Host-Parasite Relations in Uredial Infections of Peanut by *Puccinia arachidis*

Marion Cook

Former graduate student, University of the West Indies, Jamaica. Present address: c/o EMRO, c/o WHO/OMS, 1211-Geneva-27, Switzerland.

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ABSTRACT

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Germination of *Puccinia arachidis* uredospores and the subsequent penetration and infection processes on leaves of *Arachis hypogaea* were studied in detail. Resistance to infection appeared to be related to changing wettability of the leaves with age; the rate of change varied among the cultivars investigated and affected spore retention and probably germination and appressorium formation. Analyses of regressions of the densities of the characteristic structures of the different stages of infection on the density of those of the previous stage showed no significant differences among the cultivars until the uredial stage when physiologic resistance was expressed in the failure of a large proportion of chloronemic flecks to become uredia.

Additional key word: surfactant.

Details of the germination of *Puccinia arachidis* Speg. uredospores and the subsequent penetration and infection processes on peanut (*Arachis hypogaea* L.) leaves have not been reported although it has been established that penetration by the pathogen of the adaxial and abaxial surfaces of peanut leaves and subsequent infection occurs regardless of leaflet orientation (7). The germination and differentiation of the uredospores in vitro, however, have been studied (6). Therefore, these processes were investigated on peanut leaves. Concomitantly it was hoped to determine the factors that reduced infection of those cultivars found resistant in greenhouse screenings and a field trial (3) and to determine the infection stage at which physiologic resistance is expressed.

MATERIAL AND METHODS

P. arachidis uredospores were collected from peanut plantings in the parish of St. Elizabeth, Jamaica, and increased on greenhouse-grown plants of cultivar Starr.

Five peanut cultivars with diffeering susceptibilities to rust were investigated: Starr (very highly susceptible), Jamaican Valencia (highly susceptible), Virginia 61R (slightly resistant), NC 13 (moderately resistant), and Tarapoto (PI 259747, physiologically resistant). Six plants of each cultivar were grown. Seeds were sown in 20-cm diameter plastic pots in a greenhouse. Daily minimum and maximum temperatures were ~20 and 28 C, respectively. When the plants were 6 wk old, leaves that were 4, 12, 20, and 28 days old were harvested and inoculated. To ensure uniform inoculation and to control environmental factors, the leaflets were detached. The four leaflets from each leaf were placed on damp filter paper in the same petri dish; separate dishes were used for each leaf. Leaflets from three of each set of six replicate leaves were arranged with their adaxial surfaces exposed and those from the other three with their abaxial surfaces exposed.

Freshly harvested uredospores were suspended in water in a knapsack sprayer and sprayed onto the exposed leaflet surfaces until a density of approximately 100 spores/cm^2 was attained on microscope slides placed among the dishes. No surfactant was used, and because the spores were highly water repellent, they were not easily dispersed in the suspension. During assessment of spore densities, clumps were ignored. The dishes were covered and incubated in the laboratory at 25 C in daylight enhanced for 12 hr daily with ~15,000 lux from cool-white fluorescent lights.

One leaflet from each replicate leaf was cleared and stained on the first day after inoculation to assess spore retention and germination and subsequent appressorium formation. Another leaflet was cleared and stained on the fourth day to determine the development of infection foci. Leaflets were cleared by immersing the uninoculated surface for a few seconds in chloroform, allowing it to dry, and then floating it on drops of saturated chloral hydrate. Care was taken to prevent the solution from spreading across the inoculated surface. Clearing was more rapid if strips of leaflet were used and if the chloral hydrate was warmed to 50 C. The strips were mounted carefully in 0.05% cotton blue in lactophenol and examined under the high power objective of the microscope. Vertical sections were cut with the freezing microtome, stained in lactophenol cotton blue, differentiated in lactic acid at 80 C, mounted in lactophenol, and examined. To determine the extent of development of infection foci, the two-step staining method of Bell (1) was used.

After 5 days, the dishes were placed in strong, indirect light in a greenhouse. The filter papers were kept moist. Development of infection was studied by daily examination of the two leaflets remaining in each dish. Chloronemic flecks were assessed from the 8th to 12th day and uredial counts were made 14 days after inoculation.

RESULTS

Of the uredospores that germinated, most did so within 6 hr by the formation of a single unbranched germ tube that emerged from one of the equatorial germ pores in the spore wall. This germ tube was about 6 μm in diameter and grew across the leaflet surface until it made direct contact with a stoma. The germ tube pursued a moderately straight course and appeared neither to be attracted to the stomatal apertures nor to follow the grooves between epidermal cells. However, before forming an appressorium the germ tube would frequently grow along an intercellular groove (Fig. 1). The lengths of the germ tubes varied; most were ~100–200 μm, some were ~500 μm (longer than 10 epidermal cell diameters), and a few were even longer. Frequently on the adaxial surface and occasionally on the abaxial surface, a germ tube would pass near, or even over, one or more stomata before forming an appressorium. Sometimes a germ tube was observed seemingly growing indefinitely in this way, but after a time, having failed to

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make direct contact with a stoma, it would cease to grow.

After making contact with a stoma, the germ tube swelled at the tip and formed a thin-walled ellipsoidal appressorium of about the same size as the spore from which it had arisen but not quite large enough to cover the two guard cells completely (Fig. 1). A thin cross wall then formed between the germ tube and the appressorium confining the dense cytoplasm within the appressorium. Germ tube elongation and appressorium formation usually were accomplished within 12 hr of inoculation.

A narrow infection peg, elliptical in cross section, then grew from the appressorium through the stomatal aperture. This process often occurred before or at dawn. After traversing the length of the stomatal passage, the infection peg swelled and formed a vesicle in the substomatal chamber. A cross wall then formed, usually located within the stomatal passage, between the vesicle and the now virtually empty appressorium. Several infection hyphae usually grew from the substomatal vesicle within 24 hr. From these hyphae, simple knoblike haustoria developed within adjacent mesophyll cells (Fig. 2). The infection process was similar in leaves of the five cultivars studied, both for the adaxial and abaxial leaflet surfaces.

The process of uredial development was the same on detached leaflets as that determined on plants in the greenhouse (7).

High percentages of germination and subsequent infection occurred on the abaxial leaflet surfaces. The numbers of spores retained by this surface were consistent among replicates, as were the numbers germinating; these results were assessed quantitatively. Counts of infective structures on the adaxial surfaces were erratic and low; thus, these results were not assessed quantitatively.

Retention of uredospores. Fewer spores were present on the abaxial surface of older leaflets than on younger ones (Table 1) even though the leaflets had received equal spray inoculation (the mean number of spores deposited per square centimeter on the slides was 112,SD = 4.4). There appeared to be little difference among the cultivars in spore retention by the abaxial surface of 4-day-old leaflets, but there were large differences in retention by those that were 12 days old. However, by the time the leaflets were 20 days old these differences became less marked again. Analysis of variance indicated significant differences both among cultivars (P = 0.01) and leaves (P = 0.001) in the numbers of spores retained per square centimeter. The differences between the cultivar means when compared by the sequential Newman-Keuls (8) method showed that cultivars Starr and Jamaican Valencia retained significantly more spores (P= 0.05) than did NC 13 and Virginia 61R, but the retentions of the former did not differ significantly from each other. Likewise, Tarapota, Virginia 61R, and NC 13 did not differ significantly in retention. The differences between the leaf-age means were all significant (P= 0.05); the older the leaves, the fewer the spores they retained. The same general trends in retention were evident on the adaxial leaflet surfaces, although far fewer spores were retained and the differences between younger and older leaflets were not so marked.

Germination of uredospores. Whereas over 85% of the viable spores germinated on the abaxial surfaces of the youngest leaflets (96% viability), less than 80% germinated on the oldest leaflets. Analysis of covariance indicated no significant differences with respect to residual variances, slopes, or elevations among the

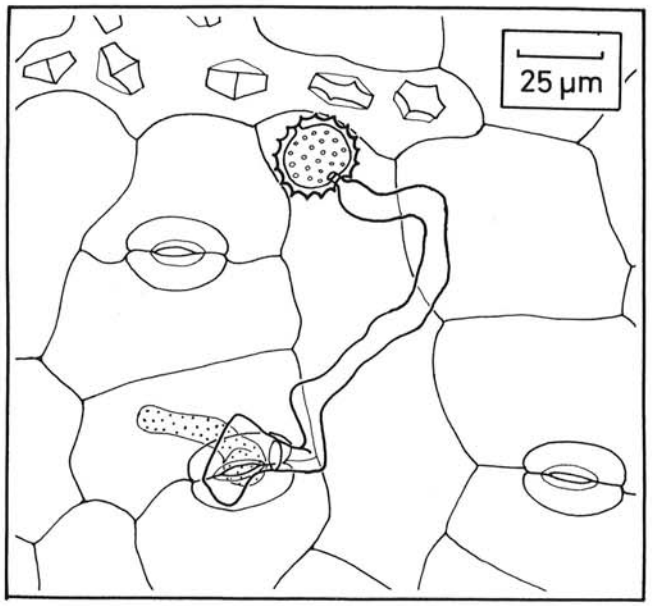

Fig. 1. Camera lucida drawing showing a germ tube from a germinated uredospore that has crossed the abaxial epidermal cells of a peanut leaf and formed an appressorium over the guard cells of a stoma. A cross wall has formed between the germ tube and the appressorium. The infection hypha is in the substomatal chamber.

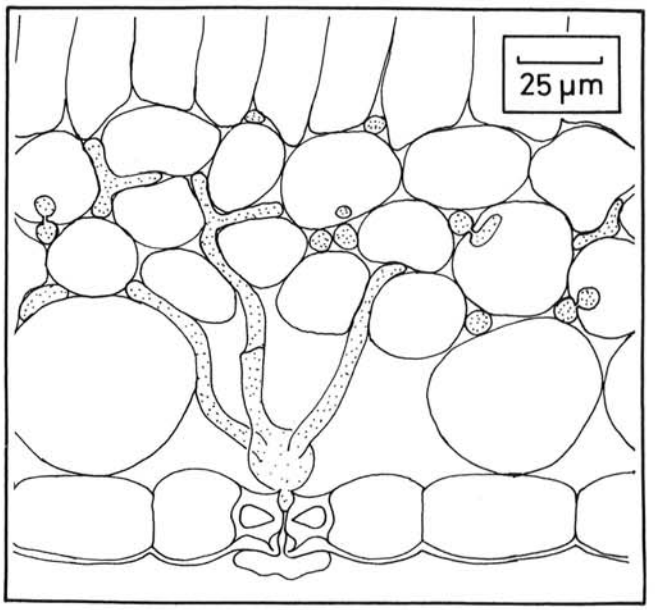

Fig. 2. Camera lucida drawing of a vertical section of a peanut leaf showing invasion by the rust pathogen. Shrivelled appressorium, substomatal vesicle, intercellular infection hyphae, and haustoria within adjacent mesophyll cells can be seen.

TABLE 1. Retention of uredospores on abaxial leaf surfaces by leaves from 6-wk-old plants of five peanut cultivars[a]

Leaf age in days	Uredospore retention per cm²[b]				
	Starr	Valencia	Virginia 61R	NC 13	Tarapoto
4	103 (3.5)	101 (3.6)	100 (4.5)	94 (3.0)	101 (3.5)
12	81 (4.5)	78 (4.7)	54 (3.8)	49 (3.8)	66 (5.5)
20	44 (3.5)	45 (4.0)	36 (4.2)	33 (3.5)	38 (3.6)
28	35 (4.0)	32 (3.6)	22 (3.1)	23 (3.1)	28 (3.5)

[a] Mean spore deposition per square centimeter was 112 (standard deviation = 4.4). Clumps and dense patches of spores were ignored in assessments of spore densities.

[b] Figures represent mean and standard deviation for three replicates.

regression lines for the cultivars of spores germinating per square centimeter on the density of viable spores. Therefore, the data were pooled for further studies on the relationship between spore density and germination. In Fig. 3A, the regressions of spores germinating per square centimeter and of the percentage of viable spores germinating on the density of viable spores have been plotted. The positive slope of the regression line for percent germination was very highly significant (P=0.001) by Student's t-test; as the range of the percentage values was not large, the arcsin transformation was not used. The extent of germination on the adaxial surfaces was erratic. It was noticed, on both leaf surfaces, that in the occasional clumps and dense patches of spores many spores failed to germinate.

Formation of appressoria. Of the spores that germinated on the abaxial surfaces, approximately 60% or more formed appressoria on the leaflets 4 and 12 days old, but less than 60% formed appressoria on the older leaflets. There were no significant differences among the regressions for the cultivars of appressoria per square centimeter on the density of germinated spores; the pooled data are plotted in Fig. 3B. The lower the density of germinated spores, the lower was the percentage that formed appressoria; this regression was very highly significant (P=0.001). Of the spores that germinated on the adaxial surfaces, extremely few formed appressoria and, although appressoria appeared to be more frequent on the youngest leaflets, no definite pattern of appressorium formation with respect to leaf age could be determined.

Establishment of mycelia. On the abaxial surfaces, 80–90% of the appressoria subsequently gave rise to infection foci; this was so even for the physiologically resistant cultivar Tarapoto. There were no significant differences among the regressions for the cultivars of infection foci per square centimeter on the density of appressoria; the pooled data are plotted in Fig. 3C. The apparent slight positive regression of percent infection foci development on the density of appressoria was significant (P = 0.05). Infection foci also formed beneath the adaxial epidermis where penetration by infection hyphae had been successful.

Formation of chloronemic flecks. Cloronemic flecks that developed on the abaxial surfaces were far fewer in number than infection foci, and the percentages of infection foci that formed chloronemic flecks were lower on younger than on older leaflets. There were no significant differences among regressions for the cultivars of chloronemic flecks per square centimeter on the density of infection foci; the pooled data are plotted in Fig. 3D. There was a very highly significant negative regression (P = 0.001) of percent chloronemic fleck formation on the density of infection foci. A very few, erratic chloronemic flecks developed on the adaxial surfaces.

Development of uredia. On leaflets of the nonphysiologically resistant cultivars, virtually all the chloronemic flecks on the abaxial surface developed into uredia; the few that failed to develop did not affect the means. On Tarapoto, however, only about half the flecks developed into uredia, the others became brown necrotic patches, and this cultivar had a significant positive regression (P=0.025) of

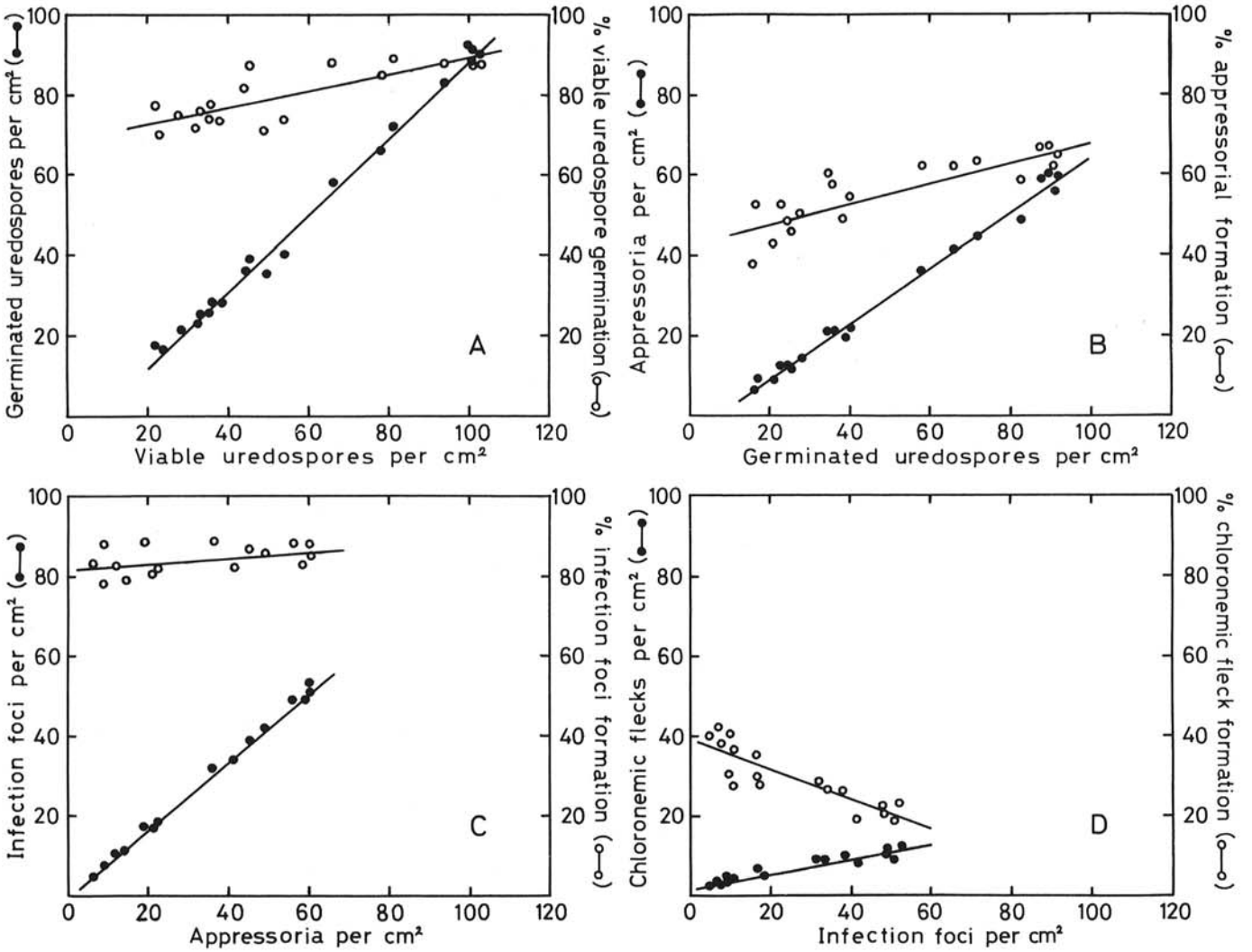

Fig. 3. The regressions of **A,** the density of uredospores germinating and percentage of viable uredospores germinating on the density of viable uredospores; **B,** the density of appressoria and percentage of germinated uredospores forming appressoria on the density of germinated uredospores; **C,** the density of infection foci and percentage of appressoria giving rise to infection foci on the density of appressoria; and **D,** the density of chloronemic flecks and percentage of infection foci developing into chloronemic flecks on the density of infection foci. Each data point represents the mean for three replicates.

percent uredial development on the density of flecks. Virtually all of the flecks on the adaxial surfaces developed into uredia, except on Tarapoto where again only about one-half turned into necrotic patches.

Relation of uredospore retention to chloronemic fleck formation. The regressions for the cultivars of chloronemic flecks per square centimeter on the density of viable spores were investigated to ascertain that small nonsignificant differences among the cultivars had not been accumulating in the interim between spore deposition and uredium formation. Analysis of covariance of the regression lines for the cultivars showed them not to differ significantly; the pooled data are plotted in Fig. 4. There was no significant regression of percent chloronemic fleck formation on the density of viable spores.

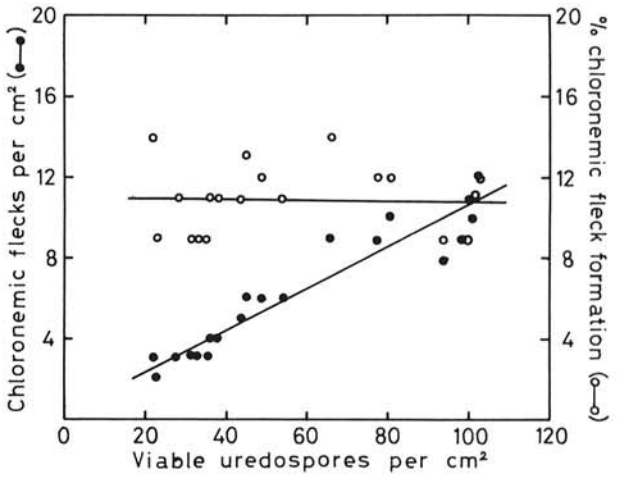

Fig. 4. The regressions of the density of chloronemic flecks and percentage of viable uredospores resulting in chloronemic flecks on the density of viable uredospores. Each data point represents the mean for three replicates.

DISCUSSION

The differential spore retention by comparable leaves of the cultivars appeared to be due to a differential rate of run-off of spray droplets containing spores, which was probably related to the wettability of the leaves. During screenings of peanut cultivars for rust resistance (3) it was noticed that whereas the adaxial surfaces of leaves were always highly water repellent, the abaxial surfaces of older leaves were less wettable than were those of younger ones. In the present results, spore retention likewise was strongly influenced by the age of the leaf; the youngest leaves retained the greatest number of spores. On very wettable leaflets almost all the droplets adhered to the abaxial surface and there was little run-off. Spraying time allowed a small proportion of the individual droplets to coalesce on this surface of these leaflets. With increasing water repellency, despite the horizontal position of the leaflets, run-off removed an increasing proportion of inoculum. This resulted in fewer spores being retained and in spores often being stranded without a surrounding film of water after the droplet receded or rolled off.

Germ tube protrusion did not occur where spores had been stranded without moisture despite a fully saturated atmosphere.

The failure of many of the spores in clumps and dense patches to germinate probably was due to the high concentration of self-inhibitor within them and in the surrounding water. This self-inhibitor was isolated by Foudin and Macko (6) and identified as methyl *cis*-3, 4-dimethoxycinnamate. They found *P. arachidis* uredospores to be more sensitive to the self-inhibitor than any other rust spores with an identified self-inhibitor. The significant positive regression of percent germination on the density of viable spores was to some extent unexpected since better germination would be expected at lower spore densities. Spore retention, however, has been shown to be related to leaf age which probably operated through a changing wettability of the surface. This wettability effect apparently overrode the anticipated effect of self-inhibition at the higher spore densities attained in this study. The apparent lack in direction of growth of the germ tubes was probably due to poor contact with the leaf surface. Dickinson (4) showed that close adhesion of the germ tubes of rust fungi to the surface is essential for directional growth.

The significant positive regression of percent appressorium formation on the density of germinated spores probably was attributable to variations in leaf wettability. If stomatal density had been the responsible factor, this regression would have been negative because stomatal counts were lower on younger than on older leaves. On leaf surfaces with low wettability, germ tubes often were seen passing over stomata without making successful contact. Troughton and Hall (9) reported that spores which germinate on a waxy surface produce hyphae which pass over stomata. The present author speculates that this phenomenon, and that of the failure of the germ tubes to make good contact with the leaf surface, could be due to the strong surface tension of water. Fogg (5) stated that the actual area of contact between surfaces of leaves with low wettability and water drops is less than the superficial area of contact owing to the presence of air films beneath the drops. Germ tubes thus probably often are prevented by the surface tension of water from making immediate contact with the leaf surface. Wynn (10) showed that with the bean rust fungus close adhesion of the germ tube tip to the leaf surface is essential for appressorium formation and it is reasonable to assume that the same factor is essential for appressorium formation by *P. arachidis*. A factor which may aid this phenomenon is that the outer walls of the guard cells tend to be slightly sunken below the level of the adjacent epidermal cells. Another possible cause of the failure of many of the germ tubes to differentiate on the adaxial surface could be that the surface itself is inimical to this process. Foudin and Macko (6) have shown that *P. arachidis* uredospores are fastidious in their requirements for differentiation. It is interesting, however, that while Castellani (2) reported that infection did not occur when the adaxial surface of peanut leaves was inoculated with *P. arachidis* uredospores, McVey (7) obtained infection every time he inoculated this surface; Castellani used no surfactant during inoculation, whereas McVey did, and Castellani sprinkled his plants with water twice daily after inoculation which could have washed the spores off this water repellent surface.

After an appressorium formed over a stoma, there was little further barrier to the establishment of the mycelium within the leaf. The percent of infection foci development was always high, and although its regression on density of appressoria was significant, it was slight. The stomata did not have to be open to allow successful penetration by the infection pegs; the process could occur in the dark.

The substantial failure of infection foci to develop into chloronemic flecks could not be accounted for solely by the coalescence of two or more adjacent foci to give a single fleck, although the negative regression of percent chloronemic fleck formation on the density of infection foci probably reflected that. Many infection foci must have become dormant or aborted.

In the nonphysiologically resistant cultivars, virtually all the chloronemic flecks proceeded through to uredial formation and uredospore release, but less than half of those on the physiologically resistant cultivar developed into uredia; the older the leaf, the more pronounced was this effect.

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