Cytology and Histology

Development of the Uredinial Thallus and Sorus in the Orange Coffee Rust Fungus, *Hemileia vastatrix*

J. W. McCain and J. F. Hennen

Postdoctoral research assistant and professor, respectively, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

Based on a portion of a Ph.D. dissertation submitted by the first author.

We gratefully acknowledge the technical advice and access to equipment provided by C. E. Bracker, F. D. Hess, Tae Soo Lee, Caroline Logan, L. D. McCabe, C. M. Montecillo, G. E. Ruhl, and E. Traylor, all of Purdue University, West Lafayette, IN. Coffee seedlings were obtained from P. Soderholm, USDA, Miami, FL, and fungal inoculum from W. B. C. Moraes, São Paulo, S.P., Brazil.

Journal paper 9644 of the Purdue University Agricultural Experiment Station. This research was supported in part by N.S.F. grant DEP-81-15592.

Accepted for publication 21 February 1984 (submitted for electronic processing).

ABSTRACT

McCain, J. W., and Hennen, J. F. 1984. Development of the uredinial thallus and sorus in the orange coffee rust fungus, *Hemileia vastatrix*. Phytopathology 74:714-721.

Orange coffee rust is a critically important disease, but the development of the pathogen within the host had not been carefully studied. Infection of coffee plants with urediniospores of *Hemileia vastatrix* resulted in radially expanding lesions containing numerous sori. Lesions were sorted into zones of pioneer hyphae, first haustoria, nutritive hyphae, protosori (unemerged incipient sori), and immature, mature, and senescent sori. The zones of nutritive hyphae and protosori corresponded with a band of chlorosis on the leaf surface. Sori were formed in about one-third of host substomatal cavities. Protosori developed from a layer of isodiametric cells, distinct from the constituent hyphae. Some protosori, although they developed in mesophyll cavities not beneath stomata, appeared to grow into the correct position for emergence. Two or three, later seven or more, sporogenous cells exited a stoma as a tight fascicle and bore spore buds in a spiral fashion. Spines were only on the upper surfaces of the urediniospores and appeared when the spores were one-fourth to one-third their full size. Developing sori were covered by a matrix that appeared to be mucilaginous. The pattern of expansion of the *H. vastatrix* mycelium through the leaf, with continual production of new sori, was the starting point for a new model of the continuum of thallus complexity levels in the rust fungi.

Additional key words: *Coffea arabica*, SEM.

Hemileia vastatrix Berk. & Br. causes a devastating rust disease of coffee (*Coffea arabica* L.). Although the most frequently used common names for the disease are common rust, leaf rust, and coffee leaf disease, we chose orange coffee rust as the most accurate and informative common name for the disease caused by *H. vastatrix*. The other coffee leaf rust disease, caused by *H. coffeicola* Maubl. & Roger, is widely known as gray coffee rust (18).

Many research papers have been published on the spread and control of orange coffee rust (15). Much less is known, however, about the anatomy of the fungus in its host plant and how the development of the fungus affects the disease cycle and the pattern of spore production. The most complete anatomical report on *H. vastatrix* is the century-old study of Ward (17), but it did not include internal spread of the fungus.

The more recent descriptions of the fungus are often contradictory (conflicting descriptions of the sori) (4,6) or in error (assuming all rust fungi must look much like *Puccinia graminis* Pers.) (16). Most researchers agree that urediniospore germination tubes of *H. vastatrix* infect coffee leaves through appressoria over stomata (17) and that sporulation occurs on special fungal cells that protrude outward through stomata (6). However, the number and mode of origin of those special cells remains unresolved.

The numerous sori are crowded together on the underside of a leaf in a circular lesion that expands radially as long as the fungus remains active (17). The term "pustule" is not strictly appropriate, because the disease spot is not a blisterlike elevation caused by

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked *"advertisement"* in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

© 1984 The American Phytopathological Society

spore formation from within but instead is produced by a group of suprastomatal sori that do not tear the host epidermis as they emerge to bear their spores outside the leaf (15).

In this experiment, we studied the arrangement of the fungus in its host and the development of the sori. Our major goal was to understand the development of the uredinial thallus of *H. vastatrix* from the spread of intercellular vegetative hyphae, through the growth of the uredinial sori that protrude through the stomata, to the maturation of the urediniospores. No probasidia (teliospores) were produced in our cultures. They are seldom seen (15), and when they are, they usually are produced in the same sorus as the urediniospores. Thus, most ontogenetic conclusions about the uredinia should be applicable also to the basidial sori (telia).

MATERIALS AND METHODS

Culture techniques. Uredinial cultures of *H. vastatrix* were grown on Caturra coffee plants in the greenhouse. Plants to be inoculated were preincubated in a dew chamber at 19 C and 100% RH for 24 hr so that the plants were covered with dew. A camel's-hair brush was wetted in a solution of 0.2–0.5% Tween 20 in deionized water and used to pick up dry urediniospores from a gelatin capsule. These spores were spread on the lower surfaces of the third and fourth pairs of leaves from the tips of branches. Inoculated plants were returned to the dew chamber for 48–72 hr in the dark to ensure infection, then moved to a cool greenhouse (17 C). Sporulation began after 35–50 days. Leaves with visible lesions were removed from the plants and fixed immediately in either formalin-acetic acid-ethyl alcohol (FAA) or 4% formaldehyde-5% glutaraldehyde in 0.1 M potassium phosphate buffer (pH 6.8) or dried in a herbarium-type drying cabinet.

Fluorescent staining. Dried coffee leaves with orange coffee rust lesions were rehydrated for 24 hr in 0.5–1% Tween 20. Specimens fixed in FAA or glutaraldehyde-formaldehyde were washed several times in deionized water to remove the fixative. For observation of internal fungal structures, the leaves were cleared for 3–7 days in lactophenol:50% ethanol (1:2, v/v) or in glacial acetic acid:95% ethanol (1:3, v/v). Fluorescent staining was done with 0.3% Uvitex BOPT dye (Ciba-Geigy, Greensboro, NC 27409) according to the method of Cartwright and Russell (3). Some specimens were sectioned with a razor blade for better penetration of the dye and observation of internal structures from a lateral perspective. The stained specimens were examined with a Zeiss IV Epifluorescence Microscope equipped with a BG 12 blue exciter filter, an LP 478 yellow barrier filter, and an FT 460 chromatic beam splitter.

Cryosections. Specimens preserved in glutaraldehyde-formaldehyde were rinsed thoroughly to remove the fixative. Specimens were frozen in Tissue-Tek II O.C.T. Compound (Lab-Tek Products, Napierville, IL 60540) and sectioned in a cryostat at −10 C. Sections 15 μm thick were collected on a microscope slide, rinsed to remove the support medium, stained with 0.1% cotton blue in lactophenol, mounted on microscope slides, and photographed.

Scanning electron microscopy (SEM). If the plane of the frozen section passed through an area of interest (as determined by microscopic examination of stained specimens), the remaining unsectioned portion of the specimen with a clean-cut face was recovered from the cryostat. These pieces were trimmed to a trapezoid shape so that the clean-cut edge was always the shorter of the two parallel sides. Samples were washed and then dehydrated through a graded ethanol series. Next, the specimens were dried in a critical-point drier, with liquid CO_2 as the transition fluid. The dried leaf pieces were mounted on their sides on SEM mounting stubs, with the short sides of the specimens up for easy examination.

In addition, some samples from dried herbarium material (Arthur Herbarium specimens, Purdue University) were hand-cut with a razor blade and mounted flat on the SEM stubs for examination of surface structures. All specimens were affixed to the mounting stubs with conductive silver paint and were coated with gold in a sputter-coater. Microscopic examination was done with either an AMR 1200 or a Jeol U-3 scanning electron microscope operated at 25 kV.

RESULTS

Thallus anatomy. The urediniospore germ tubes entered the coffee leaf from an appressorium over a stoma. With the exception of haustoria, all hyphae were intercellular and 3.5–6.2 μm in diameter. In the peripheral portions of the mycelium, only thin (3.5–4.5 μm diameter) hyphae were observed. These thinner ("pioneer") hyphae were confined to the spongy parenchyma tissue and were infrequent and widely spaced. They produced few haustoria or septa (cross walls up to 100 μm apart). The more central regions of the thallus contained mostly hyphae that were 4.7–6.2 μm in diameter. These "nutritive" or "feeder" hyphae were in the palisade as well as the spongy layer (Fig. 1) and had septa 10–20 μm apart. These hyphae produced abundant haustoria. Pioneer hyphae were seldom observed in these regions. Presumably, the feeder hyphae were derived from pioneer hyphae by enlargement of the filaments.

The pioneer hyphae branched only rarely, but the feeder hyphae were highly branched. However, the volume of hyphae as calculated from thin sections in even the most densely colonized leaf regions was usually less than 1% of the total volume of the leaf. The feeder hyphae produced an average of six haustoria per 10 host cells in the spongy tissue and seven haustoria per 10 cells in the palisade layer, as seen in cross sections, although some host cells had three observable haustoria.

On the abaxial leaf surface, a chlorotic halo 0.8–2.6 mm wide surrounded the sporulating area. The fluorescence microscope was used to determine the distribution of the internal hyphae in relation to this surface chlorosis and the location of sori. Although the Uvitex dye adhered to all parts of the fungal thallus, the haustorial parent cells, the septa of the intercellular hyphae, and the sori fluoresced much brighter than did the haustoria or the walls of the intercellular hyphae. Thus, even with a glowing dye, the pioneer

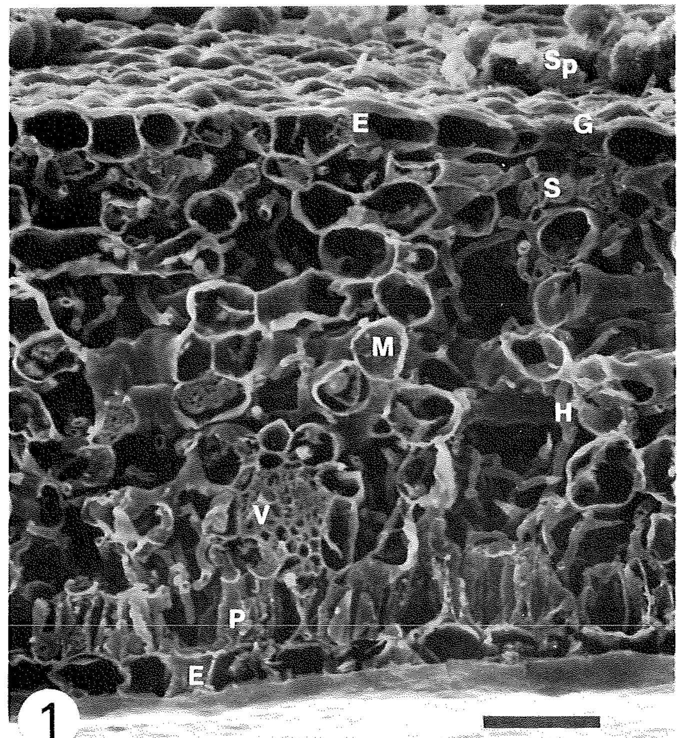
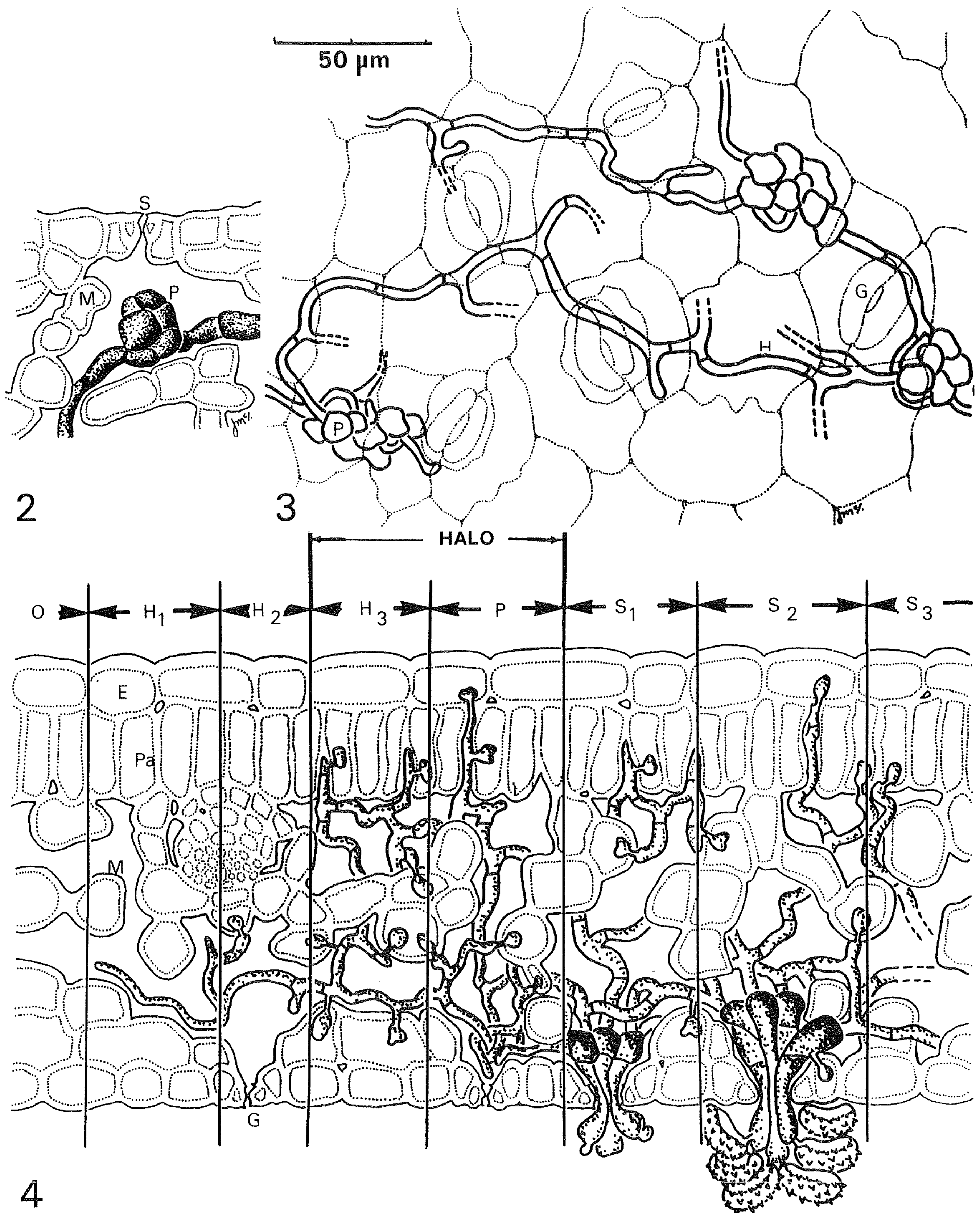


Fig. 1. Cross section of a coffee leaf showing distribution of intercellular hyphae of *Hemileia vastatrix* in the leaf tissue (scanning electron microscopy). Specimen from greenhouse inoculation, glutaraldehyde-formaldehyde fixation. Scale bar = 50 μm. S = sorus in substomatal cavity, Sp = spores on leaf surface, H = hypha, E = host epidermis, P = palisade layer, V = leaf vein, M = spongy mesophyll, and G = guard cell.



Figs. 2–4. Diagrams of protosori and thalli of *Hemileia vastatrix* in relation to coffee leaf tissues. **2,** Protosorus (P) developing below the level of the first row of coffee mesophyll cells (M). Note location of the stoma (S) and empty space in substomatal chamber. Drawn from photograph of specimen inoculated in the greenhouse, fixed and cleared in acetic acid-alcohol, hand-sectioned, and stained with cotton blue in lactophenol. **3,** Connecting hyphae (H) between three protosori (P) in a coffee leaf and their locations relative to host epidermal cells and guard cells (G), indicating that their formation is not directly beneath stomata. Drawn from a series of photographs taken as optical sections of a fluorescent-stained specimen from greenhouse inoculations. **4,** Model of the zones of the thallus, showing relationship to the chlorotic area (halo) on the lower surface of the leaf, based on interpretations of fluorescent-stained specimens from greenhouse inoculation. O = zone of healthy host tissue, H_1 = zone of pioneer hyphae, H_2 = zone of first haustoria, H_3 = zone of numerous haustoria, P = zone of protosori, S_1 = zone of immature sori, S_2 = zone of active sori, and S_3 = zone of senescent sori. Fungal cells and leaf cells drawn to scale, but lateral dimensions of zones compressed for clarity. Lesion is expanding to the left. G = guard cell, E = epidermis, M = mesophyll, and Pa = palisade layer.

hyphae were difficult to observe, which explains why they have not been described previously by other methods.

In the cross-section and fluorescent-stained specimens, incipient uredinial sori ("protosori") were seen inside the substomatal cavities (Fig. 2). Although some protosori were interspersed among mature sori (usually where lesions were coalescing), the majority were peripheral to that part of the lesion. The outermost sori that had emerged from stomata usually were younger and had smaller and less-developed urediniospores, as judged by spine development on the spores. "Runner" hyphae connected the unemerged protosori (Fig. 3).

Consequently, the entire lesion was produced from one radially expanding thallus that we arbitrarily subdivided into a series of mycelial regions or zones (Fig. 4). The transition between these zones was sometimes diffuse. As the mycelium grew, these zones moved outward, leaving behind a central region of senescent sori often hyperparasitized by *Verticillium* sp. We classified these zones as follows: O = zone of healthy, uninvaded host tissue; H_1 = zone of pioneer hyphae only, 0.2–0.4 mm wide; H_2 = zone of first haustoria (pioneer hyphae and some nutritive hyphae with occasional haustoria, about one haustorium/10 mesophyll cells), 0.3–0.5 mm wide; H_3 = zone of nutritive hyphae with abundant haustoria, 0.7–1.0 mm wide; P = zone of protosori, 0.5–1.5 mm wide; S_1 = zone of emergent but immature sori, 0.3–0.7 mm wide; S_2 = zone of mature, active sori, 0.7–4.0 (mostly 0.7–2.0) mm wide; and S_3 = zone of senescent sori.

Zone P, the part of the expanding lesion where the sori were forming beneath the stomata, could not be identified by visual examination of the leaf surface (Fig. 4). It appeared to the unaided eye as part of the chlorotic region. However, the sum of the widths of the zones (H_1–P) outside the area of visible sporulation was 1.7–3.3 mm, which is greater than the 0.8–2.6 mm measured for the surface halo. The hyphae of zones H_1 and H_2, zones of little or no direct (haustorial) contact with the host, did not cause sufficient host effects to produce chlorosis and, therefore, extended beyond the yellow area of the host leaf. The combined width (1.2–2.5 mm) of the internal zones with abundant haustoria (H_3 and P) was sufficiently close to the halo width to account for the location of that symptom. Sporulation was first visible to the unaided eye when lesions exceeded 3 mm in diameter, as also reported by Rayner (15), which is exactly the 1.5-mm minimum radius predicted here for zones H_3–S_1. Usually less than half (range 11.9–62.8%) of the area of the active portion of the thallus was devoted to sporulation.

As seen in the greenhouse, lesions with sporulating sori of *H. vastatrix* appeared to be covered by a solid mass of sori and spores. The sori could not be distinguished by the unaided eye as individual entities. However, the scanning electron microscope revealed that not all of the stomata were occluded by sori of the pathogen, even in the most densely packed lesions (Fig. 5). We found an average of 26 stomata per 0.05 mm^2 in counts from 20 coffee leaves, but no sample area of that size contained more than 11 uredinial sori of *H. vastatrix*. An average of 35% of the stomata in zone S_2 were occupied by sori in this survey, with a high value of 47% (nine sori per 19 stomata).

Protosorus anatomy. Very young, developing sori were located in substomatal cavities. Two (rarely one and sometimes up to four or five) intercellular hyphae entered the substomatal cavity to form a protosorus (Fig. 6). These hyphae did not appear different from any of the nutritive hyphae, and there was no morphological evidence that their growth was directed specifically toward a stoma. When the hyphae met in the substomatal cavity, they intertwined (Fig. 6). Several (four to 10 or more) irregular to mostly isodiametric cells, 10–15 μm (sometimes 20 μm) on each side, developed in one or two layers to form a discrete structure, the protosorus (Fig. 7). These new cells were distinct from the original hyphae, although some of the constituent hyphae remained separate and sometimes could be seen coiled about the protosorus like a garland (Fig. 3). The sporogenous cells of the sori were produced from these pseudoparenchyma-like aggregates.

By the time sporulation began, the substomatal cavity usually was filled with fungal cells. The protosori conformed to the shape of the cavity, so sometimes they were contorted. However, 10–25% of the protosori formed to one side of the stomatal opening (Fig. 8). These were commonly 20–25 μm (but some were up to 50 μm) laterally from the nearest guard cell. Apparently, at least some of these errant protosori could grow to the proper opening, sometimes by producing a hypha that extended to the substomatal chamber and produced a satellite protosorus (Fig. 8). None of these mislocated sori produced spores inside the leaf.

Sporogenous cells and sporulation. As young uredinial sori emerged through the stomata, there were only one to three emergent cells per sorus at first. Five to seven emergent sporogenous cells were visible in slightly older sori (Fig. 9). The paradermal diameters were measured for more than 100 fascicles of emergent sporogenous cells. None exceeded the size range for stomatal pore dimensions. The bundles were elliptical in cross section, with the greater axis in the direction of the longer dimension of the stomata, presumably so that as many fungal cells as possible could emerge.

Shortly after the first sorus cells emerged from the stomata, spore buds appeared near the apex of each of these sporogenous cells. As an emergent sporogenous cell grew, new buds appeared in a spiral arrangement near its apex (Fig. 9). The earlier buds, now situated

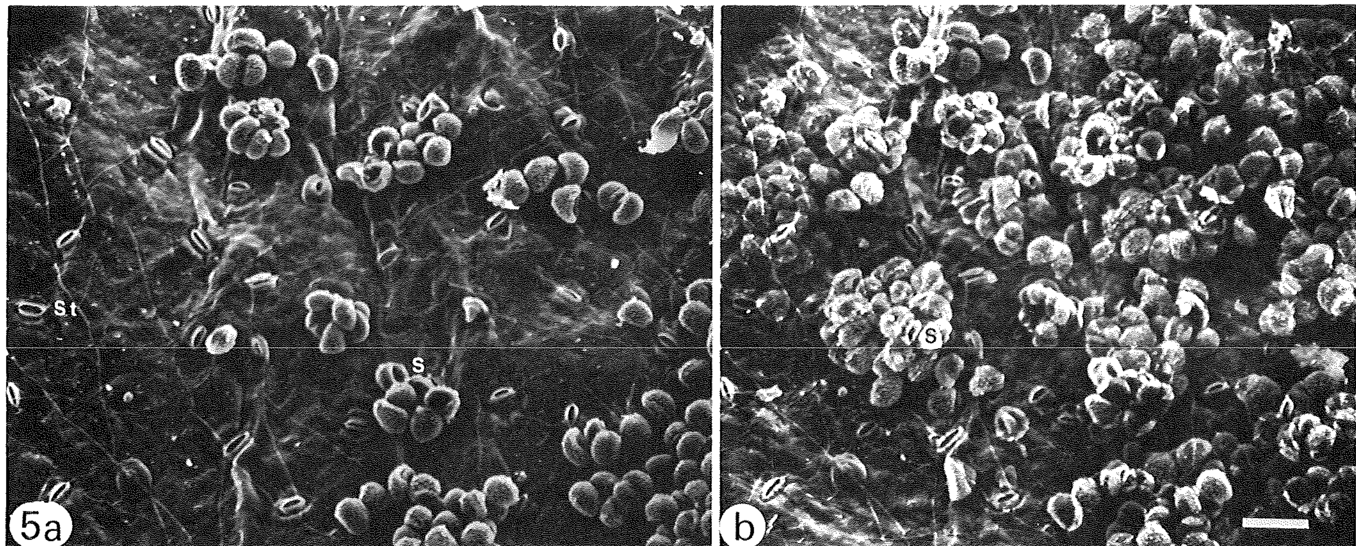
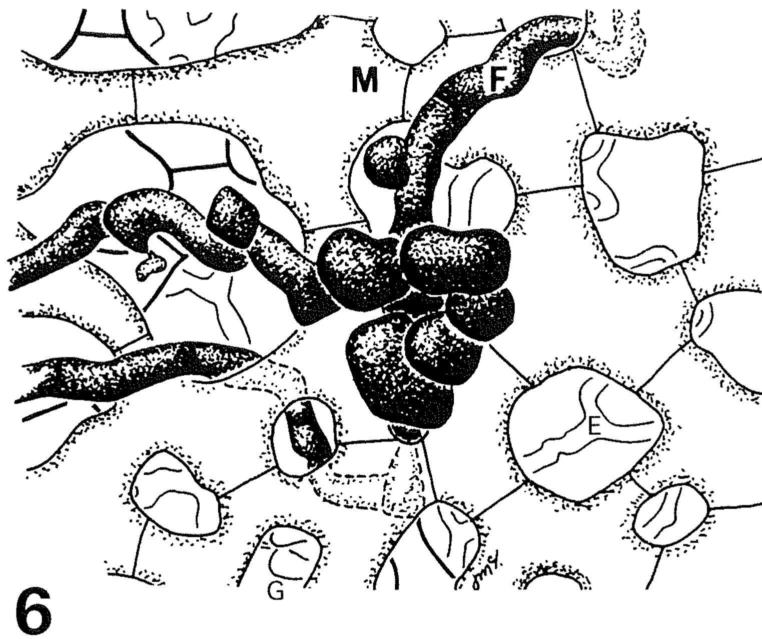
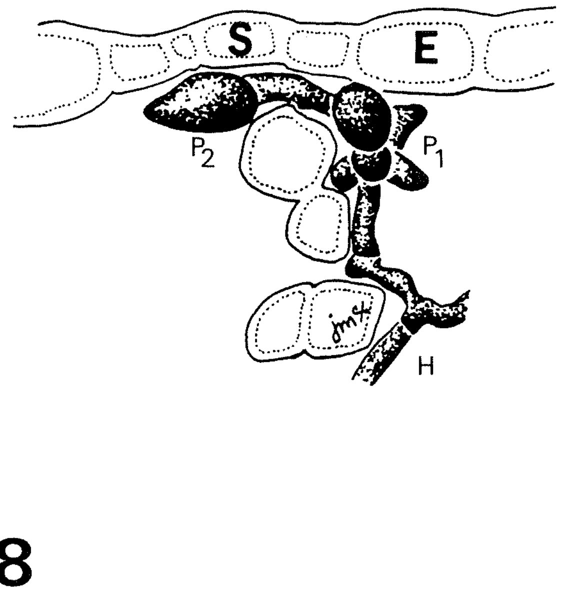
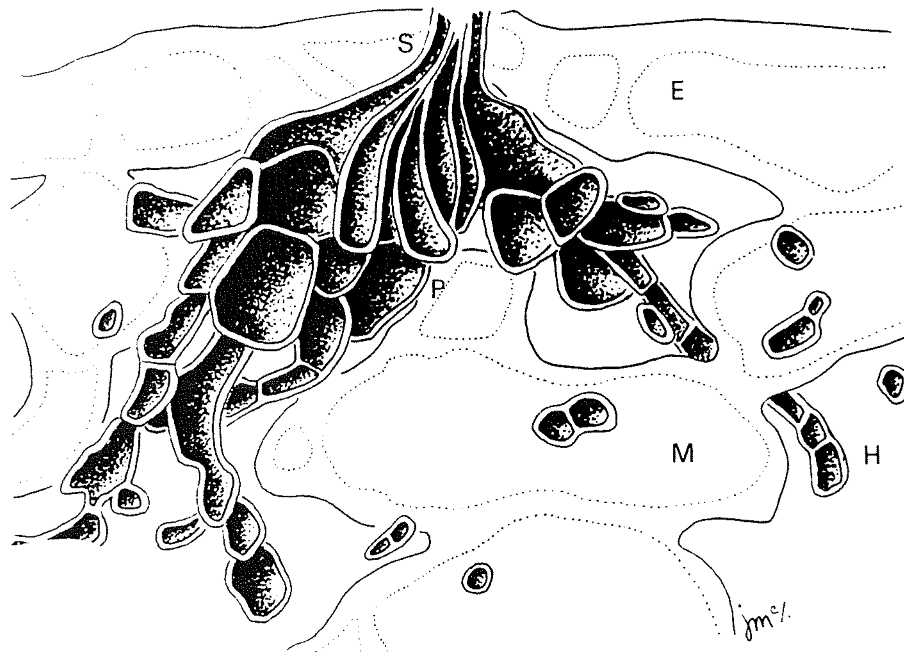


Fig. 5. Scanning electron microscopic surface view of coffee leaf showing sori (S) of *Hemileia vastatrix* from herbarium specimen. **a,** Younger sori; **b,** older sori in densely packed area of lesion. Note the numerous stomata (St) not containing sori. Scale bar = 50 μm.







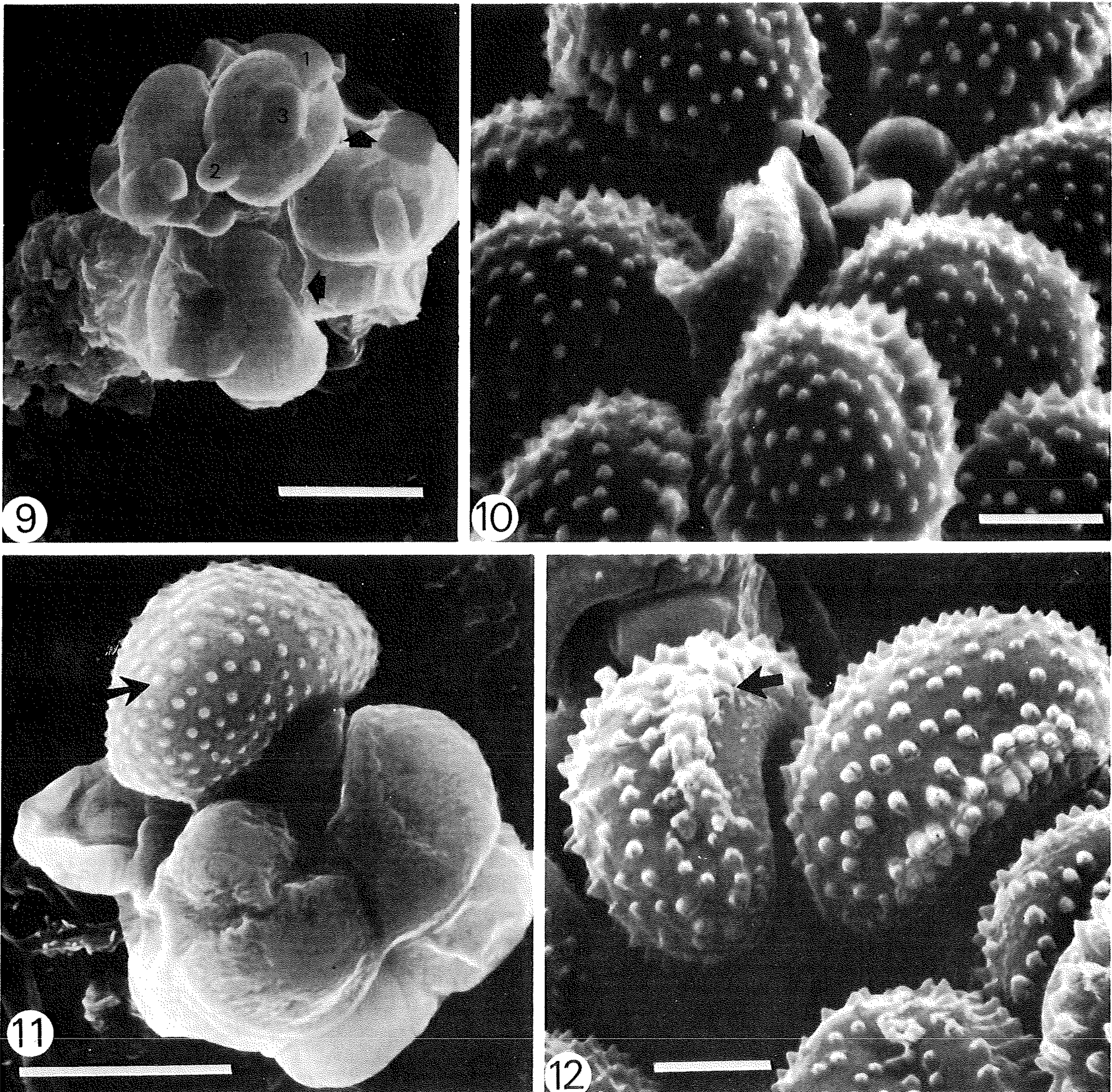
Figs. 6–8. Diagrammatic reconstructions of the structure of protosori of *Hemileia vastatrix*. **6,** Protosorus (P), model redrawn from traced photographs of serial "optical" sections of epidermal peel from specimen obtained as in Fig. 2. View of lower side of the epidermis, the first two layers of mesophyll cells, and three feeder hyphae (F). G = guard cell, M = mesophyll cell, and E = cell wall of epidermal cell. **7,** Reconstruction of sorus from serial optical sections of fluorescent-stained coffee leaves from greenhouse inoculations. S = sporogenous cell extending through stoma, P = protosorus cell, H = hyphal cell, E = host epidermal cell, and M = mesophyll cell. **8,** Protosorus that did not originate directly beneath a stoma, showing satellite protosorus. Drawn from photograph of leaf inoculated in greenhouse and then cryosectioned. Original portion (P_1, 23 μm diameter) is beneath epidermal cells. Note location of feeder hyphae (H) and that final portion (P_2, 19 μm diameter) is under a stoma. E = epidermal cell and S = subsidiary cell of stomatal apparatus.

lower on the sporogenous cell, began to enlarge. Some of these spore initials were on the inner edge of the sporogenous cell. All mature sori examined had their urediniospores facing outward like the "sections of an orange" (4), but we could not determine whether spore initials facing inward failed to mature or became realigned as they developed.

The first evidence of spine development visible by SEM was seen on the lower, larger spore initials when these spores had reached one-fourth to one-third their full size (Fig. 10). The urediniospores fulfilled the "hemi-leio" (half-smooth) syndrome by being dorsally smooth. Preliminary transmission electron microscopic (TEM) observations (J. W. McCain, *unpublished*) and some SEM data (Fig. 11) indicated that the urediniospore echinulae (spines) were developed in invaginations in the primary layer of the spore wall. At maturity, the urediniospores had 150–300 spines. Often the spines were tightly clustered along the sides of the spore, forming a continuous ridge along the boundary between the smooth and echinulate surfaces of the spores (Fig. 12).

As a sorus continued to develop, the first urediniospores matured (Fig. 13), but immature spores and spore initials were still present in the center of the sorus, indicating a basipetal pattern of spore maturation. The urediniospores were produced in flushes. As a whorl of outer spores matured, an inner cluster of new spores usually was already visible.

Two previous reports (9,10) mentioned a "membrane" that appeared to cover a young *H. vastatrix* sorus as it emerged through a stoma but was later broken down. In most young sori, we observed folds of a covering, similar to that reported by Harr (10), that connected the tips of the sporogenous cells (Fig. 9). Maturing spores extended out of this covering. In some well-developed sori



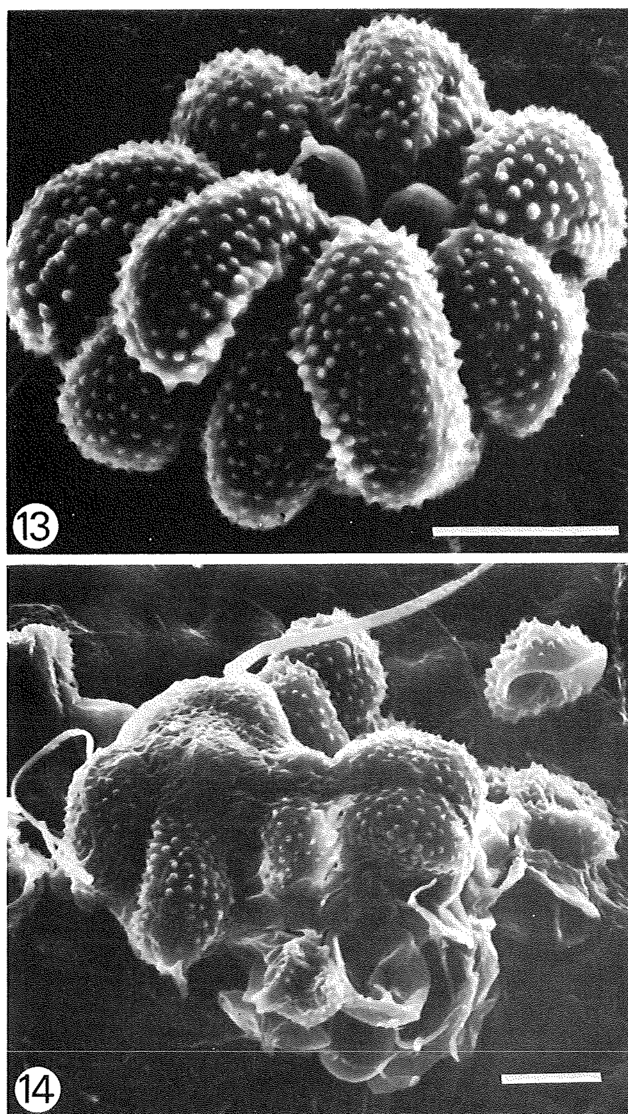
Figs. 9–12. Surface views by scanning electron microscopy of developing sori and spores of *Hemileia vastatrix*. **9,** Emergence of young sorus from leaf inoculated in greenhouse and fixed in glutaraldehyde-formaldehyde. Young sorus showing spore bud (2) on the side of a sporogenous cell that is facing the center of the fascicle of sporogenous cells. 1 = Oldest spore bud, 3 = youngest bud. Note also the apparently continuous mucilage-like covering of the sorus (arrow). Scale bar = 10 μm. **10,** Order of development of urediniospores in a sorus from a herbarium specimen, showing four sporogenous cells in center, one bearing the projection that will become a new spore (arrow). Immature spores visible, showing that spines are produced at an early point in spore enlargement. Scale bar = 10 μm. **11,** Young sorus on coffee leaf from a herbarium specimen, showing sporogenous cells. Urediniospore shows that spines apparently develop in sunken places on spore surface (arrow). Scale bar = 10 μm. **12,** Urediniospore on a herbarium specimen, showing connections between the closely bunched spines (arrow) along the lateral margin of the spore. Scale bar = 10 μm.

from herbarium material (Fig. 14), some kind of covering obscured all or parts of each urediniospore, but we could not rule out the possibility that this was artifactual. No evidence of a covering membrane or mucilage was detected in sectioned material.

DISCUSSION

Orange coffee rust urediniospores are not long-lived (15). To increase the supply of sporulating lesions in this study, urediniospores were transferred directly from active lesions to new leaves. After removal of the spores, the lesions usually resumed sporulation, as was also reported by Da Silva et al (7). We propose that this was not resumption of sporulation by the sori from which the spores were removed but that the new spores came from new sori as the lesion continued to expand.

Da Silva et al (7) also reported 40–50% differences in germinability of urediniospores from "young" compared with "old" lesions. However, if the *H. vastatrix* thallus is continually expanding, all lesions must have both young spores from zones S_1 and S_2 and old spores from zones S_2 and S_3. This explains why Nutman and Roberts (14) reported more significant germinability differences (up to 97%) than did Da Silva et al (7); Nutman and Roberts (14) attempted to collect spores only from the centers of lesions versus only from the periphery, using as a marker the often paler color of the older spores.



Figs. 13–14. Later stages in *Hemileia vastatrix* sorus development. Scanning electron microscopic photographs of herbarium specimens. **13,** Uredinial sorus, surface view. Two rows of mature urediniospores around the periphery of the sorus; apices of two sporogenous cells visible in the center of the sorus, one with a new spore bud (arrow). Scale bar = 20 μm. **14,** Mature uredinial sorus with apparent mucilaginous covering. Note how urediniospores at left are almost indistinguishable, shrouded beneath the sorus covering. Scale bar = 20 μm.

When individual sori were observed in median section in stained leaf cross sections, the walls of the cells of the fascicle of sporogenous cells overlaid one another. In such views, casual observation might suggest, as has been claimed (4), that there were as many as 30 of these emerging cells. Our comparison of fascicle measurements with stomatal sizes supported previous reports (8,15) that the bundles did not tear the epidermis as they passed between the guard cells. The host stomatal area (when open) was 29–107 μm^2. Thus, 30 emergent cells would each have to be 1–3 μm^2 or less in area to fit through such a pore. Our estimate of a cell size of 5–10 μm^2 is more consistent with the presence of only five to 10 sporogenous cells per stoma.

We conclude that the emergent cells are sporogenous cells and not pedicels as drawn by Chevaugeon (4). The protosorus is a more organized structure with larger cells than shown previously (6). We generally support the findings of Ward (17) but clarify and extend them. Some details of *H. vastatrix* sorus anatomy still remain unresolved. What is the significance of the protosori that "miss" the stomata? The sporogenous cells appear to be sympodulae (11) but this needs confirmation by TEM, as does the mode of development of the urediniospore echinulation. Preliminary observations indicate that these spines are produced by the method known for such temperate zone genera as *Puccinia* and *Melampsora* (12). The origin, nature, and function of the material that covers the sorus remains undetermined. The stretching and crinkling of this covering and its absence from outer portions of some spores seemed to indicate a mucilaginous coating that had dried up or was absorbed during spore development rather than a membrane that was broken down at sorus maturity. Perhaps it serves to ease

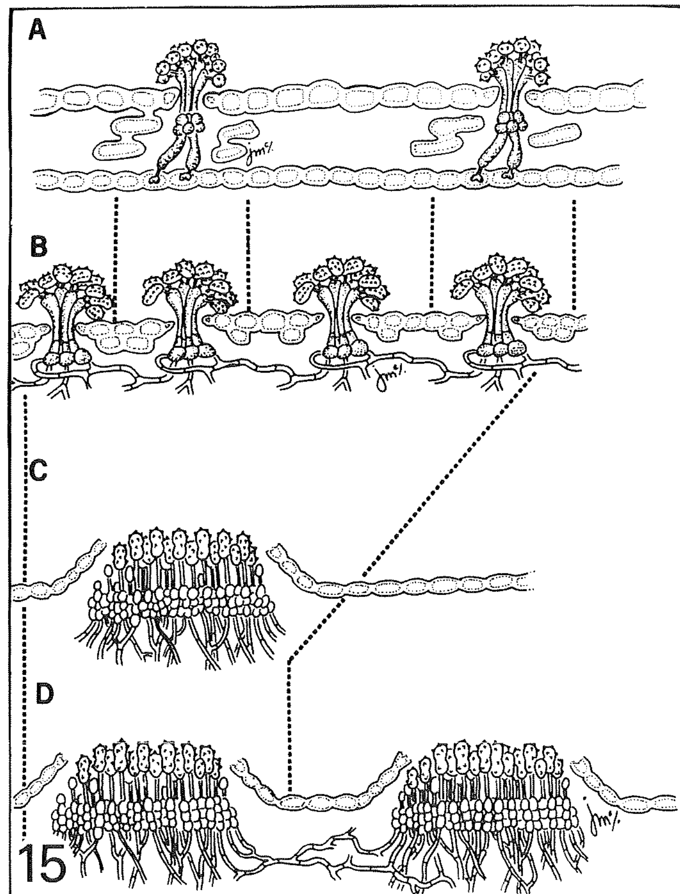


Fig. 15. Model of thallus organization in rust fungi showing four of the possible levels of thallus complexity in the rust fungi. **A,** *Desmella aneimiae,* **B,** *Hemileia vastatrix,* **C,** *Puccinia recondita,* primary sorus; and **D,** *P. recondita,* satellite sori.

passage of the sporogenous cells between the guard cells or to protect young spores from desiccation.

Our studies were the first of the whole thallus of this fungus. Hyphal connections between sori confirm earlier assumptions (15,17) that each orange coffee rust lesion consists of the numerous uredinial sori produced by one *H. vastatrix* thallus. More than one sorus results from each point of infection. The thallus enlarges, producing new sori, until it senesces after 3–5 mo, when the lesion may be 2–3 cm in diameter (15).

Because the uredinial sori of *H. vastatrix* were produced in an expanding lesion, sori of all developmental stages were present in each lesion. This eliminated the need for collecting samples at designated times after inoculation. By sectioning at intervals along a transect through a lesion, one could section in space and, in effect, also sample in developmental time.

There have been other reports of variation in size of rust fungus hyphae (1), but the size classes have not been recognized as representing particular zones of the thallus as they clearly do in *H. vastatrix*. Understanding the organization of the thallus might explain some aspects of the disease cycle. For example, among the rust fungi, there are several levels of thallus complexity (Fig. 15). In *Desmella aneimiae* H. & P. Syd., cause of a nettlesome leaf spot of florists' ferns in Brazil (2), one infection produces an extremely limited mycelium and only one small, suprastomatal sorus (5), the simplest type of rust thallus. In *H. vastatrix*, each sorus is determinate and no larger than the sori of *D. aneimiae*, but the thallus of the orange coffee rust fungus radiates from one original growing point and produces numerous sori as the hyphae encounter stomata. This is the second level of thallus complexity in the rust fungi.

P. graminis sori (pustules) are indeterminate, ie, they have no bounding structures such as peridia and each may occupy as much host tissue as an entire thallus of *H. vastatrix* and so produce a higher density of spores. *P. graminis* or *P. recondita* Rob. ex Desm. may also produce secondary pustules (1). The multiple sori of these wheat rust fungi, by their size, are one magnitude greater in developmental potential than the multiple sori of *H. vastatrix* (Fig. 15). The most complex thallus types occur in the locally systemic or gall-forming rust fungi, such as *Gymnosporangium* spp., and the long-lived, fully systemic species, eg, *Gymnoconia peckiana* (Howe) Trott. Thus, there is a range of sorus dimensions and thallus complexity in the rust fungi. Rust fungi with thallus types at either end of the scale probably spread relatively slowly to new host plants, due either to their slow growth (systemic species) or to their limited growth. In rust fungi with more than one spore stage, it may be epidemiologically significant to determine which spore stage is limited. Seldom do conidial (uredinial) stages cause hypertrophy or become systemic. When the dikaryotic mycelium of a rust fungus does produce a gall, it is typically in a species that omits the uredinial stage, eg, *Gymnosporangium clavipes* C. & P.

In this study, the smallest *H. vastatrix* sorus seen with at least one mature urediniospore also had six identifiable spore initials. The average-sized sorus had 13 spores or spore buds, but some sori had as many as 28. Four to six flushes of five to eight spores were produced over a 4- to 5-mo period, so one sorus could bear 20–50 urediniospores. Because the thallus was continually expanding, only a fraction of the lesion area was active at any one time and probably no single sorus continued to sporulate for the entire 4–5 mo. An average (18-mm-diameter) lesion would encompass 50,000–150,000 stomata. On the basis of our figure of sori developing in 35% of the stomata, such a lesion could contain 15,000–50,000 sori, which together could produce from 300,000 to 2 million spores. This agrees with Rayner (15), who collected 400,000 spores from one such lesion within only 3 mo. Thus, all the sori of one lesion taken together could be more prolific than a single pustule of *P. graminis*, which may produce 100,000 spores (13). *H. vastatrix* sori are more restricted than *P. graminis* sori (Fig. 15), but the orange coffee rust fungus compensates for the difference by its spreading thallus and its longevity.

LITERATURE CITED

1. Allen, R. F. 1926. A cytological study of *Puccinia triticina* physiologic form 11 on Little Club wheat. J. Agric. Res. 33:201-222.
2. Cardoso, R. M., Pitta, G. P. B., Figueiredo, M. B., and Hennen, J. F. 1984. *Desmella aneimiae* Syd., agente causal de ferrugem em samambaias cultivadas. O Biológico 49:(In press).
3. Cartwright, D. W., and Russell, G. E. 1981. Development of *Puccinia striiformis* in a susceptible winter wheat variety. Trans. Br. Mycol. Soc. 76:197-204.
4. Chevaugeon, J. 1956. Enquête phytopathologique dans le bassin du Cavally. Pages 57–86 in: Revue de Mycologie 21. Suppl. Colonial 2.
5. Cummins, G. B. 1940. Notes on some Uredinales. Ann. Mycol. 38:335-338.
6. Cummins, G. B., and Hiratsuka, Y. 1983. Illustrated Genera of Rust Fungi. 2nd ed. American Phytopathological Society, St. Paul, MN. 152 pp.
7. Da Silva, S. R., Martins, E. M. F., and Moraes, W. B. C. 1982. Observações preliminares quanto a viabilidade de urediniosporos de *Hemileia vastatrix* Berk. et Br. produzidos em sucessivas esporulações de um mesmo sorus (Abstr.). Page 104 in: Programa e Resumos, XV Congresso Brasileiro de Fitopatologia, São Paulo, Brazil.
8. Gopalkrishnan, K. S. 1951. Notes on the morphology of the genus *Hemileia*. Mycologia 43:271-283.
9. Guggenheim, R., and Harr, J. 1978. Contributions to the biology of *Hemileia vastatrix*, II. SEM-investigations of sporulation of *Hemileia vastatrix* on leaf surfaces of *Coffea arabica*. Phytopathol. Z. 92:97-101.
10. Harr, J. 1979. *Hemileia vastatrix* Berk. et Br.: Biology of the Fungus, Aspects of Its Control. Publ. Div. Agron. Dep. Invest. Sandos, CA. 27 pp.
11. Hughes, S. J. 1970. Ontogeny of spore forms in Uredinales. Can. J. Bot. 48:2147-2157.
12. Littlefield, L. J., and Heath, M. C. 1979. Ultrastructure of Rust Fungi. Academic Press, New York. 277 pp.
13. Melching, J. S., Bromfield, K. R., and Kingsolver, C. H. 1979. Infection, colonization, and uredospore production on Wayne soybean by four cultures of *Phakopsora pachyrhizi*, the cause of soybean rust. Phytopathology 69:1262-1265.
14. Nutman, F. J., and Roberts, E. M. 1963. Studies on the biology of *Hemileia vastatrix* Berk. et Br. Trans. Br. Mycol. Soc. 46:27-48.
15. Rayner, R. W. 1972. Micologia, Historia y Biologia de la Roya del Cafeto. Inst. Interam. Sci. Agric. Turrialba Costa Rica Publ. Misc. 94:1-68.
16. Roberts, D. A., and Boothroyd, C. W. 1972. Fundamentals of Plant Pathology. W. H. Freeman & Co., San Francisco, CA. 402 pp.
17. Ward, H. M. 1882. On the morphology of *Hemileia vastatrix*, Berk. & Br. (the fungus of the coffee disease of Ceylon). Q. J. Microsc. Soc. (n.s.) 22:1-11.
18. Wellman, F. L. 1961. Coffee: Botany, Cultivation, and Utilization. World Crops Books, Interscience Publishers Inc., New York. 488 pp.