Assessment and Etiology of Hickory Decline (NC-EM-07-01) Second Year Report

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ABSTRACT

Closer examination of widespread decline and death of hickories in multiple states of the FHM Northeastern Region continued through 2008. Field surveys and associated sampling were completed in Indiana, New York, Ohio and Wisconsin. At least three stem damage scenarios were associated with observed, major symptoms (declining crowns, crown dieback, and globose stem galls). However, rapidly declining crowns and foliar wilt on smooth bark hickories, primarily *Carya cordiformis*, were determined to be the major tree health problem. Based on insect emergence from stem sections, hickory bark beetles (HBBs) (*Scolytus quadrispinosus*) and hickory timber beetles (*Xyleborus celsus*) accounted for 91% and 8%, respectively, of the 1,344 insects obtained in 2008. *Ceratocystis* species, with the majority of isolates tentatively identified as *C. smalleyi*, were obtained from dying trees in all four states. We hypothesize that stressed hickories are attacked by HBBs carrying viable spores of *C. smalleyi* on their bodies. During aborted and successful attacks, the fungal spores are dislodged, germinate and infect the attacked host. Diffuse cankers and "wound wood" or sapwood infections resulting from the fungal colonization further stress the tree, leading to more beetle attacks. Current studies are underway to determine whether *C. smalleyi* can cause the rapid wilt, crown decline and tree death (1 to 2 years) observed during the field surveys.

Fig. 1: Declining bitternut hickory

PROJECT OBJECTIVES

- Conduct field evaluations in multiple states to
  - Determine frequencies of decline and mortality of hickories (*Carya* spp.)
  - Quantify relationships between decline and pathogen and/or insect presence
- Determine the role of two newly described *Ceratocystis* species in decline and mortality of hickories

MATERIALS AND METHODS

Field Surveys

States and Sites – In 2008, eleven field sites in four states were evaluated. Fourteen field site were evaluated in 2007.

Survey Type – Either reconnaissance (2-3 hr) or transect (6 to 8 hr) surveys were conducted.

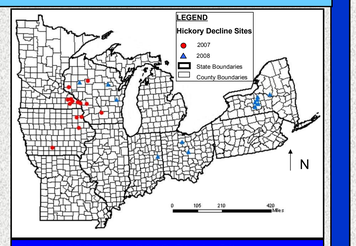

Fig. 1: 2007 and 2008 Study Sites

Field Data Collected – In the reconnaissance surveys, largely qualitative and/or categorical data on stand characteristics and level of decline/mortality were obtained from recently disturbed stands. In the transect surveys, quantitative data were collected from radial and point plots along three parallel transects.

Sampling Collection – For both survey types, three trees exhibiting the most common types of damage observed on a site were felled. Four, 0.8 m long stem sections were cut from each tree and transported to the laboratory.

Sample Processing – Insects were emerged (≤ 45 days) from two stem sections of each sampled tree and identified to genus or species. Fungal isolations were attempted from symptomatic sapwood associated with stem damage present on the other two sections.

Role of Ceratocystis: Field Pathogenicity Study

Site and Tree Selection – Twelve pole-size (5 to 11 inch DBH) bitternut hickory in a mixed hardwood stand undergoing tree stand improvement in Northeast Iowa, were selected.

Treatments – Two isolates of each of three fungal taxa (*C. caryae*, *C. smalleyi*, *Fusarium solani*,) obtained from other Iowa sites were used. Mycelial inoculum of each was placed aseptically in small, xylem-penetrating wounds.

Evaluation – Twelve months after inoculation (May 2008), all study trees were harvested and data recorded including canker incidence, extent of inner bark necrosis, and volume of sapwood discoloration. Re-isolation of the inoculated fungi was attempted.

FIELD SURVEY

Insects Associated with Hickory Decline or Dieback

Table 1. Numbers of insects emerged from stem sections from damaged hickories sampled by survey year

Survey Year	No. of Stands[1/]	No. Insects[2/]	Insect Species[3/] HBB	HTB	Others
2007	14	551	407	72	72
2008	11	1334	1223	108	13

[1/] Three trees per stand were felled and sampled
[2/] Two stem sections per tree placed in separate emergence tubes to emerge insects
3/ HBB = hickory bark beetle; HTB = hickory timber beetle; Other = several different taxa

Fig. 2: Hickory bark beetle (top) and hickory timber beetle (bottom). Photo source: www.forestryimages.org/

Together, hickory bark beetles and hickory timber beetles accounted for 87% and 99% of insects obtained in 2007 and 2008 surveys, respectively.

Fungal Taxa Isolated

Ceratocystis spp.

Table 2. Frequency of *Ceratocystis* spp. (Css) isolated from the field survey samples [1/]

Survey Year	States	Number of Sites Sampled	with Css
2007	IA, MN, WI	14	8
2008	IN, NY, OH, WI	11	10

[1/] Based on isolations from two stem sections per tree and three damaged trees per site.

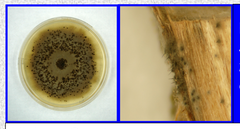

Fig. 3: Culture of *Ceratocystis smalleyi (left) obtained from fungus fruiting bodies on wood cubes (right).*

Hickory *Ceratocystis* spp. were commonly obtained from discolored sapwood associated with stem cankers.

Fusarium spp.

Table 3. Frequency of *Fusarium* spp. (Fss) isolated from the field survey samples. [1/]

Survey Year	States	Number of Sites Sampled	with Fss
2007	IA, MN, WI	14	14
2008	IN, NY, OH, WI	9	9

1/ Based on isolations from two stem sections per tree and three damaged trees per site.

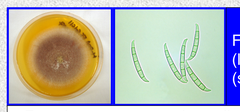

Fig. 4: *Fusarium solani* culture (left) and macroconidia (spores) (right).

- *Fusarium* spp. were universally present
  - All 2007 isolates were *F. solani*
  - 2008 isolates – species identification underway

Phomopsis spp.

Table 4: Frequency of *Phomopsis* spp. (Phm) isolated from field survey samples [1/].

Survey Year	States	Number of Sites Sampled	with Phm
2007	IA, MN, WI	14	3
2008	IN, NY, OH, WI	11	2

[1/] Based on isolations from two stem sections per tree and damaged trees per site.

Fig. 5: *Phomopsis* sp. culture (left) and globose stem gall (right).

Phomopsis species were not commonly isolated from discolored wood of galls or associated cankers.

Preliminary Conclusions

- At least three different types of damage scenarios are associated with smooth bark hickory decline, dieback and death. The problem pests and putative pathogens obtained from such trees were:
  - Hickory bark beetles and hickory timber beetles
  - *Ceratocystis* spp. (*C. caryae* and *C. smalleyi*)
  - *Fusarium* (all *F. solani* in 2007)
  - *Phomopsis*
- The most common scenario observed – (Arrows correspond to Fig. 6)
  - Hickory bark beetle attack (→)
  - Diffuse canker development and sapwood colonization by *Ceratocystis* (→)
  - Declining tree crowns (→)
  - Tree death occurs within 1 to 2 years of first crown symptom expression
- First reports of:
  - Hickory *Ceratocystis* for IN, MN, NY and OH
  - Putative Fusarium cankers on *Carya* species

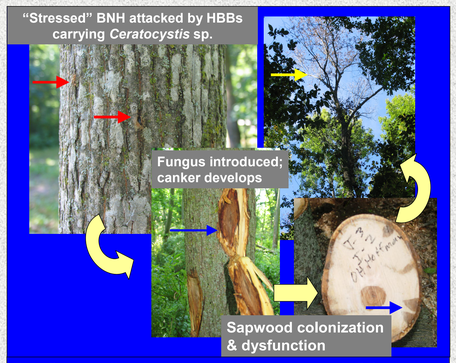

Fig. 6: Hypothosized interaction between HBB and *Ceratocystis* in the decline and death of hickory.

ROLE OF *CERATOCYSTIS* SPP.

Comparison of Ceratocystis species

Table 5. Biological and ecological traits of *Ceratocystis* species isolated from damaged hickories [1/]

Taxon	Hosts	Ecological niche	Culture morphology: perithecia	ascospore masses	Microscopic features: endoconidiophores	aleuroconidia	Culture odor
C. caryae	*Carya* spp. *Ostrya* sp. *Ulmus*	wounds into xylem (nitidulids?)	scattered or clumped on agar	pink	2 types	present	sweet, banana oil
C. smalleyi	*Carya* spp.	discolored sapwood; HBBs	concentric rings on agar	white to cream	1 type	absent	sweet

[1/] Based on original species descriptions by Johnson et al. 2005, Mycologia 97: 1067-1092

Relative Virulence

Table 6. Canker incidence, inner bark necrosis and sapwood discoloration associated with artificial inoculations of bitternut hickories (5 to 11 in. DBH).

Inoculum isolate set [/a]	Fungal isolate	No. stems exhibiting cankers[b/]	Area (ave. cm²) of inner bark necrosis: mean	SE	Volume(ave. cm³) of sapwood discoloration: mean	SE
1	*Ceratocystis smalleyi* 1952	5	22.0	3.07	44.6	14.44
	C. caryae 1412	4	7.1	1.02	7.6	2.26
	Fusarium solani G4J2	4	8.7	1.58	10.2	2.32
	Control	1	3.6	0.71	5.9	3.00
2	*C. smalleyi* 1828	4	22.8	6.06	31.7	12.34
	C. caryae 1829	3	5.2	1.25	4.5	1.63
	F. solani G 1C1A	4	6.2	0.9	7.7	2.04
	Control	2	4.4	1.27	8.4	1.58

a/ Five replicate trees per inoculum isolate set.
b/ Based on 5 inoculated stems evalatuated in late May 2008.

Preliminary Conclusions

- *The hickory Ceratocystis* species can be differentiated on the basis of visual characteristics on agar media and microscopic features.
- Relative virulence on pole-size bitternut hickory
  - *Ceratocystis smalleyi* is the more virulent pathogen, while
  - *C. caryae* and *F. solani* are much less virulent (i.e. weaker pathogens)
- Additional field studies are underway to determine the relative importance of *C. smalleyi* (vs. hickory bark beetles) in causing rapid decline and death of hickories.

OUR CURRENT UNDERSTANDING

- Bark beetle and *Ceratocystis smalleyi* are responsible for a distinct rapid decline and death of hickory. The relative roles of *Ceratocystis smalleyi* versus HBB are still under investigation.
- In addition, many other agents (including *Phomopsis* spp., *Fusarium* spp., and perhaps *C. caryae*) are involved in dieback of hickory.

ACKNOWLEDGEMENTS

- This study was partially funded by FHEM program, US Forest Service (grant no. NC-EM-07-01)
- The technical assistance of Stacey Olszewski, Greg Reynolds, Clara Shaw, and Bill Stroobants.
- The invaluable field assistance of John Juracko, Will Ayersman, Brian Beheler, John Graham, Robert Holbein, and Ed Hayes.
- Numerous cooperative landowners, land managers, and state natural resource agency specialists, who provided sites and often helped with sampling.