Infection Processes of *Puccinia recondita* in Slow- and Fast-Rusting Wheat Cultivars

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ABSTRACT

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Development of *Puccinia recondita* from spore germination to uredinia formation in 11 wheat cultivars was studied histologically to determine whether the long latent period and small uredinia in slow-rusting cultivars are due to reduced mycelial growth in leaf tissue. Six sequential samples of flag leaves (24–216 hr after inoculation) were treated with an optical brightener (Uvitex BOPT) and observed with a fluorescence microscope. Percentages of urediniospore germination, germinated urediniospores forming appressoria, and appressoria forming substomatal vesicles were the same for fast-rusting cultivars (Morocco, Suwon 92, Monon, and P72482), an intermediate slow-rusting cultivar (SW 210), and slow-rusting cultivars (Suwon 85, SW 72469, P6028, CI 13227, and L574-1), as well as for a hypersensitively resistant cultivar (P68130). However, at each sampling time the colonies in slow-rusting wheats were smaller and had fewer haustorial mother cells than colonies in fast-rusting wheats. Although the colony growth rates of *P. recondita* on fast-rusting and slow-rusting cultivars were different, formation of uredinial beds, from which uredinia develop, in both groups of cultivars began when the average colony area was 0.12–0.14 mm^2. The longer time required for colonies to reach this incipient stage of sporulation on slow-rusting cultivars would explain their long latent period.

Additional key words: durable resistance, leaf rust, resistance, *Triticum aestivum*.

The development of *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* on wheat (*Triticum aestivum* L. em. Thell) consists of several distinct stages: germination of urediniospores, formation of appressoria, formation of substomatal vesicles, formation and growth of primary and secondary infection hyphae, formation of haustorial mother cells and haustoria, formation of uredinial beds (3) and uredinia, and urediniospore production. Several of these stages are affected by resistance of the wheat host.

In monocyclic infection experiments in the greenhouse, slow-rusting cultivars exhibit a longer latent period, form fewer uredinia per square centimeter of leaf, and produce fewer spores per uredinium than fast-rusting wheat cultivars (15,20). In the field, these slow-rusting wheat cultivars retard disease development (19). The apparent infection rate and area under disease progress curves on slow-rusting wheat cultivars are lower and smaller, respectively, than those on fast-rusting wheat cultivars. Because of a negative correlation between the size of uredinia and latent period in genetic studies (9,10), we speculated that the long latent period and small uredinia are controlled by genes in common.

The present study was undertaken to investigate the interactions between wheat cultivars (fast-rusting, slow-rusting, and hypersensitive wheat cultivars) and *P. recondita* quantitatively from germination of urediniospores to uredinia formation and to determine whether the long latent period and small uredinia are due to reduced mycelial growth within infected leaf tissue.

MATERIALS AND METHODS

Quantitative histological studies of infection of 11 cultivars of *T. aestivum* by *P. recondita* were conducted. Of the cultivars used, fast-rusting cultivars Morocco, Suwon 92, and Monon, and slow-rusting cultivars P6028A2-9-5-6-1 (P6028), L574-1, and CI 13227, have been the subject of earlier reports (8,9,15,18–20). Also studied were the intermediate slow-rusting cultivar SW 210-10 and slow-rusting cultivar SW 72469-6, both from South Korea; the fast-rusting cultivar Purdue 72482G4-76 (P72482), a Purdue breeding line; and Purdue 68130A6-45-17 (P68130), a hypersensitively resistant Purdue breeding line that carries gene *Lr*19. The studies were repeated twice.

Seedlings in the one-leaf stage were vernalized in a coldroom (3 C) for 8–10 wk and then were transplanted individually into 500-cm^3 plastic pots containing standard greenhouse soil mix. Plants were grown in a greenhouse, and natural daylight was supplemented by incandescent and fluorescent light providing about 167 $\mu E \cdot m^{-2} \cdot sec^{-1}$ for 16 hr each day from transplanting to maturity.

When a plant reached the pre-boot to early-heading stage, the adaxial surface of the flag leaf was inoculated with urediniospores of culture 7434-1-1T of *P. recondita*. This culture is virulent to all wheat cultivars used in this experiment except P68130 and was described previously (8). For each inoculation, sufficient inoculum was applied with a Devilbiss atomizer, using about 0.35 $kg \cdot cm^{-2}$ of air pressure, to provide 1.2 mg of freshly collected urediniospores in 0.6 ml of distilled water per plant. The quantity of inoculum for each plant was sufficient to give more than 70% disease severity on susceptible plants according to Cobb's modified scale (16). After inoculation, the plants were sprayed lightly with distilled water, kept wet in a moisture chamber for 14–16 hr, and then returned to the greenhouse bench. The mean greenhouse temperature was 23 C. In each experiment, a randomized complete block design with five replications was used. Not all cultivars were used in every experiment.

In the spring 1980 greenhouse experiment, inoculated leaf segments were collected at four times after inoculation (24, 72, 144, and 216 hr). In the fall 1981 and spring 1982 greenhouse experiments, samples were taken at 24, 48, 96, and 144 hr after inoculation. Hereafter, the 1980, 1981, and 1982 experiments will be called the first, second, and third experiments, respectively.

Collected leaf sections were cleared and stained with Uvitex BOPT as described by Cartwright and Russell (3), except that their procedure was modified by clearing leaf sections by boiling for 2 min with lactophenol:ethanol (1:2, v/v) then washing them twice with 50% ethanol.

Stained sections were mounted in glycerol:lactophenol (19:1, v/v) and examined with a Zeiss IV Epifluorescence Microscope (HBO 50W super-pressure mercury lamp as a light source; two BG 12 blue exciter filters; yellow LP 478 barrier filter; an FT 460 chromatic beam splitter; and a BG 38 red suppression filter). Observations were made on the following phases of the infection

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process: germination, appressorial formation, substomatal vesicle formation, haustorial mother cell formation, subsequent hyphal growth, and uredinial bed formation. To measure percentage of urediniospore germination, appressorium formation, and substomatal vesicle formation, leaf sections (4 × 0.4 cm) were taken 24 hr after inoculation. Thirty random urediniospores, germinated urediniospores, or appressoria were examined to determine the percentage of germination, appressoria formation, and substomatal vesicle formation, respectively on each sample.

Sections (2 × 0.4 cm) taken 48 and 216 hr after inoculation were used to measure hyphal growth and count haustorial mother cells. To determine colony growth from a single infection site, the distance between the tips of the most extended hyphae at opposite ends of the colony was measured with a calibrated eyepiece micrometer. The length of the colony was obtained by measuring the hyphae parallel to the long axis of the leaf. Its width was obtained by measuring the hyphae perpendicular to the long axis of the leaf. To avoid a crowding effect, only isolated colonies in the leaf segment were measured.

The uredinial beds in wheat cultivars Morocco, Suwon 92, Suwon 85, SW 72469, and CI 13227 were measured in the second experiment. Colonies of *P. recondita* on fast-rusting wheat cultivars such as Morocco and Suwon 92 began to form uredinia 6 days after inoculation, and those of the slow-rusting cultivars Suwon 85, CI 13227 and SW 72469 began to form uredinia 8–9 days after inoculation. Leaf sections (4 × 0.4 cm) from fast-rusting wheat cultivars were sampled 5 days after inoculation, and those from slow-rusting cultivars were sampled 8 to 9 days after inoculation. With a calibrated eyepiece micrometer, uredinial beds were measured in 30 infection sites in two plants from each cultivar.

RESULTS

Under the fluorescence microscope, urediniospores, germ tubes, appressoria, substomatal vesicles, hyphae, and haustorial mother cells of *P. recondita* fluoresced a bright turquoise. Haustoria fluoresced less brightly and for this reason numbers of haustoria were estimated by counting haustorial mother cells. Host tissues were light blue or pale pink.

Penetration. No statistical differences in percentage germination of urediniospores were found among the wheat cultivars within each experiment ($P = 0.05$). Mean germination was 87%. Formation of appressoria and substomatal vesicle formation, averaged over all wheat cultivars, was 92% in both cases, and no differences were found among cultivars.

Development of haustorial mother cells. Haustorial mother cells in fast-rusting wheat cultivars were counted 48, 72, and 96 hr after inoculation (Table 1). Beyond this time, it was very difficult to differentiate the haustorial mother cell from urediniospore primordia. Haustorial mother cells in slow-rusting cultivars were counted 48, 72, 96, and 144 hr after inoculation. In the first experiment, 72 hr after inoculation, the average number of haustorial mother cells in slow-rusting cultivars (Suwon 85, SW 72469, and P6028) ranged from 9.0 to 9.9 and those in fast-rusting wheat cultivars (Suwon 92, Monon, and P72482) ranged from 15.2 to 18.3. In the second and third experiments, fast-rusting cultivars also had significantly more haustorial mother cells than slow-rusting and hypersensitively resistant wheat cultivars. The number of haustorial mother cells on hypersensitive wheat P68130 was far less than for slow-rusting wheat cultivars. Host cell necrosis precluded counting haustorial mother cells beyond 48 hr after inoculation, because only disintegrated haustorial mother cells or hyphae were observed beyond this point. *P. recondita* produced almost as many haustorial mother cells in CI 13227 as in Suwon 85, SW 72469, and L574-1 48 hr after inoculation, but by 144 hr after inoculation, it had produced significantly fewer haustorial mother cells in CI 13227 than in the other slow-rusting wheat cultivars. No differences in the frequency of haustorial mother cells were found among Suwon 85, SW 72469, and L574-1.

Length of colony. Up to 48 hr after inoculation, hyphae grew parallel with the axis of the leaf. By 72 hr after inoculation, the colonies had developed an elliptical shape, with the long axis of the colony oriented with the axis of the leaf. Colonies in slow-rusting wheat cultivars were significantly shorter than those in fast-rusting wheat cultivars in all three experiments (Table 2). In the first experiment, 72 hr after inoculation, the average length of the colonies in slow-rusting wheat cultivars ranged from 63 to 73 μm and those in fast-rusting wheat cultivars ranged from 119 to 131 μm. Colonies in fast-rusting wheat cultivars were consistently longer than those in slow-rusting wheat cultivars. In the second and third experiments, colonies in fast-rusting cultivars were significantly longer than those in the slow-rusting and the hypersensitively resistant cultivars.

Width of colony. In all three experiments, colonies in fast-rusting cultivars were significantly wider than those in slow-rusting cultivars (Table 3). In the first experiment, at 72 hr after inoculation, the average colony width in slow-rusting and fast-rusting wheat cultivars ranged from 45 to 48 μm and 81 to 93 μm, respectively. The colonies in the intermediately slow-rusting cultivar SW 210 were not significantly narrower than colonies in two of the fast-rusting cultivars 144 hr after inoculation.

Development of colonies and uredinia. By 144 hr after inoculation, most colonies in fast-rusting cultivars (Suwon 92, Monon, and P72482) had begun to form uredinia, while those in

TABLE 1. Number of haustorial mother cells per infection site on 11 wheat cultivars infected with *Puccinia recondita*[x]

	Experiment number and hours after inoculation							
	1		2			3		
Cultivar	72	144	48	96	144	48	96	144
Fast-rusting								
Morocco			6.0 a	99.4 a	+	7.1 a	64.3 a	+
Suwon 92	15.2 bc	+[y]	6.1 a	50.0 b	+			
Monon	16.7 ab	+						
P72482	18.3 a	+						
Intermediate slow-rusting								
SW 210	13.8 c	+						
Slow-rusting								
Suwon 85	9.9 d	38.6 a	3.8 b	20.8 c	75.4 a	3.0 b	16.1 bc	56.0 a
SW 72469	9.8 d	27.6 a	2.8 c	25.1 c	84.6 a	3.2 b	17.8 b	45.7 a
P6028	9.0 d	29.4 a						
L574-1			2.0 d	25.0 c	62.4 a			
CI 13227			1.9 d	6.4 d	32.4 b	2.2 b	10.6 c	22.2 b
Hypersensitive								
P68130			0.6 e	–[z]	–	0.5 c	–	–

[x] Each value is the mean of five infection sites from each of five replicate leaves per cultivar. Within each column, means followed by a letter in common are not significantly different according to Duncan's new multiple range test, $P = 0.05$ (21).

[y] + = too numerous to count.

[z] – = none detected.

slow-rusting cultivars (Suwon 85, SW 72469, and P6028) were still in the vegetative stage. By 216 hr after inoculation, colonies in the fast-rusting cultivars Suwon 92, Monon, and P72482 had already formed urediniospores, while those in Suwon 85 and P6028, had just begun to form uredinia. Most of the colonies in SW 72469 had not yet formed uredinial beds. In the first experiment, the rates of expansion of the colonies, between 72 and 216 hr after inoculation, in fast-rusting cultivars were similar to those in slow-rusting cultivars. During this period, colonies in fast-rusting cultivars expanded in area 55–76 times, and those in slow-rusting cultivars expanded in area by 38–84 times.

Prior to formation of uredinia, macroscopic symptoms of infection on the inoculated upper surface of flag leaves were pale-green flecks. The time of appearance of these flecks depended upon the level of resistance. Flecking on the fast-rusting cultivars first appeared about 5 days after inoculation, but it did not appear until 7–9 days after inoculation on slow-rusting wheat cultivars. Flecking on the slow-rusting wheat cultivar CI 13227, which exhibited the longest latent period in a previous experiment (19), appeared 9 days after inoculation. By the time pale-green flecks were visible on a leaf, microscopic examination revealed that the colonies had reached the stage of uredinial bed formation. Once the uredinial bed was formed, it developed into a uredinium within 1–2 days. The uredinial bed consists of primary and secondary longitudinal hyphae and masses of primordial urediniospores (3).

To investigate the sizes of uredinial beds on different wheat cultivars at the time of their formation, uredinial beds in five cultivars (Morocco, Suwon 92, Suwon 85, SW 72469, and CI 13227) were measured in the second experiment. Because of the greater time taken to reach the uredinial bed formation stage in slow leaf-rusting cultivars, leaf sections from Morocco and Suwon 92 were taken 120 hr after inoculation, sections from Suwon 85 and SW 72469 192 hr after inoculation, and those from CI 13227 216 hr after inoculation.

The average size of uredinial beds in the five cultivars were: Morocco, 0.136 mm^2; Suwon 92, 0.129 mm^2; CI 13227, 0.126 mm^2; SW 72469, 0.125 mm^2; and Suwon 85, 0.123 mm^2. No statistical differences were found among these cultivars.

DISCUSSION

Our results show that neither slow-rusting resistance nor hypersensitivity affect penetration stages of development of *P. recondita* on wheat. There were no significant differences among cultivars in frequencies of spore germination, appressorium formation, or substomatal vesicle formation. Others have reported similar results with other rust diseases (4,12,22). Some workers have noted a host-genotype effect on penetration of leaves from appressoria (2,6,17).

In this study, *P. recondita* penetrated slow-rusting and fast-rusting wheats equally well. Ohm and Shaner (15), Shaner et al (20), and Shaner and Finney (19) observed that some slow-rusting wheat cultivars such as Suwon 85, P6028, L574-1, and CI 13227 showed slightly lower receptivity when inoculated with

TABLE 2. Length of colony (μm) per infection site on 11 wheat cultivars infected with *Puccinia recondita*[y]

	Experiment and hours after inoculation								
	1			2			3		
Cultivar	72	144	216	48	96	144	48	96	144
Fast-rusting									
Morocco				82 a	355 a	826 a	76 a	269 a	621 a
Suwon 92	119 a	645 ab	1,300 a	81 a	219 b	723 b			
Monon	131 a	678 ab	1,236 a						
P72482	123 a	762 a	1,212 a						
Intermediate slow-rusting									
SW 210	96 b	592 b	908 b						
Slow-rusting									
Suwon 85	71 c	278 c	756 c	48 bc	108 d	220 d	55 b	107 c	239 b
SW 72469	73 c	271 c	514 e	44 c	151 c	279 c	52 bc	131 b	244 b
P6028	63 c	270 c	647 d						
L574-1				49 b	151 c	212 d			
CI 13227				36 d	66 e	182 d	45 c	95 c	150 c
Hypersensitive									
P68130				11 e	–[z]	–	10 d	–	–

[y] Each value is the mean of five infection sites from each of five replicate leaves per cultivar. Within each column, means followed by a letter in common are not significantly different according to Duncan's new multiple range test, $P = 0.05$ (21).
[z] – = no colonies detected.

TABLE 3. Width of colony (μm) per infection site on 11 wheat cultivars infected with *Puccinia recondita*[z]

	Experiment number and hours after inoculation						
	1			2		3	
Cultivar	72	144	216	96	144	96	144
Fast-rusting							
Morocco				218 a	364 a	169 a	338 a
Suwon 92	81 a	329 b	570 a	156 b	326 a		
Monon	93 a	366 ab	550 a				
P72482	89 a	394 a	556 a				
Intermediate slow-rusting							
SW 210	58 b	333 b	380 b				
Slow-rusting							
Suwon 85	45 b	186 c	357 bc	75 c	127 b	55 c	141 b
SW 72469	47 b	149 c	239 c	84 c	135 b	71 b	123 b
P6028	48 b	157 c	306 c				
L574-1				81 c	115 b		
CI 13227				36 d	104 b	46 c	87 c

[z] Each value is the mean of five infection sites from each of five replicate leaves per cultivar. Within each column, means followed by a letter in common are not significantly different according to Duncan's new multiple range test, $P = 0.05$ (21).

P. recondita than did the fast-rusting cultivars Suwon 92 and Monon. Therefore, we suggest that this slightly lower receptivity of slow-rusting cultivars is due to postpenetration interactions between host and rust fungus. Ashagari and Rowell (1) reported that a high percentage of host cells collapsed in slow-rusting wheat cultivars Idaed 59, Thatcher, and Lee infected with *P. graminis tritici* and that this was responsible for the apparent low receptivity. This host cell necrosis of the slow-rusting cultivars, which prevented the formation of uredinia, was very similar to that associated with hypersensitive resistance, except that it occurred only at some infection sites. Recently, Niks (13) reported that although *P. hordei* penetrated equally well on susceptible and slow-rusting barley cultivars, some colonies in slow-rusting cultivars aborted in the early phases of infection without noticeable host cell necrosis. Niks and Kuiper (14) subsequently suggested that additional colonies within the slow-rusting host aborted during later stages of development. Thus, fewer uredinia developed on the slow-rusting barley cultivars than on susceptible cultivars. In our experiments, abortion of colonies in the early phases of infection (48 hr) was observed in the hypersensitively resistant wheat cultivar P68130 but not in the slow leaf-rusting, intermediate slow leaf-rusting, and fast leaf-rusting cultivars. Abortion of colonies in P68130 could be confirmed only by observing the disorganized hyphae and haustorial mother cells. Host cells at these infection sites did not autofluoresce and appeared normal. Small colonies from slow leaf-rusting cultivars, which did not exhibit this disorganization of hyphal and haustorial mother cells, apparently did not abort and presumably continued their development.

Restricted colony development is a common host-parasite interaction in slow-rusting resistance and is not necessarily accompanied by host cell necrosis (4,11,13,14,22,23).

Even in the absence of host-cell necrosis, fungal colonies in slow-leaf-rusting wheat cultivars were significantly smaller than those in intermediate slow-rusting and fast-rusting wheat cultivars. Thus, it appears that reduced colony growth in slow-rusting wheat cultivars has a cause other than host-cell necrosis which restricts fungal development in other wheat cultivars. In our experiments, the number of haustorial mother cells (Table 1), length of colonies (Table 2), and width of colonies (Table 3) during the period from 48 to 216 hr following inoculation increased more slowly on slow-rusting than on intermediate slow-rusting and fast-rusting wheat cultivars. Even though intermediate slow-rusting wheat cultivar SW 210 showed macroscopic necrosis around the uredinia, hyphae were observed within and beyond the necrotic area. Host cell necrosis around uredinia of SW 210 did not stop the mycelial growth of *P. recondita*.

One of the difficulties in the microscopic study of colonies of *P. recondita* within the wheat leaf is relating these data to the macroscopic expression of slow-rusting. In a previous study, the slow leaf-rusting wheat cultivars exhibited a longer latent period, smaller uredinia, and lower spore production than fast-rusting wheat cultivars (15,19,20). Slow-rusting resistance seems to operate against *P. recondita* after substomatal vesicle formation and through uredinium expansion.

Because the cultivars with a longer latent period usually exhibited smaller uredinia, both of these components are apparently closely related (15,19,20). Latent period and size of uredinia of *P. recondita* on wheat cultivars are negatively correlated (9,10). Using data for uredinial expansion rates, Shaner (18) simulated epidemics on slow- and fast-rusting wheat cultivars. The simulation suggested that a slow growth rate of uredinia would be a significant factor in retarding disease development in the field.

In our experiments, although colonies in fast-rusting wheat cultivars were consistently larger than those in slow-rusting wheat cultivars, it appeared that uredinium formation began when the colony reached a certain size whether on a fast- or slow-rusting wheat cultivar. This critical size was reached sooner on the fast-rusting cultivars owing to the greater colony growth rate. Uredinial bed formation, the initial stage of uredinia formation, began 5 days after inoculation on fast-rusting cultivars and 7–9 days after inoculation on slow-rusting wheat cultivars. Initiation of uredinial bed formation on the fast-rusting and slow-rusting wheat cultivars began when average colony size was 0.12–0.14 mm^2. It has also been reported that colonies of *P. hordei* grow more slowly on slow-rusting barley cultivars than on fast-rusting barley cultivars (5). It was postulated that the fungal colonies sporulated only after attaining "a critical mass" (5,24). Shaner (18) observed that the rate of uredinial expansion on slow-rusting wheat cultivars was slower than on fast-rusting wheat cultivars. These observations would account for the difference in uredinial size between slow- and fast-rusting cultivars at any time after inoculation.

Either a long latent period or small uredinia are good criteria for selecting slow-rusting cultivars (7,9). Since a long latent period and small uredinia may both be due to slow growth of the fungus beginning with growth of intercellular hyphae from the substomatal vesicle, it is not surprising that these two components of slow-rusting are highly correlated. Either component could be used as a selection criterion in a breeding program; but, in our experience, latent period is easier to measure reliably on large populations of plants and is less sensitive to variations in the density of uredinia on the inoculated leaves.

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