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Short Communication

Epiblema minutana (Lepidoptera, Tortricidae) in Israel: Promise or Peril?

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Abstract

Parthenium weed (*Parthenium hysterophorus* L.) is a noxious weed and a flagship invasive that has been spreading throughout Israel and the Palestinian Authority since 1980. In other regions affected by this invader, parthenium weed has been managed through classical biological control releases of *Epiblema strenuana*, a stem-galling tortricid moth native to North America. More recently, a congeneric moth, *Epiblema minutana* has been identified from Israel. To better understand the implications of *E. minutana* for controlling parthenium weed, we investigated the spread of *E. minutana* within Israel and the Palestinian Authority from 2012 to 2019 and explored its host range. We used a series of no-choice experiments in field cages to evaluate damage on naturalized *Ambrosia* spp., *Xanthium italicum* and parthenium weed as well as potential non-target, commercial cultivars of sunflower (*Helianthus annuus* L.) and niger (*Guizotia abysinnica* (L. f.) Cassini). We also evaluated damage in the field, where we found substantial gall formation on *Ambrosia* spp., but none on *P. hysterophorus* or other genera. The geographical distribution increased from an estimated 424 km^2 in 2017 to 1671 km^2 in 2019. While *E. minutana* shows promise as a biological control agent of *Ambrosia* spp. and does not attack the oil crops sunflower and niger, it is not a suitable biological control agent of parthenium weed.

Key words: biological control, parthenium, weed management, biosecurity, host range, biological invasions, fortuitous biocontrol

Introduction

Parthenium weed (*Parthenium hysterophorus* (L., 1753), is a global noxious invasive weed, that has recently established and spread throughout Israel and the Palestinian Authority (Matzrafi et al. 2021). Controlling this weed has proven problematic in several parts of the world. Classical biological control, the release of specialized natural enemies from the native range of the target weed (Müller-Schärer and Schaffner 2008; Sun et al. 2022) has been identified as a promising management method for this flagship invader in different parts of the invaded range. *Epiblema strenuana*

Walker, 1863 (Lepidoptera: Tortricidae), a stem-galling tortricid moth native to North America has been released to control parthenium weed in Australia (Dhileepan et al. 2018). Releases resulted in reduced fitness, biomass and competitivity of *P. hysterophorus* (Strathie et al. 2021). In China, *E. strenuana* was introduced to control *Ambrosia artemisiifolia* DC., 1846, and in Papua New Guinea to control *Xantthium strumarium* L., 1753 (Winston et al. 2022). However, *E. strenuana* was not released in South Africa because of possible non-target effects on commercially valuable cultivars of *Guizotia abyssinnica* (L. f.) Cassini, 1829, an asteraceous oil crop in Eastern Africa (McConnachie 2015).

Epiblema strenuana was reported to be detected in Israel for the first time in 2008, feeding on the invasive weeds *Ambrosia confertiflora* Decandolle, 1830, (Asteracea), and *Ambrosia tenuifolia* Sprengel, 1830, (Asteracea) (Yaacoby and Seplyarsky 2011). However, molecular analyses revealed that the Israeli moths originally thought to be *E. strenuana* were in fact a congeneric species, the North American *Epiblema minutana* Kearfott, 1920 (Lepidoptera: Tortricidae) (Gilligan et al. 2020).

As the host range of *E. minutana* was unknown since it was recently lifted from synonym level with *E. strenuana*, this study set out to test whether this stem-galling moth can establish and develop on a group of potential target and non-target species under no-choice conditions. Host-range studies often rely on the centrifugal phylogenetic hypothesis, which posits that the likelihood that a test plant species will be attacked by an herbivore decreases with increasing phylogenetic distance between the target weed and the test plant species (Wapshere 1974). To estimate the host-range of *E. minutana* in Israel, we monitored plant species from the subtribe Heliantheae of North American origin that have established in Israel and that are closely related to recorded host plant species of *E. minutana*.

Additionally, we monitored the spread of the moth from 2012 to 2019 in Israel and the Palestinian Authority and assessed its ecological host range in the area, i.e., the range of plants attacked under field conditions.

Materials and methods

Plant species surveyed

Yaacoby and Seplyarsky (2011) reported *E. minutana*, then thought to be *E. strenuana*, on *A. tenuifolia* and *A. confertiflora*. Other host plants in *E. minutana*'s native range are presumed to be *Ambrosia artemisiifolia* DC., 1846 (Asteracea) and *Ambrosia psilostachya* DC., 1845 (Asteracea) (Gilligan et al. 2020), but these species associations are partially unclear because the information does not differentiate between *E. minutana* and *E. strenuana*. We included *Ambrosia grayi* (Nelson) Shinners, 1853 (Asteracea) in our study as it is a congeneric of the target species. All the *Ambrosia* spp. in this study are native to North America and have been introduced into Israel.

They are agricultural weeds, but have gained more notoriety because they produce highly allergenic pollen (Yair et al. 2019). We tested *P. hysterophorus* because the closely related *E. strenuana* was introduced in Australia as a biological control agent for this plant. Another plant we tested is *Xanthium italicum* (Moretti), 1825 (Asteracea). It is an agricultural weed that is widely established in Israel (Dafni and Heller 1982). Additionally, we monitored *Verbesina encelioides* (Cav.) Benth. & Hook.f., 1876, an Asteraceae that grows close to the sites of the first reports of *Epiblema* moths in Israel, therewith adding a phylogenetically more distant plant that has been naturalized in Israel to the tested plant species.

We also tested three cultivars of sunflower (*Helianthus annuus* L., 1753, Asteracea), since this is an important oil and ornamental crop, and monitored naturalized populations of wild sunflowers, and niger (*G. abyssinica*).

Epiblema minutana *life cycle*

Epiblema minutana overwinters as larvae in galls, often in dry stalks of host plants. Moths emerge, mate, and begin laying eggs on leaves within 3 days (Nussbaum 2019). First instar larvae are leaf miners that move to the apical meristem. Second larval instars then burrow into the meristem and induce gall formation where larvae develop, pupate and then emerge as adults (Yaacoby and Seplyarsky 2011). In Israel, first pupae in stems infested during the previous summer were observed as early as mid-January. Average developmental time from egg to adult is 28 days (Nussbaum 2019).

Distribution and ecological host range of E. minutana *in Israel*

We haphazardously recorded presence/absence of *E. minutana* throughout Israel between 2012 and 2017. Starting in 2017, we conducted targeted surveys in Israel and the Palestinian Authority where sites with potential host plants were identified prior to inspection. In targeted surveys, we searched at least 10 stems/host plant species/site. If more host plants were present, we searched at least 10 plants. The presence of stem galls was treated as a proxy measure for presence of *E. minutana,* but was not indicative of life-stage of the insect. To the authors' knowledge, no other gall-forming insects on the surveyed plant species are present in Israel.

We mapped survey points in QGIS and used the ConcaveHull-Plugin to determine the geographical range of *E. minutana* over the years. We then selected plants within this range as indicators for the presence of *E. minutana*, and for further verification of its host range in the field.

First caged host-range experiment

In 2017, we selected *P. hysterophorus, X. italicum*, and *H. annuus* (variety Jamanit DI3) for a no-choice experiment. This experiment was conducted in field cages at the Agricultural Research Organisation, Volcani Center in Rishon LeTsyion, Israel, from March until September 2017.

Plants were grown in 20 cm diameter pots with commercial, turf-free standard garden soil. Moths were reared on *A. confertiflora* plants in field cages. For further details, see Nussbaum (2019). We released at least two pairs of *E. minutana* adults in a 60 × 90 × 60 cm gauze-covered field cage (Bugdorm, MegaView Science Co., Ltd.) with a single test plant as follows: *H. annnuus* (N = 10), *X. italicum* (N = 6), *P. hysterophorus* (N = 16). After 90 days, plants were searched for eggs and galls.

Second caged host range experiment

In 2019, we evaluated whether *A. confertiflora*, *A. tenuifolia*, *A. artemisiifolia*, *P. hysterophorus*, *G. abyssinica*, and two varieties of *H. annuus* (the variety "Clearfield", which is used as a commercial oil crop in Europe (not in Israel), and an ornamental variety, "Orange Fire" (Sunny Me Seed Ltd., Israel)) were viable hosts for *E. minutana*. Because we could not obtain viable seeds of *A. tenuifolia* in Israel, plants of this species were grown from rhizomes collected in three different sites in Israel. All other plants were grown from seeds, that were either field collected or bought commercially. Plants were grown in 18 cm diameter pots in commercial, turf-free standard potting soil with added granulated slow-release fertilizer with drip-irrigation. Plants were between 15 and 40 cm high at the beginning of the experiment. We collected stems of *A. tenuifolia* with galls of *E. minutana* in Kfar Saba, central Israel, inserted the stems in floral sponges with a continuous water supply, and transferred them to cages. In addition, we established a rearing culture of *E. minutana* on *A. tenuifolia* plants. Moths had emerged less than four days before the start of the experiment.

The experiment was conducted at the Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture in Rehovot, in seven 60 × 60 × 90 cm netted cages (YFeng) inside a field cage from June–August 2019. One plant per species or variety was randomly assigned to one cage per replicate. We then released two pairs of the moths per cage and allowed them to lay eggs for seven days before moving the plants to a cage without moths, and dissected the plants 30 days later to search for successful gall formation. This experiment was replicated consecutively 7 times.

Statistical analysis

We tested for differences in the number of plants with and without galls per plant species with a Kruskal-Wallis test in the Agricolae package (De Mendiburu 2021). Figures were made in ggplot2 (Wickham 2011). Analyses were conducted in R version 4.1.0

Results

Geographical distribution and ecological host range

We estimated that distribution increased nearly four-fold between 2017 ($424 km^2$) and 2019 ($1671 km^2$). By 2019, *E. minutana* was observed from

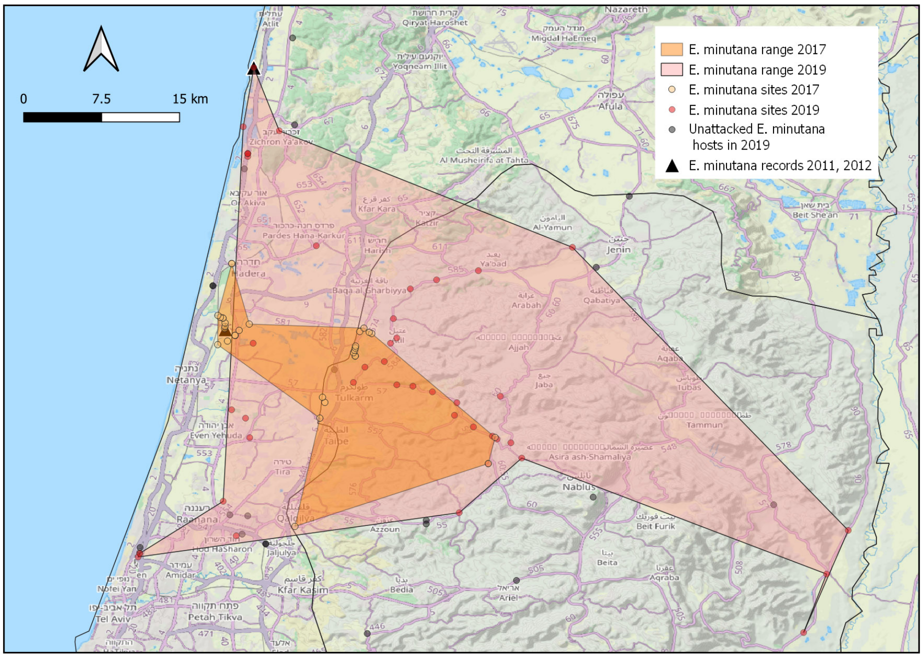

Figure 1. Spread and estimated geographical distribution of *Epiblema minutana* in Israel.

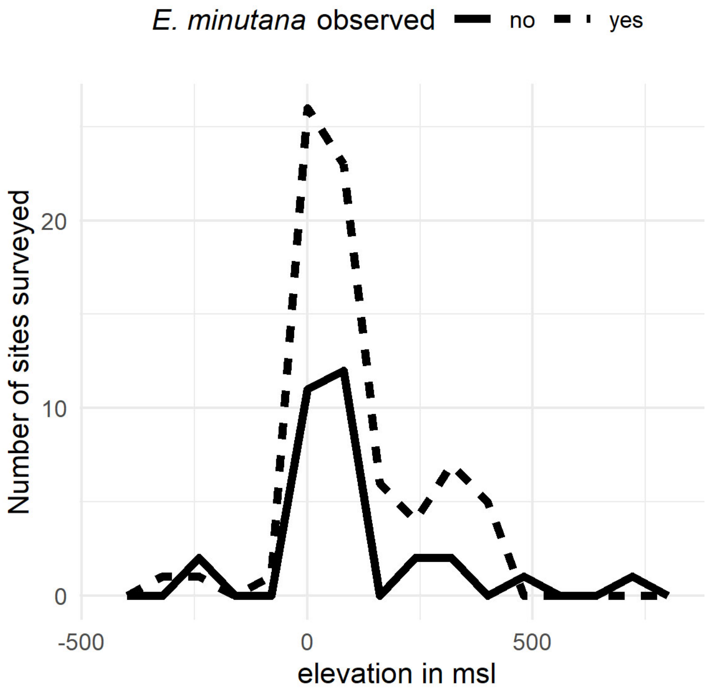

Figure 2. Altitude of sites with host plants (confirmed by no-choice experiments) surveyed. Plots depict number of sites with *Epiblema minutana* galls (dotted lines) or without (solid lines).

Habonim in the north-west of Israel, over Jenin to Maale Efraim in the south-east, and Glilot in the south-west. Moths were observed south and east of the original 2011 location records, but not to the north (Figure 1). Moths were not found in areas 500 m above sea level (Figure 2). Within the range occupied by *E. minutana*, we found galls on *Ambrosia* spp. (*A. confertiflora, A. psilostachya,* and *A. tenuifolia*), but not on *H. annuus, P. hysterophorus* and *V. encelioides* (Figure 3).

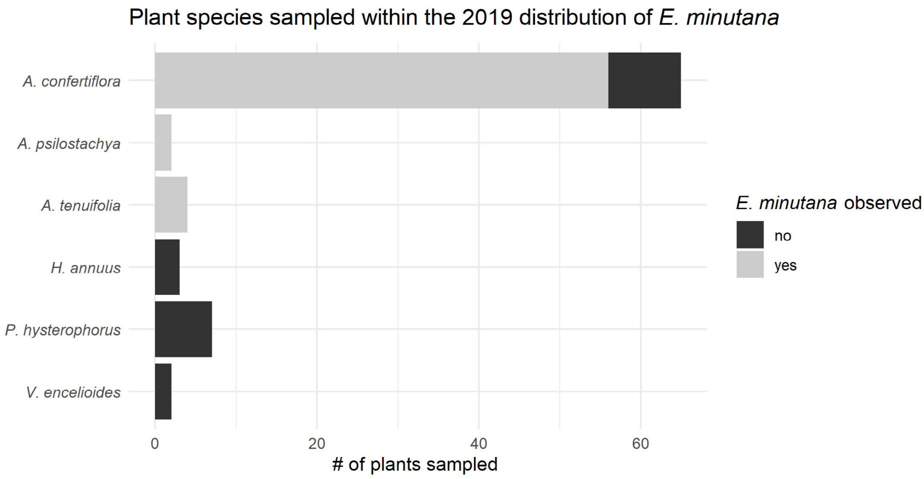

Figure 3. Ecological host range of *Epiblema minutana* within the geographical distribution in 2019.

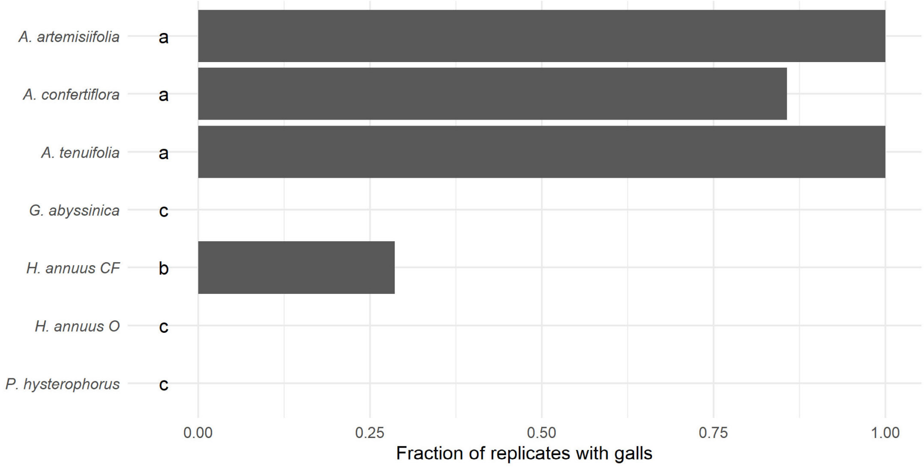

Figure 4. Preliminary fundamental host range of *Epiblema minutana* (y-axis) (different letters indicate significant differences at $p < 0.05$; Kruskal-Wallis test). *"H. annuus CF"* = *Helianthus annuus* "Clearfield", "*H. annuus O"* = *Helianthus annuus* "Orange Fire".

Fundamental host range

In the first cage experiment, no galls were found on *P. hysterophorus, X. italicum,* or *H. annuus* (variety Jamanit DI3).

In the second caged experiment, galls were observed on all *Ambrosia* spp. tested, and on two of the seven replicates of *H. annuus* ("Clearfied"). In contrast, no galls were found on the ornamental variety of *H. annuus,* on *G. abyssinica,* and *P. hysterophorus* (Figure 4). A Kruskal-Wallis test pooled the *Ambrosia* spp. together, *H. annuus* ("Clearfield") in a separate group, and then grouped *G. abyssinica, H. annuus* (ornamental), and *P. hysterophorus* together. While the *Ambrosia* spp. had at least one gall per stem, we found only one gall per *H. annuus* ("Clearfield") plant.

Discussion

The distribution of *E. minutana* in Israel has grown four-fold larger between 2017 and 2019. *Epiblema minutana* readily attacked all *Ambrosia* species provided in no-choice experiments (*A. artemisiifolia, A. confertiflora,* and *A. tenuifolia*). However, unlike its close relative *E. strenuana*, it is unlikely to be a suitable biological control agent of *P. hysterophorus*. Within the moth's range, we did not find any galls of the moth on this noxious weed. These findings were confirmed experimentally, as no gall formation was observed on *P. hysterophorus* in two separate no-choice host-range tests. Our preliminary findings thus suggest that *P. hysterophorus* is outside of *E. minutana*'s fundamental host range. This also implies that the moth is not capable of preventing further spread of *P. hysterophorus* in Israel (Matzrafi et al. 2021). Therefore, the release of other herbivore candidates should be considered, such as *Listronotus setosipennis* (Coleoptera: Curculionidae), which has a narrow host range (Weyl et al. 2021a, b) and has been introduced in similar climates to Australia and Eastern Africa and South Africa (Alemayehu and Mersie 2019; Dhileepan et al. 2018; Strathie et al. 2021).

The closely related moth *E. strenuana*was introduced as a biological control agent for *P. hysterophorus* to Australia (Dhileepan et al. 2018), but was not released in South Africa due to concerns over its safety for other African countries (McConnachie 2015). In our experiments, *E. minutana* did not attack *G. abysinnica*, and while this plant is not grown as an oil crop in Israel, this result remains important as an indicator of a different host range of the closely related moth species. From a biosecurity point of view, the potential spread of *E. minutana* from Israel to Africa should not be a concern for *G. abysinnica* farming in East Africa, especially Ethiopia, which has strong trade and cultural connections to Israel (Bishku 2015).

All *Ambrosia* spp. tested in the no-choice experiments proved suitable hosts of *E. minutana*. In the field, we confirmed that *E. minutana* attacks all *Ambrosia* spp. within the colonized distribution. We did not find galls on *A. grayii* and *A. artemisiifolia* in the field, but this is most likely due to the populations of these plant species monitored during our surveys being outside the current geographic distribution of *E. minutana.* The gall formation of this moth has been shown to reduce plant biomass of *A. confertiflora* and *A. tenuifolia* (Nussbaum 2019), but its impact on pollen production and plant fitness remains to be explored.

Epiblema strenuana has been introduced as a biological control agent against *A. artemisiifolia* in China (Winston et al. 2022), where it is also reported to feed on *X. strumarium* (Fanghao et al. 2003). It was also released for control of *X. strumarium* in Papua New Guina (Winston et al. 2022). The closely related *X. italicum* was not attacked by *E. minutana* in our experiments, giving another indication of the distinct host range of the two *Epiblema* spp.

In the Middle East, sunflower is a crop, ornamental, and an invasive plant alike (Hübner et al. 2022). Therefore, the impact of *E. minutana* on this plant would be of high concern, which was the reason for including the four different cultivars of *H. annuus* in our surveys and experiments: two frequently used for cultivation in Israel ("'Jamanit DI3", and an "Orange fire"), one that is not used commercially in Israel but planted extensively in Europe ("Clearfield"), and a wild, naturalized sunflower in our field surveys. The European agricultural cultivar ("Clearfield") was attacked in two out of 10 replicates in the no-choice experiment. However, we only found one larva per sunflower plant, while *Ambrosia* spp. showed at least one gall per branch, resulting in dozens of galls per plant. Although caged no-choice experiments usually provide an indication of fundamental host range, this generally overestimates the insect's realized or ecological host range (Cullen 1990). Furthermore, we are not aware of any records of *E. minutana* attack of sunflower fields in Israel. Therefore, damage caused by *E. minutana* to sunflower for seed or oil production in Israel is unlikely.

This study provides evidence that *E. minutana*'s fundamental and ecological host range is clearly distinct from the host range of the closely related *E. strenuana* (Gilligan et al. 2020). We found a consistent attack of plants in the genus *Ambrosia*, no attack on *G. abysinnica,* and only negligible risk to *H. annuus* grown in Israel, thus, the biosecurity concern of this moth is not justified. For managers of invasive weeds in Israel, it means that there is no promise for biological control of the weed *P. hysterophorus* by *E. minutana*. However, knowledge of the moth's impact on *Ambrosia* spp. could improve the control of these noxious and highly allergenic weeds.

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Author's contribution

BAA: research conceptualization, investigation and data collection, data analysis and interpretation, funding provision and writing, original draft. NN: investigation and data collection, writing – review and editing. YY: investigation and data collection. AH: research conceptualization. TY: research Conceptualization. HMS: research conceptualization, writing – review and editing. US: research conceptualization, sample design and methodology, writing – review and editing. BR: research conceptualization, sample design and methodology, funding provision and writing – review and editing.

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