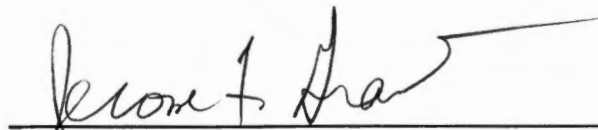
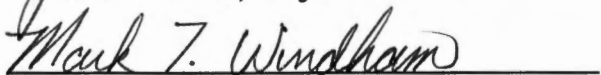


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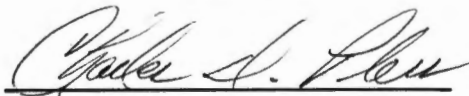


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**INCIDENCE OF ARTHROPODS INFESTED WITH CONIDIA OF
Discula destructiva Redlin IN FOREST AND CAGE ENVIRONMENTS**

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Howard Lee Holt

May 1995

AD-VET-MED.

Thesis

95

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ABSTRACT

Discula destructiva Redlin, the causal agent of dogwood anthracnose, is responsible for the decline and mortality of the eastern flowering dogwood, *Cornus florida* Link. The pathogen is believed to be disseminated by wind, rain, and man. Until recently, little research had been conducted to identify other means of dissemination (birds, deer, insects, etc.).

In an earlier study, convergent lady beetles, *Hippodamia convergens* Guérin-Méneville, acquired and carried viable conidia of *D. destructiva* on their bodies for as many as 16 days under laboratory conditions. The objectives of this research, however, were expanded into the natural environment to determine: 1) if arthropods collected from diseased trees in forested areas had viable conidia of *D. destructiva* on their bodies and 2) the effects of light intensity (sunlight and shade) on viability of conidia of *D. destructiva* over selected periods of time on lady beetles in field cages.

In 1994, 7.2% of arthropods (n=375) collected from diseased trees were infested with viable conidia of *D. destructiva*. When conidia-infested arthropods were evaluated throughout the summer, conidial infestations were greater at each site during June than on other sampling dates. The level of infestation was greatest at Rich Mountain (22.0%) followed by Jakes Creek (11.1%) and Sugarlands (8.6%) during June, when epidemics of dogwood anthracnose are normally increasing. Frequency of conidia-infested arthropods followed changes in the rate of disease severity of dogwood anthracnose, based on disease ratings using the Horsfal-Barratt scale.

Infestation of viable conidia on convergent lady beetles collected from shaded cages 1 day after release were greater than those in sunlight (20% and 3.3%, respectively). Also, beetles in the shade carried viable conidia for longer periods of time than those in sunlight (16 days and 1 day, respectively). The greatest level of infestation (ca. 12%) occurred 1 day after release and dropped significantly during the remainder of the study.

The results of this study have shown that arthropods carry viable conidia of *D. destructiva* in the natural environment. Optimal conditions for potential dissemination of conidia by arthropods were identified as: 1) viable conidia carried by arthropods within 24 h after infestation, 2) conidia transported by arthropods under shaded conditions, which are more conducive to the survivability of conidia, and 3) conidia carried by arthropods when disease severity of dogwood anthracnose was at its greatest levels. This research has demonstrated that more than one species of arthropod carried viable conidia of this fungal pathogen and suggests that arthropods may play an important role in the epidemiology of dogwood anthracnose.

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CHAPTER I

LITERATURE REVIEW

The flowering dogwood, *Cornus florida* Link., is a valuable component of the forest ecosystem. Dogwood foliage increases the organic material and calcium components of soil (Boring et al. 1981, Risley 1990). Ecologically, dogwood berries and foliage provide nutrition for more than 80 species of birds and mammals (Eyde 1988, Mitchell et al. 1988, Whitmore 1992).

In addition to their ecological importance, dogwoods are important to the economy of Tennessee where dogwood sales in 1989 were estimated to be 30 million dollars (Windham and Freeland 1990). The dogwood's springtime blooms make it a highly desirable ornamental in many residential neighborhoods and the focus of numerous festivals throughout its native range.

In the late 1970's, a disease of dogwoods was reported in southwestern Connecticut and southeastern New York (Hibben and Daughtrey 1988). Symptoms, consisting of leaf spot, leaf necrosis, and twig dieback, were concentrated in the lower crown. The disease progressed throughout the crown and the main stem where cankers formed. Pirone (1980) attributed the disease, associated with wet springs, to *Colletotrichum gloeosporioides* Penz., a fungal pathogen. Hibben and Daughtrey (1988) reported symptoms similar to those described by Pirone in several other northeastern states. Various potential pests and causal agents were considered and

eliminated. However, a fungal pathogen, *Discula* sp., was consistently associated with diseased samples (Salogga and Ammirati 1983).

Researchers in the northwestern United States reported that the pacific dogwood, *Cornus nuttallii* Audubon, displayed characteristics similar to the diseased dogwoods found in Connecticut and New York (Salogga and Ammirati 1983).

Hibben and Daughtrey (1988) compared disease symptoms and isolates of *Discula* spp. from the flowering dogwood in New York and the pacific dogwood in Washington and concluded that the same disease occurred in both locations. They named the disease dogwood anthracnose after completing Koch's postulates.

Dogwood anthracnose can attack several different plant organs. Symptomatic leaves in full sunlight are characterized by lesions with purple borders, whereas necrotic (no colored border) lesions were the dominant type on shaded trees (Parham and Windham 1992). Shoot dieback consists of shriveled bark and discoloration of terminal buds. Once terminal buds are killed and apical dominance is broken, epicormic branching occurs. The fungus can grow down the epicormic shoots, form cankers in the trunk or limbs, and kill the tree. Blighted leaves may remain on the dogwood throughout the winter and are used as a visual aid to locate diseased trees (Walton 1986, Daughtrey et al. 1988, Hibben and Daughtrey 1988, Smith 1991).

In 1991, the fungus that causes dogwood anthracnose was identified as *Discula destructiva* Redlin (Redlin 1991). Microscopic examinations revealed that conidiomata of *D. destructiva* are subcuticular and similar to conidiomata of *Discula* spp. that cause ash, oak, and sycamore anthracnose (Redlin and Stack 1988, Redlin

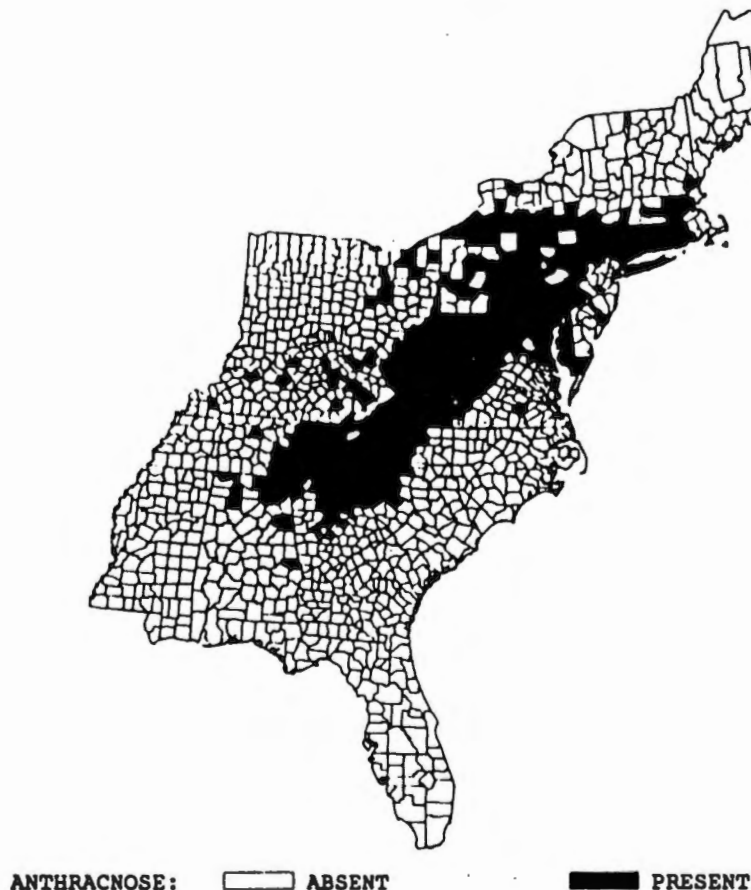
1991). Morphologically, *D. destructiva* is distinguished from other *Discula* spp. by narrow acuminate apices in the conidiogenous cells. Additionally, pure cultures of *Discula* spp. that cause ash, oak and sycamore anthracnose differ from *D. destructiva* in colony morphology.

Since the discovery of the disease in 1978, dogwood anthracnose has spread throughout the northeastern United States and southerly throughout the Appalachian Mountain range into Georgia and east central Alabama (Carey and Kelley 1991, Knighten and Anderson 1992) (Figure 1). This spread demonstrates the susceptibility of woodland understory dogwoods to this disease, even though dogwoods can be affected in urban habitats as well.

The severity of dogwood anthracnose is well illustrated by the devastation of dogwoods in Catoctin Mountain Park, MD. Mortality of dogwoods in this area increased from 33% to 79% from 1984 to 1988 (Mielke and Langdon 1986).

Site factors may influence the degree of incidence and severity of dogwood anthracnose. A higher incidence of dogwood anthracnose was found on dogwoods in an understory environment compared to those exposed to sunlight (Hibben and Daughtrey 1988, Dudt and Shure 1993, Hagan and Mullen 1993). Diseased trees in open areas survived longer than did diseased dogwoods in an understory setting. Windham and co-workers (1993) reported that disease severity was higher in experimental plots near streams than those located at least 200 m away on upland slopes. Higher humidity may result from denser vegetation in close proximity to the

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



PREPARED BY USDA FOREST SERVICE
FOREST HEALTH PROTECTION, MORGANTOWN, WV

FIGURE 1. 1993 distribution of dogwood anthracnose in the eastern United States.

stream, especially rhododendron thickets that may reduce wind velocity and increase transpiratory rates. North to northeastern aspects provided an environment conducive for growth of *D. destructiva*. This growth may be caused by lower evaporation in response to lower amounts of sunlight (Chellemi et al. 1992, Windham et al. 1993).

Predisposition of the host by environmental factors may impact incidence and severity of dogwood anthracnose. In simulated acid rain studies, decreasing the pH of rain led to an increase in deterioration of the cuticle and enhanced levels of infection (Anderson et al. 1991, Brown et al. 1994). Carey and Kelley (1991) found that drought stress was associated with infected trees; their observations were supported by Erbaugh (1992) who demonstrated that water deficits in conjunction with various treatments of light intensity (50, 10 and 2% sunlight) accelerated severity of the disease. However, trees placed in partial to full sunlight exposure with sufficient moisture were less symptomatic than trees observed in the shade (Gould and Peterson 1994).

Some of the most severe fungal diseases of woody dicots are disseminated by insects. In fact, insects aid in the spread of more than 200 plant pathogens by a variety of methods (Borror et al. 1989). Of these pathogens, 23 are fungi, and 80% of these fungal pathogens are disseminated by coleopterans (Webber and Gibbs 1989).

The ability of insects to vector pathogens depends on factors such as position of the conidia during transport, insect habits and environmental conditions (Webber and Gibbs 1989). For example, conidia of *Botrytis anthophila* Bond. and pollen grains are transferred between red clover plants by pollinating insects and deposited

on the stigma of the flower. After conidial germination, the mycelia of this pathogen proliferate within the plant. Therefore, the fungal pathogen has a means of transport, while the insect may benefit from a food source supplied by the fungus or from rotten host tissues (Webber and Gibbs 1989).

Many insects carry conidia on the surface of the exoskeleton, and ultraviolet light and desiccation may influence the viability of these conidia during flight (Webber and Gibbs 1989). Conidia of *Ophiostoma ulmi* Buisman are susceptible to desiccation when exposed to relative humidities less than 80% and ultraviolet light. Conversely, fungi that are transported inside mycangia of insects are protected from environmental stresses (Webber and Gibbs 1989). Colby (1993) demonstrated that viable conidia of *D. destructiva* are transported both externally and internally by the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), under in-vitro conditions.

An important property for dissemination of *Ceratocytis fagacearum* Bretz and *O. ulmi* is adhesiveness of conidial exudate (Dorsey et al. 1953, Norris 1953). Conidia of *D. destructiva* possess this same physical characteristic (Redlin 1991) which may be the single most important property of this pathogen in determining insect involvement in the spread of dogwood anthracnose.

Dogwoods attract a wide array of insects, such as bees, beetles, butterflies, flies, moths, and other pollinators (Hall and Sibley 1976, Douglas 1983, Barrett and Helenurm 1987, Rogers and Grant 1990, Grant 1993, Neitch et al. 1994). Thus, researchers have hypothesized that insects that visit diseased dogwoods may

accumulate conidia of *D. destructiva* and transport them to healthy dogwoods (Grant and Windham, personal communication). Eyde (1988) reported that flies as well as adrenid and halictid bees are common pollinators of dogwood. LaBerge and Ribble (1972) reported that some species of *Adrena* spp. (bees) also are associated with dogwood. As site temperatures become too cool for bees, most pollination is then done by flies.

Studies concerning dogwood anthracnose have focused primarily on site factors and physiological processes of this disease. Until recently, little information existed relating to insects associated with dogwood anthracnose. Colby (1993) demonstrated, under laboratory conditions, that the convergent lady beetle can transport viable conidia for as many as 16 days externally. Neitch and co-workers (1994) reported that peak densities of insects on dogwoods occurred in the late spring and early summer in the Great Smoky Mountains National Park when peak levels of dogwood anthracnose occurred.

Prior to these studies, two insects, the dogwood club gall midge, *Mycodiplosis clavula* Beutenmüller, and the dogwood borer, *Synanthedon scitula* Harris, were implicated in the decline of stressed dogwoods (Pless and Stanley 1967, Walton 1986, Daughtrey et al. 1988, Rogers and Grant 1990). Because arthropods transported viable conidia of *D. destructiva* (Colby 1993) in the laboratory and arthropod diversity on dogwoods in the natural environment is high (Neitch et al. 1994), arthropods have been associated with the spread of dogwood anthracnose.

Therefore, a study was initiated to determine if arthropods do carry viable conidia of *D. destructiva* in the natural environment. The specific objectives of this study were to:

1. determine the incidence of conidia-infested arthropods collected from understory dogwoods infected with *D. destructiva*,
2. identify arthropods infested with conidia of *D. destructiva* collected from infected understory dogwoods, and
3. determine effects of light intensity on the incidence of insects infested with viable conidia over selected time intervals.

CHAPTER II

INCIDENCE OF INSECTS INFESTED WITH CONIDIA OF *Discula destructiva* IN THE NATURAL ENVIRONMENT

i. INTRODUCTION

In the late 1970's, a new disease of the flowering dogwood, *Cornus florida* Link., was reported in southwestern Connecticut and southeastern New York (Pirone 1980). Symptoms included leaf spots, lower limb dieback, cankers on the main stem, and death of the tree (Daughtrey and Hibben 1983). The disease was named dogwood anthracnose, and the causal agent was identified as *Discula destructiva* Redlin (Redlin 1991). Since its discovery, dogwood anthracnose has been reported throughout the northeastern United States and southerly through the Appalachian Mountain region into south central Alabama (Carey and Kelley 1991, Knighten and Anderson 1992).

In annual crops, spread of fungal pathogens is limited by time and space (Zadoks and van den Bosch 1994); however, spread of *D. destructiva* can occur throughout most of the year because of the perennial nature of *C. florida*. Wind and rain have been suggested as factors in the rapid spread of dogwood anthracnose (Daughtrey and Hibben 1983). Colby (1993) and other researchers suggested that

insects could disperse conidia of *D. destructiva* as the insects contacted infected leaves while foraging for food.

Insects have been reported to aid in the spread of more than 200 plant pathogens of which 23 are caused by fungi (Borrer et al. 1989, Webber and Gibbs 1989). Coleopterans, especially bark beetles and weevils, are responsible for dissemination of 80% of these fungal pathogens (Webber and Gibbs 1989).

Until recently, no information existed relating to insects associated with dogwood anthracnose. Colby (1993) demonstrated that the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), could transport viable conidia externally for as many as 16 days under laboratory conditions. In the Great Smoky Mountains National Park, greater densities of insects coincided with high levels of dogwood anthracnose (Neitch et al. 1994). Thus, two factors of the dissemination process were established: the ability of an insect to accumulate and carry viable conidia and an abundance of insects during peak periods of disease severity. Previous research has suggested that insects may play an important role in the spread of dogwood anthracnose.

No information, however, is available on the incidence of conidia of *D. destructiva* on field-collected arthropods. This data would further evaluate the role of arthropods in epidemiology of dogwood anthracnose. Therefore, the objectives of this study were to: 1) determine the incidence of conidia-infested arthropods on infected understory dogwoods, 2) monitor seasonal incidence of conidia-infested arthropods, and 3) identify arthropods infested with conidia of *D. destructiva*.

ii. MATERIALS AND METHODS

Arthropods were sampled from dogwoods infected with *D. destructiva* in the Great Smoky Mountains National Park. Three sites were chosen based on their high incidence and severity of dogwood anthracnose. The three sites were located: 1) on a designated "quiet walkway" near Sugarlands Visitor Center, 2) at Jakes Creek, near Elkmont campground, and 3) at Rich Mountain overlooking Cades Cove (Figure 2). Foliage symptomatic for dogwood anthracnose was collected at each site. Infection by *D. destructiva* was confirmed by microscopic examination. At each site, insects were collected about every 3-4 weeks from June through September 1994. On each collection date, six dogwoods, ranging in height from 1 m to 7 m, were selected and sampled; the composition of dogwoods at each site was alternated on each sampling date. This sampling scheme allowed a larger sampling base and incorporated a wider range of conditions and disease characteristics associated with insect dissemination. This scheme provided a general assessment of insect involvement with the disease.

Sampling methods included hand picking and canopy agitation. Hand picking involved the placement of a clear poultry bag (20.3 cm x 10.2 cm x 45.7 cm) on the hand. After an arthropod was collected, the bag was inverted and a culture tube (17 mm x 100 mm) was placed into the bag. The arthropod was channeled into the tube, which was then capped and labelled with appropriate information (e.g., tree, location and date). Samples were placed into Thermos™ coolers and transported to the

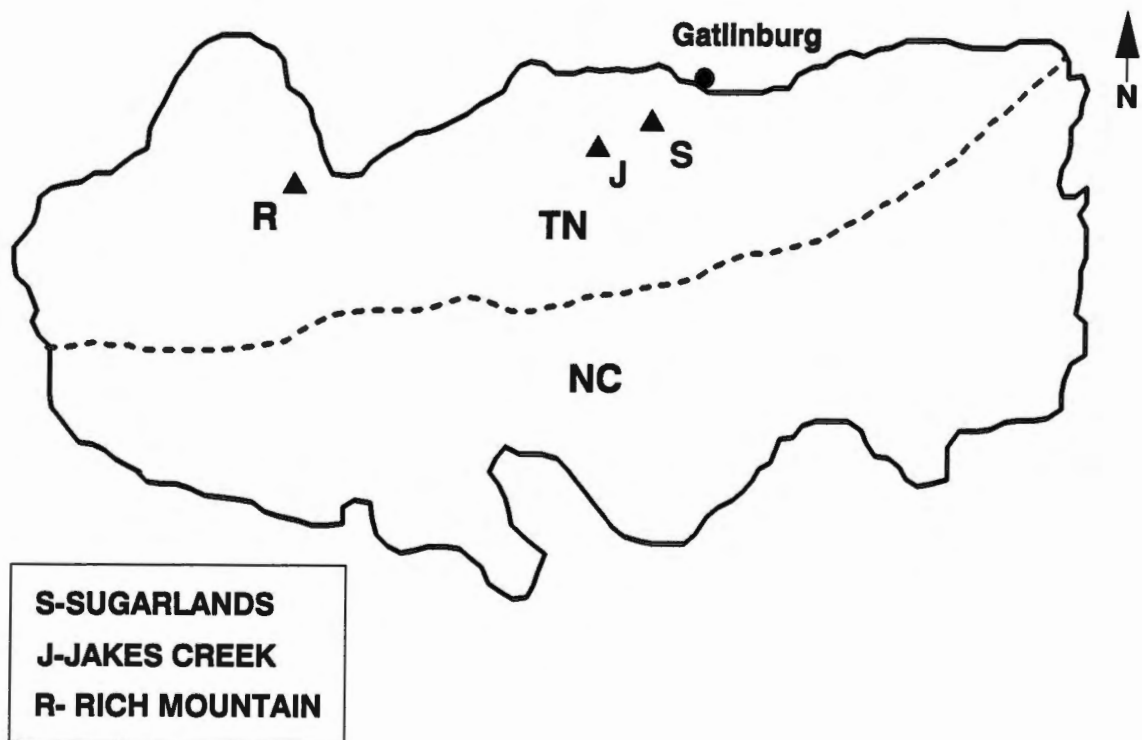


FIGURE 2. Location of sampling sites within the Great Smoky Mountains National Park, 1994.

laboratory. To prevent cross contamination during sampling, one poultry bag was used for each arthropod and then discarded. On each sampling date, hand picking was conducted for ca. 6-7 min for each tree.

Canopy agitation involved the placement of a polypropylene tarpaulin (tarp) (3.6 m x 3.6 m) under the canopy of each dogwood. The main stem was then vigorously shaken for ca. 15-30 sec. Arthropods that fell onto the tarp were then collected with forceps and placed into culture tubes (17 mm x 100 mm) (one insect/vial). After each arthropod was collected, forceps were sterilized with a 10% Clorox® solution (Clorox®/H₂O) to prevent cross contamination. Vials were capped, labelled with appropriate information, and placed into Thermos™ coolers for transportation to the laboratory.

In the laboratory, arthropods collected from infected dogwoods were assayed for viable conidia using a 10³ dilution series of sterile water. Arthropods were placed in culture tubes (17 mm x 100 mm) containing 10 ml of sterile water. Tubes were capped and agitated using a vortex machine (Baxter-Scientific Products, McGaw, IL) for ca. 8-10 sec. One ml of the water and spore suspension was placed onto a potato dextrose agar (PDA) plate amended with 30mg/L each of chlortetracycline and streptomycin sulfate. Another one ml was dispensed into the next tube in the series containing 9 ml of sterile water. The same procedure was followed for the remaining two tubes in the series.

Dilution cultures were incubated at 20°C and observed for ca. 2 weeks for sporulation of *D. destructiva*. Subculturing was often necessary to produce pure

isolates of *D. destructiva* because many other fungi and bacteria were isolated from field-collected arthropods. Wet mounts of sporulating *Discula* spp. were examined for conidia as described by Redlin (1991) and recorded when present. When colonies of *D. destructiva* were confirmed, infested arthropods were placed in appropriately labelled petri dishes (100 mm x 15 mm) and then placed in a freezer until further identification. Location and date of collection of conidia-infested arthropods were recorded.

The Horsfal-Barratt (HB) (Horsfal and Barratt 1945) scale was used on each sampling date to rate disease progression because it provided a logarithmic function necessary for scientific analysis (Campbell and Madden 1990). The site, date and number of arthropods collected from each dogwood also were recorded for analysis.

iii. RESULTS AND DISCUSSION

Arthropods (7.2%, n=375) collected from anthracnose-infected dogwoods at three sites in the Great Smoky Mountains National Park were infested with viable conidia. The percentage of arthropods infested with conidia from each site was greatest at Rich Mountain (11.4%), while conidial infestations were lower at Jakes Creek (5.9%) and Sugarlands (4.5%) (Table 1).

Conidial infestations of arthropods were greater at each site during June than on other sampling dates (Table 2 and Figure 3). The conidial infestation level was greatest at Rich Mountain (22.0%) followed by Jakes Creek (11.1%) and Sugarlands (8.6%) during June. During this time period, epidemics of dogwood

TABLE 1. The percent of arthropods (n=375) infested with viable conidia of *Discula destructiva* collected from each site from June through September in the Great Smoky Mountains National Park, 1994.

Site	Number collected	Number infested	Percent infested
Jakes Creek	119	7	5.9
Rich Mountain	123	14	11.4
Sugarlands	133	6	4.5

Table 2. Arthropods infested with viable conidia of *Discula destructiva* collected each month from three sites in the Great Smoky Mountains National Park, 1994.

Date	Site	Number Collected	Number Infested
June	Sugarlands	58	5
	Jakes Creek	63	7
	Rich Mountain	59	13
July	Sugarlands	20	0
	Jakes Creek	15	0
	Rich Mountain	21	1
August	Sugarlands	19	1
	Jakes Creek	16	0
	Rich Mountain	18	0
September	Sugarlands	36	0
	Jakes Creek	25	0
	Rich Mountain	25	0
Total	All Sites	375	27

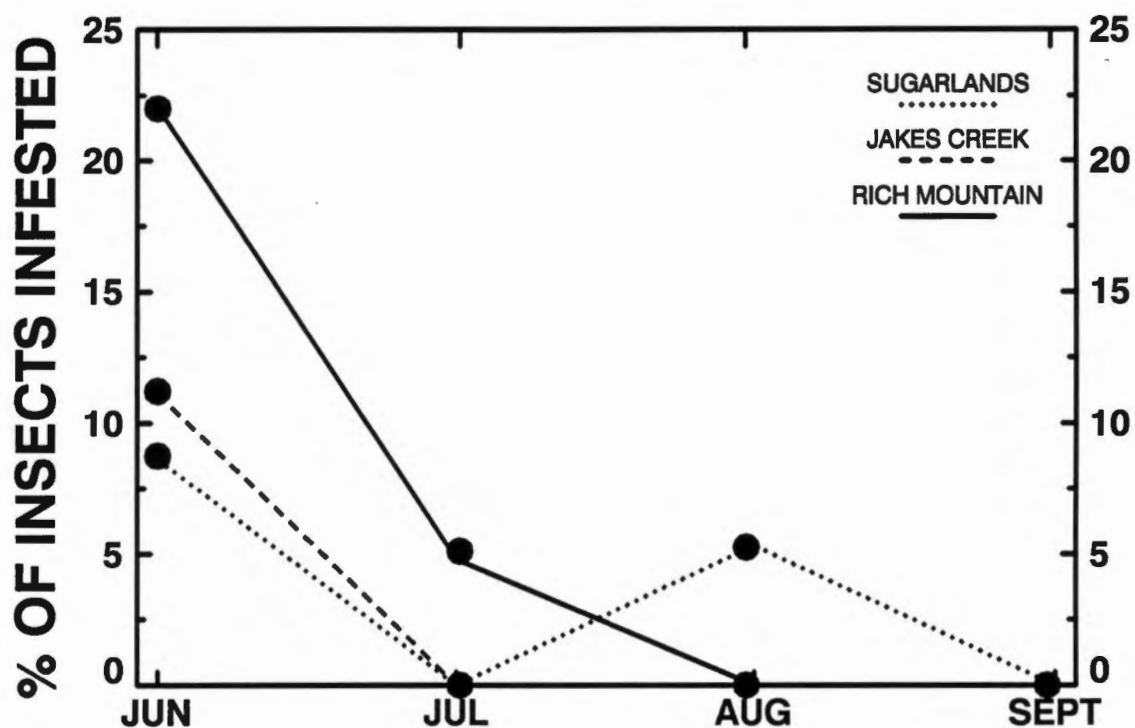


FIGURE 3. Percent of arthropods infested with viable conidia of *Discula destructiva* collected from three sites in the Great Smoky Mountains National Park, 1994.

anthracnose are normally increasing (M. T. Windham, personal communication). Dogwood anthracnose severity (rated as percent diseased foliage) was 91.0, 62.5, and 81.5% at Rich Mountain, Jakes Creek, and Sugarlands, respectively. The high level of disease severity at Rich Mountain may explain why more infested arthropods were found at that site. In another study (M. T. Windham, unpublished data), disease severity was measured at the Jakes Creek site. Increases in disease severity levels were greatest from mid-May (week 2) until early July (week 9) (Figure 4). Most arthropods infested with viable conidia were collected during this period (June 10-15) (Table 2 and Figure 3). When increases in disease severity leveled off in early July, fewer conidia-infested arthropods were collected. In fact, after June, conidial infestation levels ($< 1.0\%$; $n=2$) for all sites combined decreased abruptly and were non-existent in September. The numbers of arthropods collected also dropped after June which contributed to the collection of fewer conidia-infested arthropods (Table 2). This trend is supported by Neitch and co-workers (1994) who observed that peak arthropod densities coincided with high levels of dogwood anthracnose.

Additional research is necessary to determine whether the level of arthropod infestation was merely an index of disease severity or if disease severity was influenced by arthropod dissemination. Data from this preliminary study support the latter hypothesis, since most of the arthropods collected were able to move to other dogwoods in close proximity (Table 3). Arachnids and immature lepidopterans may not have influenced the level of disease severity because spiders remain close to their

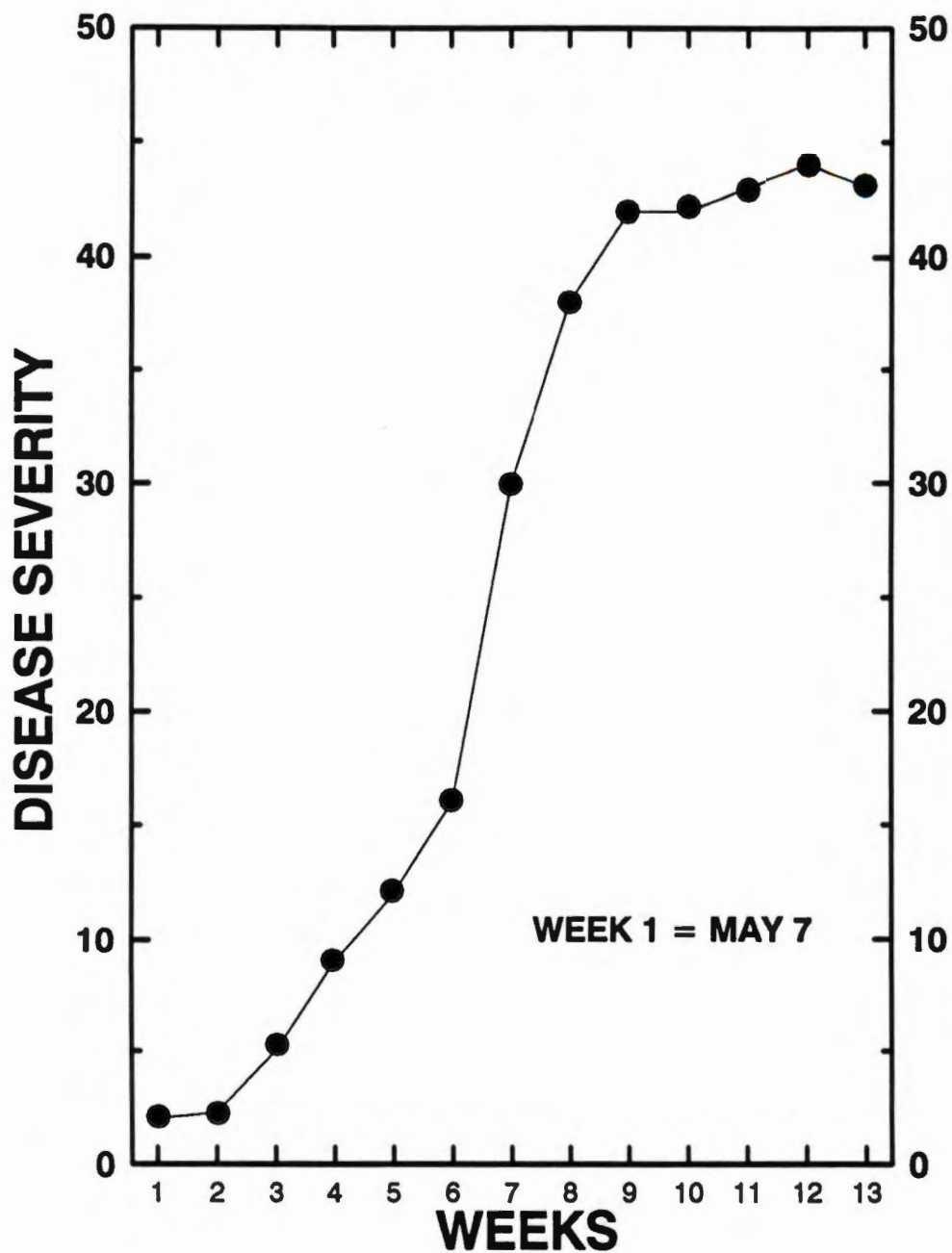


FIGURE 4. Disease severity of dogwood anthracnose in the Great Smoky Mountains National Park, 1994. Disease severity was rated using a modified Horsfal-Barratt disease rating scale (Windham, unpublished data).

TABLE 3. Percent of different taxonomic groups of field-collected arthropods infested with conidia of *Discula destructiva*, Great Smoky Mountains National Park, 1994.¹

Taxonomic Group	Number Collected	Percent ²
Arachnida	2	7.4
Coleoptera	1	3.7
Hemiptera	1	3.7
Homoptera	1	3.7
Hymenoptera	1	3.7
Lepidoptera (immatures)	7	25.9
Orthoptera (immatures)	8	29.6
Orthoptera (adults)	2	7.4
Psocoptera	3	11.1
Unidentified (damaged)	1	3.7
TOTAL	27	100

¹Of the 375 arthropods collected, 27 (7.2%) were infested with viable conidia.

²Percent breakdown of conidia-infested arthropods (n=27).

webs to capture prey while immature lepidopterans usually remain on one tree as long as there is a food source. The remaining groups are mobile enough to move from one tree to another, especially the coleopterans (e.g., curculionids), hemipterans, homopterans, hymenopterans (e.g., ants), and immature and adult orthopterans. This group of arthropods, as a whole, may intensify the severity of dogwood anthracnose in a localized area but would probably not effect the spread of dogwood anthracnose to healthy stands at a substantial rate. A more efficient method of arthropod sampling, such as the insecticidal spray used by Neitch et al. (1994), may produce a more realistic picture of the role of insects in the epidemiology of this disease. Insect sampling, using insecticidal spraying, would collect those insects that are not easily sampled by hand picking or canopy agitation. Preliminary studies would be necessary to determine the effect of insecticide, if any, upon conidial viability before this method could be utilized successfully.

Of the infested arthropods collected in this study, winged insects, especially coleopterans, dipterans, and hymenopterans, were found in low numbers (ca. 7%). These orders represent insects that can fly greater distances. Neitch and co-workers (1994) reported that these three orders comprised more than 60% of all insects collected from dogwoods in a forest environment. Collection and verification of conidial infestation levels of these "mobile" orders would be necessary to determine if insects can serve as dispersal agents of *D. destructiva*.

CHAPTER III

INFLUENCE OF SUNLIGHT AND SHADE ENVIRONMENTS ON THE VIABILITY OF CONIDIA OF *Discula destructiva* ON THE CONVERGENT LADY BEETLE, *Hippodamia convergens*

i. INTRODUCTION

Eastern flowering dogwood, *Cornus florida* Link., is a native component of eastern hardwood forests and a valuable component in the forest ecosystem. As an early successional tree in natural reforestation, the dogwood provides calcium rich foliage which supplements soil fertility, especially when soil elements may be low (Boring et al. 1981). Eyde (1988) and Whitmore (1992) reported that dogwoods provide a food source to more than 80 species of birds and mammals.

Dogwoods also are economically important especially to the nursery industry. In 1989, dogwood sales in Tennessee were estimated to be 30 million dollars (Windham and Freeland 1990). The springtime blooms of dogwoods are the focus of many festivals which attract tourists and generate additional revenue (Colby 1993).

Approximately 16 years ago, a new disease of native dogwoods in the northeastern United States was reported (Daughtrey and Hibben 1983). Redlin (1991) later reported that the cause of this decline was attributed to a fungal pathogen, *Discula destructiva* Link. Characteristic symptoms of this disease ranged from leaf spots and necrotic lesions to the coalescence of cankers around the main stem

(Daughtrey et al. 1986). Since its discovery, dogwood anthracnose has spread as far south as central Alabama (Bailey and Brown 1991, Carey and Kelley 1991, Hess 1992).

Because of the adverse effects of dogwood anthracnose upon the declining dogwood population, public interest in the dogwood has waned. Consequently, a 60% reduction in sales of this ornamental have been reported by some nurserymen (M. T. Windham, personal communication).

Insects play a major role in the dissemination of more than 200 plant pathogens (Borrer et al. 1989). Of the known insect-related diseases, 23 are caused by fungi, and coleopterans disseminate 80% of these fungal pathogens (Webber and Gibbs 1989).

Previous studies, under laboratory conditions, demonstrated that viable conidia of *D. destructiva* could be transported by the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera:Coccinellidae) (Colby 1993). However, little information is available on the role of insects as carriers of conidia of *D. destructiva* in a natural environment. Therefore, the objective of this research were to: 1) examine the effects of light intensity on viability of conidia carried by the convergent lady beetle over selected time intervals in the field and 2) evaluate the incidence of dogwoods infected with *D. destructiva* resulting from infested convergent lady beetles.

ii. MATERIALS AND METHODS

The convergent lady beetle was selected as the model insect for this study because of their availability for research purposes and they have been collected from dogwoods (J.F. Grant, personal communication). The use of the convergent lady beetle also would provide insect continuity with a previous laboratory study (Colby 1993) and enable comparisons of laboratory and field results. Adult beetles were purchased from Rincon-Vitova Insectaries, Inc. (Oak View, CA) and maintained in clear plexiglas containers (30.48 cm x 30.48 cm x 40.64 cm) with a screened opening (12 cm) for ventilation on all four sides. A mixture of honey and sugar placed on the inside wall and a cotton-plugged flask filled with water were provided for nutritional needs. Cages were placed in incubators maintained at 10-13°C and 12:12 h light:dark cycles (Colby 1993).

An isolate of *D. destructiva*, obtained from a naturally infested insect was used in this study. The isolate was verified by comparing conidia and colony morphology with similar attributes of *D. destructiva*. Stock cultures were maintained on potato dextrose agar (PDA) amended with chlortetracycline and streptomycin sulfate as described by Colby (1993).

Leaves (n=78) of *C. florida* were trimmed to fit inside a glass petri dish (90 mm x 15 mm). Leaves (8 leaves/plate) were placed inside the petri dish, separated by filter paper disks (9 cm), saturated with deionized water, and autoclaved for 1 h on two consecutive days. Sterilized leaves were placed on PDA and stored,

until needed, at 6°C. To obtain plates of sporulating *D. destructiva*, each leaf plate was inoculated with one plug (8 mm) of *D. destructiva* and incubated at 20°C with 8:16 h light:dark cycle for 2 weeks.

Two light intensities and three infestation levels were evaluated to compare their effects on conidial viability over time. This study was arranged as a 2 x 3 factorial completely randomized design with three replications. Data were analyzed by PROC GLM (SAS Institute 1985). When significant ($P \leq 0.1$) differences were observed, least square means analysis was used to determine significant ($P \leq 0.1$) differences among the means.

Light intensity, [photosynthetically active radiation (P.A.R. = μ moles of photons/m²/s²)], was measured in unrestricted sunlight, within cages in the sun, in shade restricted sunlight, and within cages in the shade. This was used to determine the effects of sunlight on conidial viability of *D. destructiva*.

Eighteen nylon-screened cages (0.9 m x 1.2 m x 1.8 m) (Lumite, Gainesville, GA) were erected at the Tobacco Experiment Station in Greeneville, TN. Nine cages were placed in full sunlight exposure and nine were located in an understory environment (shade). The three infestation levels [convergent lady beetles infested with conidia of *D. destructiva*, non-infested convergent lady beetles, and controls (beetles absent)] were randomly assigned to three cages within each light intensity. Vegetation within each cage was eliminated by application of Roundup® for immediate control and Spike® for long term control. A layer (7.6 cm) of shredded cypress mulch was then placed on the ground within each cage to prevent poisoning of

dogwoods by the herbicides. Three potted *C. florida* seedlings (ca. 0.75 m tall) were then placed into each cage and allowed to acclimate for about 1 week.

On 21 September 1994, fungal cultures and convergent lady beetles were transported to the study site. To infest adult convergent lady beetles with conidia of *D. destructiva*, adults were placed (15 adults/plate) onto leaf plates containing sporulating *D. destructiva* thalli for 1 h at 26°C. Then, 195 infested convergent lady beetles (13 plates/cage) were released into each designated cage in each light intensity. The same procedure, without the fungus, was followed with non-infested convergent lady beetles. No beetles were placed into the remaining cages, designated as the controls. To determine conidial viability over time, a 16-day sampling period was established with collection intervals of 1, 2, 4, 8 and 16 days following release of the beetles into cages. On each collection date, 10 convergent lady beetles were collected from each cage, placed individually in a culture tube, labelled, placed into a Thermos® cooler, and transported to the laboratory for processing.

All convergent lady beetles were transferred through a 10^3 dilution series of sterile H_2O . Each beetle was placed into a test tube (17 mm x 100 mm) with 10 ml of sterile H_2O . The tube was capped and agitated with a vortex machine (Baxter-Scientific Products, McGaw, IL) for ca. 8-10 secs. One ml of rinse water was placed onto a PDA plate amended with 30 mg/L each of chlortetracycline and streptomycin sulfate. Another one ml was dispensed into the next tube in the series containing 9 ml of sterile water. The same procedure was followed for the remaining two tubes in the series.

Dilution cultures were incubated at 20°C and 12:12 h light:dark cycles and observed for ca. 2 weeks for sporulation of *D. destructiva*. Wet mounts of sporulating acervuli were observed for conidia of *D. destructiva* as described by Redlin (1991) and recorded when present. Data were analyzed as previously described.

iii. RESULTS AND DISCUSSION

Conidial viability was significantly affected by light intensity and time interval (Table 4). The greatest level of infested insects occurred 1 day after release into the cages, and then decreased significantly afterwards (Table 5 and Figure 5). Insects were infested with conidia, however, for as many as 16 days.

Infestation levels differed substantially when light intensities (shade and sunlight) were examined separately (Figure 5). Average light intensities in the understory (36 PAR) cages were reduced approximately 94% when compared to the cages exposed to full sunlight (678 PAR) (Figure 6). On day 1, about 20% of the convergent lady beetles in the shaded cages were infested with viable conidia of *D. destructiva*, while approximately 3% of those in sunlight carried viable conidia. Full sunlight exposure significantly decreased conidial viability two days after release (Table 5 and Figure 5). Other studies of conidia-infested insects have shown a similar response to higher light intensities in the natural environment (Webber and Gibbs 1989). Numbers of conidia of pathogenic fungi on some insects are reported to be enormous. Scolytid beetles can transport as many as 300,000 conidia of

TABLE 4. Least square means analysis ($p \leq 0.1$) of the effects of light intensity and time interval on the viability of *Discula destructiva* infesting the bodies of *Hippodamia convergens* in cages at the Greeneville Experiment Station, 1994.

Effects	df	Probability level
Model	9	0.059
light intensity	1	0.086
time interval	4	0.048
light intensity * time interval	4	0.260

$r^2 = 51\%$

TABLE 5. Comparisons of light intensity and time interval treatments on viability of conidia of *Discula destructiva* in the natural environment.

Treatment	Variable	Mean±S.D. ¹
Light intensity ²	Shade	0.53±1.13a
	Sunlight	0.07±0.26b
Time interval ³	Day 1	1.17±1.60a
	Day 2	0.17±0.41b
	Day 4	0.00±0.00b
	Day 8	0.00±0.00b
	Day 16	0.17±0.41b

¹Means followed by the same letter are not significantly different from each other (least square means analysis, $P > 0.10$); values represent the average number of conidial infested beetles per treatment.

²Light intensity in the shade was approximately 95% lower than in full sunlight exposure.

³Conidia-infested *Hippodamia convergens* were sampled (n=10/cage) on designated days following their release into field cages.

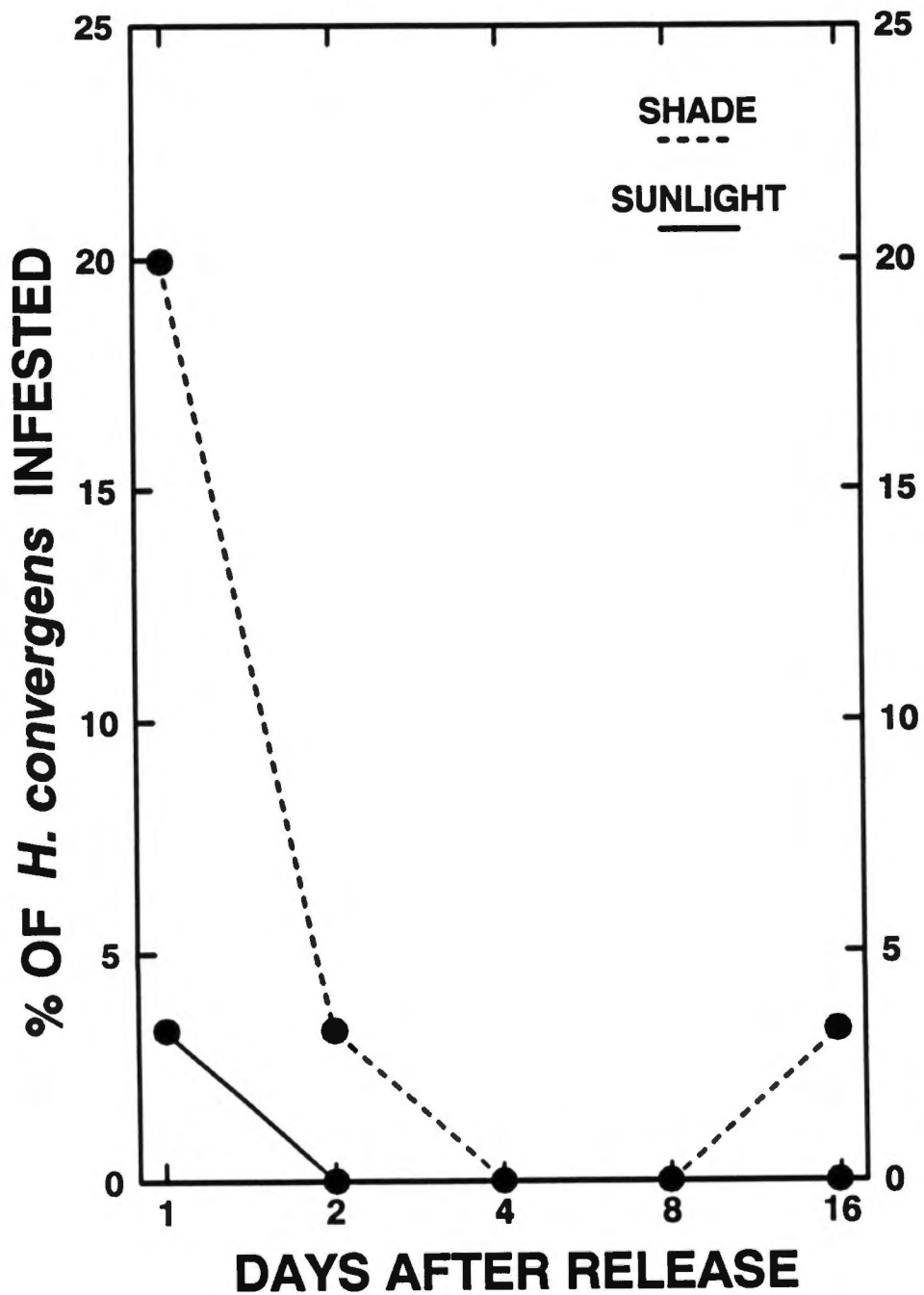


FIGURE 5. Percent of *Hippodamia convergens* infested with conidia of *Discula destructiva* in each light intensity at designated time intervals following release onto dogwoods in cages.

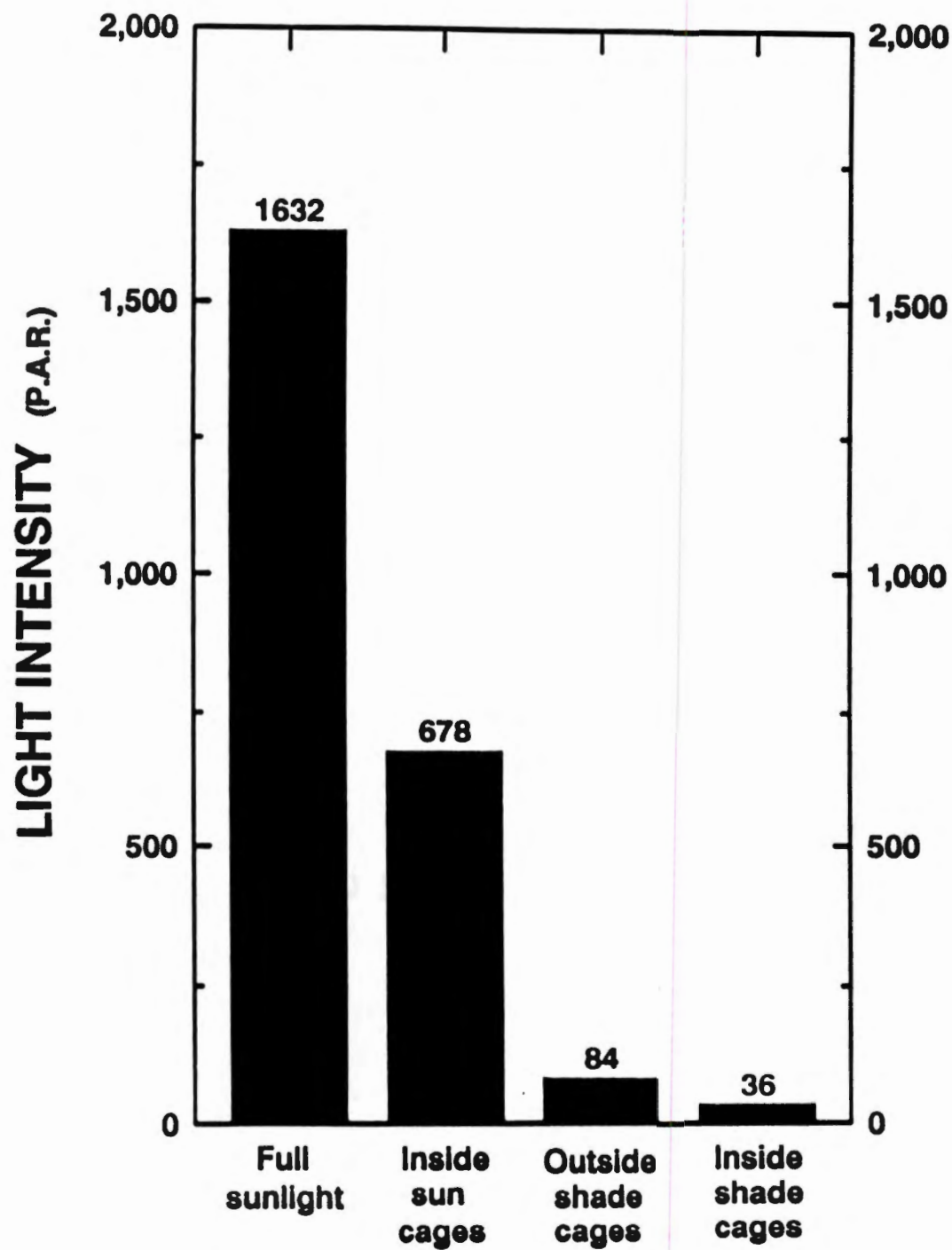


FIGURE 6. Comparison of photosynthetically active radiation (P.A.R.) intensities in unrestricted sunlight, within cages in full sunlight exposure, shade restricted sunlight, and within shaded cages.

Ophiostoma ulmi Buisman, causal agent of Dutch elm disease (Webber and Gibbs 1989). Since elm scolytids do not have specialized organs for fungal transport, conidia of *O. ulmi* are carried on the surface and within invaginations of the exoskeleton (Webber and Gibbs 1989). Many of these conidia are exposed to air currents and sunlight during flight. Studies have demonstrated that conidia of *O. ulmi* are susceptible to desiccation when RH is less than 80% and wavelengths of ultraviolet (U.V.) light are encountered (Webber and Gibbs 1989). Another study illustrated that the incidence of viable conidia of *O. ulmi* on adult scolytids was reduced from 98% to 10% after flight (Webber and Brasier 1984).

In further comparison of the two light intensities, moisture appeared to favor survival of conidia of *D. destructiva* in shade relative to those in the sun treatments. Conidial infestation was 86% greater on beetles in shaded cages than on those in full sunlight 1 day after release. The forest canopy can reduce wind velocity and light intensity resulting in higher moisture levels that may provide a more favorable environment for disease development (Chellemi et al. 1992, Windham et al. 1993). Other studies also demonstrated that the incidence of dogwood anthracnose was higher in an understory environment than in full sunlight (Hibben and Daughtrey 1988, Hagan and Mullen 1993).

Behavior of the convergent lady beetle also may play a role in low infestation levels of viable conidia of *D. destructiva* in sunlight-exposed cages. Beetles were phototropically attracted to the sunlight and congregated on the ceiling of the cage. This behavior resulted in greater exposure of the ventral surface of the insect to

sunlight. Colby (1993) reported that the ventral surface carried significantly more conidia than the dorsal surface. In addition, conidia also were observed on the setae, legs and thorax because of the adhesive nature of the encapsulating protein matrix (Colby 1993). Since a proportionately greater number of conidia are attached to this region, prolonged exposure to U.V. light may have destroyed a larger number of these conidia, when compared to the incidence of beetles carrying viable conidia in the shaded site. Beetles, in shade treatments with reduced sunlight, also remained in the uppermost areas of the cages, ventral side up. However, most U.V. light would have been blocked by foliage from overstory trees.

A decline in conidial viability also was observed in an earlier laboratory study by Colby (1993) (Figure 7). She reported that beetles were infested with viable conidia for as many as 16 days, and her results were similar to the field data from the shade cages. In both studies, infestation levels of viable conidia were highest on day 1 following beetle release. After day 1, the number of beetles carrying viable conidia sharply decreased. Initially, high levels of viable conidia found on beetles may have resulted from the effects of decreased amounts of sunlight in both studies. However, light intensity remained low for the duration of both studies indicating that time also had a significant effect on conidial viability (Table 5). The level of light (33 PAR) in the laboratory, under indirect lighting, is similar to the light level (36 PAR) in the shaded cages in this study (Figure 6). Levels of conidial infestation were generally lower than reported in previous studies (Colby 1993). Infestation levels in the natural environment were approximately 90% lower than the laboratory studies, on average,

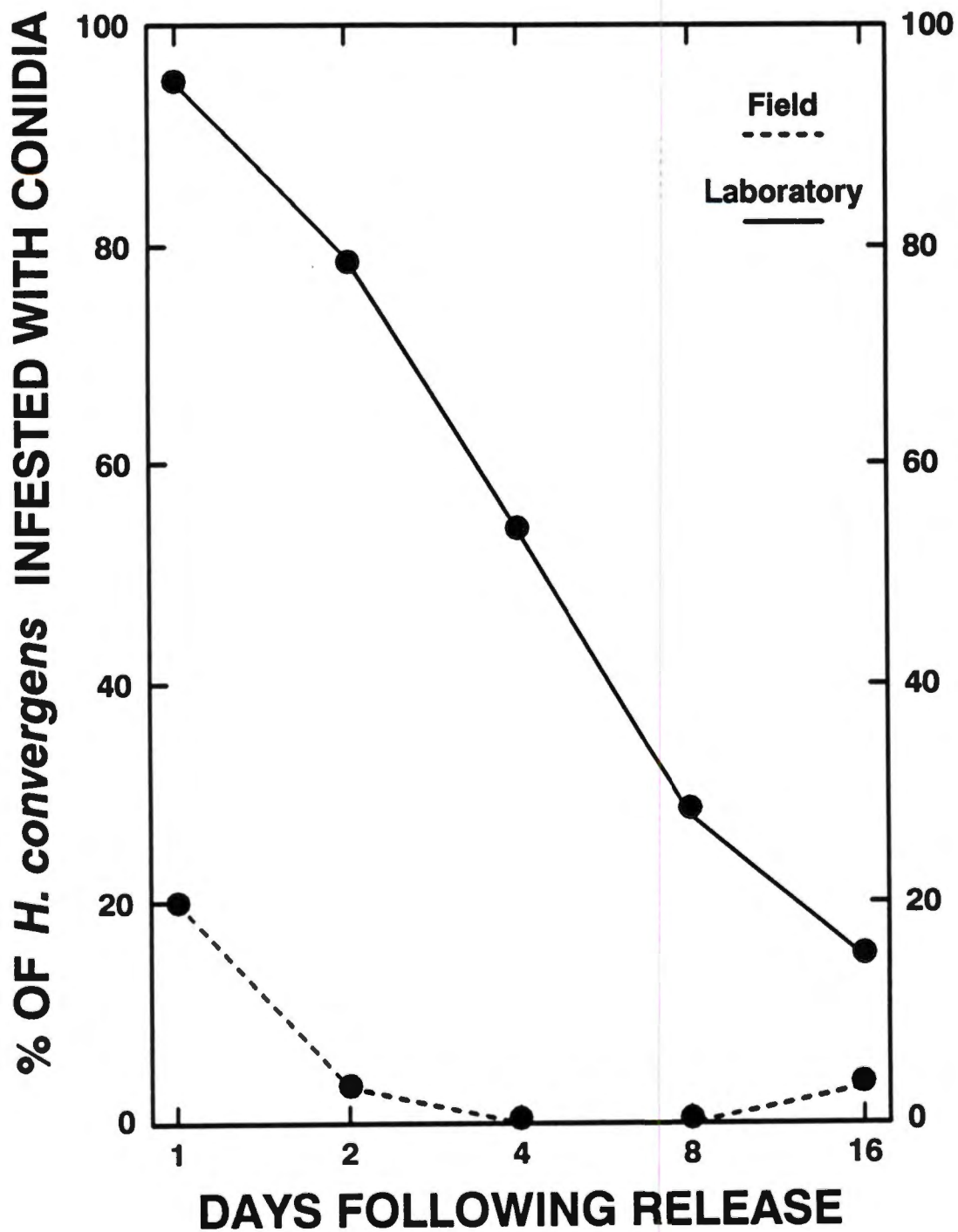


FIGURE 7. Comparison of infestations of conidia of *Discula destructiva* on *Hippodamia convergens* between the field study (shade, Figure 5) and a previously reported laboratory study (from Colby 1993).

throughout the sampling period (Figure 7). Stress regimes of the natural environment, such as sunlight and air currents, not found in the laboratory, are believed to be partially responsible for these contrasting levels (Webber and Gibbs 1989). Fewer fluctuations in temperature and humidity occur in an environmentally controlled laboratory. Constant levels of those environmental factors may have been less stressful and more conducive to survival of conidia of *D. destructiva*.

Three possible explanations account for the difference in the percent of beetles infested with viable conidia on day 1. First, all of the beetles may not have been infested initially with conidia. Second, the age of conidia may have been a factor concerning viability, as cultures of *D. destructiva* were made approximately 3.5 weeks prior to release. Based on this study and Colby's (1993) study, however, at least some conidia survive for as many as 16 days. Third, a combination of site factors may have had a detrimental effect on viable conidia-infested beetles. Site temperatures ranged from 21.5 to 27.5 C during the day, and these upper temperatures are not considered to be optimal to the health of *D. destructiva* (Roncadori 1993). Relative humidities may have been higher inside of the cages because of a reduction in wind velocity caused by the small nylon mesh on the cage. Furthermore, sunlight in the cages in the shade (36 PAR) was reduced about 57% when compared to sunlight (84 PAR) outside of those cages. This reduction in sunlight also may have increased humidity (Figure 6). Roncadori (1993) demonstrated that, as temperatures and relative humidity increased, conidial viability was reduced.

Cages were placed on a more or less western aspect in an area where there was no immediate water source, such as creeks and streams. Windham and co-workers (1993) found that severity of dogwood anthracnose was less in trees placed on the eastern, western, and southern aspects than on the northern aspect. Trees placed in areas at least 200 m from water exhibited less impact from dogwood anthracnose than those placed near a water source. If these conditions, which are conducive to disease severity, are similar for conidial survival, then their absence in this study also may have lowered conidial viability.

The second objective of this study, designed to determine the incidence of dogwood anthracnose in cages, was not accomplished because a dogwood anthracnose-free treatment could not be maintained. Dogwoods appeared healthy and disease-free when taken to the release site, but cultures taken from symptomatic trees revealed that they were infected with *D. destructiva* in non-infested and control cages during latter parts of this study. This complication did not affect the study on incidence of viable conidia on infested beetles. Non-infested beetles were sampled and processed using the same procedures as with the infested beetles. No conidia of *D. destructiva* were obtained from non-infested insects used as the control. Since non-infested beetles did not accumulate conidia from infected trees, it was determined that beetles transporting viable conidia resulted from the initial infestation procedure and not from diseased dogwoods in the cages.

Data suggest that certain biological parameters provide optimum conditions for transportation of viable conidia of *D. destructiva*. When time intervals were

evaluated for incidence of beetles infested with viable conidia, a significant difference occurred between day 1 and day 2 following infestation, when 86% fewer beetles were infested with viable conidia. This reduction illustrates that if insects can disperse viable conidia of *D. destructiva*, then most dispersal would occur within 24 h following infestation. Light intensity significantly affected the incidence of beetles infested with viable conidia. Sunlight in the shade cages was 95% lower than in the sun cages. Reduced sunlight (shade) caused the level of conidial infestation to substantially increase. An increase in beetles infested with viable conidia in the shaded cages suggests that the probability of transporting viable conidia was much greater in an understory environment than in full sunlight exposure.

CHAPTER IV

CONCLUSIONS

In the late 1970s, a new disease of the flowering dogwood, *Cornus florida* Link., was reported in the northeastern United States (Daughtrey and Hibben 1983). It was later discovered that this disease, dogwood anthracnose, was caused by the fungal pathogen, *Discula destructiva* Redlin (Redlin 1991).

Until recently, little research had been conducted on arthropods and their potential involvement in the spread of dogwood anthracnose. Colby (1993) demonstrated that the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), could carry viable conidia of *D. destructiva* for as many as 16 days under laboratory conditions. Therefore, two studies were designed to expand the laboratory research into the field. The objectives of this research were to determine if arthropods could carry viable conidia of *D. destructiva* in the natural environment and to determine the effects of light intensity (sunlight and shade) upon viable conidia of *D. destructiva* over selected periods of time.

In the first study, arthropods collected from dogwoods infected with *D. destructiva* carried viable conidia in the natural environment. Of the arthropods collected (n=375) throughout the season, 7.2% (n=27) were infested with viable conidia of this fungus. Of the collections at each site, the percentage of infested arthropods was greatest at Rich Mountain (11.4%), with lower infestations at Jakes

Creek (5.9%) and Sugarlands (4.5%). Elevated levels of infested arthropods were collected at Rich Mountain because disease conditions were more severe, averaging 91% according to the Horsfal-Barratt disease rating scale. When arthropod infestations were evaluated by sampling period, infestation levels were greatest in June for each site. Rich Mountain had the greatest level of infestation (22.0%) followed by Jakes Creek (11.1%) and Sugarlands (8.6%). During July and August, infestation levels ($<1.0\%$; $n=2$) for all sites combined decreased abruptly and were non-existent in September. Disease severity of dogwood anthracnose was at its greatest level in June, decreased significantly in July, and was maintained at low levels by abiotic factors for the remainder of the study. These results indicate that arthropods infested with conidia of *D. destructiva* were closely associated with increasing levels of severity of dogwood anthracnose.

Further research is necessary to determine whether the level of arthropod infestation was merely an index of disease severity or if disease severity was influenced by arthropod dissemination and, if so, to what degree. Results in this preliminary study support the latter hypothesis, since most of the arthropods collected were probably able to move to other dogwoods in close proximity. This group of arthropods may intensify the severity of dogwood anthracnose in localized areas. However, they would probably not substantially impact the spread of dogwood anthracnose to healthy stands.

In the second study, convergent lady beetles were sampled from cages placed in full sunlight exposure and shade to determine the effect of light intensity upon

conidial viability over selected periods of time. Infestation levels of conidia on beetles collected from shaded cages 1 day after release were greater than those in sunlight (20% and 3.3%, respectively). In addition, beetles in the shade transported viable conidia for longer periods of time than those in the sun (16 days and 1 day, respectively). Conditions, such as low light intensity and reduced wind velocity, which favor increased levels of disease severity, also were conducive to conidial viability on convergent lady beetles sampled from cages in the shade.

The results of this study have shown that arthropods carry viable conidia of *D. destructiva* in the natural environment. Furthermore, optimal conditions for the potential dispersal of conidia by arthropods were defined as: 1) viable conidia carried by insects within 24 h after infestation, 2) conidia transported by arthropods under shaded conditions, which are more conducive to the survivability of conidia, and 3) conidia carried by arthropods when disease severity of dogwood anthracnose was at its greatest levels. A large, diverse number of insects visit dogwoods (Neitch et al. 1994). In addition, this research demonstrated that more than one species of arthropod carried viable conidia of this fungal pathogen and suggests that arthropods may play an important role in the epidemiology of dogwood anthracnose. More research, however, is needed to determine the degree of arthropod involvement in the spread of dogwood anthracnose.

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VITA

H. Lee Holt was born in Humboldt, Tennessee, on November 11, 1954. He moved to Knoxville, Tennessee, with his parents in the summer of 1956. He completed his Bachelor of Science degree in Forestry Resource Management from the University of Tennessee in August 1981. After several years in the medical equipment industry, he returned to school to pursue a Master of Science degree in Entomology, again at the University of Tennessee. Under the direction of Drs. J.F. Grant and M.T. Windham, he completed the requirements for a Master of Science degree in Entomology and Plant Pathology in May 1995.

H. Lee Holt is a member of the Entomological Society of America, Tennessee Entomological Society, Gamma Sigma Delta Agricultural Honor Society, and the Entomology and Plant Pathology Graduate Student Association.