

## ORIGINAL ARTICLE

## Population fluctuation of *Melanagromyza sojae* (Diptera: Agromyzidae) on chickpea and soybean crops and update of its geographical distribution in Argentina

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### Abstract

*Melanagromyza sojae*, the soybean stem fly (Zehntner) (Diptera: Agromyzidae), is a new pest in soybean and chickpea crops in South America. The objective of this study was to determine population fluctuations of *M. sojae* in both crops using adults caught in yellow sticky traps and by sampling injured plants. Additionally, an update of its geographical distribution in Argentina is provided. Adults of this species were collected for multiple years and during the cycles of both soybean and chickpea crops, on volunteer soybean plants, and during periods when the crops were not present. Injuries caused by *M. sojae* occurred throughout the growing period of chickpea and the reproductive stages of soybean. The highest infestation level was registered in chickpea crops. Regarding the distribution of *M. sojae*, we have reported *Medicago sativa*, *Vigna radiata*, *Mellilotus* sp., *Helianthus annuus*, *Carthamus tinctorius*, *Heliotropium* sp., *Glandularia* sp., and *Parthenium* sp. for the first time as new host plants in Argentina. The presence of *M. sojae* in Chaco province (Argentina) was also reported for the first time. Finally, the highest percentage of infestation registered in chickpea crops shows that *M. sojae* is a pest in chickpea-growing areas in Argentina where this pest is widely distributed. It is of vital importance to develop effective control methods, to research and recommend efficient management strategies and control methods for *M. sojae* in chickpea.

**Keywords:** bases for IPM, invasive pest expansion, new host plants, soybean stem fly, yellow sticky traps

## Introduction

The soybean stem fly, *Melanagromyza sojae* (Zehntner) (Diptera: Agromyzidae) is a native species to East Asia (Spencer 1973). It is common in northeast Africa, Australia, Indonesia, India, Nepal, China, Japan, as well as in parts of Russia and Spain (van Den Berg *et al.* 1998; Wang and Gai 2001; Gil-Ortiz *et al.* 2010; Thapa 2012; Strakhova *et al.* 2013) and it was recently reported in South America. Its first record was in Brazil and its occurrence has been reported from the 1980s to the

present (Guedes *et al.* 2015; Arnemann *et al.* 2016; Czepak *et al.* 2018). The presence of this species has also been confirmed in Paraguay (Guedes *et al.* 2017), Bolivia (Vitorio *et al.* 2019), and was recently reported in Uruguay (Cibils-Stewart *et al.* 2024) causing damage to soybean (*Glycine max* (L.) Merr.) crops. In Argentina, it was detected for the first time causing damage on chickpea (*Cicer arietinum* L.) in 2019 (Vera *et al.* 2021) and on soybean and common bean (*Phaseolus*

*vulgaris* L.) crops in 2020 (Saluso 2020; Trossero *et al.* 2020; Murúa *et al.* 2021). Finally, the soybean stem fly was recently found developing on *Tithonia tubaeformis* (Jacq.) Cass. (Asteraceae), *Lens culinaris* (Medik) (Fabaceae) and volunteer soybeans (Fadda *et al.* 2023).

*Melanagromyza sojae* is polyphagous but prefers plants of the Fabaceae family. It was recorded on soybean, *Phaseolus radiatus* (L.) R. Wilczek, *Phaseolus calcaratus* (Thunb.) Ohwi & H. Ohashi, *Phaseolus sublobatus* Roxb., *Cajanus cajan* (L.) Huth, *Phaseolus vulgaris*, *Phaseolus mungo* (L.) Hepper, *Indigofera suffruticosa* Mill., *Indigofera sumatrana* Gaertn., *Melilotus officinalis* (L.) Pall., *Medicago sativa* L., *Medicago polymorpha* L., *Aeschynomene indica* L., *Flemingia* sp., *Swainsona galegifolia* (Andrews) R.Br., *Crotalaria juncea* L., *Pisum sativum* L., *Dolichos biflorus* (Muthira), *Vigna radiata* (L.) R. Wilczek, *Vigna angularis* (Willd.) Ohwi & H. Ohashi, *Cajanus* sp. (van der Goot 1984; Spencer 1990; Dempewolf 2004; Thapa 2012), chickpea, *Tithonia tubaeformis* (Jacq.) Cass. (Asteraceae), *Lens culinaris* (Vera *et al.* 2021; Fadda *et al.* 2023) and *Trifolium resupinatum* L. (Ferreira *et al.* 2020).

*Melanagromyza sojae* typically overwinters as pupae inside dead soybean stems, but in the northern Brazilian states, which are hotter and drier than Rio Grande do Sul, active larvae have been found inside volunteer soybean plants (plants originating from the remaining grains after harvest) as late as May (Czepak *et al.* 2018) and in host plants such as *T. resupinatum*, allowing *M. sojae* populations to survive winter conditions and hit the summer cropping season at production-threatening levels (Ferrerira *et al.* 2020). This scenario is known as green bridge, whereby overlapping crops make food resources available for polyphagous insects (such as *M. sojae*) throughout the whole year (Oliveira *et al.* 2014). There is evidence of *M. sojae* in various ecoclimatic zones on a global scale and in extensive areas planted with Fabaceae family members in the Americas (Fadda *et al.* 2023). These findings suggest that this pest has the potential to establish in a large area having the right environmental conditions throughout the continent. Fadda *et al.* (2023) identified suitable areas in South America based on a set of abiotic conditions (i.e., the ecological niche) under which the species can maintain its populations. From this analysis, the highest suitability in South America exists across the southern states of Brazil, Paraguay, central Bolivia, and northwestern Argentina. For Argentina, most of the suitable areas predicted in the models coincide with grain-producing areas in this country, so *M. sojae* could be distributed even more widely than predicted by the models.

Further, detection of this pest is often compromised by its small size and lack of external injury symptoms on the plant. *Melanagromyza sojae* has

a short lifecycle with a high oviposition rate that enables infesting populations to complete at least five generations per crop cycle (Pozebon *et al.* 2021).

Indeed, *M. sojae* has long been a problem in soybean crops in many regions of the world. Pozebon *et al.* (2021) mentions that infestation outbreaks of *M. sojae* have a positive correlation with hot, dry weather conditions. Gençer (2009) mentioned that many factors induce agromyzid leafminer outbreaks, but the loss of natural enemies due to widespread use of insecticides is one of the most influential contributors. In Brazil, infestation outbreaks have so far remained restricted to second-season soybean. In soybean-growing regions of Asia, however, early-season infestations by *M. sojae* have long been reported, with subsequent peaks in larval activity occurring periodically until the end of the hot season. In this context, research exploring El Niño impacts mentions that dry and hot years can favor the expansion of insects towards new regions (França *et al.* 2020). These impacts on biodiversity and related ecosystem functions could explain the infestation outbreaks of *M. sojae* detected in different regions of South America.

In northern Argentina, chickpea production has gained importance in recent seasons as a winter crop, and as a host it was recently detected for *M. sojae*, allowing the population development of this pest in the winter-summer sequence. The objective of this study was to determine population fluctuations of *M. sojae* in soybean and chickpea crops using adults caught in yellow sticky traps and by sampling injured plants. The distribution of *M. sojae* in Tucumán province and an update of its geographical distribution in Argentina is also provided here.

## Materials and Methods

### Sampling sites

Sampling of *M. sojae* was made in chickpea and soybean crops in San Agustín (−26.8246; −6.8576) county (Tucumán province, Argentina). The region has an average annual temperature ranging from 8.0 to 22°C and experiences mild winters. The rainfall regime is of the monsoon type, which conditions a marked seasonality of the rains from November to April. The average annual precipitation is 600 mm. In Table 1, climatic data for San Agustín during the periods evaluated in this study are shown (Solue-Gómez *et al.* 2021).

The fluctuations of adults were evaluated from May 2020 to May 2022 and infestation levels were evaluated from June to November of 2020 and 2021 in chickpea and from December to April of 2020/21 and 2021/22 in soybean growing seasons.

**Table 1.** Monthly average of maximum and minimum temperatures and total precipitation from May 2020 to May 2022 in San Agustín county (Tucumán province, Argentina)

Months	Maximum temperature [°C]			Minimum temperature [°C]			Precipitation [mm]		
	2020	2021	2022	2020	2021	2022	2020	2021	2022
January	–	32.25	35.15	–	18.59	20.45	–	74.4	91.93
February	–	29.36	31.03	–	18.04	17.55	–	156.96	81.29
March	–	27.19	27.69	–	17.61	14.92	–	78.97	84.56
April	–	26.85	25.5	–	15.5	12.8	–	34.28	76.45
May	24.13	22.84	22.05	7.22	8.31	8.14	0.25	39.09	2.29
June	21.32	19.3	–	5.87	4.59	–	4.06	14.44	–
July	19.41	22.28	–	2.22	3.08	–	0	0	–
August	24.41	25.29	–	4.99	4.62	–	0	0	–
September	28.11	28.09	–	8.93	10.49	–	0	1.53	–
October	30.55	31.57	–	13.92	12.61	–	10.66	1.27	–
November	32.2	30.59	–	16.84	15.95	–	113.03	86.62	–
December	33.55	33.53	–	17.77	20.58	–	95.49	77.46	–

## Population fluctuations

The area of study was approximately 0.25 ha. To evaluate adult fluctuations during the years, yellow sticky traps 20 × 25 cm were used according to Walczak and Roik (2010) and Allan *et al.* (2020). The traps were attached to stakes so that they were directly above the crops and were placed at each vertex of the plot. Four traps were used per week and they were replaced once a week. A total of 408 traps were used during this study.

To determine the infestation level in the area, in each crop and in the different years evaluated, 40 plants of each crop were selected at random from early vegetative to reproductive stages (until harvest) weekly.

It is important to note that in both research years, the predecessor crop of chickpea was soybean. This situation promoted volunteer soybean plants which originated from harvest losses that grew together with chickpea plants. As afore mentioned, this situation facilitates the maintenance and dispersion of *M. sojae* (Czepak *et al.* 2018; Ferreira *et al.* 2020; Fadda *et al.* 2023). Thus, for evaluation, 40 volunteer plants of soybean were sampled during 11 and 5 weeks in 2020 and 2021, respectively.

Growth stages of chickpea and soybean crops were determined based on the descriptions of Soltani *et al.* (2006) and Fehr *et al.* (1971), respectively. All samplings were made once a week during all phenological stages of both crops.

The yellow sticky traps and plants were taken to a laboratory to identify insects. The adults caught in the traps were observed with a binocular microscope (Zeiss model Stemi DV 4) equipped with

a 40× micrometer. The plants were checked for the presence of immature stages (larvae or pupae) through a longitudinal cut of the main stem and secondary branches. Through these cuts, it was possible to observe the presence of the galleries where the larvae and/or pupae of *M. sojae* were located. Regarding the gallery length forming in parts or their entire extension by advance of the larvae, Curioletti *et al.* (2018) mentioned that the gallery length measures the intensity of *M. sojae* injury. The larvae were preserved inside the stem in a glass tube until pupation. Pupae were then placed in Petri dishes with filter paper until the emergence of adults in chambers under controlled conditions (27 ± 2°C, 70–75% relative humidity, 14 : 10 h L : D photoperiod). For the identification of *M. sojae*, the immature stages were examined according to the arrangement of the posterior spiracles (Dempewolf 2004; Vera *et al.* 2021) and in the case of adults, the genitalia and wings of the males were examined (Spencer 1973; Vera *et al.* 2021).

The parameters recorded were: average number of adults caught in the yellow sticky traps, number of injured plants, number of immature stages (larvae and pupae), infestation level (percentage of injured plant) and the length of the gallery.

## Statistical analysis

An initial analysis was made to test for the normality of the data using the Shapiro-Wilk's test. Fluctuation differences of adults between years were determined by the Kruskal and Wallis (1952) test ( $p < 0.05$ ) when data were not normally distributed. The percent of injured plants, number of insects in immature stages,

and length of gallery in chickpea and soybean crops were analyzed using the Wilcoxon test for independent samples to detect differences between both crops as well as the percent of injured plants between volunteer soybean and chickpea plants. To evaluate the injured plants by *M. sojae* recorded in both crops, a  $\chi^2$  test was used. All the analyses were conducted using the software InfoStat version 2015 (Di Rienzo *et al.* 2015).

### Geographical distribution of *Melanagromyza sojae* and its host plants in Argentina

Field surveys to detect the presence of *M. sojae* were conducted as follows. Field monitoring took place in different provinces of Argentina from October 2021 to September 2023. A total of 30 sampling sites was monitored in Tucumán, Santiago del Estero, Chaco, Cordoba, Salta and Catamarca provinces. In each sampling site one hectare was sampled and five or 10 random points were selected. At each point, 10–15 host plants selected at random were monitored according to the methodology described by Fadda *et al.* (2023). The host plants monitored were: *C. arietinum*, *G. max*, *P. vulgaris*, *Vigna radiata*, *Helianthus annuus*, *M. sativa*, *Carthamus tinctorius*, and different weeds surrounding crops widely distributed in northwest Argentina. The crops were monitored during vegetative and/or reproductive stages.

The plants showing injuries were taken to the laboratory and checked for the presence of larvae or pupae through a longitudinal cut of the main stem and secondary branches as described above. For the identification of *M. sojae* pupae and adults, the same methodology mentioned above was used.

To update the presence of *M. sojae* in Argentina, reports of Saluso (2020), Trossero *et al.* (2020), Pozebon *et al.* (2021), Vera *et al.* (2021) and Fadda *et al.* (2023) were taken into account.

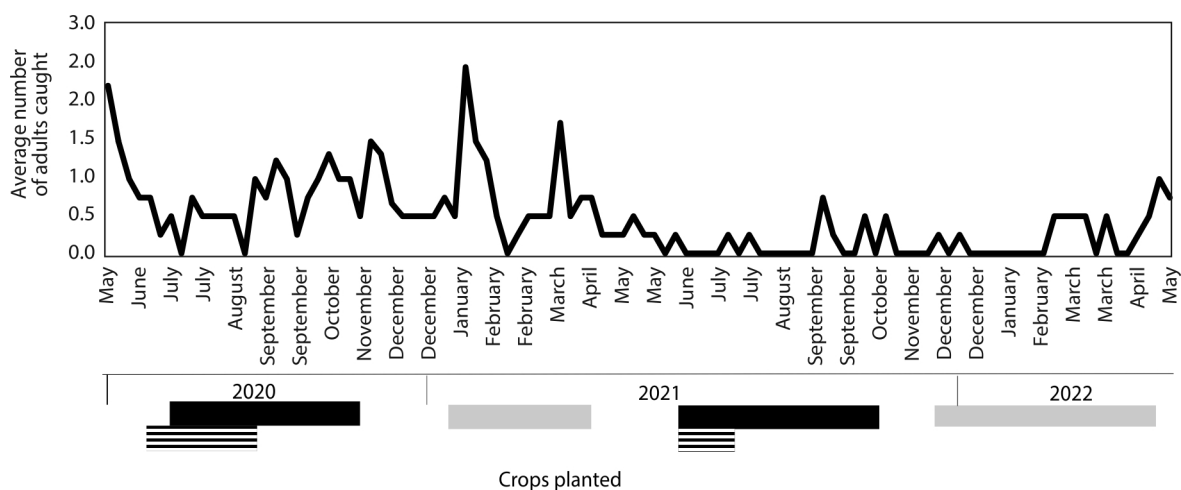
## Results

### Population fluctuations

Population fluctuation of *M. sojae* adults, using yellow sticky traps, from May 2020 to May 2022 is shown in Figure 1. Adults were caught in all the years and during the cycles of both crops, on volunteer soybean plants and at times when the crops were not present.

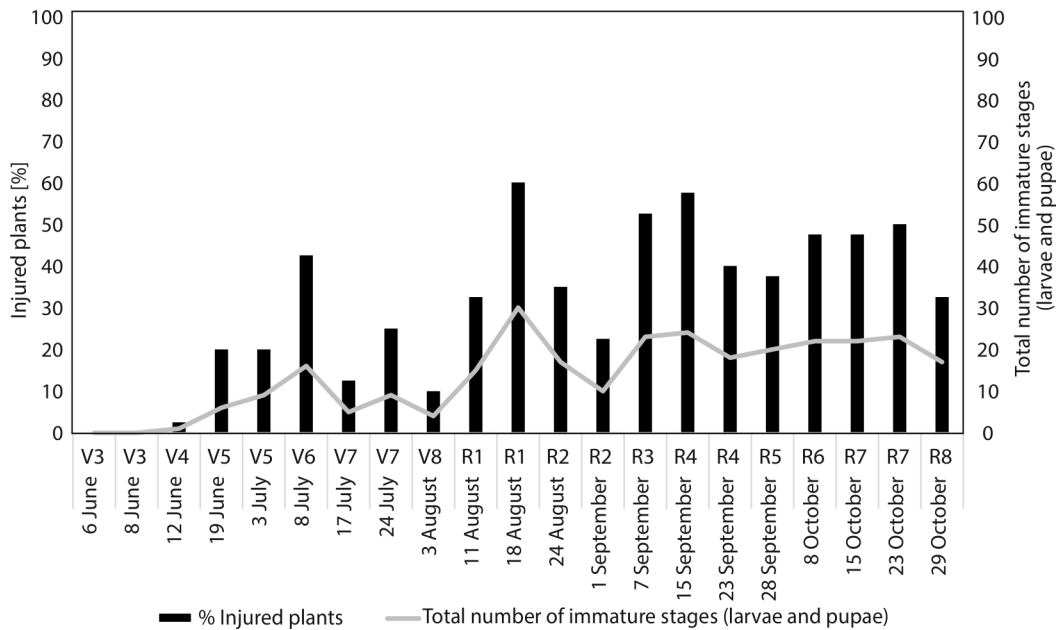
A total of 182 adults were caught in 408 sticky traps. The total average of the number of adults caught was  $2.88 \pm 0.30$  (N: 92),  $1.40 \pm 0.28$  (N: 70) and  $1.00 \pm 0.28$  (N: 20) in 2020, 2021 and 2022, respectively. The difference in the number of adults obtained during both years was statistically significant ( $H = 23.09$ ;  $p < 0.0001$ ).

In the chickpea crop the total number of plants sampled was 1675 of which 529 presented injuries caused by *M. sojae* (Figs 2 and 3). During 2020, the injured plants increased during the reproductive stage. The highest percentage of injured plants (60%) was at the beginning of the flowering stage (R1) (Fig. 2). In 2021, the injured plants were registered at the end of the vegetative stage and increased during the reproductive stage. A peak was observed at the end of the reproductive stage (R8) until the harvest of the crop. The highest percent of injured plants (85%) was observed at the final reproductive stage (R8) (Fig. 3).

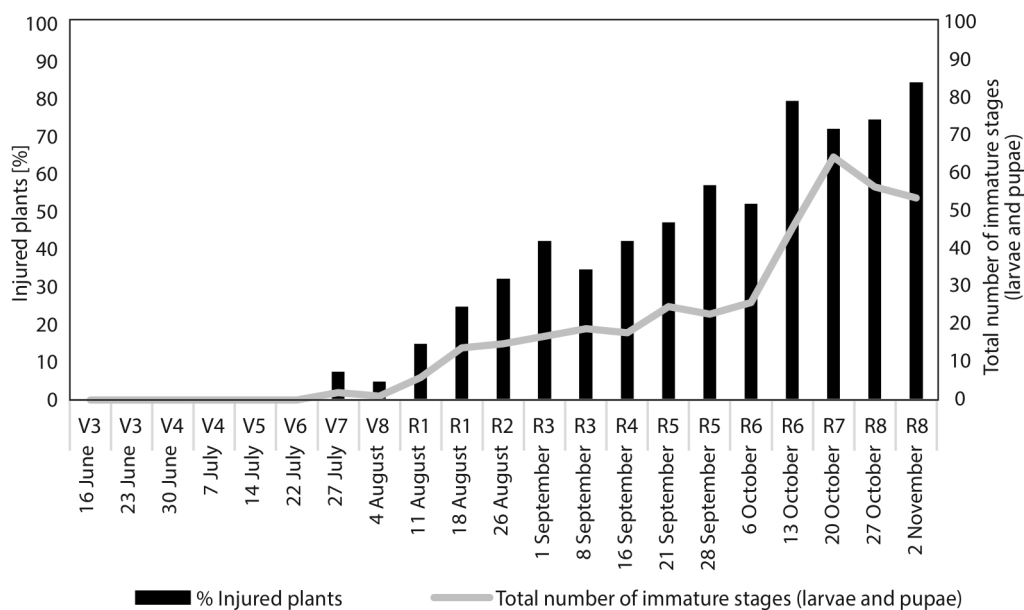


The colored bars on the X-axis represent the crops planted during the study (chickpea: black, soybean: grey, volunteer soybean plants: lined)

**Fig. 1.** Adult fluctuation of *Melanagromyza sojae* using yellow sticky traps, during the growing season of chickpea and soybean crops, on volunteer soybean plants and when both crops were not present in Tucumán, Argentina. The colored bars on the X-axis represent the crops planted during the study. Chickpea (black), soybean (grey) and volunteer soybean plants (lined)



**Fig. 2.** Percent of injured plants and immature stages of *Melanagromyza sojae* in chickpea crop in 2020 in Tucumán, Argentina



**Fig. 3.** Percent of injured plants and immature stages of *Melanagromyza sojae* in chickpea crop in 2021 in Tucumán, Argentina

The percent of injured plants in the chickpea crop in both years was not significant ( $W = 458$ ;  $p = 0.866$ ). The immature stage means (larvae and pupae) obtained were  $13.86 \pm 8.92$  (N: 291) and  $18.48 \pm 20.74$  (N: 388) in 2020 and 2021, respectively.

In 2020, immature stages were registered from early vegetative to the final reproductive stage (R8), and a peak was observed at the beginning of the flowering stage (R1) of the crop (Fig. 2). In 2021, immature stages were detected from advanced vegetative to the final reproductive stage (R8), and a peak was recorded

in the reproductive stage (R7) (Fig. 3). The range of immature stages recorded was 1–30 (2020) and 1–65 (2021). The differences found in the number of immature stages between both years were not significant ( $W = 455$ ;  $p = 0.9296$ ).

The total infestation level in volunteer soybean was 56.25 and 37.5 in 2020 and 2021, respectively. In both years evaluated, the infestation levels in volunteer soybean and chickpea were statistically significant ( $W = 102$ ;  $p = 0.0013$ ). A higher level was registered in volunteer soybean plants.

During 2020/21 and 2021/22 growing seasons in the soybean crop, the total number of plants evaluated was 1120, of which 35 presented injuries caused by *M. sojae*. In both growing seasons, injured plants were registered only in the reproductive stage (Figs 4 and 5). The highest percent of injured plants was in the fully filling pod stage (R6) (17.5%) and in filling pod (R5) (2.5%) during 2020/21 and 2021/22 growing seasons, respectively. In the first growing season, the percent of injured plants began to increase after the flowering

season (R2), reaching the maximum in fully filling pods (R6). It then decreased towards the final reproductive stage (R8) (Fig. 4). The differences in the percent of injured plants found in both growing seasons were significant ( $W = 260; p = 0.0029$ ).

The number of immature stages found was  $1.43 \pm 1.95$  in 2020/2021 (N: 20) and  $0.21 \pm 0.58$  in 2021/2022 (N: 3). In the 2020/21 growing season, immature stages were registered during flowering (R2), increasing towards fully pod filling

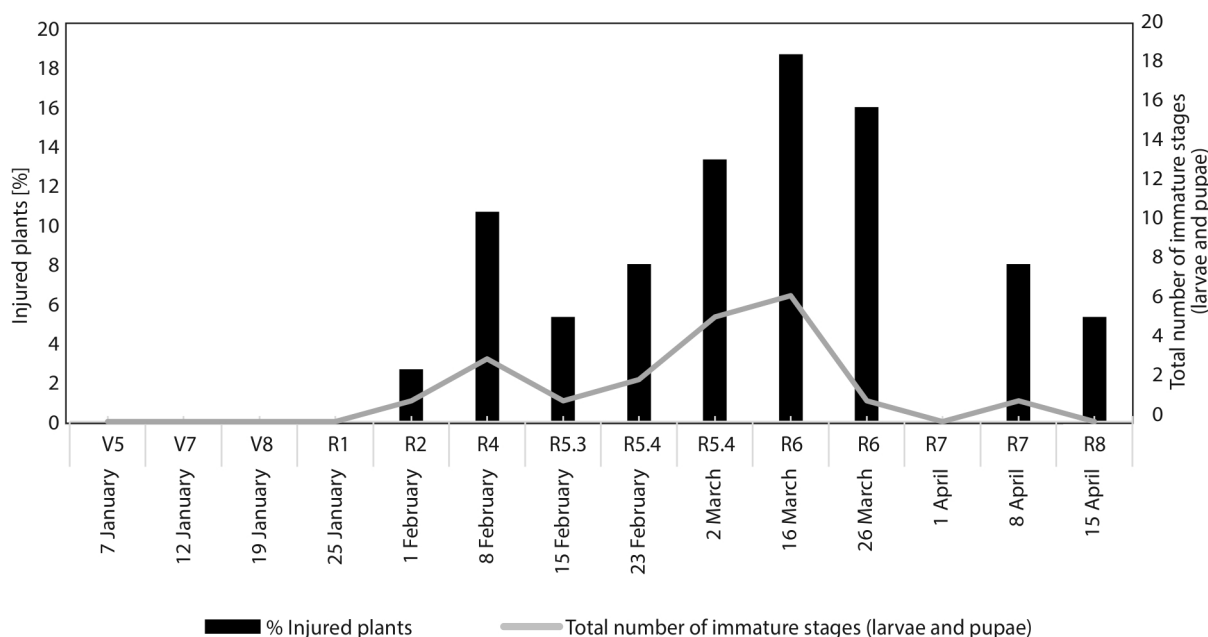


Fig. 4. Percent of injured plants and immature stages of *Melanagromyza sojae* in soybean crop in 2020/2021 growing season, in Tucumán, Argentina

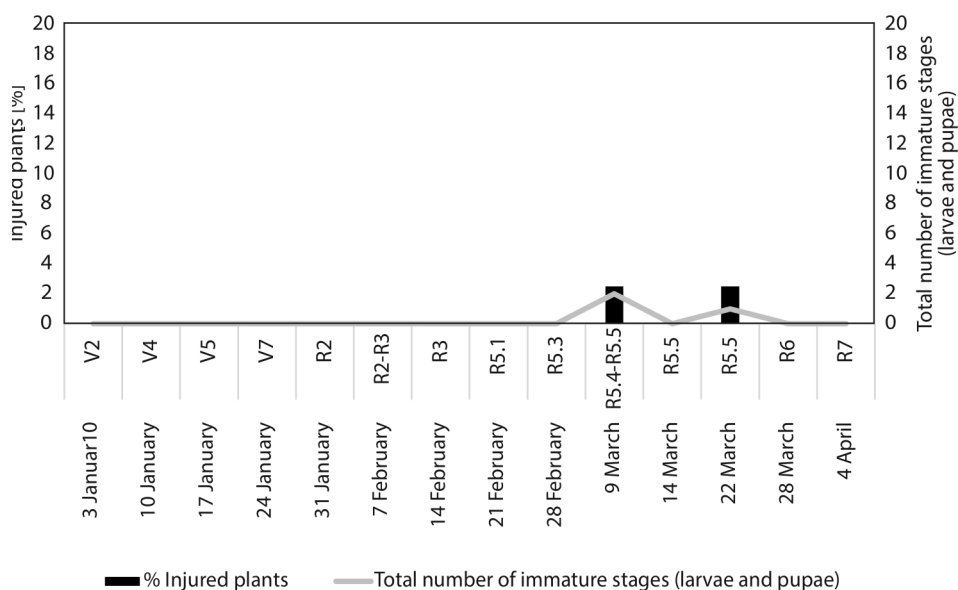


Fig. 5. Percent of injured plants and immature stages of *Melanagromyza sojae* in soybean crop in 2021/2022 growing season, in Tucumán, Argentina

(R6) and then decreasing to the final reproductive stage (R8) (Fig. 4). In the 2021/22 season, immature stages only were observed in the filling pod (R5) (Fig. 5). The range of immature stages recorded was 1–6 (2020/2021) and 1–2 (2021/2022). The differences found in the number of immature stages between both years were significant ( $W = 246,5$ ;  $p = 0.0192$ ).

The total number of injured plants recorded in both crops was compared by  $\chi^2$  test and the differences found were significant. The numbers of injured chickpea plants ( $31.49 \pm 24.71$ ) were higher than injured soybean plants ( $3.13 \pm 5.07$ ) (test  $\chi^2$ ;  $\chi^2 = 335.8$ ;  $gl = 1$ ;  $p < 0,001$ ).

The gallery length formed by advance of the larvae, detected in both crops, showed differences ( $W = 102$ ;  $p = 0.0013$ ). The chickpea gallery ( $21.41 \pm 3.42$  cm) was longer than the soybean gallery ( $8.47 \pm 1.05$  cm).

### Geographical distribution of *Meanagromyza sojae* and its host plants in Argentina

In Argentina, from the 30 surveys conducted from October 2021 to September 2023, *M. sojae* was found in all localities from Tucumán and in two localities of Santiago del Estero and Catamarca, one locality of Cordoba, Chaco, and Salta provinces. Some of these

**Table 2.** Presence of *Melanagromyza sojae* in Argentina. New records (localities and/or host plants)

State	Sampling site	Longitude	Latitude	Host plant	Month/Year
	San Agustín	-26.8249	-64.8577	<i>Heliotropium</i> sp.**	Nov 2021
	San Agustín	-26.8249	-64.8577	<i>Glandularia</i> sp.**	Nov 2021
	Vipos*	-26.4724	-65.3083	<i>Medicago sativa</i> **	Nov 2021
	Las Cejas	-26.8634	-64.7762	<i>Medicago sativa</i>	Nov 2021
	Garmendia	-26.6351	-64.5918	<i>Parthenium</i> sp.**	Feb 2022
	Garmendia	-26.6350	-64.5916	<i>Heliotropium</i> sp.	Feb 2022
	San Agustín***	-26.8229	-64.8599	<i>Phaseolus vulgaris</i> ***	Apr 2022
	San Agustín	-26.8230	-64.8599	<i>Vigna radiata</i> **	Apr 2022
	San Agustín***	-26.8255	-64.8576	<i>Cicer arietinum</i> ***	Aug 2022
	La Ramada***	-26.6993	-64.9515	<i>Cicer arietinum</i> ***	Aug 2022
	Villa Benjamín Aráoz***	-26.6044	-64.7568	<i>Cicer arietinum</i> ***	Aug 2022
Tucumán	La Virginia***	-26.7288	-64.7746	<i>Cicer arietinum</i> ***	Aug 2022
	La Cocha***	-27.7521	-65.5381	<i>Cicer arietinum</i> ***	Aug 2022
	Rumi Punco***	-27.9577	-65.5656	<i>Cicer arietinum</i> ***	Aug 2022
	La Cruz***	-26.6414	-64.8379	<i>Cicer arietinum</i> ***	Aug 2022
	El Azul***	-26.4729	-64.6391	<i>Cicer arietinum</i> ***	Aug 2022
	Garmendia***	-26.5593	-64.5933	<i>Cicer arietinum</i> ***	Aug 2022
	Los Pereyra***	-26.9301	-64.8147	<i>Cicer arietinum</i> ***	Aug 2022
	Finca Mayo*	-26.8569	-64.9970	<i>Mellilothus</i> sp.**	Sep 2022
	El Colmenar*	-26.7876	-65.1943	<i>Medicago</i> sp.	Sep 2022
	Mujer Muerta***	-27.2438	-64.8821	<i>Cicer arietinum</i> ***	Sep 2022
	Tacanas***	-27.1045	-64.7888	<i>Cicer arietinum</i> ***	Oct 2022
	San Agustín	-26.8226	-64.8556	<i>Helianthus annuus</i> **	Jan 2023
	Quimilí*	-27.5436	-62.4875	<i>Glycine max</i>	May 2022
Santiago del Estero	San Pedro de Guasayán*	-27.9298	-65.1702	<i>Glycine max</i>	Apr 2023
Córdoba	Río Primero*	-30.9819	-63.6234	<i>Carthamus tinctorius</i> **	Oct 2022
Chaco	General Capdevila*	-27.3978	-61.4103	<i>Glycine max</i>	May 2022
Salta	Chaguaral*	-24.0572	-63.9355	<i>Carthamus tinctorius</i>	Sep 2023
	El Abra***	-28,0753	-65,4365	<i>Cicer arietinum</i> ***	Sep 2022
Catamarca	Mistol ancho*	-26.0703	-65.4435	<i>Glycine max</i>	Sep 2023

\* new locality catalogued as new record for *M. sojae*

\*\* new host plant catalogued as new record for *M. sojae*

\*\*\* record of locality and plant host for *M. sojae* already recorded by Fadda et al. (2023)

localities were catalogued as new records for these provinces (Table 2).

From our field collections, *M. sojae* was recorded on chickpea, soybean, dry bean, mung bean, sunflower, alfalfa, safflower, and different weeds such as *Heliotropium* sp., *Glandularia* sp., *Parthenium* sp. and *Melilothus* sp. It is important to highlight that many of the records of *M. sojae* in soybean and chickpea in several locations registered during this study had already been reported by Fadda *et al.* (2023).

Including the reports of Saluso (2020), Trossero *et al.* (2020), Pozebon *et al.* (2021), Vera *et al.* (2021), Fadda *et al.* (2023) and our own collections, *M. sojae* was recorded in eight provinces (Tucumán, Santiago del Estero, Salta, Catamarca, Cordoba, Chaco, Santa Fe and Entre Ríos), 60 counties and on 13 host plants including *Tithonia tubaeformis* and *Lens culinaris* mentioned by Fadda *et al.* (2023) in Argentina.

## Discussion

This study reports for the first time the population dynamics of *M. sojae* in chickpea and soybean crops in Tucumán province, Argentina. Adults of this species were caught in multiple years, during the cycles of both soybean and chickpea crops, on volunteer soybean plants, and when the crops were not present. Injuries caused by *M. sojae* occurred throughout the growing period of chickpea and reproductive stages of soybean during both years and seasons evaluated, respectively. The highest infestation level was registered in chickpea crops, while in soybean crops it was very low. Our results revealed that *M. sojae* is a pest on chickpea growing areas in Argentina where chickpea is one of the most important pulse crops and, like other legumes, contains high a proportion of protein. According to Vera *et al.* (2021), the potential of *M. sojae* as a pest of chickpea crops has been demonstrated. Chickpea growing seasons could increase the chances of survival of *M. sojae*, facilitating its establishment and becoming widespread in other agricultural areas. This combination of crops and their volunteer plants originating from lost seeds contribute to dissemination of this pest in Argentina.

Adults were caught by yellow sticky traps in both years. The largest number of adults was caught when soybean was present in the first season (2020/2021). Similar observations were mentioned by Guedes *et al.* (2017), who detected a high presence of *M. sojae* adults in second season soybean. Similar to Walczak and Roik (2010), our results demonstrated that the use of yellow sticky traps was a good tool to monitor the adult fluctuation of agromyzid leafminers.

In both years evaluated, the predecessor crop of chickpea was soybean, promoting volunteer soybean plants that originated from harvest losses and grew together with chickpea plants. In this situation, *M. sojae* can complete its developmental cycle on volunteer soybean plants, and its dissemination is favored by the maintenance of these plants (Fig. 1) (Czepak *et al.* 2018; Ferreira *et al.* 2020; Fadda *et al.* 2023). This scenario is known as green bridge, whereby overlapping crops make food resources available for polyphagous insects (such as *M. sojae*) throughout the whole year (Oliveira *et al.* 2014). Because of this, the presence of damaged chickpea plants and immature stages (larvae and pupae) was observed after the winter frost that caused the death of volunteer soybean plants, allowing the transfer of this pest to chickpea plants. In our study, the total infestation percentage of volunteer soybean plants registered was 63.86 and 37.5 in 2020 and 2021, respectively. Czepak *et al.* (2018) reported that approximately 100% of the volunteer soybean plants showed damage from *M. sojae*.

The appearance of *M. sojae* coincided with the end of the vegetative stage and increased during the reproductive stage until the harvest of the chickpea crop. In both years, damaged plants and immature stages were found and the infestation percentage found was lower than that reported by Vera *et al.* (2021). According to Ferreira *et al.* (2020) and Fadda *et al.* (2023), when the infestation percentage of volunteer soybean and chickpea plants was compared, the higher percentage was registered in volunteer soybean plants. According to Ferreira *et al.* (2020) and Fadda *et al.* (2023), the eradication of post-harvest volunteer plants and the use of winter crops not suitable as hosts to *M. sojae* should be prioritized by soybean growers to avoid injury.

In the case of soybean crops, damaged plants and immature stages were registered only in the reproductive stage in both growing seasons. The mean infestation level was 6.26 and 0.36% in 2020/21 and 2021/22 growing seasons, respectively. Fand *et al.* (2017) reported that the infestations of this pest remained at relatively low to moderate levels and ranged between 4.32 and 38.13%. The peak of this species coincided with the peak flowering (R2) and pod formation (R3) stages of the crop. However, our results are not congruent with those of other authors who reported infestation levels that ranged between 10.87 to 100% for soybean crops (Singh and Singh 1990; Van den Berg *et al.* 1998; Jadhav *et al.* 2013; Gaur *et al.* 2015; Guedes *et al.* 2017; Czepak *et al.* 2018; Vitorio *et al.* 2019). Guedes *et al.* (2017) reported a moderate infestation level of *M. sojae* in the early sowing but this was high in the late growing season, reaching 100% of attacked plants in most fields in Paraguay. In Bolivia, the infestation level registered was 20–25% and 70% of early and late



sowing seasons of soybean crops, respectively (Vitorio *et al.* 2019). In Brazil, high infestation levels (95–100%) in soybean fields were observed (Pozebon *et al.* 2021). These deviances in infestation levels of *M. sojae* may be due to differences in local climatic conditions, soybean varieties grown, agronomic practices and greater diversity of legumes (Fand *et al.* 2017). In this context, Leibe (1984) evaluated the influence of temperature on development and fecundity of another species of Agromyzidae (*Liriomyza trifolii*). He found that the pupal survival was very low (9.4%) at 35°C compared to lower temperatures. Maximum oviposition rate (38.67 eggs per female per day) and fecundity (405.67 eggs per female) were attained at 30°C. Low oviposition rates and fecundity at 15°C indicated that this temperature was near the threshold of activity for the adult. According to Leibe (1984), the minimal population growth of *M. sojae* was recorded during January and February. In 2022 in our study, average maximum temperatures above 30°C may have negatively influenced the reproductive parameters of the pest. Additionally, it is important to explore El Niño impacts in the region (França *et al.* 2020) that could explain the infestation outbreaks of *M. sojae* detected in different regions of South America.

In our study, the gallery length was longer in chickpea than soybean. This observation coincides with the high levels of infestation detected in chickpea crops. Gyawali (2002) demonstrated direct correlation of gallery length with its negative impact on soybean yield. This record raises the hypothesis that the differences found between gallery length of chickpea and soybean produced by *M. sojae* could be related to the chickpea plant morphology (Ateca and Beltrami 2016). Chickpea is a small herbaceous plant with strong, deep roots, erect and hairy stems that can reach a height of about 60 cm, whose diameter is from 1.5 to 2 mm. The characteristics of the stems would favor the feeding of larvae.

Regarding the distribution of *M. sojae*, this study also provided updated information on the sites, with new records in Argentina on new localities and host plants. In congruence with Fadda *et al.* (2023), these results indicate that this species is expanding its distribution in Argentina. *Helianthus annuus*, *Carthamus tinctorius*, and weeds such as *Heliotropium* sp., *Glandularia* sp., and *Parthenium* sp. have been reported for the first time as new host plants for Argentina and globally, including the Americas. For *Medicago sativa*, *Vigna radiata*, and *Mellilotus* sp., there are new host plant records of *M. sojae* in Argentina.

The presence of *M. sojae* in Chaco province was also reported for the first time. This record of *M. sojae* in Chaco province was reported by Fadda *et al.* (2023), where the models selected showed optimal conditions for the establishment of *M. sojae* in provinces from the

north and center of the country including Jujuy and Salta, western Chaco, Tucumán, Santiago del Estero, Córdoba, southern Santa Fe, Entre Ríos, and Buenos Aires. Most of these areas predicted in both models coincide with grain-producing areas in Argentina. It is important to note that the absence of this species in other regions of Argentina may not necessarily be related to the accessibility of the area, but more likely due to its complex biology and endophytic behavior which makes detection, capture, and monitoring difficult (Guedes *et al.* 2017; Czepak *et al.* 2018; Ferreira *et al.* 2020; Pozebon *et al.* 2021; Vera *et al.* 2021; Fadda *et al.* 2023).

There are few studies on the bioecology, life cycle, crop damage, chemical control, natural enemies, host plants, or oviposition preference of *M. sojae*. Our study is the first to provide information about seasonal distribution of *M. sojae* in Argentina through adults and immature stages, and infestation levels during the growing seasons of chickpea and soybean crops. The highest percentage of infestation registered in chickpea crops shows that *M. sojae* is a pest on chickpea growing areas in Argentina where this pest is widely distributed. As aforementioned, chickpea growing seasons would increase the chances of survival of *M. sojae*, facilitating its establishment and becoming widespread in other agricultural areas. The combination of these two crops and their volunteer plants originating from lost seeds contribute to the spread of this pest in Argentina. Thus, effective control methods to research and to recommend efficient management strategies and control methods for *M. sojae* in chickpea must be developed.

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