# Appressorium Formation in Response to Topographical Signals by 27 Rust Species

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## ABSTRACT

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The formation of urediniospore appressoria in response to microfabricated topographies was observed for 27 species of rust fungi representing eight genera. Nine species, including *Uromyces appendiculatus, U. vignae, Melampsora medusae, Puccinia antirrhini, P. calcitrapae, P. carduorum, P. melanocephala, P. substriata*, and one isolate of *P. recondita*, developed appressoria on ridges within an optimal height range of approximately 0.4–0.8 μm. Appressorium formation was considerably reduced on ridge heights above and below this range. A broader range of ridge heights was observed to be inductive for appressorium formation by *P. polysora, P. menthae, P. hieracii, P. sorghi, P. arachidis*, and *Physopella zeae*. These rusts maintained high levels of appressorium formation on ridges up to 2.25 μm high. Seven species, including *Coleosporium asterum, C. tussilaginis, Phragmidium potentillae, Puccinia coronata, P. graminis* f. sp. *tritici, P. g.* f. sp. *avenae*, and *Tranzschelia discolor*, did not form appressoria on microfabricated topographies of defined heights, but they did form very low numbers of appressoria on scratched polystyrene membranes. *Phakopsora pachyrhizi* formed appressoria both on smooth membranes and in apparent association with ridges. The unique characteristics of the thigmotropic responses demonstrated by some of the rust species will be valuable in studying the mechanisms of thigmotropic sensing and appressorium formation.

Both chemical and physical (topographical) features of the host surface influence the orientation of germ tube growth of many rust fungi as well as signal the development of infection structures (8,9). Such recognition of chemical and topographic signals by fungal germ tubes or hyphae is termed chemotropism and thigmotropism, respectively (23). Appressorium formation is a prerequisite in the process of urediniospore infection by rust fungi. Various thigmotropic, chemotropic, and environmental stimuli (3,10,14,15, 20,22,25,26) signal the induction of appressorium formation among rust species. The relative importance of these stimuli appears to vary among rust genera. For example, the bean rust fungus, *Uromyces appendiculatus* (Pers.:Pers.) Unger, which has been studied in considerable detail, is triggered to form numerous appressoria equally well on a variety of substrates, such as leaf stomata or polystyrene replicas of stomata, as well as on other artificial surfaces bearing topographies of appropriate dimensions (10,25). In contrast, other rust fungi, such as *Puccinia graminis* Pers.:Pers., develop few if any appressoria in response to artificial topographies. Dickinson (3) reported appressorium formation by *P. graminis* on collodion membranes; however, subsequent researchers had little success using similar surfaces (15). Staples and co-workers (20) showed that appressoria of *P. graminis* f. sp. *tritici* Eriks. & E. Henn. form on a variety of scratched artificial substrates, but that subsequent infection structures (penetration peg, substomatal vesicle, etc.) do not develop. With few exceptions (1,2,11), urediniospore infections by rust fungi involve entry through the host stomata (6,18). The infection process of such rusts includes hydration and germination of the urediniospores, germ tube growth over the leaf surface, contact with a stoma, and subsequent serial development of specialized infection structures (8,27). The initial infection structure, the appressorium, develops only after the germ tube contacts the stomatal apparatus. The precise nature of the inductive signal provided by the stoma is still in question, but increasing evidence suggests that some physical component of the stomatal structure is responsible for triggering appressorium formation (10,24,25). In order to better understand the precise physical parameters of the signal inducing appressorium formation by *U. appendiculatus* on *Phaseolus vulgaris* L., Hoch and colleagues (10) tested the growth of urediniospore germ tubes on artificial topographies of defined heights. They showed that an abrupt change in surface topography, for example, a ridge of about 0.5 μm in height, is optimal for the induction of appressorium formation, whereas on ridges above (1.0 μm) or below (0.25 μm) this height appressorium development is significantly reduced. More refined characterization of similar topographies indicated an optimal ridge height range of 0.4–0.8 μm (E. A. Allen, *unpublished*) for appressorium formation. In addition to *U. appendiculatus* and *P. graminis* (3,17,20), at least eight other *Puccinia* spp. were reported to form appressoria in response to artificial topographic features: *P. recondita* Roberge ex Desmaz. (4), *P. coronata* Corda (5), *P. glumarum* Eriks. & E. Henn. (3), *P. triticina* Eriks. (3), *P. melanocephala* Syd. & P. Syd. (19), *P. antirrhini* Dietel & Holw. (15), *P. helianthi* Schwein. (15), and *P. sorghi* Schwein. (15). However, no attempts were made to quantify the topographical signal.

Greater understanding of thigmosensing in rust fungi may provide insight into the actual mechanisms of appressorium formation on both artificial and natural plant surfaces. Knowledge of the prepenetration development of pathogenic fungi could lead to the breeding of plants that utilize nonspecific resistance mechanisms to disrupt the normal development of infection structures, thus inhibiting fungal ingress. In addition, we question how broadly previous studies regarding thigmosensing can be applied to other rust fungi, since only a single rust species, *U. appendiculatus*, has been examined to date. Here we report findings of an examination of 27 species of rust fungi with regard to the formation of appressoria on artificial topographies of varying height.

## MATERIALS AND METHODS

Urediniospores of the 27 rust species tested were collected locally or made available by other researchers (Table 1). In some cases, more than one isolate of the same species was available for testing. Three types of substrates were used to analyze urediniospore germling growth and the potential for inducing appressoria: polystyrene with defined topographies, scratched polystyrene bearing nondefined surface features, and smooth surfaces of glass or polystyrene. Polystyrene membranes with defined topographies were produced by casting a thin film of dissolved polystyrene (Styron 685D, Dow Chemical, Midland, MI) (20% [w/v] in ethyl acetate) onto silicon wafer templates (7.5 cm in diameter) bearing microfabricated patterns produced by either optical or electron beam lithography (10). Once the solvent had completely evaporated from the polystyrene, the resulting membranes (about 25 $\mu$m thick) were floated off the templates in a 45 C water bath. Each membrane had a uniform grid pattern of ridges that were 2 $\mu$m wide and spaced 60 $\mu$m apart. For most tests, 10 different ridge heights, 0.11, 0.18, 0.23, 0.31, 0.36, 0.42, 0.5, 0.7, 0.9, and 1.24 $\mu$m, were used. In some tests, two additional ridge heights, 2.27 and 6.7 $\mu$m, were included. The heights of the ridges were confirmed by examination of test membranes with a scanning electron microscope (SEM) with a measuring program of an integrated image analysis system (FD-5000, Gould/DeAnza, San Jose, CA). Scratched polystyrene membranes were produced by rubbing the surface of a 0.1-mm-thick preformed smooth polystyrene sheet (Kings Specialty Co., Brooklyn, NY) with #0 steel wool. This created a surface with scratches of various depths and undefined dimensions. Smooth test surfaces were created by casting dissolved polystyrene onto unetched silicon wafers as above or by treating glass coverslips with 1.5% dimethyldichlorosilane (Petrarch Systems, Bristol, PA) in methylene chloride. Coverslips were soaked in the silane solution for 5 min, rinsed in methylene chloride, allowed to air dry, and baked at 100 C for 60 min.

Prior to germination, the spores were conditioned by one or more of the following methods: 1) hydration in a moist chamber for 6–12 hr at 18–22 C; 2) heat shock, in which spores were placed in a glass petri dish and floated on 40 C water for 10 min; 3) treatment with vapor of 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (= $\beta$-ionone) (Sigma, St. Louis, MO) for 20–30 min at 18–22 C; or 4) no treatment. The spores were then dusted onto 5-mm-square test membranes, cut from the membranes described above, with a sterilized camel-hair brush to a density of about 30 spores per square millimeter. To improve the adhesion of the spores to the plastic surface, the spore-laden membranes were lightly misted with distilled water, enclosed in a petri dish for 10 min, and then allowed to air dry. The membranes were then floated spore-side down on distilled water in a plastic petri dish (10 cm in diameter) and incubated for 6–24 hr (depending on the species) in the dark at 18–20 C. Variations in methods of spore preparation or incubation times were necessary for some species to achieve adequate germ tube growth and differentiation. After incubation, membranes were either fixed in 3% formaldehyde for 10 min and mounted spore-side down in 90% glycerol or were mounted directly in 0.01% toluidine blue in glycerol on a microscope slide. Each of the rust species was tested on a series of substrates consisting of three replicates of the 10 ridge heights, smooth polystyrene membranes, and scratched polystyrene membranes. Species that exhibited high rates of differentiation on 1.24-$\mu$m-high ridges were tested further on membranes bearing 2.25- and 6.7-$\mu$m-high ridges. Since these tests represented a

TABLE 1. Rust Species Tested

| Pathogen[a] | Disease | Host | Source[b] |
|---|---|---|---|
| *Coleosporium asterum* (Dietel) Syd. & P. Syd. | Aster rust | *Aster* L. sp. | 14 |
| *Coleosporium tussilaginis* (Pers.) Lév. in C. d'Orb. | Pine-bluebell rust | *Campanula* L. sp. | 14 |
| *Melampsora lini* (Ehrenb.) Desmaz. | Flax rust | *Linum usitatissimum* L. | 10 |
| *Melampsora medusae* Thuem. | Poplar rust | *Populus deltoides* J. Bartram ex Marsh. | 6 |
| *Phakopsora pachyrhizi* Syd.* | Soybean rust | *Glycine max* (L.) Merr. | 1 |
| *Phragmidium* Link. sp. | Rose rust | *Rosa* L. sp. | 14 |
| *Phragmidium potentillae* (Pers.:Pers.) P. Karst. | Strawberry rust | *Fragaria* L. sp. | 14 |
| *Physopella zeae* (Mains) Cummins & Ramachar* | Southern corn rust | *Zea mays* L. | 1 |
| *Puccinia antirrhini* Dietel & Holw. | Snapdragon rust | *Antirrhinum* L. sp. | 14 |
| *Puccinia arachidis* Speg. | Peanut rust | *Arachis hypogaea* L. | 5,13 |
| *Puccinia calcitrapae* DC. var. centaureae (DC.) Cummins* | Safflower rust | *Carthamus* L. sp. | 1 |
| *Puccinia canaliculata* (Schwein.) Lagerh.* | Nutsedge rust | *Cyperus esculentus* L. | 2 |
| *Puccinia carduorum* Jacky* | Thistle rust | *Carduus tenuiflorus* Curtis | 2 |
| *Puccinia coronata* Corda | Crown rust | *Agropyron repens* (L.) P. Beauv. | 8 |
| *Puccinia graminis* Pers.:Pers. f. sp. *avenae* Eriks. & E. Henn., race 31(6AF) | Oat stem rust | *Avena* L. sp. | 8 |
| *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn., races TLMH and MBCT | Wheat stem rust | *Triticum aestivum* L. | 8 |
| *Puccinia hieracii* (Röhl.) H. Mart. | Dandelion rust | *Taraxacum officinale* Wigg. | 14 |
| *Puccinia jaceae* Otth.* | Star-thistle rust | *Centaurea calcitrapa* L. | 2 |
| *Puccinia melanocephala* Syd. & P. Syd. | Sugarcane rust | *Saccharum* L. sp. | 7 |
| *Puccinia menthae* Pers.:Pers. | Mint rust | *Mentha* L. sp. | 11 |
| *Puccinia polysora* Underw.* | Maize rust | *Zea mays* L. | 1 |
| *Puccinia recondita* Roberge ex Desmaz., local isolates,[c] and isolates DB-66 and 89-516-YC1313 | Wheat leaf rust | *Triticum aestivum* L. | 8,14 |
| *Puccinia sorghi* Schwein. | Corn rust | *Zea mays* L. | 1,3 |
| *Puccinia substriata* Ellis & Barth. var. *indica* Ramachar & Cummins | Millet rust | *Pennisetum* Rich. ex Pers. sp. | 5 |
| *Tranzschelia discolor* (Fuckel) Tranzschel & Litv. | Stone fruit rust | *Prunus* L. sp. | 12 |
| *Uromyces appendiculatus* (Pers.:Pers.) Unger, race O | Bean rust | *Phaseolus vulgaris* L. | 14 |
| *Uromyces vignae* Barclay | Cowpea rust | *Vigna sinensis* (L.) Endl. | 4 |

[a] Nomenclature follows Fungi on Plants and Plant Products in the United States, Farr et al, APS Press. Asterisk indicates species tested at the USDA Foreign Disease-Weed Unit, Ft. Detrick, Frederick, MD.

[b] Rust spores obtained from: 1) M. Bonde, USDA, Frederick, MD; 2) W. Bruckart, USDA, Frederick, MD; 3) H. Dillard, New York State Agricultural Experiment Station (NYSAES), Geneva; 4) M. C. Heath, University of Toronto; 5) C. Mims, University of Georgia, Athens; 6) M. Ostry, U.S. Forest Service, St. Paul, MN; 7) L. H. Purdy, University of Florida, Gainesville; 8) A. P. Roelfs, USDA, St. Paul, MN; 9) R. C. Staples, Boyce Thompson Institute, Ithaca, NY; 10) G. D. Statler, North Dakota State University, Fargo; 11) W. Stevenson, University of Wisconsin, Madison; 12) B. Teviotdale, University of California, Kearney Agricultural Center, Parlier; 13) W. Wynn, University of Georgia, Athens; 14) culture collection, NYSAES, Geneva.

[c] The local isolate of *P. recondita* was tested at the USDA Cereal Rust Laboratory, St. Paul, MN, and was shown to represent a mixture of races NBB-10, NBB-18, and LBB-18.

second and separate experiment, membranes with 0.7- and 1.24-μm-high ridges also were included as a measure of comparison with the first test. In all tests, 200 germ tubes were examined on each of the three replicate membranes and assessed for appressorium formation in association with ridges. Means and standard deviations of the replicates were calculated and expressed as percent differentiation (percentage of germ tubes that bore appressoria). If sufficient quantities of spores were available, the entire experimental series was repeated two or three times.

Germling growth and response of some of the rusts was tested on polystyrene replicas of leaf surfaces prepared as reported by Wynn (25). Briefly, silicon rubber (RTV-11, General Electric Co., Waterford, NY) was polymerized by catalysis with stannous octoate (0.025 ml/g RTV-11) and spread over the leaf surface. After 20 min the polymerized silicon rubber was peeled off the leaf, forming a negative impression of the leaf surface. Dissolved polystyrene was spread over the silicon rubber template, dried, and peeled off, resulting in a positive replica of the leaf surface.

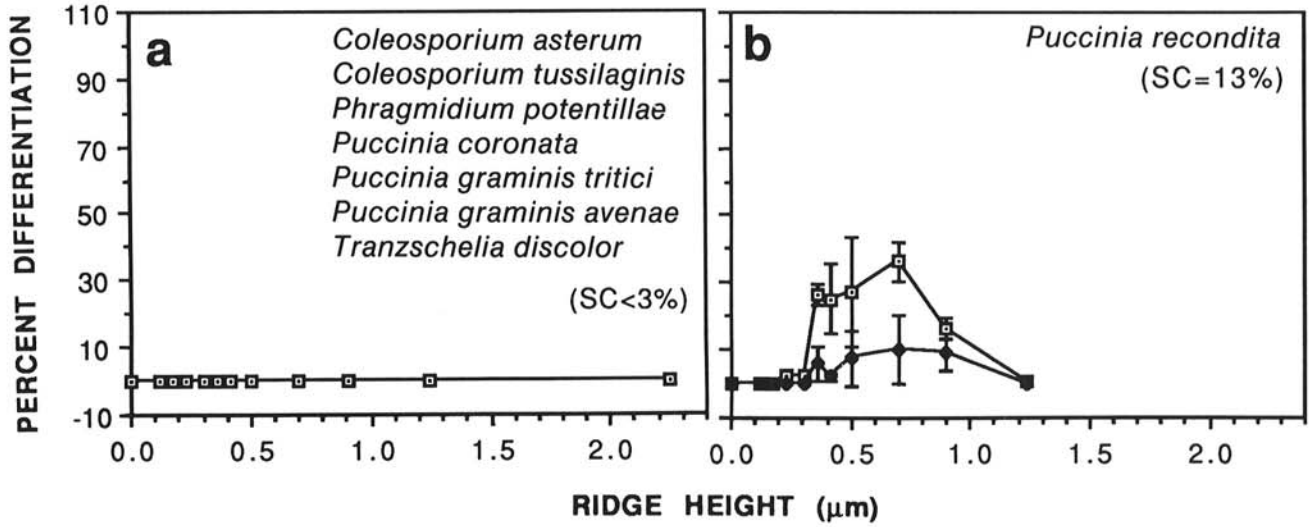


**Fig. 1.** Percent differentiation (appressorium formation) in response to polystyrene ridges ranging in height from (**A**) 0.11 to 2.24 μm and (**B**) 0.11 to 1.24 μm. SC denotes response to scratched polystyrene membrane. ⊡ = Local isolate; ◆ = isolate 89-516-YC1313.

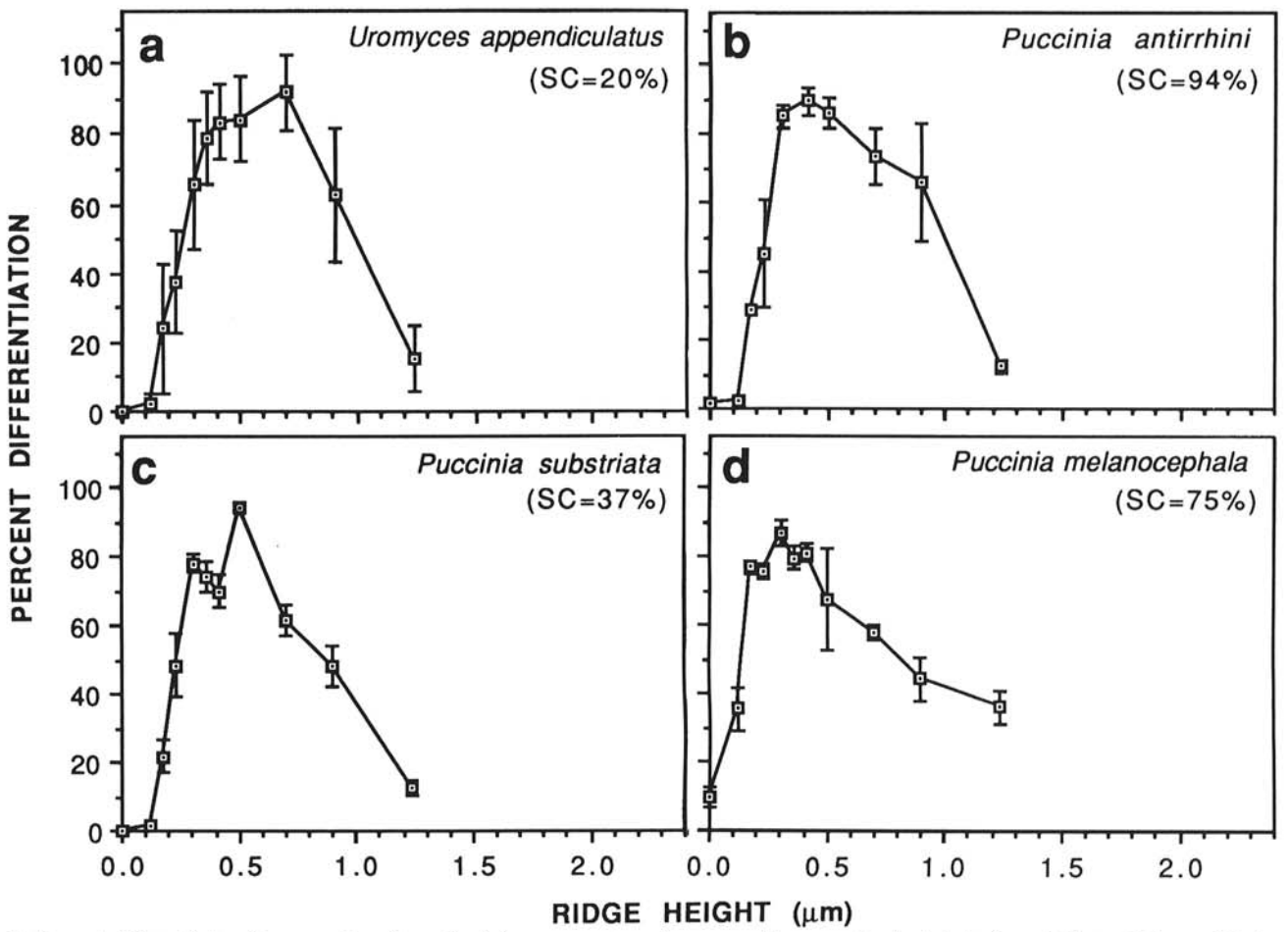


**Fig. 2.** Percent differentiation (appressorium formation) in response to polystyrene ridges ranging in height from 0.11 to 1.24 μm. SC denotes response to scratched polystyrene membrane.

These polystyrene leaf replicas were dusted with spores and incubated in the same manner as the other test membranes.

Appressorium formation was assessed and photographed with either a Zeiss Axiophot or a Zeiss Photomicroscope III by phase-contrast, differential-interference contrast, or fluorescence illumination. To visualize appressoria on scratched membranes, germlings were stained with 0.1% aqueous Calcofluor (Polysciences Inc., Warrington, PA), rinsed in water, and examined with UV illumination (excitation wavelength, 365 nm; dichroic mirror, 395 nm; barrier emission wavelength, 420 nm). Occasionally, putative appressoria of some species were difficult to assess because of their shapes. However, since most rust urediniospore germlings are binucleate before appressorium formation and have four nuclei following appressorium formation, the number of nuclei was used as a guide. To visualize nuclei, germlings were fixed in a 3% formaldehyde solution, rinsed and stained with an aqueous solution (1 μg/ml) of the DNA-specific fluorochrome, 4,6-diamidino-2-phenylindole (DAPI, Sigma, St. Louis, MO) (7,21), and examined with the same UV filter combination described above.

For SEM examination, specimens were fixed in 2% aqueous osmium tetroxide overnight at 5 C, dehydrated in a graded ethyl alcohol series, critical-point dried, and coated with gold-palladium. Specimens were observed with a Hitachi S-530 SEM operating at 25 kV.

## RESULTS

Urediniospore germling growth and the formation of appressoria on polystyrene membranes bearing specific topographies varied among the rust species tested. We grouped them into broad categories based on their tendencies to form appressoria on specific ridge heights.

In group 1, no appressoria were formed on any ridged or smooth membranes. Rusts that responded in this manner included *Coleosporium asterum, C. tussilaginis, Phragmidium potentillae, Puccinia coronata, P. g. tritici, P. g.* f. sp. *avenae*, and *Tranzschelia discolor* (Fig. 1A). Of the two isolates of *Puccinia recondita* obtained from the Cereal Rust Laboratory, isolate DB-66 formed virtually no appressoria on ridges of any height (Fig. 1B), whereas isolate 89-516-YC1313 erratically formed a few appressoria in association with ridges. In contrast, all of the other tested rust fungi developed significant numbers of appressoria in association with ridges of at least some height.

In group 2, the differentiation response was characterized by a curve that was low on low ridges, increased to a height range where appressorium formation was optimal, and then declined as the ridge height increased further. Rust species that followed this pattern included *U. appendiculatus, U. vignae, Melampsora medusae, Puccinia antirrhini, P. calcitrapae, P. carduorum, P. melanocephala, P. substriata* (Figs. 2A–D and 3A–D), and an isolate of *P. recondita* collected locally near Geneva, New York (Fig. 1B). Optimal ridge heights for appressorium formation varied among rust species but generally fell in the range of 0.4–0.8 μm; exceptions to this range were also found. For example, *P. substriata, P. antirrhini*, and *U. appendiculatus* all showed less than 20% differentiation on 1.24-μm ridges, whereas *U. vignae* showed greater than 50% differentiation at this height (Fig. 3B). The formation of appressoria by *U. vignae*, however, was reduced to 8% on 2.24-μm-high ridges.

A third type of response was produced by group 3, which consisted of *Puccinia polysora, P. menthae, P. hieracii, P. sorghi,*

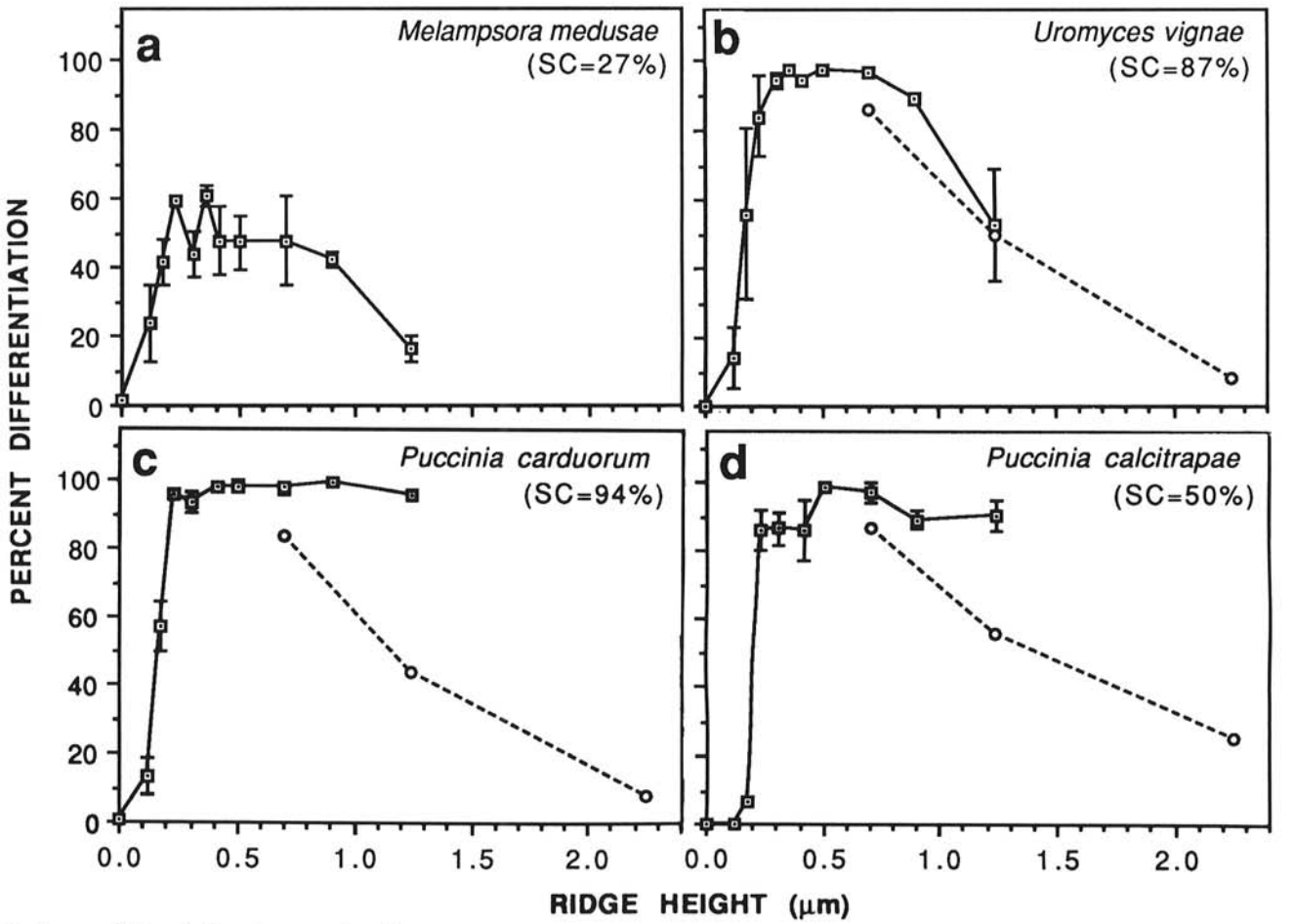


**Fig. 3.** Percent differentiation (appressorium formation) in response to polystyrene ridges ranging in height from 0.11 to 2.24 μm. SC denotes response to scratched polystyrene membrane. Dotted lines in **B**, **C**, and **D** represent a second test that included ridge heights of 0.7, 1.24, and 2.24 μm.

*P. arachidis*, and *Physopella zeae* (Fig. 4A–F). These species also responded to ridges but continued to form appressoria on ridges with increasing height. Most of the latter species in this group showed more than 80% differentiation on 1.24-$\mu$m-high ridges and showed little decrease in the number of appressoria formed on 2.24-$\mu$m-high ridges. *P. hieracii* continued to form appressoria on ridges as high as 6.7 $\mu$m, but at a somewhat reduced level (77%) (Fig. 4B). Other rust species formed appressoria in association with 6.7-$\mu$m-high ridges (Table 2), but not all of the appressoria were formed on these actual ridges. *P. sorghi* and *P. arachidis*, for example, produced appressoria on ridges up to 1.24 $\mu$m in height but formed them against rather than on higher ridges. A similar trend was observed with *Physopella zeae*, which produced appressoria beside ridges 0.8 $\mu$m high and higher. *Puccinia menthae* developed appressoria on ridges, as well as against both the front and the back sides of 6.7-$\mu$m-high ridges (Fig. 5B inset). *P. hieracii* differed from other species in this group in that it formed appressoria on ridges at all heights tested (Figs. 4B and 5c). During initial observations of *P. hieracii* and *P. arachidis*, it appeared that appressoria were formed in high numbers on both smooth polystyrene membranes and membranes with 0.11-$\mu$m-high ridges (Figs. 4A and B). When retested on silane-coated glass coverslips, surfaces that were observed with SEM to be very smooth, *P. hieracii* did not form appressoria. SEM examination of the polystyrene membranes showed that appressorium development was associated with minor flaws or cracks about 0.01 $\mu$m high or deep in the membrane surface. The formation of appressoria by *P. arachidis* occurred on both types of smooth membranes, but in virtually all cases, appressoria formed immediately adjacent to other germ tubes growing on

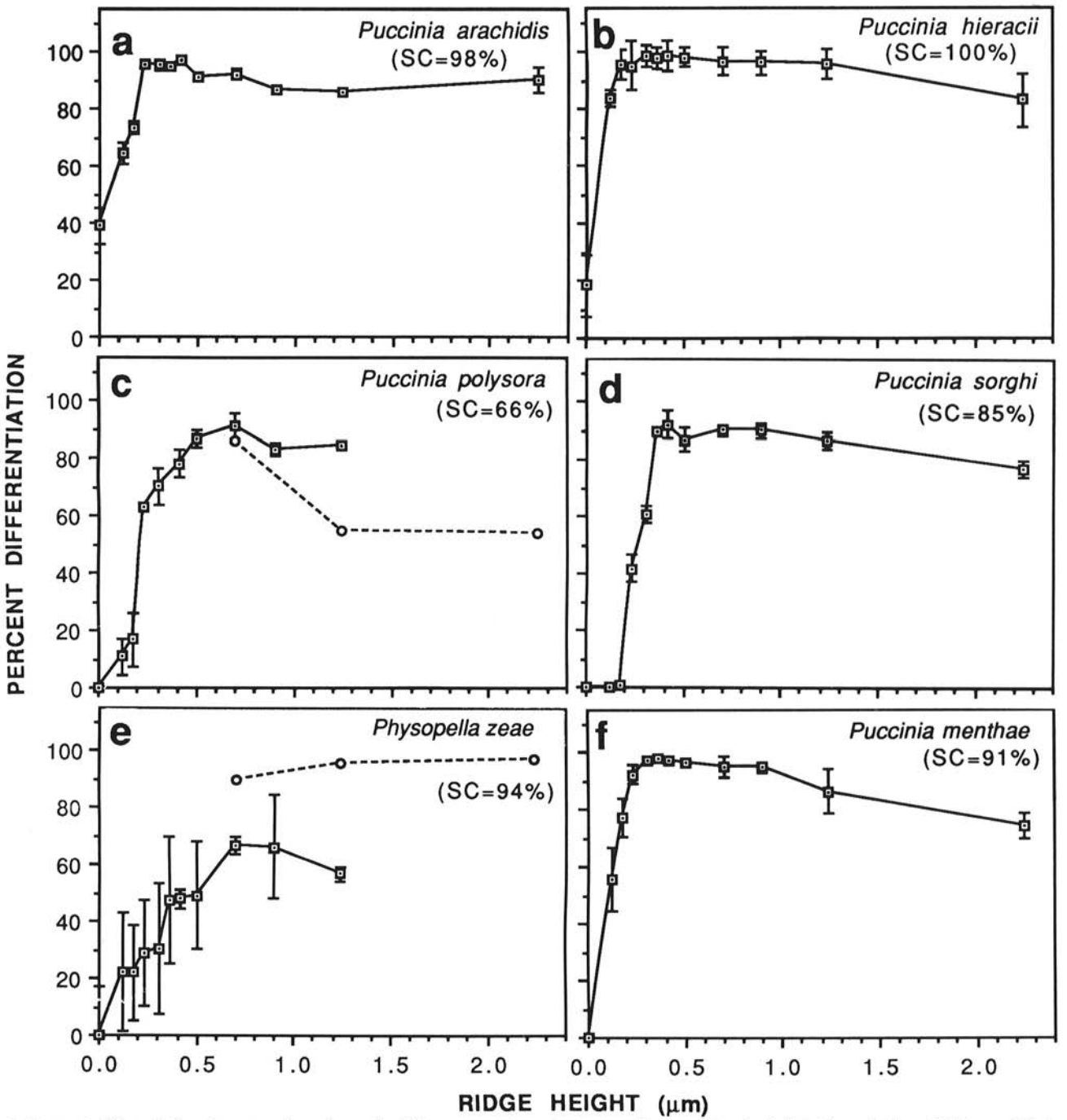


**Fig. 4.** Percent differentiation (appressorium formation) in response to polystyrene ridges ranging in height from 0.11 to 2.24 $\mu$m. SC denotes response to scratched polystyrene membrane. In **A,** appressoria on membranes with no ridges (i.e., 0 $\mu$m height) formed adjacent to germ tubes. In **B,** appressorium formation on membranes with no ridges was attributed to flaws in the membrane surface. Dotted lines in **C** and **E** represent a second test that included ridge heights of 0.7, 1.24, and 2.24 $\mu$m.

the surface (Fig. 6A).

Interestingly, the development of appressoria after contact with ridges was delayed in some of the rusts tested, the most extreme example being *P. arachidis*. Germ tubes frequently grew over and past the inductive ridges by as much as 50 μm before apical extension ceased. However, development of the appressorium subsequently occurred over the ridge as the cytoplasm was retracted into the swelling; septa were formed on both sides of the newly formed appressorium (Fig. 6B). Overgrowth of ridges by other rust species was generally not more than 5–10 μm.

TABLE 2. Rust species induced to form appressoria on 6.7-μm-high ridges

| Rust species | Percent differentiation | Appressorium position |
|---|---|---|
| *Puccinia hieracii* | 77 | On ridges |
| *P. arachidis* | 60 | Beside ridges |
| *P. sorghi* | 26 | Beside ridges |
| *P. carduorum* | 3 | Beside ridges |
| *P. menthae* | 11 | On ridges |
| *P. polysora* | 23 | Beside ridges |
| *Physopella zeae* | 81 | Beside ridges |

The final type of germling response observed in this study was that of *Phakopsora pachyrhizi* (group 4). Total appressorium formation was high on ridges of all heights (90–100%), as well as on both smooth polystyrene membranes (75%) and silane-treated glass (97%). Appressoria formed on smooth areas between, beside, and on ridges in approximately equal proportions (Fig. 7). Urediniospores that were germinated on polystyrene replicas of both *Glycine* and *Phaseolus* leaf surfaces formed appressoria in the "valleys" formed at the junctions of epidermal cells (Fig. 8).

Scratched polystyrene was used as a comparative surface for the induction of appressoria, especially for rust species that did not readily form appressoria on ridges of simple design. Generally, with the exception of *P. recondita*, rust species in group 1, which exhibited little or no response to ridges (Fig. 1A and B), rarely developed appressoria on scratched membranes. Conversely, rust species that developed appressoria in response to ridges (Figs. 2–4) readily formed appressoria on the scratched surfaces. Furthermore, the rust species of group 3 that formed appressoria in high numbers on high ridges also formed appressoria very efficiently (100%) on scratched membranes (Fig. 4). A greater range of responses to scratched membranes was observed for the rust species of group 2, for which appressorium formation was reduced on high ridges. The rare instances of group 1 rusts forming appressoria on scratches were associated with very deep scratches.

The shapes of appressoria differed among the rusts examined (Fig. 9). The most common shape, variously ovate to obovate, was exemplified by *U. appendiculatus* and *P. hieracii* (Fig. 9A and B). Appressoria of *P. sorghi* were sometimes of this form (Fig. 9C) but also were commonly elongate (Fig. 9C inset), particularly in association with ridges 1.24 μm high or higher. Germ tubes terminating in what appeared to be one bifurcate or two

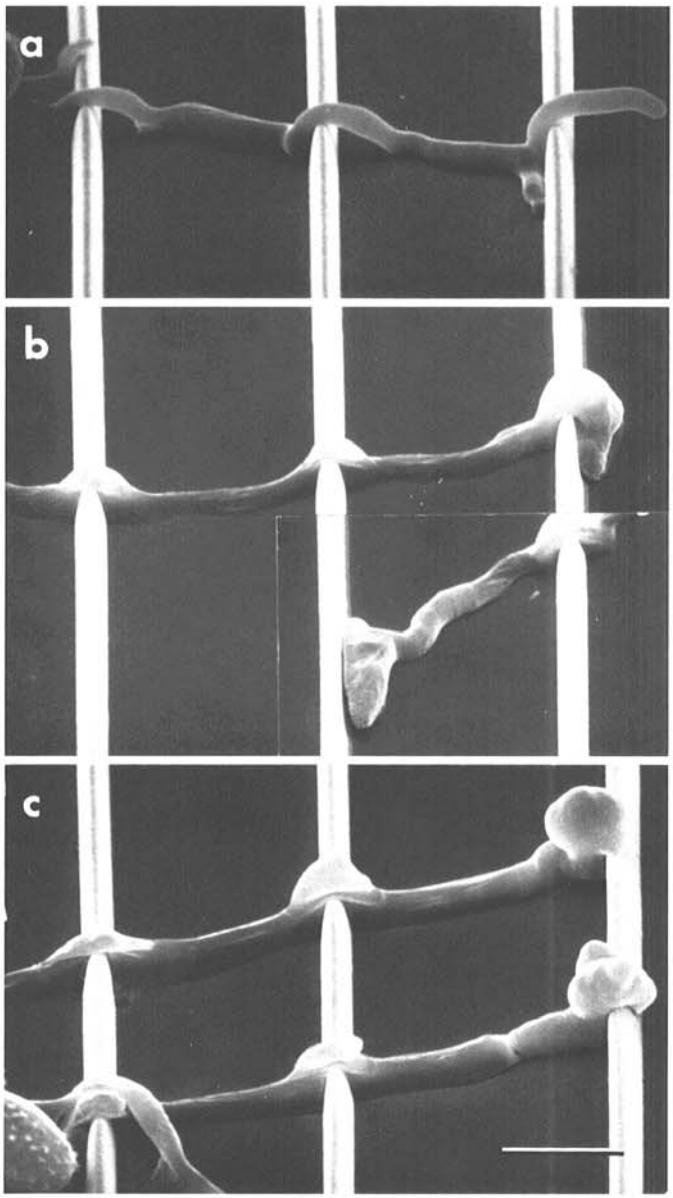


**Fig. 5.** Urediniospore germ tube growth and appressorium formation in association with 6.7-μm-high ridges. **A,** *Uromyces appendiculatus* did not form appressoria on these topographies. The germ tube exhibited poor adhesion to the back side of the ridges. **B,** *Puccinia menthae* germ tubes adhered closely to all surfaces of the ridges. Appressoria formed on front and back sides (inset) of ridges. **C,** *P. hieracii* germ tubes adhered closely to all surfaces. Appressoria formed over ridges. Magnification bar = 20 μm.

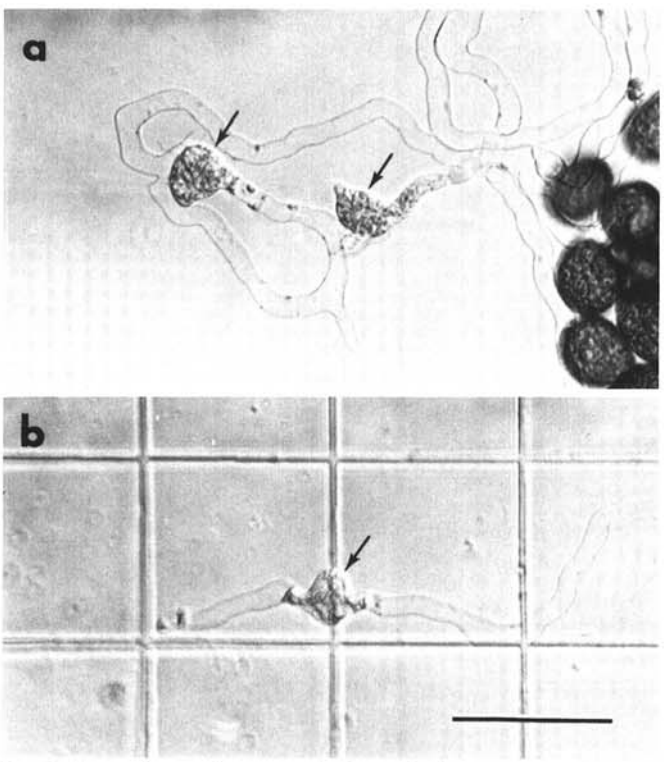


**Fig. 6.** Germ tubes and appressoria (arrows) of *Puccinia arachidis*. **A,** On polystyrene membranes with no ridges, appressoria formed immediately adjacent to germ tubes. **B,** The germ tube apex grew past a 0.7-μm-high inductive ridge and subsequently formed an appressorium. Magnification bar = 50 μm.

distinct appressoria were often formed by *P. sorghi* (about 5%) (Fig. 9C and inset). Such bifurcate appressoria were also formed, but less commonly, by *P. hieracii*. Irregularly shaped appressoria with multiple lobes were produced by *M. medusae* and *P. recondita* (Fig. 9D and E).

Germ tubes of all rust species appeared to adhere equally well to all test substrates. Exceptions were observed when the the growth of germ tubes of *U. appendiculatus, P. sorghi*, and *P. hieracii* grew over 6.7-μm-high ridges. Germ tubes of *U. appendiculatus* (Fig. 5A) adhered closely to the polystyrene substrate and to the front sides of ridges but did not maintain contact with the back sides. In contrast, *P. menthae* (Fig. 5B) and *P. hieracii* (Fig. 5C) maintained close contact with all ridge surfaces. Such close adhesion appeared to be correlated with appressorium formation in association with high ridges by these two species.

Urediniospores of several rust species obtained for testing germinated poorly or were not available in quantities necessary for replicate testing on all ridge heights. However, some data were collected regarding their general response to topographical signals. For example, appressoria of *Puccinia jaceae, P. canaliculata*, and the *Phragmidium* species from rose formed only on ridges. In another case, germ tubes of *M. lini* produced irregularly lobed swellings, similar to those of *M. medusae* (Fig. 9D), in association with ridges. However, it was difficult to determine whether these structures were in fact appressoria, despite tests to determine the nuclear condition and the presence of septa.

## DISCUSSION

All the rust fungi tested developed appressoria with varying efficiency in response to either ridged or scratched topographies.

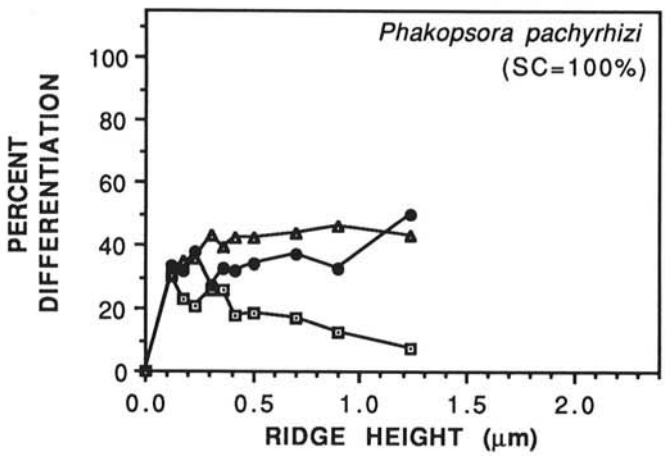


**Fig. 7.** Percent differentiation (appressorium formation) by *Phakopsora pachyrhizi* in response to polystyrene ridges ranging in height from 0.11 to 1.24 μm. ⊡ = Beside ridges; ● = on smooth areas between ridges; △ = over ridges. SC denotes response to scratched polystyrene membrane.

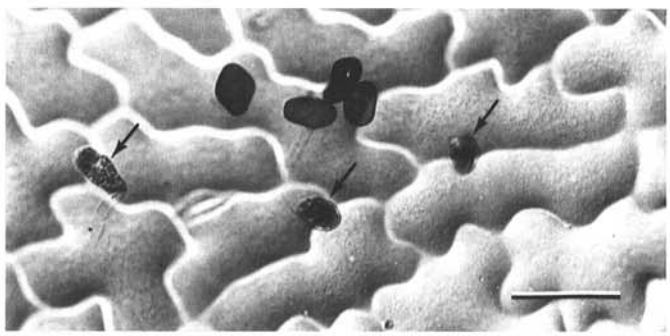

**Fig. 8.** Urediniospores and appressoria (arrows) of *Phakopsora pachyrhizi* on a polystyrene replica of a *Phaseolus vulgaris* leaf. Appressoria formed over anticlinal wall junctions between epidermal cells. Magnification bar = 50 μm.

The nature of the response fell into four categories: group 1, with no differentiation on single ridges and low differentiation on scratched membranes; group 2, with differentiation on ridge heights within a distinct optimum range and variable differentiation on scratched membranes; group 3, with differentiation even on the highest ridges tested and high differentiation on scratched membranes; and group 4, with differentiation in loose association with the signal (ridge) and high differentiation on scratched membranes.

It is not clear whether the groupings by signal response reflect any fungal taxonomic relationships. For example, in the genera *Coleosporium* and *Uromyces*, the responses of the two species tested fell into the same categories, group 1 and group 2, respectively. Yet within the most broadly tested genus, *Puccinia*, three of the four recognized response types were observed. More species of each genus will have to be tested to resolve this point. The groups do not seem to reflect any feature of the hosts' taxonomies

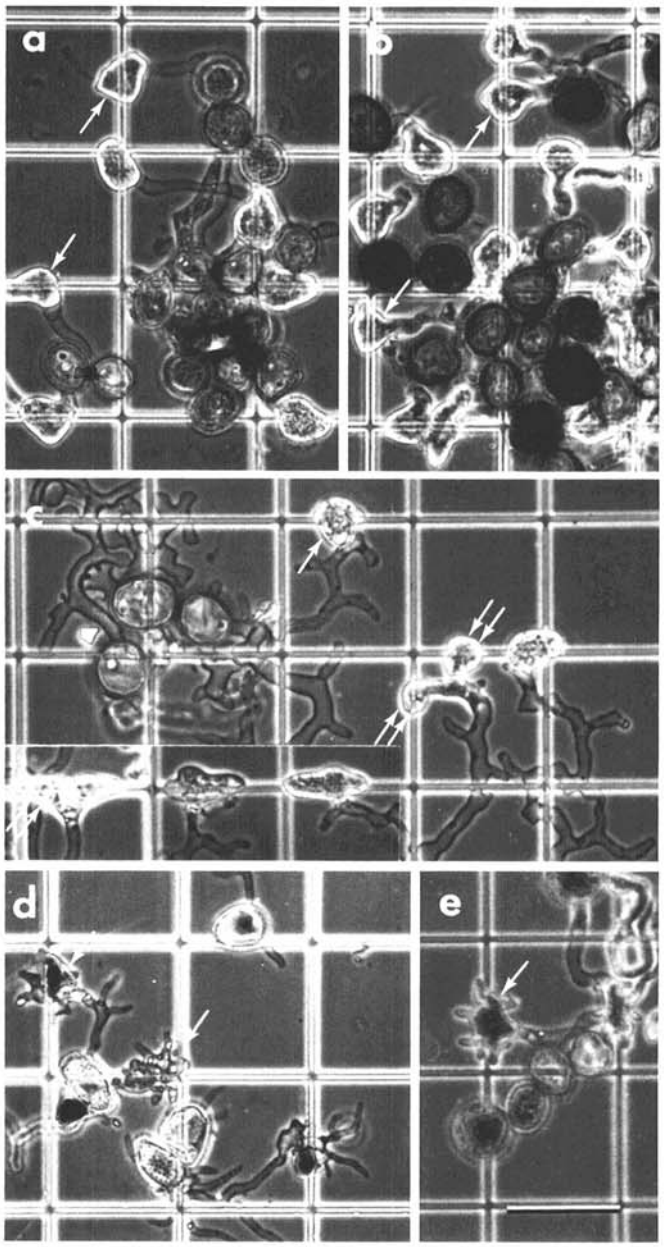


**Fig. 9.** Variation in urediniospore germling growth and shape of appressoria (arrows) on inductive 0.7-μm-high polystyrene ridges. **A,** *Uromyces appendiculatus*; **B,** *Puccinia hieracii*; **C,** *P. sorghi*. Double arrows indicate bifurcate appressoria near a ridge intersection and on a straight section of a ridge (inset). Appressorium shape is often elongate (inset). **D** and **E,** Lobed, irregularly shaped appressoria of *Melampsora medusae* and *P. recondita*, respectively. Magnification bar = 50 μm.

or growth habits, although *M. lini, P. coronata, P. g. tritici, P. g. avenae*, and most isolates of *P. recondita*, all cereal rusts, tended to differentiate poorly on the artificial substrates. It is possible that leaf surface features of the grass hosts are poorly represented by the relatively simple artificial topographies. The other monocot rusts, *P. melanocephala, P. polysora, P. sorghi*, and *Physopella zeae*, however, formed appressoria efficiently on both scratches and ridges. A study of host stomatal architecture of these large-leaved grasses might reveal characteristics that could explain these different responses.

Although the within-group relatedness is unclear, there are strong differences in responses between groups. These differences may indicate that the ridges mimic the stomatal signal poorly for species with poor differentiation (group 1) or only partially for species with persistently high rates of differentiation, even on very high ridges. There is a strong indication that a component of the signal is lacking. Two of the rusts, *Phakopsora pachyrhizi* and *Physopella zeae*, do not penetrate stomata, but enter directly through the epidermis (1,2). On both of their respective hosts, *Glycine* and *Zea*, the site of penetration is generally above the junctions between the anticlinal walls of epidermal cells. Certainly some component of thigmotropic sensing appears to be operative, because penetration site specificity was observed on both leaf surfaces and polystyrene replicas of leaf surfaces and both rusts formed appressoria in association with ridges and scratches.

The behavior of *P. pachyrhizi* on ridged topographies can be interpreted in two ways. First, since appressoria developed on, beside, and between ridges in approximately equal proportions on all ridge heights, and since differentiation efficiency was high (about 75%) on smooth surfaces, the conclusion might be made that appressoria form primarily in response to surface contact. Such an interpretation is consistent with the results of previous studies (12,13) that describe a nonspecific thigmotropic response for this rust. A second approach, however, recognizes that appressoria are preferentially formed over epidermal cell junctions on both leaves and leaf replicas, indicating that *P. pachyrhizi* does sense surface topography. If the values for appressorium formation on and beside ridges are pooled and considered a thigmotropic response, then appressorium formation in association with ridges (63%) approximates that observed over epidermal cell junctions on leaves (85%) (2). Therefore, although *P. pachyrhizi* is capable of forming appressoria in the absence of topographical signals, surface topography is likely involved in the location of penetration sites. In contrast, *Physopella zeae* appears to require a greater topographical stimulus to induce appressorium formation, as evidenced by its significant response to the ridge topographies used in this study.

The characteristics required for inductive signals vary among rust fungi. For example, *P. graminis* appears to respond primarily to chemotropic stimuli to complete infection structure development (20), whereas other rusts, such as *P. recondita*, may respond to both chemotropic and topographic stimuli. Topographic signals appear to play an even more prominent role in infection structure formation of the other rust species tested in this study. While these other rust species demonstrated significant thigmotropic responses, there were striking differences in their abilities to sense specific ridge heights. *P. hieracii* and *U. appendiculatus* exemplify such extremes. Whereas a significant reduction in appressorium formation was observed for *U. appendiculatus* on ridge heights below 0.18 $\mu$m and above 1.24 $\mu$m, *P. hieracii* developed appressoria efficiently (over 80%) on these ridge heights. Of particular interest is the formation of appressoria by *P. hieracii* on the "smooth" polystyrene surface. The extreme thigmotropic sensitivity of this species may be useful in studies concerned with elucidation of the mechanisms responsible for sensing topographical features. *P. arachidis* was also able to sense very low ridge heights (about 70% differentiation of ridges less that 0.18 $\mu$m in height). Observations of leaf surfaces that have shown misplaced appressorium formation over epidermal wall junctions (16) may be explained by this sensitivity. Such responses do not appear to be beneficial to the fungus; since direct penetration is not possible, infection attempts by the misplaced appressoria are aborted. Another interesting characteristic of *P. arachidis* is the overgrowth of germ tubes past the inductive signal and the subsequent subapical formation of appressoria. This phenomenon is not fully understood but may provide insight into the distribution of thigmotropic sensing sites along the germ tube.

This study has provided some clues to the cytological basis of signal reception. The SEM observations suggest that strong adhesion to the substrate is necessary for the induction of appressorium formation, at least on the highest ridges, and support the suggestion (10) that topographical signaling involves a simple deformation of the plasma membrane. Once induced, the speed of appressorium formation may vary, as indicated by the ridge overgrowth of *P. arachidis*. In addition, the actual site of signal reception may vary with species and may not always be in the tip region. Some fungi required many hours to reach maximum appressorium formation, during which time they grew over a number of ridges without responding. It is possible that a change in metabolic state, such as the nutrient level, causes an increase in sensitivity to topography in these fungi. Alternatively, the topographies that we have microfabricated may not fully represent the same signaling topographies that occur on host leaves.

The functional significance of the variations in the thigmotropic responses exhibited by the rust fungi in this study is not clear. We may speculate, however, that a requirement for a more complex signal or combination of thigmotropic and chemotropic signals could be beneficial by improving identification of appropriate sites for appressorium formation. Such a fastidious approach might be exemplified by the rust species that did not form appressoria on test membranes but are known to locate and penetrate through stomata. Similarly, the rust species that responded to a relatively narrow range of topographic heights may have a recognition specificity that improves the likelihood that appressoria are properly located. Conversely, the wider thigmotropic sensitivity shown by *P. arachidis*, for example, although disadvantageous by increasing the probability of misplaced (and therefore nonfunctional) appressoria, could be beneficial in adapting to a wider host range or to variable characters within a given host genus. Further study of fungal behavior on the host surface is under way to address some of these ideas.

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