Hippodamia convergens (Coleoptera: Coccinellidae) Dissemination of Dogwood Anthracnose Fungus (Melanconiales: Melanconeacae)

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ABSTRACT The fungal pathogen, *Discula destructiva* Redlin, has been responsible for the decline or death, or both, of flowering dogwood, *Cornus florida* L., in the eastern United States. Our research addressed the role of a selected insect in transportation and dissemination of *D. destructiva*. Densities of conidia on adults of our model insect, the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville, were quantified using scanning electron microscopy. Significantly more conidia were attached to the ventral body surface than to the dorsal surface. Also, conidia were deposited around trichomes of dogwood leaves exposed to infested convergent lady beetles. Our data demonstrate that our model, the convergent lady beetle, is capable of transporting conidia of *D. destructiva*. Because conidia may remain viable for 16 d, insects may play an important role in the epidemiology of dogwood anthracnose.

KEY WORDS *Hippodamia convergens*, dogwood anthracnose, insect dissemination of fungi, *Discula*, insect–plant interactions

THE FOLIAGE and berries of the flowering dogwood, *Cornus florida* L., native to the eastern United States, provide nutrients for >80 species of birds and mammals (Eyde 1988, Whitmore 1992). The lipid-rich berries are especially important to neotropical birds (Whitmore 1992). Dogwoods are also among the 1st trees to reestablish in disturbed forests (Boring et al. 1981). Aside from their ecological importance, dogwoods maintain an equal or greater economic distinction within the tourist and nursery industries. In the southeastern United States, spring blooms attract many tourists to national parks and town festivals. For example, the Dogwood Arts Festival in Knoxville, TN, contributes $12 million to the local economy (B. Neel, Dogwood Arts Festival, Knoxville, TN, personal communication). In 1987, wholesale dogwood sales in Tennessee were estimated to be $30 million (Windham and Freeland 1990) and attest to their popularity as an attractive addition to urban landscapes.

Dogwood anthracnose, a disease caused by the fungus *Discula destructiva* Redlin, has generated decline and mortality of native dogwoods in both woodland and urban habitats since the late 1970s (Daughtrey 1983, Redlin 1991). Disease symptoms are first observed as purple-rimmed, necrotic lesions on leaves in early spring or as conditions become conducive for fungal growth. Disease incidence and severity are dependent on temperatures of 25°C or less and 85% RH (Britton 1991, Parham and Windham 1992, Roncadori 1993). *D. destructiva* conidia in cirri often exude from subcuticular acervuli located below trichomes (Redlin 1991). Inoculum from infected leaves and branches is dispersed onto epicormic shoots. Invasion of the trunk through this young tissue induces canker formations that may coalesce and girdle the tree (Daughtrey et al. 1986, Hibben and Daughtrey 1988). Little attention has been directed toward research on dissemination of this pathogen. Although wind and water are considered mechanisms of dispersal of fungal conidia (Daughtrey et al. 1986), no data have been published defining dispersal mechanisms for this pathogen. Researchers have speculated that insects and birds may be involved in dissemination.

Insects spread some plant-pathogenic fungi. Several of the most destructive diseases of woody plants (for example, chestnut blight, Dutch elm disease, and oak wilt) are caused by insect-disseminated fungal pathogens (Webber and Gibbs 1989). During sporulation of *D. destructiva*, cirri which contain conidia within a protein matrix arise from acervuli. The adhesive properties of this matrix may foster dissemination of conidia by insects. Because insects do come in contact and may become infested with conidia, we designed a study to assess their role in epidemiology of dogwood anthracnose. Our specific objectives were to observe and quantify conidia of *D. destructiva* on insect body regions by way of scanning electron microscopy

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(SEM) and to observe conidia of *D. destructiva* deposited onto dogwood leaf surfaces by infested insects.

Materials and Methods

We selected the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville, as our model insect for this research. This species was chosen as a model because it was readily available and colonies are easily maintained in the laboratory. Convergent lady beetle also is encountered in urban and forested areas and has been collected from native dogwoods (J.F.C., unpublished data). Adult convergent lady beetles were obtained from Ricon-Vitova Insectaries (Oak View, CA). Colonies were maintained in clear Plexiglas cages (30.48 by 30.48 by 40.64 cm) with a screen-covered opening (12 cm) on each side. Convergent lady beetles were fed a mixture of honey and sugar, and water was provided by way of a cotton-plugged flask (250 ml). Cages were held in incubators maintained at 10–13°C and a photoperiod of 12:12 (L:D) h.

Stock cultures of *D. destructiva* isolate Va 17b were sustained on potato sucrose agar (Dhingra and Sinclair 1985) and dogwood leaf tissue. Cultures were maintained at 20°C in incubators with a photoperiod of 8:16 (L:D) h.

Two leaves from greenhouse-grown dogwoods were trimmed to fit within a glass petri dish (100 by 15 mm). Leaves, separated by a filter paper disk (9 mm), were placed into the dish which was then filled with deionized water. Leaves were autoclaved for 1 h for 2 consecutive days. Sterilized leaves were then placed on potato sucrose agar plates and inoculated with 1 plug (8 mm) of *D. destructiva* per dish from stock cultures. Cultures were incubated until sporulation occurred on leaf surfaces (≈2–4 wk).

To determine if conidia were carried on the exterior of insects, 5 convergent lady beetles were placed on each of the 2 *D. destructiva*-infested leaves for 1 h at room temperature (≈24°C). Adults were then etherized, placed in 2.5% glutaraldehyde and 0.1 M potassium phosphate buffer, pH 6.8, refrigerated for 24 h, and aspirated in a vacuum desiccator for 60 min or until beetles submerged. Adults were then rinsed in 0.1 M sodium cacodylate buffer, pH 7.2, fixed in a 4% osmium tetroxide aqueous solution for 2 h, rinsed in distilled water, and transferred through a 25, 50, and 75% acetone dehydration series for 24 h in each solution. Convergent lady beetles were then critical point dried and attached to SEM specimen mounts (5 dorsal aspect and 5 ventral aspect). Six convergent lady beetles, not exposed to *D. destructiva,* were also fixed and prepared for observation.

Body surfaces were observed under a SEM to quantify conidial densities on different body regions (dorsal aspect–head, pronotum, scutellum, right elytron, and left elytron; ventral aspect—mouthparts, prothorax, thorax, legs, and abdomen). The study was arranged as a completely randomized design with 5 replications per body aspect (dorsal and ventral). Each replication consisted of 1 infested beetle. A 6 factor rating scale was devised to quantify conidia in specific body regions (0 = no conidia, 1 = 1–10, 2 = 11–25, 3 = 26–50, 4 = 51–75, and 5 ≥ 76 conidia). Data were analyzed by general linear model and Waller–Duncan ratio *t*-test (SAS Institute 1985). Statistical analyses were conducted to determine significant ($P < 0.05$) differences between conidia densities on dorsal and ventral aspects; when significant differences were found, Waller–Duncan ratio *t*-tests were performed to determine significant differences among means (SAS Institute 1985).

To determine if convergent lady beetles could disseminate conidia of *D. destructiva,* infested beetles were allowed to walk on washed dogwood leaves. Six dogwood leaves from greenhouse-grown trees were placed under a No. 18 standard testing sieve and rinsed in tap water for 10 min each side to remove various types of fungal spores that had been deposited by air currents in the greenhouse. Leaves were placed in individual petri dishes, adaxial surface up. Twenty convergent lady beetles (5 per leaf), infested according to previously stated procedures, were allowed to walk on leaves for 4 h at room temperature. Four disks (11 mm) were cut with a No. 7 cork borer from each leaf and prepared for observation on a SEM according to methods used for convergent lady beetle preparation. Leaf disks were photographed to document areas where conidia were deposited. The remaining 2 leaves served as controls and were not exposed to infested beetles. Disks were also cut from these leaves and evaluated in the same manner as described above.

Results and Discussion

Convergent lady beetles carried conidia of *D. destructiva* on their external body surfaces and deposited these conidia onto dogwood leaves. The ventral aspect of the body surface had significantly more conidia than the dorsal aspect ($F = 3.79$; df = 13, 35; $P < 0.05$ level, *t*-test) (Table 1). Regions rated as 5 often had a conglomerate of conidia, cirral slime matrix and setae massed together. These masses made conidia impossible to separate and prevented us from calculating an exact conidial count per region.

Numbers of conidia were not significantly different among ventral body regions. Convergent lady beetles were observed to groom their conidia-laden appendages, which would partially explain the conidia-encrusted mouthparts. Conidia were also observed among setae, singularly or in clumps, sometimes incorporated within a cirral slime matrix on legs, thorax, and abdomen (Fig. 1).

On the dorsal aspect, the pronotum had significantly more conidia than other dorsal regions. The

Table 1. Mean rating comparisons of densities of *D. destructiva* conidia on specific body regions of *H. convergens*

Body aspect	Location	Mean	Range
Dorsal	Head	1.0c	0–3
	Pronotum	5.0a	5–5
	Scutellum	3.0b	0–5
	Left elytron	3.0b	1–5
	Right elytron	4.0ab	2–5
Ventral	Mouthparts	3.6ab	1–5
	Prothorax	5.0a	3–5
	Thorax	4.8ab	4–5
	Legs	4.6ab	3–5
	Abdomen	4.0ab	2–5

Values within a column followed by the same letter are not significantly different ($P > 0.05$, Waller–Duncan k ratio t-test). Mean rating was determined by assessing conidial densities on insects using the following scale: 0 = none, 1 = 1–10, 2 = 11–25, 3 = 26–50, 4 = 51–75, and 5 ≥ 76. Range, minimum and maximum ratings for each body region ($n = 5$).

dorsal head area possessed the least number of conidia of any region, dorsally or ventrally. Conidia were scattered across the pronotum and found in pits and crevices along the junction of the elytra. Conidia on the scutellum were often observed in setal sockets. The dorsal surface came in contact with conidia as convergent lady beetles moved over top of one another or fell from the petri dish lid onto the leaf. Presence of conidia within regions on the dorsal surface verify that such transportation can occur; however, conidia on this surface are more vulnerable to desiccation than those carried on the ventral surface. Conidia on the dorsal surface were not observed embedded in matrix as on the ventral surface which may help to retard the effects of environmental factors on conidial viability. Therefore, conidia on the dorsal surface are less likely to be a virulent source of inoculum. No conidia were observed on the controls (that is, convergent lady beetles not exposed to *D. destructiva*).

Our observations were similar to those reported for the bark beetle *Scolytus scolytus* F. carrying the fungus *Ophiostoma ulmi* Buisiman, the pathogen causing Dutch elm disease. This bark beetle was reported to carry fungal spores of *O. ulmi* randomly scattered or lodged in setal pits on their body surfaces (Webber and Gibbs 1989). Also, spores of *0. ulmi* are contained within a sticky mucus droplet (Wilson et al. 1970), which may help them adhere to their insect vector. Likewise, conidia of *D. destructiva* are embedded in a sticky slime matrix that oozes from acervuli during sporulation.

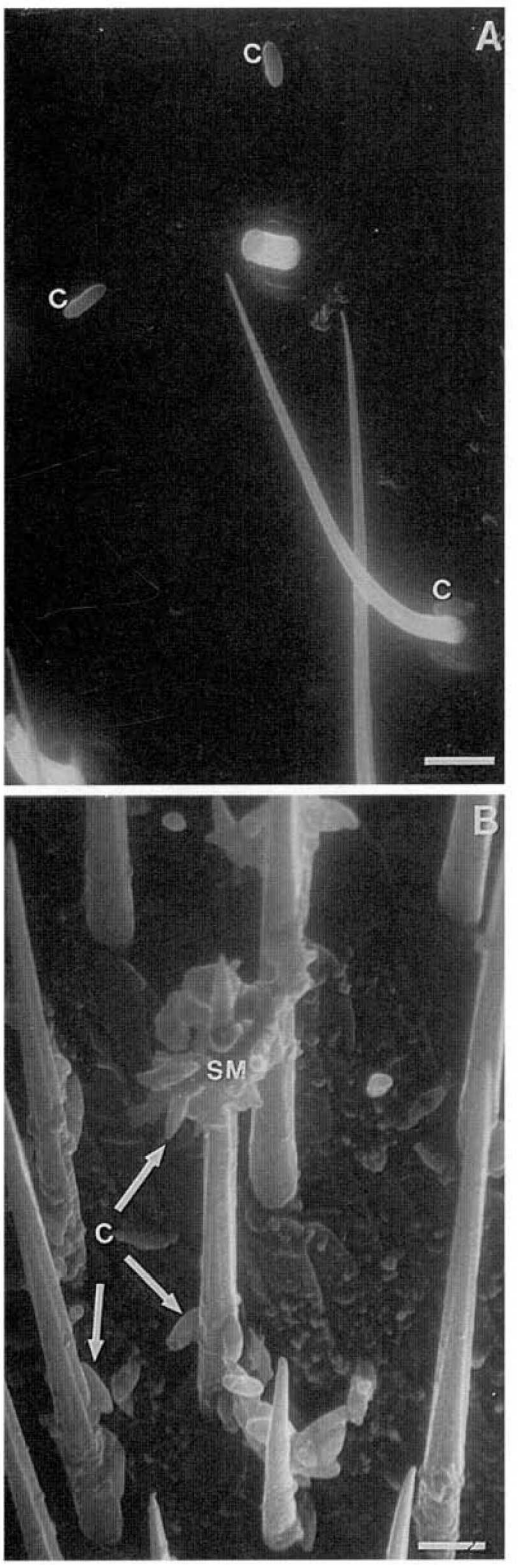

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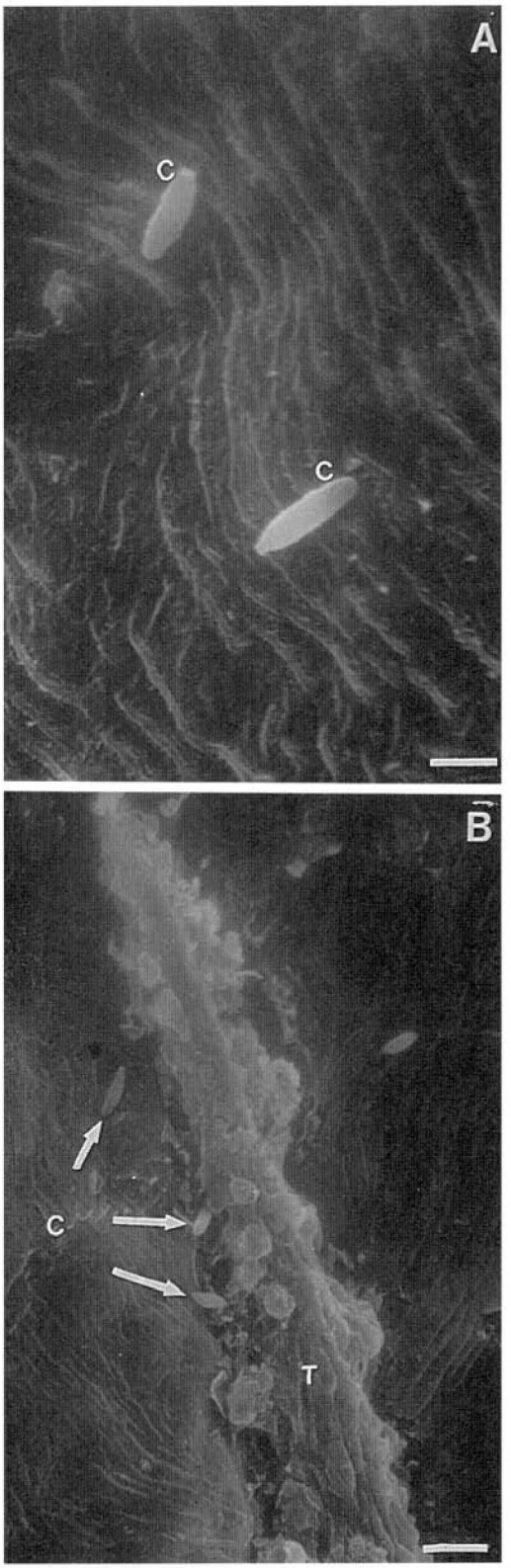
Fig. 1. SEM of *D. destructiva* conidia on the body surface of *H. convergens*. (A) Conidia on mesosternum (800×, bar = 12 μm). (B) Acervular slime mass, containing conidia, speared by seta; and, conidia scattered on surface of mesothoracic tibia (640×; bar = 16 μm). C, conidium; SM, slime mass.



Convergent lady beetles did disseminate conidia to the leaf surface. Most conidia were deposited around trichomes with many conidia located beneath trichomes (Fig. 2). Leaves not exposed to infested convergent lady beetles had no signs of contamination. The raised, 2-armed morphology of dogwood trichomes may mechanically dislodge conidia from the beetle. Depressions in the leaf epidermis around the base of a trichome may provide a suitable environment for conidia germination or penetration, or both. Because the base of a trichome is an area where deposited conidia persist, the location may be susceptible to fungal invasion.

Conidia were carried on the dorsal and ventral body surfaces of adult convergent lady beetles and were deposited onto dogwood leaves. Most conidia were probably disseminated from regions on the ventral surface (that is, mouthparts, legs, thorax, and abdomen) to trichomes on the adaxial leaf surface. Conidia on the ventral surface may be shielded from desiccation and UV radiation for longer periods of time, thereby increasing the probability for deposition of viable inoculum to a susceptible host. Our results conclusively demonstrated that our model insect, the convergent lady beetle, was capable of acquiring, transporting, and depositing conidia of *D. destructiva*. Other studies have shown that convergent lady beetles can disseminate viable conidia externally or internally, or both, for as many as 16 d in a laboratory environment (Colby 1993). Also, inoculation of healthy *C. florida* trees by *D. destructiva*-infested convergent lady beetles, in a climate-controlled greenhouse chamber, resulted in infection of both wounded and nonwounded tissue (Colby 1993). Thus, our data predicate dissemination of conidia by other insect species and support the idea that insect species may play an important role in the localized spread of dogwood anthracnose. More importantly, these results underscore the need for evaluation of exposed and nonexposed insect species in regard to dissemination potential. Additional research will further our understanding of the role of insects in the epidemiology of dogwood anthracnose.

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Fig. 2. SEM of *D. destructiva* conidia deposited by *H. convergens* to the leaf surface of *C. florida*. (A) Conidia scattered across leaf surface (1600×; bar = 6 μm). (B) Conidia around and under trichome (680×; bar = 13 μm). C, conidium; T, trichome.

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