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Source: Florida Entomologist, 98(4) : 1244-1246

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.098.0436

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First report of an egg parasitoid reared from *Neomusotima conspurcatalis* (Lepidoptera: Crambidae), a biological control agent of *Lygodium microphyllum* (Schizaeales: Lygodiaceae)

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Native predators and parasitoids within the introduced range of a biological control agent often find and attack the agent, a novel resource (Hill & Hulley 1995; Paynter et al. 2010; Christensen et al. 2011). Biotic interference or resistance can decrease the efficacy of a biological control agent, and may even prevent biological control agents from establishing in the introduced range (Goeden & Louda 1976; Van Driesche et al. 2008 and references therein; Paynter et al. 2010). For example, predation by the generalist *Plagiognathus politis* Uhler (Hemiptera: Miridae) decreased the effectiveness of *Galerucella calmariensis* L. (Coleoptera: Chrysomelidae), on *Lythrum salicaria* L. (Myrtales: Lythraceae), purple loosestrife, and predation by spiders, fire ants, and birds contributed to the failure of *Spodoptera pectinicornis* Hampson (Lepidoptera: Noctuidae) to establish on *Pistia stratiotes* L. (Alismatales: Araceae), waterlettuce (Dray et al. 2001; Hunt-Joshi & Blossey 2005).

Old World climbing fern, *Lygodium microphyllum* (Cavanilles) R. Brown (Schizaeales: Lygodiaceae), is native to tropical and subtropical regions of the Old World (Pemberton 1998; Smith et al. 2006). It escaped cultivation in Florida and was first reported as naturalized in 1965 (Beckner 1968; Pemberton & Ferriter 1998). *Lygodium microphyllum* is now widespread in wetland and mesic habitats in south and central Florida, with several isolated populations in north Florida (EDDMaps 2015). This aggressive indeterminate vine can climb 20 m or more into trees and can extend horizontally, smothering native vegetation and reducing plant diversity and ecosystem services (Gordon 1998; Pemberton & Ferriter 1998). *Lygodium microphyllum* produces vast numbers of spores that are windborne and opportunistically self-compatible (Lott et al. 2003; Volin et al. 2004).

The rapid spread of *L. microphyllum*, its impact on native communities, and the lack of effective long-term control using conventional management techniques prompted the start of a classical biological control program in 1997 (Goolsby et al. 2003; Hutchinson et al. 2006). The ongoing search for potential biological control agents has focused on Asia and Australia (Pemberton 1998; Goolsby et al. 2003).

The moth *Neomusotima conspurcatalis* Warren (Lepidoptera: Crambidae) was approved for release as a biological control agent of *L. microphyllum* in 2007 (Boughton & Pemberton 2009). Field releases began in 2008 and large populations developed quickly at some sites causing "brown out" events where heavy defoliation by the larvae caused large areas of *L. microphyllum* to turn brown (Boughton & Pemberton 2009). Field collections of *N. conspurcatalis* larvae to check for parasitism began approximately 8 mo after the first release (Boughton et al. 2012). In total, 1,100 *N. conspurcatalis* larvae were collected from Jonathan Dickinson State Park, Hobe Sound, Florida, USA, between 2008 and 2010 (Boughton et al. 2012). Four species of hymenopteran and one species of dipteran parasitoids were reared from these larvae (Kula et al. 2010; Boughton et al. 2012).

Here we report the first egg parasitoid reared from *N. conspurcatalis* in its introduced range. This is the first recorded egg parasitoid for *Neomusotima* at the generic level (Noyes 2015). *Neomusotima conspurcatalis* females lay eggs singly or in clutches of 20 or more eggs, usually on the underside of *L. microphyllum* foliage (Solis et al. 2004; Boughton & Pemberton 2012; personal observation). The eggs are relatively flat, may overlap like shingles, and are translucent pale yellow in color (Solis et al. 2004; Boughton & Pemberton 2012; personal observation). The egg turns more yellow as the larva develops, and the dark head capsule becomes visible before the 2 mm long larva emerges. Healthy egg masses are difficult to see with the naked eye due to their color and small size. In contrast, parasitized egg masses are more visible because the chorion of parasitized eggs turns a grayish color with black spots, making these egg masses stand out against the *L. microphyllum* foliage. In Mar 2013, a dark *N. conspurcatalis* egg mass was observed on *L. microphyllum* collected from a field site in Martin County, Florida, USA. This egg mass was held in the laboratory and microhymenopterans emerged.

Parasitized egg masses have since been recovered at multiple sites located up to 125 km apart in southeast Florida. These egg masses were found by observers searching foliage in the field or were detected in the laboratory on field-collected *L. microphyllum* that was examined visually prior to use in the *N. conspurcatalis* mass rearing operation. Individual parasitized egg masses were left in situ on foliage of *L. microphyllum* and held in a plastic vial (Thorton Plastics CO, Salt Lake City, Utah, USA) at approximately 24 °C until parasitoids emerged. The microhymenopterans and remains of the egg mass were then preserved in 95% ethanol and sent to the U.S. Department of Agriculture, Agricultural Research Service, Systematic Entomology Labora-

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tory for identification. Males were identified by antennal features, and slide mounts were made using the methods outlined by Platner et al. (1999). Specimens collected from 2 sites in south Florida (26.070815°N, 80.277655°W; 25.958249°N, 80.340957°W) were identified as *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) using Pinto (1997).

Subsequently, specimens were keyed through the revision of North American species of *Trichogramma* (Pinto 1999), but a satisfactory result was not obtained. Additional males are needed, particularly larger specimens, in order to observe the ridges and processes of the genital capsule (via scanning electron microscopy) that are difficult to discern with slide mounted material (Pinto 1999). Further, additional slide mounts will allow for accurate determination of the presence of basiconic peg sensilla on the flagellum (Pinto 1999). With the continued efforts outlined above, we hope to arrive at a positive identification. However, it may be that this species is extralimital or, possibly, new. In either case, the results would be reported separately.

Trichogrammatid wasps are egg parasitoids and the genus *Trichogramma* includes numerous biological control agents (Triplehorn & Johnson 2005). Multiple eggs within a single *N. conspurcatalis* egg mass are often parasitized and parasitism rates can reach 100%. Parasitism rates by *Trichogramma* species can vary depending on environmental conditions, such as temperature and humidity, and by host density (Bourchier & Smith 1996; Quayle et al. 2003; Kalyebi et al. 2005). Romeis et al. (2005) reviewed additional factors that can alter parasitism rates by *Trichogramma*, including habitat type and host food plant species and structure. Further research is needed to determine how these factors influence the interaction between *N. conspurcatalis* and its *Trichogramma* sp. parasitoid. It also remains unclear how parasitism affects the population dynamics of *N. conspurcatalis* and its efficacy as a biological control agent of *L. microphyllum*.

We thank the many land managers who permitted us to conduct research on their *L. microphyllum* populations. Tracey Wasylik, Alex Chapman, and Cristina Martinez provided assistance in the field and laboratory. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA). USDA is an equal opportunity provider and employer. Funding was provided by the South Florida Water Management District and the U.S. Army Corps of Engineers as part of the Comprehensive Everglades Restoration Plan (CERP).

Summary

Neomusotima conspurcatalis Warren (Lepidoptera: Crambidae) was first released in Florida, USA, as a biological control agent of *Lygodium microphyllum* (Cavanilles) R. Brown (Schizaeales: Lygodiaceae), Old World climbing fern, in 2008. The first egg parasitoid, a *Trichogramma* sp. (Hymenoptera: Trichogrammatidae), was reared from *N. conspurcatalis* in 2013. The parasitoid is widely distributed in south Florida, where its egg mass parasitism rates can reach 100%.

Key Words: egg parasitism; *Trichogramma*; Old World climbing fern; weed biological control

Sumario

Neomusotima conspurcatalis Warren (Lepidoptera: Crambidae) fue liberado por primera vez en la Florida, EE.UU., como un agente de control biológico de *Lygodium microphyllum* (Cavanilles) R. Brown (Polypodiales: Lygodiaceae), el helecho trepador del Viejo Mundo, en 2008. El primer parasitoide de huevos, un *Trichogramma* sp., fue criado de *N. conspurcatalis* en 2013. El parasitoide se distribuye ampliamente en el sur de Florida, donde sus tasas de parasitismo de masas de huevos pueden llegar al 100%.

Palabras Clave: parasitismo de huevos; *Trichogramma;* helecho trepador de Viejo Mundo; control biológico de malezas

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