Is the brown marmorated stink bug parasitoid *Trissolcus japonicus* (Ashmead) already in Germany?

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Abstract

During a survey on bug egg parasitoids in the region of Lake of Constance (Germany), parasitized egg clusters of Pentatoma rufipes *(Hemiptera: Pentatomidae) were collected in an organic apple orchard. After adult emergence, the egg parasitoid species has been identified in a rapid determination as* Trissolcus spec. *probably* japonicus *(Ashmead) (Hymenoptera: Scelionidae). This would be not only the first record for this parasitoid species in Germany but also the first record of* P. rufipes *as its host under natural conditions. Egg clusters of* Halyomorpha halys *and* Nezara viridula *were used as potential hosts for subsequent culture of the parasitoid under laboratory conditions, revealing these pentatomids as alternative hosts for* Candidatus T. japonicus.

Keywords: *Trissolcus spec., Pentatoma rufipes, Nezara viridula, Halyomorpa halys*.

Introduction

The Brown marmorated stink bug (*Halyomorpha halys* Stål) (Hemiptera: Pentatomidae) is the most dangerous pest among stink bugs, posing an imminent and serious threat to a wide variety of tree fruit, nut, vegetable, and field crops (Rice et al., 2014). Moreover, it is considered a recent invasive pest in Europe and North America (Rice et al., 2014, Wermelinger et al., 2008, Haye et al., 2015). Callot and Brua (2013) reported that populations of *H. halys* has been established in Europe.

Up to date, the management of *H. halys* in Europe depends on the application of broad-spectrum insecticides (Haye et a., 2015), because mass releases of native European egg parasitoids, such as *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae) and *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae), is considered insufficient to effectively suppress this pest, yet (Haye et al., 2015, Stahl et al., 2018). In its native range East-Asia, *H. halys* is attacked by more than ten native species of parasitoids (Yang et al., 2009, Talamas et al., 2015a), among these *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) was the most promising biocontrol candidate with reported parasitisation levels up to 90% (Yang et al., 2009).

In Germany, the effect of parasitoids on stink and forest bugs is poorly known. Thus, a survey to examine parasitisation levels and species composition of parasitoids attacking the eggs of *Pentatoma rufipes* (L.) (Hemiptera: Pentatomidae) was conducted in regions with recorded abundance or fruit damages by this pentatomid pest.

Material and Methods

Surveys of egg parasitoids of the red-legged shieldbug (*P. rufipes*) were conducted in 2019 in apple orchards in the region of Lake Constance (Germany), where *P. rufipes* or damage by shield-bug has been recorded.

Egg masses of *P. rufipes*, which are usually laid on the underside of leaves, were collected in September and October 2019. The egg clusters were then each put into a Petri dish (10

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cm), on a filter paper with absorbent cotton ball, brought into the laboratory and then reared in climatic chambers (25 ±1°C, 65% RH, 16:8 L: D). They were checked daily until parasitoids or bug nymphs emerged. Emerged adult parasitoids were preserved in 95% EtOH for later taxonomic determination.

Other newly emerged parasitoids were provided with 2-4 days old eggs of *Halyomorpha halys* (Stål) and *Nezara viridula* (L.) to test whether these pentatomids could possibly also serve as hosts.

Primary identification

Morphological identification of parasitoids

Specimens were identified and photographed using a ZEISS Stemi DV4 stereomicroscope and take photographs. A scanning electron microscope (SEM) was used to take photographs of body sections of taxonomic importance, such as the head, antennae, thorax and abdomen. The parasitoids were morphologically identified following the key of Talamas et al. (2017).

Molecular confirmation

Five specimens were selected for DNA extraction by using a chelex DNA extraction protocol. Universal primers LCO-1490 and HCO-2198 (Folmer et al., 1994) were used for amplification and sequencing of the DNA barcode (Gariepy et al. 2012).

PCR products were verified by gel electrophoresis and cleaned for sequencing with Cycle Pure Kits (C-Line), following the manufacturer's instructions and sent for sequencing in two directions using the forward primer to an external service.

The sequence obtained in all samples has been compared with published sequences available in the GenBank database using the Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

According to our survey of the literature, *T. japonicus* has not been reported from the eggs of *P. rufipes*. This is the first record of this parasitoid infecting the egg masses of this forest bug in the nature worldwide. Specimens are corresponding with the description of *T. japonicus* by Talamas et al. (2015b, 2017).

In the molecular determination, the five field specimens generated a unique barcode sequence of 635-bp in length. A BLAST search showed that these specimens shared only 96 % and 97% sequence similarity with voucher *T. japonicus* collected from established populations in the USA and in Asia (Accession No. MH919759). However, a more significant similarity would be helpful to be on the safe side of species determination as the genus *Trissolcus* comprises some cryptic species hard to distinguish. Thus, **Morphological** and **Molecular Re-identification is in progress now.**

Future research must focus on the description of the reproductive biology, life cycles, and a better understanding of ecological interactions between *P. rufipes* or other pentatomids and *T. japonicus* under laboratory conditions for a sound risk assessment on *T. japonicus*.

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