Surface Morphology, Wall Structure, and Initial Adhesion of Conidia of the Powdery Mildew Fungus *Uncinuliella australiana*

C. W. Mims, K. A. Liljebjelke, and E. A. Richardson

First and second authors: Department of Plant Pathology, University of Georgia, Athens 30602; and third author: Department of Botany, University of Georgia, Athens 30602.

We thank JEOL Corporation for technical assistance and Ron Clay for assistance with gold-labeling procedures. Supported by State and Hatch Funds allocated to the Georgia Agricultural Experiment Station. Accepted for publication 7 December 1994.

**ABSTRACT**


A combination of light microscopy, low-voltage scanning electron microscopy, and transmission electron microscopy was used in this study of conidia of the powdery mildew fungus *Uncinuliella australiana*, a pathogen of crape-myrtle (*Lagerstroemia indica*). These conidia were ellipsoid-cylindrical in shape and virtually transparent when viewed with bright field light microscopy. Each conidium possessed a thin wall that stained moderately for polysaccharides. Results from wheat germ agglutinin-gold labeling procedures indicated that chitin was present in the wall. Conidia possessed minute longitudinal surface ridges that extended along most of the length of each conidium. These ridges were formed by thickened, slightly raised portions of the wall. The surface of each conidium was coated with a thin networklike layer of extracellular mucilage. When strips of moist dialysis membrane were touched to conidia, this material spread instantly from conidial surfaces onto the membrane forming deposits referred to here as adhesion pads. Formation of an adhesion pad between the underside of a conidium and the membrane surface appeared to be the initial step in the process of conidial adhesion.

Powdery mildew is a common and troublesome disease of crape-myrtle (*Lagerstroemia indica* L.), a popular ornamental widely grown in the southern United States. This disease has been attributed principally to either *Erysiphe lagerstroemiae* E. West or *Uncinuliella australiana* (McAlpine) Zheng & Chen (1,10,23). However, in a recent monograph of powdery mildew fungi, Braun (4) rejected *E. lagerstroemiae* as a species and suggested that *U. australiana* is the cause of powdery mildew disease of crape-myrtle.
All of the samples we collected during the course of this study of infected crape-myrtle plants consisted only of asexual (conidial) stages, and all conformed to the description of the asexual stage of *U. australiana* provided by Braun (1). For this reason, we refer to the pathogen examined here as *U. australiana* even though we never collected its sexual (ascocarp) stage. Ascocarps are, of course, required for unequivocal identification of most species of powdery mildew fungi.

The initial objective of this study was to characterize the surface morphology and wall structure of conidia of *U. australiana* using a combination of light and electron microscopic techniques. However, the project was expanded to examine the initial aspects of the adhesion of conidia to strips of dialysis membrane. The work reported here is timely in view of the current high level of interest in the very early stages of plant infections by fungi. As noted by numerous authors (3,6,8,19,29,33), it is apparent that spores of many plant pathogenic fungi are active participants not only in the process of adhesion to host surfaces, but also in the preparation of the infection court. To better understand the initial interactions between fungal spores and host surfaces, it is necessary to know more about spore surface morphology as well as the nature of the spore wall. In the case of powdery mildew fungi, surprisingly little is known about these details even though some of these organisms are among the most extensively studied of all fungal plant pathogens.

**MATERIALS AND METHODS**

The conidia of *U. australiana* used in this study were collected from May to August in 1993 and 1994, principally from infected crape-myrtle plants growing on the campus of the University of Georgia in Athens. Light microscopic observations were made using a Nikon microscope equipped with differential interference contrast (DIC) optics and epifluorescence attachments. Living, unstained conidia from freshly collected leaves were examined in water mounts using DIC microscopy. Conidia also were examined using epifluorescence microscopy (excitation 380 nm, mirror 400 nm; barrier 400 nm) following staining with calcofluor (5). In this case, pieces of infected leaves were submerged in 0.3% calcofluor for 1 min and transferred to a distilled water wash for 5 min. Conidia were scraped off the leaf into a drop of water on a microscope slide and examined.

Surface features of conidia were examined using low-voltage scanning electron microscopy (SEM). Small pieces of fresh, unfixed infected leaves were attached to specimen stubs using double-stick carbon tape and placed directly into a JEOL 5400 LV microscope, where they were examined without coating at 1.5 kV.

Conidia were prepared for study with transmission electron microscope (TEM) using freeze substitution fixation. Thin (1-2 mm) strips were cut from portions of infected leaves bearing colonies of the pathogen and frozen by plunging into liquid propane. These samples were processed to the point of embedment according to the procedures of Hoch (11). After infiltration with 100% Epon resin, the sharpened end of a wood applicator stick was used to gently dislodge conidia from leaf strips into drops of fresh 100% resin resting on a rectangular (35.5 × 40 mm) piece of plastic cut from either the lid or bottom of a Lux Permanox disposable culture dish. The leaf tissue was discarded, a similar piece of plastic was lowered onto the droplets, and the Epon was polymerized for 48 h at 60°C. Epon did not react with the plastic of the Lux Permanox dishes, and the use of a release agent was not required. A razor blade was used to separate the two pieces of plastic, and the one to which the thin layer of polymerized resin containing the samples remained attached was affixed to a glass microscope slide with strips of tape. Light microscopy was used to select what appeared to be well-preserved conidia for thin sectioning (26). In all, a total of more than 50 well-preserved conidia were examined using TEM.

To examine the interaction of conidia with a solid surface, small pieces of moist, sterile dialysis membrane (Fisher Scientific, Cat. No. 8-667E) held with forceps were touched gently to fungal colonies on infected leaves and immediately plunged into liquid propane. Membranes and adhering conidia then were processed for TEM and flat-embedded between rectangular pieces of plastic as described above.

Ultrathin sections of conidia and conidia adhering to pieces of dialysis membrane were sectioned with a diamond knife, collected on gold slot grids, and allowed to dry down onto formvar-coated aluminum bridges using the procedure of Rowley and Moran (32). Sections for routine observation were poststained with uranyl acetate and lead citrate and examined with a Zeiss EM 902A microscope operating at 80 kV. Other sections were treated with a periodic acid, thiocarbohydrazide, silver proteinate poststain designed to localize polysaccharides or with either a two-step incubation procedure using wheat germ agglutinin (WGA) and ovomucoid gold complexes (2,7) or concanavalin A (ConA) gold complexes (7). For polysaccharide staining, a modified version of the Thierry (34) procedure described by Lingle (16) was used prior to poststaining with uranyl acetate and lead citrate. As a control for the poststaining stain, thiocarbohydrazide was deleted. The control for the WGA labeling procedure

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**Fig. 1.** Light micrographs of conidia of *Uncindiaustraliana*. A, Differential interference contrast (DIC) micrograph showing the typical appearance of conidia in a water mount. Nuclei (arrowheads) and vacuoles (arrows) are identified in some conidia. B, DIC micrograph of conidia trapped in an air bubble in a water mount. Longitudinal surface striations are barely visible on the conidia shown at the arrowheads. C and D, Epifluorescence micrographs of conidia stained with calcofluor. All micrographs are the same magnification. Bar in D = 10 μm.
involved a two-step incubation with WGA previously absorbed with N, N', N"-triacetylchitotriose followed by incubation with the ovomucoid-gold complex. Control samples for ConA labeling were incubated with 2 μg of glycogen mixed with the ConA gold complexes.

RESULTS

Light microscopy. Conidia of *U. australiana* examined in this study were ellipsoid-cylindrical in shape and measured 28–38 × 13–18 μm. Conidia were virtually transparent with transmitted light, but by using DIC microscopy it was possible to resolve various structures within individual conidia. The most prominent structures were vacuoles and the nucleus (Fig. 1A). The nucleus characterized was located very near the longitudinal midpoint of the conidium and was spherical in shape with a prominent, eccentrically positioned nucleolus. Large vacuoles generally were prominent near the ends of each conidium, although not always visible in the same focal plane. Small vacuoles were numerous, and some of these could easily be mistaken for the nucleus.

By focusing on the conidial surface, it was possible to detect minute longitudinal surface striations. These were most prominent on conidia trapped in air bubbles in water mounts. Although these striations are difficult to photograph with light microscopy, some evidence them of can be detected on some of the conidia visible in Figure 1B.

Conidia fluoresced under ultraviolet light after exposure to calcofluor (Fig. 1C and D), indicating the presence of β-glucans. Some conidia fluoresced brightly over their entire surfaces (Fig. 1C), while others were brighter at their ends (Fig. 1D). No evidence of the surface striations noted with DIC microscopy was apparent with epifluorescence microscopy. However, as described below, the existence of these structures was confirmed with both SEM and TEM.

SEM. Low-voltage SEM of freshly collected, unfixed conidia gave excellent results (Fig. 2A–D). It was, however, important to examine and photograph samples quickly, as surface features become altered soon after exposure to the electron beam and the vacuum conditions within the column of the microscope. SEM revealed that conidia of *U. australiana* developed at the tips of

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![Figure 2](image-url)

**Fig. 2.** Low-voltage scanning electron micrographs of conidia of *Uncinula australiana*. A, Typical appearance of conidia. A portion of one conidiophore (arrow) bearing two conidia is visible. Note the slightly raised longitudinal surface ridges on the conidium and the material (arrowheads) between the two conidia whose sides are touching. B, Higher magnification of a portion of Figure 2A. Note the material (arrowhead) connecting the sides of the two conidia. The rounded end of one conidium is visible at the asterisk. C, Illustration of the extracellular mucilage (arrowheads) present on the surface of a conidium. D, Higher magnification view of extracellular mucilage near the ends of two conidia. The rounded ends of the conidia are visible at the asterisks. Bars in A–C = 4 μm; bar in D = 2 μm.
upright conidiophores (Fig. 2A). Once a conidium was delimited from its conidiophore, it was displaced by a younger conidium. Conidia were never observed in long chains, even when living samples on leaves were viewed with a dissecting microscope. Typically, no more than two conidia were observed on any single conidiophore (Fig. 2A), and one of these was always the developing conidium still attached to the conidiophore tip.

Both mature and developing conidia possessed the longitudinal surface striations mentioned earlier. The structures (Fig. 2A-D) appeared as ridges that extended along most of the length of each conidium. These ridges were not, however, present on the slightly rounded ends of conidia (Fig. 2B and D). At higher magnifications (Fig. 2C and D), it was also possible to resolve a thin networklike film of extracellular mucilage on the surface of each conidium. This material was most prominent at the ends of conidia near the points at which the ridges terminated (Fig. 2D). In some cases, it also could be seen between the sides of conidia that were touching one another (Fig. 2A and B).

**TEM.** The quality of preservation obtained with freeze-substituted conidia varied greatly. Badly damaged and/or poorly preserved conidia usually could be identified and avoided by examining flat embedded samples with light microscopy. However, some conidia that looked very good at the light microscopic level sometimes showed either variations in the appearance of cellular components or some type of mechanical damage when sectioned and examined with TEM. The most variation we observed related to the appearance of glycogen particles and vacuoles (Fig. 3A-C). In the most well-preserved conidia, glycogen particles appeared as electron-dense deposits (Fig. 3A and B), while vacuoles (Fig. 3B) were rounded with smooth and intact tonoplasts. In slightly less well-preserved conidia (Fig. 3C), glycogen particles were electron transparent, and vacuoles sometimes showed evidence of mechanical damage. However, in almost all of the adequately preserved conidia sectioned and examined in this study, the conidial wall was well-preserved.

When viewed in cross section (Fig. 3A), the conidial wall had a distinctive serrate appearance because of alternating thin and thick regions, the latter of which tapered to raised points. These thickened areas created the longitudinal surface striations or ridges visible on the surfaces of conidia. The outer portion of the conidial wall and the bulk of each ridge were slightly more electron dense than the inner portion of the wall (Fig. 3A). Differences in the electron densities of the inner and outer portions of the wall also were apparent in longitudinal sections of the wall (Fig. 3D and E). In Figure 3E, the point at which the plane of section left a thin portion of the wall and entered a ridge is quite apparent.

The conidial wall could be labeled with WGA/ovomucoid gold complexes (Fig. 3F) but not with ConA gold complexes. A control sample for the WGA gold labeling procedure is shown in Figure 3G. The wall also stained moderately for polysaccharides using the modified Thiry procedure (Fig. 4A). This procedure also stained glycogen within conidia (Fig. 4A). Figure 4B is a control sample for the polysaccharide staining procedure. Although not illustrated here, ConA gold complexes showed a high affinity for the glycogen deposits.

Material thought to correspond to the extracellular mucilage observed on conidia with SEM also was seen with TEM. In most instances, this material appeared as a thin, irregular, electron-dense layer not in direct contact with the conidial wall (Fig. 3C and D). Although it was never observed to be continuous around the entire surface of any conidium, at least some evidence of this material was found on each conidium examined in this study. In a few instances, we observed extracellular material in direct contact with the conidial wall (Fig. 4C and D). Such material always was observed near the ends of conidia in areas where mucilage was most prominent on conidia examined with SEM (see Fig. 2D).

Light microscopic examination of flat embedded pieces of dialysis membrane that were touched to fungal colonies immediately prior to plunge freezing revealed the presence of numerous conidia. By examining sections cut at right angles to the surfaces of these membranes, it was possible to examine the interfaces between conidia and their underlying membranes. Figure 4E is typical of what we saw in these sections. Coating the membrane surface immediately below each conidium was a deposit of material referred to here as an adhesion pad (Fig. 4E and F). As evident in Figure 4F, the adhesion pad was composed of finely fibrillar material in which various other types of inclusions were present. The material comprising the pad spread out onto the dialysis membrane a short distance from the conidium, clearly continuous with the thin layer of extracellular material associated with the conidium (Fig. 4F). This thin layer appeared to correspond to the layer present on conidia prepared for study by plunge freezing leaf strips. While we never found a conidium that was in direct contact with its adhesion pad, profiles of sectioned pads always conformed exactly to the curved surface of the nearby conidium (Fig. 4E). At higher magnification, it was apparent that the ridges present on a conidium matched perfectly with depressions present in the surface of the adhesion pad (Fig. 4G). This, of course, indicates that conidia were in direct contact with adhesion pads at the time of freezing and subsequently separated from them.

Polysaccharide staining had little if any effect on the material comprising the adhesion pad. In addition, this material did not label with either WGA or ConA.

**DISCUSSION.**

Results from WGA/ovomucoid gold labeling indicated that chitin was present in the conidial wall of *U. australiana*. This is in agreement with the observations of Rohringer et al. (31) for conidia of the powdery mildew fungus *Erysiphe graminis* f. sp. *hordei*. The results from the polysaccharide staining procedure were not particularly informative for *U. australiana*, as the bulk of the wall stained uniformly for polysaccharides. This is in contrast to the results obtained with conidia of *Colletotrichum graminicola*, where this same procedure greatly enhanced the appearance of a finely fibrillar layer that coated the surface of the conidial wall (24).

Whether longitudinal surface ridges similar to those noted in this study are present on conidia of other species of powdery mildew fungi is unknown. Braun (4) has stated that, within this group of fungi, conidial surfaces vary from smooth, faintly rough, or verrucose to strongly verrucose, but to date few workers have examined these conidia using modern scanning electron microscopic techniques. Kunoh et al. (14) and Nicholson et al. (30) did, however, use cryo-techniques to study conidia of *E. g. hordëi*. These workers reported that these conidia exhibited a distinct pattern of reticulation interspersed with spinelike projections, but made no mention of any type of extracellular mucilage on the conidial surface prior to contact with either the host surface or a collenchyma membrane. However, within 10 min after contact, this network became less and less distinct and eventually disappeared, apparently as a result of the deposition of a filmlike layer of material on the conidial surface. By about 30 min after contact, material was released from conidia and could be detected on the membrane beneath them. In our opinion, these deposits appear to correspond to the adhesion pads noted in *U. australiana*.

In an early TEM study, McKeen et al. (18) described a 1-µm-thick gelatinous layer surrounding individual conidia of *Erysiphe cichoracearum*. However, this layer was not convincingly illustrated. In a subsequent study, Martin and Gay (17) detected the presence of a much thinner "osmiophilic cuticle" on conidia, conidiophores, and hyphae of *Erysiphe pisi*. This cuticle appeared as two dark lines closely appressed to the surfaces of conidia, conidiophores, and hyphae. To us, neither this cuticle nor the gelatinous layer reported by McKeen et al. (18) corresponds to the extracellular mucilage on the conidium of *U. australiana*.

The results obtained from low-voltage SEM of conidia of *U. australiana* rival the best published results from frozen hydrated fungal specimens that we are aware of. In particular, the ability to visualize the extracellular mucilage coating conidia was remarkable. The meniscus-like profile of the mucilage visible in Figure 2B suggests that this material was in a liquid state. This idea is
supported by our observations that the extracellular mucilage flowed instantly from conidia onto strips of dialysis membrane. The time interval between touching the membranes to conidia and plunging the membranes into liquid propane was no more than 5–10 s. We suggest that the contact of this material with the dialysis membrane was the initial step in the adhesion process in *U. australiana*.

The material comprising the adhesive pad was shown to be

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**Fig. 3.** Transmission electron micrographs of *Uncinula australiana* conidia. A, Portion of a cross section of a conidium showing the serrate appearance of the wall (W). The raised, slightly pointed areas (arrowheads) correspond to the surface striations visible with light and the scanning electron microscope. In this conidium, glycogen particles (G) appear as electron-dense structures. B, Examples of a vacuole (V) in an extremely well-preserved conidium. The tonoplast is visible at the arrowhead. Glycogen (G) is present both in the vacuole and in the surrounding cytoplasm. C, A longitudinal section of a conidium in which the glycogen appears as electron-transparent areas in the cytoplasm and vacuoles (V). Two vacuoles are ruptured (arrowheads). The electron-transparent regions (asterisks) in the damaged vacuoles are holes in the section. The nucleus (N) with its eccentrically positioned nucleolus (Nu) is visible near the middle of the conidium. Also visible are the wall (W) and a thin layer of extracellular material (arrows) covering part of the conidium. D and E, Higher magnification views of longitudinal sections of the conidial wall (W). The point at which the section shown in E began to enter a ridge is shown at the arrowhead. Extracellular material is shown at the arrows. F, Conidial wall (W) labeled with WGA/ovomucoid gold complexes. Extracellular material is shown at the arrow. G, A control sample for the gold labeling of the wall (W). Bar in C = 3.6 μm; all others = 0.5 μm.
Fig. 4. Transmission electron micrographs of Uncinula australiana conidia. A, A portion of a cross section of a conidium stained using the modified Thiéry procedure. Visible are the wall (W) and glycogen particles (G). Some separation of the plasma membrane from the wall is visible at the arrows. B, Control sample for the modified Thiéry staining. The wall is shown at W and glycogen particles at G. C and D, Portions of longitudinal sections near the rounded ends of two conidia. Note the extracellular material (arrows) that is in direct contact with the wall (W) of each conidium. E, Cross section of a conidium on a piece of dialysis membrane (DM). Note the extracellular material or adhesion pad (arrowheads) that has been deposited on the dialysis membrane. F, Higher magnification view of the lower left part of E showing the fibrillar nature of the material comprising the adhesion pad (arrowhead) in contact with the dialysis membrane (DM). Note that this material appears to be continuous with the thin layer of extracellular material (arrows) that extends up along the side of the conidium. Various inclusions (asterisks) are visible in the material comprising the adhesion pad. G, Higher magnification view of the extracellular material of an adhesion pad associated with a conidium on a piece of dialysis membrane. The arrows indicate areas where the ridges on the conidial surface made imprints in the extracellular material. Bar in E = 2.0 μm; all others = 0.5 μm.
continuous with the thin layer of extracellular material associated with each conidium. The fact that this thin layer was separated from the conidial surface in samples examined using TEM was probably a fixation artifact resulting from either the freezing process or the subsequent manipulation of specimens during infiltration and embedding. However, considering the apparent liquid nature of this material, it is remarkable that any of it remained on conidia prepared for study with TEM.

Adhesion of ungerminated conidia has been reported in a variety of plant pathogenic fungi, including Nectria haematococca (6, 13, 15), Cochliobolus heterosporus (3), Magnaporthe grisea (9), and various species of Colletotrichum (20–22, 24, 33). However, unlike the situation in U. australiana, the adhesive materials in the species noted above do not appear to be present on conidia when they initially contact host or artificial substrates. For example, mature macroconidia of N. haematococca initially are nonadherent (6) but become adhesion competent after a 5-min exposure to zucchini fruit extract, apparently as a result of the release of mucilage from the tips of the conidia (15). Conidia of C. heterosporus begin to adhere about 20 min after hydration and inoculation (3). This ability to adhere also is correlated with the appearance of an extracellular matrix at the tip of the conidium. Conidia of M. grisea adhere by means of a spore tip mucilage that is released from the conidium by a breaking of the wall within 20 min of the onset of hydration (9). In the case of conidia of Colletotrichum graminicola, maximum adhesion on host leaves and hydrophobic surfaces occurs within 30 min of the time of contact (20, 22).

While successful results from plunger freezing have been reported for a few types of larger fungal samples (25, 28), the usefulness of this procedure generally is limited to samples no more than a few micrometers in diameter (11). We were, therefore, pleasantly surprised with the overall quality of preservation obtained for the rather large conidia of U. australiana. Additionally, very little ice crystal damage was observed in any of our samples, even though conidia of powdery mildew fungi were reported to have a very high water content compared to airborne spores of other fungi (12, 35). In this regard, it is possible that the large amount of glycocon present in each conidium may have served as a natural cryoprotectant during freezing. Overall, the quality of fixation we obtained compared favorably to that seen in a variety of different types of fungal spores prepared for study using similar protocols (24, 25, 27, 28).

LITERATURE CITED