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Taxonomic Paper

First record of *Trissolcus basalis* (Hymenoptera: Scelionidae) parasitizing *Halyomorpha halys* (Hemiptera: Pentatomidae) in the United States

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Abstract

Background

A parasitoid wasp, *Trissolcus basalis* (Wollaston), was recorded parasitizing eggs of the invasive stink bug *Halyomorpha halys* (Stål) in the United States. This is the first record of this species parasitizing fresh and frozen eggs of *H. halys* in the United States.

New information

First record of *Trissolcus basalis parasitizing Halyomorpha halys* eggs in the United States.

Keywords

Parasitoid wasp, endoparasitoid, brown marmorated stink bug

Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stål), 1855 (Hemiptera: Pentatomidae) (BMSB) is a native of China, Taiwan, South Korea and Japan. Unfortunately, this invasive insect pest has spread to the United States (Lee et al. 2013), where it is both an urban nuisance pest (Inkley 2012) and a serious economic pest of orchard, field and vegetable crops (Leskey et al. 2012a, Leskey et al. 2012b, Rice et al. 2014). The first known *H. halys* populations in the United States were reported in 1996 from Allentown, PA (Hoebeke and Carter 2003). It has now been found in 44 states (StopBMSB 2018).

In the south-eastern U.S., populations of *H. halys* are continuing to expand into the Piedmont and Coastal Plains regions of Georgia and Alabama. *Halyomorpha halys* was first detected in Alabama in 2010. One year later, urban pest management professionals began reporting overwintering brown marmorated stink bugs in homes in the metropolitan Atlanta area. Currently, the brown marmorated stink bug threatens peaches, plums, blueberries, apples, wine grapes, kiwifruit, soybean, cotton, pecan and tomatoes in both states. The tree of heaven, *Ailanthus altissima* (Mill.) Swingle, a tree with seed pods that are a favourite non-crop food source for *H. halys*, also occurs in both states.

Presently, 18 species of hymenopteran endoparasitoids in the genera *Anastatus* Motchulsky (Eupelmidae), *Trissolcus* Ashmead, *Telenomus* Haliday and *Gryon* Haliday (Scelionidae) have been reported to parasitize eggs of *H. halys* in the U.S. (Abram et al. 2014, Rice et al. 2014). As the impact of stink bug parasitoids on this pest was unknown in Georgia and Alabama, a survey to examine parasitism and species composition of parasitoids attacking sentinel egg masses of *H. halys* was conducted in 2017 in regions where populations of *H. halys* had become established.

Materials and methods

Laboratory-reared *H. halys* egg masses were laid on knit cloth (97% cotton, 3% spandex). On 24 June 2018, 30 fresh egg masses (≤ 24 h old) were hung as sentinels on tomato plants for 72 h. Some egg masses (≤ 12 h old) were frozen and held at -20ºC for 1–4 d. On 18 October, 30 frozen egg masses were hung as sentinels on plants in cotton and soybean for 72 h. In the laboratory, the collected egg masses were held for emergence of adult parasitoids and emergent wasps were identified using the key of Talamas et al. (2015). Voucher specimens of parasitoids are deposited in the Florida State Collection of Arthropods, Gainesville, Florida (FSCA 00090444, FSCA 00090269).

All egg masses were dissected for dead, immature parasitoids. Determination of *T. basalis* immature stages, mainly third instars, prepupae and pupae, were based on descriptions of *T. basalis* immatures in Volkoff and Colazza (1992) and on descriptions of *H. halys* eggs, parasitised by *T. basalis* every 24 h from oviposition to pupation (G. Tillman, unpublished data).

Two dried point-mounted specimens were selected for DNA extraction and mitochondrial cytochrome c oxidase I (COI) fragment sequencing. Specimens were softened in 70% ethanol for two hours, then DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen). The DNA samples were quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific). At least 20 ng of genomic DNA was used per PCR. The 5'-COI region was PCR-amplified using the primers LCO1490 and HCO2198 (Folmer et al. 1994). PCRs were performed at 25 µl volumes using HiFi HotStart DNA Polymerase (Kapa Biosystems). PCR thermocycle conditions were: 1) initial denaturing at 95°C for 2 min followed by 32 cycles of steps 2–4, 2) 98°C for 30 seconds, 3) 50°C for 30 seconds, 4) 72°C for 40 seconds and 5) final extension at 72°C for 7:00 minutes. PCR products were verified by gel electrophoresis and cleaned for sequencing with QIAquick Gel Extraction Kits (Qiagen). Purified PCR products were Sanger-sequenced in both directions using BigDye Terminator v3.1 (Applied Biosystems) chemistry on a SeqStudio Genetic Analyzer (Applied Biosystems). Sequence reads were trimmed and sequence contigs were assembled in Sequencher 5.4.6 (Gene Codes Corporation). COI sequences, generated during this study, were deposited in GenBank (MK720833, MK720834).

Taxon treatment

Trissolcus basalis (Wollaston) 1858

a. scientificName: *Trissolcus basalis*; scientificNameID: urn:lsid:biosci.ohio-state.edu:osuc_names:3189; kingdom: Animalia; phylum: Arthropoda; class: Hexapoda; order: Hymenoptera; family: Scelionidae; genus: *Trissolcus*; specificEpithet: *basalis*; country: United States; stateProvince: Alabama; county: Tuscaloosa; locality: Tuscaloosa, Tuscaloosa Co., AL, U.S.A.; decimalLatitude: 33.21; decimalLongitude: -87.57; georeferenceSources: GNIS-USGS; samplingProtocol: reared from egg; eventDate: 06/17/2017; verbatimEventDate: Jun-17-2017; fieldNotes: [USA: AL: Tuscaloosa. Tomato 6-2, ex. fresh BMSB eggs 17-JUN-2017, Coll. Rammohan Balusu]; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: FSCA 00090269; recordedBy: Balusu, R. (Rammohan); identifiedBy: Talamas, E. J. (Elijah Jacob); dateIdentified: 2019; language: en; institutionCode: Florida State Collection of Arthropods, Gainesville, FL (FSCA); collectionCode: Insects; basisOfRecord: PreservedSpecimen; source: http://hol.osu.edu/spmInfo.html?id=FSCA%2000090269; occurrenceID: urn:lsid:biosci.ohio-state.edu:osuc_occurrences:FSCA__00090269

b. scientificName: *Trissolcus basalis*; scientificNameID: urn:lsid:biosci.ohio-state.edu:osuc_names:3189; kingdom: Animalia; phylum: Arthropoda; class: Hexapoda; order: Hymenoptera; family: Scelionidae; genus: *Trissolcus*; specificEpithet: *basalis*; country: United States; stateProvince: Alabama; county: Tuscaloosa; locality: Tuscaloosa, Tuscaloosa Co., AL, U.S.A.; decimalLatitude: 33.21; decimalLongitude: -87.57; georeferenceSources: GNIS-USGS; samplingProtocol: reared from egg; eventDate: 07/23/2017; verbatimEventDate: Jul-23-2017; fieldNotes: [USA: AL: Tuscaloosa, Tomato 3-6, ex. fresh BMSB eggs 23-JUL-2017, Coll. Rammohan Balusu]; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: FSCA 00090444; recordedBy: Balusu, R. (Rammohan); identifiedBy: Talamas, E. J. (Elijah Jacob); dateIdentified: 2019; language: en; institutionCode: Florida State Collection of Arthropods, Gainesville, FL (FSCA); collectionCode: Insects; basisOfRecord: PreservedSpecimen; source: http://hol.osu.edu/spmInfo.html?id=FSCA%2000090444; occurrenceID: urn:lsid:biosci.ohio-state.edu:osuc_occurrences:FSCA__00090444

Diagnosis

Trissolcus basalis can be identified from Nearctic congeners by the combination of the following characters: vertex without hyperoccipal carina, netrion sulcus incomplete, mesopleuron with episternal foveae shallowly impressed, metapleuron without setation and without well-defined paracoxal sulcus; T2 striate (Figs 1, 2, 3) (Talamas et al. 2015).

Figure 1. doi

Trissolcus basalis, female (FSCA 00090269), dorsal habitus.

Figure 2. doi

Trissolcus basalis, female (FSCA 00090269), lateral habitus.

Figure 3. doi

Trissolcus basalis, female (FSCA 00090269), anteroventral habitus.

Distribution

Trissolcus basalis is found worldwide (http://hol.osu.edu/map-large.html?id=3189).

Ecology

At the Tuscaloosa site, *T. basalis* parasitised four of the 30 sentinel egg masses; at the other two sites, only one of the 30 egg masses was parasitised by *T. basalis*. Overall, percent parasitism per egg mass was moderately high (62.7%). In general, percent immature mortality was slightly higher for frozen egg masses (48.2%) than for fresh ones (35.4%). Overall, 38.0% of the parasitoids emerged as adults. A female biased sex ratio of 4F:1M was observed for emergent parasitoids.

Biology

Additional host associations of *T. basalis*, provided by Johnson (1985), are *Nezara viridula* (L.), *Euschistus servus* (Say), *Euthyrhynchus floridanus* (L.), *Piezodorus hybneri* (Gmelin) and *Plautia affinis* (Dallas). Additional host associations, provided by Talamas et al. (2015), are *Aelia acuminata* (L.), *Aelia cognata* Fieber, *Aelia germari* Küster, *Agonoscelis rutila* (Fabricius), *Calidea dregeii* Germar, *Carpocoris fuscispinus* (Boheman), *Coleotichus blackburniae, Cuspicona simplex* Walker, *Dolicoris baccharum* (L.), *Eurydema ornata* (L.), *Eurygaster austriaca* (Schrank), *Eurygaster integriceps* Puton, *Graphosoma semipunctata* (Fabricius), *Halyomorpha annulicornis* (Signoret),

Odontotarsus grammicus (L.), *Oechalia schellenbergi* Guérin-Méneville and *Raphigaster* Laporte.

Taxon discussion

The CO1 sequences of the two specimens were identical to each other and to the 7 CO1 sequences of *T. basalis* in Genbank. These sequences derive from specimens collected in Italy, Japan and the United States and their invariance indicates that this gene is not informative for identifying populations within the species.

Discussion

Multiple species of *Trissolcus* are known to oviposit into the eggs of *H. halys* despite a physiological inability to develop in them, creating an evolutionary trap (Abram et al. 2014, Haye et al. 2015, Abram et al. 2017). Our records of *T. basalis* are mainly from fresh BMSB eggs, providing evidence that *T. basalis* finds live BMSB eggs acceptable and suitable as a host. *Trissolcus basalis* belongs to the *basalis*-species group (sensu Johnson 1985, Talamas et al. 2017), whereas the species reported to successfully parasitise BMSB in its native range belong to the *flavipes*-group (sensu Talamas et al. 2017). Our records demonstrate that species in the *basalis* group also can successfully parasitise BMSB. In the Umbria Region of Italy, *T. basalis* emerged from frozen eggs of *H. halys* in soybean (Rondoni et al. 2017). This parasitoid was also reported parasitising *H. halys* in Lengquan, China, at very low frequency (Zhang et al. 2017). Peach leaves with naturally-laid egg masses, either fresh or stored at 10°C for no more than 48 h to prevent further development, were deployed as sentinels in the field. At the temperature used, the stored eggs were likely alive when deployed, for BMSB egg masses need to be held in a refrigerator at a minimum of 3.9°C for 12 h to kill the eggs (G. Tillman, unpublished data). At present, reports of parasitism of BMSB by *T. basalis* are rare.

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