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PARASITOIDS ATTACKING THE EMERALD ASH BORER (COLEOPTERA: BUPRESTIDAE) IN WESTERN PENNSYLVANIA

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ABSTRACT

Field surveys of the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, and associated parasitoids were conducted in Cranberry Township, PA from 11 Mar to 23 Oct 2008. Several species of parasitic Hymenoptera were collected from EAB-infested green ash trees or reared from late-instar EAB larvae, prepupae, and/or pupae. These included *Balcha indica* (Mani & Kaul), *Eupelmus pini* Taylor (Eupelmidae), *Dolichomitus vitticrus* Townes (Ichneumonidae), and 2 additional unidentified ichneumonids, *Orthizema* sp. Townes and *Cubocephalus* sp. Townes. Together, these parasitoids caused about 3.6% parasitism of EAB in the field. The most abundant species was *B. indica* accounting for 82% of all parasitoids recovered during our survey. Subsequent laboratory assays confirmed that *B. indica* and *E. pini* are solitary ectoparasitoids of EAB larvae, prepupae, and/or pupae. In addition, both *B. indica* and *E. pini* reproduce through thelytokous parthenogenesis; i.e., virgin females produce daughters. These parasitoids may be complementary to current classical biological control programs against EAB in North America, which have been focusing primarily on the introduction of exotic larval and egg parasitoids from China.

Key Words: *Agrilus planipennis*, parasitoid, *Balcha indica*, *Eupelmus pini*, biological control

RESUMEN

Se realizaron sondeos de campo para el barrenador esmeralda de fresno (BEF), *Agrilus planipennis* Fairmaire, y los parasitoides asociados en el pueblo de Cranberry, PA desde el 11 de marzo hasta el 23 de octubre de 2008. Varias especies de parasitoides del orden Hymenoptera fueron recolectadas de árboles de fresno verde infestados de BEF o criadas de los últimos estadios de las larvas, prepupas y/o pupas de BEF. Estos incluyen *Balcha indica* (Mani & Kaul), *Eupelmus pini* Taylor (Eupelmidae), *Dolichomitus vitticrus* Townes (Ichneumonidae) y 2 ichneumónidos no identificados adicionales, *Orthizema* sp. Townes y *Cubocephalus* sp. Townes. Juntos, estos parasitoides causaron aproximadamente 3.6% del parasitismo de BEF en el campo. La especie mas abundante fue *B. indica* que represento 82% de todos los parasitoides recuperados durante el sondeo. Ensayos de laboratorio subsiguientes confirman que el *B. indica* y *E. pini*, son ectoparasitoides solitarios de larvas, prepupas y/o pupas de BEF. Además, ambos *B. indica* y *E. pini* se reproducen por medio de la partenogénesis de thelytokia, donde hembras vírgenes producen hijas. Estos parasitoides pueden complementar los programas de control biológico clásico actuales contra el BEF en Norteamérica, los cuales han sido enfocados principalmente en la introducción de parasitoides exóticos de China sobre larvas y huevos.

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, is a serious invasive pest that has killed millions of ash trees in North America since its discovery in 2002 in Michigan and Ontario (Haack et al. 2002; Poland & McCullough 2006). Regulatory efforts to contain the pest's spread via early detection, quarantine, and removal of infested ash trees, have had limited effects on containing this pest (Cappaert et al. 2005), and chemical control cannot be used to protect native ashes in forest ecosystems because of prohibitive cost and general impracticality (Poland & McCullough 2006). Currently, EAB is established in Michigan, Indiana, Illinois, Ohio, Pennsylvania, Maryland, West Virginia, Wisconsin in the US, and Ontario and Quebec in Canada. An isolated infestation was detected in southeastern Missouri as recently in summer of 2008 (NAPPO Phytosanitary Alert System 2008).

Following the discovery of EAB in Michigan, classical biological control programs were initiated against this invasive pest. The programs have primarily focused on the introduction and release of exotic parasitoids from China, the probable origin of the pest (USDA APHIS 2007; Liu et al. 2007; Bauer et al. 2008). Recent field surveys in Michigan and Ontario, however, indicate that some indigenous parasitoids have already become associated with EAB and may play a role in suppressing the local populations of EAB (Bauer et

al. 2004; Cappaert & McCullough 2008; Lyons 2008). In the present study, we investigated extant parasitoid guilds associated with emerald ash borers in western Pennsylvania, where the pest was first discovered in 2007. We also conducted laboratory tests with the recovered parasitoid species against the larvae, prepupae, and/or pupae of EAB to further confirm their associations.

METHODS

Field Survey

A total of 41 green ash (*Fraxinus pennsylvanica* Marshall) trees with an average diameter breast height (DBH) of 21.5 cm (range 10-45 cm) with symptoms of EAB infestation such as woodpecker feeding, thin canopy, epicormic shoots, and bark splits (Cappaert et al. 2005) were searched for and located in Cranberry Township, PA (Fig. 1) from 11 Mar to 23 Oct 2008. On each sampling occasion in the months of Mar, Apr, Jun, Sep, and Oct, we removed the bark from the boles of 3 to 12 trees from the ground to the height of 2 m using draw knives. To avoid damaging immature EAB larvae, prepupae and/or pupae under the bark, we first cut the bark using the draw knife to reach the cambium and the surface of the wood tissue, and then peeled down the bark to expose immature EAB and parasitoids. Exposed immature EAB and parasitoids were collected with soft aluminum forceps, placed in cells of plastic culture plates (12 or 24 cells per plate, each lined with wet filter paper) or plastic tubes with ventilated caps, and returned to the USDA ARS Beneficial Insects Introduction Research quarantine facility (Newark, DE) to rear the parasitoids to the adult stage. Most of the parasitoids collected by debark sampling were immature stages (larvae and/or pupae), but a few were emerging adults. Adult parasitoids either directly collected from the field or recovered later from rearing in the laboratory were sent to the USDA ARS Systematic Entomology Laboratory for identification to species, and voucher specimens were deposited there.

Laboratory Exposure Assay

To evaluate parasitoid-host associations, we presented adult parasitoids to EAB late-instars, prepupae, or pupae that were inserted into ash sticks (about 10 cm long × 1 cm diameter) freshly-cut from green ash trees by methods modified from Liu & Bauer (2007). Our modified method involved cutting a thin flap (about 5 cm long x 0.5 cm wide) of the outer bark from the top of the stick with a utility knife, leaving the end of the flap attached in the middle of the stick. We then cut a narrow groove (3-4 mm deep) in the exposed wood of the stick underneath the flap with a wood-carving V-shaped chisel. An EAB larva, prepupa or pupa was then placed in the groove, covered with the flap, and secured with thin bands of parafilm at the top and bottom of the flap. The sticks were placed, with the insertion-end up, into cells of a 12-well tissue culture plate, which was placed inside a ventilated plastic box (17.6 × 12.6 × 10 cm). To maintain moisture in the ash sticks, water was added to the cells of the culture plate so that the base of each stick was submerged in about 1/4 cm of water. Parafilm was used to cover the surface of the plate to prevent spillage of water.

The adult parasitoids used in the laboratory assay originated from the field-collected immature stages of EAB or ash bark infested with EAB. At least 7 d after emergence from the immature (cocoon or pupa) stages, 1 to 6 adult parasitoids were released into the plastic box containing EAB-infested ash sticks at parasitoid to host ratios ranging from 1:4 to 1:6. The exposure duration was 3 to 7 d, depending upon the longevity of the test parasitoids. At the end of each trial, exposed ash sticks containing immature EAB were incubated in a growth chamber at 20-26°C; 55% - 65% RH, and 16:8 h (L:D) photoperiod for recovery of F_1 parasitoid progeny. When no adult parasitoids emerged from the exposed ash sticks after 4 weeks of incubation, we dissected them and transferred parasitoids or hosts to wells of 12-well tissue cell plates lined with moist filter paper; these were incubated for 8 to 12 weeks of incubation or until adult parasitoids or EAB emerged. Approximately equal proportions of the EAB late instars (3^{rd} to 4^{th}), prepupae, and pupae were tested across different trials for each species of the parasitoid tested. Percentage parasitism for each trial was calculated as proportion of immature EAB successfully attacked, as evidenced with the presence of parasitoid progeny produced by the test parasitoid.

RESULTS AND DISCUSSION

Five species of hymenopteran parasitoids were recovered from 1,091 EAB larvae, prepupae, and/or pupae collected in the field survey, including *Balcha indica* Mani & Kaul (Eupelmidae), *Eupelmus pini* Taylor (Eupelmidae), *Dolichomitus vitticrus* Townes (Ichneumonidae), and 2 unidentified ichneumonids, *Orthizema* sp. Townes and *Cubocephalus* sp. Townes. These parasitoids together parasitized 3.6% of the sampled EAB hosts (Table 1). *Balcha indica* was the most abundant species of parasitoid recovered from the field survey, accounting for 82% of the parasitoids recovered. While all the *B. indica* adults (n = 32) recovered from the field survey were females, both sexes of *D. vitticrus* (n = 2), and *Cubocephalus* sp. (n = 3) were recovered. Only 1 individual female was recovered from the field survey for both *E. pini* and *Orthizema* sp.

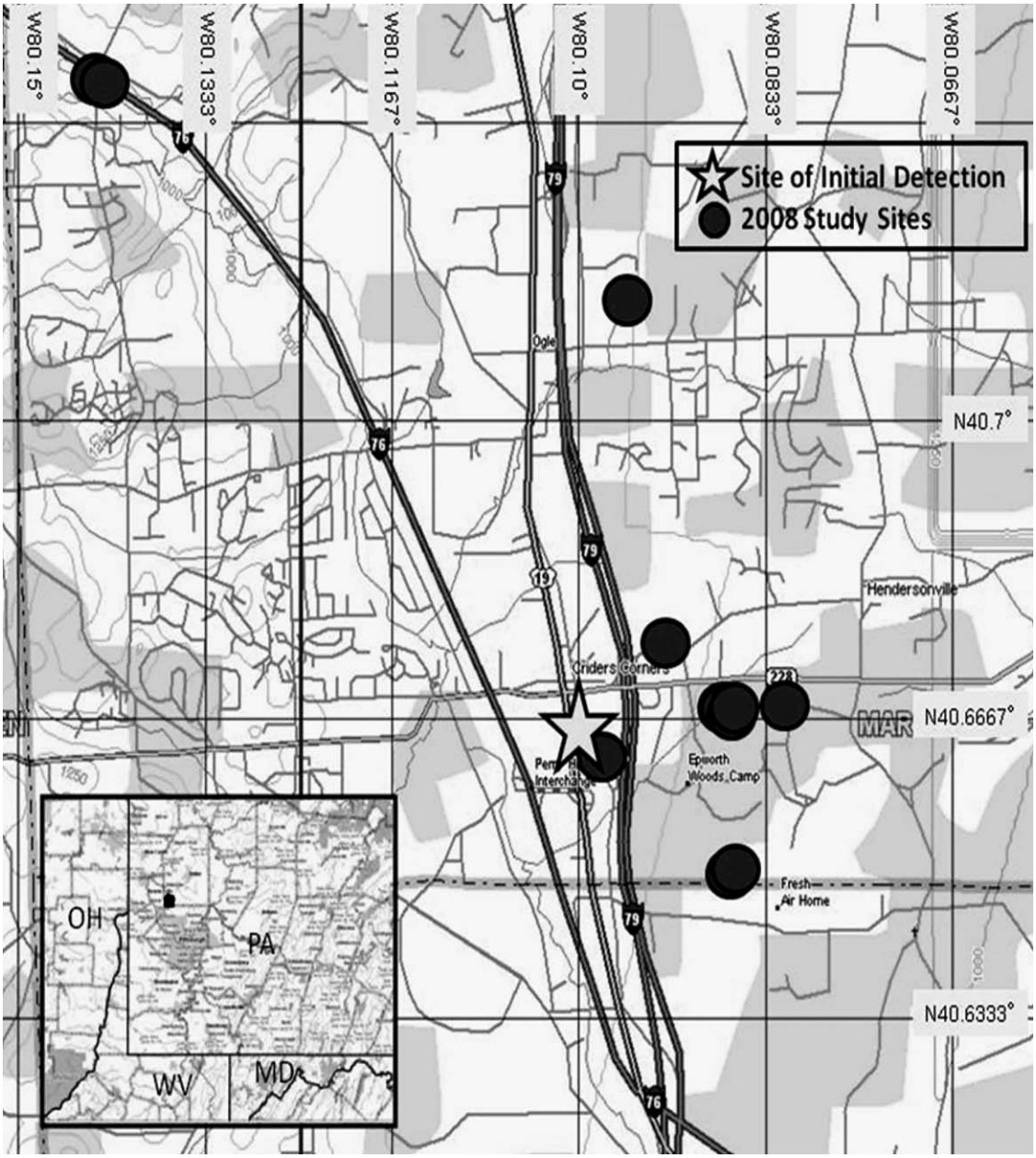

Fig 1. Locations where the first EAB were discovered and field survey samples were taken in Cranberry Township, PA.

Larval stages of *B. indica* were observed in association with remains of emerald ash borer larvae, prepupae, and/or pupae in the field. However, the association of *E. pini, D. vitticrus*, *Cubocephalus* sp., and *Orthizema* sp. with the emerald ash borer could not be positively confirmed from field observations as stages of these parasitoids had already progressed to pupae and/or pharate adults and their host remains were not recognizable. Thus, all the recovered female adult parasitoids were further tested in the laboratory assay against EAB late-instars, prepupae, and pupae.

Results from our laboratory assays demonstrated that both field-recovered eupelmids, *B. indica* and *E. pini*, successfully attacked and developed from EAB late-instars, pre-pupae, and/or pupae inserted into the ash sticks, while the other parasitoids did not (Table 2). All the progeny pro-

TABLE 1. PARASITOIDS RECOVERED/COLLECTED FROM EAB-INFESTED ASH TREES, THEIR RELATIVE ABUNDANCE AND PARASITISM (11 MAR-24 OCT). A TOTAL OF 1091 EAB LARVAE, PREPUPAE/PUPAE WERE COLLECTED FROM THE SURVEY.

Parasitoid Taxon	No. Individuals Recovered	Relative Abundance (%)	% Parasitism of EAB[1]
Balcha indica	32	82.0	2.9
Eupelmus pini	1	2.6	0.1
Dolichomitus vitticrus	2	5.1	0.2
Orthizema sp.	1	2.6	0.1
Cubocephalus sp.	3	7.7	0.3

[1]Parasitism calculated as percent of the total parasitoids recovered relative to the total number of the EAB (1091) and parasitoids (39) throughout the entire survey.

duced by both virgin *B. indica* and *E. pini* were females, indicating that both species had reproduced through thelytokous parthenogenesis.

Although EAB was just recently discovered in western PA, the extent of the damage to ash trees in the area and the size of the infestation suggest EAB has been present at this site for several years (Pennsylvania State University 2009). During that time, 2 locally extant parasitoids, *B. indica* and *E. pini,* became associated with EAB, exerting about 3% parasitism (Table 1). However, the association of the 3 ichneumonids, *D. vitticrus, Cubocephalus* sp. and *Orthizema* sp., with EAB could not be confirmed from our field survey and laboratory experiments. It is possible that these ichneumonid parasitoids collected from our field samples were from insects other than EAB infesting ash tree bark.

Gibson (2005) reported the first collection of *B. indica* in Virginia in 1994 and suggests this parasitoid was probably introduced to the U.S. from Southeast Asia in the 1960s. *Balcha indica* was next found in 2003 in Michigan parasitizing EAB (Bauer et al. 2004, 2005) and in Maryland from wood-boring beetles in a cherry tree (*Prunus* sp.) (Gibson 2005). Only females have been collected, and Gibson (2005) proposed that *B. indica* was likely parthenogenetic. Data from our laboratory assay indicates that *B. indica* reproduces via thelytokous parthenogenesis, and successfully parasitizes larvae, prepupae, and/or pupae of EAB (Table 2).

We collected a single female of *E. pini* associated with EAB-infested ash trees at our field sites, and it was our least abundant parasitoid species (Table 1). It was also recovered from EAB-infested ash in southeast Michigan from 2002 to 2004 (Bauer et al. 2004). Using laboratory assays, we have now confirmed that this species reproduces via a thelytokous parthenogenesis on EAB larvae, prepupae, and/or pupae (Table 2). Surprisingly, *E. pini* previously was only recorded parasitizing late-instars of the white pine weevil, *Pissodes strobi* (Peck), in eastern US (Taylor 1929; Harman & Kulman 1967) and Quebec (Williams & Langor 2002). Although there are no records of *E. pini* attacking buprestids, species from Eupelminae are more niche-specific than host-specific, and *Eupelmus* spp. generally parasitize a wide range of insect larvae or pupae in concealed locations (G. Gibson, Agriculture and Agri-Food Canada, personal communication).

These eupelmid species may be complementary to the ongoing EAB biological control efforts in the U.S., which include 1 egg and 2 larval parasitoids that attack EAB in China (USDA APHIS 2007; Liu et al. 2007). Another native ectoparasitoid found to attack EAB larvae, *Atanycolus hico-*

TABLE 2. RESULTS OF EXPOSURE ASSAYS OF PARASITOIDS AGAINST IMMATURE EAB (LARVAE/PRE-PUPAE/PUPAE) CONTAINED IN GREEN ASH TWIGS IN THE LABORATORY.

Parasitoid Taxon	No. Trials	Parasitoid: Host ratio	Total No. Host Exposed[1]	% Parasitism[2]	Parasitoid Progeny produced[3]
Balcha indica	15	1:4	130	16.9	22
Eupelmus pini	58	1:4	58	13.8	8
Dolichomitus vitticrusz	3	1:5	15	0	0
Orthizema sp.	4	1:6	24	0	0
Cubocephalus sp.	8	1:4	42	0	0

[1]Parasitized host stages (EAB larvae, prepupae and pupae) were pooled for calculation of percent parasitism.
[2]Approximately equal proportions of late instars (3rd to 4th), prepupae, and pupae were presented in different trials.
[3]All progeny produced by virgin *B. indica* (F_0) and *E. pini* (F_0 to F_2) are females.

riae (Braconidae) (Bauer et al. 2004, 2005), is being evaluated for possible use as a biocontrol agent of EAB (Cappaert & McCullough 2008). Studies of *B. indica* and *E. pini* are ongoing in our laboratory, where we are focusing on reproductive and developmental biology, host finding and selection behavior, and the eventual development of mass rearing methods for use in augmentative biological control agents against EAB in the U.S.

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