Table 1. Characteristics of the NOAA/AVHRR systems. The NOAA-7 satellite was launched in June 1981 and taken out of operation 28 January 1985. NOAA-9 was launched in December 1984 and is still operational.

Characteristic	Measure
Coverage cycle	9 days
Scan angle range	±56°
Ground coverage	2700 km
Orbit inclination	98.9°
Orbital height	833 km
Orbital period	102 min
Ground resolution	1.1 km (nadir); 2.4 km (maximum off-angle along track) 6.9 km (maximum off-angle across track)
Equatorial crossing	Descending mode, 1430 hours Ascending mode, 0230 hours

sponding period in the two subsequent years (F and I, Fig. 4). The December 1982 visual image correlates with the highest NDVI and PVAF values seen at point C (Figs. 2 and 3) and coincides with the only RVF viral activity detected.

The positive correlation between NDVI and rainfall is evident in both ecological zones examined, even though other parameters controlling vegetation production, such as temperature and evapotranspiration, were not considered. In addition, the correlation between NDVI and mosquito populations, as reflected by our collection data, further corroborates the relation between NDVI and rainfall, as mosquito population levels are known to be highly related to rainfall patterns.

The ability of the PVAF to detect, with precision, RVF viral activity in ecological zones 2 and 3 in our study areas during an interepizootic period suggests that RVF epizootics could be detected reliably from this statistic. The isolation of virus in mosquitoes represents the earliest stages in a RVF epizootic. Detection of viral activity at this point in the RVF viral life cycle could allow time for specific control operations before an epizootic occurs. The PVAF generates rapid knowledge about potential viral activity conditions in ecologically equivalent areas and consolidates NDVI data, effectively reducing the operational decision-making process as it relates to control strategies. The ground studies and remote sensing technology discussed here for RVF virus will certainly have an application to other diseases that decimate the continent and are ecologically linked, either directly or through transmission vectors.

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Signaling for Growth Orientation and Cell Differentiation by Surface Topography in *Uromyces*

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The dimensions of the topographical signals for growth orientation and infection structure formation, a cell differentiation event that includes nuclear division, were determined for the stomatal penetrating rust fungus *Uromyces appendiculatus*. The differentiation signal was found to be a simple ridge on the substrate surface that had a markedly optimum height of 0.5 micrometer. Such ridges were microfabricated on silicon wafers by using electron-beam lithography. A similar ridge, in the form of a stomatal lip, was found associated with the stomatal guard cells of the bean (*Phaseolus vulgaris*) leaf. Ridge elevations greater than 1.0 micrometer or less than 0.25 micrometer did not serve as effective signals. Germ tubes of the fungus were highly oriented by ridge spacings of 0.5 to 6.7 micrometers. The data indicate that the fungus is able to distinguish uniquely minute differences in leaf surface topography in order to infect the host plant.

A BROAD RANGE OF EUKARYOTIC cells sense surface signals (*1, 2*), but none display a more precise and unique recognition phenomenon than that exhibited for topographical perception by many of the obligate fungal plant pathogens (*3–5*). For example, the bean rust fungus, *Uromyces appendiculatus* (Pers.) Unger, germinates from a spore and grows on the leaf surface as a hypha in a precisely oriented direction toward a stomate where it ceases growth and develops a series of specialized infection structures necessary for leaf colonization (*5, 6*). The developmental sequence involves gene expression (*7*), mitosis, and distinct morphological changes (*8*). The first of these infection structures, termed an appressorium, forms directly over the stomate (Fig. 1) through which it must eventually enter the leaf to develop other infection structures, for example, vesicles, haustorial mother cells, and haustoria. Using chemically inert plastic replicas of the leaf surface, Wynn (*9*) showed that the precise positioning of the appressorium over the stomate was solely in response to topographical features inherent on the stomatal guard cells. It was thought that a sequence of topographical signals was required to trigger this event (*4*); however, Staples *et al.* recently demonstrated that only a single scratch in the substrate was necessary (*5*). The nature, size, and location of these topographical signals have until now only been surmised.

During investigations to create artificial substrates that are reproducibly inductive for appressorium formation, we discovered that *U. appendiculatus* recognizes, to a very high degree, a topography formed by the leading edge of a sharply inclined raised surface. The inductiveness of the elevated surface edge was directly related to its height. We further determined that a similar topography is present on the stomatal guard cell, and undoubtedly is the signal that the fungal cell perceives to undergo cell differentiation before invasion of the host.

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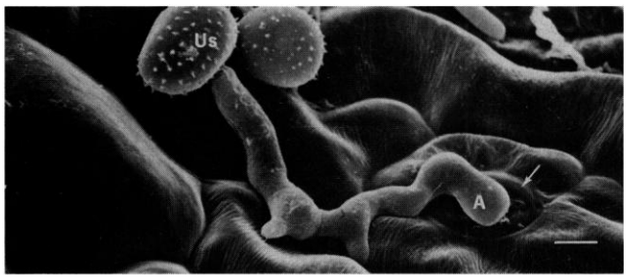

Fig. 1. Germinated uredospore (Us) of *U. appendiculatus* on the leaf surface of a bean (*P. vulgaris*) leaf. The germling grew oriented to the stomate (arrow) where it underwent cell differentiation and developed a primary infection structure (an appressorium) (A), necessary for eventual invasion of the leaf tissues. Bar scale, 10 μm.

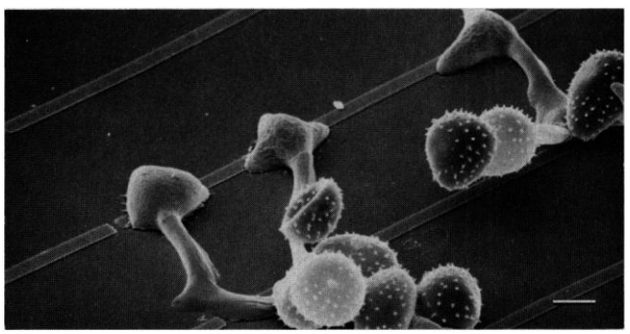

Fig. 2. Polystyrene replicas containing ridges 0.5 μm high by 4.0 μm wide, made from precision ion-etched silicon wafer templates, were highly inductive for infection structure formation in *U. appendiculatus* germlings. Bar scale, 10 μm.

Table 1. Cell differentiation by *U. appendiculatus* in response to topographical ridges of varying dimensions. Percentage of cell differentiation (appressorium formation) was determined microscopically 5 hours after initiation of germination of uredospores on polystyrene replicas made from silicon templates. The data represent observations of 100 counts per replica (*n*) of each treatment.

Ridges height × width (μm)	Differentiation (mean %)	*n*	SD
0.03 × 2.0	0.00	5	0.00
0.1 × 2.0	5.75	8	5.67
0.25 × 2.0	4.75	8	2.43
0.5 × 2.0	75.35	8	4.55
1.0 × 2.0	8.75	8	3.49
2.0 × 2.0	0.00	8	0.00
5.0 × 2.0	0.00	8	0.00
0.5 × 0.5	71.20	5	2.95
0.5 × 1.0	69.00	5	3.80
0.5 × 2.0	72.60	5	5.41
0.5 × 4.0	76.40	5	1.81
0.5 × 100.0*	69.40	5	4.98
5.0 × 100.0*	0.20	5	0.45

*Polystyrene replicas of 0.5 or 5.0 μm by 100 by 100 μm.

Uredospore germlings of *U. appendiculatus* grown (*10*) on polystyrene replicas (*11*) of ion-etched silicon wafer templates (*12*) having specific surface topographies exhibited maximum cell differentiation (70 to 75%) in response to ridges or plateaus 0.5 μm high (Table 1). Appressoria (cell differentiation of the germling) that formed on these ridges (Fig. 2) were morphologically and functionally similar to those formed over a stomate (Fig. 1). The percentage of cell differentiation observed for germlings responding to ridges more than 1.0 μm or less than 0.25 μm in height was significantly less than that for the 0.5-μm-high ridges. The hyphal diameter of uredospore germlings ranges between 5.0 and 8.0 μm; thus the optimum signal height for cell differentiation lies between 1/10 and 1/16 of the cell size.

The width of the ridge did not constitute a parameter of the signal for cell differentiation (Table 1). This was especially apparent when the 100 by 100 μm plateaus, arranged in a checkerboard pattern, were examined. Germlings growing either onto or off of the plateaus differentiated only at the edge of the plateaus, and only when they were 0.5 μm high. Furthermore, essentially no cell differentiation (0.20%) occurred on replicas of 5.0-μm-high plateaus. Taken together, these data indicate that the signal for cell differentiation in *U. appendiculatus* is an elevation change of approximately 0.5 μm and that two acute angles must be present. One angle, such as that associated with the top or bottom of the 5.0-μm plateaus or ridges, does not signal cell differentiation. Instead, the germlings simply grow over the obstacle, usually with poor contact on the leading edge (Fig. 3).

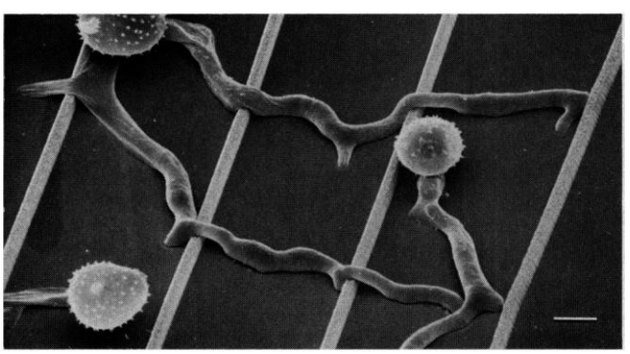

Fig. 3. Polystyrene replicas similar to those in Fig. 2, but with ridges 5.0 μm high by 4.0 μm wide, were not inductive for infection structure development. Instead, the fungus germlings grew across ridges, at an angle of about 90°. Bar scale, 10 μm.

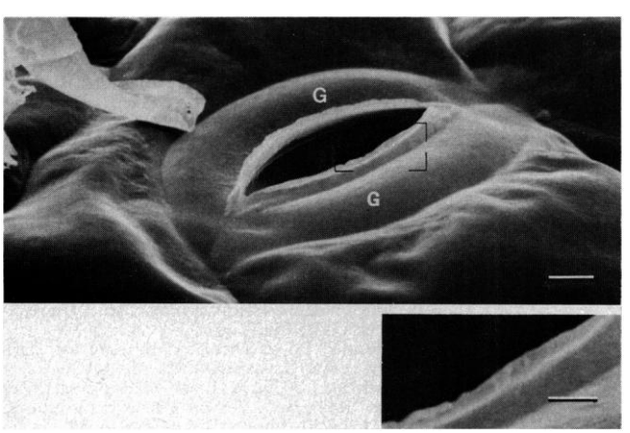

Fig. 4. Scanning electron micrograph of bean leaf stomatal guard cells (G) having prominent erect lips (depicted in inset, from bracketed area) that serve as the signal for appressorium formation in *U. appendiculatus*. The fungus eventually enters the leaf through the stomatal opening where infection of the host cell occurs. Bar scale, 2 μm; inset bar scale, 1 μm.

After we found that the signal for cell differentiation in *U. appendiculatus* consists of a sharp change in the elevation of the substrate surface by 0.5 μm, we reexamined the morphology of the bean (*Phaseolus vulgaris*) leaf stomatal guard cell to determine if similar topographies exist. Previous reports (*9*), as well as our own observations of bean leaves conventionally prepared for scanning electron microscopy (*13*), showed a small rounded lip at the edge of the guard cell that did not appear particularly prominent. Bean leaf specimens that were quick frozen and then freeze-dried (*14*), however, revealed

Table 2. Cell differentiation by *U. appendiculatus* in response to the distance between ridges 0.5 μm high by 1.4 μm wide after 5 hours of growth. The data represent observations of 100 counts per replica (*n*) of each treatment.

Distance between ridges (μm)	Differentiation (mean %)	*n*	SD
62	50.76	9	6.82
30	46.00	9	7.38
15.5	44.46	9	6.52
6.7	30.09	9	8.33
2.4	25.27	8	11.96
0.5	2.51*	8	7.09

*These counts were attributed to a single replica (value, 20.02%) with a damaged pattern that gave rise to a few appressoria; otherwise no appressoria formed on the internal areas of similar profiles.

prominent guard cell lips oriented nearly perpendicular to the cell surface (Fig. 4, inset), especially when examined with a stage tilt of 90° compared to a more conventional tilt angle of 35° to 45°. The lip frequently appeared to be somewhat ragged along the outer edge and nonuniform in height. The mean height of the lip, as determined from four measurements (*15*) for each of 16 separate guard cells, was 0.487 μm (SD, 0.07 μm). This height corresponds closely to the optimum height of 0.5 μm that signaled for maximum cell differentiation on polystyrene replicas of the silicon wafers (Table 1).

Not only must the fungus germling recognize the appropriate site (stomate) at which it must cease growth and develop the first infection structure, but also it must find this site without expending unnecessary energy growing aimlessly over the leaf surface (*5, 16*). Thus, growth is oriented toward the stomatal guard cells in many of the rust fungi, including the bean rust fungus (*9, 17*) (Fig. 5). Using polystyrene replicas of silicon wafers containing uniformly but variously spaced topographical ridges, we found that *U. appendiculatus* germlings responded with oriented growth (Fig. 6). Germlings grew very straight and perpendicular to ridges (0.5 μm high by 1.4 μm wide) spaced 0.5 μm apart (Fig. 6a). Growth was also perpendicular when the ridges were spaced 2.4, 6.7, and 15.5 μm apart; however, the straightness of the germlings diminished as the spacing between the signals was increased (Fig. 6b). Signal spacings of more than 30 μm generally did not induce continued perpendicular-oriented growth. Eighty-seven percent (SD, 7.4) of the germlings having a nonoriented growth pattern exhibited corrected (perpendicular) growth once they contacted the topographical signal (Figs. 3 and 6c). When the spacing of the signals was very close, growth was highly oriented, but cell differentiation was low

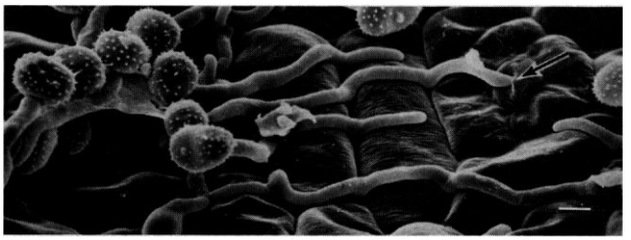

Fig. 5. Growth of *U. appendiculatus* germlings is frequently directed to a stomate (arrow) by the surface topography represented as depressions located above the anticlinal walls of the epidermal cells of the bean leaf. Bar scale, 10 μm.

(Table 2). Cell differentiation on the closest (0.5 μm apart) spacing patterns occurred only when the cell grew onto or off of the patterned area. The germlings thus responded to the entire area as they did to the plateaus.

What topographical features on the leaf surface orient the germling to the stomatal guard cells? Examination of bean leaves on which *U. appendiculatus* was germinated revealed that surface depressions located at the anticlinal walls of the epidermal cells could serve as such signals (Fig. 5). Since these depressions are spaced 15 to 30 μm apart, germling orientation is not as strong as that observed with the closest spacings of the polystyrene replicas. Wandering germling growth is, however, frequently corrected once the cell grows over the depression, no matter what the spacing. Height or depth of the signal for growth orientation is not as critical as it is for cell differentiation since orientation occurred on ridges ranging in height from 0.1 to 5.0 μm. Similar growth orientation of uredospore germlings of *Puccinia graminis*, a closely related rust fungus, occurs on leaves of wheat. The germlings grow across the longitudinally oriented epidermal cells in order to have a better chance of contacting stomata, which are arranged in rows (*4, 5*).

Signaling for both cell differentiation and growth orientation was equally efficient for grooves as for ridges. The important parameters were height or depth of the topographical feature and its separation from neighboring signals. The mechanisms by which the fungus perceives these signals are not clearly understood. It has been postulated, however, that a temporary depolymerization or break in the continuity of the cytoskeleton in the region of the cell overlying the signal is involved in mediation for cell differentiation in *U. appendiculatus* (*18*). Similar involvements of the cytoskeleton in signal mediation have been reported for other organisms (*2*). Recent evidence that differentiation is inhibited when microtubules, but not actin microfilaments, are depolymerized in the tip region of the germ tube (*19*) supports this hypothesis. Alternatively, treatments known to promote microtubule stabilization have been shown to enhance cell differentiation in *P. graminis* (*20*). Sig-

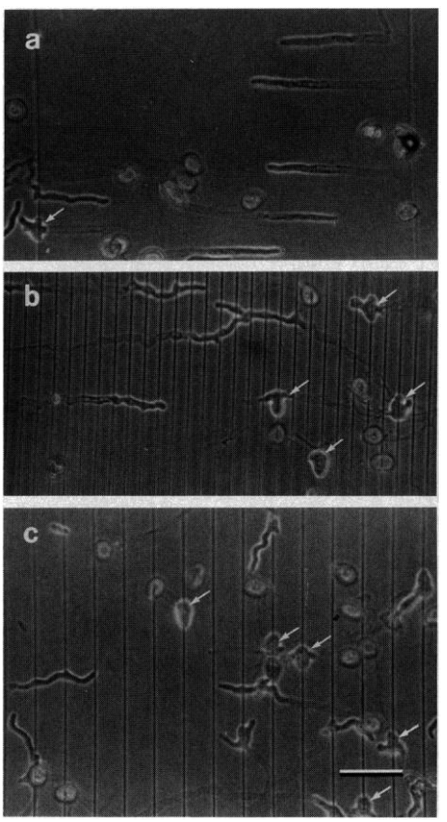

Fig. 6. Growth of *U. appendiculatus* on polystyrene replicas with ridges 0.5 μm high by 2.0 μm wide, by 0.5 μm (**a**), 15.0 μm (**b**), and 30.0 μm (**c**) apart, respectively. Closely spaced ridges directed hyphal growth perpendicular to the ridges and did not signal for cell differentiation except at the edge (arrow) of the ridged pattern (a). As the ridges are spaced farther apart, growth becomes less oriented, but cell differentiation is signaled (arrows) (b and c). Bar scale, 60 μm.

nal reception may be initiated through the extracellular matrix (*21*) or through the plasmalemma by changes in membrane potentials, and these possibilities are currently being explored. Growth orientation, on the other hand, is likely governed through some different type of mechanism in which the cluster of apical vesicles (*Spitzenkörper*) (*22*) that is responsible for cell growth is maintained in a tight and precise position in the cell apex.

Precision-made, highly reproducible surfaces such as those described here can now be used to elucidate further the mechanisms involved in signal reception from both the

cytological and biochemical standpoints in this plant pathogen. Other races of *U. appendiculatus*, or other rust fungi that penetrate the stomate, could be examined to determine the specificity of the size parameters of the signal. Additional parameters, such as the acuteness of the two angles of the signal as well as a more precise determination for the optimum signal height for cell differentiation, could also be studied. Information from the discovery of the specific size parameter and the confirmation of the involvement of the stomatal lip in signaling for the appressorium will contribute to the search for *P. vulgaris* phenotypes having stomatal lips of a size that are not inductive for cell differentiation in *U. appendiculatus*.

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10. Uredospores of *U. appendiculatus* were treated with β-ionone for 20 minutes to overcome the germination self-inhibitor, then dusted onto the polystyrene test substrates contained in plastic petri plates at a density of about 600 spores per square centimeter. The spore-laden membranes were then misted with distilled water, incubated and humidified at 17°C for 5 hours, after which they were fixed with a 3% solution of formaldehyde buffered with 50 m*M* potassium phosphate, *p*H 7.0. The membranes were subsequently examined by phase-contrast microscopy and enumerated for cell differentiation.
11. Replicas were made by spreading dissolved polystyrene (15 g of polystyrene per 100 ml of ethyl acetate) over the silicon templates, allowing them to dry, then floating off the replicas in water.
12. Templates used in this study were fabricated from silicon wafers (7.6 cm in diameter) by electron-beam lithography and reactive ion etching. The wafers were first coated with 100 nm of chromium by thermal evaporation and then spin-coated with 400 nm of polymethyl methacrylate (PMMA) by spinning on a 6% solution of methyl isobutyl ketone at 4×10^3 rev/min. PMMA is a positive-acting electron resist. The resist was selectively exposed to a 20-kV, 10-nA electron beam in a Cambridge EBMF 6 (electron beam microfabricator) (Cambridge Instruments, Cambridge, England) under computer control to create specified patterns. After exposure, the resist was developed in a 1:1 solution of methyl isobutyl ketone and isopropanol for 2 minutes and rinsed in isopropanol. The chromium areas that were thus opened by development were then etched for about 1 minute in a solution of ceric ammonium nitrate with acetic acid (Transene Company, Rowley, MA). The created pattern was then transferred to the underlying silicon by reactive ion etching in a SF_6/O_2 plasma [M. Zhang, J. Li, I. Adesida, E. D. Wolf, *J. Vac. Sci. Technol. B* **1**, 1037 (1983)]. The specified conditions were 10/10 (SF_6/O_2) standard cm^3/min flow rates, a pressure of 20 mtorr, and a power density of 0.20 W/cm^2. These conditions resulted in a vertical etch rate of 100 nm/min and produced a vertical profile with sharp corners in the template. Etch depth was varied by controlling the etch time, since the depth versus time is linear. Since the chromium mask etch rate was <2 nm/min, mask erosion was insignificant. After the silicon wafer was etched to the desired depth, the remaining PMMA was removed with a pure oxygen plasma while in the same chamber. The chrome mask was removed with the chromium etching solution. The resulting wafers have planar structures with vertical walls. All surfaces are clean silicon; however, periodic cleaning of the wafers with buffered hydrofluoric acid (Transene Company, Rowley, MA) was necessary to remove SiO_2 that formed on exposure to the atmosphere.
13. Eight-day-old primary pinto bean leaves were fixed in a 3% solution of glutaraldehyde buffered with 75 m*M* potassium phosphate, *p*H 6.8, post-fixed with 2% OsO_4, dehydrated through an acetone series, and dried at the critical point with acetone-CO_2 before being sputter-coated with a thin layer of gold and palladium to prevent charging of the sample.
14. Leaf segments of 1 cm^2, similar to those in (*13*), were quickly plunged into a eutectic propane-ethane mixture that was cooled to −193°C [H. C. Hoch, in *Ultrastructure Techniques for Microorganisms*, H. C. Aldrich and W. J. Todd, Eds. (Plenum, New York, 1986), pp. 183–212], then transferred to liquid nitrogen and freeze-dried before being sputter-coated with a layer of gold and palladium.
15. Measurements were made from scanning electron micrographs by using a cursor-controlled "line" program operated through a video-interfaced image processor (FD-5000, Gould/DeAnza, Fremont, CA).
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