Physiology and Biochemistry

Postpenetration Development of *Puccinia coronata avenae* in Slow- and Fast-Rusting Cultivars of *Avena byzantina*


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ABSTRACT


Development of *Puccinia coronata* in slow-rusting (Red Rustproof 14) and fast-rusting (Fulghum) cultivars of *Avena byzantina* was compared by using fluorescence microscopy. The first visible response was fluorescence in mesophyll cell walls of the slow-rusting cultivar. This reaction occurred about 18 hr after inoculation, and seemed to depend upon development of substomal vesicles. The linear growth of hyphae in the slow-ruster was significantly less than in the fast-ruster 48 hr after inoculation. The growth of the parasite at individual infection sites in the slow-ruster was inversely related to the degree of host response, which was expressed as the number of fluorescing cells. A rapid and severe host reaction resulted in the arrest of growth of the parasite beforehaustorial mother cells formed, but slow host response merely retarded hyphal growth. The arrest of growth was associated with a reduction in the number of uredinia, while the retardation of hyphal growth reduced size of the uredinia and increased the latent period (LP6). Thus, fluorescence of the mesophyll cell walls was correlated with these three components of slow-rusting. In some sites, the host did not react to invasion by the parasite. When this happened, the hyphae grew at about the same rate in both cultivars. A few infection sites in the fast-rusting cultivar also exhibited a reaction in the mesophyll cells, but only 8% reacted quickly enough to arrest the growth of the parasite. Seven days after inoculation, the average area of the uredinia of the fast-ruster was about three times larger than that of the slow-ruster.

Additional key words: horizontal resistance, reduced pustule number.

The effect of slow-rusting on the retardation of crown rust (*Puccinia coronata* f. sp. *avenae* Fraser & Lwed.) development on oats (*Avena byzantina* C. Koch) was reported in 1889 (22). Early workers also noted that certain types of *A. byzantina* exhibited a susceptible reaction but rusted so slowly that they did not suffer any yield loss (20). Slow-rusting in Red Rustproof oats was observed to retard the onset and to impede the rate of development of the epidemic (10).

During the past century, much has been written about slow-rusting, but very little is known about the basic nature of this unusual phenomenon. Reduced penetration by the parasite was considered responsible for a reduction in the amount of disease (4,5), but other workers (12–14,18) have suggested that the major factors that controlled slow-rusting occurred after penetration. Restricted colonization of host tissue has also been reported as the major factor affecting slow-rusting (3,9,21). We recently reported that events that controlled development of the pathogen in a slow-rusting cultivar of *A. byzantina* occurred after penetration (11).

In this article we now describe the host-pathogen interactions that occurred and assess the effects of host reaction on the major components of slow-rusting.

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MATERIALS AND METHODS

Field-grown plants were used because they exhibited a more pronounced form of slow-rusting than those grown in a growth chamber (11). Red Rustproof-14 (CI 4876) (slow-rusting) was planted 5 November 1981 and Fulghum (CI 708) (fast-rusting) was planted 3 December 1981. Several tests were conducted in the spring of 1982. Plants in growth stage 5 (8) were transferred to 20.5-cm-diameter clay pots, and placed in a greenhouse for 2 days. The lower leaves were removed, and the youngest fully mature leaves (leaf 5 or 6) were inoculated in a settling tower (2). About 250 spores per square centimeter were deposited on leaves. Only race 264B4 of crown rust was used because, in previous tests, the slow-rusting cultivar reacted the same to all races as which it had been tested (10). After spore application, plants were maintained in a dew chamber for 16-18 hr at 20-22° C. Inoculated plants were placed in a greenhouse where the temperature ranged from 10-31° C and maximum light was ~64,500 lux. Beginning 10 days after inoculation, uredinia were counted at 24-hr intervals on both leaf surfaces without visual aids.

Leaves from five different plants of each cultivar were removed, 24, 48, 72, and 96 hr after spore application and prepared for fluorescence microscopy according to a procedure described by Kuck et al (6). We modified their procedure by extending the period in chloroform-methanol to 16-18 hr and used a 0.3% solution of fluorescence brightener 124 (Tinopal BSA) obtained from the Dyestuffs and Chemical Division, Ciba-Geigy Corp., Greensboro, NC 27409. Leaf pieces (2 x 5 cm) were mounted on microscope slides in lactophenol and examined with a Zeiss standard microscope fitted with epifluorescence equipment (light source HBO 50 W, exciter filter BP 490-440, chromatic beam-splitter FT 460, and barrier filter LP 470).

Tests were conducted in the spring of 1983 to determine the earliest histological response of the host and to describe the host-parasite interactions that occurred later in the disease cycle. Planting dates were similar to the 1981 planting. Materials and methods described above were used in these tests. Leaves were excised and prepared for fluorescence microscopy 16, 18, 20, and 168 hr after spore application.

RESULTS

The first visible reaction of the slow-ruster to the parasite was observed 18 hr after spore application, or about 6-8 hr after formation of the substomatatal vesicle. The initial host response detected was fluorescence in mesophyll cell walls (Fig. 1). Parvin et al. (11) found that the slow-rusting cultivar Red Rustproof was about 2 days longer that than the fast-ruster, and the L.P.0 of the slow-rusting cultivar in the epidermis of Red Rustproof was about 7 days longer than that of the fast-rusting cultivar Fulghum. In this paper, the L.P.0 is defined as the time (in days) from spore application until 50% of the uredinia were visible. This value is similar to the L.P.0 defined by Parlevliet (15) and the T.S defined by Shaner (17). Although the linear growth of the hypha of Red Rustproof was less than that of Fulghum 24 hr after spore application, the difference between the growth of the parasite in these cultivars was not statistically significant until 48 hr after spore application (Table 1). Over a 72-hr period (24-96 hr after inoculation) the linear growth of the hypha in the slow-ruster increased about twofold and that in the fast-ruster about fourfold.

Seven days (168 hr) after inoculation, examination with the fluorescence microscope indicated that 45 and 89%, respectively, of the infection sites of the slow- and fast-rusting cultivars contained spores. Not all of the spore-containing infection sites in the slow-ruster produced uredinia that were macroscopically visible. The ranges of the maximum lengths of the sporulating areas (uredinia) were 60-540 μm and 150-780 μm for the slow- and fast-rusters, respectively. Seven days after inoculation, the area of the uredinia of the fast-rusting cultivar was about three times larger than that of the slow-ruster (Table 1). There was a wide range in the size of uredinia in the slow-rusting cultivar.

<table>
<thead>
<tr>
<th>Test no. and host</th>
<th>Uredinia/cm² post-inoculation:</th>
<th>Length of hypha (μm)² at:</th>
<th>Uredinia (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 days</td>
<td>13 days</td>
<td>24 hr</td>
</tr>
<tr>
<td>Test 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Rustproof</td>
<td>0.4</td>
<td>2.2</td>
<td>46</td>
</tr>
<tr>
<td>Fulghum</td>
<td>16.2</td>
<td>18.2</td>
<td>52</td>
</tr>
<tr>
<td>Test 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Rustproof</td>
<td>0.2</td>
<td>0.6</td>
<td>42</td>
</tr>
<tr>
<td>Fulghum</td>
<td>8.7</td>
<td>9.4</td>
<td>58</td>
</tr>
</tbody>
</table>

*Red Rustproof is slow-rusting and Fulghum is fast-rusting.

**In test 2, this was 14 days.

°Length of hypha is the distance from the end of the substomatatal vesicle to the tip of the longest hypha. Measurements were made at specified intervals after inoculation. Asterisks * and ** indicate significant difference, P = 0.05 and 0.01, respectively, between cultivar at each time and each test (19). ns = not significant.

The uredinial area is the product of the maximum length and width of the sporulating area 7 days after inoculation (9 March and 1 April 1983 in tests 1 and 2, respectively). Each datum is an average of 100 uredinial measurements.

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Fig. 1. Host-parasite interactions of a slow-rusting cultivar of *Avena byzantina* inoculated with race 264B of *Puccinia coronata avenae*. A, mesophyll cell reaction category 1 (<10 cells fluorescing); B, category 2 (10–19 cells fluorescing); C, category 3 (>19 cells fluorescing); D, category 0 (no cells fluorescing); (A, B, C, and D ×740); E, 0 and 3 type host reactions under adjacent stomata (×460); (A, B, C, D, and E 72 hr after inoculation); F, arrest of growth of hypha 7 days after inoculation. Note no collapse of mesophyll cells (×1,156) (a = appressoria, sv = substomatal vesicle, gt = germ tube, arrows = infection hyphae).
DISCUSSION

There are several components of slow-rusting (general or horizontal) resistance of cereal hosts: feweruredinia per unit area, longer latent periods, smaller uredinia, and fewer spores per uredinia. These and some minor components have been characterized for several cereal rusts (3,7,11,15). In most reports, the authors have presented only the general effects of a component with little consideration for the rate and intensity of host reaction to invasion by the parasite. For the \textit{P. coronata-A. byzantina} system, we characterized three components of slow-rusting by the intensity of the reaction in mesophyll cell walls. In a severe host reaction, at some infection sites, the growth of the pathogen was arrested (Fig. 1C) and this presumably resulted in fewer uredinia being formed. In less severe reactions the pathogen continued to grow at reduced rates (Fig. 1A,B). We believe that the reduction in the rates of growth resulted in a lengthened latent period and smaller uredinia. These observations substantiate a prescient hypothesis by Shaner (18) who suggested that a common underlying mechanism that extends latent period and reduces uredinia size would explain the observed correlation between these two components of slow-rusting. Thus, the reaction in mesophyll cell walls that results in fluorescence seems to qualify as the underlying mechanism proposed by Shaner.

Mares (12) and Niks (13,14) investigated slow-rusting in wheat and barley. Mares concluded that resistance was triggered by recognition between the first haustorium and the affected host mesophyll cell, but Niks reported that resistance was initiated immediately after the formation of the first haustorial mother cell. Mares and Niks both suggested that resistance mechanisms were initiated before disruption of host cells. Rowell (16), working with slow-rusting wheat, stated: “Thus, the very low receptivity to infection observed in adult plants of \textit{L. dactylifera} results from the high frequency of penetrants that cease development when the primary haustorial mother cells are attached to necrosed host cells.” But he also noticed that another mechanism slowed the development and growth of the fungus. In the \textit{P. coronata-A. byzantina} system, the resistance mechanism was triggered about 18 hr after inoculation. This was similar to the times for activation of the resistance mechanism suggested by both Mares and Niks, but earlier than onset of resistance suggested by Rowell (16).

Rowell (16) and Ashagari and Rowell (1) considered low receptivity to be a major feature of slow-rusting in wheat. They noted host cell necrosis and placed considerable emphasis on it. Mares (12) and Niks (13,14) working with other forms of slow-rusting (adult plant resistance and partial resistance) did not observe host cell necrosis until 5 or 6 days after inoculation, and did not place much emphasis on its importance. We did not find cell
collapse (host cell necrosis) in our system 7 days after inoculation (Fig. 1F). It is difficult to compare our results with those of Ashagari and Rowell (low receptivity), because their most important reaction type (highly incompatible) was a manifestation of hypersensitivity (cell-collapse) and we did not observe this reaction. The differences in our results and those of Ashagari and Rowell may be due to vertical genes that were carried by their test cultivars but which were absent in our system.

The growth of hyphae in slow-rusters has been reported to be less than in fast-rusters (3,9,14,16,21). We found a significant reduction in the growth of the hyphae of the slow-rusting cultivar 48 hr after inoculation, and noticed a relationship between growth retardation and LPso. In our tests, gradations in growth of hyphae in the slow-ruster formed a continuum ranging from 50 to 250 μm (Fig. 3, 96 hr). We believe that variation in growth of the parasite controlled the extent of the LPso of the slow-ruster.

In some infections, hyphae grew beyond the fluorescing cells. When this occurred, additional visible host response was not triggered by the hyphae that grew beyond the reacting area. We suspect that host reaction was not triggered by the hyphae but rather by the substomatal vesicle. Fluorescence of mesophyll cells did not occur until substomatal vesicles were formed. Moreover, contact between the hyphae and mesophyll cells was not necessary to trigger this host reaction. We therefore speculate that the substomatal vesicles produce a compound that diffuses among the mesophyll cells and causes the host responses illustrated in Fig. 1. We believe that almost all host mesophyll cells are capable of response to the pathogen, but some cells in a given leaf are more sensitive than others. Mesophyll cells are not activated (do not express fluorescence) in some infection courts (Fig. 1D) because they are not very sensitive and are not stimulated by the amount of activator compound produced by the fungus. When mesophyll cells are not activated, the hyphae grow as fast in the slow-ruster as they do in the fast-ruster. If host response is delayed, the parasite grows beyond the area of host cell reaction and ultimately develops into sporulating lesions. Intermediate host responses slow the growth and may be responsible for the long LPso and smaller uredinia observed in the slow-ruster. Quick response and intense host reaction stops growth before the formation of the first haustorial mother cell (Fig. 1F) and is presumably responsible for reduced pustule number. Thus, the time of response and the intensity of reaction are postulated to be key elements in the slow-rusting phenomenon. These observations indicate that a reaction in the mesophyll cell walls results in reduced numbers of uredinia, smaller uredinia, and increased LPso. This present hypothesis is in partial agreement with Kuhn et al (7), who speculated that two components of slow-rusting (uredinium size and latent period) were under common genetic control.

LITERATURE CITED