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Curculionid beetles as phoretic vectors of *Geosmithia morbida* – the causal agent of thousand cankers disease

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ABSTRACT

Thousand cankers disease (TCD) is a pest complex formed by the association between the walnut twig beetle (WTB), *Pityophthorus juglandis* (Coleoptera: Curculionidae: Scolytinae), and the fungal pathogen *Geosmithia morbida*. TCD has caused the widespread death of walnut trees throughout the West, and has recently been confirmed in seven eastern states within the native range of black walnut (*Juglans nigra*). However, little is known about the capacity of walnut colonizing insects to transmit the disease. In 2014, we reared insects from stem and branch sections of four TCD-symptomatic trees growing in Butler Co., Ohio to determine the extent to which other insects might transmit the pathogen. Eight beetle taxa emerged from symptomatic trees sections. We recovered *G. morbida* from WTB, two ambrosia beetles, and a weevil. It is now clear that a suite of insect species colonizes black walnut as TCD develops and may be capable of transmitting *G. morbida*. Characterizing the assemblage of scolytine beetles and weevils recovered from TCD symptomatic eastern black walnut is essential in the understanding of the etiology and epidemiology of this disease complex in the native range of black walnut.

INTRODUCTION

- Thousand cankers disease (TCD) is caused by a weak pathogenic fungus, *Geosmithia morbida*, and vectored by the walnut twig beetle (WTB), *Pityophthorus juglandis*
- TCD is responsible for widespread decline of black walnut, *Juglans nigra*, throughout the West (Fig. 1)
- TCD was detected in Knoxville, Tennessee in August, 2010 – the first discovery of the disease within the native range of black walnut. It is now found in seven eastern states (Fig. 2)



Fig. 1: TCD symptomatic black walnut



Fig. 2: Current distribution of TCD

- There are regional differences in the progression and severity of TCD, with the disease in the East progressing slower, and some trees even recovering altogether (Griffin 2014)
- In 2010, *G. morbida* was recovered from three specimens of a weevil, *Stenomimus pallidus*, reared from black walnut samples harvested from Yellowwood State Forest in Brown Co., Indiana
- Bark and ambrosia beetles are capable of vectoring numerous species of phytopathogenic fungi such as *Fusarium solani* (Kasson 2013)
- Assessment of the insects associated with TCD-symptomatic walnut is essential in understanding the etiology TCD in the East

OBJECTIVE

- Determine the extent to which walnut colonizing beetles other than WTB may vector *G. morbida*

METHODS

- Sixteen main stem sections (30 cm x 20–23 cm dia.) and sixteen branch sections (30 cm x 3–8 cm dia.) were harvested from each of four TCD symptomatic black walnut trees in Butler Co., OH on September 9, 2014 (Fig. 3)
- Half of the main stem samples and half of the branch samples (eight main stem and branch samples per tree) were placed in emergence buckets to collect emerging insects

Insect Rearing and Fungal Assay

- Sections were suspended in individual 18.9-L rearing buckets with a dry collection cup (Rubbermaid Twist n' Seal, Atlanta, Georgia) to collect emerged insects (Fig. 4)
- Emerged insects were collected biweekly, placed in individual 1.5-ml microcentrifuge tubes and stored at -20°C prior to identification
- Specimens were identified to species (Fig. 5) using a complete taxonomic monograph of the scolytinae (Wood 1982)
- Identified specimens were assayed for *G. morbida* using a dual serial dilution plating/PCR analysis described in Juzwik et al. 2015



Fig. 3: TCD symptomatic trees in Butler Co. OH



Fig. 4: 18.9-L insect rearing buckets



Fig. 5: Identification of emerged insects

RESULTS

Table 1: Total number of insects emerged from the main stems of two TCD symptomatic black walnut trees in Butler Co., OH.

Taxonomic group	Species	Number of specimens
Ambrosia Beetles	<i>Xyleborus affinis</i>	1
	<i>Xylosandrus crassiusculus</i>	26
	<i>Xyleborinus saxeseni</i>	73
	<i>Monarthrum mali</i>	1
Bark Beetles	<i>Pityophthorus juglandis</i>	1
Weevils	<i>Himatium errans</i>	2
	<i>Stenomimus pallidus</i>	47
Ironclad Beetles	<i>Synchyta fuliginosa</i>	1

RESULTS

- G. morbida* was recovered from specimens of the following beetle species emerged from TCD symptomatic black walnut trees in Butler Co., OH:



17 of 26 specimens of *Xylosandrus crassiusculus*



15 of 68 specimens of *Xyleborinus saxeseni*



13 of 47 specimens of *Stenomimus pallidus*

CONCLUSIONS

- There appears to be a suite of insect species colonizing black walnut as TCD develops.
- This is the first discovery of *G. morbida* on *X. crassiusculus* and *X. saxeseni*, two ecologically and economically destructive invasive exotic species
- The role of these insect species in the etiology of TCD in the East warrants further examination

FUTURE DIRECTIONS

- Determine the extent to which these beetles are attracted to TCD-symptomatic walnut trees
- Establish an invasion sequence of insect colonization of TCD symptomatic trees and determine dispersal and phoresy rates of potential *G. morbida* vectors

REFERENCES

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