibility (experiment 1)

An experiment was conducted to investigate the relationship between the macroscopically determined infection density, i.e., the number of uredia per cm^2 leaf area, and the microscopically observed colony abortion in stages after appressorium formation and to quantify abortion in the respective phases of development. Four host genotypes were studied: 'L94' and 'Vada' as controls and C-41 and C-118 as lines which were previously shown to be considerably less infectible than 'Vada' (Niks and Parlevliet 1979). Two flats, one with 7 or 8 seedlings and the other with about 20 seedlings per genotype were planted. The plants in the first flat were used to determine the infectibility of the genotypes microscopically. The plants in the second flat were inoculated without the use of the settling tower while the primary leaves were in upright position. These plants were used to determine the infectibility of the genotypes macroscopically, using the method of Parlevliet and Kuiper (1977). Per leaf the infection density was determined in a segment of at least 3 cm in length. The experiment was conducted in three consecutive series.

Qualification and quantification of colony abortion in seven barley genotypes (experiment 2)

The general validity of the results from experiment 1 was investigated by performing components analyses on six more C-lines: C-92, C-29, C-70, C-123, C-120 and C-197, which were thought to have low infectibility. Two flats were planted each with three of the lines and with 'Vada' as control. Each line was represented by eight leaves. Before sampling the leaf segments, the number of uredia per cm^2 of leaf area was determined. After preparation, each

of the eight leaf segments per line was scanned for 20 to 25 infection units. These were classified according to their stage of development. The experiment was conducted in two consecutive series.

Qualification and quantification of colony abortion of eight leaf rust isolates (experiment 3)

To verify whether results with other isolates would be consistent with those obtained with isolate 121A, an experiment with seven additional isolates of diverse origins (Table 1) was set up. Four flats were planted with 'Julia' and 'Vada' lacking hypersensitivity genes to any of the isolates under study. Each flat was divided in two, one half receiving the inoculum of one isolate, while the other was covered by a sheet of paper. After the appearance of uredia five leaf segments per cultivar/isolate combination were collected and processed as usual. Each segment was scanned as a whole; all infection units were scored and classified according to their stage of development. The experiment was conducted without replication.

The infection cycle of Puccinia hordei in barley

For a good understanding of the results, successive phases of fungal development are distinguished, as recognized by the morphological structures that are formed (Table 2). Infection units can be arrested in any phase of development, so that different types of abortion can be recognized (Table 2, Figs. 1-5).

RESULTS

Macroscopically and microscopically determined infectibility (experiment 1)

The average numbers of uredia per cm^2 of leaf area of the lines and the microscopically determined proportions of successful infection units were converted to relative values with corresponding infection data on 'L94' set at 100%. This enabled a comparison of the results between the series and the two methods. The experimental

Table 2. The phases of the infection process of leaf rust in barley, the mycelial structures that are formed therein and the designation of the types of abortion if the corresponding structure is the last one that is formed.

Phase of development	Successive mycelial structures	Designation of the types of abortion
Germination	Germ tube	-
Appressorium formation	Appressorium over stoma	Non-penetration
Stoma penetration and substomatal vesicle formation	Infection peg, substomatal vesicle (SSV)	SSV abortion
Establishment	First infection hyphae, haustorial mother cells (HMC)	Early abortion
Colonization	Hyphae and possibly sporogenic tissue	Late abortion
Reproduction	Mature urediospores (successful infection)	-

units for the statistical analyses were the infection density and the proportion of successful infection units per leaf. Genotypic differences were tested for significance using non-parametric tests (Siegel 1956).

Results of the macroscopically and microscopically obtained infectibility assessments (Table 3) are similar with regard to the ranking of the genotypes: 'L94' and C-41 are highly infectible and 'Vada' and C-118 are of about equally low infectibility. As to the size of the genotypic differences, the two methods are not equivalent: the differences between the genotypes for relative infection density are larger than for relative proportion of successful infection units.

Qualification and quantification of colony abortion in partially resistant barley (experiments 1 and 2).

The aborted colonies in experiment 1 were classified according to the phase of development in which their growth stopped (Table 4).

Table 3. Relative infectibilities of four barley genotypes with leaf rust isolate 121A, the infectibility of 'L94' being set at 100%, determined macroscopically and microscopically (experiment 1).

Infectibility measured as:	Barley genotype	Series I	Series II	Series III	Mean
Infection density†	'L94'§	100[a*]	100[a]	100[a]	100
	C-41	76[a]	61[a]	72[a]	70
	'Vada'	49[b]	33[b]	50[b]	44
	C-118	39[b]	19[b]	44[b]	34
Proportion successful infection units()	'L94'§§	100[a]	100[a]	100[a]	100
	C-41	100[a]	97[a]	99[a]	99
	'Vada'	71[b]	75[b]	68[b]	71
	C-118	77[b]	75[b]	74[b]	75

* Per subcolumn different letters indicate a significant difference ($P \leq 0.02$) according to the non-parametric Mann-Whitney test in pairs.

† About 80 seedlings per flat, inoculated without settling tower.

() About 32 seedlings per flat, inoculated in a settling tower.

§ On 'L94' the average infection density amounted to 11.8, 6.7, and 10.8 uredia/cm^2 in the respective series.

§§ On 'L94' the average proportion of successful infection units amounted to 0.90, 0.89, and 0.94 in the respective series.

The proportion of infection units aborted in one of the four phases of development (Tables 4,5; Fig. 6) has been calculated as follows. The number of infection units aborted in a given phase was divided by the total number of infection units that entered that phase.

The non-penetrating appressoria (Fig. 1) were easily recognized by the absence of a substomatal vesicle (SSV). A genotypic effect on the degree of non-penetration was not detected.

Only a low number of SSVs, sometimes surrounded by necrotic and collapsed host cells, failed to develop infection hyphae (Fig. 2). Thus SSV abortion does not show significance for the level of infectibility.

The most pronounced genotypic effect concerns the degree of early abortion. A colony was considered to be 'early aborted' if up to five or six haustorial mother cells (HMC) were formed and if the infection hyphae were not or little branched (Figs. 3,5). Such aborted colonies were rarely associated with the collapse of host cells. Often the infection hyphae were highly emaciated. In the low infectible genotypes, 'Vada' and C-118, about a quarter of the in-

Table 4. Proportions of arrested infection units of leaf rust isolate 121A in four barley genotypes (experiment 1). The infection units are classified in four types of abortion. Per genotype per series 7 or 8 leaves were screened with at least 200 infection units in all.

Type of abortion	Barley genotype	Series I	Series II	Series III	Significance of effects§ Genotype	Significance of effects§ Series
Non-penetration	'L94'	.08	.08	.04		
	C-41	.06	.09	.04		
	'Vada'	.11	.11	.03	-	**
	C-118	.09	.08	.05		
SSV abortion	'L94'	.00	.01	.00		
	C-41	.01	.00	.01	*	-
	'Vada'	.00	.00	.00		
	C-118	.02	.01	.01		
Early abortion	'L94'	.01	.01	.01		
	C-41	.00	.03	.02	**	-
	'Vada'	.23	.23	.32		
	C-118	.17	.21	.23		
Late abortion	'L94'	.01	.02	.01		
	C-41	.02	.03	.02	**	-
	'Vada'	.06	.02	.04		
	C-118	.08	.06	.04		

§ According to the Kruskal-Wallis test:
- not significant
* significant ($P \leq 0.05$)
** significant ($P \leq 0.01$)

fection units was stopped by this type of abortion (Table 4).

Once established, few colonies aborted. Established colonies that had not reached the reproductive phase at the day of sampling were classified as 'late aborted' colonies. This category contained really aborted infection units and possibly also some infection units that eventually would have formed uredia, although the sampling was carried out several days after the appearance of the majority of the uredia. The late aborted colonies were strongly branched and bore more HMCs than the early aborted colonies (Fig. 4), but generally no sporogenic tissue had been formed. Late abortion was not necessarily associated with host cell necrosis. The cultivars showed no large differences in this late abortion (Table 4).

In experiment 2 infection densities of the six lines and 'Vada' showed a significant genotype effect, although all genotypes were assumed to be of low infectibility (Table 5). As in experiment 1 the differences in infection density are explained mainly by differences in the degree of early abortion. Again, in the host genotypes with low infectibility early aborted colonies were rarely associated with autofluorescent or collapsed host cells (Table 6). If so, there usually were one or two necrotic cells. In the genotypes with little early abortion the association with host cell necrosis was stronger.

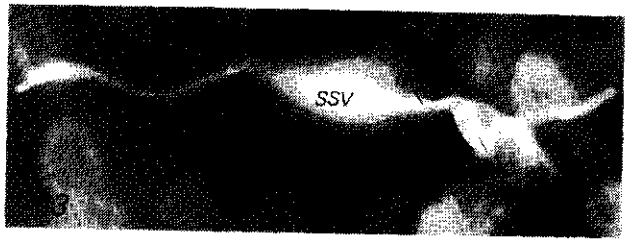
No significant genotype effects for the degree of non-penetration and SSV abortion were found.

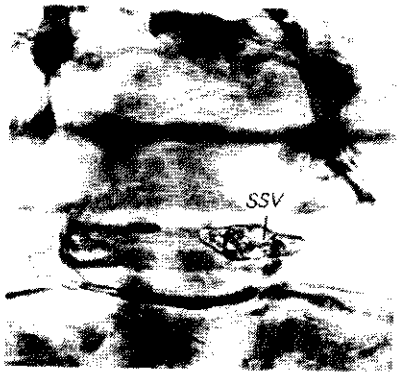
Regarding the degree of abortion during the colonization phase, the behaviour of C-120 attracts attention: its low infectibility is to a high degree due to late abortion (Table 5). Often such arrested colonies were associated with autofluorescent host cells. It must be mentioned, however, that C-120 did not meet the definition of PR in this experiment: the uredia were associated with some chlorosis (infection type 6 on the scale of McNeal et al. 1971). The other genotypes, which showed fully susceptible infection types, did not differ significantly among themselves for the degree of late abortion.

Qualification and quantification of colony abortion of eight leaf rust isolates (experiment 3)

The results of the components analysis of the eight isolates on 'Vada' and 'Julia' are presented in Fig. 6. As a result of unknown causes, only 3 to 13% of the urediospores gave rise to an appressorium, so that the conclusions about some of the isolates were based on a small number of infection units. The results with isolates F, 5 and 22 are based on 60 infection units or less per cultivar, those with isolates 121A, 18, and 201 on at least 100 per cultivar.

According to the Kruskal-Wallis test (Siegel 1956) 'Vada' and 'Julia' differed only for the degree of early abortion. Also the isolate effect regarding this type of abortion proved to be significant. The Everyman's Contingency Table Analysis (Everitt 1977)


SSV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4


5

Table 5. Infection densities in uredia per cm^2 and proportions of succesful and aborted infection units of leaf rust isolate 121A in 'Vada' and six C-lines (experiment 2). Each entry is the mean of eight seedlings. The classification of the aborted colonies is given (n = 170 - 190 infection units per genotype per series)

Series	Barley genotype	Infection density	Proportion successful infections	Proportions of infection units arrested by: Non-penetration	SSV abortion	Early abortion	Late abortion
		*	*			*	
	'Vada'	19.9 a	.56 a	.11	.01	.35 a	.02
I	C-92	20.7 a	.62 ab	.04	.03	.31 a	.04
	C-29	37.1 b	.78 b	.07	.05	.08 b	.03
	C-70	40.3 b	.80 b	.06	.01	.13 b	.03
	'Vada'	27.2 a	.40 a	.03	.01	.54 a	.08
II	C-92	35.9 a	.53 a	.05	.01	.35 b	.14
	C-29	58.5 b	.72 b	.03	.01	.17 bc	.09
	C-70	56.7 b	.74 b	.06	.00	.15 c	.07
	'Vada'	35.4 a	.65 a	.06	.01	.26 a	.05
I	C-123	36.3 a	.66 a	.02	.02	.26 a	.01
	C-120	41.4 a	.70 a	.03	.01	.09 b	.25
	C-197	55.1 b	.76 a	.06	.04	.09 b	.06
	'Vada'	31.7 a	.38 a	.03	.00	.59 a	.03
II	C-123	23.3 a	.42 a	.05	.01	.56 a	.00
	C-120	45.5 b	.49 a	.06	.02	.17 b	.36
	C-197	57.9 c	.81 b	.04	.02	.12 b	.02

* Per subcolumn different letters indicate a significant difference ($P \leq 0.01$) according to Duncan's multiple range test.

Figs. 1-5. Infection units of Puccinia hordei, aborted in different phases of development.

Fig. 1. Non-penetrating appressorium. x 415.

Fig. 2. Aborted substomal vesicle (SSV). Note the collapse of surrounding host cells. x 510.

Fig. 3. Early aborted infection unit. The substomatal vesicle (SSV) has formed infection hyphae (IH) and a few haustorial mother cells (HMC). x 470.

Fig. 4. Late aborted infection unit. Note the excessively branched hyphae. x 400.

Fig. 5. Part of a sporulating colony and an early aborted colony (arrow). x 375.

Figs. 1,3,4, and 5 are fluorescence micrographs. Fig. 2 is a white light micrograph.

Table 6. Proportions of early aborted infection units of leaf rust in seven barley genotypes, which were not associated with host cell necrosis. The data are obtained from the second replication of experiment 2.

Barley genotype	Proportion of early aborted infection units without necrosis	Number of infection units studied
'Vada'	.95	88
C-123	.93	83
C-92	.87	61
C-120	.67	43
C-29	.33	79
C-197	.27	40
C-70	.27	59

does not indicate a significant interaction between cultivars and isolates for early abortion (critical level: 0.36). Absence of interaction between cultivars and isolates in disease development is by definition (Van der Plank 1968) characteristic of horizontal resistance.

The isolate effect for non-penetration was not significant. Again, the degree of SSV abortion was so low that it was epidemiologically insignificant, despite a statistically significant isolate effect. Generally, the level of late abortion was low.

Variation in infectivity among isolates apparently is based mainly on differences in early abortion, as is variation in infectibility due to PR among barley lines. The degree of early abortion is not related to the number and identity of the avirulence alleles of the isolates (Table 1).

DISCUSSION

There is a large variation in infectibility to leaf rust among seedlings of barley genotypes despite the fact that the infection types indicate susceptibility (Parlevliet and Kuiper 1977; Niks and Parlevliet 1979). The variation in infection density is at least in part explained by variation in the degree of colony abortion. In experiment 1 the genotype differences in relative infection density were larger than those for the relative proportion of successful infection units. Also the formerly observed low infectibilities of some of the lines, e.g., C-41 and C-70 (Niks and Parlevliet 1979) were not confirmed by present results. Possibly differences in

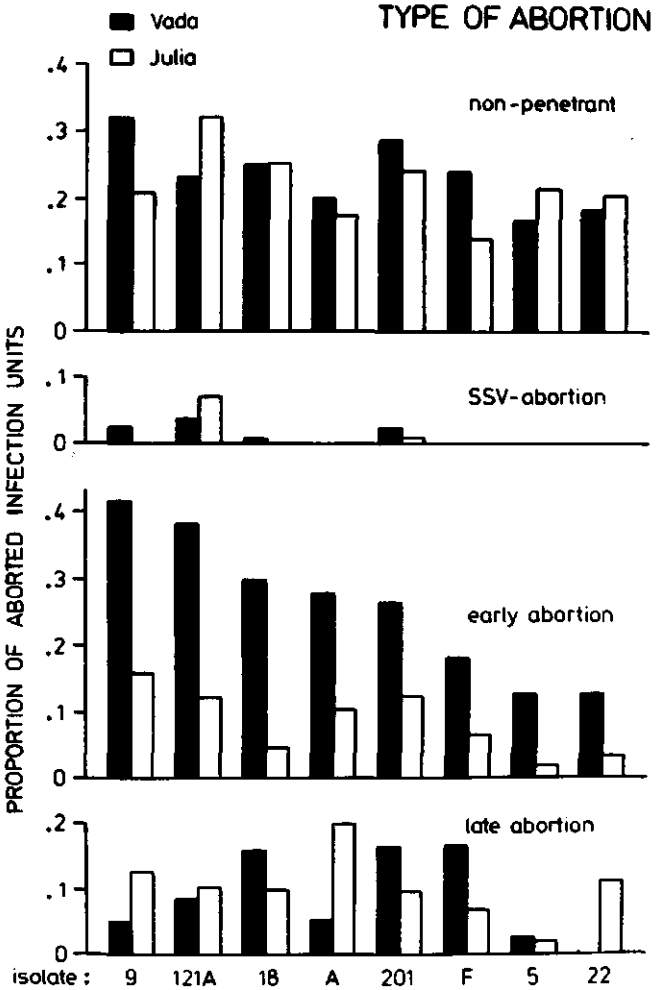

Fig. 6. Proportions of aborted infection units of eight isolates of leaf rust in seedlings of the barley cultivars 'Vada' and 'Julia'. Four types of abortion are distinguished according to the stages of development of the infection units.

inoculation and incubation techniques are responsible for these discrepancies.

In the histology of low infectibility owing to PR of barley seedlings to leaf rust, early abortion turned out to be the most important factor. Genes for PR do not appear to affect appressorium formation on seedlings (Niks 1981). The present results did not show a relationship between the level of PR and the degree of non-penetration and SSV abortion.

Early abortion probably occurs when the first haustoria are about to be formed, i.e., approximately 20 h after the onset of the infection process. Unfortunately, the fluorescence staining method does not permit observation of the haustoria. Accordingly, it is not clear whether the infection units are arrested before or after the formation of the first haustoria. Early abortion seldom is accompanied by host cell necrosis. Low infectibility owing to early abortion does not appear a peculiarity of a special host genotype: a high degree of early abortion occurred in several unrelated barley genotypes ('Vada', C-118, C-123, C-92). Similarly, differences in infectivity of isolates are explained by differences in early abortion. It is not clear whether the variation in infectivity is genetic or physiological in nature. Possibly, the isolates used in experiment 3 differ in their environmental requirements. The isolate effects may also have been caused by differences in inoculum quality: the spores were collected in different periods.

Early abortion has not been recognized before as the primary factor which determines infectibility of barley to leaf rust. According to Clifford (1972), the low infectibility of 'Vada' to leaf rust should, at least in part, be due to a significant SSV abortion. In a recent study (Clifford and Roderick 1981) also with 'Vada', no colony abortion in the early infection phases was mentioned. Studying the histology of the infection process on some other cultivars Clifford and Roderick (1978) stated: "The biological processes that result in a proportion of the colonies failing to sporulate is not clear from histology". The present results, which show a consistently low incidence of SSV abortion, seem to contradict Clifford's (1972) earlier results. Possibly, in his studies the colonies that were arrested early were overlooked. Like almost all workers on the histology of resistance, he used dyes (cotton

blue and acid fuchsine) that stain the fungal cytoplasm. Early aborted colonies tend to become unstainable quickly with these types of dyes.

The occurrence of abortion at the time of the first haustorium formation, which is not accompanied by host cell necrosis, has also not been reported for other cereal/rust systems. From a histological study of the postpenetration phenomena in some wheat cultivars with a 'low receptivity' to infection by *P. graminis* f.sp. *tritici*, Ashagari and Rowell (1980) concluded that the quantitative differences in the receptivity of the cultivars apparently resulted from the rapidity and frequency of the necrotic reaction of the host. In a similar investigation, Martin et al. (1977) indicated 'slow rusting' could be related with host cell necrosis while the fungal growth did not cease within 24 h. Recently, Cartwright and Russell (1980) showed that the majority of the infection units of *P. striiformis* in 'Little Joss' (a wheat cultivar supposed to exhibit a durable resistance against this pathogen) are arrested during or shortly after the SSV formation. Here again, a serious collapse of host cells accompanied colony abortion. It is not clear whether these and other reports deal with PR *sensu* Parlevliet (1978); often the infection types are not mentioned or they are not of a fully susceptible type.

Probably the only account of early abortion on a host with a susceptible reaction has been given by Heath (1977). Describing non-host reactions, she made a passing reference to the cessation of fungal growth of 23% of the infections of *P. helianthi* on sunflower (cv. Sunrise) after the formation of the first haustorium. This abortion was not necessarily associated with a collapse of the surrounding host cells. Remarkably, the early abortion of leaf rust colonies in partially resistant barley seedlings resembles the so-called non-host reaction: on a plant of a non-host species the infection units usually are arrested just between the HMC formation and the first haustoria formation and generally no substantial collapse of host cells occurs (Heath 1977).

With the hypersensitive type of resistance, the colonies usually stop when several haustoria and a fair amount of mycelium have been formed (e.g., Hilu 1965; Zimmer 1965; Littlefield and Aronson 1969; Sood and Sackston 1970; Mendgen 1978; Rohringer et al. 1979). In

all instances the interaction is associated with host cell necrosis. Possibly the reaction of line C-120 (Table 5) should be attributed to an incompletely expressed gene for hypersensitivity: this line inoculated with isolate 121A showed an intermediate infection type, while a considerable proportion of infection units was arrested after establishment. Often such colonies were surrounded by slightly autofluorescent cells. The low proportions of late aborted colonies in 'Vada', C-118, C-123 and C-92 (Tables 4 and 5) suggest that PR genes hardly cause the blocking of infection units during the colonization stage in barley seedlings.

In the literature two systems are proposed to be operative in host-pathogen interactions: basic compatibility which controls the establishment of the pathogen, and hypersensitivity presumed to be superimposed on basic compatibility (e.g., Day 1976; Loegering 1978). The system of hypersensitivity has been investigated thoroughly in many reports, but research on the system of basic compatibility has hardly started (e.g., Gabriel et al. 1979). The histological resemblance of early abortion of leaf rust colonies in partially resistant barley with the non-host reaction suggests that these genes are part of the system of basic compatibility.

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3. Histology of the relation between minor and major genes for resistance of barley to leaf rust

by

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ABSTRACT

Pa7 and Pa3, two major genes which confer hypersensitive resistance in barley to leaf rust, *Puccinia hordei*, were introduced into three genetic backgrounds with different levels of partial resistance (PR) to study the interaction between both types of resistance. The growth rate and the degree of abortion of the colonies in the genotypes were determined by fluorescence microscopy. The degree of host cell necrosis was recorded. The PR genes affected the success of colony establishment in the host and reduced the growth and development rate of colonies after establishment. This effect was also apparent in presence of Pa7, the effects of which were seen relatively late in the infection process. Apparently, PR genes and Pa7 acted independently and consecutively. Pa3 acted shortly after the establishment of the colonies and largely obscured the effect of the PR genes. Yet the level of PR can be assessed in the presence of Pa3 by determining the proportion of early aborted colonies not associated with host cell necrosis.

INTRODUCTION

Since Van der Plank (24) introduced the concept of vertical and horizontal resistance (VR and HR), these two types of resistance have been compared in many publications. One host genotype may possess alleles for both HR and VR, but many authors surmise that the expression of HR is obscured if the host also possesses effective alleles for VR (eg, 20). As a consequence, selection for HR, which is desirable because of its assumed durability, would be possible only in the absence of VR.

In the barley/leaf rust (*Puccinia hordei* Otth) relationship, the major genes conferring a hypersensitive reaction with avirulent races are considered VR genes. These genes are designated Pa-genes (13). The minor genes conferring partial resistance (PR) are presumed to belong to the type of HR genes (8, 14). PR is characterized by a reduced epidemic build-up, despite a susceptible infection type (IT) (15). Both types of resistance can occur in one genotype (17).

In this paper, a histological study is presented on the relation between both types of resistance.

MATERIALS AND METHODS

A back-crossing procedure was used to introduce the dominant alleles Pa7 of Cebada Capa and Pa3 of Rika x (Rika x Baladi) (see 13) into three recipient cultivars of barley: L94, Zephyr and Vada. These have undetectable, moderate and high PR, respectively (19). From each of the six major gene/recipient combinations one hypersensitive plant was singled out after the fourth backcross (B4). After selfing, each B4 plant produced a population which segregated for the major gene. These populations were screened for IT with isolate 121A, which is avirulent to Pa7 and Pa3. The seedlings with the lowest IT were assumed to be homozygous for the dominant allele of the major gene. The progenies from two selfed very hypersensitive and two selfed fully susceptible plants (ie, second generation after B4) for each major gene/recipient combination were used in this study. The two donor cultivars, indicated respectively as CC and Rika, were also studied. The lines are designated according to

the presence (P) or absence (p) of the dominant allele of the Pa gene (7 or 3). The genetic background of the line, which resembles that of the recipients (L94, Zephyr or Vada) is indicated by L, Ze or Va (see also Table 1).

The 24 lines were grown in 12 flats. Each flat contained one line derived from a hypersensitive plant and one fully susceptible sister line. Each line was represented by 16 seedlings. CC and Rika were grown in an additional flat. The primary leaves were inoculated with urediospores of isolate 121A, using a settling tower. Between 80 and 190 spores per cm^2 were applied, of which 50% formed an appressorium over a stoma. Central segments of the leaves were sampled 2½, 4½, 6½ and 14½ days post inoculation (d.p.i.), but for the lines with a Vada background the final sampling was 16½ d.p.i. because of the reduced rate of uredial development. Four segments per line were collected per sampling day. The leaf pieces were stained (22), with Blancophor BA 267% (Bayer, Leverkusen) replacing the Calcofluor. The observations were carried out with a Zeiss epifluorescence equipment NXL. Details of the foregoing procedures were given elsewhere (10).

Infection units (each unit representing the thallus developing from a single urediospore [25]) were classified according to their developmental stage and by the measurement of colony lengths. Here, germ tubes were not observed, nor were germination and appressorium formation studied. The developmental phases of the infection process and the designations of the corresponding infection units are given in Table 2 (see also 10). Each leaf was screened for as many infection units as were necessary to find 20 measurable and established colonies which were either aborted late or successful. The size of the colonies was assessed with an eyepiece micrometer, measuring the length of the projection of the colony on the long axis of the leaf. Intertwined colonies were considered immeasurable. They were, however, recorded to determine the proportions of infection units per developmental phase.

Host cells were classified as necrotic if they showed one or more of three qualities: browning of the cell contents, collapse of cell walls and autofluorescence. The latter criterion has been used also by Samborski et al (23). The number of autofluorescent host

Table 1. Average colony length (in µm) of Puccinia hordei in 26 different barley lines at four measuring times.

Host genotype	Time of sampling [u]				
	2½	4½	6½	14½	16½
P7-L[v]-1	174[x] g[y]	330 f	429 fg	973 f	-
P7-L -2	174 g	326 f	447 g	940 f	-
p7-L -1	178 g	419 gh	768 j	1640 j	-
p7-L -2	172 g	385 g	773 j	1436 i	-
P3-L -1	134 def[z]	174 bc	230 cd	446 c	-
P3-L -2	133 de	187 bcd	262 d	490 c	-
p3-L -1	171 g	430 h	816 j	1490 i	-
p3-L -2	178 g	400 gh	761 j	1433 i	-
P7-Ze-1	141 ef	257 e	379 ef	780 e	-
P7-Ze-2	142 ef	255 e	330 e	772 e	-
p7-Ze-1	143 ef	328 f	572 h	1188 gh	-
p7-Ze-2	141 ef	314 f	585 h	1194 gh	-
P3-Ze-1	94 a	123 a	126 a	220 ab	-
P3-Ze-2	117 bc	125 a	133 ab	169 a	-
p3-Ze-1	151 f	310 f	651 i	1279 h	-
p3-Ze-2	144 ef	273 e	553 h	1290 h	-
P7-Va-1	143 ef	206 bcd	241 d	-	656 d
P7-Va-2	137 ef	200 bcd	255 d	-	740 de
p7-Va-1	128 cde	217 d	379 ef	-	1118 g
p7-Va-2	135 def	209 bcd	341 e	-	1229 gh
P3-Va-1	109 b	-	116 a	-	299 b
P3-Va-2	120 bcd	116 a	128 a	-	168 a
p3-Va-1	141 ef	211 cd	401 fg	-	1210 gh
p3-Va-2	135 ef	209 bcd	405 fg	-	1185 gh
Cebada Capa[w]	135 def	194 bcd	182 bc	287 b	-
Rika[w]	105 ab	113 a	112 a	211 ab	-

[u] Expressed in days after inoculation.

[v] P indicates presence, p absence of the dominant allele of the major gene; 7 and 3 refer to Pa7 and Pa3, respectively; L, Ze and Va indicate the repicient genotypes, respectively L94, Zephyr and Vada.

[w] Cebada Capa is the donor of the Pa7 allele, Rika ((= Rika x (Rika x Baladi))) is donor of the Pa3 allele.

[x] Each entry is the mean length of 80 established colonies in four primary leaves unless indicated otherwise.

[y] Per column, entries with a common letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

[z] Underlined entries are averages of the lengths of early aborted and of established colonies, since the latter could not be recognized unambiguously.

Table 2. Designation and definition of the infection units of Puccinia hordei in barley, according to their stage of development.

Developmental phase	Designation of the infection unit	Definition
Pre-penetration	Non-penetrant[z]	Appressorium over stoma without formation of a substomatal vesicle
Substomatal vesicle (SSV) formation	Aborted SSV[z]	SSV without hyphae
Establishment	Early aborted colony[z]	SSV with primary infection hyphae and up to six haustorial mother cells
Colonization	Established colony or Late aborten colony[z]	Branched hyphae, six or more haustorial mother cells; sporogenic tissue may be present
Reproduction	Successful colony	At least some uredio-spores are formed

[z] Arrested in the respective developmental phase

cells per infection unit was counted or assessed. With large colonies the proportion of the colony area with cell browning was estimated.

In the statistical analyses, the experimental units were (the means of) the responses per leaf.

RESULTS

One of the P3-Va lines segregated for hypersensitive resistance. The observations on this line were discarded as for the leaves collected at 4½ d.p.i., since the results suggested that the leaves sampled on that day belonged to susceptible segregants. No segregation for hypersensitivity occurred in the other lines, indicating that these lines descended from parents that were homozygous for this character.

Colony growth. The average lengths of the established colonies at the four times of sampling are presented in Table 1. The effect of Pa3 on the colony growth was pronounced: a significant lag in colony length was apparent as early as 2½ d.p.i. The growth re-

tarding effect of Pa7 was less serious. At 2½ d.p.i. the colonies in the P7 lines did not prove to be smaller than in the p7 lines. The effect of Pa7 became only detectable when the colonies in the susceptible counterparts averaged at least 300 μm.

Four susceptible lines per recipient genotype were available to compare the effect of the minor genes for PR on colony growth. No systematic differences between p3 and p7 lines were found within a recipient genotype. In accordance with the level of PR of the recipients, the colonies in the susceptible L-lines had a higher rate of growth than those in the susceptible Va-lines. The colonies in the Ze-lines grew at an intermediate rate.

The effect of the PR genes on colony growth in the lines with Pa7 was evident. At 6½ d.p.i. there was a significant interaction effect between the Pa7 allele and the PR-background (ANOVA, critical level $P < 0.01$), indicating that the larger the average colony length in the susceptible line, the larger the relative arrears in the hypersensitive counterpart. In the lines carrying the Pa3 allele, the colonies in the L-lines reached the largest size, as expected. The colony lengths in Ze- and Va- lines showed no significant differences; in both types of lines the average colony length hardly increased with time. The growth retarding effect of PR genes was obscured by the strong expression of Pa3.

Colony abortion. The proportion of infection units blocked as non-penetrants and as aborted substomatal vesicles (SSV) were calculated from the data obtained from all 16 leaves per line. The mean proportion of non-penetrants was 6%, without conspicuous differences between the lines (Kruskal-Wallis non-parametric test, $P = 0.14$). The differences in SSV-abortion were not significant either (Kruskal-Wallis, $P = 0.10$) and too low to be of interest (average proportion 0.9%).

For the degree of early abortion, pronounced differences between the lines were found (Table 3, Fig. 1). In all the Va-lines and in the P3-L and P3-Ze lines a high proportion of colonies was found that fitted the (morphological) definition of early abortion as given in Table 2 (Table 3). Many other colonies in the Pa3 carrying lines were blocked little beyond the establishment stage: these possessed more branched hyphae suggesting a successful establishment, but had only five to eight haustorial mother cells. As a conse-

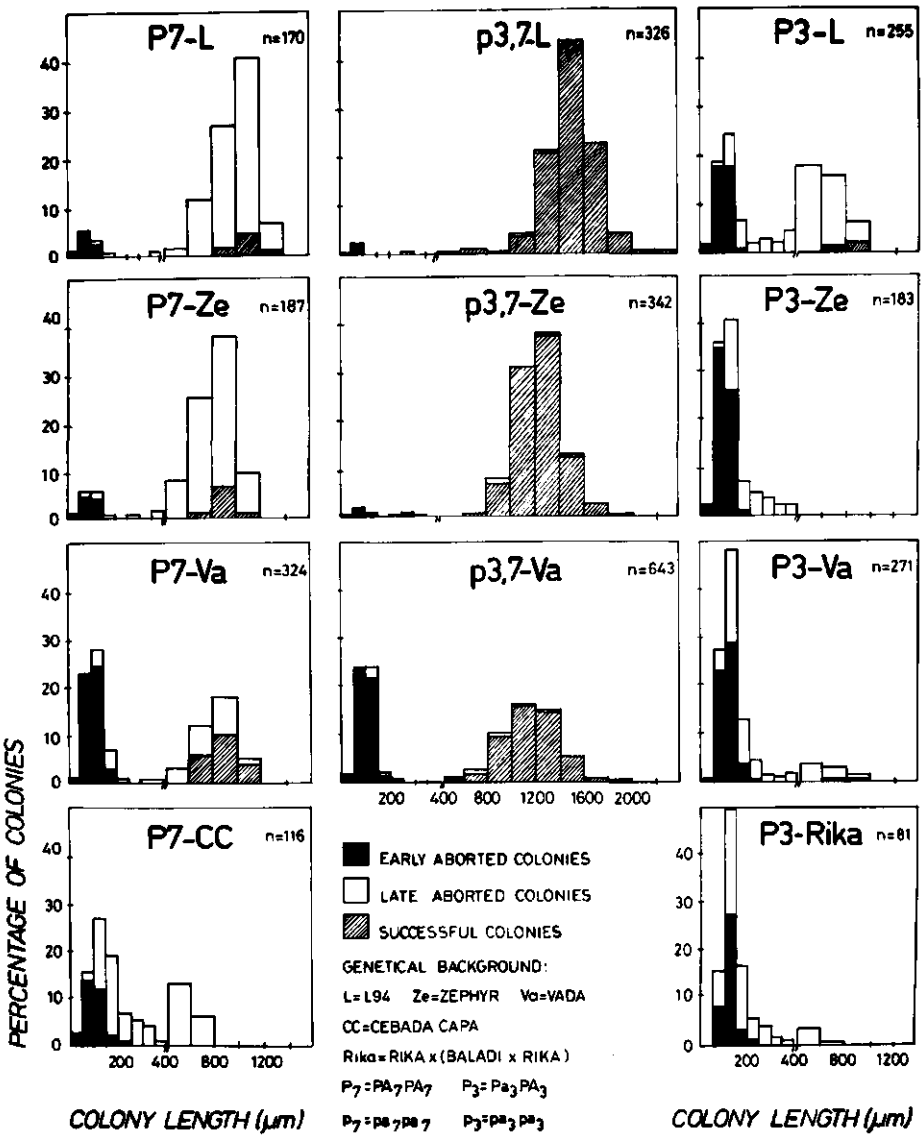

Fig 1. Frequency distribution of the colony length of Puccinia hordei in seedlings of barley genotypes differing in genetical background and in the presence (P) or absence (p) of a major gene (Pa7 or Pa3) for resistance. The leaves were collected 16½ (Vada background) or 14½ (the others) days after inoculation. Each graph is based on the measurements of n colonies. The three classes of colonies are defined in Table 2. Note the change of scale at 400 µm.

Table 3. Percentage of early aborted colonies of Puccinia hordei in barley seedlings with different genotypic backgrounds and different alleles of major genes for resistance.

Genotypic background	Line	Alleles of the major genes[y]			
		Pa7	pa7	Pa3	pa3
L94	1	10 bc[z]	4 ab	32 de	4 ab
	2	2 a	3 a	33 ef	2 a
Zephyr	1	7 abc	3 a	50 gh	5 ab
	2	12 c	4 ab	22 d	3 a
Vada	1	52 h	55 hi	54 hi	42 fg
	2	37 ef	42 fg	33 def	43 fg
Cebada Capa		31 de			
Rika				63 i	

y Pa7 and Pa3 cause a hypersensitive reaction, pa7 and pa3 a susceptible reaction.

z Entries with a common letter are not significantly different ($P \leq 0.05$) based on Duncan's multiple range test.

Table 4. Percentage of late aborted colonies of Puccinia hordei in barley seedlings with different genotypic backgrounds and different alleles of major genes for resistance.

Genotypic background	Line	Alleles of the major genes[x]			
		Pa7	pa7	Pa3	pa3
L94	1	88[y] de[z]	0 a	99 e	1 a
	2	99 e	2 a	91 de	2 a
Zephyr	1	97 e	2 a	100 e	2 a
	2	86 d	3 ab	100 e	2 a
Vada	1	64 c	5 ab	96 de	14 b
	2	56 c	8 ab	100 e	10 ab
Cebada Capa		100 e			
Rika				100 e	

x Pa7 and Pa3 cause a hypersensitive reaction, pa7 and pa3 a susceptible reaction.

y Entries are the average percentages of established colonies that failed to form urediospores. The data are obtained from leaf segments collected 16½ (Vada-background) or 14½ (the others) days after inoculation.

z Entries with a common letter are not significantly different ($P \leq 0.05$) based on Duncan's multiple range test.

quence, a distinction between established and early aborted colonies often was impossible with these lines. The presence of the Pa3 allele did not raise the level of early abortion in the Vada background. In contrast with Pa3, the Pa7 allele had scarcely any effect on the level of early abortion.

The degree of late abortion was assessed from the leaf segments of the final sampling date. The colonies that had not reached the reproduction phase at this date were classified as late aborted colonies. It must be borne in mind that the level of late abortion may depend on the date of sampling. Almost all established colonies in the genotypes without a dominant allele for hypersensitivity had reached the reproduction phase at the final sampling date (Table 4, Fig. 1). Those that had not, were not likely to have the potential to become reproductive. The susceptible Va-lines showed a barely higher level of late abortion than the L-and Ze-lines.

Of the Pa7 containing genotypes the level of late abortion was highest in Cebada Capa. No reproduction of the pathogen was observed in this cultivar (Table 4) and the colony growth ceased at a rather small colony size (Table 1, Fig. 1). In the other genotypic backgrounds the Pa7 gene did not prevent reproduction completely. Especially in the P7-Va-lines a substantial proportion of the established colonies succeeded in the formation of at least a few urediospores (Fig. 1).

With the genotypes carrying Pa3, late abortion generally occurred at smaller colony sizes than with Pa7. Except for the P_3-L-lines, the majority of the late aborted colonies were arrested just beyond the establishing phase (see above). Of the colonies that continued growth, only a few had reached the reproduction phase at the final sampling date. In the P_3-L lines relatively numerous colonies reached lengths of over 400 µm (Table 1, Fig. 1).

Host cell necrosis. Generally, small colonies were associated with the necrosis associated with autofluorescence, large colonies with browned and collapsed cells which were non-autofluorescent.

In the genotypes with a high level of early abortion due to PR (Va-lines and CC) (Table 3) the early aborted colonies were rarely associated with host cell necrosis (Table 5). The early abortion due to Pa3 (Table 3) on the contrary, was almost always associated with necrotic host cells (Table 5).

Table 5. Percentage of early aborted colonies of Puccinia hordei associated with at least one autofluorescent host cell in barley seedlings with different genotypic backgrounds and different alleles of major genes for resistance.

Genotypic background	Alleles of the major genes[z]			
	Pa7	pa7	Pa3	pa3
L94	32	23	88	16
Zephyr	10	14	92	10
Vada	5	3	29	3
Cebada Capa	11			
Rika			88	

z Pa7 and Pa3 cause a hypersensitive reaction, pa7 and pa3 a susceptible reaction.

Pa7 and Pa3 differed in the degree to which host cells became necrotic in established colonies. At 2½ d.p.i. the established colonies in Pa7 carrying lines were associated with cell necrosis as much as or little more than the susceptible lines (Table 6). From 4½ d.p.i. on, the Pa7 gene induced a browning of host cells which progressed until at the final sampling date in the larger colonies cell browning occurred over 25-50%, and in CC up to 100% of the colony area. These colonies were macroscopically visible as small dark spots on the leaves.

Pa3 induced cell necrosis around the majority of the colonies in the P3-L, P3-Ze and Rika genotypes as early as 2½ d.p.i. (Table 6). In P3-Va the degree of necrosis appeared less, probably owing to the early abortion caused by PR genes, which seldom goes with host cell necrosis. The colonies seemed to be 'knocked down' by Pa3 just at or shortly after establishment. The cell necrosis mostly concerned the cells that were associated with the haustorial mother cells of the young colony.

In all genotypes carrying Pa3, especially in the P3-L lines, part of the colonies seemed to recover from the early inhibitory effects of Pa3. Their mycelium passed by the necrotic host cells and continued growth without provoking further cell necrosis. These larger colonies were associated with only a few browned host cells

Table 6. Average percentage of established colonies of Puccinia hordei associated with at least one necrotic (= autofluorescent, browned or collapsed) host cell, 2½ days after inoculation.

Genotypic background	Line	Alleles of the major genes[x] Pa7	pa7	Pa3[y]	pa3
L94	1	48 (1.4)[z]	49 (1.9)	84 (1.6)	40 (1.3)
	2	55 (1.3)	21 (1.2)	95 (2.1)	51 (1.5)
Zephyr	1	33 (1.5)	21 (1.4)	88 (2.4)	20 (1.1)
	2	36 (1.4)	35 (1.3)	98 (2.2)	20 (1.6)
Vada	1	28 (2.0)	6 (1.3)	25 (1.3)	10 (1.3)
	2	29 (1.4)	20 (1.2)	35 (1.4)	5 (1.5)
Cebada Capa		15 (1.2)			
Rika				75 (1.7)	

[x] Pa7 and Pa3 cause a hypersensitive reaction, pa7 and pa3 a susceptible reaction.

[y] In the lines carrying Pa3 the established colonies could not be recognized unambiguously from the early aborted colonies. The data concern all colonies which formed at least one haustorial mother cell.

[z] Within brackets the average number of necrotic cells associated with the colonies concerned are given.

in the centre of the colony. The browning seldom occupied more than 25% of the colony area. Since Pa3 causes a macroscopically visible chlorosis and necrosis of the host tissue around the infection sites, it appears that the larger colonies also were associated with host cell alterations that were not detectable with the methods used here.

In the susceptible lines, particularly the L- and Ze-lines, many established colonies were associated with autofluorescent cells (Table 6), but the number of these cells per colony was small and the amount of necrosis was negligible in relation to the colony area.

DISCUSSION

As is known from macroscopical investigations, the minor genes for PR to leaf rust in barley cause a reduced rate of development of the colonies (8, 12). In the present experiment, the uredia on the susceptible lines with the partially resistant Vada background appeared a few days later than on the lines with L94 and Zephyr background. In connection with the reduced rate of development, the growth rate of the colonies is affected by the PR genes (Table 1), as has been reported before (2, 4, 5). In addition, minor genes for PR can reduce the number of uredia per cm^2 leaf area (18) by causing a substantial early abortion of colonies which seldom is associated with host cell necrosis (10) (see the data collected from the p7-Va and p3-Va lines in Tables 3 and 5).

The dominant alleles of the two major genes for resistance to leaf rust considered (Pa7 and Pa3) clearly differed in their mode of action, irrespective of the genetic background. The action of Pa7 became manifest several days after the establishment of the fungus by a gradual growth retardation associated with an extensive cell browning. In contrast, Pa3 caused an early cessation of the fungal growth associated with host cell necrosis. Part of the colonies continued growth without provoking further necrosis of host cells. Similar differences in action between major genes for resistance were reported, eg, for flax and flax rust (9), wheat and stem rust (21) and wheat and powdery mildew (7). The genetic background may modify the mode of action of the hypersensitivity alleles, although the general characteristics of the resistance reaction are not lost (6, 21). In the present study, the genetic background also influenced the expression of the major genes. With Pa7, relatively many established colonies reached the reproductive phase in the Vada background, whereas in the donor cultivar Cebada Capa no successful colonies were found (Fig. 1). Since both Cebada Capa and Vada possess a high gene dose for PR (17), it is likely that the mitigation of the action of the Pa7 allele in the Vada background is attributable to other than PR genes. Apparently, the PR genes and Pa7 act independently and consecutively: only the colonies that are not arrested by early abortion due to PR have to cope with the barrier raised by Pa7. These results confirm the findings of

Parlevliet (16) who demonstrated that the more minor genes for low infectibility are present, the fewer necrotic flecks appear in the presence of Pa7. Clifford (3) also found a good correlation between the level of PR in the genetic background and the number of necrotic spots due to hypersensitivity. He investigated cultivars which were recessive for Pa7, but dominant for at least Pa2. The growth retarding effect of PR genes remained unimpeded in the presence of Pa7 (Table 1), in accordance with the slower appearance of necrotic flecks in lines with Pa7 in a high PR background found by Parlevliet (16).

The effects of the PR genes were less easily recognized in the presence of Pa3, since this major gene acts about as early as the PR genes. Differences in growth rate due to PR genes were largely obscured by the substantial abortion of colonies due to Pa3 at or shortly after establishment. The results suggest that Pa3 acts even more intensely in the Zephyr background than in the donor cultivar Rika. Despite the low degree of reproduction in the P3-lines at the final sampling date, it cannot be excluded that more colonies would have come to reproduction if the sampling of the leaves had been postponed some days. J.E. Parlevliet (unpublished) observed a fair degree of sporulation in the three Pa3 carrying recipients.

It could be demonstrated that the early abortion by Pa3 occurs shortly after the formation of the first haustoria (11). The cells in which these haustoria are formed become necrotic. This explains the high association of early abortion due to Pa3 with host cell necrosis in genotypes with little PR (Table 5). Considering the formation of the first haustoria as successful establishment, it is more appropriate to speak of early hypersensitivity. Early abortion by PR genes occurs before the formation of haustoria as a failure of establishment (11). This means that the PR genes and Pa3 again act consecutively but with a short time interval. Only the colonies not arrested by PR genes (without necrosis) may be arrested by Pa3 (with necrosis). The level of PR in genotypes with an early acting allele for hypersensitivity can be assessed by the degree of host cell necrosis. This implicates that Rika does not have an appreciable level of PR. A similar difference in the degree of host cell necrosis between race non-specific and race-specific resistance was reported for oats and powdery mildew (1).

In the presence of a late acting major gene for resistance the level of PR in the genetic background can be estimated much easier than in the presence of an early acting major gene. The results suggest that minor and major genes for resistance acted independently and constituted different defence systems. Plants combining hypersensitive resistance with a high proportion of small aborted colonies lacking host cell necrosis should be promising parental material, because they may carry a high level of possibly durable resistance in their genetic background.

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4. Comparative histology of partial resistance and non-host reaction to leaf rusts in barley and wheat seedlings

by

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ABSTRACT

The histological responses involved in partial resistance (PR) and non-host reaction were compared in three barley and three wheat genotypes inoculated with *Puccinia hordei* and *P. recondita tritici*, the leaf rust pathogens of barley and wheat. Non-host and PR reactions to the leaf rust pathogens were characterized by a high proportion of colonies that were arrested early, (i.e., immediately after the formation of the first haustorial mother cells) and were associated with little or no plant cell necrosis. Eight barley genotypes, four with a low and four with a high level of PR to *P. hordei* were inoculated with the leaf rusts *P. hordei*, *P. recondita secalis* and *P. recondita tritici*. The latter pathogen produced reproductive structures on all four barley genotypes with a low level of PR to *P. hordei*, suggesting that alleles for low PR to *P. hordei* also reduced the effectivity of the reaction to *P. recondita tritici*.

INTRODUCTION

Apart from the hypersensitive resistance, another and less conspicuous type of resistance is known in the barley/leaf rust (*Puccinia hordei*) relation, which is characterized by a slow rate of epidemic development despite the presence of a susceptible infection type. This resistance has been designated 'partial resistance' (PR) (15). Although the epidemiological and genetical aspects of PR are well documentated (6, 16), little is known about the underlying mechanisms. In a histological study of the infection process of the leaf rust fungus in barley seedlings, development of a large proportion of leaf rust colonies in partially resistant genotypes was arrested just after the first infection hyphae and a few haustorial mother cells were formed (11). This 'early abortion' resembles the non-host reactions described by Heath (5).

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

MATERIALS AND METHODS

In the first experiment the histology of effects associated with PR and non-host reaction were compared. Two plant boxes with three barley and three wheat genotypes were grown. One box was inoculated with monospore culture 121A of *P.hordei*, the other with a monospore culture of *P. recondita tritici*. The barley genotypes were L94, Vada and 139-4. L94 is highly infectible to *P. hordei*, Vada and 139-4 have a high level of PR. The wheat cultivars, Saratovskaja 210 (S210), Adonis, and Duri, are fully susceptible to the isolate of *P.recondita tritici* used, but their level of PR to this pathogen was unknown. The experiment was carried out in two consecutive series. In each genotype/pathogen combination, six or seven primary leaves were inoculated with urediospores utilizing a settling tower. Segments of the cental parts of the leaves were collected 200 (in the first series) or 225 (in the second series) hours post inoculation (h.p.i.). They were cleared and stained for fluorescence microscopy (11). The infection units in the leaves were

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

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

RESULTS

The three leaf rust pathogens could be distinguished by the morphology of their substomatal vesicles (SSV) (Fig. 1). Generally, the SSV of *P. hordei* was cigar-shaped with a transverse septum in the middle. From the SSV two infection hyphae developed, growing parallel to the long axis of the leaf. The SSV of *P. recondita*

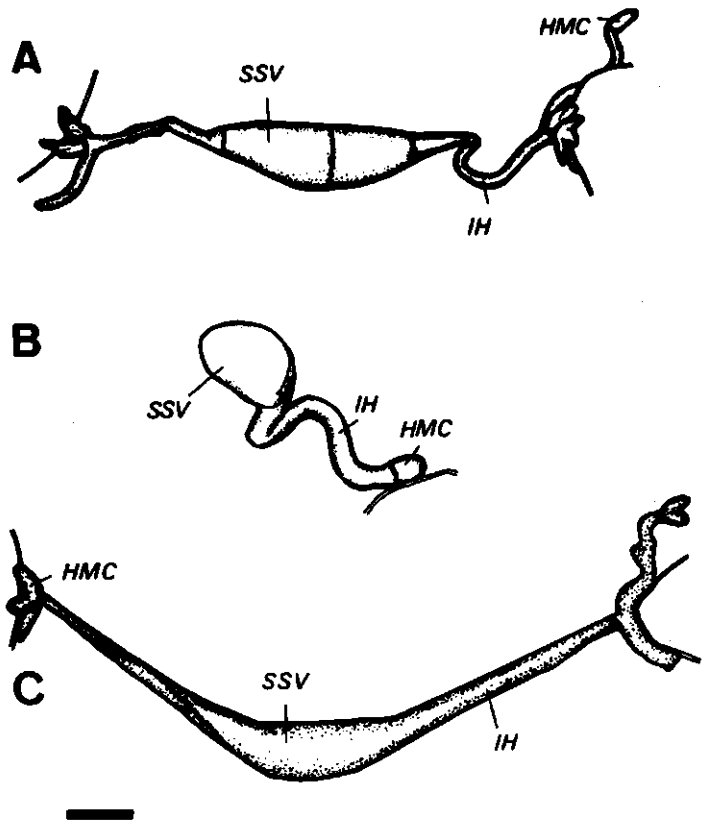

Fig. 1. Typical shape of early aborted colonies of Puccinia hordei (A), P. recondita tritici (B), and P. recondita secalis (C) in primary leaves of barley. Indicated are substomatal vesicles (SSV), infection hyphae (IH), and haustorial mother cells (HMC). Part of the walls of barley mesophyll cells are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

secalis was more slender and the septum was either absent or hardly visible. The SSV of *P. recondita tritici* was egg-shaped and developed one infection hypha, which tended to grow transversely to the long axis of the leaf. The thickness of the hyphae of *P. hordei* was about half of that of the two other leaf rusts (ca. 2.5 and 5.0 µm diameter, respectively).

In the first experiment, uredia of a low infection type (IT 5 on the scale of McNeal et al [8]) appeared on barley genotype L94 in-

oculated with *P. recondita tritici*. The colonies had hyphae of ca. 5.0 µm diameter. Urediospores from these uredia were transferred to a few plants of barley (cv. L98) and wheat (cv. Kaspar) by means of a brush. After incubation of these plants, only Kaspar showed a susceptible reaction (IT 9). *P. recondita tritici* did not give visible symptoms in Vada and 139-4, nor did *P. hordei* in the three wheat genotypes.

There were no consistent differences among the plant/rust species combinations in the proportions of penetrations of appressoria through the stomata (Table 1). There was a tendency towards a reduced stoma penetration on non-hosts (e.g., Duri, Vada), but the opposite also occurred (S210, series 2). Often, the proportions of non-penetrating appressoria of either pathogenic or non-pathogenic rust species on a plant genotype hardly differed (e.g., 139-4). The degree of non-penetration on the partially resistant genotypes Vada and 139-4 was not higher than on the highly infectible L94.

There was little SSV abortion in either the host and non-host combinations.

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

The leaf segments were sampled before the latent period had elapsed. Consequently, the percentage of colonies that formed uredia could not be assessed. As can be concluded from Table 2, the proportions of established colonies (i.e., 1 - the proportion of early aborted colonies) was high in the host/pathogen combinations with L94, S210, Adonis, and Duri and moderate in the partially re-

Table 1. Average proportions of non-penetrating appressoria when the primary leaves of three barley and three wheat genotypes were inoculated with urediospores of Puccinia hordei and P.recondita tritici.

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

[a] Each entry is based on observations of six or seven leaves.

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

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

[a] Calculated as proportion to the total number of colonies that formed at least one haustorial mother cell.

[b] Calculated as proportion to the number of early aborted colonies.

Table 3. Average proportions of non-penetrating appressoria and aborted substomatal vesicles (SSV) of three leaf rust pathogens in seedlings of eight barley genotypes.

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

sistant Vada and 139-4 with *P. hordei*. Of the combinations considered as non-host combinations, L94/*P.recondita tritici* gave the highest proportion of established colonies.

In the second experiment the eight barley genotypes showed no reproducible differences in degree of non-penetration and SSV abortion in any of the leaf rust pathogens. Therefore, per leaf rust pathogen the proportions of these types of abortion were averaged over the genotypes (Table 3). The degrees of non-penetration and SSV abortion of the non-pathogenic *P.recondita tritici* and *P.recondita secalis* were not higher than of the pathogenic *P.hordei*.

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

All the colonies of *P.recondita secalis* were arrested before they had produced visible symptoms in barley. Arbitrarily, colonies were considered 'established' when at least eight haustorial mother cells (HMC) were observed. Such colonies usually were more branched than shown in Fig. 1C; They were much smaller than the established colonies of *P.recondita tritici* in barley and mostly without cell necrosis. Colonies with seven or less HMCs were considered 'early aborted'. The proportion of early aborted colonies of *P.recondita secalis* was high in all the barley genotypes (Table 4). In the first series, the genotypic differences for early abortion were in-

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

Barley genotype	Proportion of early abortion					Length of established and successful colonies				Infection type		
	P.hordei[w]	P.recondita tritici		P.recondita secalis		P.hordei		P.recondita tritici		P.hordei	P.recondita tritici	P.recondita secalis
		I[x]	II	I	II	I	II	I	II			
L94	0.09	0.86	0.88	0.96	0.92	1848 a[y]	1869 a	254	820	9	1-5	0
L92	0.11	0.83	0.65	0.92	0.96	1747 a	1648 b	506	712	9	5	0
Akka	0.16	0.96	0.99	0.94	1.00	1742 a	1489 bc	358	603	9	0-5	0
L98	0.48	0.88	0.97	0.99	1.00	1541 b	1415 bc	305	95	9	5	0
Vada	0.49	0.98	0.98	0.94	0.98	1373 c	1140 d	140	232	9	0	0
C-118	0.56	0.99	1.00	0.99	1.00	1350 c	1455 bc	181	-	9	0	0
C-123	0.58	0.98	1.00	0.99	1.00	1382 c	1377 c	146	-	9	0	0
C- 92	0.48	0.99	1.00	0.97	0.99	1381 c	1305 cd	251	-	9	0	0
Significance of genotype effect (Kruskal-Wallis)[z]	*	*	*	-	*							

[w] No significant series effect (Kruskal-Wallis, $P < 0.01$).

[x] The experiment was carried out in two series.

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

[z] * indicates significance ($P \leq 0.01$); - non-significance.

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

The colonies of *P. recondita tritici* could be classified unambiguously into the two groups 'early aborted' and 'established'. Also with this fungus, a high proportion of the colonies aborted early, i.e., after the formation of one to four HMCs (Table 4). It could not be established whether a similar deposit of callose containing material occurred as with *P. recondita secalis*, since the infection hyphae of *P. recondita tritici* grew into the deeper mesophyll layers. In all genotypes less than 20% of the early aborted colonies were associated with necrosis of mesophyll cells. Established colonies were more branched than early aborted ones, and usually they were associated with cell browning. In four of the genotypes, one or more established colonies of *P. recondita tritici* produced urediospores. Such uredia were of IT 5 or lower (Table 4) and had thicker hyphae than the few *P. hordei* colonies that appeared, due to contamination.

On Akka only some uredia were produced in the first series. Particularly on L92 *P. recondita tritici* succeeded in producing uredia: 6 (series 1) and 10% (series 2) of the infection units that developed beyond the SSV stage produced urediospores. The barley genotypes on which *P. recondita tritici* formed uredia were the four that were chosen as representative of genotypes with a low level of PR to *P. hordei*.

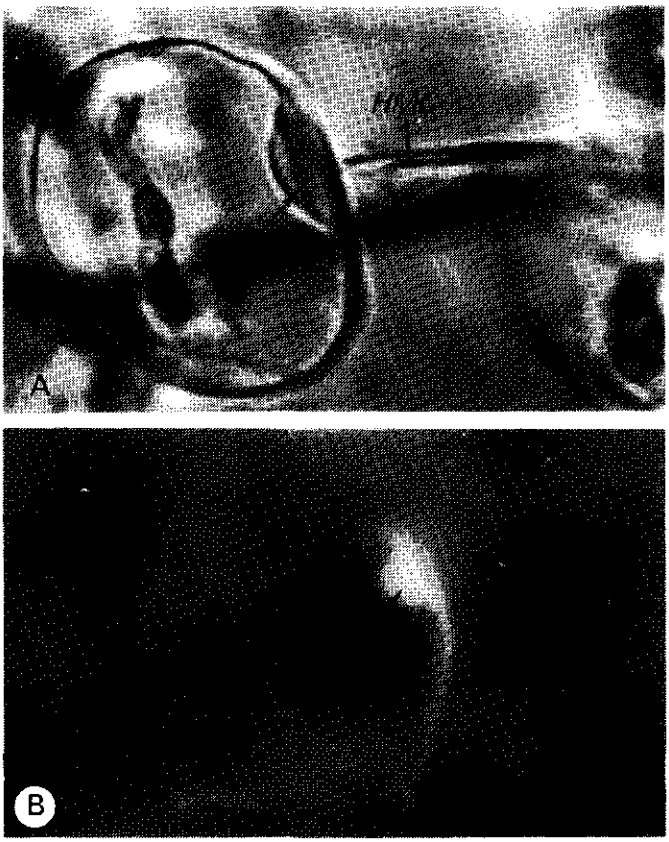

Fig. 2. Deposit of callose containing material (arrow) in barley seedling adjacent to a haustorial mother cell (HMC) of Puccinia recondita secalis. A, White light micrograph in whole mount preparation prepared for fluorescence microscopy; B, Fluorescence micrograph after staining in 0.005% aniline blue in 0.07 M K_2HPO_4, pH 8.9. The fluorescence of the deposit is clearly visible.
Both figures x 1560.

DISCUSSION

There is a remarkable resemblance between the histological responses involved in PR of barley (to *P. hordei*) and the non-host reactions of wheat (to *P. hordei*) and barley (to *P. recondita secalis* and *P. recondita tritici*). Neither PR of barley nor non-host reaction of wheat to *P. hordei* result from reduced appressorium formation (10). With both PR and non-host reactions, the stoma pene-

tration, SSV and infection hyphae formation were not affected (Tables 1, 3). With cowpea rust, induced by *Uromyces phaseoli* var. *vignae*, reduced stoma penetration and SSV formation in some non-host species was reported (4, 5). Rust pathogens of grasses, however, formed readily appressoria and SSVs in graminaceous non-hosts (14, 21). PR and non-host reactions to leaf rusts were associated with a high degree of early abortion with little or no cell necrosis (Tables 2, 4) and a reduced size of the established colonies at the time of sampling (Table 4). In the non-host reaction these effects were more extreme than with PR. Not only were the proportions of early abortion higher in non-host reactions, but the deposition of callose containing material at the contact points with HMCs of *P. recondita secalis* suggested a more extreme reaction to infection by a non-pathogen. A substantial colony abortion immediately after the formation of the first HMCs was mentioned also for other non-host combinations (5, 9, 21). Deposition of callose as part of a non-host reaction does not seem to be common (5). The resemblance between PR and non-host reactions was furthermore expressed by the fact that the early abortion of *P. hordei* colonies in both non-host wheat and partially resistant barley Vada was associated with a failure in the formation of haustoria (12).

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

The level of PR of the barley genotypes was not obviously related with differences in nature of non-host reaction to *P. recondita secalis*, but the results suggested a relationship between PR and the nature of reaction to *P. recondita triciti*. The barley genotypes

in which *P. recondita tritici* produced urediospores were all highly infectible to *P. hordei* in this study, except for L98, but this genotype had a low level of PR in previous studies (18, 20). This suggests that alleles for low PR to *P. hordei* also reduce the effectivity of the reaction to *P. recondita tritici*. There is, however, evidence that slow rusting genes or genes for horizontal resistance are pathogen species-specific (3, 17, 22). Therefore, more barley genotypes have to be tested with more *P. recondita tritici* isolates to establish whether the relation between level of PR to *P. hordei* and level of non-host reaction to *P. recondita tritici* suggested by the present study is real or merely due to coincidence.

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5. Haustorium formation of *Puccinia hordei* in leaves of hypersensitive, partially resistant and non-host plant genotypes

by

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ABSTRACT

In barley genotypes with early acting hypersensitivity or with partial resistance and in non-host species like wheat, a large proportion of the colonies of *Puccinia hordei* aborted after the formation of the first infection hyphae and a few haustorial mother cells. The early abortion without collapse of plant cells was associated with a failure in the formation of haustoria. This was particularly evident in partially resistant barley. Early abortion with host cell necrosis occurred after the formation of the first haustorium. This type of abortion was important in barley with the early acting Pa3 gene for hypersensitivity. In non-host wheat most of the infection units aborted before the formation of the first haustorium.

INTRODUCTION

There are three types of resistance associated with early cessation of colony growth of the leaf rust pathogen of barley, *Puccinia hordei* Otth. This phenomenon has been called 'early abortion' (6) and is characterized by arrested growth just after the formation of the first infection hyphae and a few haustorial mother cells (HMC). It was found in seedlings of partially resistant barley genotypes (6), in barley genotypes carrying an early acting gene for hypersensitivity such as Pa3 (8), and in non-host species such as wheat (7). Since a clearing and staining technique was used in those studies which did not permit observations of haustoria, research was initiated to determine whether early abortion occurred before or after the formation of the first haustoria.

MATERIALS AND METHODS

For each type of resistance (6-8) one representative genotype was used: wheat (*Triticum aestivum* L.) cultivar Duri (non-host [7]) and barley (*Hordeum vulgare* L.) genotypes Vada (high level of partial resistance [6]), P3-Ze-2 (carrying the Pa3 allele for hypersensitivity in a background with a moderate level of partial resistance [8]) and L94 (no detectable host resistance, control). The plants were grown in a 37 x 39 cm flat. The primary leaves were inoculated with urediospores of *Puccinia hordei*, isolate 121A. This isolate is avirulent to Pa3 and causes no symptoms in wheat cultivar Duri. Seven of the 12 primary leaves per genotype were densely inoculated with a brush. These leaves were used for the observations on the haustorium formation. The remaining leaves received an inoculum dose of about 100 spores per cm^2 using a settling tower. These leaves were investigated after preparation for fluorescence microscopy (6,8), to assess the degree of early abortion and the degree of host cell necrosis associated with the early abortion. The moderate inoculum density was necessary to prevent intertwinement of the growing colonies. The plants were transferred to a greenhouse compartment where during the first night the relative humidity was kept at saturation.

Sections of upper epidermis and mesophyll of the densely inoculated leaves were sampled 20, 35 and 45 hours post inoculation (h.p.i.). They were hand sectioned in a plane parallel to the leaf surface. The sections of four to six leaves per genotype were processed together. They were fixed in acetic acid: ethanol (1:3, v/v) for 12 h, boiled in 0.03% aniline blue in lactophenol: ethanol (1:2, v/v) for ca 15 min and cleared in a nearly saturated solution of chloral hydrate (5:2, w/v) for ca 45 min at room temperature. The sections were mounted in glycerol and the observations were made by means of a phase contrast microscope (x 1200). The stomatal cavities were screened for the presence of *P. hordei* colonies. Per colony, two infection hyphae developed antipodally forming two poles which were oriented parallel to the long axis of the leaf. The number of haustoria was determined per pole. Colonies developed from doubly penetrated stomata were discarded. Observations were made on 80 colonies per genotype and per sampling time, if available.

The five or six leaves that had been inoculated less densely, were sampled ca 90 h.p.i. Fifty penetrated infection units per leaf segment were screened for developmental stage.

The experiment was repeated once.

RESULTS AND DISCUSSION

To determine whether or not early abortion occurs before the formation of the first haustoria, the proportion of colonies failing to produce haustoria had to be compared with the frequency of early abortion. In all four genotypes, some colonies did not form haustoria (Table 1). At 20 h.p.i. this proportion tended to be higher than at 35 h.p.i., indicating that a part of the colonies produced their first haustorium between 20 and 35 h.p.i. At 45 h.p.i. counting of haustoria was difficult in L94 due to the large amount of mycelium and in P3-Ze-2 and Duri because of an excessive uptake of stain by collapsing mesophyll cells.

No detectable host resistance. At 35 h.p.i. less than 10% of the colonies had not formed at least one haustorium in L94 (Table 1). This proportion was in agreement with the low degree of early abortion in the leaves that were investigated using fluorescence microscopy (Table 1). The colonies without haustoria had the typical

Table 1. Proportion of colonies of Puccinia hordei not forming at least one haustorium in the primary leaves of three barley and one wheat genotype, representing three types of resistance and the proportion of colonies that aborted early without provoking host cell necrosis.

Genotype	Type of resistance	First experiment			Second experiment		
		Proportion of colonies not forming haustoria		Proportion of early abortion without necrosis	Proportion of colonies not forming haustoria		Proportion of early abortion without necrosis
		20 h.p.i.[a]	35 h.p.i		20 h.p.i	35 h.p.i.	
L94	No resistance	0.25	0.06	0.03	0.29	0.09	0.06
P3-Ze-2	Hypersensitivity (Pa3)	0.35	0.05	0.11	0.43	0.36	0.26
Vada	Partial resistance	0.63	0.61	0.58	0.80	0.60	0.64
Duri (wheat)	Non-host reaction	0.76	0.64	0.86	0.77	0.76	0.75

[a] h.p.i.: hours post inoculation.

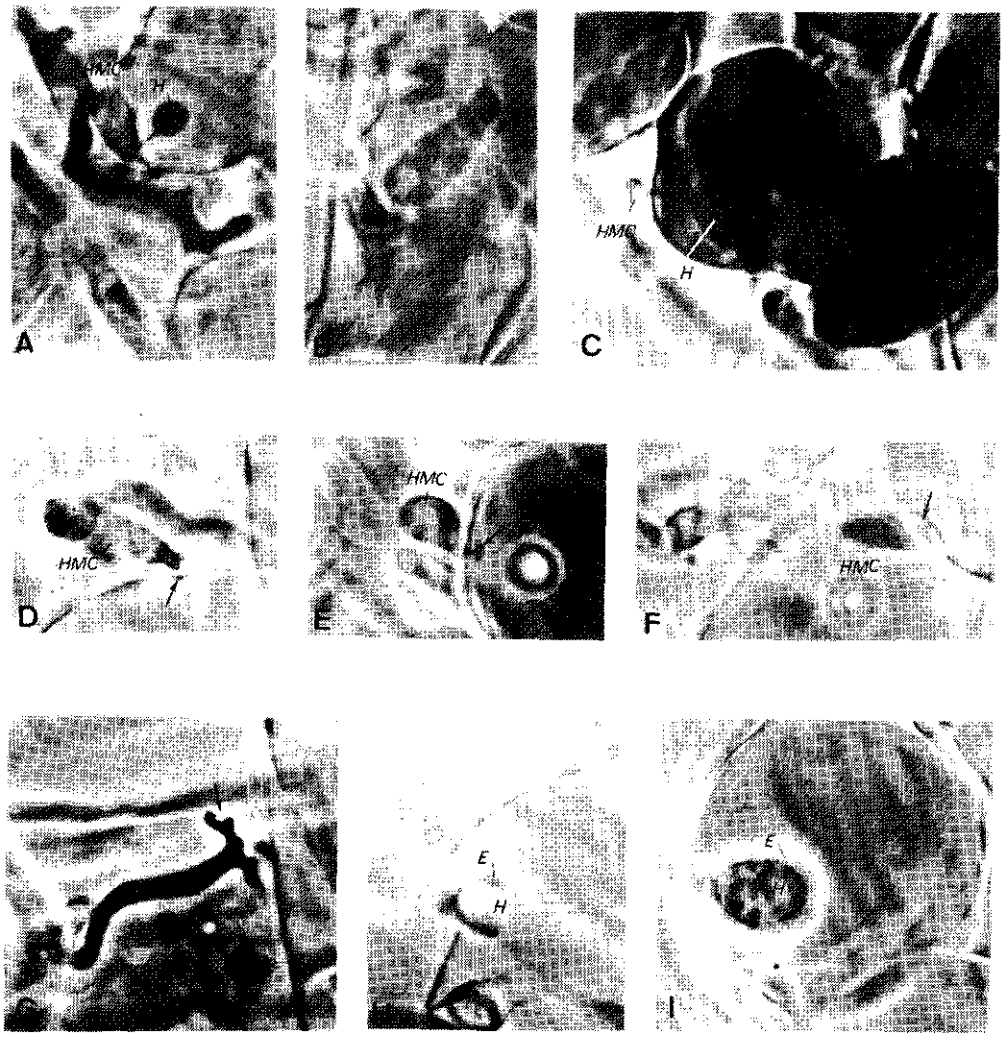

Fig. 1. Reactions to haustorium formation by Puccinia hordei; A, young haustorium and B, mature haustorium in barley cultivar L94 (no detectable resistance); C, deeply stained collapsing mesophyll cell of barley cultivar P3-Ze-2 (hypersensitivity) containing a haustorium; D, E, failed haustorium formation in barley cultivar Vada (partial resistance) and F, in L94 (no detectable resistance). The cell wall shows a slight thickening (arrow); G, lobed hyphal tip (arrow) in Vada; H, partially encased, and I, completely encased haustorium in wheat cultivar Duri (non-host). Indicated are: H, haustorium; HMC, haustorial mother cell; E, encasement. All figures x 1085.

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General discussion

The objective of the investigations presented in this thesis was, to deepen the insight in the nature of partial resistance (PR) in barley to leaf rust (*Puccinia hordei* Otth). This resistance is thought to be horizontal sensu Van der Plank (18,19), and the underlying principles may also apply for horizontal resistance (HR) in other host/pathogen relationships.

In the experiments, the histology of PR has been investigated in relation to other defence mechanisms of plants to rust fungi. Three important barriers were discernable, acting in three distinct phases of the infection process (Fig. 1).

First, the plant may lack the stimuli that are required to direct the germ tubes of a rust fungus towards the stomata. The germ tubes grow randomly, and seldom find stomata to form appressoria over. This barrier was demonstrated in the first paper (13) with lettuce and *P. hordei*. It may be the most common defence mechanism of (non-host) plants to rust fungi. Failing appressorium formation did not appear to be the cause of low infectibility due to PR in barley seedlings.

Second, an important barrier occurs at the interphase of the haustorial mother cell (HMC) and the first mesophyll cell wall to be penetrated. Failing formation of the first haustoria leads to early abortion of colonies: the colonies are arrested after the formation of a few HMCs, without provoking host cell necrosis. It could be demonstrated that PR of barley seedlings to *P. hordei* rests on such a hampered haustorium formation by the rust colonies (second and fifth paper [14,16]). In partially resistant adult plants, early abortion of colonies is also an important feature (unpublished results). This barrier is also pertinent for the infection units of rust that succeed in penetrating stomata of non-host species (fourth and fifth paper [15, 16]).

Third, a barrier may occur during the colonization phase, after the formation of at least one haustorium. Abortion of colonies in this phase (late abortion) is important with the simply inherited

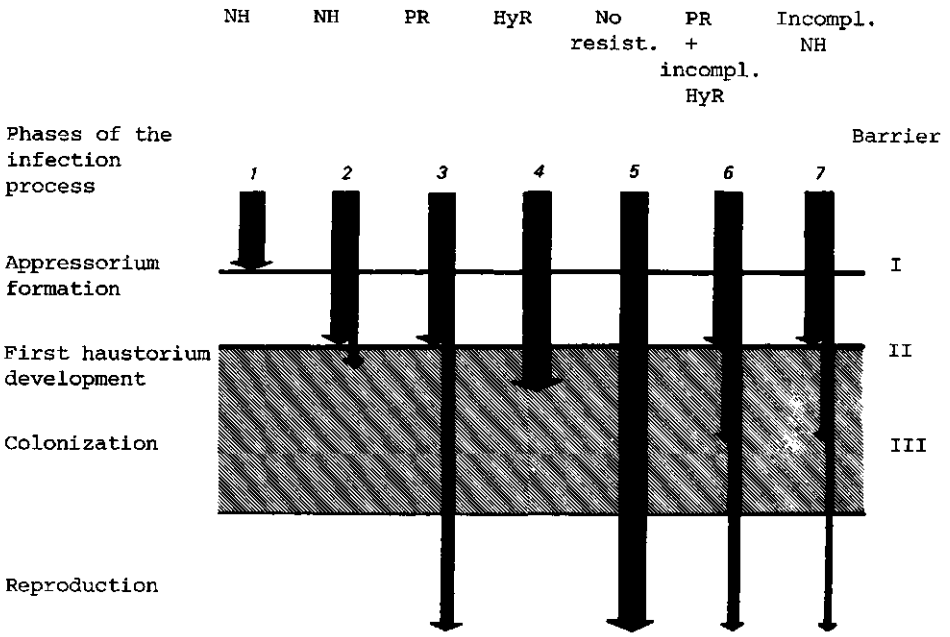

Fig. 1: Diagrammatic representation of the barriers a pathogen may encounter while invading a plant. The widths of the arrows are proportional to the fractions of the colonies encountering the respective barriers.
Abbreviations: HyR, hypersensitive resistance; NH, non-host reaction; PR, partial resistance.
The following host/pathogen systems are given as examples:
1. Lettuce/P. hordei (13).
2. Wheat cv. Duri/P. hordei (16).
3. Barley cv. Vada/P. hordei (14, 16).
4. Barley cv. Rika x (Rika x Baladi)/P. hordei, 121A (17).
5. Barley cv. L94/P. hordei (14, 16).
6. Barley P7-Va/P. hordei, 121A (17).
7. Barley cv. L92/P. recondita tritici (15).

hypersensitive resistance (third and fifth paper [16, 17]), but it occurs also with colonies in non-hosts that succeed to pass the second barrier. Late abortion in non-host combinations may (wheat/*P. hordei*) or may not (barley/*P. recondita secalis*) be associated with hypersensitivity. In partially resistant barley seedlings abortion of established *P. hordei* colonies is relatively rare, and usually not associated with host cell necrosis. In adult plants,

late abortion probably is a more important component of PR (unpublished results).

PR, assumed to represent HR, and hypersensitive resistance, assumed to represent vertical resistance (VR) (18, 19) in the barley/*P. hordei* relation, appear to be essentially different defence systems. They can be distinguished histologically, HR being 'pre-haustorial incompatibility', VR being 'post-haustorial incompatibility' in the terminology of Heath (8). There is neither a reason to consider a division of both types of resistance artificial (2) nor to consider them representing the same kind of resistance (12). Also HR does not seem to be an 'artifact' sensu Ellingboe (5).

The data presented here suggest that treatises in which HR and VR are compared (1, 2, 4, 10, 12) are of little relevance in gaining a better understanding of the mechanisms causing PR in barley to *P. hordei*. PR appears to be more akin to non-host reaction at barrier II. There is no essential difference between the histology of fungal development in a partially resistant host with incompletely acting hypersensitive resistance and that in an incomplete non-host (Fig. 1, arrow 6 and 7). It is also conceivable that when enough alleles for PR are compiled into one barley genotype, a barley variety is obtained that is a 'non-host' to *P. hordei* because of a complete blocking of colonies at barrier II.

Treatises that relate HR to non-host reactions have not yet come to the authors notice. In a recent paper, however, Parlevliet (11) stated that "The former (ie, stable pathogen-specific resistance genes, REN) consists of resistance genes, that are assumed to operate within the basic resistance-pathogenicity system and confer partial resistance. The latter (ie, very unstable pathogen-specific resistance, REN) genes are thought to act within an incompatibility system giving low IT reactions, superimposed upon this basic system...". The concept of a basic resistance-pathogenicity system on which VR is supposed to be superimposed has been delt with by several authors (3, 6, 9). Day (3) connects the concept to non-host reaction, but none of the authors relate the system to HR, as Parlevliet (11) does. If PR of barley to *P. hordei* acts within the basic system indeed (and the histological observations indicate so), the statement of Gabriel et al. (7) that there are no examples of naturally occuring variability in genes belonging to this system

does not hold true. Genotypic differences with respect to the basic system are definitely present in barley as well as in *P. hordei* (second paper [14]).

The results presented in this thesis are mainly of importance as a contribution to the understanding of the nature of HR. There are, however, implications for resistance breeding. The discovery that HR and VR in the barley/*P. hordei* relation are histologically discernable, suggests that a screening of breeding material for HR by fluorescence microscopy may be worthwhile. The methods are too laborious to justify large scale screening, but easy enough to be used in projects that are aimed to compile alleles for HR into important parents, to be used in a crossing program.

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Samenvatting

In de gerst (*Hordeum vulgare*) - dwergroest (*Puccinia hordei*) relatie zijn twee typen van resistentie te onderscheiden, namelijk overgevoeligheidsresistentie en partiële resistentie. Deze typen kunnen beschouwd worden als voorbeelden van Van der Plank's verticale en horizontale resistentie respectievelijk. Laatstgenoemd type is vermoedelijk beduidend duurzamer dan de overgevoeligheidsresistentie, maar over het mechanisme dat aan partiële resistentie ten grondslag ligt is nog maar zeer weinig bekend.

Partiële resistentie (PR) wordt gekenmerkt door een verminderde epidemie opbouw in het veld ondanks een vatbaar reactietype tussen waard en pathogeen. Uit macroscopisch onderzoek is gebleken dat een lange ontwikkelingsduur van de schimmel, een lage infectiedichtheid en een lage sporenproduktie per urediosorus de belangrijkste componenten van PR zijn.

Onderzoek naar de histologische achtergrond van de lage infectiedichtheid in partieel resistente genotypen vormt het onderwerp van de eerste twee artikelen van dit proefschrift. In de laatste drie artikelen wordt door middel van histologisch onderzoek PR vergeleken met overgevoeligheidsresistentie en met de niet-waard reactie.

Het eerste artikel betreft een onderzoek naar de appressorium vorming door *P. hordei* op zaailingen van de zeer vatbare gerstlijn L94 en van 11 gerstlijnen die een matig tot hoog niveau van PR bezaten. De 11 weinig vatbare gerstlijnen uit deze studie bleken niet reproduceerbaar te verschillen van L94 in het aantal door appressoria bezette huidmondjes per vierkante centimeter bladoppervlak. Dit wijst erop dat infectiedichtheidsverschillen ten gevolge van PR veroorzaakt worden door mechanismen die werken na de appressoriumvorming. Het bleek dat zelfs op de niet-waardsoort tarwe, waarop *P. hordei* geen symptomen veroorzaakt, niet minder appressoria werden gevormd dan op L94. Gerst en tarwe hebben een soortgelijke

epidermisstructuur welke waarschijnlijk de stimuli verschaft die de schimmel in staat stellen de huidmondjes te vinden en er appressoria op te vormen. Op een sla genotype trad geen appressoriumvorming op. Op deze niet-waardsoort, met een epidermisstructuur die sterk verschilt van die van Gramineae, ontbreken waarschijnlijk de stimuli die de schimmel in staat stellen huidmondjes te vinden.

In een onderzoek beschreven in het tweede artikel werd gebruik gemaakt van fluorescentie microscopie om de ontwikkeling van *P. hordei* in zaailingen van partieel resistentie gerstlijnen te bestuderen. Het bleek dat de infectiedichtheidsverschillen tussen de partieel resistente genotypen en de zeer vatbare L94 berusten op verschillen in frequentie van "vroege abortie" van *P. hordei* kolonies. Deze vorm van abortie trad op wanneer één tot zes haustoriummoedercellen waren gevormd. Vroege abortie in partieel resistente lijnen ging slechts zelden samen met necrose van mesophyl cellen in de waard. Er werd aangetoond dat ook verschillen in aggressiviteit tussen dwergroest isolaten hoofdzakelijk berusten op verschillen in frequentie van vroege abortie. Het feit dat een hoge frequentie van vroege abortie werd gevonden in verscheidene niet verwante gerstgenotypen wijst erop dat het mechanisme dat aan PR ten grondslag ligt deel uitmaakt van een algemeen in gerst voorkomend systeem.

Het effect van genen voor PR werd vervolgens bestudeerd bij aan- en afwezigheid van genen voor overgevoeligheid (derde artikel). Het bleek dat de vroege abortie ten gevolge van PR niet alleen optrad bij afwezigheid van een overgevoeligheidsreactie maar ook bij overgevoeligheid ten gevolge van het resistentiegen Pa7. Ook het vertragende effect van PR genen op de schimmelgroei bleef merkbaar bij aanwezigheid van Pa7. Wanneer een overgevoeligheidsreactie ten gevolge van het Pa3 gen optrad, waren de effecten van PR genen minder duidelijk waarneembaar. De reactie van Pa3 is veel eerder in het infectieproces waarneembaar dan die van Pa7. Pa3 veroorzaakt evenals PR genen abortie van een belangrijk deel van de kolonies direct na de vorming van één tot zes haustoriummoedercellen. Een belangrijk verschil tussen de vroege abortie door PR genen en het Pa3 gen is dat de abortie in het laatste geval geassocieerd is met necrose van waardcellen.

Het onderzoek beschreven in het vierde artikel toonde aan dat er een grote overeenkomst bestaat in histologie van de PR reactie en de niet-waard reactie bij dwergroest en bruine roest in gerst en

tarwe. Beide reacties werden gekarakteriseerd door een hoge frequentie van vroege abortie met weinig of geen necrose van mesophylcellen. Bij *P. hordei* op tarwe en *P. recondita secalis* op gerst bestond de niet-waard reactie uit een vroege abortie van vrijwel alle kolonies. *P. recondita tritici* was, ondanks een hoge frequentie van vroege abortie, in staat tot reproductie te komen op vier gerstlijnen met een zeer laag niveau van PR tegen *P. hordei*, maar niet op vier lijnen met een hoog niveau van PR. Mogelijk gaat een laag niveau van PR tegen *P. hordei* samen met een minder effectieve niet-waard reactie tegen *P. recondita tritici*.

Onderzoek naar de haustoriumvorming door *P. hordei* in vatbare, partieel resistente en overgevoelige gerst zaailingen en de niet-waardsoort tarwe (vijfde artikel) bevestigde de overeenkomst tussen de PR en niet-waard reacties. Bij beide afweersystemen bleek vroege abortie geassocieerd te zijn met een mislukken van de haustoriumvorming zonder necrose van de mesophylcel. De vroege abortie met necrose ten gevolge van het Pa3 overgevoeligheidsgen bleek op te treden kort na de vorming van het eerste haustorium.

Uit de studies kan geconcludeerd worden dat PR en overgevoeligheidsresistentie essentieel verschillende afweersystemen zijn. PR is, evenals de niet-waard reactie, voornamelijk een "pre-haustorium incompatibiliteit", overgevoeligheidsresistentie een "post-haustoriu incompatibiliteit". Men neemt wel aan dat overgevoeligheidsgenen werken binnen een systeem dat gesuperponeerd is op een basis resistentie-pathogeniteitssysteem. De niet-waard reactie zou berusten op een niet-herkenningsreactie in dit basis systeem. De resultaten van het onderzoek waarop dit proefschrift is gebaseerd wijzen erop dat PR genen behoren tot het basis resistentie-pathogeniteitssysteem.