

Erysiphe graminis hordeoi の第一発芽管と付着器からの寄主細胞侵入

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Primary Germ Tubes and Host Cell Penetrations from Appressoria of *Erysiphe graminis hordei**

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久能 均** · James R. Aist*** · Herbert W. Israel***: *Erysiphe graminis hordei* の第一発芽管と付着器からの寄主細胞侵入*

Abstract

Conidia of *Erysiphe graminis hordei* usually produce multiple germ tubes on epidermal cells of barley coleoptiles. Primary germ tubes precede appressorial germ tubes by several hours and ordinarily send penetration pegs into the host cell walls, eliciting papilla formation. We investigated by interference contrast microscopy the *in vivo* time courses of selected events from 0 to 24 hr after inoculation to determine whether or not host responses to primary germ tubes might influence the penetration efficiency of the appressoria that subsequently develop from appressorial germ tubes. Cytoplasmic aggregates were associated with 86% of the primary germ tubes, and appressorial germ tubes emerged from 88% of those conidia that had primary germ tubes. In only 16.5% of the conidia with mature appressoria did the appressoria produce haustoria in the absence of cytoplasmic aggregates below the primary germ tubes. Whenever cytoplasmic aggregates were associated with primary germ tubes, these aggregates always preceded the development of appressoria from appressorial germ tubes of the same conidia. For time course analyses, conidia that attempted penetrations from appressoria were divided into groups defined by the timing of primary germ tube-associated cytoplasmic aggregate initiation and termination, appressoria emergence, and whether or not haustorial central bodies were formed. The interval from primary germ tube-associated cytoplasmic aggregate initiation to appressorial germ tube emergence remained almost constant. The interval between primary germ tube-associated cytoplasmic aggregate initiation and termination was longer when appressoria failed to produce haustoria than when they succeeded. Late termination of primary germ tube-associated cytoplasmic aggregates correlated with a reduction in subsequent haustorial formation.

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Introduction

Kunoh and associates^{8,9)} have shown by scanning electron microscopy of chemically fixed materials that the first of several non-appressorial germ tubes, termed "primary germ tube (PGT)", that develops from *E. graminis hordei* and *tritici* conidia 0.5 to 6 hr after inoculation is associated with a penetration pore and/or papilla in the underlying barley host cell. Papilla deposition generally follows (and may derive from) formation of a host cytoplasmic aggregate that occurs in response to the activity of the appressorium on the host surface^{1,2,3)}. The present study was undertaken to determine whether or not host cytoplasmic aggregates (CA) occur

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in response to PGT's and to better document early stages of pathogen development and host responses.

Materials and methods

A single compatible parasite-host system was used throughout this study; the host has been shown to have some resistance to penetration by the parasite^{12,14}. Procedures for growing, inoculating, and mounting coleoptiles were similar to those described by Aist and Israel¹¹: barley (*Hordeum vulgare* L. 'Proctor') was grown from seed at 65% RH in a chamber kept at 22C during lighted periods and 18C during the dark. The plants were illuminated with 19,000-30,000 lux from mixed incandescent-fluorescent lamps for 16 hr per day. *Erysiphe graminis* DC. f. sp. *hordei* (race B7, formerly referred to as race Ao^{1,2,14}) was maintained in an environmental control chamber on 'Proctor'.

Seven-day-old coleoptiles were partially dissected and mounted between two plastic wafers. Within 30 min after mounting, the coleoptiles were inoculated with freshly harvested conidia that were dusted onto the coleoptiles. Within minutes, these inoculated coleoptiles were observed with a Zeiss Photomicroscope II and the individual locations of apparently ungerminated, turgid conidia were recorded. The coleoptiles were incubated in the dark for 1 hr at 18C. For this period, the coleoptiles were placed on small 0.01M Ca (NO₃)₂-containing reservoirs in petri dishes maintained at 65% RH by addition of a 75% aqueous solution of glycerol to the filter paper in the bottoms of the chambers. Following this, a small piece of No. 1 glass coverslip was placed over the central observation port in the upper plastic element of each coleoptile mount and the conidia previously located were viewed *in vivo* (a heat-reflecting filter was used at all times) at $\times 500$ magnification with interference contrast optics. For each coleoptile, ten to fifteen conidia were each observed at 10-min intervals between 1 and 6 hr after inoculation, and the times of PGT-associated CA initiation and termination, and appressorial germ tube (AGT) emergence were recorded. The coleoptiles were then returned to the dark and further incubated 18 hr. Thereafter PGT's and AGT's which had been located earlier were further observed, and the penetration efficiency (PE) of the appressoria that developed was calculated. Data were accumulated from ten separate, but replicated, experiments performed over a period of 2 months. Penetration efficiency was defined as the quotient obtained by dividing the number of fully formed haustorial central bodies by the number of AGT's observed, multiplied by 100%.

For the time course analyses, the 128 conidia that attempted penetrations from appressoria were divided into groups designated by the timing of PGT-associated CA initiation and termination and appressorial emergence, whether or not the appressoria penetrated. Differences in PE between groups were analyzed by the χ^2 test¹¹. To detect significant time differences between events within a given group, the paired t-test¹³ was used. Differences in the time of occurrence of the same event or in the interval between events in different groups were evaluated by the non-paired t-test¹³.

Results

Germination and development of conidia

Twenty to thirty minutes were needed to microscopically select ungerminated, turgid conidia and to record their locations on the coleoptiles. Five to seven percent

of the conidia were already germinated when inoculated, and because nearly 30% of the remainder germinated during the recording interval, the exact timing of PGT emergence was difficult to determine. Thus we focused our attention on, and selected only, ungerminated conidia.

Among 204 test conidia, 162 (79.4%) produced at least one germ tube within one hour after inoculation (Fig. 1,a) and developed further (Fig. 1,b-f). Of the remainder, 26 (12.7%) conidia germinated but collapsed after their PGT's burst at the apices, and 16 (7.9%) conidia eventually collapsed after showing no signs of germinating.

Initiation of a CA was usually the first visible response of a host cell to the presence of a PGT (Figs. 1,b & c). The CA was seen below only one of the short germ tubes emerging from each conidium, and this germ tube was designated the PGT, as before^{8,9}. Duration of CA's varied from 10 to 200 min. Sometimes papillae were seen below PGT's (Fig. 1,d-f), but because of their small sizes, it was difficult to determine the timing of their initiation.

Appressorial germ tubes (AGT's) emerged from most conidia between 180 and 205 min after inoculation (Fig. 1,d). Frequently, AGT's emerged from conidia prior to termination of the PGT-associated CA (Fig. 2, lines 1 & 4); in other cases they emerged after termination of the CA (Fig. 2, lines 2 & 5). By 190 min after inoculation the AGT's were partitioned from the conidia by septa (Fig. 1,e); by 242 min, immature appressoria were formed (Fig. 1,f).

Time-course studies

To discover what, if any, correlations exist between and among PGT-associated CA's, AGT's, and appressorial PE, we grouped the 128 conidia that attempted penetr-

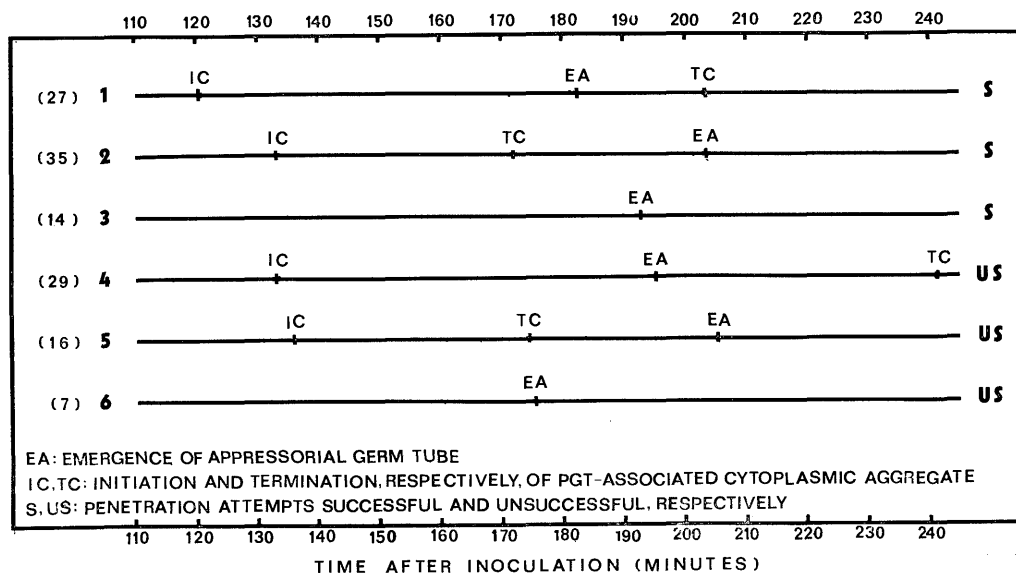


Fig. 2. Time-lines showing the courses of events related to primary germ tube (PGT) and appressorial germ tube (AGT) emergence from 128 turgid *Erysiphe graminis hordei* conidia on barley (*Hordeum vulgare*) coleoptile cells. The 128 conidia are grouped according to relative times of initiation and termination of PGT-associated cytoplasmic aggregates, AGT emergence and appressorial penetration attempts. The number of conidia in each group is shown in parentheses, and the means of the absolute times of events are indicated on the lines. Groups 1-3 penetrated and groups 4-6 did not.

ations from appressoria as described above, and then derived time lines for them (Fig. 2). Among the 76 conidia that effected penetration (Fig. 2, lines 1-3), 81.6% had PGT-associated CA's (Fig. 2, lines 1 & 2). In 43.5% of these cases, AGT's emerged from conidia prior to termination of the PGT-associated CA's (Fig. 2, line 1), and 46.5% did so afterwards (Fig. 2, line 2). Among the 52 conidia that did not penetrate host cells (Fig. 2, lines 4-6), 86.5% had PGT-associated CA's (Fig. 2, lines 4 & 5). In 55.8% of these cases, AGT's emerged prior to termination of PGT-associated CA's (Fig. 2, line 4), and in 44.2% of the cases they did afterwards (Fig. 2, line 5). Regardless of the timing of PGT-associated CA termination, the interval from PGT-associated CA initiation to AGT emergence (Fig. 2, lines 1, 2, 4, & 5) was not significantly ($P=0.25$) different. A PGT-associated CA was never initiated after an AGT emerged from any given conidium. When PGT-associated CA termination occurred after AGT emergence, appressorial PE was significantly ($P=0.05$) less than when CA termination occurred prior to AGT emergence. This relationship was obtained because PGT-associated CA's persisted marginally ($P=0.10$) longer when appressorial penetrations did not occur than when they did (Fig. 2).

Relationships between the presence of PGT-associated CA's and appressorial PE

As shown in Table 1, for all 162 conidia that produced PGT's, CA's were associated with 85.8% of the PGT's, and of the 139 conidia that produced CA's, 122 (87.8%) also produced appressoria. However, 15 (12.3%) of these appressoria had collapsed by 24 hr after inoculation.

Among the 76 turgid conidia whose appressoria penetrated host cells, 62 (81.6%) had PGT-associated CA's, and among the 52 turgid conidia that did not penetrate, 45 (86.5%) had them (Fig. 2). This difference (4.9%) in CA incidence was not significant ($P=0.50$).

Discussion

These results show that CA's form in response to PGT's within 1-1.5 hr after inoculation, which is agreement with a previous report of early papilla formation associated with PGT's^{8,9}. Thus, PGT's elicited host responses—CA's and papillae—sufficiently early to trigger alterations in host resistance¹⁰ and metabolism⁴.

Appressorial germ tubes emerged from conidia between 170 and 205 min after inoculation irrespective of the time of PGT-associated CA termination. In several earlier studies on conidial germination of *E. graminis* it was surmised that appressorial germ tubes emerged from conidia simultaneously with non-appressorial germ tubes^{5,6,7,8}. In the present study, however, we clearly established that appressorial germ tubes always emerged later than primary germ tubes.

As shown in Table 1, the PGT-associated CA's were not seen in association with 16.4% of the conidia that developed PGT's and AGT's. Kunoh *et al.*⁸ pointed out that PGT's emerging from directly beneath conidia could not be viewed unless the conidia were first moved aside by micromanipulation. In a separate study by micromanipulation (Kunoh, Aist, and Israel, unpublished data) we found that nearly 10% of the PGT's emerged from beneath conidia and could not be viewed until they had been moved. Thus, some of the CA's in question may have been missed in the present light microscopic study.

The intervals from PGT-associated CA initiation to AGT emergence from the same conidia were very consistent among groups in all possible comparisons. the other hand, the intervals between initiation and termination of PGT-associated CA's

Table 1. Relationships between primary germ tube(PGT)-associated cytoplasmic aggregates (CA's) and appressorial penetration efficiencies (PE's) of *E. graminis hordei* conidia that formed PGT's on coleoptiles of barley

Condition of		No. of		PE
PGT-associated CA's	Appressoria	Conidia	appressorial penetrations	
Present	Present, turgid	107	62	57.9 ^{a)}
	Present, collapsed	15	0	0
	Not present	17	0	0
	Sub-totals	139	62	44.6 ^{b)}
Not present	Present, turgid	21	14	66.7 ^{a)}
	Present, collapsed	1	0	0
	Not present	1	0	0
	Sub-totals	23	14	60.9 ^{b)}
Totals		162	76	46.9 ^{c)}

a) $\frac{\text{No. of penetrations}}{\text{No. of conidia}} \times 100$; The numbers marked a) are not significantly different by χ^2 analysis ($P=0.25$).

b) $\frac{\text{Sub-total no. of penetrations}}{\text{Sub-total no. of conidia}} \times 100$;
The numbers marked b) are significantly different by χ^2 analysis ($p=0.25$).

c) $\frac{\text{Total no. of penetrations}}{\text{Total no. of conidia}} \times 100$.

were marginally longer when appressoria did not penetrate than when they did. Similarly, late termination of PGT-associated CA's was associated with lowered appressorial PE. Aist and Israel¹⁾ reported comparable findings for appressorium-associated CA's and appressorial PE of this same fungus. Whether a cause-and-effect relationship exists here remains to be determined. Further discussion awaits critical studies on the time course of incompatible interactions and experimental manipulation of PGT's in incompatible interactions.

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和 文 摘 要

Erysiphe graminis hordei の第一発芽管と付着器からの寄主細胞侵入

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Erysiphe graminis hordei の分生胞子は、通常、複数の発芽管を生じ、そのうちの短い一本の発芽管（第一発芽管）は、付着器より数時間早く寄主侵入を行ない、パピラ形成を誘導する。本実験では、オオムギ子葉鞘上に分生胞子を接種し、0 から24時間までの胞子の生長過程と寄主細胞の反応を干渉顕微鏡によって経時的に観察し、第一発芽管が誘導する寄主反応が付着器侵入に影響を及ぼすかどうかを検討した。観察した胞子総数の86%が第一発芽管下に細胞質凝集を誘導し、これらの胞子の88%が付着器発芽管を生じたが、成熟付着器を形成した胞子のうち、16.5%は第一発芽管下に細胞質凝集を誘導しなかった。さらに、大部分の第一発芽管は付着器発芽管が発達する以前に細胞質凝集を誘導することが確認された。本菌の分生胞子は、接種後複数発芽管をほぼ同時に生じ、その中の一本が発達して付着器になると従来考えられていたが、本実験の観察によって、付着器になる発芽管は常に第一発芽管より1-2時間遅れて生じてくることが明らかになった。第一発芽管下の細胞質凝集の開始から付着器発芽管の形成開始までの時間は、いずれの胞子でもほぼ一定であった。付着器が吸器を形成する胞子よりも吸器を形成しない胞子で、第一発芽管下の細胞質凝集はより長く継続した。

Explanation of plate

Figure 1, a to f. A time-lapse series of interference contrast photomicrographs showing progressive events involving a single conidium of *Erysiphe graminis hordei* on a living *Hordeum vulgare* coleoptile cell. By 60 min after inoculation, a primary germ tube had emerged from the conidium (a). At 98 min a cytoplasmic aggregate was seen below the primary germ tube (b). At 120 min the cytoplasmic aggregate reached maximum size (c). At this time an appressorial germ tube emerged (d). At 190 min a septum was formed at the base of the appressorial germ tube near the conidium (e). By 242 min the appressorial germ tube had formed an appressorium (f). agt=appressorial germ tube, app=appressorium, ca=cytoplasmic aggregate, pa=papilla, pgt=primary germ tube, spt=septum. Magnification: $\times 950$. Calibration=10 μ m.

