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Natural Enemies of the Walnut Twig Beetlein Eastern Tennessee

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ABSTRACT

Sixteen species of natural enemies were found to be associated with the walnut twig beetle. Of these, 14 coleopteran species representing five families were collected and identified as potential predators of the walnut twig beetle in eastern Tennessee. Of the six clerid species collected, larvae of three species (*Enoclerus nigripes* (Say), *Madoniella dislocatus* (Say), and *Pyticeroides laticornis* (Say)) were observed to feed on walnut twig beetle larvae within the galleries. Consumption rates for these three clerid species were recorded. Behavioral comparisons for *M. dislocatus* and *P. laticornis* consisted of predator recognition time, time between attacks, total feeding time, and preening behavior. In addition to the 14 coleopteran species, two parasitoid species (*Neocalosoter pityophthori* and *Theocolax* sp. (Cerocephalinae)) emerged from walnut twig beetle infested black walnut bolts. Natural enemies of the invasive walnut twig beetle may be useful as biological control agents of the walnut twig beetle within newly infested areas.

Keywords: Predators, parasitoids, walnut twig beetle, thousand cankers disease

INTRODUCTION

Black walnut, *Juglans nigra* L., is native to eastern North America where it occurs in forest coves and well-drained river bottoms (1, 2).Within the last decade, western states have experienced increasing mortality of *J. nigra* (3,4). Tree deaths are attributed to the fungal species, *Geosmithia morbid* Kolařík (2,3,4).The only known vector of *G. morbida* is the walnut twig beetle (WTB), *Pityophthorus juglandis* Blackman (2,5). This newly recognized disease/insect complex was named Thousand Cankers Disease (TCD) due to the number of coalescing cankers produced by *G. morbida* (4).

Like several other bark beetles, WTB bores into the phloem-cambial region of twigs, branches, or trunks of walnuts. The sub circular, minute entrance/exit holes (0.64-0.75mm) in the bark are a good indication of infestation by WTB (6). In 2010, WTB/TCD was discovered in Knox Co., TN representing the first documentation of this disease complex east of the Mississippi River (7). Infestations have since been documented in IN, MD, NC, OH, PA, and VA (8, 9).Various management methods are currently being investigated to prevent further spread of this disease including chemical studies to evaluate various insecticide products and application techniques for controlling WTB (10). While the status and usefulness of insecticides for control of WTB remain unclear, natural enemies of this pest may provide the most feasible and sustainable method of suppressing pest populations. Although little is known about the life history or the occurrence and impact of natural enemies on this invasive pest, generalist predators have been documented to reduce pest populations of bark beetles (11).

Some generalist predaceous beetles in the families Laemophloeidae, Monotomidae, and Trogossitidae were found to be associated with WTB on walnut in California, as well as two Hymenoptera species in the families Bethylidae and Pteromalidae (12). In 2010, research was initiated in eastern Tennessee to search for potential biological control agents for use against WTB or that may play a role in regulating WTB populations.

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MATERIALS AND METHODS

Study Sites and Experimental Design

Three field sites in eastern Tennessee were selected based on preliminary observations of WTB-infested walnut trees and surveyed for predators and parasitoids of WTB from September 2010 to November 2014. Walnut bolts (30-35cm long and 10-14cm diameter) were cut from infested *J. nigra* obtained from two sites (Lakeshore (35˚55'23.59"N; 83˚59'29.47"W) and Beaver Dam Baptist Church, Emory Road (36˚5'5.33"N; 83˚55'54.98"W) (Knox Co., TN). Samples of potential natural enemies from the third site located at Strong Stock Farm (36˚ 3'7.1526"N; 83˚ 47'23.3802"W) in Knox Co., TN were obtained using two soda bottle (2-liter) traps hung in the upper and lower canopies of six mature black walnut trees. Samples were taken to the laboratory and maintained in plexi-glass containers at 22°C. Containers were observed daily for emergence of native natural enemies of WTB from August 2010-August 2012.

Trees selected for evaluation were located either on the edge of forested areas or in clumps in open fields. Twelve soda bottle traps were each baited with a black walnut bolt (~15cm long and ~4cm diameter), with a 1.27 cm hole drilled to a depth of 11.5 cm into the center of each bolt and filled with 95% ethanol. Traps were coupled with Nalgene®250mL wide mouth bottles screwed into the opening of the soda bottle lid. The bottoms of the Nalgene® bottles were removed and replaced with mesh netting fastened with hot glue to allow rain water to pass through without drowning the specimens. Two holes (10-12 cm diameter) were cut on either side of the soda bottles to allow insects to enter the trap. Traps were placed on the outer edge of limbs and monitored every other day for potential native predators.

Collected predators were placed into 118ml plastic specimen cups and taken to the laboratory for observation. Each predator was identified and individually placed in a Petri dish (9 cm) provided with a sheet of filter paper, a wet cotton ball and adult WTB (2-5) to sustain predators. Specimens of WTB were reared in the laboratory to establish a colony by placing fresh black walnut bolts (20-50 cm) into acrylic glass boxes (size 30.5 cm x 30.5cm x 40.5cm) containing WTB-infested bolts and maintained at 22°C. These acrylic containers had a round opening (12.4 cm) on each side of the container; and were covered with a fine mesh glued over it to allow for air circulation and prevent WTB from escaping.

Of the predators collected, two species, *Madoniella dislocatus* (Say) and *Pyticeroides laticornis* (Say), were selected to assess their feeding behavior, to determine if adults from the two clerid species exhibited a preference for live or dead WTB and if they were generalist feeders. Experiments were conducted under laboratory conditions, and were designed as choice tests replicated seven times, consisting of exposing three live and three dead WTB to predators for 2 weeks. Prior to each test, predators (n=14) selected for the behavior tests were starved for 24 hours prior to evaluation. Data catalogued from these tests included: predator recognition time, time between attacks, total feeding time, preening behavior, and consumption rates. Each test was terminated after 15 minutes if the specimen failed to feed (13). Prey escape behavior was noted in addition to predator feeding and preening behavior. Tests were timed using a stop watch and specific behavior activity was recorded in seconds. Consumption rates (number of prey captured and consumed/feeding unit) were recorded for *Enoclerus nigripes* (Say), *M. dislocatus*, and *P. laticornis*. To assess generalist feeding, two separate experiments were conducted. A choice/preference test was conducted with *P. laticornis* and *M. dislocatus* in which each of the predators were fed seven WTB and seven *Xylosandrus crassiusculus* (Motschulsky) adults for 10 days. A similar choice test was performed for 10 days using seven *Tribolium confusum* Jacquelin du Val and seven WTB as potential prey for *M. dislocatus* and *P. laticornis*.

Data Analysis

Shapiro-Wilks W test of normality and Levene's test of homogeneity of variances were used to verify that the behavioral data (species, number of specimens, prey recognition time, time between attacks, total feeding time, consumption rates, total preening time and feeding preference) collected conformed to the assumptions of the student's *t*-test (14).

RESULTS AND DISCUSSION

Fourteen predators and two parasitoid species were collected associated with WTB on *J. nigra* in Tennessee (Table 1). The six clerid species collected represented 58.9% (n=128) of all predators captured (217 specimens). Two clerid species, *M. dislocatus* and *P. laticornis,* captured in soda bottle traps were observed to feed on WTB in the laboratory. These two species represented 54.8% of all predaceous specimens collected. Three specimens of *E. nigripes* were collected from WTB-infested bolts confined in acrylic glass containers in the laboratory. From these three specimens, one consumed 198 WTB in 89 days for a potential consumption rate of 2.23 live WTB per day. *E. nigripes* is a native species distributed throughout the eastern and central U.S. (19) with a range extending from Maine to Minnesota and from South Carolina to Oklahoma. This predaceous species has been recorded to feed on adults, pupae, and larvae of wood-boring beetles on hardwoods (15).We observed specimens attacking and feeding on adult WTB. Three additional species of clerids, *Enoclerus ichneumoneus* (F.), *Placopterus thoracicus* (Olivier), and *Priocera castanea* (Newman), were captured in soda bottle traps, but not in sufficient numbers to evaluate their consumption of WTB. Larval and adult specimens of these clerids were observed on the bark of WTB-infested logs, while only clerid larvae were observed within WTB galleries in black walnut bolts maintained in the laboratory. In one instance, a clerid larva completely consumed a WTB larva by lifting it up in the air and pinching its side with its mandibles.

Eight additional coleopteran species that are potential predators of WTB emerged from infested bolts held in the acrylic glass containers. These included *Leptophloeus angustulus* (LeConte) (Laemophloeidae), a native predator in the eastern U.S. and Canada with a range extending from Maryland to Mississippi and from Florida to Canada (15, 16). *L. angustulus* is often found in scolytine galleries on hardwoods. Adult *Narthecius grandiceps* LeConte (Laemophloeidae) are predaceous and are native to the southern and eastern U.S. ranging from Oklahoma to Florida and north to Pennsylvania and Michigan (15, 17).The predator *Tenebroides marginatus* P. Beauv. (Trogossitidae) is also native to the U.S. (15). *Bitoma quadriguttata* (Say) (Zopheridae) is native to the eastern U.S. ranging from Florida, New Hampshire, and Texas (15). *B. quadriguttata* is reported as a saproxylic beetle from oak (18) and perceived to feed on conidia within galleries. *Aeletes floridae* (Marseul) (Histeridae) is widespread throughout the eastern U.S. ranging from Ohio south to Florida and Texas. This species appears to develop underneath the bark of dead or dying trees and documented to feed on larvae of scolyids (15). We observed this species within the larval gallery. *Dysmerus basilas* Casey and *Corydium lineola* Say and unidentified staphylinid specimens, were collected from infested WTB bolts maintained within the laboratory. All of these coleopteran species emerged from *J. nigra* and are known to feed on prey in the subfamily Scolytinae.

Table1. *Potential Predators and Parasitoids of the Walnut Twig Beetle Collected in Knox County, TN.*

Family	Genus	species	Author	Collection Method*	Habits	U.S. Distribution
Cleridae	*Enoclerus*	*nigripes*	(Say)	EWB	Feeds on all life stages of bark beetles. (19). Len. 5-7mm.	DC,DE, FL, GA, IL, IN, KS, MA,ME, MD, MI, MN, MO, NC, NH, NY,OH, OK, PA, SC, TN, VA, WI(2019)
Cleridae	*Enoclerus*	*ichneumoneus*	(F.)	SBT	Predator of bark beetles (19). Len. 8-11mm.	AL,AR,CO, DC, FL, GA, IA, IN, IL, KS, KY, LA,MD, MS,NM, NV, NY, OH, PA, SC, TN, TX, VA, WI, WV(19,20)
Cleridae	*Madoniella*	*dislocatus*	(Say)	SBT	Generalist predator, feeds on bark beetles (19). Len. 3.5-6mm	CO, FL,GA, IN, IL, KS, NY, NJ, OH, PA, SC, TN,TX,WI, WV(19)
Cleridae	*Priocera*	*castanea*	(Newman)	SBT	Predator on bark beetles	Eastern U.S., ON to FL, KS to NY (20)
Cleridae	*Pyticeroides*	*laticornis*	(Say)	SBT	Generalist predator, feeds on bark beetles (19). Len. 4-7mm.	CT, FL,GA, IL, IN, MD, NC, NJ, NY, OH, PA, SC,TN,VA,WV (19,20)
Cleridae	*Placopterus*	*thoracicus*	(Olivier)	SBT	Predator of bark beetles (19). Len. 5-8mm.	FL,GA, IL, IN, MD,MI,MI,**NC**,NY, NJ, OH, PA,SC,SD,TN,VA (19,20)
Histeridae	*Aeletes*	*floridae*	(Marseul)	. EWB	Predator (possibly of eggs).	Eastern US:MD to OH south FL to TX.(15)
Laemophloeidae	*Dysmerus*	*basalis*	Casey	EWB	Found in Scolytine burrows in oaks. Len. 1.7mm	AL, DC,FL,NJ(20)
Laemophloeidae	*Leptophloeus*	*angustulus*	(LeConte)	EWB	Scolytine predator especially in oaks. Len. 1.6-2.0mm.	FL, IN, MD, MI, MO,NH, OH, OK(16,20)
Laemophloeidae	*Narthecius*	*grandiceps*	LeConte	EWB	Scolytine predator L. 3.0mm.	FL,IN.,MI,NE,OK, PA (21)

Pteromalidae	*Neocalosoter*	*pityophthori*	Ashmead	EWB	Scolytine parasitoid of *Pityophthorus consimilis, Styphlosoma granulatum, Thysanoes fimbricornis* (22).	NC, FL, Nearctic (22,23,24,25)
Pteromalidae	*Theocolax*	unidentified		EWB, Pupae WTBlarval tunnels.	Found in WTB tunnels. Parasitoids of bark beetles (26)	China and U.S. (27,28)
Staphylinidae	Unidentified			SBT	Predator	Unknown
Trogossitidae	*Tenebroides*	*marginatus*	P.Beauv.	EWB	Larvae are predacious and hunt in galleries of wood boring insects. Adults are fungivores and feed on dead trees. (20)Len. 4.3-7mm.	AL, FL, GA, IA. IL, KY, KS, LA, MI, MS, NC, NY,OH, PA, SC, TX, WV(20)
Zopheridae	*Bitoma*	*quadriguttata*	(Say)	EWB, Found in WTB tunnels.	Found under the bark of dead hardwoods and pines.(29)	CT, DE, FL, GA, IN, MD, MI, NC, NJ, NH, NY, OH, OK,PA, SC, TN, TX,VA,WV (29)
Zopheridae	*Colydium*	*lineola*	Say	EWB	Predator on ambrosia and scolyid beetles (30).	AL,AR,AZ,CA,CO,DC, DE,FL,GA,ID,IL,IN, LA,, MA,MD,MI,MO,MS,NC,NJ,NY,NM, NV,NY,OH,OK,OR, PA, SC, SD,TN, TX, UT,VA,WA, WV(29)

**EWB: specimens emerging from walnut bolts in laboratory; SBT: specimens collected from soda bottle traps on black walnut trees.*

In the behavioral tests conducted on *M. dislocatus* and *P. laticornis*, both similarities and differences in feeding and preening behaviors were found between the two species. For example, both *M. dislocatus* and *P. laticornis* strongly (*t* test; t=1.45; df = 2; P< 0.05) prefer live rather than dead WTB prey (Figure 1). WTB exhibited thanatosis, death feigning, as a defense mechanism against predator attacks. WTB would tuck its legs and antennae close to its body and lay still. The predator would struggle to pick up its prey and after a few seconds cease its attack and wander away from its prey. In addition to WTB, both *M. dislocatus* and *P. laticornis* were observed to exhibit death feigning behavior when being removed from their Petri dish. Both clerid species would tuck their legs and antennae close to their body and lay on their dorsal side.

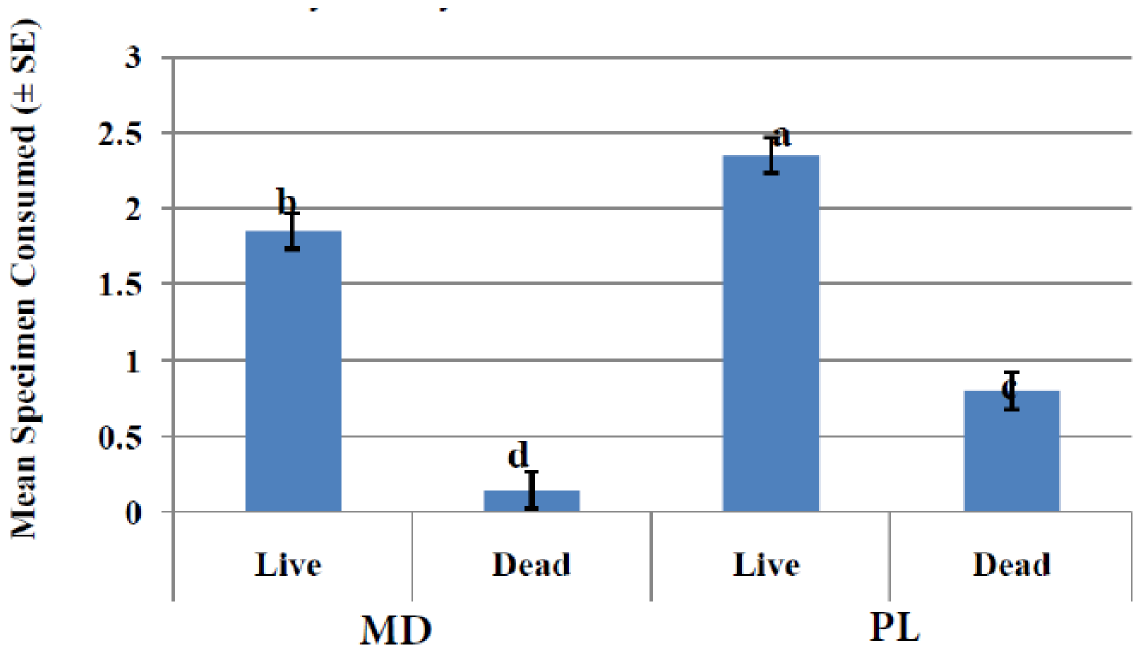

Figure1. *Mean (± SE) live and dead specimens consumed by Madoniella dislocatus (MD) and Pyticeroides laticornis(PL). Treatments with different lowercase letter(s) above the bars are significantly different (t-test; P< 0.05).*

Pyticeroides laticornis was observed to feed on dead prey and is more likely to exhibit a scavenger behavior than *M. dislocatus*, although its scavenger behavior in the laboratory may be attributed to its higher consumption rate, indicating it may not have been provided sufficient live prey to satiate its hunger or it was unable to capture sufficient prey. *P. laticornis* consumed a significantly (*t*-test; t =1.45; df = 2; P< 0.05) greater numbers (27% more) of WTB in 2 weeks than *M. dislocatus*. This difference is probably due to *P. laticornis* being larger than *M. dislocatus* and requiring more food to sustain life functions. In addition, *M. dislocatus* was significantly (*t*-test; t = 1.42; df = 2; P< 0.05) faster in prey response time, time between attacks, and total feeding time than *P. laticornis*. *M. dislocatus*; however, did spend significantly (*t*-test; t=1.66; df = 2; P<0.05) more time preening than *P. laticornis* (Figure 2).

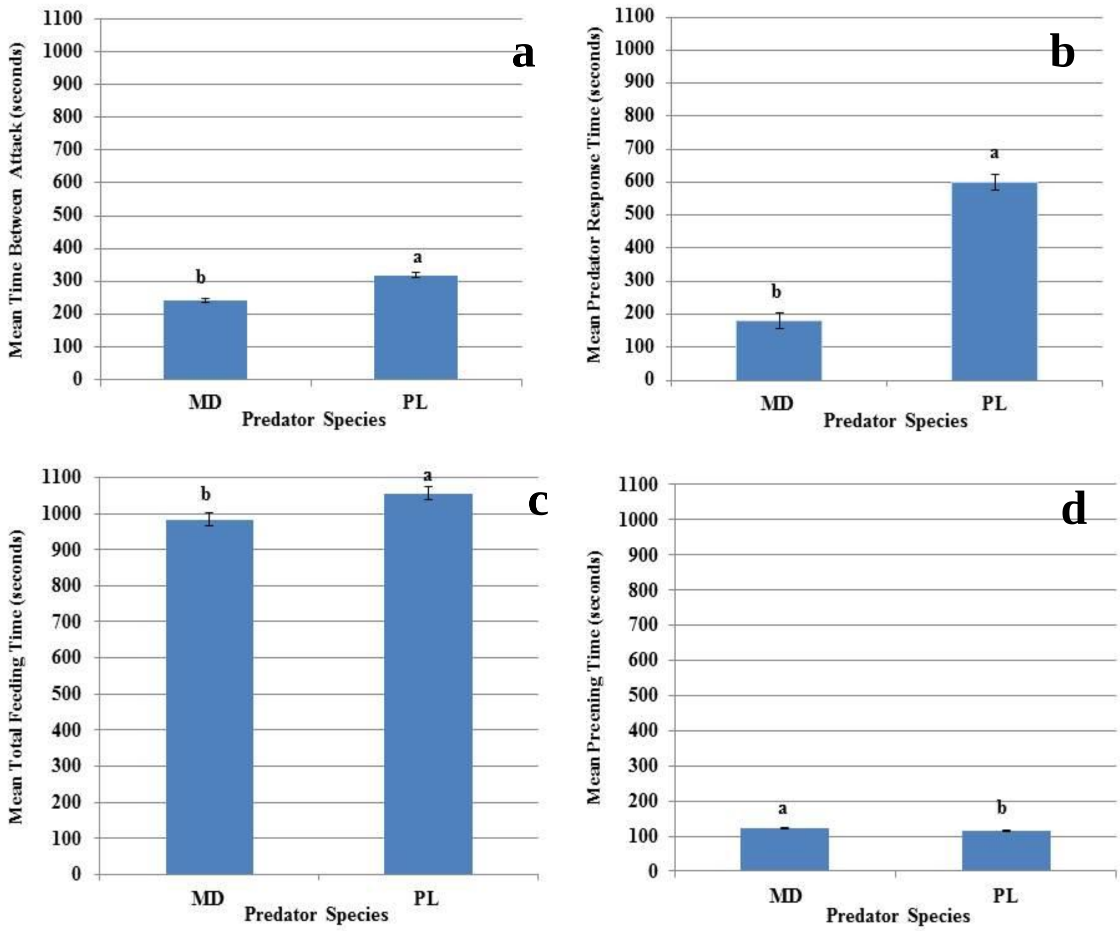

Figure2. *(a) Mean (±SE) time between attacks (seconds) by Madoniella dislocatus (MD) and Pyticeroides laticornis (PL); (b) Mean (±SE) predator response time by M. dislocatus (MD) and P. laticornis(PL); (c) Mean (±SE) total feeding time (seconds) by M. dislocatus(MD) and P. laticornis(PL); (d) Mean (±SE) total preening time (seconds) by M. dislocatus(MD) and P. laticornis(PL). Treatments within a grouping followed by different lowercase letter(s) above the bars are significantly different (t-test; P< 0.05).*

Neither species consumed *T. confusum* in the choice test between WTB and *T. confusum* (Figure 3). The lack of consumption of *T. confusum* may have been due to the relative size between *T. confusum* and both clerid species tested. *T. confusum* is similar in size (3.2mm) to both clerid species, which may have made it unsuitable as a prey species. In the choice test between WTB and *X. crassiusculus*, *P. laticornis* significantly (*t*-test; t = 1.85; df = 2; P< 0.05) preferred WTB (1.5-1.9mm) over *X. crassiusculus* (2.1-2.9mm) (Figure 3) (31). These findings support earlier conclusions by researchers that prey size can influence prey preference (32, 33). However, *P. laticornis* did feed on *X. crassiusculus,* while *M. dislocatus* did not feed on *X. crassiusculus*. *X. crassiusculus* was present in black walnut trees and was collected in the same soda bottle traps used to collect the clerid species. From collection data, we found specimens of *X. crassiusculus* to be present within the same trees as *P. laticornis* and *M. dislocatus*. Again, size appears to play a significant behavioral role in the clerid’s feeding dynamic as *M. dislocatus* is too small to eat *X. crassiusculus*, while *P. laticornis* can manage to feed on *X. crassiusculus*, when necessary. The consumption rate of *X. crassiusculus* was low compared to WTB; however, *P. laticornis* may not need to feed on as many *X. crassiusculus* to reach satiation due to the larger size of *X. crassiusculus*. Both *M. dislocatus* and *P. laticornis* exhibited feeding and hunting behaviors similar to *Thanasimus dubius* (F.) (13). Upon contact with a potential prey source, both predators moved their antennae in an up-and-down motion while walking rapidly in search of prey.

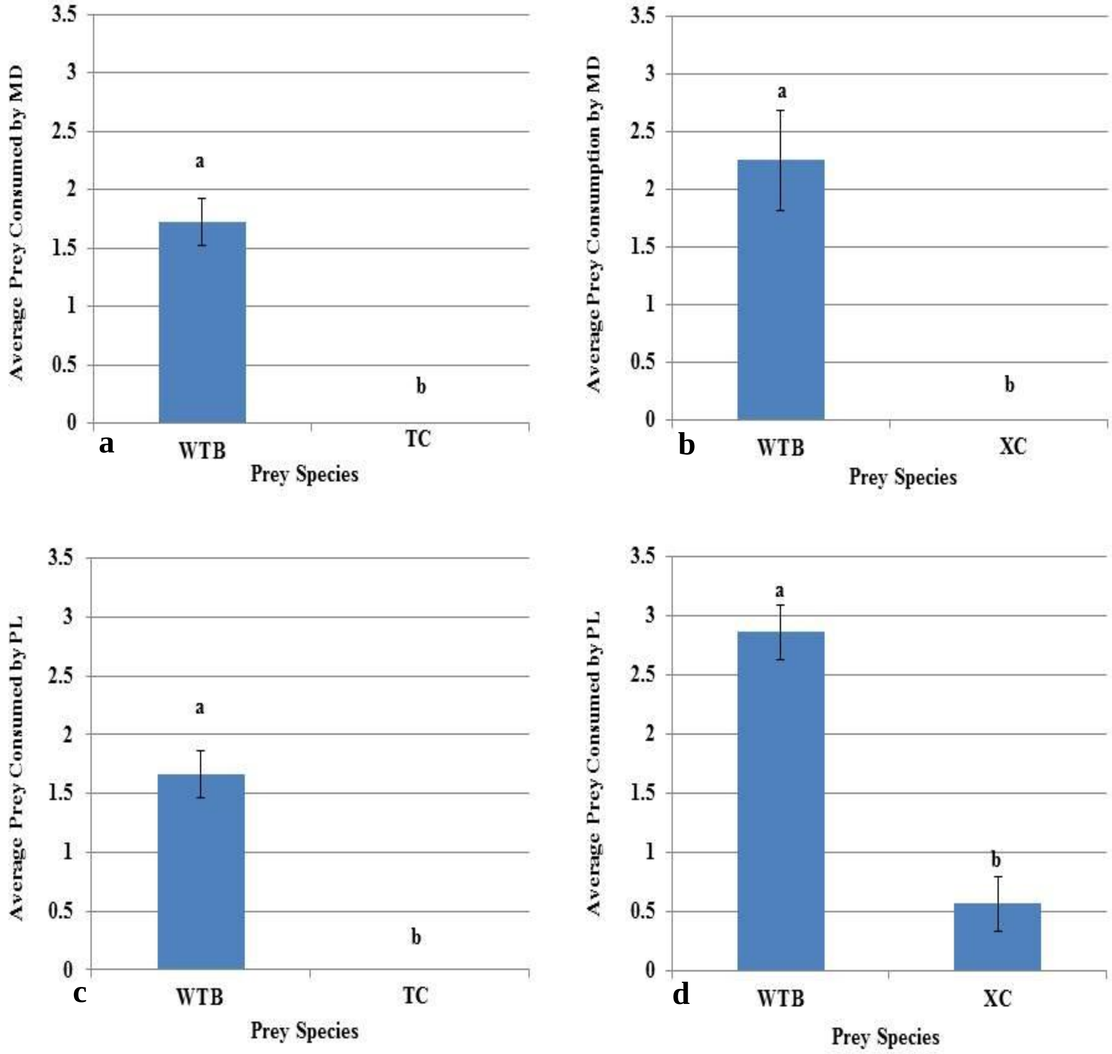

Figure3. *(a) Mean (±SE)Pityophthorus juglandis (WTB) and Tribolium confusum (TC) specimens attacked by Madoniella dislocatus(MD); (b) Mean (±SE)Pityophthorus juglandis (WTB) and Xylosandrus crassiusculus (XC) specimens attacked by Madoniella dislocatus (MD); (c) Mean (±SE)Pityophthorus juglandis (WTB) and Tribolium confusum (TC) specimens attack by Pyticeroides laticornis(PL); (d) Mean (±SE)Pityophthorus juglandis (WTB) and Xylosandrus crassiusculus (XC) specimens attacked by Pyticeroides laticornis(PL). Treatments within a grouping followed by different lowercase letter(s) above the bars are significantly different (t-test; P< 0.05).*

Based on their rapid recognition of the prey source and their actions, it appears these predators respond to bark beetle chemical and visual cues. Once the predator recognized the prey source and attacked, it seized the prey, positioned the prey on its back with the underside of the prey's head aligned with the predator's mandibles, and began feeding. The predators used their pro and mesothoracic legs to manipulate the prey and their metathoracic legs to stabilize and balance their body during feeding. Both *P. Laticornis* and *M. dislocatus* fed on soft tissue - usually in the cervix region of the prey. Both predator species frequently decapitated the prey before proceeding to feed on the internal tissue. The predator often left the head capsule, elytra and wings uneaten. Grooming or preening behaviors occurred either between attacks or at the end of the feeding period. Preening behavior consisted of the predator grooming their prothoracic legs with their mandibles and/or pulling down their antennae with their prothoracic legs one at a time and grooming them with their mandibles. *P. laticornis* and *M. dislocatus* expressed different temperaments in the laboratory. *M. dislocatus* specimens appeared calmer, less active and easier to care for because they were not constantly active and trying to escape. *P. laticornis* specimens often tried to fly away when food was dropped into their Petri dish, which sometimes resulted in damage to the specimens.

Two species of parasitoids (Cerocephalinae) also emerged from WTB-infested bolts of *J. nigra* (Table 1).The dominant parasitoid collected was the ectoparasitic *Neocalosoter pityophthori* Ashmead

(Figure 4).Larvae of *N. pityophthori* were observed feeding on larvae within the WTB galleries. This species, which pupated within the larval galleries of WTB, emerged in high numbers in infested walnut bolts implying a possible parasitoid relationship with WTB in black walnut. Also, the larvae of an undetermined ectoparasitoid, possibly a new species (Crystal McEwen, communication) in the genus *Theocolax* were observed feeding on larvae within the WTB galleries. Members of this genus are known to be associated with bark beetles (26, 28). Several adult *Theocolax* specimens were found searching female WTB galleries possibly for a suitable place to lay their eggs. However, only a few specimens of this species were recovered from our traps and from the field-cut walnut bolts maintained in the laboratory.

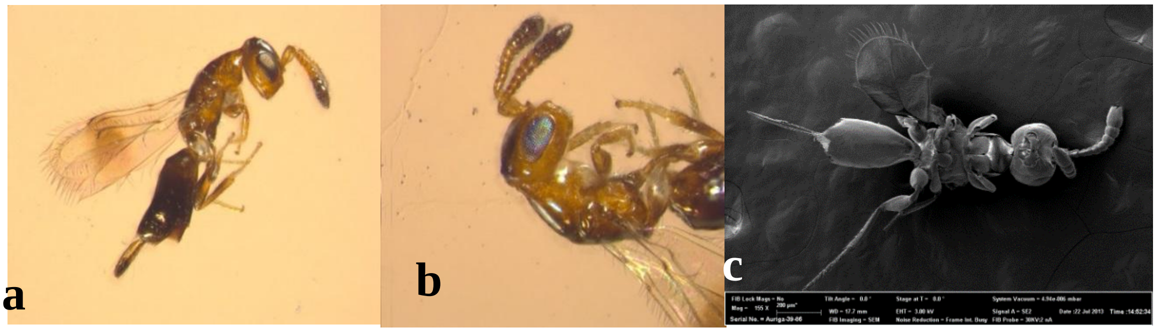

Figure4(a-c): *Neocalosoter pityophthori species found emerging from J. nigra bolts: (a-b) Lateral view of N. pityophthori; (c) Ventral view of N. pityophthori.*

CONCLUSIONS

Fourteen predaceous beetle species and two parasitoid species were collected over three years from traps placed on WTB-infested black walnut trees or from black walnut bolts in the natural range of black walnut. Native predators were confirmed to feed on WTB from cut bolts and in laboratory consumption tests. The most common predators collected from baited traps on black walnut were *M. dislocatus* and *P. laticornis.* In observations on *M. dislocatus* and *P. laticornis*, significant behavioral differences were noted when feeding on WTB. Due to size differences, it appears *M. dislocatus* may be a more effective predator because it focuses on smaller prey close to the size range of WTB. In addition, *M. dislocatus* is easier to maintain in the laboratory due to its calmer disposition compared with *P. laticornis*. The discovery of these predators and parasitoids provides the means to develop potential biological control agents for suppressing WTB populations in the future. Native predators and parasitoids are currently interacting with WTB and thus, impact their population size. However, the overall effect these natural enemies have on the WTB population has not been investigated.

Two parasitoid species were observed to infest WTB adults within the galleries. The presence of these predators and parasitoids may be that they are associated with the native scolyid, *P. lautus*, also found on *J. nigra*. Because these indigenous parasitoids are widespread throughout the eastern U.S. covering the range of black walnut, they should be further investigated as potential biological control agents against WTB to suppress TCD. The ectoparasitoids, *Neocalosoter pityophthori* and an unidentified species of *Theocolax*, were both discovered feeding on WTB larvae within the larval galleries. *N. pityophthori* specimens were dominant and are being investigated in studies on its biology. A comprehensive study of predator and parasitoid populations in forested areas is critical to understanding the risk that WTB will have on native black walnut populations. If predator and parasitoid populations are already in place in forested areas, then the spread of TCD could be lessened and the forests better protected against this new insect/disease complex.

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