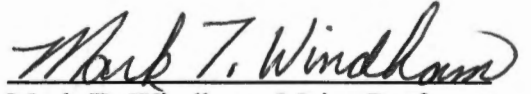
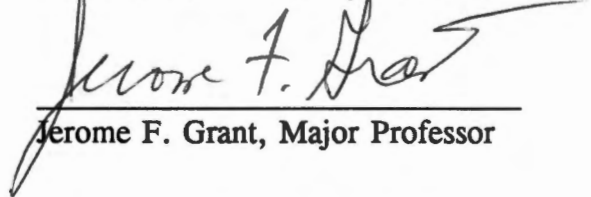


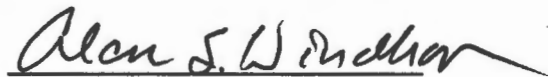
To the Graduate Council:

I am submitting herewith a thesis written by Deanna M. Colby entitled "Arthropod Dissemination of *Discula destructiva* Conidia on *Cornus florida*." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

  
Mark T. Windham, Major Professor

  
Jerome F. Grant, Major Professor

We have read this thesis  
and recommend its acceptance:





Accepted for the Council:

  
Associate Vice Chancellor  
and Dean of The Graduate School

**ARTHROPOD DISSEMINATION OF *Discula destructiva***  
**CONIDIA ON *Cornus florida***

A Thesis  
Presented for the  
Master of Science  
Degree  
The University of Tennessee, Knoxville

Deanna M. Colby

December 1993

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## ABSTRACT

In 1978, a fungal disease, identified as *Discula destructiva* Redlin sp. Nov., was discovered killing native flowering dogwoods, *Cornus florida* Link., in the northeastern United States. Since then, the pathogen has spread southward along the Appalachian mountain range to Georgia and Alabama, infecting dogwoods on more than 2.3 million hectares.

Known methods of pathogen dissemination include wind and rain. Other possible mechanisms for dispersing fungal conidia involve animal vectors, such as arthropods which may carry conidia externally and/or internally. The objective of this research was to determine if arthropods could disseminate viable *D. destructiva* conidia. The adult convergent lady beetle (CLB), *Hippodamia convergens* Guerin-Ménèville (Coleoptera: Coccinellidae), was selected as the model insect for this research.

In experiments testing for viability of conidia carried externally and internally, CLBs exposed to TN 8 carried viable conidia externally and internally, 100% of the time. All beetles infested with VA 17b carried viable conidia externally and 95% internally. These results were supported by data from time trials in which infested CLBs carried and deposited viable conidia as many as 16 d after exposure to the inoculum.

Scanning electron microscopy of CLB body surface showed significantly more conidia were attached to the ventral surface. No difference in conidia densities were

observed among body regions on the ventral surface; however, regions within the dorsal surface did have significantly different amounts of conidia.

Greenhouse studies to determine if infested CLBs could initiate infection of healthy *C. florida* trees produced significant differences when infection by non-inoculated CLBs, no CLBs, and inoculated CLBs were compared. Ninety percent of trees treated with inoculated CLBs displayed symptoms of *D. destructiva* infection. Scanning electron micrographs of leaves exposed to infested CLBs were observed for locations of deposited conidia. Conidia were deposited around trichomes on the adaxial *C. florida* leaf surface.

Insects are abundant and active organisms that inhabit the forest/urban ecosystems and that, indirectly, may play a role in epidemiology of dogwood anthracnose. Results from this research have indicated that insects can disseminate viable *D. destructiva* conidia and may be involved in the rapid movement of the pathogen along the eastern United States throughout *C. florida*'s habitat range.

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## CHAPTER I

### INTRODUCTION

*Cornus florida* Link. is the flowering dogwood native to eastern hardwood forests. The foliage and berries provide nutrients for more than 80 species of birds and mammals. Migrating birds are especially dependent on the lipid rich berries as a high energy food source (Eyde 1988, Whitmore 1992). Dogwoods are also among the first trees to reestablish in disturbed forests and augment soil fertility with the calcium in their leaves (Boring et al. 1981).

Aside from the ecological importance of *C. florida*, these trees maintain an equal or greater economic distinction with the nursery industry. In the southeastern United States, spring blooms attract many tourists to national parks and town festivals to admire the beauty of the floral bracts amid the forest understory or along scenic drives. Annual dogwood sales in excess of \$100 million attest to their popularity as an attractive addition to urban landscapes (K. Tilt, Auburn Univ., personal communication).

In 1978, a lower twig dieback was documented on the western flowering dogwood, *Cornus nuttallii* Audubon, in Washington and was named dogwood anthracnose (Hibben and Daughtrey 1988, Salogga and Ammirati 1983). Concurrently, a decline in tree health, in both native and cultivated *C. florida* was observed in New York (Daughtrey 1983). By 1993, the disease had spread

throughout the northeastern United States, down the Appalachian Mountain range to northern Alabama and Georgia (Figure 1). In the southeast, devastation of native stands was reported on 2.3 million hectares (Anderson 1991). The disease was most severe in cool, wet forests at elevations of 914 m and above, and little to no disease was found along southern coastal regions (Anderson 1991, Bruck and Grand 1993, Chellemi and Britton 1992, Windham et al. 1992).

The disease was first believed to be caused by *Colletotrichum gloeosporioides* Penz. (Pirone 1980). However, shortly after, the pathogen was correctly identified as a member of the *Discula* genus (Hibben and Daughtrey 1988) and was later described as *Discula destructiva* Redlin sp. Nov. (Melonconeaceae). The sexual stage, if existent, would be similar to Ascomycetes in the genera *Apiogonomia* or *Gnomoniella* (Redlin 1991).

First observed are purple-rimmed, necrotic lesions on leaves. These lesions begin to appear in early spring or as conditions are conducive to fungal growth. Disease incidence and severity are dependent upon temperatures of 25 C or less and RH of 85% (Britton 1991, Parham and Windham 1992, Roncadori 1993). Leaves that become completely necrotic do not senesce in autumn months. This retention of leaves has served as a primary indicator of infection and has served as a "tool" for researchers to monitor disease spread. Growth of epicormic shoots is initiated by loss of apical dominance due to infection and subsequent death of meristematic buds. Inoculum from infected leaves and branches is dispersed onto the shoots.

**DISTRIBUTION OF DOGWOOD ANTHRACNOSE  
IN THE  
EASTERN UNITED STATES  
March 5, 1993**



Figure 1. 1993 Distribution of dogwood anthracnose in the eastern United States.

The succulent tissue provides direct access to the trunk. This invasion induces canker formations which may coalesce and girdle the tree (Daughtrey et al. 1986, Hibben and Daughtrey 1988).

Even though much information has been discovered about the pathogen since it was first reported, few studies have addressed issues of dissemination and transmission of *Discula*. Two methods of dissemination of *D. destructiva* have been documented: wind and rain. For example, conidia can be dispersed from infected tissues by strong winds or rain droplets during storms to susceptible hosts (Daughtrey 1983). Dissemination via animals that feed on diseased dogwoods or insect "vectoring" may also occur. Cases in which insects disperse fungi have proven to be some of the most destructive and widespread diseases of woody plants (e.g., Dutch elm disease, oak wilt, chestnut blight, black pod of cocoa, and various diseases of conifers) (Webber and Gibbs 1989). Arthropod dissemination of fungal pathogens is not limited to any one group of insects. However, 80% of the 23 diseases where insects disseminate a fungal pathogen to a woody plant involves members of the order Coleoptera. Location of the fungus on the host plant and habits of insects that visit the plant regulate the type of insect that is exposed to the inoculum (Webber and Gibbs 1989).

Effective dissemination of a pathogen by an insect to a suitable host is determined by morphological and behavioral characteristics of the insect, physical properties of the fungus, environmental conditions, and host plant predisposition (Webber and Gibbs 1989). Most fungi are disseminated on the insect's exterior.

Some arthropods have specialized organs, mycangia, which facilitate conidia transport. However, the majority of insects does not possess mycangia; thereby, dissemination is dependent upon physical properties of the fungus. For instance, Dutch elm disease caused by *Ophiostoma ulmi* Buisman is transported on the external body surface of a bark beetle, *Scolytus scolytus* Fabricius, to elm. Conidia of this fungus are embedded in a sticky, mucus droplet and adhere to the beetle's body in pits and crevices. Another example involves nitidulid beetles which are responsible for transporting *Ceratocytis fagacearum* Bretz, the fungal pathogen for oak wilt disease. Ascospores of this fungus are also exuded in a sticky droplet (Alexopoulos and Mims 1979). They may be carried externally similar to that of *O. ulmi*, or the spores can be carried internally, through the gut (Webber and Gibbs 1989).

Environmental conditions such as temperature and moisture are key factors in dissemination of a pathogen, regulating both the type of insect involved and the viability of the fungus (Webber and Gibbs 1989). For example, dogwoods are pollinated mainly by syrphid flies and andrenid and halictid bees. However, in areas where the temperature is too cool for bees, syrphids are responsible for most of the pollination (Eyde 1988). Temperature and moisture levels must also be suitable for fungal growth and sporulation.

Host plant predisposition determines the susceptibility of the infection court to pathogen invasion. Healthy dogwoods may be predisposed to *D. destructiva* invasion by environmental factors or injury (Erbaugh et al. 1993). Several acid rain studies have demonstrated that decreased pH ( $\leq 5$ ) deteriorates the leaf cuticle and increases

the possibility of infection (Anderson et al. 1989, 1991). Insect damage, such as that caused by the dogwood borer, *Synanthedon scitula* Harris, may predispose trees to infection and/or increase symptom severity once infected (Walton 1986).

The objective of this study was to determine if the convergent lady beetle (CLB), *Hippodamia convergens* Guerin-Ménèville (Coleoptera: Coccinellidae), could disseminate viable *D. destructiva* conidia. The adult CLB was selected as the model insect in this research because they are found in almost every field and forest habitat in North America and have been collected from dogwoods (Borror and White 1970, White 1983, J.F. Grant, unpublished data). The potential and evident devastation *D. destructiva* has caused in native dogwood stands was realized by both scientific and public domains shortly after its discovery (Anderson 1991, Bailey and Brown 1991).

In 1991, 94% (n=100) of the counties surveyed in seven southeastern states had diseased trees (Anderson et al. 1991). An urgency exists to locate and identify *C. florida* germplasm resistant to *D. destructiva* before native stands are depleted (Graham and Windham 1993). To reduce the impact and spread of dogwood anthracnose in urban areas, homeowners are requested to purchase trees from reputable nurseries, use proper planting and maintenance techniques, and apply fungicides when necessary (i.e., chlorothalonil, propiconazole, or thiophanate-methyl + mancozeb) (Stipes 1992). Research on insect involvement in dissemination of the pathogen may provide some insight as to potential "vectors" and/or modes of conidia dispersal and may lead to further, more effective means of control.

## CHAPTER II

### DISSEMINATION OF *Discula destructiva* CONIDIA BY CONVERGENT LADY BEETLE, *Hippodamia convergens*

#### i. INTRODUCTION

Eastern flowering dogwood, *Cornus florida* Link., is often found in urban landscapes and parks. The dogwood's early spring bloom attracts many tourists to national parks and festivals throughout the southeastern United States. Beginning in the late 1970's, dogwood anthracnose, caused by the fungus *Discula destructiva* Redlin sp. Nov., was reported to cause dieback and decline of native dogwoods in forests in the northeastern United States (Daughtrey 1983, Redlin 1991). Characteristic symptoms range from purple-rimmed leaf spots to trunk cankers (Daughtrey et al. 1986). Recent accounts of disease progression have documented infected trees as far south as Alabama and Georgia (Bailey and Brown 1991, Hess 1992).

In the eastern United States, dogwood sales reach approximately \$100 million annually, contributing \$35-40 million to the Tennessee nursery economy (K. Tilt, Auburn Univ., personal communication). Adverse publicity about dogwood anthracnose sparked public concern and lessened interest in dogwoods as an ornamental. Consequently, nurserymen have noted up to a 60% reduction in dogwood sales across the United States (M.T. Windham, personal communication).

The objectives of this research were to determine if insects could carry viable *D. destructiva* conidia externally and/or internally, and, if so, to determine the length of time viable conidia could be carried and deposited by insects. The convergent lady beetle (CLB), *Hippodamia convergens* Guerin-Ménèville (Coleoptera: Coccinellidae), was chosen as the model insect for this study. These insects, readily available for research purposes, have been collected from dogwoods (J.F. Grant, personal communication).

## ii. MATERIALS AND METHODS

CLBs purchased from Ricon-Vitova Insectaries, Inc. (Oak View, CA 93022) were maintained in clear plexiglas cages (30.48 x 30.48 x 40.64 cm) with a screen-covered opening (12 cm) on one side. CLBs were fed a mixture of honey and sugar, and 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.

Two isolates of *D. destructiva*, TN 8 (type 1) and VA 17b (type 2), were used as the sources of inoculum. Isolates of *Discula* were type grouped on the basis of mycelial growth patterns in culture. Type 1 isolates tended to grow with irregular borders; whereas, type 2 isolates grew in concentric rings. Also, further studies have shown type 2 isolates to be different from type 1 by the lack of catechol oxidases (Trigiano et al. 1991). *Discula* of the type 2 lineage are suspected to be native to the eastern United States. More numerous genomic variations existed within members of the type 2 group as compared to those in type 1. These genetic differences led

researchers to hypothesize that the presence of type 1 in the United States most likely occurred due to introduction at a port location (Trigiano et al. 1993).

Stock cultures of isolates were maintained on potato sucrose agar (PSA) (Dhingra and Sinclair 1985) and *C. florida* leaf tissue. Leaves from greenhouse-grown *C. florida* trees were trimmed with scissors to fit inside glass petri plates (100 x 15 mm). Six leaves/plate were placed adaxial side down and separated from each other by filter paper disks (9 mm). Plates were filled with deionized water, autoclaved for 1 h, allowed to incubate at room temperature (24 C) for 24 h, and autoclaved for another 1 h. Leaves were then placed, adaxial side up, on PSA and inoculated with one *Discula* plug/PSA dish (8 mm). Cultures were maintained at 20 C in incubators with 8:16 h of light:dark. Plates were ready to be used for infesting CLBs when sporulation occurred (ca. 2 wk); unused plates were replaced monthly.

Procedures for infesting CLBs with *D. destructiva* were the same for both dilutions and time interval experiments. Adult CLBs, 10/PSA dish, were placed on the sporulating *Discula* and allowed to walk inside the dish for 1 h at 24 C.

#### Surface dilutions

Infested CLBs (n=20/isolate type) were surface rinsed in sterile water for removal of external conidia. Individual CLBs were transferred through a 10<sup>3</sup> dilution series of sterile water. An infested CLB was placed in a test tube (17 x 100 mm) with 10 ml of sterile water. The tube was capped and vortexed for 10 s. One ml of the rinse water was dispensed onto a PSA plate amended with 25 mg/L each of chlortetracycline and streptomycin sulfate. Another 1 ml was put into the next test

tube in the series, containing 9 ml of sterile water. The same steps were repeated for the remaining two dilutions.

Dilutions were incubated until *Discula* sporulation was observed. After the first week of growth, subculturing was necessary from some of the PSA dilution plates. Wet mounts from sporulating acervuli were viewed for *D. destructiva* conidia as described by Redlin (1991).

#### Maceration dilutions

CLBs from the previous experiment were surface disinfested, macerated and plated to detect growth of viable conidia carried internally. CLBs were placed singly into 5 ml of Clorox <sup>®</sup>, vortexed for 1 min, rinsed and vortexed in 5 ml of sterile water for 30 s, removed, and macerated with mortar and pestle in 10 ml of sterile water. Dilutions continued in the same manner as those for external rinsing with each dilution being plated onto PSA with antibiotics. Dilutions were incubated and verified for *Discula* growth as stated previously.

#### Time trials

To determine the length of time CLBs could carry and deposit viable conidia, a 32 d period, consisting of nine time intervals (i.e., 0, 6, 12 h, and 1, 2, 4, 8, 16, and 32 d), was designated to be the duration of the experiment. Five infested CLBs/interval/isolate type were each placed in petri dishes lined with a filter paper disk and labeled as to time interval. Filter paper was moistened and a drop of honey served as the food source. Live CLBs, at each interval, were placed on PSA, for 4

h. CLBs were then discarded, and PSA was incubated. Fungal cultures were verified microscopically for *D. destructiva*.

PROC ANOVA was used to interpret data from time interval studies. A regression equation which best fit the data was determined (SAS Institute 1985). Data points were plotted on a graph to depict the percentage of CLBs depositing viable conidia over time intervals.

### iii. RESULTS AND DISCUSSION

#### Surface and maceration dilutions

All CLBs transported viable conidia externally. Ingested conidia were viable and/or transported 95% and 100% of the time for VA 17b isolate and TN 8 isolate, respectively. Isolate type did not affect *Discula* growth on PSA. Growth of VA 17b and TN 8 colonies was 97.5 and 100%, respectively (n=40). It was noted that colony growth and sporulation occurred sooner (ca. 1 wk) with conidia carried internally, than externally; however, exact times were not determined. It is possible that enzymes in the CLB's digestive tract dissolve the protein matrix which envelops *Discula* conidia thereby decreasing the time required to germinate (Dillon and Charnley 1991).

#### Time trials

Viable conidia were carried and deposited for as many as 16 days after infestation (Figure 2). Immediately after exposure to the fungus (0 h), 100% of the CLBs deposited viable conidia to PSA. At the 16 d interval, only 15% were depositing conidia and by day 32, the beetles had either died or were not carrying

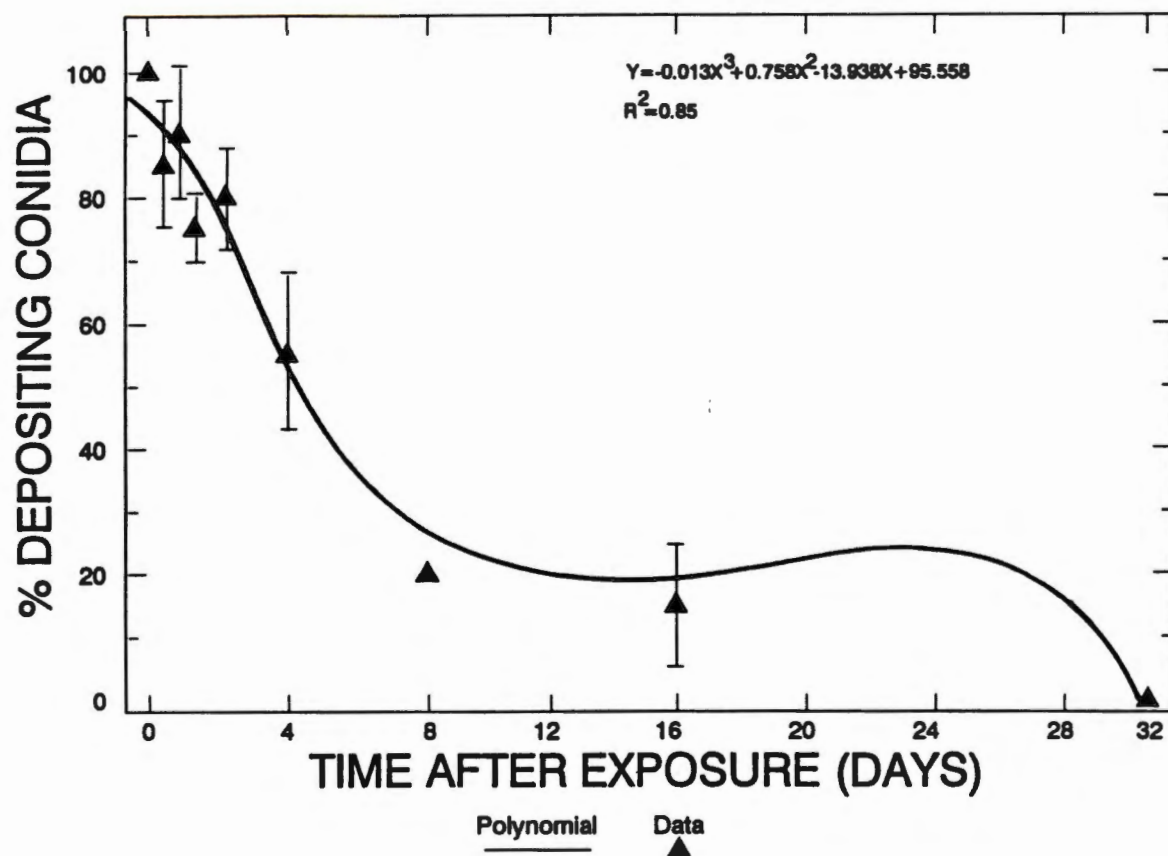


Figure 2. Percentage of *Hippodamia convergens* depositing viable *Discula destructiva* conidia at specific time intervals after infestation.

viable conidia. A polynomial regression equation best fit the data  $Y = -0.013X^3 + 0.758X^2 - 13.938X + 95.558$ , an  $R^2 = 0.85$ . VA 17b data only are represented in Figure 2. In a repeated study comparing both isolate types, all beetles died by 8 d. CLBs used in the second study had been stored in conditions which encouraged hibernation for several months. However, it was noted that in all intervals up to 8 d, no differences existed in dissemination or viability of the two isolate types.

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 which are 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 (Webber and Gibbs 1989). Quantity, however, does not indicate viability. For example, in a study involving bark beetles, *Scolytus scolytus* Fabricius, these insects were found to carry more than 300,000 spores of *Ophiostoma ulmi* Buisman, the causal pathogen of Dutch elm disease. The percentage of beetles carrying viable spores after flight was reduced from 98% to 10%. Spores of *O. ulmi* were found to desiccate at relative humidities below 80% and under UV light encountered during daylight hours (Webber and Gibbs 1989).

Research on *Discula* survivability at different temperature and humidity levels revealed that conidia would remain viable for 16 d at 20 C with RH 51-60%, but as humidity increases up to 90%, viability decreases from 90 to 10% (Roncadori 1993). Results from the time trial experiment may have been influenced by the moistened

filter paper in the petri dishes. It is possible that if no water had been added to the dishes, conidia may have remained viable on the CLBs bodies for more than 16 d or a greater percentage of the beetles would have deposited viable conidia. However, without a source of moisture, CLBs would probably not have endured past 8 d. These in vitro conditions were not designed to mimic, nor are they indicative of, environmental conditions encountered in vivo; however, data obtained from this study suggest that insects have the potential to disseminate viable inoculum.

## CHAPTER III

### EXAMINATIONS OF CONVERGENT LADY BEETLE, *Hippodamia convergens*, INFESTED WITH *Discula destructiva* AND CONTAMINATED *Cornus florida* LEAF SURFACES FOR PRESENCE OF CONIDIA

#### i. INTRODUCTION

Dogwood anthracnose, a disease caused by the fungus *Discula destructiva* Redlin sp. Nov., has caused decline and mortality of native *Cornus florida* Link. stands in both woodland and urban habitats since the late 1970's (Daughtrey 1983, Redlin 1991). Little attention has been directed toward research on dissemination of the pathogen. The two known mechanisms (i.e., wind and rain) for dissipating the fungal conidia do not account for the widespread movement of the pathogen along the Appalachian Mountain range (Daughtrey et al. 1986).

Insects have been reported to "vector" more than 200 plant pathogens (Borror et al. 1989). In fact, some of the most severe diseases of woody plants are caused by insect-disseminated fungal pathogens (e.g., chestnut blight, Dutch elm disease, and oak wilt) (Webber and Gibbs 1989). Representatives from all major insect orders have been collected from healthy and diseased *C. florida* trees (Costa and Crossley 1991, Eyde 1988, Risley 1990, J.F. Grant, unpublished data). Therefore, the objectives of this study were to observe and quantify *D. destructiva* conidia on body

regions of the adult convergent lady beetle (CLB), *Hippodamia convergens* Guerin-Ménèville (Coleoptera: Coccinellidae), via scanning electron microscopy (SEM), and also, to observe with SEM, *D. destructiva* conidia deposited to *C. florida* leaf surfaces by infested CLBs. Adult CLBs are encountered in forested and urban areas and have been collected from native dogwoods (Borror et al. 1989, J.F. Grant, unpublished data).

## ii. MATERIALS AND METHODS

CLBs were obtained from Ricon-Vitova Insectaries, Inc. (Oak View, CA 93022). Colonies were maintained in clear plexiglas cages (30.48 x 30.48 x 40.64 cm) with a screen-covered opening (12 cm) on one side. CLBs were fed a mixture of honey and sugar, and 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.

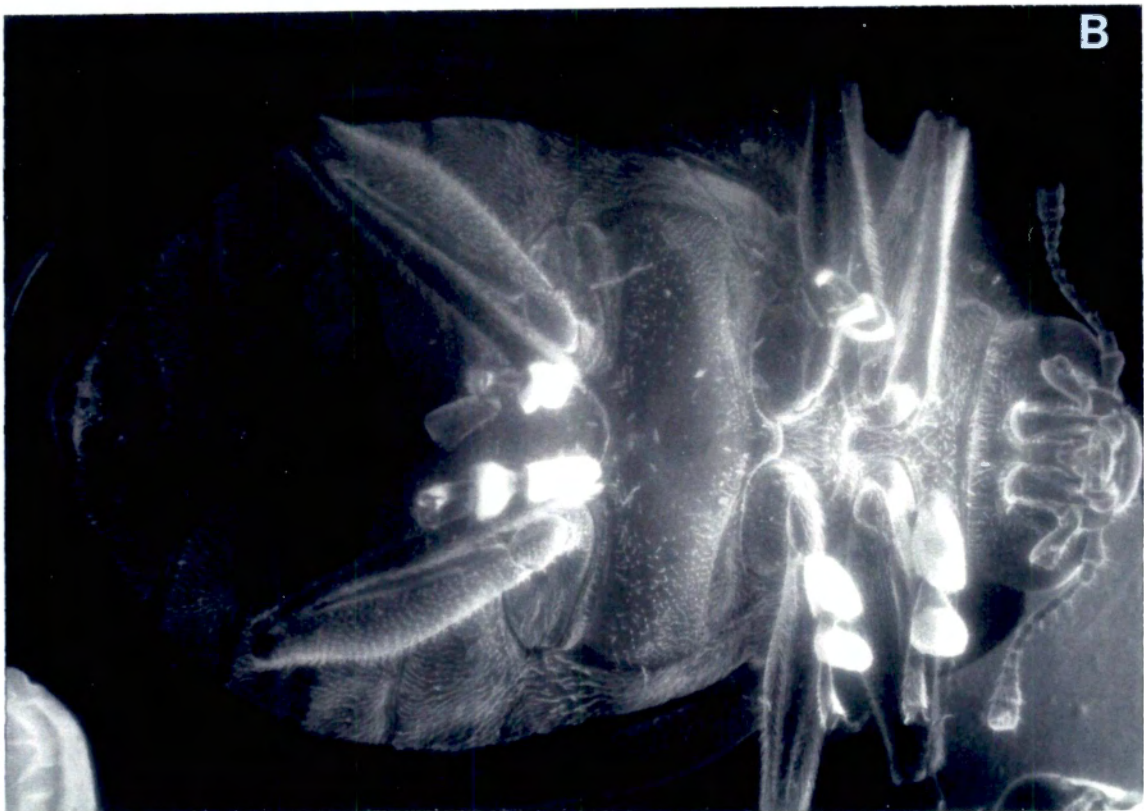
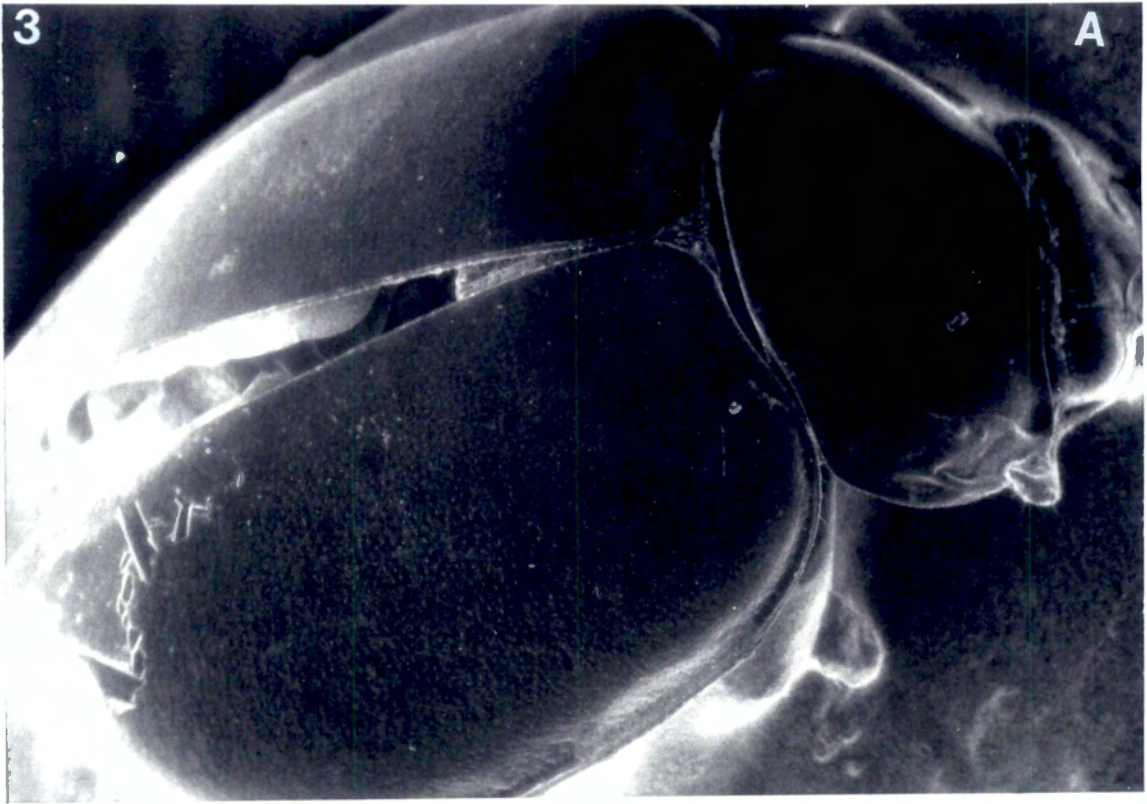
Stock cultures of *D. destructiva* isolate Va 17b (type 2) were maintained on potato sucrose agar (PSA) as described by Dhingra and Sinclair (1985) and *C. florida* leaf tissue. Cultures were maintained at 20 C in incubators with 8:16 h of light:dark.

Two *C. florida* leaves from greenhouse trees were trimmed to fit within a glass petri plate (100 x 15 mm). Leaves, separated by a filter paper disk (9 mm), were placed in the plate which was then filled with deionized water. Leaves were autoclaved for 1 h, incubated at room temperature (24 C) for 24 h, and autoclaved for another 1 h. Sterilized leaves were then placed on PSA plates and inoculated with one *Discula* plug/plate (8 mm) from stock cultures. Leaf plates were incubated until sporulation occurred on leaf surfaces.

To evaluate if conidia were carried on the insect's exterior, 10 CLBs, 5/leaf plate, were placed on *Discula*-infested leaves for 1 h at room temperature (ca. 24 C). CLBs were then etherized, placed in 2.5% glutaraldehyde and 0.1 M potassium phosphate buffer with a pH of 6.8, refrigerated for 24 h, and aspirated in a vacuum desiccator for 60 min or until beetles submerged. CLBs were then rinsed in 0.1 M sodium cacodylate buffer with a pH of 7.2, fixed in a 4% osmium tetroxide aqueous solution for 2 h, and rinsed in distilled water. Fixed insects were transferred through a 25, 50, and 75% acetone dehydration series for 24 h in each solution. Adults were then critical point dried, attached to SEM specimen mounts (5 dorsal view and 5 ventral view), and observed under SEM.

Body surfaces were observed and photographed to quantify inoculum densities in specific body regions. The study was arranged as a completely randomized design with 10 infested beetles; 5 positioned dorsally and 5 positioned ventrally (Figure 3). Five body regions/view were observed for conidia. On the dorsal surface, the head, pronotum, scutellum, and right and left elytra were rated. The mouth, prothorax, thorax, legs, and abdomen were observed and rated on the ventral surface. A six 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= $\geq$ 76). Data were analyzed by General Linear Model and Waller-Duncan *K*-Ratio *t* test (SAS Institute 1985). Statistical analyses were conducted to determine significant differences between conidia densities on dorsal and ventral surfaces; when significant differences were

Figure 3. Scanning electron micrographs of *Hippodamia convergens*: (A) Dorsal view (150x), (B) Ventral view (13x).



found, Waller-Duncan *K*-ratio *t* tests were performed to determine significant differences among means (SAS Institute 1985).

To ascertain if CLBs could disseminate *D. destructiva* conidia, infested beetles were allowed to walk on washed *C. florida* leaves. Four *C. florida* leaves from trees grown in the greenhouse were placed under a #18 Standard Testing Sieve and rinsed in cool tap water for 10 min each side. Leaves were placed in individual petri plates, adaxial surface up. Twenty CLBs (5/leaf), infested according to previously stated procedures, were allowed to walk on leaves for 4 h at room temperature. Four disks (11 mm) were randomly cut from each leaf and prepared for observation on SEM according to methods used for CLB preparation. Leaves were photographed to document areas where conidia were deposited.

### iii. RESULTS AND DISCUSSION

CLBs carried *D. destructiva* conidia on their external body surfaces and deposited conidia on *C. florida* leaves. However, the ventral body surface had significantly more conidia than the dorsal body surface ( $F=3.79$ ;  $df=13, 35$ ;  $P=0.0008$  level, *t* Test). Numbers of conidia were not significantly different among ventral body regions (Table 1). CLBs were observed to groom their conidia-laden appendages which would explain the conidia-encrusted mouth parts (Figure 4). Conidia were also observed among setae, singularly or in mass, sometimes contained by the protein matrix on legs, thorax, and abdomen (Figure 5).

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

| Body Aspect | Location     | Mean <sup>1,2</sup> | Range <sup>3</sup> |
|-------------|--------------|---------------------|--------------------|
| Dorsal      | Head         | 1.0 c               | 0-3                |
|             | Pronotum     | 5.0 a               | 5-5                |
|             | Scutellum    | 3.0 b               | 0-5                |
|             | Left elytra  | 3.0 b               | 1-5                |
|             | Right elytra | 4.0 ab              | 2-5                |
| Ventral     | Mouth parts  | 3.6 ab              | 1-5                |
|             | Prothorax    | 5.0 a               | 3-5                |
|             | Thorax       | 4.8 ab              | 4-5                |
|             | Legs         | 4.6 ab              | 3-5                |
|             | Abdomen      | 4.0 ab              | 2-5                |

<sup>1</sup> Mean rating was determined by assessing conidial densities on insects using the following scale: 0=no conidia, 1=1-10, 2=11-25, 3=26-50, 4=51-75, and 5 ≥ 76.

<sup>2</sup> Values within a column followed by the same letter are not significantly different ( $P > 0.05$ , Waller-Duncan  $K$ -Ratio  $t$  Test).

<sup>3</sup> Range=minimum and maximum numbers of conidia found on each body region (n=5).

Figure 4. Scanning electron micrographs of *Hippodamia convergens* mouthparts encrusted with *Discula destructiva* conidia: (A) Ventral view, side, of mouthparts (128x), (B) Labial palp, labrum, and maxillary palp (160x), (C) Conidia on maxillary palp (320x), (D) *D. destructiva* conidia (1280x) [LP - labial palp, L - labrum, MP - maxillary palp, C - conidium].

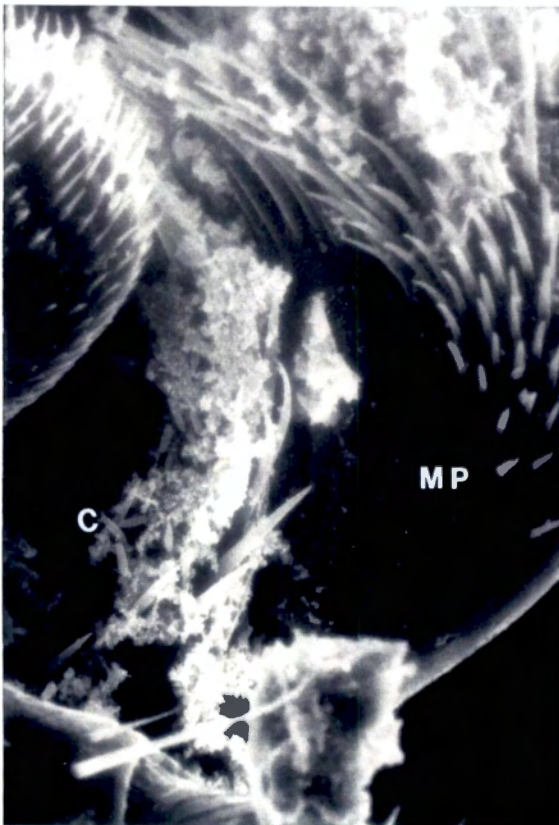
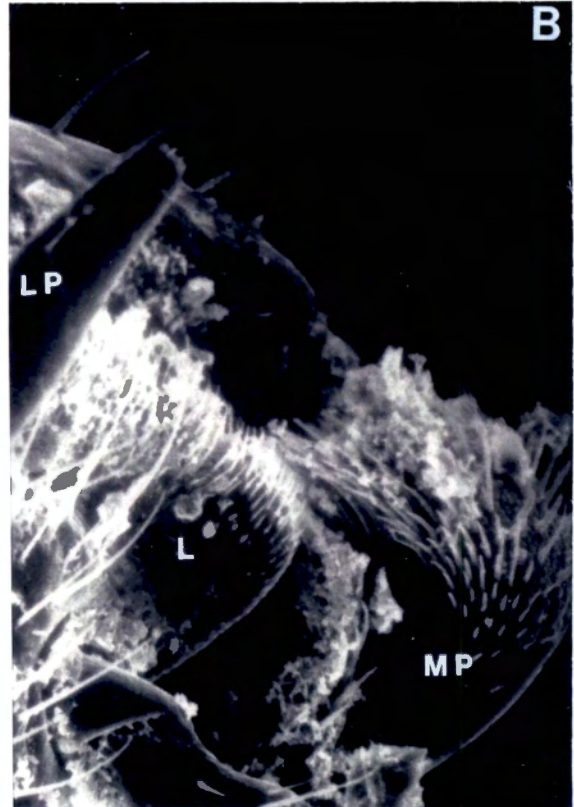
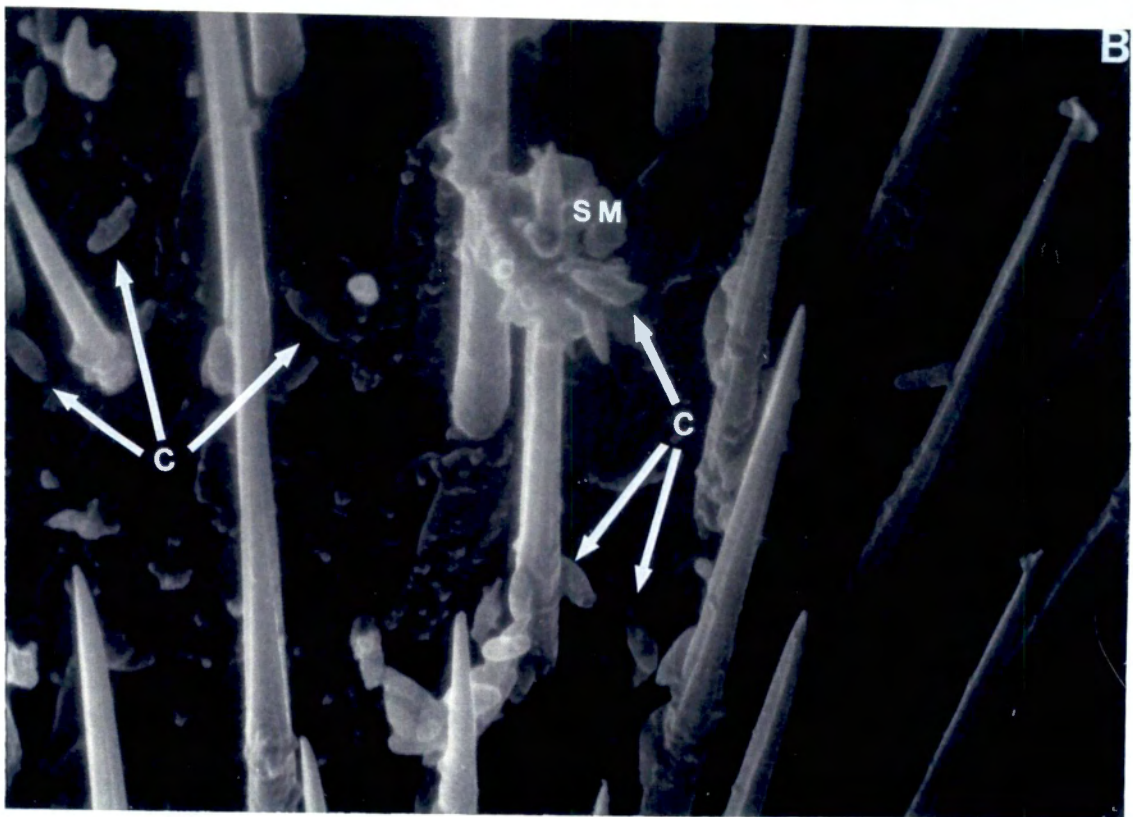
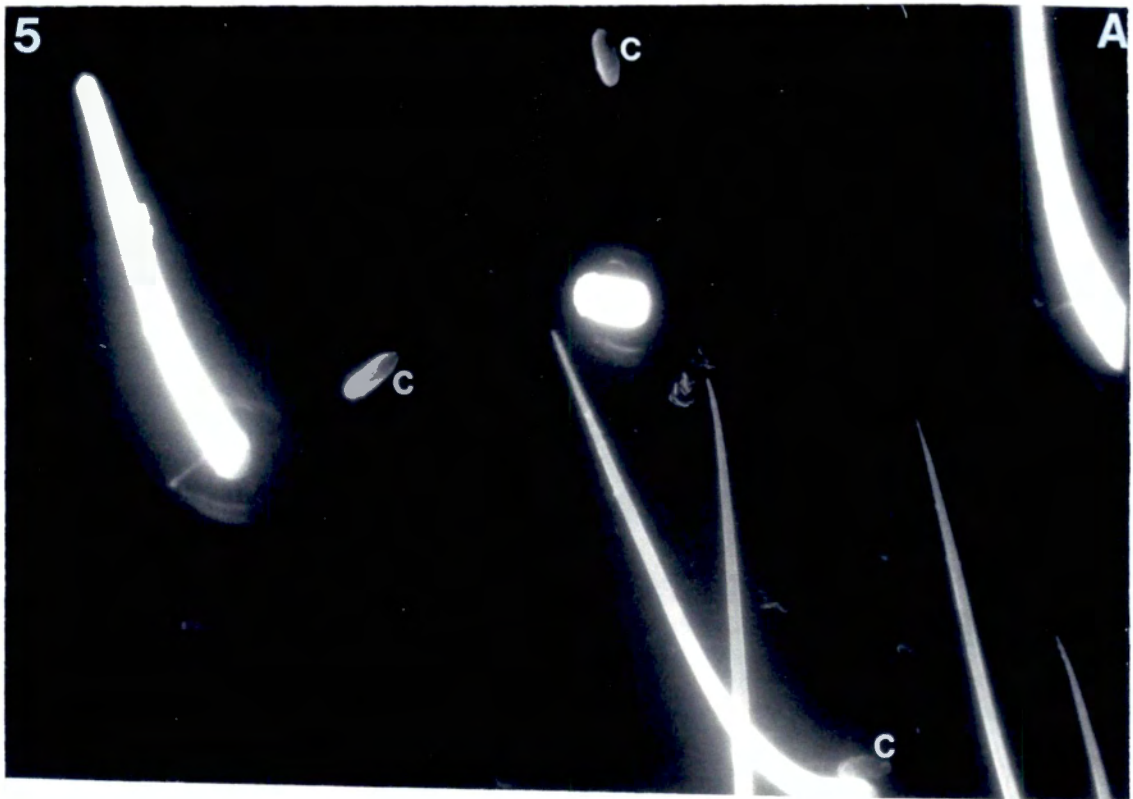


Figure 5. Scanning electron micrographs of *Discula destructiva* conidia on the body surface of *Hippodamia convergens*: (A) Conidia on mesosternum (800x), (B) Acervular slime matrix, containing conidia, speared by seta; also, conidia scattered on surface of mesothoracic tibia (640x) [C - conidium, SM - slime matrix].



On the dorsal surface, the pronotum had significantly more conidia than other dorsal regions (Table 1). The dorsal head area possessed the least number of conidia for any region, dorsal or ventral. Conidia were scattered across the pronotum and nestled in pits and crevices along the junction of the elytra. Conidia on the scutellum were often observed in setal sockets (Figure 6). Insects which do not possess mycangia may transport fungal spores, randomly scattered or lodged in setal pits, on their body surfaces. *Scolytus scolytus* Fabricius was reported to carry fungal spores of *Ophiostoma ulmi* Buisman in a similar manner (Webber and Gibbs 1989). Spores of *O. ulmi* are contained within a sticky mucus droplet (Wilson et al. 1970) which may help them adhere to their insect vector. *Discula* conidia are also embedded in a sticky protein matrix that ooze from acervuli during sporulation.

CLBs did disseminate conidia to the leaf surface. Most conidia had been deposited around trichomes with many conidia wedged beneath trichomes (Figure 7).

The raised, two-armed morphology of dogwood trichomes may precipitate the disposal of conidia from the beetles' bodies as the setae are jarred by the trichomes. Depressions in the leaf epidermis around the base of the trichome may provide a suitable environment for conidia germination and/or penetration. Because the location of hyphal penetration is not known, and the base of the trichome is an area where deposited conidia may persist, the location may be vulnerable to fungal invasion.

*D. destructiva* was observed to sporulate sooner when in the presence of leaf tissue versus cultures grown on PSA only. These observations were not substantiated by data nor was any study conducted to determine differences in sporulation times.

Figure 6. Scanning electron micrographs of *Discula destructiva* conidia on the body surface of *Hippodamia convergens*: (A) Junction of elytra, scutellum, and pronotum (120x), (B) Conidia in setal socket on scutellum (1200x) [C - conidium, SC - scutellum].

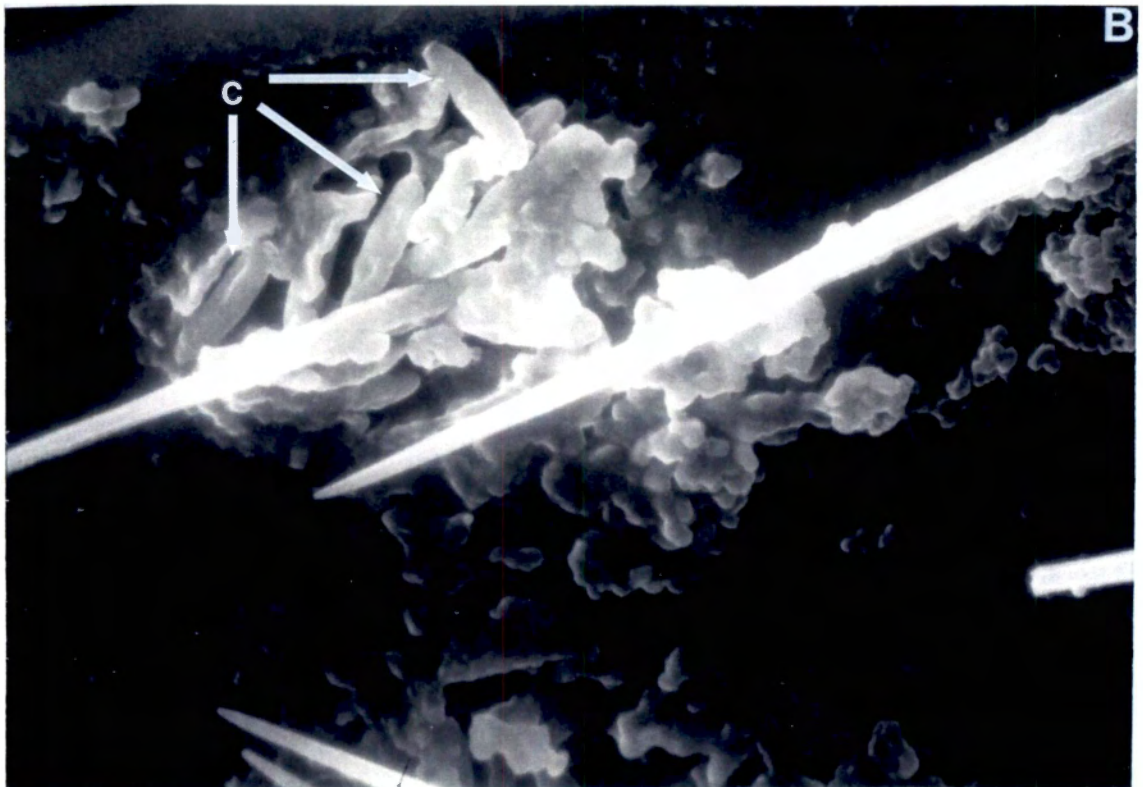
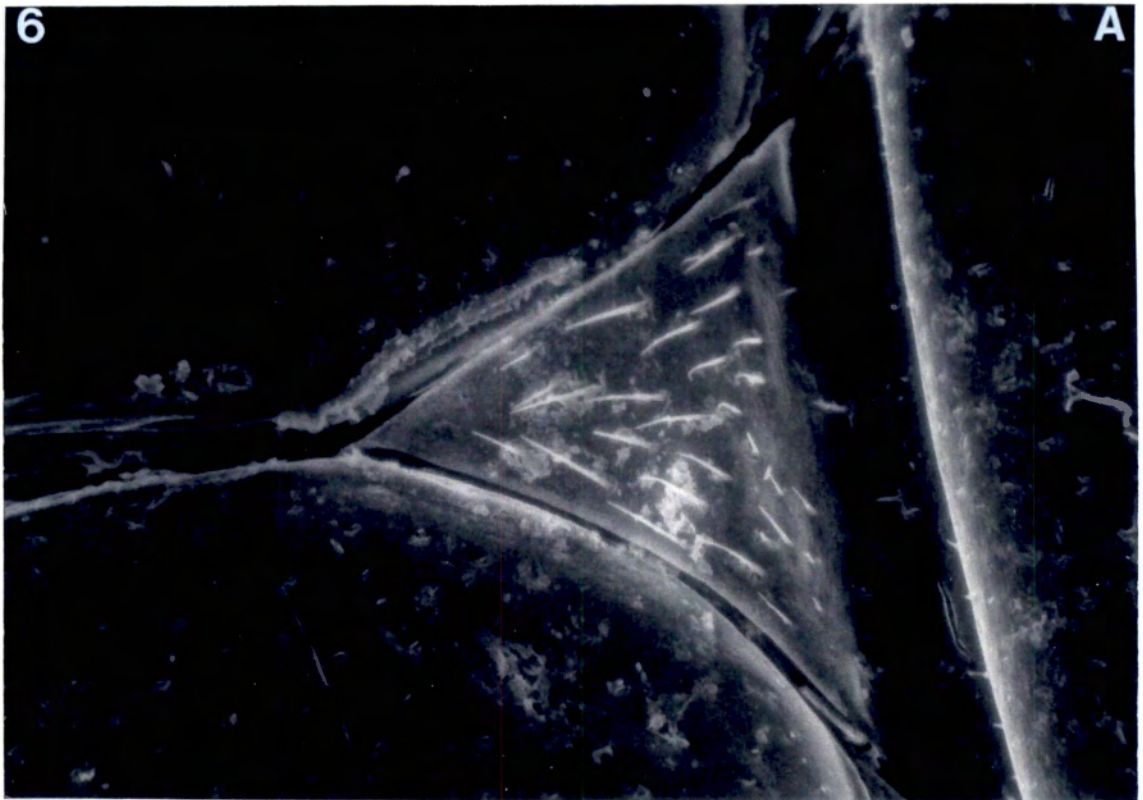
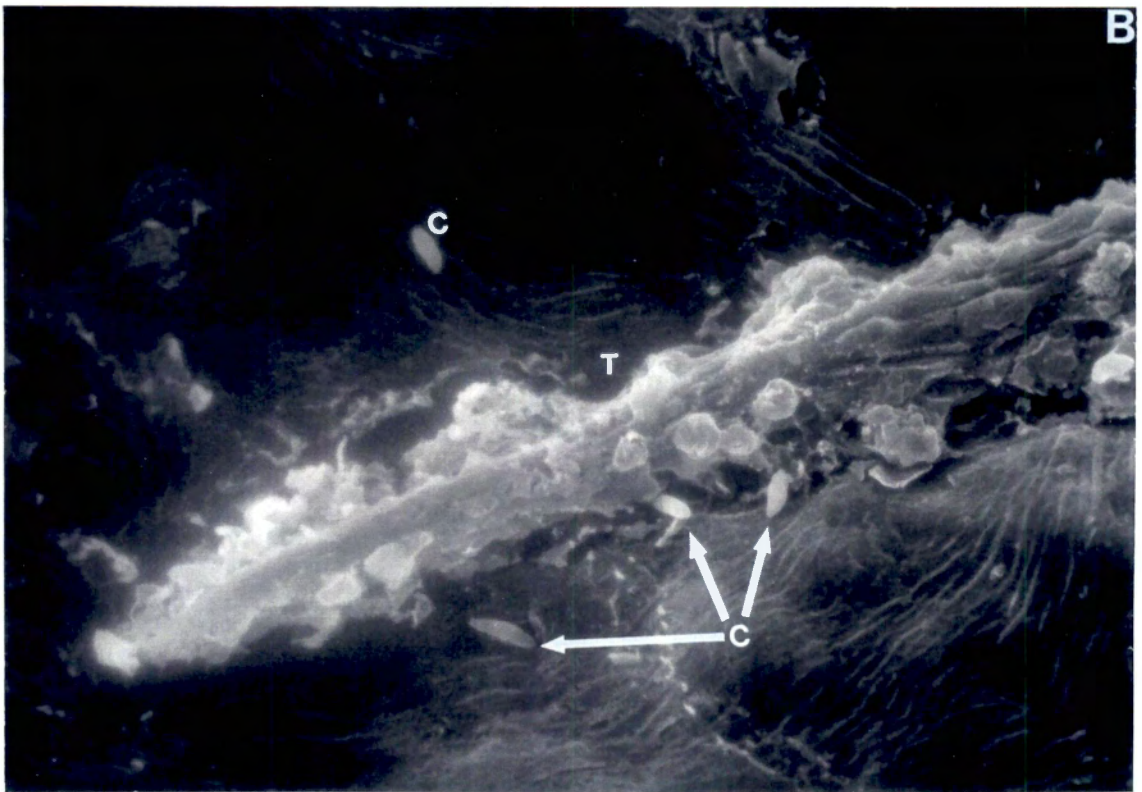
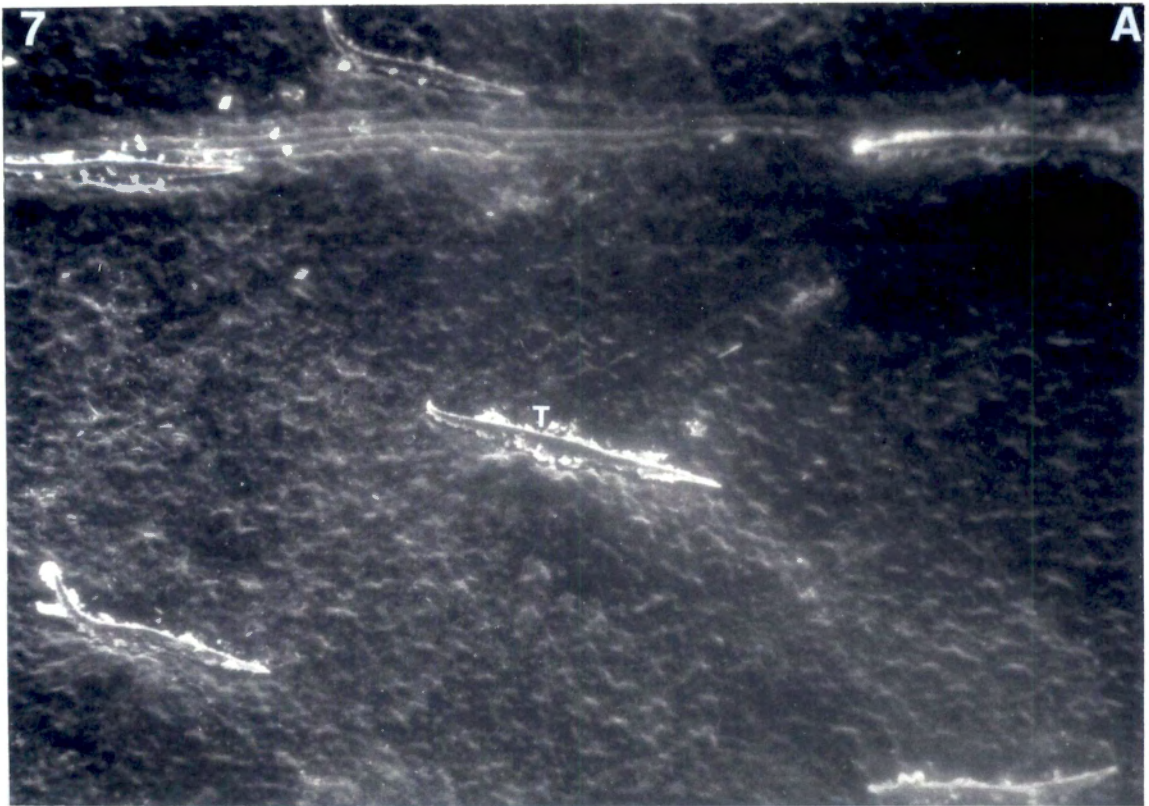


Figure 7. Scanning electron micrographs of *Discula destructiva* conidia deposited by *Hippodamia convergens* to a *Cornus florida* leaf surface: (A) Adaxial leaf surface with two-armed trichomes (72x), (B) Conidia deposited near and under trichome (680x) [C - conidium, T - trichome].



The intention for using whole, autoclaved dogwood leaves was to provide CLBs with a firm surface to walk across while on PSA.

Conidia were carried on the dorsal and ventral body surfaces of adult CLBs and were deposited to *C. florida* leaves. Most conidia were probably disseminated from regions on the ventral exterior (i.e., mouthparts, legs, thorax, and abdomen) to trichomes on the adaxial leaf surface. Conidia on the ventral surface may be shielded from desiccation by heat and sunlight during flight for longer periods of time; thereby increasing the probability of deposition of viable inoculum to a susceptible host.

## CHAPTER IV

### INFECTION OF HEALTHY *Cornus florida* IN A GREENHOUSE MOISTURE CHAMBER BY *Discula destructiva* CONIDIA FROM INFESTED LADY BEETLES

#### i. INTRODUCTION

Eastern flowering dogwood, *Cornus florida* Link., has been susceptible to infection by the fungus *Discula destructiva* Redlin sp. Nov. for more than 15 years (Daughtrey 1983, Pirone 1980, Redlin 1991). Symptoms of this disease, known as dogwood anthracnose, were first observed in New York on dogwoods exhibiting limb dieback (Hibben and Daughtrey 1988). Conidia can be dispersed from infected branches and leaves during rain storms by strong winds or rain droplets onto vulnerable dogwood tissue (Chellemi and Britton 1992, Daughtrey et al. 1988, Windham et al. 1992). Although the epidemiology of this disease has been studied, the mechanism(s) responsible for the rapid dispersment of the disease in the southern Appalachian mountains remains unknown (Daughtrey et al. 1988, Bruck 1993).

Insects are responsible for disseminating some of the most severe fungal diseases of woody dicots (e.g., chestnut blight, Dutch elm disease, and oak wilt) (Webber and Gibbs 1989). Arthropods representing all major orders have been identified in association with dogwoods (Eyde 1988, Risley 1990, Costa and Crossley 1991). Therefore, the objective of this study was to determine if insects, specifically,

the convergent lady beetle (CLB), *Hippodamia convergens* Guerin-Ménéville (Coleoptera: Coccinellidae), could disseminate infective *D. destructiva* conidia to healthy *C. florida* in a greenhouse environment.

## ii. MATERIALS AND METHODS

CLBs were purchased from Ricon-Vitova Insectaries, Inc. (Oak View, CA 93022). Beetle colonies were maintained in clear plexiglas cages (30.48 x 30.48 x 40.64 cm) with a screen-covered opening (12 cm) on one side. They were provided a diet of honey, sugar and water. Cages were held in incubators, maintained at 10-13 C with 12:12 h light:dark cycle.

Two isolates of *D. destructiva* were used for inoculum. TN 8, a type-1 isolate, and VA 17b, a type-2 isolate, are differentiated from one another by growth patterns on artificial media, physiological distinctions (i.e., type 2 lack catechol oxidase), and genetic variations. The isolate types are believed to be related, but type 2 is believed to be native; whereas, type 1 is suspected to have been imported (Trigiano et al. 1993).

Stock cultures of both isolates were sustained on potato sucrose agar (PSA) as described by Dhingra and Sinclair (1985), along with *C. florida* leaf tissue. Dogwood leaves (n=13) from greenhouse-grown trees were trimmed to fit inside a glass petri dish (100 x 15 mm). Leaves were placed adaxial side down and separated from each other by filter paper disks (9 mm). Deionized water was added until disks were saturated. Dishes were autoclaved for 1 h, incubated at 24 C for 24 h, and autoclaved for another 1 h. Sterilized leaves were placed adaxial side up on PSA

inoculated with one *Discula* plug/PSA dish (8 mm). Equal numbers of isolate types were used. Cultures were incubated at 20 C with 8:16 h light:dark cycle for two wk or until acervuli covered the leaves.

This study required the use of a greenhouse inoculation chamber to mimic optimum environmental conditions for *D. destructiva* growth. The chamber consisted of a wooden frame (437 x 141 x 118 cm) covered in two sheets of plastic, with five cm of air space between them. An 80% shade cloth covered the entire chamber. The inoculation chamber was equipped with an air conditioner in one corner and a humidifier in the center (Figure 8).

Ten greenhouse-grown *C. florida* wild seedlings (each ca. 138 cm tall) were placed inside the chamber one wk prior to treatment. Adult CLBs (n=138), 10/PSA plate, were exposed to the sporulating fungus for one h. Beetles infested with *Discula* isolates were placed on the trees (5 trees/isolate type) in the following treatment conditions: wounded leaves (W), wounded leaves with non-infested lady beetles (WB), wounded leaves with infested lady beetles (WIB), nonwounded leaves (NW), nonwounded leaves with non-infested lady beetles (NWB), and nonwounded leaves with infested lady beetles (NWIB). Each treatment (ca. 10 leaves/treatment) was randomly placed on a different branch. Leaf wounding was accomplished by gently puncturing the leaf surface with a piece of cork containing 100 insect pins. All treated branches were covered with plastic bags and sealed. To maintain live CLBs in the appropriate treatment bags for 24 h, any dead CLBs were replaced with live ones. After 24 h, CLBs were removed and the bags resealed. Temperatures inside

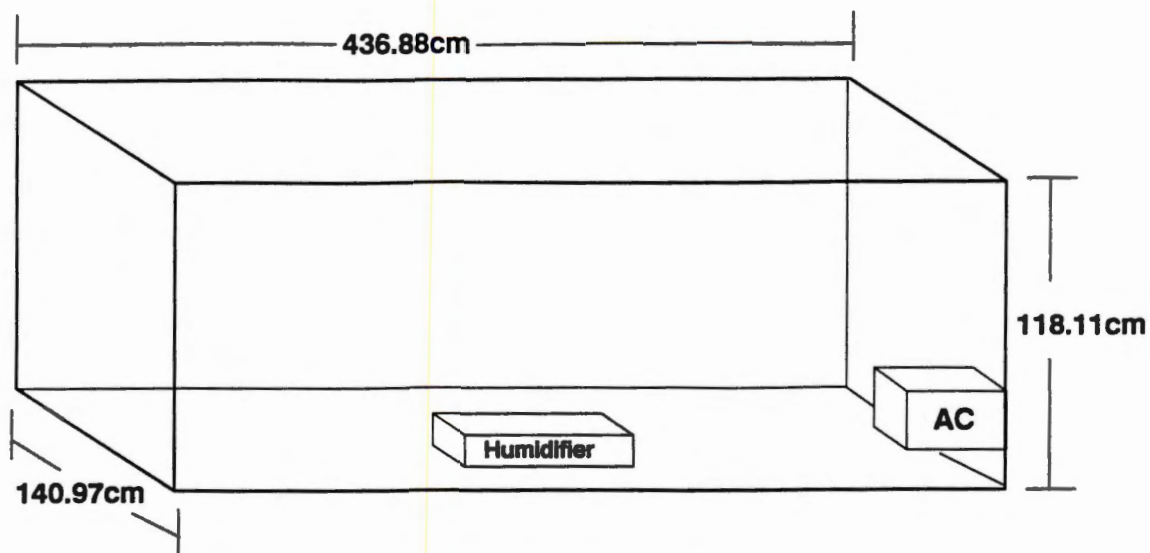


Figure 8. Greenhouse inoculation chamber equipped with air conditioner (AC) and humidifier.

the chamber fluctuated between 18 - 27 C with RH of 100% inside the bags. Foliage within the bags was observed for 4 wk to monitor *Discula* infection.

After 4 wk, all leaves were removed from the bags and placed on moistened filter paper in petri dishes. Dishes were parafilmed and incubated at room temperature (24 C) for one wk. Conidia from infected leaves were observed microscopically. Isolations onto PSA amended with streptomycin sulfate and chlortetracycline (25 mg/ea) were made from leaves exhibiting *Discula*-like acervuli. These cultures were incubated and verified for *Discula*.

The greenhouse experiment was designed as a 2 x 2 x 3 factorial with isolate type, wound type, and beetle condition as treatment variables. Analysis of variance was calculated between isolate types and wound types and among beetle conditions (SAS Institute 1985).

### iii. RESULTS AND DISCUSSION

Similar studies, without insects, where trees were mechanically predisposed by piercing the leaves, spraying them with spore suspensions, and bagging the branches, met with limited success. Non-wounded leaves typically did not become infected (Bruck and Grand 1993, Schreiber et al. 1993). However, results from this study demonstrated that infection was not significantly different among wound types ( $F=45.35$ ;  $df=13, 46$ ;  $P=0.0001$ ). Symptoms did not appear on non-wounded leaves until one week after wounded leaves had become infected. However, by the end of the fourth week, symptom severity was equal in both treatments.

Leaf infection was not significantly different between isolates. Leaf infection, however, was significantly different among beetle treatments (Table 2). No infection was observed in bags subjected to non-inoculated beetles or that contained no beetles. *Discula* was present in 90% (18 of 20) of the bags where leaves were exposed to inoculated beetles.

This experiment has shown that adult CLBs can initiate infection of healthy *C. florida* by disseminating *D. destructiva* conidia. The cohesive properties of the sticky matrix surrounding the conidia enable them to become attached to and transported by insects that may frequent infected and noninfected dogwoods. Also, use of CLBs was an effective means of inoculating healthy trees which did not require injury to the plant nor dependence on climatic variables.

Table 2. Percent of two-year-old *Cornus florida* trees infected with *Discula destructiva* obtained with each treatment (isolate/wound treatment/beetle treatment) combination.

| Treatments     |                      | % Infected |
|----------------|----------------------|------------|
| Isolate VA 17b |                      |            |
| Nonwounded     |                      |            |
|                | Infested beetles     | 100        |
|                | Non-infested beetles | 0          |
|                | No beetles           | 0          |
| Wounded        |                      |            |
|                | Infested beetles     | 100        |
|                | Non-infested beetles | 0          |
|                | No beetles           | 0          |
| Isolate TN 8   |                      |            |
| NonWounded     |                      |            |
|                | Infested beetles     | 60         |
|                | Non-infested beetles | 0          |
|                | No beetles           | 0          |
| Wounded        |                      |            |
|                | Infested beetles     | 100        |
|                | Non-infested beetles | 0          |
|                | No beetles           | 0          |

## CHAPTER V

### CONCLUSIONS

*Discula destructiva* Redlin sp. Nov., the fungal pathogen that causes dogwood anthracnose, has affected reproduction and survival of the flowering dogwood, *Cornus florida* Link., on more than 2.3 million hectares of forest in the southeastern United States alone (Anderson 1991). Native dogwoods in some areas of the Great Smoky Mountains National Park where the disease was severe were reported to be endangered (Windham et al. 1992).

Researchers of dogwood anthracnose now know that the disease is characterized by certain environmental factors, such as temperatures of 25 C or lower, shade, and RH of 90% or greater (Anderson et. al 1991, Chellemi and Britton 1992, Parham and Windham 1992). This information was useful especially for nurserymen and homeowners, but where forest-dwelling dogwoods were concerned, this knowledge only indicated trees that were highly susceptible to infection. Regardless of the available information, dogwood anthracnose continues to spread; researchers have discovered little about the mechanisms of conidia dispersal or the mode of fungal invasion.

Therefore, the objective of this study was to determine if a selected insect, the convergent lady beetle (CLB), *Hippodamia convergens* Guerin-Ménèville (Coleoptera: Coccinellidae), could disseminate viable *D. destructiva* conidia. This objective was

accomplished through a series of experiments designed to address specific questions involving insect dissemination of a fungal pathogen. First, the potential for CLBs to carry viable conidia on their external body surface and/or internally was determined. Then specific areas on the bodies of CLBs were observed with scanning electron microscopy (SEM) to determine if conidia were present. The length of time conidia could be carried and deposited without desiccating was also determined. Areas on the *C. florida* leaf surface were observed for conidia after exposure to infested CLBs. Lastly, the ability of infested CLBs to initiate infection on healthy trees was investigated.

No significant differences existed between isolate types (TN 8 - type 1 and VA 17b - type 2) in external and internal dilution experiments. TN 8 colonies were present on 100% of both external and internal dilution plates (n=40). Growth of VA 17b occurred on 100% of the external dilution plates (n=20) and on 95% of internal dilution plates (n=20).

Data from scanning electron micrographs of infested CLB body surfaces showed significant differences in numbers of conidia carried on the ventral surface compared with those on the dorsal surface. The abundance of conidia was not significantly different among specific regions on the ventral surface. However, regions within the dorsal surface did have different amounts of conidia; the pronotum had the most and the head had the least conidia of all body regions. Viable conidia were carried and deposited as many as 16 d after CLBs were exposed to sporulating *Discula*. The percentage of CLBs depositing viable conidia did decrease over time.

However, 100% (n=5) of the CLBs disseminated conidia immediately after infestation.

Results from inoculation of healthy dogwoods via CLBs indicated no significant differences in infection between isolate types or wound/nonwound treatments. Significant differences did exist when infection by non-inoculated CLBs, no CLBs, and inoculated CLBs were compared. Ninety percent of the branches treated with inoculated CLBs had symptoms of and displayed signs of *D. destructiva*. Micrographs of leaves exposed to infested CLBs revealed that conidia were deposited onto leaf surfaces and that most deposited conidia were associated with trichomes on the adaxial surface.

Few studies that focus on insect-disseminated fungal pathogens have been conducted. Results from this research, as well as similar studies, demonstrate that insects can disperse viable conidia to susceptible hosts. The CLB was chosen as a model insect for this research mostly for convenience but also the possibility exists for these types of arthropods to be involved in the spread of dogwood anthracnose. No one particular group of insects is solely responsible for disseminating fungal pathogens. However, any insect, whether a pollinator, predator, scavenger, or other type, which visits a diseased dogwood, has the potential to disseminate viable *D. destructiva* conidia to other dogwoods and continue disease progression.

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