Transportation and Viability of Conidia of *Discula destructiva* on *Hippodamia convergens*

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ABSTRACT

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This research was designed to evaluate the role of insects in dissemination of *D. destructiva* conidia. Results confirmed that our model insect, the convergent lady beetle, *Hippodamia convergens,* could transport viable conidia externally and internally. Also, conidia carried externally remained viable for as long as 16 days. This research suggests that insects may play a role in localized spread of dogwood anthracnose.

Additional keywords: *Cornus florida,* dogwood

Flowering dogwood, *Cornus florida* Link, is threatened by dogwood anthracnose, caused by the fungus *Discula destructiva* Redlin, in the eastern United States (3,8). Little information has been generated about mechanisms for dispersal of the fungus beyond that of water-splash dissemination (6). Researchers have hypothesized that insects, partially because of their abundance and diversity, may be involved in the spread of the pathogen. Therefore, the objectives of this research were to determine if insects could carry viable conidia of *D. destructiva* externally and/or internally and, if so, to determine the length of time viable conidia could be carried and deposited by insects.

MATERIALS AND METHODS

The convergent lady beetle (CLB), *Hippodamia convergens* Guérin-Méneville, was chosen for the model insect because of its availability for research purposes, easy maintenance in the laboratory, and its presence in urban and forested areas (J. F. Grant, unpublished). Adult CLBs were used in all experiments and purchased from Ricon-Vitova Insectaries, Inc. (Oak View, CA). Colonies of CLBs were maintained in clear Plexiglas cages (30.48 × 30.48 × 40.64 cm) with a screen-covered opening (12 cm) on one side and fed a mixture of honey and sugar. Water was dispensed via a cotton-plugged flask (250 ml). Cages were held in incubators maintained at 10 to 13°C and 12:12 h light/dark cycles.

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Culture preparation and beetle infestation. Stock cultures of *Discula* isolates VA 17b (obtained from J. Knighten, U.S. Forest Service, Asheville, NC) and TN 8 (local isolate) were maintained on potato sucrose agar (PSA) (4) and dogwood leaf tissue. Leaves from greenhouse-grown dogwoods were trimmed to fit within glass petri dishes (100 × 15 mm) that were filled with deionized water, autoclaved for 1 h on two consecutive days, and placed on PSA. A plug (8 mm) of one of the two fungal isolates was placed in each dish adjacent to leaf tissue. Cultures were maintained at 20°C in incubators with 8:16 h of light/dark for about 3 weeks, when sporulation usually occurred.

Procedures for infesting CLBs with *D. destructiva* were the same for both dilution and time interval experiments. Adult CLBs were placed on the sporulating cultures of *D. destructiva* (10 per PSA dish) and allowed to walk inside the dish for 1 h at 24°C.

Surface dilutions. Infested CLBs (n = 20 per isolate) and noninfested CLBs (n = 5 per control) were surface rinsed with a 10^3 serial dilution of sterile water for removal of external conidia. Each CLB was placed in a test tube (17 × 100 mm) with 10 ml of sterile water. The tubes were shaken on a S/P Vortex Mixer for 10 s. One-milliliter aliquots of the water were dispensed onto PSA amended with 25 mg/liter each of chlortetracycline and streptomycin sulfate. One milliliter of the water was also put into the next test tube in the series. The same steps were repeated for the remaining two dilutions.

Dilutions were incubated until sporulation of *D. destructiva* was observed. After the first week of growth, subculturing was necessary. Sterile dogwood leaf pieces were added to the dishes to encourage sporulation. Wet mounts from sporulating acervuli were viewed for *D. destructiva* conidia as described by Redlin (10).

Maceration dilutions. CLBs from the previous experiment were surface disinfested, macerated, and placed on PSA to detect growth of viable conidia carried internally. CLBs were placed singly into 5 ml of Clorox and vortexed for 1 min. Adult CLBs were then transferred to 5 ml of sterile water, vortexed for 30 s, removed, and macerated with mortar and pestle in 10 ml of sterile water. Dilutions were performed in the same manner as those for external rinsing. Plates were incubated and examined as described above.

Time trials. To determine the length of time CLBs could carry and deposit viable conidia, a 32-day period consisting of nine time intervals (0, 6, 12 h, and 1, 2, 4, 8, 16, and 32 days) was designated as the duration of the experiment. For each isolate, five infested CLBs per interval were individually placed in petri dishes on moistened filter paper. A drop of honey, which served as the food source, was added to each petri dish. Live CLBs, at each interval, were then removed from their dishes, placed on PSA, and allowed to move freely within the dish. After 4 h, CLBs were discarded and the plates were incubated. Three noninfested CLBs per interval, subjected to the same procedures, were used as controls. Presence of *D. destructiva* was confirmed microscopically. The initial experiment was conducted with only isolate VA 17b. The experiment was then repeated with isolates 17b and TN 8.

PROC ANOVA (SAS Institute, Cary, NC) was used to interpret data from time interval studies. The percentage of CLBs depositing viable conidia at specific time intervals after infestation was calculated, and a regression equation that best fit the data was determined.

RESULTS AND DISCUSSION

Surface and maceration dilutions. All CLBs transported viable conidia externally. As the CLBs walked around on the leaves in the petri dishes, they came in contact with the sporulating acervuli of *D. destructiva* and conidia adhered to their body parts. Ingold (9) stated that "Most insect-disseminated conidia are slimy." *D. destructiva* was no exception; the protein matrix that envelops the conidia appeared to be viscid, thus allowing conidia to re-

main attached to CLB body parts. The adhesive property of the matrix held conidia to CLB bodies during the fixing process for microbial observation. Scanning electron microscopy showed conidia on both dorsal and ventral body aspects and many conidia were observed on mouth parts, as well as on other appendages (1,2).

Viable ingested conidia were detected 95% (VA 17b) and 100% (TN 8) of the time. No colonies of *D. destructiva* were isolated from control CLBs in either test. Colony growth and sporulation occurred sooner (approximately 1 week) with conidia carried internally, than externally; however, exact times were not determined. Enhanced growth and sporulation observed for internally carried conidia in this study may partially be explained by Dillon and Charnley (5), who reported that enzymes in digestive tracts of insects could dissolve the protein matrix that envelops conidia and thus decrease the time required for spore germination. Research on arthropod dissemination of the fungus, *Phytophthora palmivora* Butl., that causes black pod of cocoa, indicated that certain beetle species, caterpillars, and millipedes contained viable spores in their fecal material (7). Although conidia of *D. destructiva* were found internally, these experiments did not distinguish between internal body parts and waste products. Further research is necessary to assess the presence of viable conidia *D. destructiva* in insect feces.

Time trials. Viable conidia were carried and deposited for as many as 16 days after infestation (Fig. 1). Immediately after exposure to the fungus (0 h), 100% ($n = 5$) of the CLBs deposited viable conidia to PSA. At the 16-day interval, 15% were depositing conidia, and by day 32 the beetles had either died or were not carrying viable conidia. A polynomial regression equation best fit the data ($Y = -0.013X^3 + 0.758X^2 - 13.938X + 95.558$, and $R^2 = 0.85$). VA 17b data only are represented in Figure 1. In a second study comparing both isolate types, all beetles died by 8 days. CLBs used in the second study had been stored in conditions that encouraged hibernation for several months. However, in all intervals up to 8 days, no differences existed in dissemination or viability of the two isolate types. PSA plates exposed to control CLBs showed no signs of growth of *D. destructiva.*

In insect-disseminated fungal diseases of trees where the disease cycle is known, insects have the capacity to vector several hundred thousand spores. Nitidulid beetles, responsible for the spread of oak wilt, have been reported to carry 760,000 spores on both the external body surface and via the gut (12). Quantity, however, does not indicate viability. For example, in a study involving bark beetles, *Scolytus scolytus* F., these insects were found to carry more than 300,000 spores of *Ophiostoma ulmi* Buisman, the pathogen of Dutch elm disease. Spores of *O. ulmi* desiccate at relative humidities below 80% and under UV light encountered during daylight hours. Thus, the percentage of beetles carrying viable spores after flight was reduced from 98 to 10% (12).

Conidia of *D. destructiva* remained viable for 16 days at 20°C with relative humidity 51 to 60%, but as humidity increased up to 90%, viability decreased from 90 to 10% (11). Results from the time trial experiment may have been influenced by moistened filter paper in the petri dishes. If no water had been added to the dishes, a greater percentage of beetles may have deposited viable conidia at the 16-day interval. However, without a source of moisture, CLBs may not have survived longer than 8 days. It is unlikely that many CLBs, if any, would have carried viable conidia for more than 16 days, based on other research (11).

In a related study, CLBs were found to deposit conidia onto dogwood leaf surfaces, and those leaves were symptomatic for dogwood anthracnose several weeks later (1). This research confirmed that the model insect could transport viable conidia externally or internally and for as many as 16 days. Therefore, insects could carry viable spores for sufficient time to be effective vectors of dogwood anthracnose. Further research is necessary to determine if other types of insects can transport viable conidia and to determine the incidence of conidia of *D. destructiva* on field-collected insects. This information will enable us to better define the role of insects in localized spread of dogwood anthracnose.

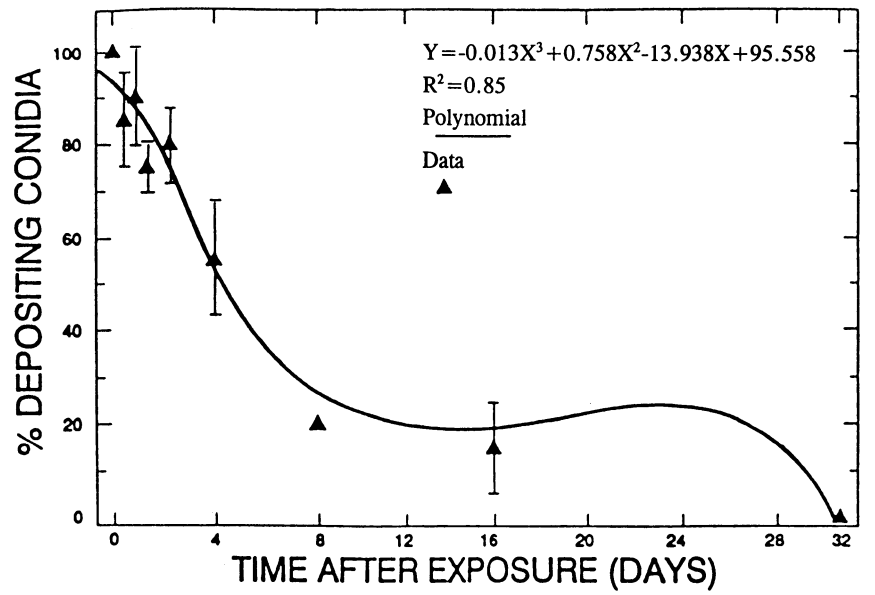

Fig. 1. Percentage of *Hippodamia convergens* depositing viable conidia of *Discula destructiva* at specific time intervals after infestation.

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