CHAPTER 9.

Assessment and Etiology of Hickory (*Carya* spp.) Decline in the Midwest and Northeastern Regions

(Project NC-EM-07-01)

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INTRODUCTION

Hickory decline, particularly decline in bitternut hickory (*Carya cordiformis*) and to a lesser extent in shagbark hickory (*C. ovata*), has recently been noted in Iowa, Maryland, Missouri, New York, Pennsylvania, West Virginia, and Wisconsin (Steinman 2004). Periodic episodes of hickory mortality have been recorded from Wisconsin to Vermont and south-central Georgia since the early part of the 20th century (Hopkins 1912; St. George 1929; USDA Forest Service 1956, 1972). Within the first decade of that century, thousands of hickory trees died in central New York State (Hopkins 1912). Subsequent periodic episodes were reported through the rest of the century. For example, in Wisconsin, episodes of hickory decline or dieback were reported in the late 1960s, late 1980s, and early 2000s (Wisconsin Department of Natural Resources 2005).

Widespread mortality of hickory has historically been attributed to outbreaks of the hickory bark beetle (*Scolytus quadrispinosus*) during extended periods of drought. This insect is considered the most important pest of the species group. In 1994, a fungus species new to science was reported in discolored wood and sunken bark cankers associated with hickory bark beetle attacks. This fungus and a related species were recently described as *Ceratocystis smalleyi* and *C. caryae*, respectively (Johnson and others 2005). Both species were pathogenic on 2-year-old *Carya* spp. in greenhouse studies.

This project was conducted between 2007 and 2011. The planned objectives of the overall project were to (1) conduct field evaluations to (a) determine frequencies of decline and mortality of bitternut, shagbark, and other hickory species in appropriate forest cover types where deviations from expected levels of mortality have been observed; (b) quantify relationships between decline incidence and pathogen, insect pest presence, or both; and (c) quantify relationships between decline incidence and prior land use, fire history, soil fertility, drought, or some combination of the four; and (2) determine the role of two newly described *Ceratocystis* spp. in decline and mortality of hickory.

In this summary, we will briefly describe the findings under the assessment objective (1) and in more detail describe the results of the etiology objective (2). The specific objectives of the etiology portion of these investigations were expanded to include (a) determine the predominant fungi isolated from stem damage observed on hickory trees with declining crowns in surveyed stands, (b) determine the pathogenicity of the predominant fungi, (c) characterize the interactions between the hickory bark beetle and *C. smalleyi* on bitternut hickory exhibiting active crown decline, and (d) determine the role of *C. smalleyi* in development of hickory crown decline. Portions of these results have been published (Juzwik and Banik 2011; Juzwik and others 2008a, 2008b; Park 2011; Park and Juzwik 2012; Park and others 2009, 2010, 2013).

METHODS AND RESULTS

Assessment (objective 1)

Survey methods and results of the assessment portion of the project were summarized in the synthesis of evaluation monitoring projects for 1998–2007 (Ambrose 2012). A survey of 27 stands in six States (Indiana, Iowa, Minnesota, New York, Ohio, and Wisconsin) was conducted between 2007 and 2009. The most widespread and predominant insect pest associated with declining bitternut and pignut (*C. glabra*) hickory was the hickory bark beetle. The hickory timber beetle, *Xyleborus celsus*, was the second most common pest documented. Of 1,334 insects that emerged from sections taken from 33 felled declining hickories in 2008, 91 percent were hickory bark beetle and 8 percent were hickory timber beetle. Entry or exit holes of different diameters were the most common type of stem damage observed during point-plot surveys in 21 stands with actively declining bitternut hickory. Cankers and globose galls were the second most common type of stem damage (12 and 11 percent, respectively). Fungi were suspected to be the cause of the cankers and galls observed based on related reports in the literature.

Etiology: determination of predominant pathogens associated with stem cankers and galls (objective 2a)

***Methods*—**Stem sections for fungal isolation were cut from felled hickory trees in 27 stands in six States (fig. 9.1). Three trees were felled in each stand; in total, 299 sections were obtained from 87 trees. Stem sections were examined for the presence of cankers, galls, xylem discoloration, and insect damage. Subsamples of the damaged stems from diseased bark, sapwood, or both were used for fungal isolation. Isolations were attempted in two ways. Small wood cubes placed in moist chambers fostered sporulation of some fungi, from which some isolates were obtained. Other isolates were obtained by plating wood or bark chips from margins of the diseased-healthy tissue following surface sterilization. Identification of pure isolates was based on cultural and morphological characteristics as well as results of DNA sequencing of the internally transcribed spacer region, the translation elongation factor 1-α gene, or both.

***Results*—**Most sections were taken from trees with either diffuse cankers (50 percent) or annual cankers (38 percent), regardless of insect damage occurrence. Stem galls were present

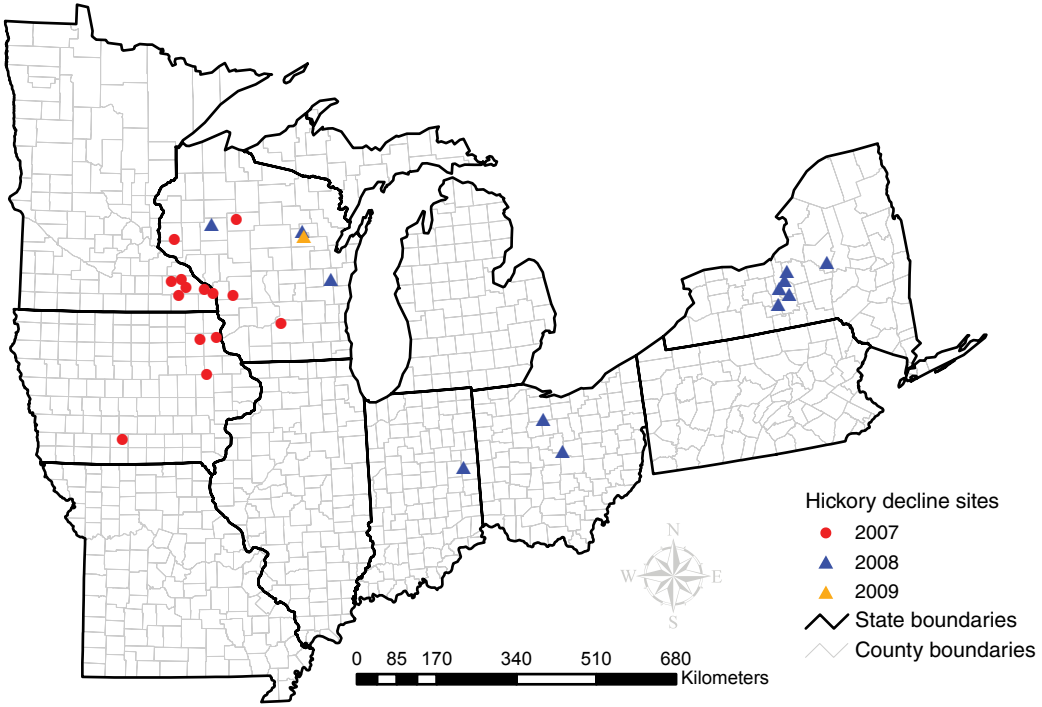

Figure 9.1—Geographical distribution of forest stands surveyed for hickory decline in six of the North Central and Northeastern States between 2007 and 2009. Source: Paul Castillo (USDA FS).

on 9 percent of the stem samples while hickory bark beetle damage only (no cankers) was present on 4 percent of the samples. *C. smalleyi* and *Fusarium solani* were commonly isolated while *Phomopsis* sp. was infrequently isolated from the stem samples. One or more of these fungal species were obtained from 132 of the 299 stem sections assayed. *Ophiostoma quercina* and *Penicillium* spp. were found infrequently. Incidences of *C. smalleyi* and *F. solani* were similar for surveyed stands in the six States. *C. smalleyi* was the most commonly isolated fungus from diffuse cankers (149 sections) obtained from 33 trees with declining crowns. *F. solani* was the most commonly isolated fungus from annual cankers (113 sections) obtained from 23 trees

exhibiting top dieback. The isolation frequencies of *C. smalleyi* and *F. solani* were significantly associated with different stem canker types ($p < 0.0001$). *Phomopsis* sp. was the most commonly isolated fungus from upper main stem galls (26 sections) from 7 trees; crown galls typically were not associated with top dieback.

Etiology: pathogenicity of predominant fungi (objective 2b)

***Methods*—**Pathogenicity tests with *C. smalleyi* were conducted with local isolates on healthy bitternut hickory (13 to 18 cm diameter at breast height [d.b.h.]) in forest stands in Iowa, Minnesota, and Wisconsin. Either fungus-colonized agar plugs (Iowa) or spores suspensions (Minnesota and Wisconsin) were placed in drilled holes to the outer sapwood of study trees and the holes were covered with sterile moist cotton and laboratory film. Water agar plugs (Iowa) or sterile water (Minnesota and Wisconsin) served as a control treatment. Similar pathogenicity tests with *F. solani* were conducted in the same States and sites. Pathogenicity of *Phomopsis* sp. was tested in only the Iowa sites, using fungus-colonized agar disks inoculated into 2- to 4-cm diameter branches of 10- to 15-cm d.b.h. bitternut hickory trees.

***Results*—**Inoculations with *C. smalleyi* resulted in reddish brown bark necrosis and sapwood discoloration typical of the diffuse cankers found on trees with declining crowns. *C. smalleyi* was re-isolated from all fungus-inoculated trees but not from the controls. Stem inoculations with two haplotypes (BB and BC) of *F. solani* resulted in inner bark lesions whose length varied by study site (Park and Juzwik 2012). Sunken or open cankers (average 30 and 38 mm long for Minnesota and Wisconsin, respectively) resulted from all BC isolate inoculations and cankers were similar to those found on trees with top dieback. The BB isolate inoculations resulted in relatively small and callus-bounded cankers (average 20 and 34 mm long for Minnesota and Wisconsin, respectively). We reisolated the same haplotype fungus from cankers in each location; no *F. solani* was obtained from the control wounds. No cankers or galls resulted within 12 months following branch inoculation with *Phomopsis* sp.

Etiology: *S. quadrispinosus*-*C. smalleyi* interaction on declining trees (objective 2c)

***Methods*—**Two bitternut hickory with 40 and 55 percent crown decline in Marathon County, Wisconsin, and one with 80 percent decline in Chippewa County, Wisconsin, were felled in June 2009. The bark was stripped from the main stem of each tree using a drawknife. Presence and extent of stem colonization by hickory bark beetles and presence of visible bark cankers and xylem lesions with or without associated beetle damage were recorded. In addition, attacking beetles were collected directly from trees in Minnesota and Wisconsin locations in late August and early September, and stem sections from trees felled in late May or early June were placed in emergence tubes to yield "emerged beetles." Teneral adult bark beetles collected by excavation also were obtained from one site.

Serial dilution plating and DNA-based assays (DNA extraction, polymerase chain reaction, and cloning) of attacking and of emerged hickory bark beetles were used to detect presence of *C. smalleyi* on the beetles.

Results—The extent of hickory bark beetle colonization of the main stems of the three actively declining trees ranged from aborted attacks (probing holes only) to successful colonization; i.e., egg gallery with radiating larval tunnels. Successful colonization accounted for 92, 53, and 80 percent of the documented hickory bark beetle damage on the declining trees (40, 55 and 80 percent decline rating, respectively). Fewer than 40 percent of the documented bark beetle attacks had accompanying bark cankers, xylem lesions, or both. The margins of the measured lesions always extended beyond the ends of the bark beetle larval tunnels when the damage types co-occurred. The average lengths of the xylem lesions of each tree were 8.2, 5.1, and 7.1 cm, respectively, on the bark-stripped trees. Numbers of bark cankers and xylem lesions varied by tree; i.e., 113 for the 80-percent decline rating tree, 585 for the 55-percent decline rating tree, and 26 for the 40-percent decline rating tree.

C. smalleyi was commonly isolated (88 and 93 percent, respectively, for 60 and 61 adult bark beetles from two sites) from hickory bark beetles captured by probing entry tunnels on six bitternut hickories in northern Wisconsin locations. The pathogen was isolated from fewer (11 percent of 21 adults) bark beetles obtained from the Minnesota site. Although the DNA assay was conducted with only a subset of bark beetles from the Wisconsin sites, similar results (92 percent of 24 assayed adults) to the serial dilution plating results were obtained. In contrast, *C. smalleyi* was infrequently isolated from the excavated bark beetle adults (7 percent of 43 beetles) and not isolated from any of the 120 emerged bark beetles. The same result (i.e., 0 percent positive for *C. smalleyi)* was found when molecular assay for DNA of *C. smalleyi* was conducted for 21 emerged bark beetles.

Etiology: Role of *C. smalleyi* in crown decline (objective 2d)

Methods—For sap-flow experiments, healthy bitternut hickory between 13 and 28 cm d.b.h. were inoculated at 50 points with either a spore suspension of *C. smalleyi* or sterile water. Stems of study trees (6 trees in Minnesota, 11 in Wisconsin) were examined for presence of visible cankers 13 to 14 months later. Granier-type thermal dissipation probes and their associated system were used to monitor sap-flow rate of each study tree. In Minnesota, probes were installed at 4.3 to 4.6 m height on three fungus-inoculated, one water-inoculated, and two noninoculated trees in mid-September 2009. Signals from the probes were recorded by a datalogger for 18 days. In Wisconsin, probes were installed above inoculated columns at 4.3 to 4.6 m height on five fungus-inoculated, three water-inoculated, and three noninoculated trees in late July 2010. Following sap-flow measurement, the bark around each inoculation point was stripped using a drawknife and the extent of inner bark necrosis recorded. The

proportion of the total stem area (between 1.8 and 4.0 m) with cankered tissues was calculated for each tree. The presence of *C. smalleyi* in necrotic tissue was verified by isolation. Two sapwood cubes were taken from above and below each probe location, and thin cross sections were obtained using a sliding microtome. For each probe location, 250 vessels for each cube were examined, diameter was measured, and tylose presence or absence was recorded for each vessel.

Results—The fungus-inoculated sap-flow study trees exhibited large bark cankers and accompanying xylem lesions compared with very small necrotic areas on water-inoculated trees 13 to 14 months after inoculation in both sites. Differences in canker sizes, however, were found between study sites. The mean size (area) of the cankers on trees in Wisconsin (64.3 ± 2.2 cm^2) was larger than that on trees in Minnesota (15.3 ± 0.6 cm^2) ($p < 0.0001$). The calculated proportions of *C. smalleyi*-inoculated stems with bark cankers (≤ 11.5 percent in Minnesota and ≤ 41.3 percent in Wisconsin) were much larger than necrotic bark areas on water-inoculated control trees in both sites (< 2.2 percent). Diurnal trends in sap-flow rate were similar for all the trees in both sites (fig. 9.2). Rates were highest in early afternoon and lowest at night. Maximum sap-flow rates, however, were lower for fungus-inoculated trees compared with maximum rates for water-inoculated and noninoculated control trees. Specifically, the maximum sap-flow rates for fungus-inoculated trees were 10.7 to 18.1 g $m^{-2}s^{-1}$ in Minnesota and 5.7 to 27.2 g $m^{-2}s^{-1}$ in Wisconsin, whereas those of the control trees were in the range of 26.3 to 30.0 g $m^{-2}s^{-1}$ in Minnesota and 34.2 to 49.3 g $m^{-2}s^{-1}$ in Wisconsin. In more general terms, the average maximum sap-flow rate for four fungus-inoculated trees (14.0± 2.1 g $m^{-2}s^{-1}$) was 51 percent lower than the average maximum rate for three control trees (28.6 ± 1.1 g $m^{-2}s^{-1}$) ($p = 0.009$) in Minnesota. Similarly, the average maximum sap-flow rate for five fungus-inoculated trees (15.3 ± 3.8 g $m^{-2}s^{-1}$) was 64 percent lower than the average maximum rate for six control trees (41.9 ± 2.2 g $m^{-2}s^{-1}$) ($p= 0.0001$) in Wisconsin. The consistently lower sap-flow rates obtained in Minnesota compared with rates in Wisconsin were attributed to the timing of the measurements, which were done in early fall in Minnesota and mid-summer in Wisconsin (Park 2011, Park and others 2013).

For all fungus-inoculated trees in both studies, *C. smalleyi* was isolated from margins of necrotic tissue after sap-flow measurements were completed. The fungus was not isolated from inoculation wounds on any of the water-

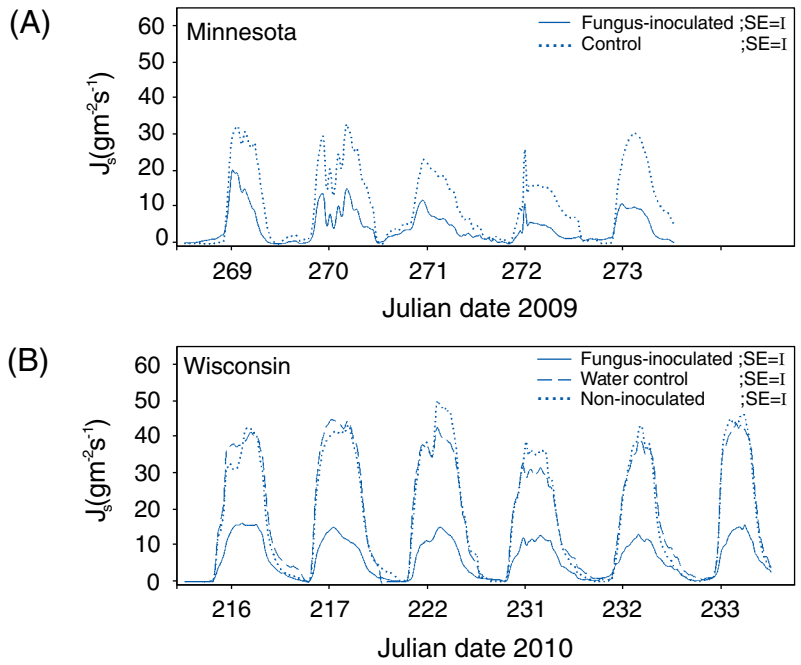

Figure 9.2—Diurnal changes in sap flow velocity (J_s) in Ceratocystis smalleyi*-inoculated trees versus water-inoculated and non-inoculated controls on selected days during the study period (Park 2011). (A) In Minnesota, data were averaged for fungus-inoculated trees and for one water-inoculated and two non-inoculated trees (= control). (B) In Wisconsin, data were averaged for five fungus-inoculated trees, three water-inoculated trees, and three non-inoculated healthy trees for each treatment. Ticks in x-axis indicate noon on each day. Bars indicate standard errors of the mean peak sap flow velocity values for each treatment.*

inoculated control trees. Vessel diameters and vessel diameter distributions were similar ($p = 0.23$) for the fungus-inoculated and the control trees in each location. As expected, tyloses were observed within vessels of all the study trees. They were more abundant in the fungus-inoculated trees, however, than in the control trees ($p < 0.006$) in both the 2 outer annual rings (30 to 56 percent, fungus-inoculated; 9 percent, controls) and the 9 to 10 outer annual rings (37 to 59 percent, fungus-inoculated; 25 to 42 percent, controls). Within the treatments, tyloses were more abundant in the deeper sapwood location than the outer sapwood annual rings. In addition, more vessels were found plugged in the 9 to 10 outer annual rings of *C. smalleyi*-inoculated trees than in the control trees ($p < 0.006$) for both sites. Significant interactions were found between (1) the average maximum sap-flow rate and tylose abundance in the two outer annual rings ($p = 0.0084$) for both sites combined, and (2) tylose abundance in the same sapwood location and the proportion of cankered bark area ($p = 0.0045$) based on correlation analyses. Based on linear regression analyses, an inverse relationship was found between the proportion of cankered bark area and average maximum flow rates in both sites ($R^2 = 0.90$, $p = 0.0042$, Minnesota; $R^2 = 0.90$, $p < 0.0001$, Wisconsin).

DISCUSSION AND CONCLUSIONS

Hickory decline—i.e., unhealthy crowns, per Steinman (2004)—is a complex of at least three diseases, two of which involve insect interactions. In one of these diseases, top dieback is caused by heavy gall formation attributed to *Phomopsis* sp. on branches and main stems of smooth-bark hickories. In a second disease, numerous annual cankers caused by *F. solani* on the upper stem and main branches cause top dieback. Ambrosia beetles (e.g., hickory timber beetles) and hickory bark beetles are known to carry *F. solani*. These insects may create infection courts for the fungus and serve as transmitters as well. In the third and most common disease scenario observed in our study, hickory bark beetles and *C. smalleyi* are the predominant biotic agents that cause rapid declining crowns and tree mortality.

The synergistic interaction between hickory bark beetles and *C. smalleyi* results in numerous bark cankers and debilitating xylem lesions on stems of bitternut hickory that we hypothesize leads to rapid crown decline and tree death, especially following predisposing abiotic events such as drought. The bark beetle provides the entry and infection court for *C. smalleyi* on susceptible hickories and is hypothesized to be responsible for disseminating and inoculating the fungus into the wounded tissues. Anatomical observations of fungus-inoculated trees in the sap-flow studies revealed *C. smalleyi* infections in the sapwood of bitternut hickory are correlated with increased tylose formation in xylem vessels. Reduced sap-flow rates in bitternut hickory with numerous (tens to hundreds) stem infections are attributed to waterflow obstruction induced by tyloses in outer annual ring vessels and accumulation of gels in late-wood vessels and adjacent parenchyma cells. Numerous fungus infections and resulting host response can logically explain the symptoms of rapid crown decline, including foliage wilt, observed in affected trees.

We hypothesize that the most recent occurrences of hickory decline we investigated are similar in causes to those of historical episodes reported in the literature. The presence of bark cankers and xylem lesions associated with hickory bark beetle-attacked trees was likely overlooked or considered insignificant in historical investigations. Furthermore, the discovery of the new pathogen, *C. smalleyi*, was made only after the first report of the cankers on such trees (USDA Forest Service 1994). The relatively widespread occurrence of *C. smalleyi* (Park and others 2010) and the occurrence of a sister species suggests that the pathogen is native to the two regions.

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