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Uromyces viciae-fabae haustorium formation in susceptible and resistant faba bean lines

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Abstract

Haustorium formation by the faba bean rust (*Uromyces viciae-fabae*) was studied on susceptible and resistant faba bean lines. The resistant lines showed incomplete resistance, based on late acting hypersensitivity or on non-hypersensitive resistance acting before haustorium formation. Histological observations on infected leaves showed that both the number of haustoria per infection unit and their developmental stage was reduced in both resistant lines. Isolation of haustoria confirmed that both the number and the size of haustoria were reduced in resistant lines, irrespective of whether the resistance was associated with hypersensitivity. Plant age had no detectable effect on both parameters.

Faba bean rust, caused by *Uromyces viciae-fabae* (Pers.) Schroet., is a disease of worldwide distribution. It is important in the Mediterranean region where moderate to substantial yield losses occur, particularly if the disease starts early in the season. Varying levels of incomplete resistance have been reported. They result in a reduction of disease severity due to a prolonged latency period (LP) and reduced colony size (Rashid and Bernier, 1986; 1991; Sillero et al., 2000). This incomplete resistance is not associated with macroscopically visible necrosis in most of the cases reported. Recently, some lines have been identified in which incomplete resistance is associated with a moderate level of necrosis (Sillero et al., 2000) due to a late acting hypersensitive response (Sillero and Rubiales, 2002).

The infection is initiated by a urediospore germling. It differentiates an appressorium over a stoma, from which an infection peg develops and enters the plant through the stomatal pore. Then, a substomatal vesicle is formed that develops intercellular infection hyphae. Haustorium mother cells are differentiated in contact with mesophyll cells, and haustoria are formed in the host cell by invagination of the plasma membrane. Development of haustoria is essential for a successful infection. In addition to uptake of nutrients, haustoria are likely to carry out functions that are involved in recognition, specificity, and induction or suppression of host cell defence responses. The zone between the haustorium surface and surrounding host cell plasmalemma, the extrahaustorial matrix, represents the most intimate area of contact between host and parasite (Staples, 2001).

In the present study, we compared haustorium formation by *U. viciae-fabae* in living cells of susceptible and resistant faba bean lines by histological observations and by isolation of haustoria, in order to determine the effect of both types of resistance and of plant age on number and size of haustoria.

Faba bean lines V-1273, with incomplete resistance associated with late hypersensitivity, BPL-261, with incomplete non-hypersensitive resistance (Sillero et al., 2000), and the susceptible control line VF-176 were studied. Seedlings with the second leaf fully expanded and older plants with the eighth leaf fully expanded were inoculated by dusting rust spores diluted in talcum powder (1 : 10). For all the experiments, freshly collected spores of single-spore isolate 96-Cord-02 of *U. viciae-fabae* multiplied on VF-176 susceptible

faba bean line were used. After incubation for 24 h at 20 °C in complete darkness and 100% relative humidity, plants were transferred to a growth chamber at 20 °C under a 14 h light : 10 h dark photoperiod. Three consecutive experiments were carried out with three plants per line in each experiment.

Latency period, the period of time between inoculation and sporulation of 50% of the pustules, was determined by counting daily the number of uredosori visible in a 1.5 cm^2 marked area on the leaves, using a pocket lens (magnification 7×), until the number of uredosori no longer increased. At 14 days after inoculation (d.a.i.), reaction type was assessed on remaining leaves according to the 0–4 Infection Type (IT) scale of Stakman et al. (1962). The susceptible control VF-176 and line BPL-261 displayed a susceptible reaction (IT 4) in both seedling and mature plants (Table 1). These lines differed, however, in the LP, which was significantly longer in BPL-261 line. Line V-1273 displayed a much longer LP than both VF-176 and BPL-261, but this was associated with a moderate reduction in IT. The latent period was longer in mature plants than in seedlings (215 vs. 173 h).

Haustorium formation was studied on leaf segments cut 2 d.a.i. and stained with Trypan blue (Sillero and Rubiales, 2002). Three leaf segments (2–3 cm^2) were harvested per genotype per replication. For each leaf piece, 15–20 randomly chosen colonies were studied using a phase contrast Leica DM LS microscope at 400× magnification. The number of haustoria per colony were counted. The relative maturity of each haustorium was recorded. Small spherical and dark-staining haustoria were considered young; large oblong and vacuolated haustoria were considered mature.

The number of haustoria formed per colony and their relative stage of maturity differed between the host lines. Significantly fewer haustoria were formed in lines BPL-261 and V-1273 than in the control VF-176, in both seedlings and adult plants. In addition to that, haustoria formed on BPL-261 and V-1273 tended to retain an immature appearance: small spherical and dark staining haustoria were more frequent than mature, irregularly lobed haustoria. No significant differences in number of haustoria per colony and relative stage of maturity were observed between BPL-261 and V-1273 at any of the plant maturity stages. Fewer haustoria per colony were formed in mature plants than in seedlings (2.9 vs. 3.9, respectively, in the control). The number of haustoria per colony was particularly low in mature plants of V-1273, but differences with BPL-261 were not significant. V-1273, the line displaying late acting hypersensitivity allowed formation of fewer haustoria per colony and the haustoria tended to be smaller than those of the control, even at 2 d.a.i. when hypersensitivity was still a rather rare event. Hypersensitivity in faba bean is more evident microscopically from day 4 after inoculation and maximum by day 8 (Sillero and Rubiales, 2002). This suggests that in addition to the late acting hypersensitive resistance, V-1273 carries other genes for a level of non-hypersensitive resistance comparable to that in BPL-261.

Seven days after inoculation, haustoria were isolated following the procedure described by Tiburzy et al. (1992) Observations on number and size of haustoria were made by fluorescence microscopy at 1000× magnification after staining by adding a drop of 0.1% Uvitex 2B (Ciba Geigy) in 0.1 M Tris/HCl buffer. The number of haustoria was determined by use of a Thoma counting chamber and calculated as the number of haustoria per fresh leaf weight. The length and average width of 100 haustoria were measured and haustorium size was calculated. Isolated haustoria with

Table 1. Reaction of faba bean lines to rust (*Uromyces viciae-fabae*) 14 days after inoculation (d.a.i.) and microscopical observation on haustoria formation 2 d.a.i.

Line	Seedling						Adult plant					
	14 d.a.i.		2 d.a.i.				14 d.a.i.		2 d.a.i.			
	Infection type	Latency[1] period	No. of haustoria/ colony	Mature haustoria (%)	Medium haustoria (%)	Young haustoria (%)	Infection type	Latency period	No. of haustoria/ colony	Mature haustoria (%)	Medium haustoria (%)	Young haustoria (%)
VF-176	4	100 c[2] (172.9 h)	3.9 a	39.6 a	40.1 a	20.3 a	4	100 b (215.1 h)	2.9 a	40.7 a	39.0 a	20.3 a
BPL-261	4	113.9 b	1.8 b	24.7 b	48.9 b	26.4 a	4	119.5 ab	1.5 b	18.8 b	51.3 a	29.9 a
V-1273	2–2+	136.6 a	2.4 b	23.8 b	47.1 b	29.1 a	2	139.5 a	0.9 b	16.0 b	57.3 a	26.7 a

All data are the averages of three replicates.

[1]Latency period expressed as values relatives to VF-176 (=100%). The actual values (hours) are presented in brackets.

[2]Data with the same letter in each column are not significantly different ($p < 0.05$, Duncan's multiple range test).

Table 2. Uromyces viciae-fabae haustorium size and number of haustoria per milligram of infected faba bean leaves, 7 days after inoculation

Line	Haustorium size (μm^2)		No. of haustoria/mg leaf tissue	
	Seedlings	Adult plants	Seedlings	Adult plants
VF-176	107.3 a	107.4 a	618 a	548 a
BPL-261	94.3 b	85.3 b	293 ab	260 b
V-1273	81.2 b	75.2 c	248 b	198 b

Data with the same letter in each column are not significantly different ($p < 0.05$, Duncan's multiple range test). All data are the averages of three replicates.

ball-shaped, bean-shaped or lobed bodies, with the haustorial neck still connected to the body, exhibited a bright fluorescence after staining with Uvitex 2B. Transmission light microscopy revealed that haustoria retained their granular cytoplasmic contents. The haustoria maintained their typical shape after the isolation procedure, even after a week in isolation medium under refrigeration. This is in agreement with previous observations on isolated haustoria (Hahn and Mendgen, 1992; Cantrill and Deverall, 1993) and suggests a rather rigid wall structure.

Measurement of isolated haustoria confirmed the histological observations: average haustorium size was significantly reduced in both resistant lines (Table 2) with respect to the control, both in seedlings and in mature plants. Haustoria in adult plants line V-1273 were significantly smaller than those in BPL-261, but the difference in the seedlings was not significant. In the susceptible check there were no differences in haustorium size harvested from seedling and mature plants. The number of haustoria per unit leaf weight was also reduced in the resistant lines. Differences from the control were significant in adult plant in both lines but in seedlings only in line V-1273. As with the haustorium size, the number of haustoria was slightly reduced, although not significantly, with the plant age.

The isolation of haustoria could help to facilitate molecular, genetic and biochemical studies of the host–parasite interaction. In studies with faba bean, haustoria have been isolated to construct a c-DNA library and several plant-induced genes have been identified (Hahn and Mengden, 1992). New studies could be carried out where proteins can be isolated from haustoria and their function in the host–parasite interaction investigated.

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