Relation of Postpenetration Events in Idaed 59 Wheat Seedlings to Low Receptivity to Infection by *Puccinia graminis* f. sp. *tritici*

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ABSTRACT

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Seedlings of wheat (*Triticum aestivum*) cultivar Idaed 59 and the differential line W2691SrTt-1 with the specific resistance governed by *Sr*Tt-1 had about 35% less receptivity to infection by the putatively virulent race 15-TLM of *Puccinia graminis* f. sp. *tritici* than did seedlings of the wheat cultivar Baart. In all three wheats a small percentage of penetrants ceased development after formation of substomatal vesicles, primary infection hyphae, primary haustorial mother cells, or the secondary hyphal branches. Penetrants that failed to develop into colonies when primary haustorial mother cells were attached to necrosed host cells also were present in the three wheats, but the probability of this event was significantly greater in Idaed 59 and W2691SrTt-1 than in Baart. The greater frequency of this event probably accounted for most of the reduction in uredial number on the seedling leaves of Idaed 59 and W2691SrTt-1. In Baart, there were no colonies with one or more secondary haustorial mother cells attached to necrosed host cells, but in Idaed 59 and W2691SrTt-1 the frequency of such colonies increased with time to comprise almost all colonies at 160 hr after inoculation. Apparently most colonies with necrosed host cells produced uredia of a high infection type on the seedling leaves, but growth ceased in a few with many necrosed host cells to produce a fleck infection type. The maximum linear growth of secondary hyphae was similar in colonies with and without necrosed host cells in Idaed 59 and W2691SrTt-1 and was significantly less than in Baart. A significant lag in the formation of primary infection hyphae and primary haustorial mother cells also occurred in the wheats with low receptivity. Thus, the dominant gene associated with *Sr*Tt-1 in Idaed 59 that conditions low receptivity to infection of adult plants by races virulent for *Sr*Tt-1 also conditions a resistance in seedlings with a mixture of reactions similar to the mesothetic infection type.

Additional key words: wheat stem rust, general resistance, slow-rusting, hypersensitivity.

The low receptivity to infection by a putative virulent culture of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. of adult plants of 'Idaed 59' wheat (*Triticum aestivum* L.) apparently is governed by a single gene closely linked to the specific resistance governed by the gene *Sr*Tt-1 (8).

A histologic study (2) of postpenetration development of a single-spore isolate of race 15B-2 (race 15-TLM by Cereal Rust Laboratory nomenclature [5]), revealed that the low receptivity of adult plants of Idaed 59 and W2691SrTt-1 (the differential for the resistance of *Sr*Tt-1 in race identification [5]) is associated with a high frequency of incompatible colonies and a low frequency of compatible colonies of significantly reduced size. The incompatible colonies induced a hypersensitive host response similar to that associated with specific gene resistance.

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The seedling resistance of *Sr*Tt-1 to stem rust races in North America is expressed as various low infection types ranging from types 0 to ; with isolates of races 151-QSH, 56-MBC, and 32-RSH and from types ;1$^+$ to X$^-$ with isolates of races 151-QFB and -QCB (6). The high infection type observed with all supposedly virulent isolates of races such as 15-TLM, 15-TNM, and 113-RKQ consists of a reduced number of type 3 to 4 uredia with an occasional type ; infection present (6). No isolates with a higher virulence have been found in North America (2).

In the present study, the receptivity of seedlings of Idaed 59 and W2691SrTt-1 to the same isolate of race 15-TLM used previously (2) was examined and the sequence of postpenetration events leading to colonization of the host was analyzed.

MATERIALS AND METHODS

The test wheats were Baart (CI 1697), Idaed 59 (CI 13631), and W2691SrTt-1 (CI 17385). Six test seedlings were grown in a mixture of field soil, sand, peat, and manure (3:3:3:1, v/v) in 7.5-cm-diameter clay pots in a growth chamber at constant 24 C and a 12-hr fluorescent light cycle of about 16,500 lx. The area of the first true seedling leaf was measured with a portable area meter (Lambda Instrument Corp. Model LI-3000) when the leaf was fully expanded at 7 days after planting. Then test plants were inoculated in a calibrated spray chamber (9) with 0.2 ml of fresh uredospores carried in a light mineral oil. The single-spore isolate of race 15-TLM used in this study was prepared in an earlier study (2). Inoculated plants were incubated in a dew chamber for 12 hr in darkness at 18 C followed by 4 hr of light at about 15,500 lx at 24 C. After slow drying of the leaves for 3 hr, the plants were returned to the growth chamber at a constant 21 C.

Three sets of four replicate randomized pots were used for the diverse observations on each cultivar. One set was inoculated with 9 mg of spores per milliliter and sampled at 40 hr after inoculation for observations of the prepenetration processes of infection and at 46, 68, and 92 hr after inoculation for observations on the post-penetration sequence of histological events (stages) in the infection process. A second set was inoculated at 3 mg/ml and sampled at 113, 137, 160, and 184 hr after inoculation for observations on the advanced stages of postpenetration development with minimal interference between infections. A third set was inoculated with 0.6 mg of spores per milliliter to determine the number of uredia produced per square centimeter.

The prepenetration development of germlings was determined on a single leaf from each replicate pot of the first set after the leaf surfaces were sprayed with a mixture of acid fuchsin and cotton blue (1). Fifty spores were examined on each side of the leaf for germ tube production, appressorium formation, and appressorial emptying by microscopic examination with an epicondenser of adjacent transits across the leaf at 4 cm from the leaf tip.

Segments, 1.5 cm long, were cut 3 cm from the leaf tip of a single leaf in each replicate pot for examination at the previously indicated sampling times for sets one and two. Segments were processed by the method for fluorescence microscopy of Rohringer et al (7) and examined with a Reichert microscope equipped with an Incident Light Fluorescent Illuminator fitted with an OSRAM HBO-200 light source, exciter filter #5970 with peak emission at 363 nm, and a barrier filter in position 45 that excludes emissions $\leqslant$400 nm. In these preparations the vesicles, infection hyphae, secondary hyphae, haustorial mother cells, and hypersensitively necrosed host cells were clearly visible, but the haustoria were not. All penetrants of the abaxial and adaxial surfaces in the segment were examined and scored. Linear growth from the end of the vesicle to the maximum limit of secondary hyphal extension in the host was measured with a calibrated eyepiece micrometer. Detailed observations were not made on the 184-hr sample because the largest colonies had incipient sporulation that concealed the initiating vesicle thereby preventing precise measurement of linear growth, and overgrowth by adjacent colonies obscured many aborted penetrants.

Uredia were counted 14 days after inoculation on leaves in the third set and the mean number of uredia per square centimeter was calculated from the leaf area measured before inoculation.

Analysis of variance was used to test for significant differences between cultivars of the number of uredia per square centimeter and of the arc sine of the percentages of spore germination, appressorium formation, and appressorium emptying. A modification of the binary pathway developed by Johnson et al (4) for analysis of primary infection and host response in populations of powdery mildew fungi was used to trace the events of postpenetration development leading to colonization of the host by *P. graminis* f. sp. *tritici*. Differences in the probability of an event leading to colonization between cultivars and between different times of sampling within a cultivar were tested for significance in a test for the difference of attributes by use of the normal deviate (11).

RESULTS

Uniform inoculation of seedlings with an inoculum concentration of 0.6 mg spores per milliliter produced significantly fewer uredia on Idaed 59 and W2691SrTt-1 than on Baart (Table 1). Examination of prepenetration development from spores on leaves of the three wheats inoculated simultaneously with 9 mg spores per milliliter revealed no significant difference in the percentages that germinated, formed appressoria, or emptied appressoria.

Histological examination by incident fluorescent microscopy of the penetrants in heavily inoculated segments of seedling leaves showed that the sequence of events for fungal development was similar in the three wheats (Fig. 1). In the diagram the right-hand branches indicate the progressive events that led to successful

TABLE 1. Number of uredia produced and percentage of uredospores producing germ tubes, appressoria, and empty appressoria on seedlings of wheats with and without the resistance of gene *Sr*Tt-1 after uniform inoculation with race 15-TLM of *Puccinia graminis* f. sp. *tritici*

Host	Genotype	Uredia/cm^2[a]	Percentage of spores producing:[b] Germ tubes	Appres-soria	Empty appressoria
Baart	*sr*Tt-1	2.95	84.0	53.0	10.5
Idaed 59	*Sr*Tt-1	1.94*	82.0	43.5	12.5
W2691SrTt-1	*Sr*Tt-1	1.81*	89.5	39.5	18.5
LSD ($P = 0.05$)[c]		0.95	NS[d]	NS	NS

[a] Inoculum contained 0.6 mg spores per milliliter. * Significantly different ($P = 0.05$) from Baart.
[b] Inoculum contained 9 mg spores per milliliter.
[c] LSD = least significant difference.
[d] NS = not significant.

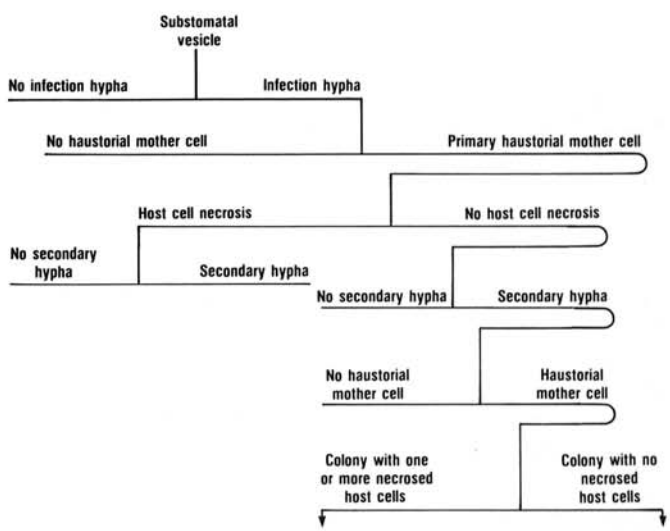

Fig. 1. Sequence of histological events observed by fluorescent microscopy in the postpenetration development of *Puccinia graminis* f. sp. *tritici* race 15-TLM in seedling leaves of Baart, Idaed 59, and W2691SrTt-1.

colonization of the host. The left-hand branches indicate the events at which fungal development ceased without colony formation as indicated by comparable frequencies of such penetrants at all times of sampling after 46 hr.

Some penetrants ceased development after vesicles formed. Most of the remaining penetrants developed a single infection hypha from one end of the vesicle, but 5.4% formed infection hyphae from both ends. Scoring of penetrants with two infection hyphae was based on the hypha that had progressed farthest in the colonization process. A few of the infection hyphae failed to produce haustorial mother cells. Haustorial mother cells attached to epidermal cells were rarely observed in these materials in contrast to those observed in other race/cultivar combinations (3). Among penetrants that formed the primary haustorial mother cell, some were attached to necrosed mesophyll cells in the three wheats. This group of penetrants either made no further development or produced a secondary branch hypha without haustorial mother cells. A few of the penetrants with primary haustorial mother cells attached to living host cells also ceased development, but most of these penetrants produced secondary branch hyphae. A few penetrants with these secondary hyphae developed no further. The remaining penetrants formed the initial secondary haustorial mother cell and developed into colonies of widely varying size. In these colonies either all attacked host cells appeared normal or one to many had necrosed.

In the largest colonies, hyphae had penetrated the leaf to the opposite epidermis by 113 hr, sporophytic hyphae were formed by 160 hr, and initial sporulation was present at 184 hr.

The percentage of penetrants that advanced to these events (Table 2) and the probability for events associated with the colonization of seedling leaves (Table 3) in the three wheats are presented only for the samples taken at 46, 92, and 160 hr. The data for samples taken at 68, 113, and 137 hr did not differ significantly in any respects from those from samples taken at 92 and 160 hr from the same cultivar. Detailed observations on many penetrants in the 184-hr samples were prevented by overgrowth from the larger colonies.

The frequency of penetrants that advanced to the various stages in colonization of the host shows that rust development was similar in Idaed 59 and W2691SrTt-1 (Table 2). Rust development differed in these wheats from Baart in that more penetrants caused necrosis of host cells attacked by primary haustorial mother cells and many colonies had necrosed host cells. That these differences were significant is shown by the corresponding data for the probability of events in colonization of the host (Table 3).

The probability that a penetrant would advance to the next event in all steps associated with successful colonization of seedling leaves of the three wheats was less than one, except all penetrants in Baart with secondary haustorial mother cells produced colonies in which no necrosed host cells were present (Table 3). Idaed 59 and W2691SrTt-1 were significantly lower than Baart in the probabilities that either the primary or secondary haustorial mother cells were attached to living host cells. Significant differences in the probability of events existed between the 46 hr and subsequent sampling times in the production of infection hyphae in Idaed 59, and the formation of the primary haustorial mother cell in Idaed 59 and W2691SrTt-1, which indicated that pathogen development was slower in these wheats than in Baart. The significant differences in the probabilities at 46 hr and subsequent samplings for colonies with secondary haustorial mother cells attached to necrosed host cells in Idaed 59 and W2691SrTt-1 are due to the increased number of attacked host cells as colonies enlarge.

The mean linear growth of colonies with secondary haustorial mother cells as measured in a straight line from the end of the vesicle to the maximum limit of hyphal extension into the host was significantly less in Idaed 59 and W2691SrTt-1 than in Baart (Table 4). In Idaed 59 and W2691SrTt-1 at 92 hr, the colonies with no necrosed host cells did not differ significantly in mean linear growth from those in the same host that had necrosed host cells present. Too few colonies with no necrosed host cell present were observed for a similar analysis of the samples taken at 160 hr.

The frequency distribution (Fig. 2) of colonies in classes of similar size show that the three wheats had similar ranges in linear

TABLE 2. Frequency of penetrants of *Puccinia graminis* f. sp. *tritici* race 15-TLM that advanced to the various events (stages) associated with colonization of seedling leaves of Baart, Idaed 59, and W2691SrTt-1 wheat

	Percentage of penetrants								
	Baart			Idaed 59			W2691SrTt-1		
Event	46 hr	92 hr	160 hr	46 hr	92 hr	160 hr	46 hr	92 hr	160 hr
Infection hypha	86	89	97	78	93	97	86	93	95
Primary haustorial mother cell	74	84	91	66	87	83	68	85	85
First-attacked host cell not necrosed	74	83	90	53	66	63	57	59	54
Secondary hypha	65	79	87	44	57	61	44	54	51
Secondary haustorial mother cell	61	78	84	40	56	58	42	52	49
Colonies with no necrosed host cells	61	78	84	38	10	4	39	13	2

TABLE 3. Probability for events associated with colonization of seedling leaves of Baart, Idaed 59, and W2691SrTt-1 wheat by *Puccinia graminis* f. sp. *tritici* race 15-TLM

	Probability of event[a]								
	Baart			Idaed 59			W2691SrTt-1		
Event	46 hr	92 hr	160 hr	46 hr	92 hr	160 hr	46 hr	92 hr	160 hr
Infection hypha	0.86±0.03	0.89±0.06	0.97±0.05	0.78±0.15‡	0.93±0.09	0.97±0.04	0.86±0.07	0.93±0.06	0.95±0.07
Primary haustorial mother cell	0.87±0.05	0.95±0.04	0.95±0.04	0.79±0.09†	0.97±0.05	0.86±0.05	0.78±0.10‡	0.94±0.06	0.89±0.07
First-attacked host cell not necrosed	1.0	0.99±0.02	0.99±0.03	0.79±0.19*	0.71±0.24**	0.72±0.13*	0.88±0.10	0.71±0.13*	0.66±0.11*
Secondary hypha	0.88±0.03	0.95±0.06	0.97±0.04	0.83±0.19	0.83±0.08	0.94±0.09	0.79±0.08	0.89±0.11	0.93±0.12
Secondary haustorial mother cell	0.93±0.07	0.99±0.03	0.98±0.04	0.95±0.06	0.98±0.06	0.94±0.09	0.95±0.07	0.95±0.08	0.93±0.12
Colony with no necrosed host cells	1.0	1.0	1.0	0.97±0.05‡	0.22±0.16*	0.04±0.08*	0.96±0.07‡	0.15±0.12*	0.04±0.10*

[a]*The value differs significantly ($P = 0.05$) from the comparable sample from Baart.
†The value differs significantly ($P = 0.05$) from the 92-hr sample of that wheat.
‡The value differs significantly ($P = 0.05$) from the 92- and 160-hr samples of that wheat.

growth. The distribution of colonies in these classes was similar for Idaed 59 and W2691SrTt-1 with the majority of colonies falling into the lower classes; whereas in Baart, most of the colonies fell into the higher classes. At 92 hr, colonies with and without host cell necrosis in Idaed 59 and W2691SrTt-1 had similar distributions in size.

DISCUSSION

Controlled inoculation of seedlings of wheat cultivars Idaed 59 and W2691SrTt-1 with the putative virulent culture of race 15B-TLM of *P. graminis* f. sp. *tritici* produced about one-third fewer uredia of high infection type than on the fully susceptible cultivar Baart. The low receptivity to infection of these cultivars (2,8) operated to a lesser extent in seedlings than in adult plants in which only a few uredia are produced. Previous studies of postpenetration development of putative virulent cultures in Idaed 59 and W2691SrTt-1 indicated that low receptivity resulted from a high proportion of incompatible penetrants that reacted with the host in a manner similar to the hypersensitive reaction (2). The few uredia produced on adult plants were ascribed to a few penetrants that developed compatibly at reduced growth rates. The present study of postpenetration development of a culture of race 15-TLM in seedlings gave a more detailed view of the host-pathogen interaction associated with the low receptivity of Idaed 59 and W2691SrTt-1.

No significant differences were observed in the prepenetration stages of pathogen development on the three wheats. In postpenetration development, a small, comparable proportion of penetrants aborted in the three wheats at the stages preceding the formation of infection hyphae, primary haustorial mother cells, secondary hyphal branches, or secondary haustorial mother cells. If these failures in the infection process are due to host barriers against pathogen attack, they appear to act to a similar degree in these hosts with high and low receptivity.

Idaed 59 and W2691SrTt-1 differed from Baart in the frequency with which penetrants induced hypersensitive necrosis of host cells. Most of the reduction in number of uredia produced on seedling leaves of Idaed 59 and W2691SrTt-1 probably resulted from penetrants that failed to develop a colony when the primary haustorial mother cell was attached to a necrosed host cell. The very low frequency of colonies without host cell necrosis in Idaed 59 and W2691SrTt-1 at 160 hr, however, indicates that many uredia developed from colonies with one or more secondary haustorial mother cells attached to necrosed host cells. About 50% or more of the attacked host cells had necrosed at 160 hr in 29 and 42% of these colonies in Idaed 59 and W2691SrTt-1, respectively. Some of these colonies with severe host cell necrosis apparently ceased development and caused the occasional flecks observed on seedling leaves. Thus, the seedling reaction to race 15-TLM is a mixture made up mostly of infections of type 3 to 4 uredia, lesser amounts of type 0 infections that were halted with the collapse of the first attacked host cell, and a few type ; infections from colonies in which most of the attacked host cells collapsed. These results indicate that the dominant gene for low receptivity linked with *Sr*Tt-1 in Idaed 59 and W2691SrTt-1 conditions a seedling resistance expressed by a mesothetic infection type.

In the previous study of adult plants (2) the few uredia that developed on Idaed 59 were ascribed to colonies free of necrosed cells at 96 hr. The frequency of these colonies, 25% of the examined penetrants, was greater than the frequency of uredia observed as well as the frequency of such colonies observed in the present study. I have examined segments of leaf blades from adult plants of Baart and Idaed 59 inoculated in a large settling tower at inoculum rates much less than rates used in the previous study. Fungal structures and necrosed cells in this adult tissue did not fluoresce after treatment by the method of Rohringer et al (7) presumably due to the absorption of UV light by the heavy cuticle. When the tissue fixed in lactophenol was cleared for 2 hr in saturated chloral hydrate and washed with water before further processing, the infection events were observed. In Baart, 95% of the penetrants developed primary haustorial mother cells, 2% had primary haustorial mother cells attached to necrosed host cells, 79% formed colonies free of host cell necrosis, and 0.3% formed colonies with some host cell necrosis present. In Idaed 59 the percentages of penetrants were: 91% with primary haustorial mother cells, 76% with primary haustorial mother cells attached to necrosed host cells, 0.4% with colonies free of necrosed host cells, and 0.9% with colonies with necrosed host cells. These differing results suggest that the massive inoculation rates used in the previous study altered the host response to infection and increased the frequency of colony formation. Thus, the very low receptivity to infection observed in adult plants of Idaed 59 results from the high frequency of penetrants that cease development when the primary haustorial mother cells are attached to necrosed host cells.

In addition to the hypersensitive response of the host cells, another mechanism apparently slows the development and growth of the rust penetrants in Idaed 59 and W2691SrTt-1. The significant reduction in the maximum limit of extension of secondary hyphae

TABLE 4. Maximum linear growth of hyphae from vesicle into host tissue of *Puccinia graminis* f. sp. *tritici* race 15-TLM in seedling leaves of Baart, Idaed 59, and W2691SrTt-1

Host	Host cell necrosis	Distance (μm)[a] 92 hr	160 hr
Baart	None	187.9	1,066.4
Idaed 59	None	99.2**	
	Some	130.5**	553.3**
W2691SrTt-1	None	128.3**	
	Some	140.5*	460.6**
LSD[b] $P = 0.05$		33.25	154.8
LSD $P = 0.01$		48.36	234.4

[a] LSD = least significant difference.
[b] * Significantly different ($P = 0.05$) from Baart and ** significantly different ($P = 0.01$) from Baart.

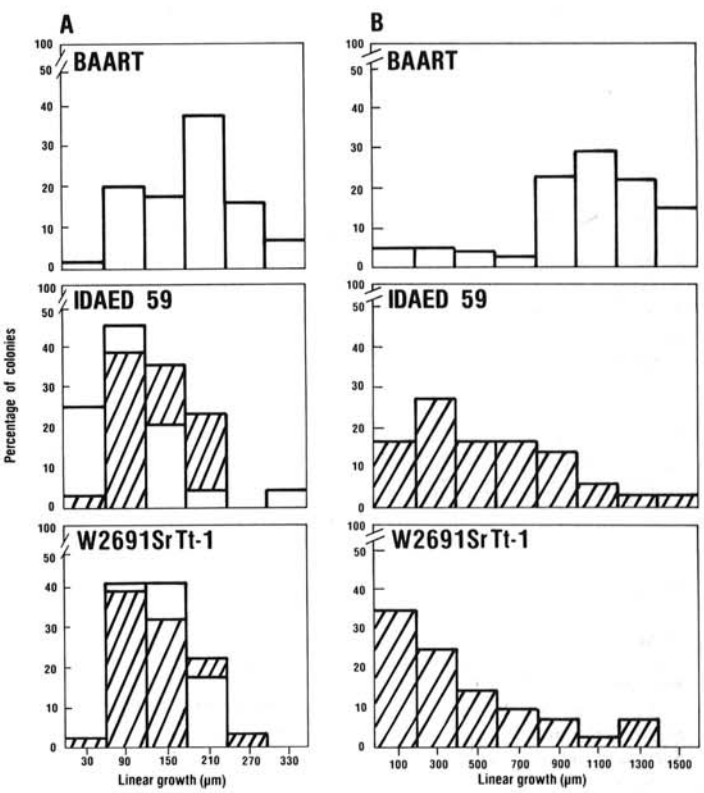

Fig. 2. Frequency distributions of colonies of *Puccinia graminis* f. sp. *tritici* race 15-TLM into classes of similar linear growth in seedling leaves of Baart, Idaed 59, and W2691SrTt-1 wheat. **A**, 92 hr after inoculation. **B**, 160 hr after inoculation. Open bars—no host cell necrosis present. Hatched bars—some host cell necrosis present.

in colonies with no necrosed host cells at 96 hr may result either from an intracellular mechanism or from an extracellular incompatible interaction not evident as hypersensitive necrosis of host cells. That host cell necrosis is not an immediate response to attack is evident in the significant change in the colonies without cell necrosis between 46 and 92 hr in Idaed 59 and W2691SrTt-1. The significant delay in the formation of infection hyphae in Idaed 59 and in primary haustorial mother cells in Idaed 59 and W2691SrTt-1 suggests inhibition of pathogen development by some extracellular mechanism.

If the above interpretation of the observations on pathogen development is assumed to be correct, then the following hypothesis may explain the resistance that causes low receptivity in Idaed 59 and W2691SrTt-1. The hypersensitive necrosis of attacked host cells is a time-dependent general response of host cells to pathogenic attack. When the invading penetrant grows and develops a haustorium rapidly, host cell necrosis does not occur, but it does occur when the cell is invaded slowly. Necrosis of the host cell attacked by the primary haustorial mother cell halts penetrant development before a second haustorial mother cell is formed, but necrosis of a host cell attacked by a secondary haustorial mother cell does not halt further pathogen development. The significantly greater frequency of necrosed host cells attacked by primary haustorial mother cells in Idaed 59 and W2691SrTt-1, therefore, results from slow penetrant development induced by the inhibitory mechanism. A similar relationship may operate in the temperature-sensitive resistance governed by gene *Sr6* (10) in which more rapid cell invasion at high temperatures could reduce the frequency of host cell necrosis. The occasional necrosed host cell attacked by a primary haustorial mother cell observed in Baart could be due to the slowest growing penetrants. Note that these were observed in the 92- and 120-hr samples, but not at 46 hr. Although no host cell necrosis was observed in colonies with secondary haustorial mother cells in seedlings of Baart in this experiment, this event has been observed very infrequently in Baart in other experiments. Other research workers (10) have observed in wheat stem rust the occasional necrosis of host cells associated with infections by a virulent culture in a susceptible host.

LITERATURE CITED

1. Anderson, A. S., and Rowell, J. B. 1962. Duration of protective activity in wheat seedlings of various compounds against wheat rust. Phytopathology 52:909-913.
2. Ashagari, D., and Rowell, J. B. 1980. Postpenetration phenomena in wheat cultivars with low receptivity to infection by *Puccinia graminis* f. sp. *tritici*. Phytopathology 70:624-627.
3. Harder, D. E., Rohringer, R., Samborski, D. J., Rimmer, S. R., Kim, W. K., and Chong, J. 1979. Electron microscopy of susceptible and near-isogenic (*sr6*/*Sr6*) lines of wheat infected with *Puccinia graminis tritici*. II. Expression of incompatibility in mesophyll and epidermal cells and the effect of temperature on host-parasite interactions in these cells. Can. J. Bot. 57:2617-2625.
4. Johnson, L. E. B., Bushnell, W. R., and Zeyen, R. J. 1979. Binary pathways for analysis of primary infection and host response in populations of powdery mildew fungi. Can. J. Bot. 57:497-511.
5. Roelfs, A. P., and McVey, D. V. 1975. Races of *Puccinia graminis* f. sp. *tritici* in the U.S.A. during 1974. Plant Dis. Rep. 59:681-685.
6. Roelfs, A. P., and McVey, D. V. 1979. Low infection types produced by *Puccinia graminis* f. sp. *tritici* and wheat lines with designated genes for resistance. Phytopathology 69:722-730.
7. Rohringer, R., Kim, W. K., Samborski, D. J., and Howes, N. K. 1977. Calcofluor: An optical brightener for fluorescence microscopy of fungal plant parasites in leaves. Phytopathology 67:808-810.
8. Rowell, J. B., and McVey, D. V. 1979. A method for field evaluation of wheats for low receptivity to infection by *Puccinia graminis* f. sp. *tritici*. Phytopathology 69:405-409.
9. Rowell, J. B., and Olien, C. R. 1957. Controlled inoculation of wheat seedlings with urediospores of *Puccinia graminis* var. *tritici*. Phytopathology 47:650-655.
10. Samborski, D. J., Kim, W. K., Rohringer, R., Howes, N. K., and Baker, R. J. 1977. Histological studies on host-cell necrosis conditioned by the *Sr6* gene for resistance in wheat to stem rust. Can. J. Bot. 55:1445-1452.
11. Snedecor, G. W., and Cochran, W. G. 1967. Statistical Methods. The Iowa State University Press, Ames. 593 pp.