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Brief Communication

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First Reports of *Macrosaccus morrisella* (Lepidoptera: Gracillariidae) Feeding on Soybean, *Glycine max* (Fabales: Fabaceae)

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Abstract

Macrosaccus morrisella (Fitch) is a tiny leaf-mining moth native to North America. In this publication, we provide first reports of this insect feeding on soybean, *Glycine max* (L.) Merr., in Canada and the United States, describe its injury to soybean, and briefly review leaf miners associated with soybean. Further efforts related to *M. morrisella* should focus on its geographic extent of infestation of soybean, impacts to soybean, and ecology in the agricultural landscape.

Key words: leaf miner, host range, pest, soybean

Soybean, *Glycine max* (L.) Merr. (Fabales: Fabaceae), is an important crop to North American agriculture. Because soybean is a non-native crop, the herbivorous insects feeding on it in North America represent native species that have adapted to soybean and exotic species that have invaded (Kogan 1981, Kogan and Turnipseed 1987). Here we provide first reports of infestations of soybean in Canada and the United States by a native leaf-mining moth *Macrosaccus morrisella* (Fitch) (Lepidoptera: Gracillariidae).

Macrosaccus morrisella is widely distributed across eastern North America (Braun 1908, Davis and De Prins 2011). The identification of its life stages is detailed by Braun (1908) and Davis and De Prins (2011). The adults are tiny moths measuring 6–7 mm (Braun 1908). The front wings are patterned with orange, white and gray-black markings (Davis and De Prins 2011). Larvae reach about 4.7 mm in length and are pale green to white in color (Davis and De Prins 2011). The pupae are about 3.6 mm in length (Davis and De Prins 2011). *Macrosaccus* spp. feed on plants in the Fabaceae family (Davis and De Prins 2011). In particular, *M. morrisella* is known to feed on American hogpeanut, *Amphicarpaea bracteata* (L.) Fernald (Fabales: Fabaceae), and sickleseed fuzzybean, *Strophostyles leiosperma* (Torr. & A. Gray) Piper (Fabales: Fabaceae), which are both vining plants with trifoliate leaves (Davis and De Prins 2011). The observations presented below suggest that *M. morrisella* has adapted to feed on soybean.

Sample Collection and Identification of Insects

In Québec, Canada, white-colored blotch-type leaf mines were observed over several years. On 9 August 2016, heavily mined soybean leaves (e.g., Figs. 1 and 2) were collected from a field in Saint-Paul and sent to the Laboratoire d'Expertise et de Diagnostic en Phytoprotection (LEDP) for identification. During the following week, infested leaves were collected in Saint-Ambroise-de-Kildare (15 August 2016), Berthierville (18 August 2016), and Nicolet (18 August 2016), and sent to the LEDP. More than 10 mines could be seen on some leaflets. Additional soybean leaves with blotch-type leaf mines were collected from a field in Princeville on 8 July 2019 and from fields in Saint-Gervais and Yamachiche in 2021. Adult insects were reared from infested soybean leaves from each of these locations in Québec.

In Minnesota, United States, leaf mines similar to those in Québec were observed in soybean fields near St. Paul and Rosemount on 13 and 17 August 2021, respectively (e.g., Fig. 3). At both locations, infestations were light with only scattered plants showing symptoms of infestation. From the St. Paul and Rosemount fields, soybean leaves with blotch-type mines were collected. For each leaf, the number of mines, and length and width of each mine was recorded. Then, each mine was inspected under a dissecting scope to determine the presence and life stages of insects. The tip of an insect pin was used to carefully open intact mines. Pupae were reared to adults. Among the 45 infested leaves collected from these two fields in Minnesota, 37 leaves had one mine, five leaves had two mines, and three leaves had three mines. These mines average 12.5 ± 0.6 (±SEM) mm long and 7.3 ± 0.3 mm wide. Of the 56 mines observed across these leaves, 31 mines were empty, 19 mines had larvae (18 mines with one larva each, and one mine with three larvae), and six mines had pupae (five mines with one pupa each, and one mine with two pupae). Five adults later emerged from the seven pupae.

Morphological identification of adult specimens was performed using descriptions from Davis and De Prins (2011). Male genitalia were dissected and placed in 85% lactic acid and heated for 25 min at about 90°C, and observed using a Zeiss Discover V20 at 150×magnification. In addition, specimens from each location were subjected to DNA barcoding. DNA extraction was performed using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Following DNA extraction, amplification of the COI gene was conducted with primers LCO1490 and HCO2198 in reactions containing: 25.00 µl Invitrogen Platinum Hot Start PCR 2× MasterMix, 19.00 µl H_2O, 0.5 µl 10 µM forward primer, 0.5 µl 10 µM reverse primer, and 5 µl template DNA. Thermal cycler conditions were: 95°C for 3 min, 45 cycles of 95°C for 45 s, 53°C for 45 s, and 72°C for 45 s, and a final extension of 72°C for 3 min. Amplification success was verified using QIAxcel Advanced System (Qiagen). Purification and Sanger Sequencing were completed at the SANGER Sequencing Platform of the CHU de Québec-Université Laval Research Center on a ABI 3730xl DNA Analyzer. The sequences were trimmed, aligned, and assembled using Geneious v2021.2.1.

Observations and Significance

The leaf-mining insects obtained from soybean leaves collected in Québec and Minnesota were determined to be *M. morrisella* based on morphological identification and DNA barcoding. The blotch-type leaf mines of *M. morrisella* observed here on soybean (Figs. 1–3) were unlike the mines of *Odontota horni* Smith (Coleoptera: Chrysomelidae) (Kogan and Kogan 1979, Buntin 1994, Hodgson et al. 2012). Davis and De Prins (2011) described the mines of *M. morrisella* as: 'The mine begins as an elongate serpentine track on the abaxial side of the leaflet. This enlarges to an elongate-oval, whitish blotch which eventually becomes

Fig. 1. Blotch-type leaf mines caused by *Macrosaccus morrisella* in a soybean field. Image credit: J. Moisan-De Serres.

Fig. 3. Intact blotch-type leaf mine caused by *Macrosaccus morrisella* on abaxial (lower) side of a soybean leaflet. Image credit: R. L. Koch.

Fig. 2. Soybean leaves heavily infested with *Macrosaccus morrisella*. Image credit: J. Moisan-De Serres.

strongly tentiform'. The mines from *M. morrisella* in soybean did not cross the midribs or main lateral veins of the leaves, and one or more margins of individual mines were often defined by the midrib or lateral veins (Figs. 1–4). The lower (i.e., abaxial) surface of the mines was generally white in color and very thin (Figs. 1–3, 4B, and C). Sometimes the upper (i.e., adaxial) surface of the mine was slightly raised (i.e., tentiform) (Figs. 2 and 4A). In addition, the outer adaxial surface of the mines often had numerous small light-colored spots (Figs. 2 and 4A), which appeared to be caused by small feeding pits from larvae feeding on the inner side of that leaf surface (Fig. 5A). This spotted appearance was less apparent or absent for mines with small larvae. The inside of intact mines contained dark-colored frass from the larvae (Fig. 4C). Silken enclosures were found in mines with large larvae preparing to pupate and mines containing pupae (Fig. 5B). Most of the empty mines contained remnants of these silken enclosures and sometimes pupal exuviae, suggesting the adults had already emerged.

Leaf-mining insects are known to have physical and physiological impacts on their host plants (Liu et al. 2015). Three native leaf miners

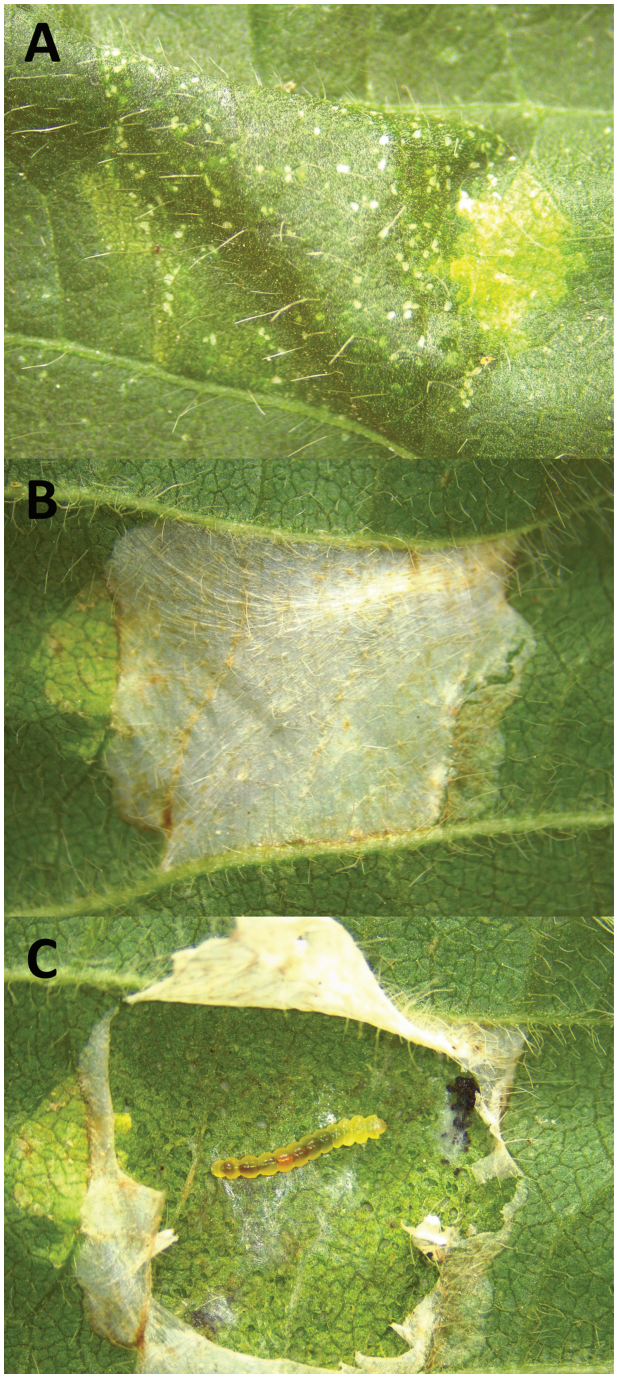

Fig. 4. Injury on the adaxial (upper) side (A), and intact (B) and open leaf mine showing a *Macrosaccus morrisella* larva (C) on the abaxial (lower) side of a soybean leaflet. Image credit: R. L. Koch.

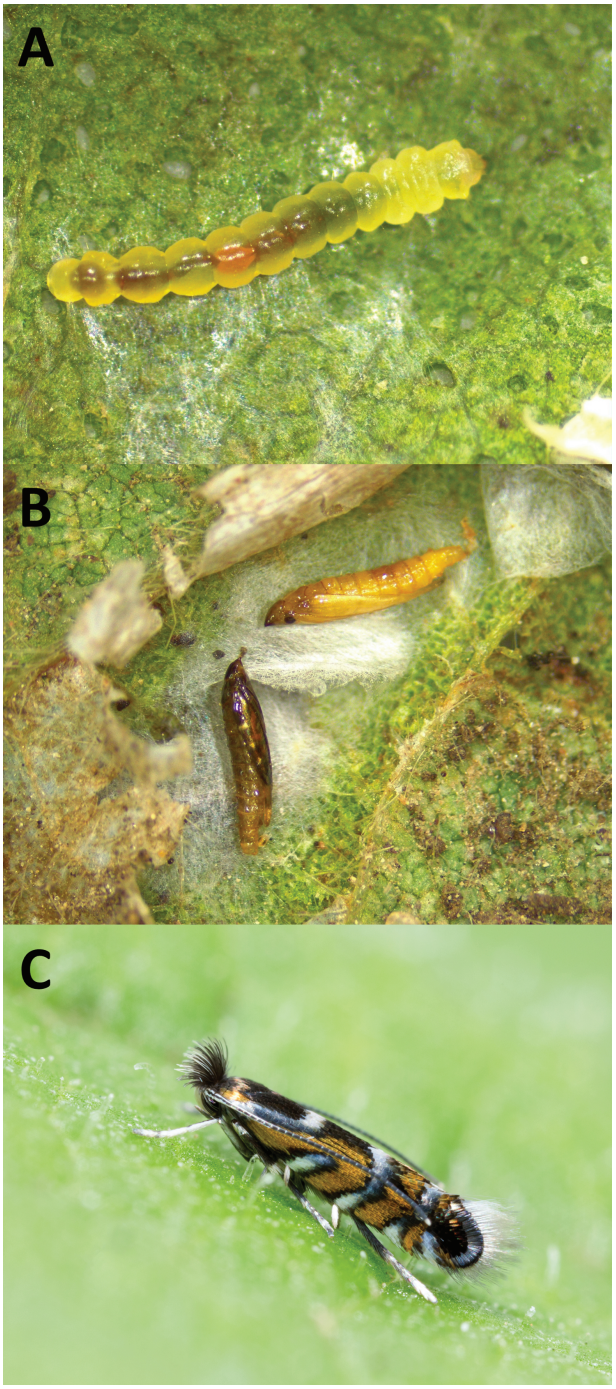

Fig. 5. Larva (A), pupae (B), and adult (C) of *Macrosaccus morrisella* from soybean. Image credits: R. L. Koch for (A) and (B); J. Moisan-De Serres for (C).

(i.e., soybean leaf miner, *Odontota horni* Smith, locust leaf miner, *O. dorsalis* (Thunberg), and *Sumitrosis rosea* (Weber) (Coleoptera: Chrysomelidae)) are known to feed on soybean in North America (Buntin and Pedigo 1982, McPherson and Ravlin 1983); however, these leaf miners are rarely of economic significance to soybean production (Buntin 1994). In contrast, the leaf-mining insect *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) is a significant pest of soybean production in Africa, Asia, and Australia (Gaur and Mogalapu 2018, Buthelezi et al. 2021). Furthermore, within the family Gracillariidae (Lepidoptera), *Caloptilia soyella* (Deventer) in China and Japan, *Phodoryctis caerulea* (Meyrick) in Indonesia, *Porphyrosela homotropha* Vári in Ethiopia (De Prins and De Prins 2006–2021), and *Porphyrosela* (*Lithocolletis*) *aglaozona* (Meyrick) (Bailey 2007) have been reported to feed on soybean. Because the leaf-mining niche in North American soybean appears underutilized (Buntin 1994), the potential adaptation to soybean by a leaf-mining lepidopteran like *M. morrisella* requires further examination (e.g., Kogan 1981).

Relatively little is known about the ecology and management of *M. morrisella*. However, this species is known to be attacked by two parasitoids, *Pediobius albipes* (Provancher) and *Sympiesis marylandensis* Girault (Eulophidae: Hymenoptera) (Peck 1985, Maier 1988, De Prins and De Prins 2006–2021). Knowledge of the impacts and management of the chrysomelid leaf miners of soybean in North America (Buntin 1994) and lepidopteran leaf miners of soybean in Africa, Asia, and Australia (Gaur and Mogalapu 2018, Buthelezi et al. 2021) could provide a foundation for development of management plans for *M. morrisella*, if infestations persist and intensify. Future research should assess its geographic extent of infestation of soybean, impacts to soybean, and ecology in the agricultural landscape.

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