Morphological Development of *Physopella zeae* on Corn

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ABSTRACT

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Free moisture on the surface of leaves for 1–2 hr at 22 C induced uredospore germination of *Physopella zeae*. By 5 hr, numerous large appressoria had developed, usually over anticlinal walls of leaf epidermal cells. Leaf penetration was directly through the leaf cuticle and was first observed at 12 hr following inoculation and placement of plants into dew chambers. A primary hypha (average maximum width 9.8 μm) grew from the penetration peg and by 28 hr averaged 18 μm in length. Secondary, narrow (avg 4.3 μm wide) hyphae grew from primary hyphae by 24 hr. The pathogen colonized the epidermis and mesophyll tissues as secondary intracellular hyphae. Although penetration was never observed before 12 hr (ie, absent in 5- to 10-hr samples), plants removed from dew at 5–6 hr and placed in a greenhouse still developed pustules. Apparently, once appressoria reach a certain maturity, visible free moisture is not required for subsequent penetration.

Additional key words: *Zea mays*, corn rust, Uredinales.

Tropical corn rust, which is incited by *Physopella zeae* (Mains) Cumm. & Ramachar, occurs in the warm, humid areas of Mexico, the Caribbean, Central America, and the northern part of South America to latitude 5° south (9). Two races of *P. zeae* were distinguished by Robert (11) among three cultures tested (one race was obtained in Peru, the other in Nicaragua).

We became interested in the morphological development of *P. zeae* during our earlier studies on another rust organism, *Phakopsora pachyrhizi*, the causal agent of soybean rust. *P. pachyrhizi* was shown in our laboratory (1,7), and in at least three other laboratories (4,5,10), to penetrate soybean leaves directly through the cuticle following uredospore germination and appressorium formation. This mode of penetration from uredospores has rarely been described (eg, 3).

Because of the very close taxonomic relationship between the genera *Phakopsora* and *Physopella* (2), we extended our histological studies on *P. pachyrhizi* to include a species of *Physopella*. Our initial objective was to determine whether or not *P. zeae* penetrates and colonizes maize leaves in the same manner as *P. pachyrhizi* penetrates and colonizes soybean. Before our present studies, little was known of the morphological development of *P. zeae*. This information should be useful for identifying the pathogen and disease.

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MATERIALS AND METHODS

Corn plants (DeKalb hybrid XL-43 or Pioneer hybrid 3369A), grown in 10-cm-diameter clay pots, two plants per pot, were inoculated at the four- to six-leaf stage in a turntable tower (8) with either fresh uredospores or uredospores from liquid nitrogen storage of race 1 (isolate ERL Pz1) from Nicaragua. Two consecutive 20-μg releases of dry spores were made per set of eight pots. In a few experiments, to obtain higher spore densities per unit leaf area, spores were brushed by means of cotton swabs onto the midsection of the second and third (or third and fourth) formed leaves of individual seedlings. Immediately after inoculation, all plants were placed in a dark dew chamber (6) at an air temperature of 22 C. Uninoculated control plants were placed in a second dew chamber at 22 C.

Prepenetration development of the pathogen was determined on leaf pieces collected at 0, 1, 2, 3, 4, or 5 hr after inoculation while plants were in the dew chamber. The leaf pieces were placed over 38% formaldehyde in a closed jar for at least 1 hr to halt fungal development. Following the formaldehyde treatment, leaf segments (1–4 cm^2) were placed on glass microscope slides with the upper (adaxial) surface facing up. One or two drops of 0.1% cotton blue in lactophenol were applied, and the leaf surface was observed microscopically at ×100 or ×473 magnification.

For microscopic observations of development of the pathogen following penetration, leaves were excised from plants after 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, or 24 hr in dew or from plants which received at least an 8-hr dew period and were then placed in the greenhouse for disease development. Leaf pieces (1–4 cm^2) were cut

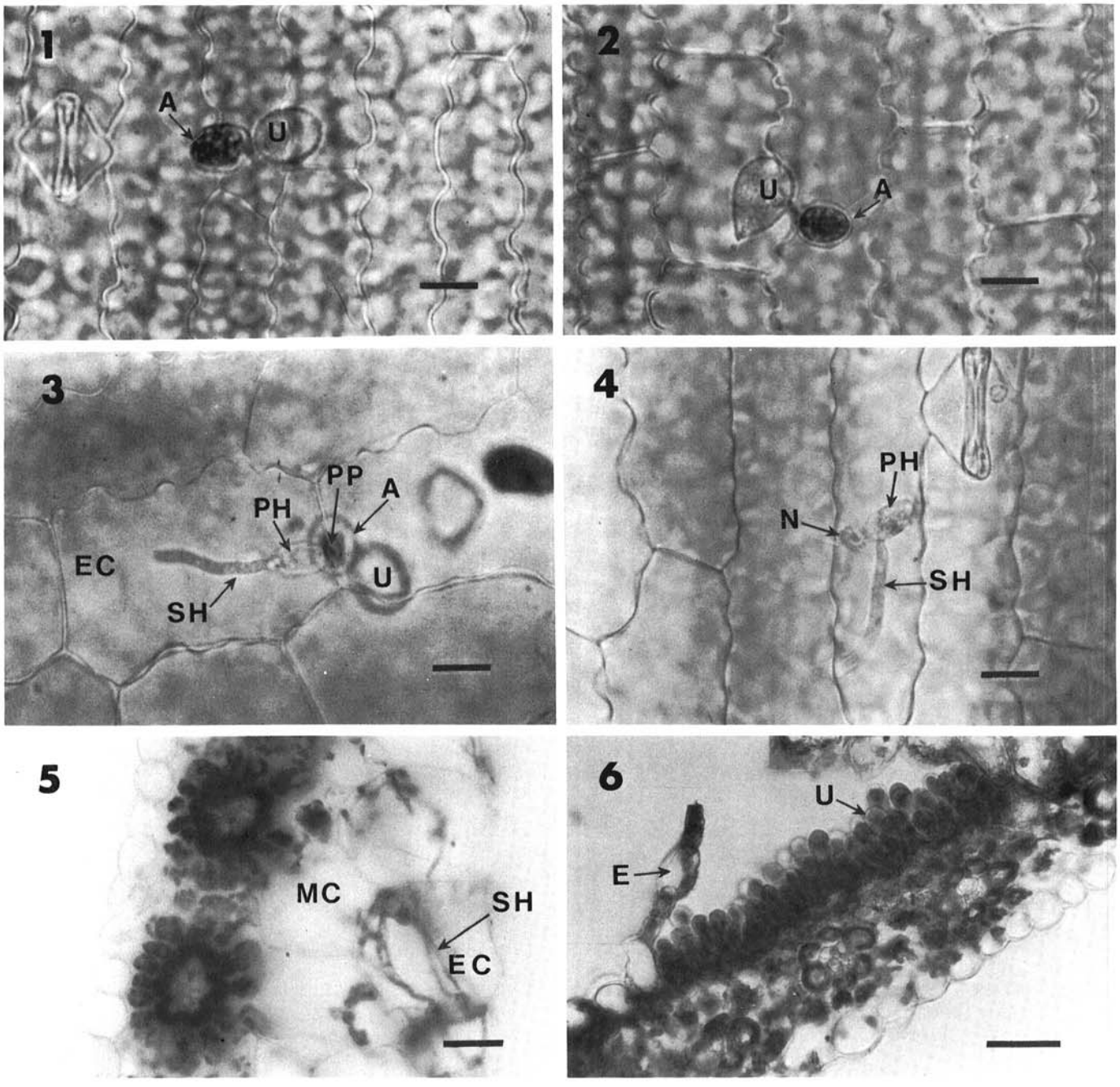
from inoculated areas and were fixed in absolute ethanol-acetic acid (2:1, v/v). After at least 24 hr in the fixing solution, the leaf pieces were cleared for 24 hr in lactophenol, stained for 24 hr in 0.1% cotton blue in lactophenol, and mounted in lactophenol on glass slides for microscopic observations.

Some cross sections of fixed leaf tissue were made by means of a Hooker Plant Microtome (Lab-Line Instruments, Inc., Melrose Park, IL). These were stained in 0.1% cotton blue in lactophenol and mounted either in the cotton blue solution or in clear lactophenol.

To determine the minimum dew duration required for pustule development, inoculated plants in some experiments were not sampled but were removed from the dew chambers after various lengths of exposure and held in the greenhouse with a normal temperature fluctuation from 21 to 28 C; on occasion the temperature peaked as high as 40 C shortly after noon.

RESULTS AND DISCUSSION

Observations of the surface of leaves held in a formaldehyde-saturated atmosphere after collection revealed that germination of *P. zeae* uredospores initially occurred 1–2 hr after inoculation and placement of plants into the dew chamber at 22 C. By 5 hr, numerous large appressoria had developed, usually over anticlinal



Figs. 1–6. Development of *Physopella zeae* on leaves of DeKalb XL-43 corn plants. **1** and **2,** Germinated uredospores (U) 9 hr after inoculation on the surface of cleared leaves showing appressoria (A) over anticlinal walls separating adjacent epidermal cells. Host reaction (darkening around site where penetration will occur) is apparent in 2. **3,** Penetration peg (PP) ringed by host reaction, primary hypha (PH), and secondary hypha (SH) in penetrated epidermal cell (EC) 28 hr after inoculation. Penetration initially occurred down through anticlinal wall and into the epidermal cell from the end. A primary hypha grew from the penetration peg and a secondary hypha has grown from the tip of the primary hypha. Uredospore (U) and appressorium (A) on the leaf surface are evident. **4,** Secondary hypha (SH) extended from near the tip of a primary hypha (PH) 28 hr after inoculation. Host nucleus (N) is evident. **5,** Secondary hyphae (SH) in leaf epidermal cells (EC) and mesophyll cells (MC). **6,** Cross section through pustule on leaf 7 days after inoculation. Uredospores (U) in sorus are evident beneath strips of host epidermis (E). Scale bar = 20 μm in Figs. 1–5 and 40 μm in Fig. 6.

walls of leaf epidermal cells (Figs. 1 and 2). Germ tubes often were very short (Figs. 1 and 2) although they averaged 35 μm in length at 28 hr.

Fully grown appressoria were nearly the size of their parent spores. Data for spore and appressorium dimensions were obtained from 24- and 28-hr samples. The average length and width of 54 spores were 23 (SD = 2) and 17 (SD = 2) μm, respectively, and the average length and width of 55 appressoria were 20 (SD = 2) and 16 (SD = 2) μm, respectively.

The earliest leaf penetration observed histologically was at 12 hr following inoculation and placement of plants in dew. In a few rare instances, penetration pegs at 12 hr had grown from the underside of appressoria (one penetration peg per appressorium) and entered the leaf at the junctures between leaf epidermal cells. From there, the penetration peg at 12 hr had proceeded to enter one of the epidermal cells through the anticlinal wall and formed a primary hypha, usually elliptical in profile. By 28 hr, these primary hyphae averaged 18 μm in length and 9.8 μm in width (Figs. 3 and 4).

Although penetration almost always occurred at the juncture between leaf epidermal cells, in one experiment 8% of the observed penetrations were directly into epidermal cells at places other than at anticlinal walls. The reason for penetration occurring at a different site in this single experiment is not known.

Secondary hyphae (Figs. 3 and 4) averaging 4.3 μm in width were first observed growing from primary hyphae at 24 hr. At 24 hr, these secondary hyphae were always short (maximum length 5 μm). By 40 hr, they were usually long and considerably branched; however, the fungus usually was still present only in the first cell penetrated and had not grown to adjacent host cells. By 72 hr, the fungus had spread to adjacent epidermal and mesophyll cells (Fig. 5) and at 6–7 days uredial pustules erupted through the epidermis (Figs. 6 and 7).

The minimum dew duration required for subsequent pustule development varied among experiments from 5 to 7 hr.

In one experiment, plants were removed from dew at hourly intervals from 5 to 8 hr, and subsequently at 2-hr intervals. Immediately following the dew treatment, the plants were placed under Sylvania 1,000-W Metalarc high-intensity lamps for drying in the greenhouse; dew disappeared in a maximum of 20 min, essentially the same duration required for dew formation when plants were placed in the dew chambers.

In this experiment, no penetration occurred in 10 hr and only a few penetrations occurred between 10 and 12 hr in continuous dew. However, the presence of dew for 5 hr (and removal to the greenhouse) was sufficient for some pustules to develop, and a period of 8 hr allowed the development of many pustules. These results suggest that penetration was occurring after plants were removed frcm dew and that once appressoria reach a certain maturity, visible free moisture was not required for subsequent penetration.

The actual difference in time required for penetration and duration of dew required for eventual pustule formation is probably less than the apparent 5–7 hr. This is because of a greater sensitivity in detecting pustules than in detecting penetrations per unit leaf area.

P. pachyrhizi and *P. zeae* are similar in that they both penetrate via uredospores, following appressorial formation, directly through the leaf cuticle. A major difference between *P. pachyrhizi* and *P. zeae* is in their modes of colonizing their respective hosts. *P. pachyrhizi*, after penetrating an epidermal cell and forming a transepidermal vesicle, colonizes the leaf mesophyll tissue as intercellular hyphae (1). In contrast, *P. zeae*, upon leaf penetration, forms a primary hypha in the initially penetrated epidermal cell and then colonizes both the epidermis and mesophyll tissues as secondary intracellular hyphae. Intercellular hyphae were not observed. Of the four rust pathogens known to us that penetrate directly from uredospores after appressorial formation (1), only *P. zeae* infects a monocotyledonous plant species. Further studies may show that direct penetration from uredospores is not uncommon for pathogens of either the monocots or dicots.

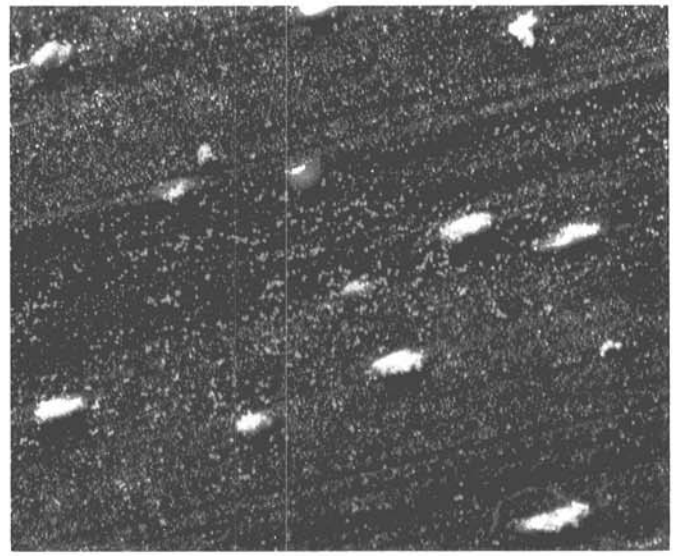


Fig. 7. Sporulating uredia of *Physopella zeae* on the surface of a corn leaf. The pustules are elongated parallel to the leaf veins; masses of uredospores are white to buff-tan in color.

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