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

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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 underlay it (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 humudifier. 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

Table 1. Relative number of stomata per cm² 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	Series II	Series III	$\bar{x}$	Barley genotype	Series I	Series II	Series 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 cm² (=100%)	164	129	283			194	104	287	
Genotype effect ($P \leq 0.05$, ANOVA)	yes	no	no			yes	no	no	

Tabel 1. Relatief aantal huidmondjes per cm² blad oppervlak beszet door appressoria van Puccinia hordei op het primaire blad van 12 gerstgenotypen. Per herhaling is het totaal gemiddelde gesteld op 100%.

density averaged 250 to 450 urediospores per cm².

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 mm² 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.

Table 2. Relative number of stomata per cm^2 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 cm^2 on L94 has been set at 100%.

Species	Cultivar	Series			
		I	II	III	IV
Barley (host)	L94	100a[1]	100 c	100a	100 b
Wheat (non-host)	Duri	132a	64 b	127a	130 c
	Adonis	112a	45a	138a	100 b
	S210	102a	38a	118a	72a
Lettuce (non-host)		0	0	–	–
Mean number of occupied stomata per cm^2 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.

Tabel 2. Relatief aantal huidmondjes per cm^2 blad oppervlak bezet door appressoria van Puccinia hordei op primaire bladeren van een gerstcultivar en drie tarwecultivars en op de eerste echte bladeren van een sla-genotype. Per herhaling is het gemiddelde aantal bezette stomata per cm^2 op L94 gesteld op 100%.

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 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 (Mlodzianovski 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 urediospoes, 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 dicotyledonous 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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Samenvatting

Appressoriumvorming van Puccinia hordei op partieel resistente gerst en op twee niet-waardsoorten

Eén van de componenten van partiële resistentie van gerst tegen dwergroest, *Puccinia hordei,* is een verminderde infectiedichtheid. Het mechanisme, dat hieraan ten grondslag ligt, is onbekend. Een experiment werd uitgevoerd om na te gaan of bij partieel resistente rassen een verminderde appressoriumvorming optreedt. Na inoculatie in een inoculatietoren en een zorgvuldig uitgevoerde incubatie werd het aantal huidmondjes per cm^2 bladoppervlak bepaald dat bezet was door appressoria van *P. hordei.* De elf weinig vatbare gerstlijnen uit deze studie bleken niet reproduceerbaar te verschillen van de zeer vatbare gerstlijn L94 in de mate van appressoriumbezetting. Dit wijst erop dat infectiedichtheidsverschillen t.g.v. partiële resistentie veroorzaakt worden door mechanismen die werken na de appressoriumvorming. In een tweede experiment werd aangetoond dat zelfs op de niet-waardsoort tarwe, waarop *P. hordei* geen symptomen veroorzaakt, niet minder appressoria worden gevormd dan op L94. Op een sla-genotype trad echter geen appressoriumvorming op. Op deze laatste niet-waardsoort ontbreken waarschijnlijk de stimuli die de schimmel in staat stellen huidmondjes te vinden.

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