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Germination and appressorium formation of wheat leaf rust on susceptible, partially resistant and resistant wheat seedlings and on seedlings of other Gramineae

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Abstract

Germination and appressorium formation of wheat leaf rust urediospores were studied in two experiments. No consistent differences could be detected between 11 genotypes of wheat (*Triticum aestivum*), two of barley, one of *Triticum dicoccum*, one of *T. dicoccoides*, one of *T. boeoticum* and one of *Aegilops squarrosa*. Host genotypes and non-hosts gave similar results, suggesting that the phases before penetration of the host leaf are not affected by the resistance mechanisms operating in hosts and non-host genotypes.

Additional keywords: Puccinia recondita f.sp. *tritici*, partial resistance.

Introduction

Prepenetration stages in the development of wheat leaf rust (*Puccinia recondita* f.sp. *tritici* Robb. ex Desmon., syn. *Pucc. triticina* Erikss.) urediospores are germination, germ tube growth and formation of an appressorium over a stoma. Chemical and physical stimuli affect growth of the germinating rust spore (Hoch and Staples, 1987; Staples and Macko, 1984). It is believed that germination and germ tube growth require less stimuli than appressorium formation (Goodman et al., 1986). Major gene resistance is often not judged as an important factor which influences appressorium formation (Hoch and Staples, 1987). However, prepenetration inhibition of a leaf pathogen has been reported (Evans and Pluck, 1978; Stubbs and Plotnikova, 1972). Hooker (1984) cited Peletz who reported that significantly more corn rust spores germinated on the leaf surface of susceptible than of resistant plants. Poyntz and Hyde (1987) reported a difference in germination but not in appressorium formation of wheat leaf rust between a susceptible and a resistant wheat genotype. Not much is known about the effects of genes for partial resistance on the prepenetration phases.

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

Materials and methods

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

Experiment 1. In the first experiment of five leaves per genotype, leaf segments from the central part of the leaf, each 4.0 cm long, were harvested approx. five and ten hours after the estimated point of darkness (19.00 h), gently boiled in lactohenol : ethanol (1 : 2) and placed in 0.05% Blancophor for 5 min (Niks, 1981). Care was taken not to loose germinated urediospores. The glycerol mounted segments, were observed under an epi-fluorescence microscope. Each leaf segment, the number of germinated urediospores without and with an appressorium were assessed.

Urediospores were noted as germinated if the length of the germ tube exceeded the diameter of the spore. The area of the leaf segments was measured. The numbers of germinated urediospores and formed appressoria were converted to numbers per cm^2 for statistical analysis. The ratio of appressoria and germinated urediospores was calculated.

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

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

Experiment 2. Experimental procedures were identical to those of Experiment 1. Differences are described below.

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

The experiment consisted of two series. Seedlings in the first series were sown and inoculated a week earlier and showed longer leaves than seedlings in the second series. Each genotype, five leaves were used. From each leaf two segments, each 4.0 cm long, were harvested after one night incubation with a relative humidity of 100%. One segment included the top, the other segment was harvested from the central part of the leaf.

A priori assumption were:

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

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

Results

Experiment 1. Estimated latency periods ranged from four to eight days (Table 1). Most genotypes studied showed a susceptible infection type. The barley genotype Vada and *Triticum boeoticum* did not show any visible response after inoculation with wheat leaf rust. In the barley genotype L94 small colonies with chlorosis developed. The mean number of spores assessed per genotype was 247 five hours after inoculation and 146

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

Nr	Genotype	LP50	IT	5 hours		10 hours	
				germ.	ratio	germ.	ratio
1	L94 (barly)	8	3	23.4 a*	0.52 a	17.3 a	0.39 ab
2	Vada (barley)	–	0	18.3 a	0.49 a	21.5 a	0.38 ab
3	Saratovskaja 36	4	9	29.9 a	0.50 a	25.0 a	0.38 ab
4	Melchior	7	9	16.2 a	0.44 a	33.4 a	0.44 ab
5	Bonza Sib	7	9	33.3 a	0.50 a	25.1 a	0.44 ab
6	Kaspar	6	9	24.6 a	0.55 a	28.8 a	0.54 ab
7	CI 9321	6	9	25.5 a	0.41 a	42.3 a	0.35 ab
8	Downy	6	9	24.3 a	0.44 a	32.9 a	0.20 a
9	*Triticum dicoccum*	8	8	31.0 a	0.53 a	28.7 a	0.51 b
10	*Triticum dicoccoides*	8	9	35.4 a	0.55 a	42.5 a	0.54 b
11	*Aegilops squarrosa*	4	9	25.9 a	0.35 a	48.3 a	0.50 ab
12	*Triticum boeoticum*	–	0	21.5 a	0.30 a	34.9 a	0.40 ab
Average				25.8	0.47	31.7	0.42

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

Table 2. Latency period relative to *Aegilops squarrosa* (RLP50), number of germinated urediospores per cm^2 (germ.) and the ratio of appressoria and germinated urediospores of wheat leaf rust on 4.0 cm long segments from the top and central part of seedling leaves of wheat and other gramineae.

Nr	Genotype	RLP50	Series 1				Series 2			
			top		middle		top		middle	
			germ.	ratio	germ.	ratio	germ.	ratio	germ.	ratio
1	Vada	–	65.4 a	0.77 a	61.8 a	0.70 a	164.0 ab	0.56 a	203.5 c	0.58 a
2	Melchior	110.5	54.6 a	0.78 a	64.3 a	0.87 a	169.2 ab	0.54 a	171.9 bc	0.46 a
3	Kaspar	113.3	63.3 a	0.84 a	60.6 a	0.83 a	181.5 ab	0.67 a	169.7 ab	0.70 a
4	CI 9321	107.2	69.8 a	0.78 a	76.0 a	0.67 a	163.6 ab	0.44 a	98.2 ab	0.68 a
5	Akabozu	126.8	54.2 a	0.81 a	94.4 a	0.73 a	147.0 a	0.63 a	174.8 ab	0.61 a
6	Canthatch	106.5	58.4 a	0.81 a	83.1 a	0.84 a	159.3 ab	0.49 a	211.2 bc	0.66 a
7	Thatcher	107.6	72.2 a	0.84 a	61.7 a	0.79 a	175.3 b	0.39 a	192.6 ab	0.76 a
8	Lr 19	–	81.5 a	0.81 a	82.8 a	0.83 a	165.9 ab	0.58 a	194.0 abc	0.65 a
9	*Triticum dicoccum*	109.2	92.4 a	0.81 a	80.2 a	0.85 a	125.5 ab	0.54 a	198.3 ab	0.61 a
10	*Aegilops squarrosa*	100.0	76.9 a	0.78 a	119.0 a	0.72 a	152.4 a	0.82 a	98.5 a	0.65 a
Average			68.9	0.80	78.4	0.78	160.4	0.57	171.3	0.64

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

ten hours after inoculation. The mean number of germinated urediospores per genotype was 25.8 per cm^2 five hours after inoculations and 31.7 five hours later (Table 1). The mean number of appressoria averaged 12.2 per cm^2 after 5 hours and 13.8 after 10 hours. None of the *a priori* contrasts was significant. In the first experiment there were no significant differences in number of germinated urediospores and appressoria per cm^2 between the genotypes. Five hours after inoculation the ratio of appressoria and germinated urediospores did not show significant differences. Ten hours after inoculation some differences in the ratio were observed. The data do not show a relation between latency period or infection type on one hand and number of germinated urediospores per cm^2 or the ratio of appressoria and germinated urediospores on the other.

Experiment 2. The infection types of all genotypes were high (8-9) except for Lr 19 and the barley genotypes L94 and Vada. Relative latency period ranged from 100 for *Aegilopus squarosa* to nearly 127 for the partially resistant wheat genotype Akabozu (Table 2). The mean number of spores assessed per genotype was 490 (top) and 636 (middle) in series 1, 1139 (top) and 1541 (middle) in series 2. The mean number of germinated urediospores per cm^2 in series 2 was considerably higher than in series 1. This was probably caused by differences in inoculum density. The two tested *a priori* contrasts were not significant. In the first series no genotypic differences in number of germinated urediospores per cm^2 and the ratio of appressoria and germinated urediospores have been observed (Table 2). In the second series the numbers of germinated urediospores showed significant differences, however, no significant differences between the genotypes of hosts and non-hosts were observed for the ratio of appressoria and germinated urediospores. No relation between latency period and number of germinated urediospores without or with an appressorium appeared from the data.

Discussion

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

Other workers reported no significant differences in germination and appressorium formation neither between susceptible and slow leaf rusting wheat genotypes (Chang and Line, 1983; Gavinlertvatana and Wilcoxson, 1978; Lee and Shaner, 1984) nor between near-isogenic lines with different hypersensitivity resistance genes to wheat leaf rust (Plotnikova et al., 1985). Effects of a gene for hypersensitive reaction on the early stages of infection could not be detected in stem rust (Gousseau and Deverall, 1985). The present study indicates that there were no consistent (measurable) differences in germination and appressorium formation between hosts and non-hosts. This study also indicates that genotypes with genes coding for partial resistance or hypersensitive resistance to wheat leaf rust do not seem to influence the pre-penetration phases.

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Samenvatting

Kieming en appressoriumvorming van bruine roest op vatbare, partieel resistente en resistente tarwekiemplanten en op kiemplanten van andere gramineeënsoorten

Kieming en de vorming van appressoria is bestudeerd in twee experimenten. Er zijn geen systematische verschillen waargenomen tussen 11 genotypen van tarwe, twee van gerst, een van *Triticum dicoccum*, een van *T. dicoccoides*, een van *T. boeoticum* en een van *Aegilops squarrosa*. Waard en niet-waard genotypen gaven overeenkomstige resultaten, dit suggereert dat de fasen voor penetratie van het gastheerblad niet beïnvloed worden door de resistentiemechanismen werkzaam in waard en niet-waard.

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