# Haustorium Formation by *Puccinia hordei* in Leaves of Hypersensitive, Partially Resistant, and Nonhost Plant Genotypes

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## ABSTRACT

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In barley genotypes with early acting hypersensitivity or with partial resistance and in nonhost 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 *Pa*3 gene for hypersensitivity. In nonhost wheat, most of the infection units aborted before the formation of the first haustorium.

Three types of resistance are associated with early cessation of colony growth of the barley leaf rust pathogen, *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 *Pa*3 (8), and in nonhost species such as wheat (7). Since a clearing and staining technique, which did not permit observations of haustoria, was used in those studies, the research reported here 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. 'Duri', a nonhost [7]) and genotypes of barley (*Hordeum vulgare* L. 'Vada', which has a high level of partial resistance [6], 'P3-Ze-2' (carrying the *Pa*3 allele for hypersensitivity in a background with a moderate level of partial resistance [8]), and 'L94' (no detectable host resistance, a susceptible control). The plants were grown in a 37 × 39-cm planting box. The primary leaves were inoculated with urediospores of *Puccinia hordei* isolate 121A, which is avirulent to *Pa*3 and causes no symptoms in wheat cultivar Duri. Seven of the 12 primary leaves per genotype were densely inoculated with a brush. Sections of these leaves were used for observing haustorium formation. The remaining leaves received an inoculum dose of about 100 spores per square centimeter applied in a settling tower. The degree of early abortion and the degree of host cell necrosis associated with the early abortion in these leaves were investigated by using fluorescence microscopy (6,8). The moderate inoculum density was necessary to prevent early entwinement 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 hr postinoculation (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 hr, boiled in 0.03 % aniline blue in lactophenol-ethanol (1:2, v/v) for ~15 min, and cleared in a nearly saturated solution of chloral hydrate (5:2, w/v) for ~45 min at room temperature. The sections were mounted in glycerol and the observations were made by means of a phase-contrast microscope (×1,200). The stomatal cavities were screened for the presence of *P. hordei* colonies. Per colony, two infection hyphae developed antipodally forming two poles that 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, if available, at each sampling time.

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

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## RESULTS AND DISCUSSION

To determine whether 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 by using fluorescence microscopy (Table 1). The colonies without haustoria had the typical shape of early aborted colonies (6): sparsely branched infection hyphae and one to three HMCs per pole.

**Hypersensitivity.** In the first experiment, only 5% of the colonies of *P. hordei* in P3-Ze-2 had not formed one haustorium at 35 h.p.i.; in the second experiment, this proportion was 36%. These percentages agreed fairly well with the degree of early abortion not associated with host cell necrosis (Table 1). The other colonies produced conventional haustoria of the same type as formed in L94 (Fig. 1A and B), but fewer. At 20 h.p.i., no sign of resistance reaction was observed, but at 35 h.p.i. many colonies were associated with one or two deeply stained and sometimes collapsed host cells. Such cells always contained a haustorium (Fig. 1C). Fluorescence microscopy showed that about 25 (experiment 1) and 20% (experiment 2) of the penetrated infection units were early aborted in association with host cell necrosis. A high degree of early abortion with cell necrosis in P3-Ze-2 was observed also in a previous study (8). The results of the study on the haustorium formation suggests that early abortion associated with cell necrosis occurred after the formation of the first haustorium. The action of

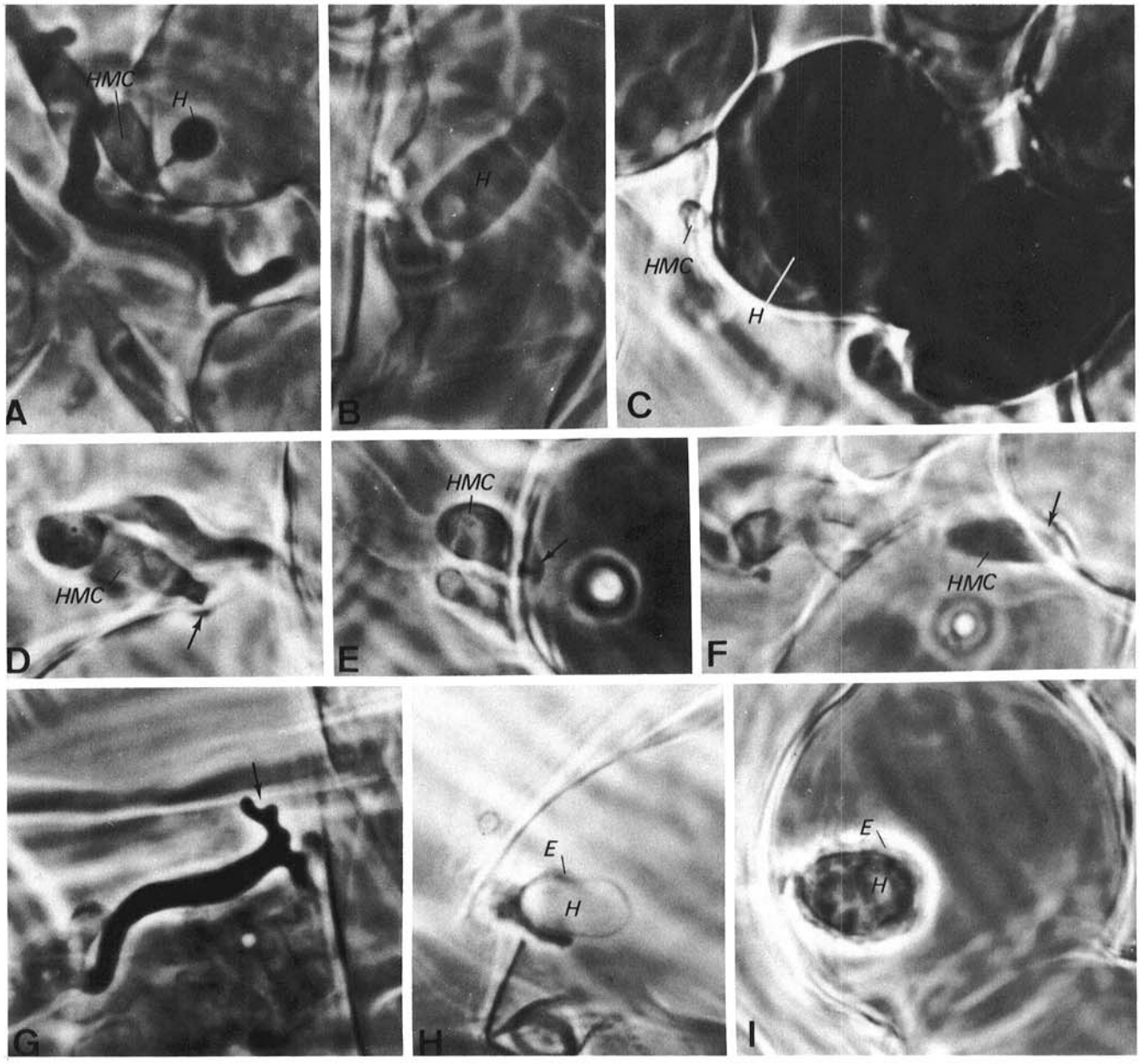


**Fig. 1.** Reactions to haustorium formation by *Puccinia hordei*: **A,** young haustorium and **B,** mature haustorium in barley cultivar L94 (no detectable resistance); **C,** deeply stained collapsing mesophyll cell of barley cultivar P3-Ze-2 (hypersensitivity) containing a haustorium; **D and E,** 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 (nonhost). Symbols represent: H = haustorium; HMC = haustorial mother cell; E = encasement. All figures ×1,560.

TABLE 1. Proportion of colonies of *Puccinia hordei* not forming at least one haustorium in the primary leaves of three barley genotypes 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. | |
| Barley | | | | | | | |
| 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 |
| Wheat | | | | | | | |
| Duri | Nonhost reaction | 0.76 | 0.64 | 0.86 | 0.77 | 0.76 | 0.75 |

[a]h.p.i. = Hours postinoculation.

the *Pa3* allele to *P. hordei* ressembles the early hypersensitivity of wheat (*Sr5*) to stem rust (9), of bean to bean rust (4), and of cowpea to cowpea rust (1), in which it also has been established that the reaction occurs after the formation of the first haustorium.

**Partial resistance.** The chance of successful haustorium formation by *P. hordei* in partially resistant Vada was much lower than in the highly infectible L94. The haustorium formation in Vada was nearly as poor as in the nonhost wheat cultivar Duri. At 35 h.p.i., about 60% of the colonies were still without haustorium (Table 1), and at 45 h.p.i. this proportion was still the same. The high proportion of colonies without haustorium coincided with the high proportion of early abortion in this experiment (Table 1). Often, a slight thickening of the cell wall at the place of contact with the HMC was visible (Fig. 1D and E). In the three other genotypes, failure of haustorium formation often was associated with similar reactions (Fig. 1F). No cell collapse, excessive cell staining, or other cell alterations were observed. The colonies seemed to stop growth after the formation of one to three HMCs per pole. Sometimes unusual lobed HMCs and hyphal tips were found (Fig. 1G), as if one HMC had tried to penetrate the cell wall at more than one place. The haustoria formed in Vada were typical.

**Nonhost reaction.** In the wheat cultivar Duri, the majority of the colonies produced no haustoria (Table 1). The reaction of the mesophyll cells was similar to that in Vada. Also other nonhost reactions are reported to be characterized by a blocking of the fungus before the haustorium development (2,3,10), possibly because of a failing recognition reaction at the interface of the HMC and the plant cell wall (5). At 20 h.p.i., part of the colonies had produced one haustorium of a normal appearance. At 35 h.p.i., some of these haustoria were encased in granular-looking material (Fig. 1H and I). Part of the haustorium-containing cells became deeply stained, particularly at 45 h.p.i. The colonies with haustorium often were somewhat more branched than the colonies without. The investigations by fluorescence microscopy on the moderately densely inoculated leaves revealed that in both experiments all penetrated infection units were arrested by early abortion. Part of these colonies were associated with necrotic mesophyll cells. Such colonies often were more branched than those without necrosis, and therefore probably had formed a haustorium.

With regard to the poor formation of first haustoria, nonhost reaction resembled partial resistance. The relatively few colonies that had formed haustoria, however, were arrested by a hypersensitive-type of reaction that is associated either with necrosis of the invaded mesophyll cell or with encasement of the haustorium. Both reactions have been reported for major gene resistance and nonhost reactions (1,3). Also the established colonies of *P. recondita tritici* in four genotypes of barley were associated with hypersensitive reactions (7).

Heath (2) stated that "non-host responses typically occur during the earliest stages of infection" and that "varietal resistance does not appear to make use of these effective forms of pre-haustorial incompatibility, but is characterized by responses induced after the formation of the first haustorium." The observations in Duri (nonhost) and P3-Ze-2 (early hypersensitivity) are in accordance with this view. Results of the present study, however, demonstrate that partial resistance, as displayed by Vada, is a type of "varietal" resistance that rests on such a prehaustorial incompatibility.

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