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The soybean stem fly found on Persian clover as an alternative wintering host in the soybean belt of South America

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ABSTRACT. The soybean stem fly (*Melanagromyza sojae*) is a widely distributed and highly damaging soybean pest, recently introduced to Brazil and neighboring countries. The bioecology of this pest under South American growing conditions is largely unknown, including how infesting populations survive throughout the winter. Fly larvae collected in August 2019 from Persian clover *Trifolium resupinatum* in Santa Maria, southern Brazil, were identified as *M. sojae* via molecular characterization. Persian clover is commonly grown as a cattle forage crop in this region. This is the first report of *M. sojae* occurring on *T. resupinatum*, and of any overwintering host available to the pest in the New World. This finding will help understand the bioecology of *M. sojae* populations in Brazil, so that adequate pest management strategies can be planned for this invasive pest.

Key words: Soybean stem fly; Persian clover; Agromyzidae; Diptera; Molecular characterization

INTRODUCTION

The soybean stem fly (SSF) *Melanagromyza sojae* (Diptera: Agromyzidae) is an important soybean pest native to Asia and widespread throughout Africa and

Oceania (Spencer, 1973; Dempewolf, 2004). Its occurrence has been reported in the soybean belt of South America since 2015 in Brazil (Arnemann et al., 2016a; Czepak et al., 2019), rapidly spreading to Paraguay in 2016 (Guedes et al., 2017) and Bolivia in 2018 (Vitorio et al., 2019). This pest can complete five cycles per year, with females laying up to 170 eggs cycle^{-1} (Wang, 1979). Oviposition occurs on young soybean leaves, and after hatching the larva feeds on mesophyll tissue reaching the leaf's main vein; from there, it bores towards the plant stem via the petiole, feeding there during seven days (larva phase duration) (Lee, 1962). Before pupation begins, the larva bores an exit hole for the adult (Figure 1), covering it with debris for protection (Van der Goot, 1930). The total cycle takes 16 to 26 days to complete (Spencer, 1990).

Figure 1. Exit hole of *Melanagromyza sojae* in *Trifolium resupinatum*. Experimental area of Irrigated Rice Research Group, Federal University of Santa Maria, RS, Brazil. Photo by Jonas Arnemann.

SSF larvae injure soybean plants by boring in the main stem and tunneling through the pith to feed (Figure 2), consequently impairing water and nutrient translocation, reducing plant height, number of flowers, number of pods, number of grains and dry matter accumulation (Talekar, 1989; Van den berg et al., 1998). The finding of tunnels along the pith is the foremost indicator of the pest's occurrence and must be thoroughly scouted throughout the crop's cycle (Gangrade and Kogan, 1980).

Soybean yield losses due to SSF attack can reach up to 40%, according to the growth stage at which infestation begins and the damage level (Talekar and Chen, 1985; Jadhav et al., 2013).

Figure 2. Injury caused by *Melanagromyza sojae* larvae in *Trifolium resupinatum.* Experimental area of Irrigated Rice Research Group, Federal University of Santa Maria, RS, Brazil. Photo by Jonas Arnemann.

Known plant hosts of *M. sojae* include 12 Fabaceae species from different genera (Spencer, 1990; Dempewolf, 2004). The most common are *Cajanus indicus* (pigeon pea), *Medicago sativa L.* (alfalfa), *Medicago polymorpha* (burr medic), *Phaseolus vulgaris* (common bean), *Pisum sativum* (common pea), *Melilotus officinalis* (yellow sweet clover), *Aeschynomene indica* (Indian joint vetch), *Indigofera suffruticosa* (Guatemalan indigo), *Vigna angularis* (adzuki bean), *Crotalaria juncea* (brown hemp) and the main host, *Glycine max* (L.) Merr. (soybean) (Spencer, 1990).

Although numerous SSF infestations have been reported throughout the first and second soybean seasons in Brazil (Pozebon et al., 2020), no other plant hosts have been reported to date, whether in summer or winter seasons. The ecological behavior of SSF during wintertime in the New World remains unknown, as well as the alternative plant hosts suitable for the pest to survive throughout the year in this landscape. As in other countries, SSF individuals can overwinter as pupae inside dead soybean stems (Ziaee, 2012), while others complete their development cycle on volunteer soybean plants originated from harvest losses. The objective of this work was to document and identify the presence of SSF on winter host plants.

MATERIAL AND METHODS

The plants were collected at the Irrigated Rice Research Station of the Federal University of Santa Maria in Rio Grande do Sul, Brazil (29°43'18.9"S, 53°43'31.9"W, 113 m altitude) on August 21, 2019. Five larvae and one pupa were sampled from T. resupinatum plants for molecular analysis. Individual samples were submitted to gDNA extraction, using the Qiagen DNasy Blood and Tissue DNA Extraction Kit (Qiagen, Hilden, Germany). Amplification of the cytochrome oxidase I (COI) mitochondrial gene was done via PCR, using M. sojae-specific primers and PCR conditions as described by Arnemann et al. (2016b). The amplified PCR products were sent for sequencing at ACTGene, Porto Alegre, Brazil. Sequence analysis was carried out using Pregap and Gap4, from the Staden package (Staden and Bonfield, 2000) and Geneious R9 (Biomatters Ltd., New Zealand).

RESULTS

Molecular analysis of all samples identified the species as *M. sojae* and the haplotype as MSOJ–COI–02, which was previously reported in Australia (Arnemann et al., 2016a, GenBank accession number KT821484), Brazil (Arnemann et al., 2016a), Paraguay (Guedes et al., 2017) and Bolivia (Vitorio et al., 2019), being one of the most frequent haplotypes found in the New World. The successful spread of this haplotype throughout several South American countries indicates adaptation to the environmental conditions within this geographical range, including colonization of new plant hosts.

Overwintering hosts are key components for the population dynamics of *M. sojae*, with the Persian clover (*T. resupinatum* L.) standing out as a feasible and previously unidentified alternative for the species' development. *T. resupinatum* is a cold-season, annual crop from the Fabaceae family, well adapted to poorly-drained and hydromorphic soils due to its hollow stems, which provide extra aeration to the roots (Bortolini et al., 2012). Because of these characteristics and high quality as forage for cattle feeding, Persian clover has been increasingly grown as a winter crop in many regions of southern Brazil.

Successful establishment of an exotic species in a new environment is primarily dependent on number of transported propagules (or individuals), genetic variability and frequency of the incursion flows, which directly affects the species' population growth and spread (Grevstad, 1999; Ahlroth et al., 2003; Memmott et al., 2005), as well as availability of suitable hosts (Pozebon et al., 2020). Factors such as high initial propagule pressure (Simberloff, 2009) or multiple and continuous incursions of this haplotype (MSOJ–COI–02) into the continent may have favored its predominance over others, as already reported for other invasive pest species in Brazil, such as *Bemisia tabaci* MED ('Mediterranean' species, previously known as *B. tabaci* Q-biotype; De Barro et al., 2011; Barbosa et al., 2015), *Helicoverpa armigera* (Arnemann et al., 2019) and *Thaumastocoris peregrinus* (Barbosa et al., 2020).

The imposition of a sanitary break in the Rio Grande do Sul state, lawfully forbidding the cultivation of soybean after the main summer season, has been discussed as an alternative to reduce infestation by invasive pathogens and pests, such SSF and Asian soybean rust (*Phakopsora pachyrhizi*). While soybean rust management could benefit greatly from such a measure, the presence of alternative overwintering hosts for *M. sojae* (as *T. resupinatum*) makes it an ineffective strategy to reduce the pest's incidence in soybean fields.

Identifying alternative plant hosts is a major step towards the development of a comprehensive understanding of *M. sojae's* bioecology, population dynamics, and prediction of occurrence during first and second soybean seasons in Brazil (Lopes et al., 2008). The eradication of post-harvest volunteer plants and the use of winter crops not suitable as hosts to SSF should be prioritized by soybean growers to avoid injury. At a global scale, there is scarcity of data relating to evolutionary genetics, ecology parameters and resistance profile associated with integrated pest management practices and insecticide responses in SSF. Further investigations should be devoted to the development of management strategies for this pest in South America, as done by Curioletti et al. (2018).

Usual control measures for SSF in the Old World include the use of resistant cultivars and sowing dates outside the population peaks (Talekar, 1989). Seed or foliar insecticide sprayings have also been reported (Adak, 2012; Jadhav et al., 2013). However, continuous insecticide spraying targeting *M. sojae* does not represent the best management strategy for this pest in the long term, since risks of selection of tolerant individuals and harmful effects on natural enemies remain uncertain. Alternatively, the use of endemic beneficial insects represents a feasible biological strategy for SSF control (Talekar, 1990; Van der Berg, 1998), with one parasitoid species (*Syntomopus parisii*) already identified in Brazil and Paraguay (Beche et al., 2018).

Our study contributes to a better understanding of the ecology and population dynamics of the SSF in the New World, assisting in the development of appropriate management strategies for this important soybean pest.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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