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Differentiation of aecidiospore- and uredospore-derived infection structures on cowpea leaves and on artificial surfaces by *Uromyces vignae*

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Aecidiospores and uredospores are the two dikaryotic spore forms of the cowpea rust fungus *Uromyces vignae*. After germination they can be induced to develop a series of infection structures including appressoria, infection hyphae, and haustorial mother cells. Haustoria are then formed within host cells. The differentiation of infection structures was compared on polystyrene membranes with defined topographies, on scratched polyethylene membranes, and in planta. On polystyrene membranes with defined topographies both sporelings showed highest rates of differentiation on ridges 0.3 μm high but aecidiosporelings responded less efficiently to this stimulus than uredosporelings. On scratched polyethylene membranes, almost 90% of both sporelings differentiated appressoria, but only 10% formed haustorial mother cells; haustoria were not observed. On the host plant, by contrast, only 50% of the sporelings differentiated appressoria, but most of these formed haustorial mother cells and haustoria. In planta haustorial mother cell development occurred approximately 6 h earlier than on inductive membranes. Infection structures formed on artificial membranes and on host plants were similar in morphology and nuclear condition.

Key words: cowpea rust fungus, nucleus, appressorium.

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L'écidiospore et l'urédospore sont deux types de spores dicaryotiques qu'on retrouve chez le champignon de la rouille du pois chiche, l'*Uromyces vignae*. Après germination, on peut les amener à développer une série de structures d'infection incluant des appressoriums et des cellules mères d'haustéries. Par la suite les haustéries se forment à l'intérieur des cellules de l'hôte. La différenciation des structures d'infection a été comparée sur membranes de polystyrène avec topographies définies, sur membranes de polyéthylène rayées et sur la plante. Sur les membranes de polystyrène avec topographies définies, la germination des deux types de spore montre les plus forts taux de différenciation en présence de raies hautes de 0,3 μm, mais les germes des écidiospores réagissent moins fortement à ce stimulus que ceux des urédospores. Sur les membranes de polyéthylène rayées, environ 90% des deux types de spore conduisent à la différenciation d'appressoriums, mais seulement 10% ont formé des cellules mères des haustéries; aucune haustérie n'a été observée. Sur la plante hôte, au contraire, seulement 50% des spores germées ont donnée des appressoriums mais la plupart de celles-çi ont formé des cellules mères des haustéries ainsi que des haustéries. Dans la plante, le développement de la cellule mère de l'haustérie survient environ 6 h plus tôt que sur membranes inductrices. Les structures d'infection formées sur membranes artificielles et sur plantes hôtes montrent une morphologie et un arrangement nucléaire identiques.

Mots clés : rouille du pois chiche, noyau, appressorium.

[Traduit par la rédaction]

Introduction

The cowpea rust fungus, *Uromyces vignae* (Barclay), is an autoecious rust fungus that produces two dikaryotic spore types, aecidiospores and uredospores. Both spore forms differentiate a series of infection structures to penetrate the host plant via stomata (Littlefield and Heath 1979). Germ tubes of *Uromyces appendiculatus* (Pers.: Pers.) Unger uredospores elongate until an appropriate thigmotropic stimulus is perceived. Then germ tube growth ceases and the formation of appressoria is initiated (Hoch et al. 1987; Hoch and Staples 1991). The topographic characteristics of stomata, inductive for thigmo-differentiation of uredosporelings, can be mimicked by oil–collodion membranes, scratched polyethylene membranes, or microfabricated topographies (for review, see Read et al. 1992). Dimensions of microfabricated topographies that induce maximal rates of appressorium differentiation correspond closely to dimensions of the lips of stomatal guard cells (Terhune et al. 1991). After appressorium formation a penetration hypha produces a substomatal vesicle in the substomatal chamber of the leaf that then elongates to form a young infection hypha (Mendgen and Deising 1993). After the formation of haustorial mother cells, haustoria develop in host cells.

In contrast with the well-studied process of infection structure differentiation of uredospores, only a few reports on aecidiospore germination and infection structure formation are available. In these studies differentiation of infection structures of *Arthuriomyces peckianus* (E. Howe) Cummins and Y. Hirutsaka (syn. *Gymnoconia interstitialis* Lagerh.) (Pady 1934) and germination of *Cronartium quercuum* (Berk.) Miyabe ex Shirai f.sp. *fusiforme* (syn. *Cronartium fusiforme* Hedge. & N. Hunt) (Walkinshaw et al. 1967) were described by light and electron microscopy. More recently Swann and Mims (1991) reported on the presence of four nuclei in the appressoria of the autodemicyclic rust fungus *A. peckianus* grown in vitro. However, there is a lack of sufficient morphological information of nuclear behavior and infection structure development of aecidiospores. Since *U. vignae* can reliably be cultivated under greenhouse conditions and since teliospores of *U. vignae* do not require a resting period (Freytag et al. 1988), we performed this study with aecidiospores and uredospores of the cowpea rust fungus.

Our work shows that infection structures formed by aecidiospores and uredospores in planta or on artificial membranes are identical in morphology, sequential development, nuclear behavior, and response to topographical signals. However, the timing and efficiency of differentiation steps are different in both

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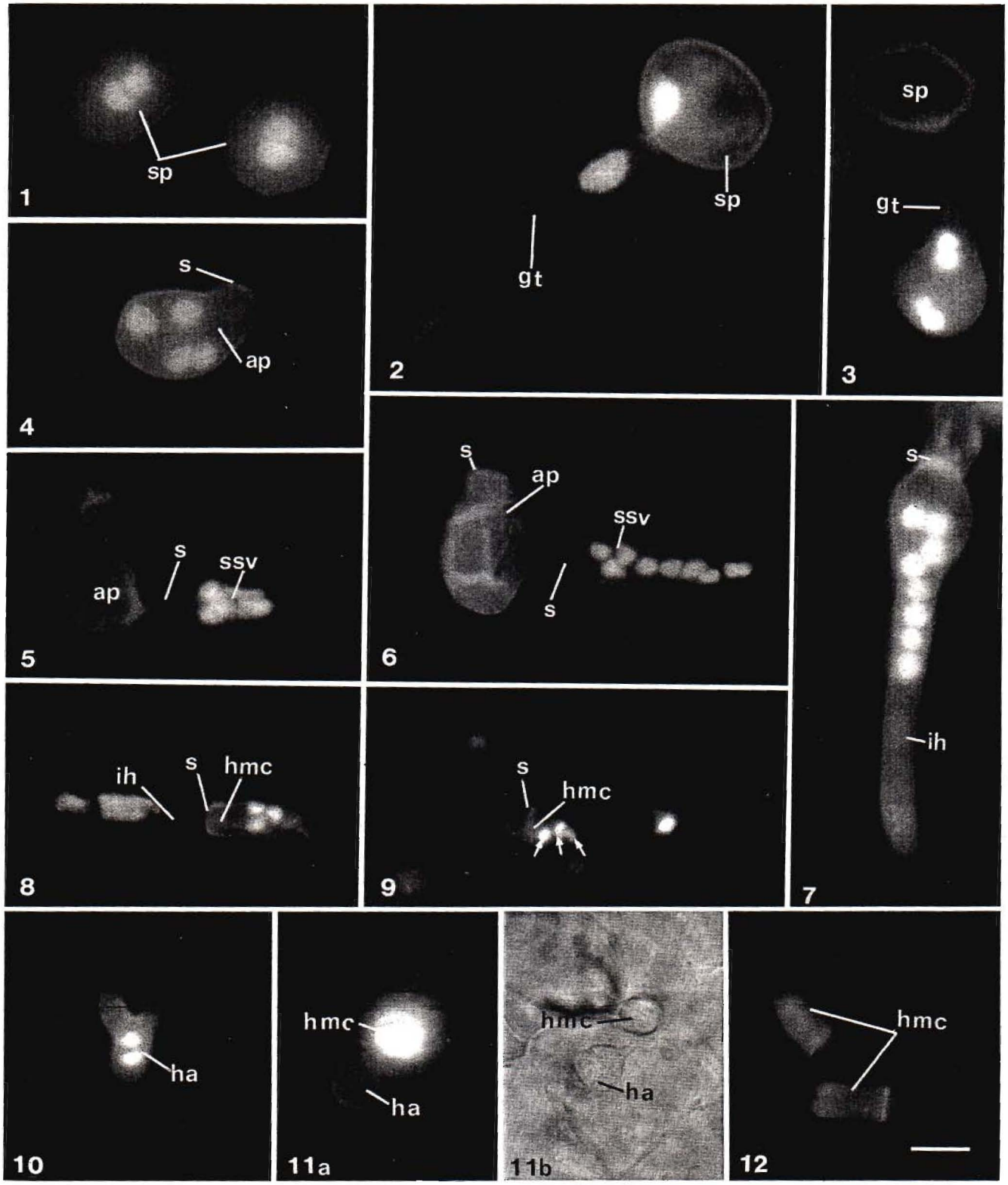

FIGS. 1–12. Nuclear conditions of aecidiospores and aecidiospore-derived infection structures shown by fluorescence microscopy after staining with DAPI–diethanol on scratched polyethylene membranes (Figs. 1–8) and in the host plant (Figs. 9–12). Fig. 1. The aecidiospore is binucleate. Fig. 2. The two nuclei migrate into the germ tube. Fig. 3. The germ tube tip swells and the two nuclei start to divide. Fig. 4. The appressorium contains four nuclei, and a septum separates it from the germ tube. Fig. 5. In the substomatal vesicle a second round of mitosis takes place. Fig. 6. A substomatal vesicle with eight nuclei. Fig. 7. An infection hypha elongates from the substomatal vesicle. Fig. 8. A trinucleate haustorial mother cell is delimited from the infection hypha by a septum. Fig. 9. A haustorial mother cell with three nuclei (arrows), observed in the host tissue. Fig. 10. A binucleate haustorium isolated from leaf tissue. Fig. 11. A haustorium and a haustorial mother cell observed in the host tissue 20 h. after inoculation under (*a*) UV light and (*b*) interference contrast microscopy. Fig. 12. Secondary haustorial mother cells in the host tissue 24 h. after inoculation. Scale bar = 10 μm. *sp*, spore; *gt*, germ tube; *s*, septum; *ap*, appressorium; *ssv*, substomatal vesicle; *ih*, infection hypha; *hmc*, haustorial mother cell; *ha*, haustorium.

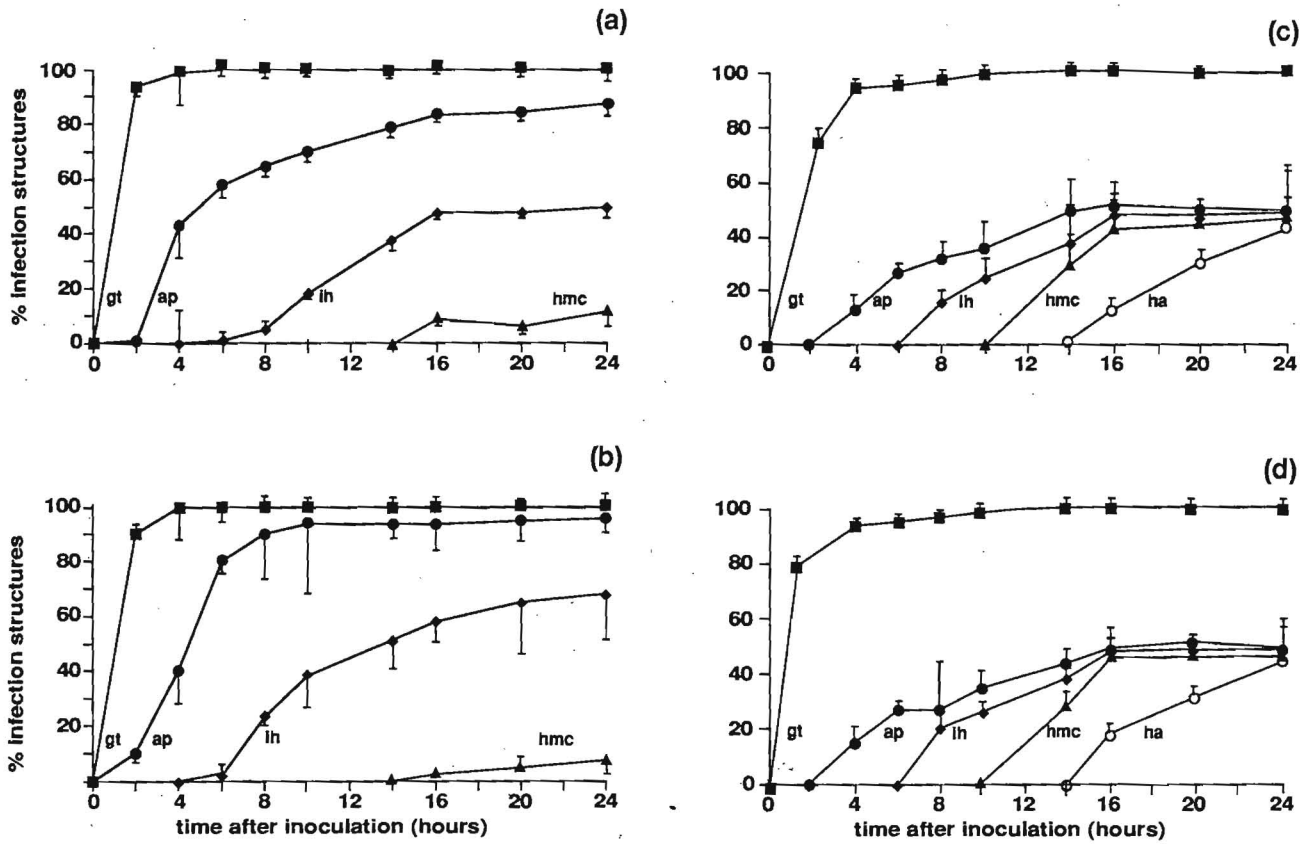

FIG. 13. Sequential differentiation of *Uromyces vignae* infection structures. (*a*) Aecidiospore-derived infection structures on scratched polyethylene membranes. (*b*) Uredospore-derived infection structures on scratched polyethylene membranes. (*c*) Aecidiospore-derived infection structures in planta. (*d*) Uredospore-derived infection structures in planta. A minimum of 400 sporelings per point of time of four different experiments were counted. Vertical lines represent mean deviations. *gt*, germ tube; *ap*, appressorium; *ih*, infection hypha; *hmc*, haustorial mother cell; *ha*, haustorium.

spore forms in planta and on scratched polyethylene membranes.

Materials and methods

Propagation of rust spores

Initials of the uredinial culture of *U. vignae* (Barclay) were kindly provided by M.C. Heath, University of Toronto. Teliospores were increased on susceptible cowpea (*Vigna sinensis* L. cv. California Blackeye) in growth chambers as previously described for the bean rust fungus (Gold and Mendgen 1983). To induce their germination, teliospores were rehydrated by moist filter paper and incubated with a daily photoperiod of 16 h at 21°C and 100% RH. Three days after rehydration, the filter paper with activated teliospores was mounted onto the lid of a Petri dish on top of primary leaves of 14-day-old host plants so that basidiospores were released from the germinated teliospores onto the host leaves. After 10 days pycnia were observed, and after 21 days aecia had developed. Aecidiospores were collected and stored at −70°C.

Induction of infection structures

To obtain infection structures in vitro two types of inductive membranes were prepared: scratched polyethylene membranes and polystyrene membranes. Polyethylene membranes (Melitta-Werke Bentz & Sohn, Minden, Germany) were scratched with a brass brush. Scratches on polyethylene membranes were arranged 2−40 μm apart and had a roughly semicircular shape, with scratch heights ranging from 0.1 to 5 μm. Polystyrene membranes were kindly provided by H.C. Hoch. Polystyrene membranes were prepared as replicas from silicon wafers bearing corresponding negative topographies that had been produced by electron beam lithography according to Allen et al. (1991*a*, 1991*b*). Polystyrene membranes with seven different ridge heights, i.e., 0.18, 0.23, 0.3, 0.42, 0.69, 0.89, and 1.53 μm, were used. Each membrane had a uniform pattern of a single ridge height, 2 μm wide and 60 μm apart in a grid pattern. Aecidiospores or uredospores were dusted either on primary leaves of the host plant, or onto membranes, and lightly misted with water. Samples were incubated at 20°C and 100% RH in the dark for 4−24 h. Haustoria were isolated from infected plants (5 days after inoculation) according to the method described by Hahn and Mendgen (1992).

Microscopy

Infection structures on membranes were studied with differential interference contrast microscopy after staining with 0.1% trypan blue in lactophenol−glycerol−water (1:1:1). Infection structures in planta were studied by fluorescence microscopy using a modified method of Kuck et al. (1981) with the exception that the fungus was stained with 0.2% (w/v) diethanol (Uvitex 2B, Ciba Geigy, Basel, Switzerland). Nuclei were stained after fixation with 2% glutaraldehyde in 0.05 M phosphate buffer, pH 7.2, for 30 min, with 0.05% (w/v) 4,6-diamidinophenyl-indol · 2HCl (DAPI; Serva, Heidelberg, Germany) in the same buffer for 5 min, and subsequently treated with 0.02% (w/v) diethanol for 2 min and mounted in 50% (v/v) glycerin. Observations were made with a Zeiss incident fluorescence microscope. A 365-nm excitation filter and 420-nm barrier filter were used. The diameter of the nuclei was measured with an ocular-micrometer.

Results

Development of infection structures

On scratched polyethylene membranes, uredospores (results not shown) and aecidiospores (Fig. 1) produced germ tubes

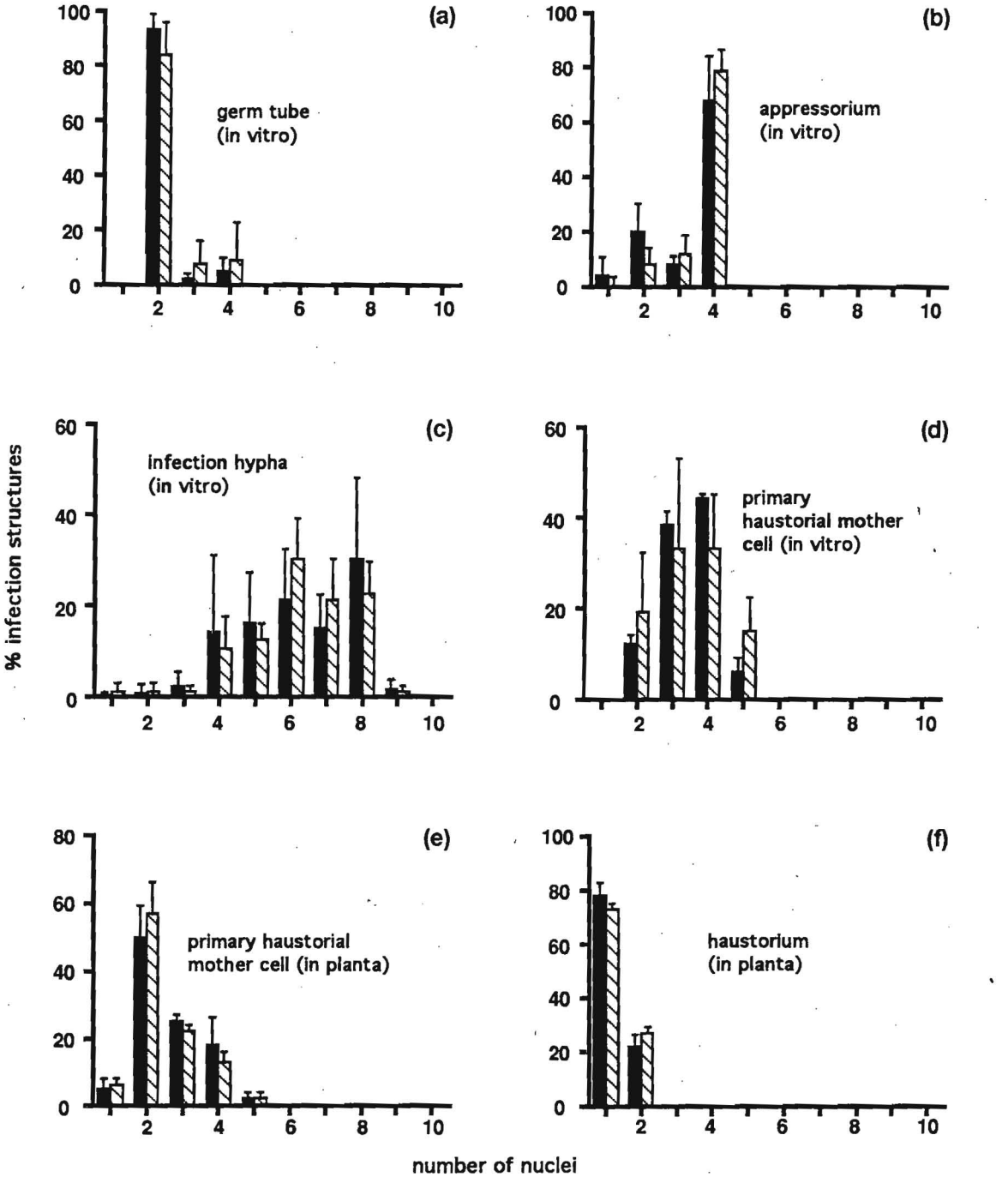

FIG. 14. Distribution of nuclei in aecidiospore and uredospore infection structures. At each point of time at least 200 infection structures (100 in *e*) of four different experiments were evaluated for their nuclear condition. Solid bars indicate aecidiospore-derived infection structures, hatched bars indicate uredospore-derived infection structures, and vertical lines represent mean deviations.

into which both nuclei migrated (Fig. 2). After perception of a thigmotropic stimulus the germ tube tip swelled to form an appressorium, and the two nuclei underwent the first round of mitosis (Fig. 3). Appressorium development was accompanied by septum formation between germ tube and appressorium (Fig. 4). Next the penetration hypha developed and grew through the stomatal opening to form the substomatal vesicle. The appressorial nuclei migrated into the substomatal vesicle, and a septum was formed between penetration hypha and substomatal vesicle (Fig. 5). Then the substomatal vesicle elongated to form a young infection hypha. After a second round of nuclear division the infection hypha contained up to eight nuclei (Figs. 6 and 7). Primary haustorial mother cells contained similar numbers of nuclei, not only on scratched polyethylene membranes (Fig. 8) but also in the host mesophyll tissue (Fig. 9). Haustoria (Figs. 10 and 11) and secondary haustorial mother cells (Fig. 12) were observed in planta.

The series of infection structures derived from both aecidiospores and uredospores occurred in a similar sequence (Fig. 13). There were differences between infection structure development on scratched polyethylene membranes (Figs. 13*a* and 13*b*) and on the host plant (Figs. 13*c* and 13*d*). On scratched

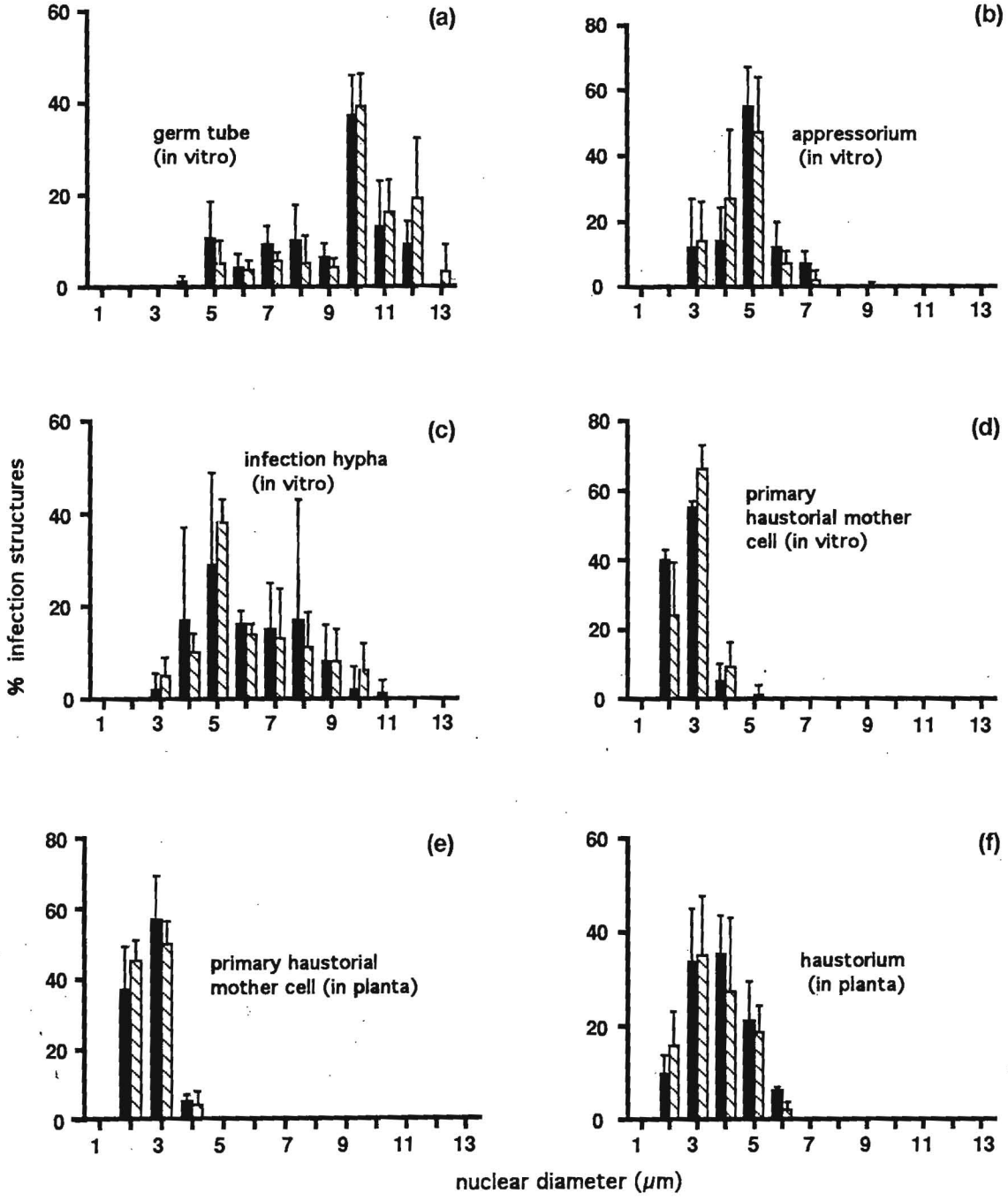

FIG. 15. Size of nuclei within the different infection structures. From a minimum of 200 nuclei per infection structure of four different experiments the maximal diameter was measured. Solid bars indicate aecidiospore-derived infection structures, hatched bars indicate uredospore-derived infection structures, and vertical lines represent mean deviations.

polyethylene membranes nearly 90% of the sporelings developed appressoria, whereas on the host plants, only 50% of the sporelings developed appressoria. Infection hyphae were formed between 8 and 16 h after inoculation. On the host plant almost every infection hypha produced haustorial mother cells and haustoria. On scratched polyethylene membranes only 10% of the infection hyphae differentiated haustorial mother cells. Differentiation of haustorial mother cells occurred in vivo approximately 6 h earlier than in vitro. On scratched polyethylene membranes, no haustoria were observed. In planta, haustoria and secondary haustorial mother cells were formed 16 h after inoculation.

The number of nuclei in uredospore- and aecidiospore-derived infection structures is shown in Fig. 14. About 20% of the primary haustorial mother cells in planta and about 40% of the primary haustorial mother cells on scratched polyethylene membranes contained three or four nuclei (Figs. 14*d* and 14*e*). Haustoria in planta contained one or two nuclei (Figs. 10 and 14*f*). In vitro the nuclear diameter was reduced from an average of 10 μm in germ tubes to an average of 5 μm after the first round of mitosis in the appressoria (Figs. 15*a* and 15*b*). This size remained similar after the second round of mitosis in the infection hyphae (Fig. 15*c*). In haustorial mother cells the size of nuclei was 3 μm in vitro and in vivo (Figs. 15*d* and

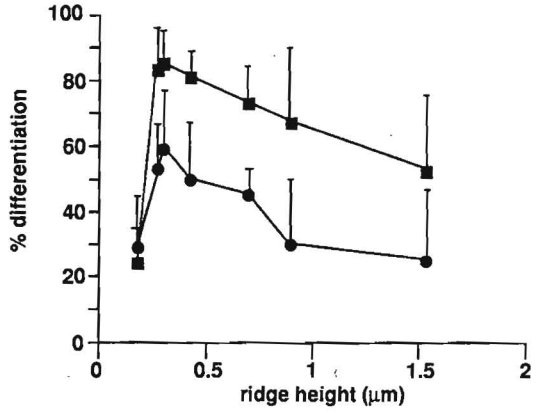

FIG. 16. Formation of appressoria in response to polystyrene ridges ranging in heights from 0.18 to 1.53 μm. At least 300 germlings were evaluated for each ridge height in three different experiments. ■, uredospores; ●, aecidiospores.

15*e*). Nuclei of haustoria, on the other hand, had a diameter of about 4 μm (Fig. 15*f*).

Determination of optimal signal dimension for differentiation of appressoria

The optimal response for both types of sporelings to differentiate appressoria was best at a ridge height of about 0.3 μm (Fig. 16). There was a difference in efficiency of appressorium formation between the two types of spores. About 85% of the uredospore-derived germlings but only about 60% of the aecidiospore-derived germlings differentiated appressoria on polystyrene replicas with the optimal ridge height. On host plants, however, about 50% of both aecidiospore- and uredospore-derived germlings produced appressoria (Fig. 13*c* and 13*d*).

Discussion

Appressorium formation can be induced in vitro by chemical, temperature, or physical stimuli (Hoch and Staples 1987). Examples for thigmotropically effective membranes are scratched polyethylene membranes or microfabricated topographies (Allen et al. 1991*b*; Read et al. 1992). Infection structures induced by various triggers differ in several aspects. They exhibit different expression patterns of proteins after heat shock or thigmotropic induction (Bhairi et al. 1990). D_2O and taxol are inhibitory to chemically induced but not to thigmotropically induced cell differentiation (Hoch and Bourett 1986). Therefore in planta and on inductive membranes infection structure development and nuclear behavior may also differ. Our results indicate that scratched polyethylene or ridged polystyrene membranes result in a higher degree of appressorium differentiation than the host-specific stomatal lips of guard cells. This might be due to a higher density of inductive stimuli on artificial membranes compared with the lower surface of primary leaves of *V. sinensis*. Ridges on polystyrene membranes were arranged in a 60-μm grid pattern, whereas stomatal guard cells on the host plant were separated from each other by two epidermal cells. Thus the probability for aecidiospore and uredospore germlings of finding an inductive stimulus is much higher on artificial membranes than on the host plant.

Compared with uredospores, aecidiospores showed a lower efficiency of appressorium differentiation on microfabricated polystyrene membranes (Fig. 16). On scratched polyethylene membranes aecidiosporelings and uredosporelings differentiated similar rates of appressoria (Figs. 13*a* and 13*b*). This difference between the two artificial surfaces might be due to well-defined steplike, rectangular ridges with 0.18–1.53 μm ridge heights on polystyrene membranes and roughly semicircular scratches on polyethylene membranes with scratch heights ranging from 0.1 to 5 μm.

The results concerning the appressorium differentiation rate of uredospore germlings on polystyrene membranes (Fig. 16) closely correspond to those obtained by Allen et al. (1991*a*).

On the host plant appressorium differentiation almost inevitably led to formation of infection hypha, haustorial mother cell, and haustorium. Under in vitro conditions few haustorial mother cells and no haustoria were formed, and the haustorial mother cells appeared approximately 6 h later than under in vivo conditions. This might be due to premature senescence or even death of the haustorial mother cells (Heath and Perumalla 1988) because they lack mono- and di-saccharides, amino acids, or other nutrients present in the leaf apoplast (Kaminskyj and Day 1984). Differentiation of haustoria on artificial membranes could only be induced by addition of autoclaved driselase or certain sugars (Heath 1989, 1990).

Kuck et al. (1981) describe staining methods with diethanol to visualize fungal hyphae in the host tissue. These methods include clearing of host tissue before staining, and therefore DAPI-stained nuclei can hardly be identified. By fixing the host tissue without clearing before staining with DAPI and diethanol, we were able to identify nuclei of infection structures growing within the host tissue. Since haustoria were not stained by this procedure, they were isolated from infected plants (Hahn and Mendgen 1992) prior to staining and analysis (Fig. 10).

Our results indicate similar nuclear conditions in both aecidiospore- and uredospore-derived infection structures. The nuclear behavior and septum formation in appressoria of *U. vignae* resembled that described for *U. appendiculatus* (Staples et al. 1984; Bourett and Hoch 1987; Kwon and Hoch 1991). A second round of mitosis always occurred in the young infection hypha. Here we report for the first time on multinucleate primary haustorial mother cells studied in vivo. Multinucleate haustorial mother cells have been observed mostly in vitro (Deising et al. 1991; Maheshwari et al. 1967), and only Chong et al. (1992) reported on multinucleate secondary haustorial mother cells in *Puccinia striiformis* Westend. in planta. Our studies indicate that multinucleate primary haustorial mother cells are not an artifact of in vitro conditions (Figs. 14*d* and 14*e*). Aecidiospore- and uredospore-derived primary haustorial mother cells exhibited a different distribution pattern of the number of nuclei (Figs. 14*d* and 14*e*).

Studies on nuclear diameter (Fig. 15) revealed a reduction of size after the first round of mitosis. This size is maintained within appressoria and during the second round of mitosis in the infection hypha. The diameter of nuclei in infection hyphae revealed a very broad distribution pattern. We interpret this as the consequence of nuclei being in different stages of the replication cycle (Staples et al. 1984). Haustorial mother cells contained smaller nuclei with a maximal nuclear diameter of 3 μm (Figs. 15*d* and 15*e*) and haustoria contained nuclei with diameters of 4 μm. This confirms studies of Heath and Heath (1978) who observed similarly small nuclei in haustorial mother cells and haustoria.

Artificial surfaces are generally used to investigate fungal appressorium differentiation (Read et al. 1992) and differenti-

ation of fungal proteins (Deising et al. 1991). Therefore it was important to test if there were any observable differences between infection structure development in planta and infection structure development on artificial surfaces. Our study shows that the higher density of inductive stimuli on artificial surfaces compared with the lower surface of the leaf might be the reason for a higher differentiation rate of appressoria. As soon as the rust fungus is within the host tissue its development is obviously supported by the plant cells.

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Allen, E.A., Hazen, B.E., Hoch, H.C., *et al.* 1991*a*. Appressorium formation in response to topographical signals by 27 rust species. Phytopathology, **81**: 323–331.

Allen, E.A., Hoch, H.C., Stavely, J.R., and Steadman, J.R. 1991*b*. Uniformity among races of *Uromyces appendiculatus* in response to topographic signaling for appressorium formation. Phytopathology, **81**: 883–887.

Bhairi, S.M., Lacetti, L., and Staples, R.C. 1990. Effect of heat shock on expression of thigmo-specific genes from a rust fungus. Exp. Mycol. **14**: 94–98.

Bourett, T., and Hoch, H.C. 1987. Association of the microtubule cytoskeleton with the thigmotropic signal for appressorium formation in *Uromyces*. Mycologia, **79**: 540–545.

Chong, J., Kang, Z., Kim, W.K., and Rohringer, R. 1992. Multinucleate condition of *Puccinia striiformis* in colonies isolated from infected wheat leaves with macerating enzymes. Can. J. Bot. **70**: 222–224.

Deising, H., Jungblut, P.R., and Mendgen, K. 1991. Differentiation-related proteins of the broad bean rust fungus *Uromyces viciae-fabae*, as revealed by high resolution two-dimensional polyacrylamide gel electrophoresis. Arch. Microbiol. **155**: 191–198.

Freytag, S., Bruscaglioni, L., Gold, R.E., and Mendgen, K. 1988. Basidiospores of rust fungi (*Uromyces* species) differentiate infection structures *in vitro*. Exp. Mycol. **12**: 275–283.

Gold, R.E., and Mendgen, K. 1983. Activation of teliospore germination in *Uromyces appendiculatus* var. *appendiculatus*. I. Aging and temperature. Phytopathol. Z. **108**: 267–280.

Hahn, M., and Mendgen, K. 1992. Isolation by ConA binding of haustoria from different rust fungi and comparison of their surface qualities. Protoplasma, **170**: 95–103.

Heath, M.C. 1989. *In vitro* formation of haustoria of the cowpea rust fungus, *Uromyces vignae*, in the absence of a living plant cell. I. Light microscopy. Physiol. Mol. Plant Pathol. **35**: 357–366.

Heath, M.C. 1990. *In vitro* formation of haustoria of the cowpea rust fungus *Uromyces vignae* in the absence of a living plant cell. II. Electron microscopy. Can. J. Bot. **68**: 278–287.

Heath, M.C., and Heath, I.B. 1978. Structural studies of the development of infection structures of cowpea rust, *Uromyces phaseoli* var. *vignae*. I. Nucleoli and nuclei. Can. J. Bot. **56**: 648–661.

Heath, M.C., and Perumalla, J. 1988. Haustorial mother cell development by *Uromyces vignae* on collodion membranes. Can. J. Bot. **66**: 736–741.

Hoch, H.C., and Bourett, T.M. 1986. Inhibition of cell differentiation in *Uromyces* species with D_2O and taxol. Eur. J. Cell Biol. **41**: 290–297.

Hoch, H.C., and Staples, R.C. 1987. Structural and chemical changes among the rust fungi during appressorium development. Annu. Rev. Phytopathol. **25**: 231–247.

Hoch, H.C., and Staples, R.C. 1991. Signaling for infection structure formation in fungi. *In* The fungal spore and disease initiation in plants and animals. *Edited by* G.T. Cole and H.C. Hoch. Plenum Press, New York. pp. 25–46.

Hoch, H.C., Staples, R.C., Whitehead, B., *et al.* 1987. Signaling for growth orientation and cell differentiation by surface topography in *Uromyces*. Science (Washington, D.C.), **235**: 1659–1662.

Kaminskyj, S.G., and Day, A.W. 1984. Chemical induction of infection structures in rust fungi. I. Sugars and complex media. Exp. Mycol. **8**: 63–72.

Kuck, K.H., Tiburzy, R., Hänssler, G., and Reisener, H.J. 1981. Visualization of rust haustoria in wheat leaves by using fluorochromes. Physiol. Mol. Plant Pathol. **19**: 439–441.

Kwon, Y.H., and Hoch, H.C. 1991. Temporal and spatial dynamics of appressorium formation in *Uromyces appendiculatus*. Exp. Mycol. **15**: 116–131.

Littlefield, L.J., and Heath, M.C. 1979. Ultrastructure of rust fungi. Acadamic Press, New York.

Maheshwari, R., Allen, P.J., and Hildebrandt, A.C. 1967. Physical and chemical factors controlling the development of infection structures from uredospore germ tubes of rust fungi. Phytopathology, **57**: 855–862.

Mendgen, K., and Deising, H. 1993. Infection structures of fungal plant pathogens — a cytological and physiological evaluation. New Phytol. **124**: 193–213.

Pady, S.M. 1934. Aeciospore infection in *Gymnoconia interstitialis* by penetration of the cuticle. Phytopathology, **25**: 453–471.

Read, N.D., Kellock, L.J., Knight, H.S., and Trewavas, A.J. 1992. Contact sensing during infection by fungal pathogens. *In* Perspectives of plant cell recognition. *Edited by* J.A. Callow and J.R. Green. University Press, Cambridge. pp. 137–172.

Staples, R.C., Gross, D., Tiburzi, R., *et al.* 1984. Changes in DNA content of nuclei in rust uredospore germlings during start of differentiation. Exp. Mycol. **8**: 245–255.

Swann, E.C., and Mims, C.W. 1991. Ultrastructure of freeze-substituted appressoria produced by aeciospore germlings of the rust fungus *Arthuriomyces peckianus*. Can. J. Bot. **69**: 1655–1665.

Terhune, B.T., Allen, E.A., Hoch, H.C., *et al.* 1991. Stomatal ontogeny and morphology in *Phaseolus vulgaris* in relation to infection structure initiation by *Uromyces appendiculatus*. Can. J. Bot. **69**: 477–484.

Walkinshaw, C.H., Hyde, J.H., and Van Zandt, J. 1967. Fine structure of quiescent and germinating aeciospores of *Cronartium fusiforme*. J. Bacteriol. **94**: 245–254.