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# The role of genetically engineered soybean and *Amaranthus* weeds on biological and reproductive parameters of *Spodoptera cosmioides* (Lepidoptera: Noctuidae)

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# ABSTRACT

**BACKGROUND:** In soybean fields containing insect and herbicide resistant genetically engineered varieties, some weed species have increasingly become difficult to be managed and may favor the population growth of secondary pests like *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidae). To test this hypothesis, we measured life-history traits, population growth parameters, and adult nutrient content of *S. cosmioides* reared on the foliage of four *Amaranthus* species, of Cry1Ac Bt and non-Bt soybean varieties, and on meridic artificial diet.

**RESULTS:** Larvae reared on *A. palmeri* and *A. spinosus* had a shorter development time (5–7 days) than on the soybean varieties and *A. hybridus*. The armyworm survival probability was zero on *A. viridis* and highest (80 and 71%) on soybeans and *A. palmeri*. This latter and the artificial diet produced the heaviest larvae and pupae, contrariwise the non-Bt soybean variety. Body nutrient content diverged mostly for the artificial diet reared adults in relation to those on the soybean varieties. The intrinsic rate of population increase (i.e., overall fitness) was 27.88% higher for the armyworms on *A. palmeri*, Cry1Ac Bt soybean, and artificial diet than on non-Bt soybean, *A. spinosus*, and *A. hybridus*.

**CONCLUSIONS:** Cry1Ac soybean fields infested by some *Amaranthus* weeds, especially *A. palmeri,* are conducive for the population growth of *S. cosmioides*. Integrated Pest Management programs may be needed to properly manage *S. cosmioides* in soybean fields, with surveillance for population peaks and judicious control measures when needed.

**Keywords**: Black armyworm, GM soybean, host quality, agricultural landscape, life-history traits, adult nutrient content.

# 1 INTRODUCTION

Soybean, *Glycine max* (L.) Merrill, is one of the most important agricultural crops in South America.[1] Genetically modified soybean has been available in Argentina, Brazil, Paraguay and Uruguay since 2013.[1, 2] This technology combines the transformation events MON 87701 (expressing the Cry1Ac protein from the bacterium *Bacillus thuringiensis* [Berliner]) and MON 89788 (glyphosate tolerance),[2] and is provided to growers as high-yield cultivars.[3] However, the adoption of these varieties in vast scale has selected herbicide-resistant weeds, which has increased the need for applications of herbicide with different mechanisms of action and alternative weed control measures.

Weeds compete with crops for water, light, nutrients and space, and they can act as a reservoir for insect pests and pathogens. *Amaranthus hybridus* L. (Caryophyllales: Amaranthaceae) is considered the most problematic weed of recent times in Argentina due to its high resistance to conventional herbicides. The introduction of *A. palmeri* S. Watson was officially reported in 2013,[4, 5] apparently due to contamination from seeds imported from the USA.[6] This species may have already been present in Argentina since 2004, being confused with the native species *A. hybridus*.[5] In the last years, the expansion of *A. palmeri* was relatively rapid, with most populations resistant to glyphosate and acetolactate synthase inhibiting herbicides.[4, 7] In consequence, *A. palmeri* is currently considered as an invasive weed, which requires a more complex and expensive combination of herbicides for its effective control.[4] This scenario where herbicide-resistant-weeds and a monoculture are present may favor increase in the population of insect pests.

Cry1Ac Bt soybean is effective to control populations of noctuid and erebid pest species, including *Anticarsia gemmatalis* (Hübner), *Chrysodeixis includens* (Walker), *Helicoverpa gelotopoeon* (Dyar), *Rachiplusia nu* (Gueneé), and other economically less important species. The high level of control efficacy against these target species generally requiring less use of broad-spectrum insecticides may have favored the occurrence of non-target pests of Bt toxins. Thus, ecological niches that were not accessible, because of interspecific competition or the application of

synthetic insecticides, are now occupied by other phytophagous species that tend to increase their population levels and may become economically important pests. For instance, the abundance of armyworms of the *Spodoptera* Guenée genus in soybean crops has increased in the last years, and this change coincides with the greater use of Bt varieties.[8-10] The black armyworm, *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidae), is a frequently encountered species occurring in several countries in South America, such as Brazil, Paraguay, and Argentina.[9-12] This polyphagous noctuid presents a high leaf consumption rate causing damage to economically important crops and weeds.[9, 13, 14] The most updated list of host plants for *S. cosmioides* includes 126 plants of 40 families, many of which are considered weeds.[15] In Argentina, *S. cosmioides* is commonly known as pigweed caterpillar, as a reference to its association with weeds belonging to the *Amaranthus* L. genus, which would reflect particular host specificity.

Nutrients in food sources play a key role in the survival and fitness of phytophagous insects, and balanced consumption of macronutrients (proteins, carbohydrates and lipids) can improve their developmental and biological parameters as well as their immune system and reproductive capacity.[16-18] The need to fulfill nutritional requirements can condition the behavior and mobility of insects in the field [19] and determine host preference. In cases where larvae feed on a substrate that is not nutritionally optimal, the life cycle can be longer, with reduced survival, weight gain, weight, and fecundity.[20] The energy transfer by larvae to adults can be reflected in the amount of macronutrients stored in the pupal or adult body, as has been studied in other species of lepidopteran pests and vector insects.[21, 22] Furthermore, the quality of the food consumed by the adult stage can also influence its performance.[23, 24] Although host effect on development, reproduction and building up of reserves is more visible in monophagous insects, there are several studies with polyphagous insects, including *S. cosmioides*, which show the impact of host species on insect survival, biology and reproductive capacity and, in consequence, hosts can be sorted into different categories.[15-19, 25-29]

Survivorship, development, and fecundity are fundamental parameters affecting the insect population dynamics. Measuring key life-history traits such as survival rate, growth, and reproduction

allow to estimate the potential population growth of a herbivorous species on host plants, helping understand the population dynamics of agricultural insect pests.[30] Several studies have evaluated the suitability of different host species and their contribution to the population growth of pests, such as *Helicoverpa armigera* (Hübner), *H. zea* (Boddie) and *Plutella xylostella* (L.).[31, 32] For the *Spodoptera* genus, many studies analyzed the impact of different host species to determine host-plant quality and even predict the potential population growth.[15, 19, 25, 27-29, 33-36] However, these studies have focused only on the host agricultural crops, neglecting weeds that are often present in the soybean agroecosystem. Only Zamora-Belli et al.[28] compared the development of *S. cosmioides* larvae reared on Bt soybean and its main host, *Amaranthus* sp., but neither the insect reproductive parameters nor the weed species identification were provided in the study.

The large-scale adoption of Bt crop varieties makes fundamental the analysis of the role of the "Bt soybean–refuge–weed" agricultural scenario in order to better understand how different host-plant species can impact the life history and population fitness of a pest species. In this study, we determined the life-history traits and estimated population growth parameters of *S. cosmioides*, on four *Amaranthus* species, Cry1Ac Bt and non-Bt soybean varieties, and a meridic artificial diet. Additionally, we investigated the moths' nutrient content as an indicator of the host-plant quality larvae fed on. Because *S. cosmioides* is a highly polyphagous species, we hypothesized that the larvae would grow and develop adequately on the different host plants although with a variable population fitness depending on the host plant species.

## 2 MATERIAL AND METHODS

### 2.1 *Spodoptera cosmioides* population

Approximately 250 larvae were collected in soybean commercial fields with high infestation of *Amaranthus* weeds in Villa Benjamín Aráoz, Tucumán, Argentina (26°38'46.2"S 64°42'21.5"W). The larvae were brought to the laboratory, placed in Petri dishes with artificial diet [37] consisting of soybean flour, wheat germ and yeast, and maintained under controlled conditions (25 ± 2°C, 14:10 h light: dark and no humidity control) until pupation. The emerged adults, 25 females and 25 males per

cage, were placed in cylindrical polyvinyl chloride oviposition cages (40 cm high x 25 cm diameter) covered with a transparent plastic film. The internal cage walls were covered with sulphite paper as oviposition substrate. A piece of cotton soaked in a solution of 10% honey and sugar (with the addition of ascorbic acid, nipagin and vitamin E) was used to feed the adults. The cages were inspected daily to collect the egg masses, which were placed in a Petri dish and checked daily until hatching.

## 2.2 Rearing substrates

Seven rearing larval substrates were evaluated: four weed species belonging to *Amaranthus* genus (*A. palmeri*, *A. hybridus*, *A. spinosus* and *A. viridis*), two soybeans varieties (one expressing the Cry1Ac protein, DM 60i62 variety, which is referred here to as Bt soybean; and other non-Bt soybean, NS 8282 variety, being both resistant to glyphosate and widely used in the region) and one artificial diet. The artificial diet used for the bioassays was the same as that used for the larval rearing (see section 2.1). *Amaranthus* species and soybean varieties were sown in the experimental field from Facultad de Agronomia y Zootecnia, Universidad Nacional de Tucuman (FAZ – UNT; 26°50'20.6"S 65°16'26.1"W). The plants were grown without the application of fertilizer, insecticides and fungicides throughout all the vegetative and reproductive stages. Only one herbicide application (glyphosate 2 L ha$^{-1}$) was made during the vegetative stage to keep the experimental field free of other weed species. Voucher samples were deposited in the laboratory of Cátedra Terapéutica Vegetal, FAZ – UNT, Tucuman, Argentina.

## 2.3 Developmental and biological parameters

Neonate larvae (24 h after hatching) from the F1 generation were used to perform experiments. Two larvae were placed with a fine brush in a glass assay tube (12 cm x 1.5 cm; length x diameter) on either a piece of leaf (4 cm x 4 cm) or a piece of artificial diet (1.5 x 1.5 x 1 cm) depending on the corresponding substrate. The assay tubes were covered with a cotton plug and maintained under previously described conditions. Leaves were cut from the plant at the time of bioassay and a drop of water was placed inside the tube to conserve humidity. A total of 100 larvae

per rearing substrate were evaluated during the entire life cycle. Leaves were provided *ad libitum* and replaced every 48 h in order to maintain them fresh; the artificial diet was also provided *ad libitum* and replaced when necessary. When the larvae reached 1.5 cm in length, they were transferred to Petri dishes with one larva per Petri dish. The following response variables were recorded: larval survival (recorded every 48 h), duration of egg, larva, pupa, and adult stages, and the whole life span (the time elapsed from egg to adult); larval weights at 7, 15 and 21 days; pupal weights after 48 h pupal formation and sex ratio. Larval and pupal weights were measured using a high precision analytical balance (KERN ACS 220-4). Duration of egg stage was estimated using data recorded from egg masses collected from female adults obtained from this experiment and used to calculate the reproductive and population growth parameters (see below).

## 2.4 Reproductive and population growth parameters

To determine parameters related to population growth potential, several reproductive traits were measured on adults recovered from larvae reared on the different substrates. Sex of adults was determined at the pupal stage, according to the shape and position of genital openings. Then, male and female pupae were paired for each substrate (treatment). Number of pairs evaluated depended on availability of moths. In the case of *A. viridis*, we were not able to obtain pupae due to large delay in development time and the concomitant lack of available substrate. The pairs were placed inside plastic cages (15 cm high x 10 cm in diameter) covered on top with a transparent plastic film held with a rubber band. The internal cage walls were covered using sulphite paper as oviposition substrate. A piece of cotton soaked in a solution of 10% honey and sugar (as previously described) was used to feed adults. Cages were checked daily to measure adult mortality and number of oviposited eggs. In addition, egg masses were photographed for subsequent area calculation using the Image J program (public domain software, National Institute of Health) and transferred to a plastic bag until hatching. A sample of 79 hatched eggs masses were used to record the number of neonates. To avoid counting all them, the number of oviposited eggs (hatched + unhatched eggs) per egg mass was estimated from area calculation of egg masses.

**2.5 Nutrient content**

Nutrient content of males and females was determined using newly emerged moths (≤ 24 h) following standard biochemical techniques. Protein content was determined with the Bradford method [38] using Coomassie brilliant blue G-250 reagent (Biopack®, Buenos Aires, Argentina). Lipid and carbohydrate contents were determined with the Van Handel method [39]. Lipid content was measured with vanillin reagent (Sigma-Aldrich, St. Louis, USA) whereas total carbohydrates and glycogen contents were measured with anthrone reagent (Cicarelli, Santa Fe, Argentina). Optical density was measured on a ZL-5100 Zeltee Spectrophotometer. The number of individuals evaluated per rearing substrate depended on availability of moths and priority was given to fecundity assessment. Dry weight of moths was recorded with a precision scale (OHAUS Corporation, USA) to standardize nutrient content by individual weight prior to statistical analysis.

**2.6 Statistical Analyses**

Duration of egg, larval and pupal stages, and life span, were analyzed using generalized linear models (GLM) with a Poisson distribution and a log-link function, using the substrate as a fixed factor. To contrast differences between substrates, we performed a likelihood-ratio Chi-square test using the "Anova" function provided by the "car" package of R software version 4.1.0.[41]. A GLM was also fitted to adult longevities of males and females, adding to the model the interaction between sex and substrate. Larval at 7, 14 and 21 days of age, and pupal weights were modelled through GLM based on the Tweedie family of distributions for continuous data, which allow modelling distribution/link combinations that are disallowed by the R "glm" function. As link function, we utilized log-link and the substrate was the only fixed factor used to build the model. To contrast differences in all models, we performed a likelihood-ratio Chisquare test. Post-hoc, we carried out all pairwise multiple comparisons on least square means using a Holm-Sidak correction method (P ≤ 0.05). Additionally, we were carried out a Spearman correlation analysis between the larval weights at 21 days and the pupal weights per substrate in order to know whether larval weight is a good predictor of pupal weight. Survival curves of larvae and adults were obtained using nonparametric

Kaplan–Meier survival analysis.[40] Non-parametric log rank tests were conducted to determine whether larval rearing substrates had a significant effect on the survival of larvae and adults. Possible departure from a 1:1 sex ratio was subjected to a chi-square goodness of fit test on the number of pupae of each sex.

The offspring produced per female per day was analyzed with zero-inflated negative binomial mixed model with log-link function using "glmmTMB" package of R as the data were both zero-inflated and overdispersed. The significance of the differences was determined using the likelihood-ratio Chi-square test, and the pairwise multiple comparisons on least square means were conducted using a Holm-Sidak correction method. The gross reproductive rate (*GRR*), the net reproduction rate (*RO*), the mean generation time (*T*), the intrinsic rate of increase ($r_m$), the finite rate of increase (λ) and doubling time (*DT*) were estimated using the jackknife procedure within the "bootstrap" package of R. Then, we carried out Kruskal–Wallis tests due to assumptions of normality and/or homoscedasticity were not met. Finally, we performed pairwise comparisons of groups using the criterium Fisher's least significant difference (LSD).

Nutrient content was analyzed using the Tweedie distribution GLM with log-link function. The rearing substrate and the sex of emerging adults were used as explanatory variables and the interaction between them was tested. We used the likelihood-ratio Chi-square test followed by all pairwise multiple comparisons using a Holm-Sidak correction method. Possible grouping associated to nutrient content was assessed using a non-metric multidimensional scaling analysis (NMDS) based on Bray-Curtis distance matrix in R software.

## 3 RESULTS

### 3.1 Growth, development, and survival

The rearing substrates had a contrasting effect on the duration of the immature stages (Table 1). The duration of the egg and pupa stages was not significantly different among substrates ($\chi^2$ =2.08, df =5, *P* =0.84 and $\chi^2$ =7.81, df =5, *P* =0.17, respectively), whereas the larval development time was different ($\chi^2$ =72.89, df =5, *P* < 0.001). Larvae reared on the soybean varieties and *A. hybridus* had a

longer development time than those reared on artificial diet, *A. palmeri* and *A. spinosus*. The interaction between substrate and sex of moths was not significant ($\chi^2$ =0.83, df =5, $P$ =0.97), indicating that the sex ratio did not depend on the rearing substrate. Adult longevity was affected by both substrate ($\chi^2$ =15.98, df =5, $P$ =0.01) and sex ($\chi^2$ =15.19, df =1, $P$ <0.001). Adults reared on Cry1Ac soybean lived longer than on *A. palmeri*, and females were longer-lived than males. The total life span was also significantly shorter for individuals reared on *A. palmeri* than those reared on *A. hybridus* and Cry1Ac soybean ($\chi^2$ =20.75, df =5, $P$ <0.001).

**Table 1**

Larval survival also varied with the rearing substrate ($\chi^2$ =192, df =6, $P$ <0.001). Armyworms fed on *A. spinosus* showed lower survival probability than those reared on the soybean varieties (Fig. 1). Moreover, *A. hybridus* and *A. spinosus* presented the lowest pupation percentages (21% and 26%, respectively), whereas *A. palmeri*, artificial diet and soybeans exhibited the highest pupation percentages (71%, 80% and 79-80%, respectively). No pupae were recovered from larvae fed on *A. viridis* leaves. In addition, there was a high mortality rate (37.5%) in *A. hybridus* due to parasitism by Tachinidae flies. This phenomenon occurred at the pupal stage, when nine parasitoid pupae were recovered from *S. cosmioides* pupae (one per pupa). The percentage of moth emergence was influenced by substrate; Bt and non-Bt soybeans had the highest percentages (88.75% and 83.54%), followed by *A. palmeri* (76.06%), artificial diet (66.25%), and *A. hybridus* (57.14%) and *A. spinosus* (46.15%), which had the lowest one.

**Figure 1**

Larval and pupal weights were affected by the different rearing substrates (Table 2). At 7 days of larval development, the greatest weight gain was recorded for the larvae reared on *A. palmeri* and *A. spinosus*, whereas the lowest weight gain was recorded for armyworms fed on *A. viridis* and Bt soybean ($\chi^2$ =603.75, df =6, P <0.001). At 15 days, the larvae reared on *A. spinosus* weighed 40 times more than those fed on *A. viridis*, and five times more than those fed on Bt soybean ($\chi^2$ =1661.4,

df =6, $P$ <0.001). At 21 days, the larvae reared on *A. palmeri* had the greatest weight gain, whereas those on *A. viridis* had the lowest one ($\chi^2$ =1882.6, df =6, $P$ <0.001). The pupae on the artificial diet were the heaviest, whereas those on non-Bt soybean were the lightest pupae ($\chi^2$ =1082.2, df =5, $P$ <0.001). No significant correlation was observed between larval weights at 21 days and pupal weights for the different substrates, with exception of Bt soybean (*A. spinosus*: $\rho$ = 0.23, $P$ = 0.4709; artificial diet: $\rho$ = 0.09, $P$ = 0.4561; *A. palmeri*: $\rho$ =0.09, $P$ = 0.4929; Bt soybean: $\rho$ =-0.27, $P$ = 0.01545; non-Bt soybean: $\rho$ =-0.07, $P$ = 0.4932). The larval rearing substrates did not affect the sex ratio ($\chi^2$ =1.48, df =1, $P$ =0,223); the overall mean ± SE sex ratio values were 1:1.06 ± 0.11 (females: males). The survival of males and females adults was not affected by the rearing substrates ($\chi^2$ =10.8, df =5, $P$ = 0.06 and $\chi^2$ =6.1, df =5, $P$ = 0.30).

**Table 2**

**3.2 Reproductive and population growth parameters**

The rearing substrates significantly affected the offspring produced per female per day (interaction effect: $\chi^2$ =14.35, df =5, $P$ = 0.01). The females from Cry1Ac soybean, *A. palmeri,* and the artificial diet were more fecund than those from *A. spinosus* (Fig. 2). In all cases, the females presented a pre-oviposition period of approximately 2 days, and the egg-laying peak occurred 5–7 days. Parameters related to population growth potential significantly differed among the treatments (Table 3). The gross reproductive rate (total eggs / female) was higher than 2000 eggs for females from Bt soybean variety and *A. hybridus*. However, the percentage of non-viable eggs oviposited by females reared on *A. hybridus* and *A. spinosus* leaves were the highest (69% and 55%, respectively), in contrast to non-Bt soybean, artificial diet, *A. palmeri* and Bt soybean (36%, 35%, 17% and 13%, respectively). The net reproductive rate (i.e., the number of times the population multiplies per generation) was 3.66 times higher for individuals on Bt soybean than on *A. spinosus.* The mean generation time was significantly shorter (4-6 days) on *A. spinosus* and *A. palmeri* than on *A. hybridus* and Bt soybean. The intrinsic rate of population increase was 27.88% higher for individuals on artificial diet, Cry1Ac

Bt soybean, and *A. palmeri* than on non-Bt soybean, *A. spinosus*, and *A. hybridus*. The finite rate of increase was 1.02 times higher for females on artificial diet and *A. palmeri* than for those reared on *A. hybridus*. Finally, the doubling time was 1-day longer for individuals reared on *A. hybridus* than on Cry1Ac soybean and *A. palmeri*.

**Figure 2**

**Table 3**

**3.3 Nutrient content**

Few adults were obtained from *A. hybridus* and *A. spinosus,* thus the nutrient content was only evaluated for the other treatment. These conditioned differentially the content of nutrient in both sexes (Table 4). The interaction between substrate and sex was only significant for carbohydrate content ($\chi^2$ =12.48, df =3, $P < 0.01$). Females reared on *A. palmeri* had the lowest carbohydrate content, whereas males reared on Bt and non-Bt soybean had the highest one. The glycogen content showed significant differences only for substrate ($\chi^2$ =44.62, df =3, $P < 0.001$). Individuals reared on *A. palmeri* had lower glycogen content than those reared on artificial diet. As above, the lipid and protein contents showed significant differences only for rearing substrate ($\chi^2$ =10.02, df =3, $P = 0.02$ and $\chi^2$ =12.05, df =3, $P < 0.01$, respectively). Individuals reared on artificial diet and *A. palmeri* had lower lipid contents than on non-Bt soybean, whereas individuals reared on artificial diet had lower protein contents than on non-Bt soybean. Taking into account all nutrients, the adults on the artificial diet were the most distant individuals, contrastingly to those from the soybean varieties. The adults from *A. palmeri* were the most similar individuals in their nutrient content (Fig.3).

**Table 4**

**Figure 3**

**4 DISCUSSION**

In spite of being a polyphagous species, *S. cosmioides* life history and population fitness varied in the seven larval food sources assessed here. The armyworm survival rate values, developmental time, and population growth parameters were similar on the soybean varieties,

artificial diet, and *A. palmeri*. In contrast, the larvae on *A. hybridus* and *A. spinosus* had reduced reproductive capacity, and those on *A. viridis* did not complete development. This variable performance is likely related to the nutritional food quality, although qualitative and quantitative differences in the allelochemicals of *Amaranthus* species cannot be excluded.[42] The distinct nutrient profiles in the emerging adults may reflect the variable nutritional food quality to they were subjected during their immature stages.

The larvae on the exotic *A. palmeri* developed faster than on the native *A. hybridus* and the soybean varieties, which extended the armyworm life cycle in one week, consistent with previous studies comparing dry beans, cotton, oats, wheat, corn, and weeds of the *Amaranthus* genus.[19, 28, 43] Regarding body size, the larval weights at 7, 15, and 21 days of age were not a good predictor of pupal weight; despite the average size of the larvae reared on the artificial diet, their pupae were the heaviest. On Cry1Ac soybean, the larvae took longer to develop but reached pupal weight as high as or higher than those reared on *A. palmeri* and *A. spinosus* or *A. hybridus*. In contrast, on the non-Bt soybean variety, the larvae extended the development time, but generating the lightest pupae. Although in previous studies with *S. cosmioides* these pupal weight differences were not observed,[33, 44] they were with *S. eridania* [29] and here, indicating that Cry1Ac soybean can improve weight gain of *Spodoptera* larvae in some cases. Another study found that *S. cosmioides* reared on the foliage of *Amaranthus* spp., had pupal weight greater than on Cry1Ac soybean.[28] These discrepancies challenge the extent to which pupal weight *per se* is a reliable indicator of insect performance and host-plant quality. Perhaps there may be a threshold for pupal weight, given that the heaviest pupae (on artificial diet) had a higher mortality rate.

Several polyphagous insect herbivores can minimize the effects of suboptimal food quality by selecting different host plants and even moving between vegetative and reproductive structures (flowers and pods) of the same plant [42], as reported for larvae of *S. cosmioides*.[12, 19, 20] At the organismal level, Rabelo *et al.*[44] showed that the larvae descendants of parents undergoing stress inherited the negative impacts during the first days after eclosion; however, they later recovered

without affecting their reproductive traits. In our study, despite having a longer development time and lower weight gain, the larvae fed on the soybean varieties compensated for the presumed nutritional imbalance without reducing fertility and population growth. In some cases, the compensatory response (i.e., changes in the physiological system seeking homeostasis in the organism) can increase performance as observed in the larvae reared on the soybean varieties. This was not the case for the larvae on the native *Amaranthus* species, on which the insects were unable to maintain the regular population potential. In field settings, several questions remain about the realized insect population performance and the compensation process; the availability of different host plants can affect the larval foraging behavior, not to mention the various direct and indirect ecological interactions.

Trophic interactions between immature lepidopterans and their host plants play a key role in survival and reproduction.[45] The larvae fed on *A. hybridus* and *A. spinosus* were unable to develop successfully. The lower larval survival of *A. hybridus* and *A. spinosus* was associated with viral infection symptoms such as flaccid body, melanization, and fluid leaking from the integument. Tachinidae parasitoid pupae were also recovered from armyworms on *A. hybridus*. Tachinidae females lay microtypic eggs on the leaf surface, and neonates actively search for hosts.[46] Emerged parasitoids were only recovered in larvae fed on *A. hybridus* even though all the plants species originated from the same experimental field. This outcome may be associated with a tri-trophic interaction, in which specific signals released by *A. hybridus* could act as host location cues for the parasitoid.[42] We hypothesize that in the evolutionary history, the armyworm herbivory may have posed a selective pressure on the native *A. hybridus* and *A. spinosus*, such that they now harbor entomopathogenic virus and signal to parasitoid flies as an indirect defense against *S. cosmioides*; the exotic *A. palmeri* and soybeans may not present this defense system, allowing more remarkable armyworm survival. In contrast, *A. viridis* delayed the growth and development of armyworm, perhaps due to specific secondary metabolites, which may be interesting to investigate.

Host-plant quality emerges from the interaction between nutritional content, physical properties, and secondary metabolites.[42] Adults of *S. cosmioides* had different nutrient profiles according to the larval food substrate. Those reared on *A. palmeri* showed lower glycogen levels compared to non-Bt soybean variety and lower levels of lipids when compared to the Cry1Ac Bt soybean variety. The levels of protein and carbohydrates in the moths were equal for the three plant substrates analyzed. The significant interaction between substrate and sex was likely due to the high amount of carbohydrates in males reared on plants (soybean or *Amaranthus*) and females on the artificial diet. Carbohydrates in males may be associated with an energetic need to disperse, search and compete for receptive females.[47] Moreover, the high contrast between the carbohydrate-protein ratio between moths emerging from the artificial diet and those from the host plants evaluated here suggests that the artificial diet differs in nutrient composition compared to the host plants. This different nutritional quality in the artificial diet probably impacted pupal viability, given that adult emergence for that treatment was lower than for soybeans and *A. palmeri*. Therefore, nutrient content indicated that the diet is probably not balanced; it may have had a carbohydrate-protein relationship biased towards carbohydrates, requiring the larvae increased consumption to satisfy their protein needs.[48] Given that the artificial diet we used was modified from the one for a crambid species,[37] further studies adjusting nutrients proportions may help understand the nutritional needs of *S. cosmioides* and optimize their performance in rearing facilities.

Regardless of the rearing substrate, the female armyworm moths showed a brief pre-oviposition period like in other lepidopterans,[26, 49] with unimodal oviposition curves. Conversely, the food substrates influenced the oviposition peak and the number of oviposited eggs per day, supporting our hypothesis that the life history of this armyworm would be altered according to host plant. This variability caused by the host plant or artificial diet was also observed in the population growth parameters. Individuals reared on Cry1Ac soybean had the highest *RO* values (3.66 times more than *A. spinosus*), and together with those reared on *A. palmeri*, presented the highest values of the intrinsic rate of population increase (27.88%). As regards the number of total eggs/female (GRR), Cry1Ac Bt

soybean and *A. hybridus* provided the individuals with the highest fecundity, although this second substrate had the highest percentage of non-viable eggs (69%), which seems to support our hypothesis that this native weed may have a more developed defense system than the exotic weed *A. palmeri*. As mentioned earlier, the potential for population growth may not be fully be realized in the field because the conditions are different, however our results showed that resistant weeds to conventional herbicides and distinct soybean varieties, which are present in a field scenario, favored the population parameters of this species. Moreover, our findings are consistent with field records where defoliation is observed in both soybean varieties and *Amaranthus* weeds, in addition to the fact that the armyworm larvae have the possibility of feeding on pods, a question that has not yet been evaluated in conditions of laboratory. Therefore, intensive agricultural systems using monocultures and large-scale adoption of insect- and herbicide-resistant transgenic varieties may select resistance in weed populations/species and change the status of pest arthropods in the community of interacting species.[45, 50]

In summary, the research shows that soybean agroecosystem infested by herbicide resistant *Amaranthus* weeds may supply a suitable scenario for the population increase of *S. cosmioides*. Within an IPM context, alternative strategies will be necessary to control of insects and weeds when outbreaks occur on the Cry1Ac Bt soybean system, including peak population monitoring and correct application of insecticides when necessary.

## 6. ACKNOWLEDGMENTS

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**Figure 1.** Survival curves of *S. cosmioides* larvae reared on different rearing substrates. The signs (+) represent points where larvae molted to pupae.

**Figure 2.** Mean number (± standard error) of oviposited eggs per day by *S. cosmioides* females emerged from larvae reared on different rearing substrates.

**Figure 3.** Non-metric multidimensional scaling analysis (NMDS) for nutrient contents of *S. cosmioides* adults discriminated by different rearing substrates (geometric shapes) and sex of moths (colors). Web centers represent the weighted centroids for substrates and sexes. Ellipses grouped substrates with a 95% confidence interval.
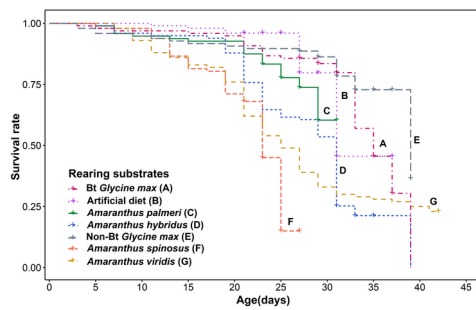
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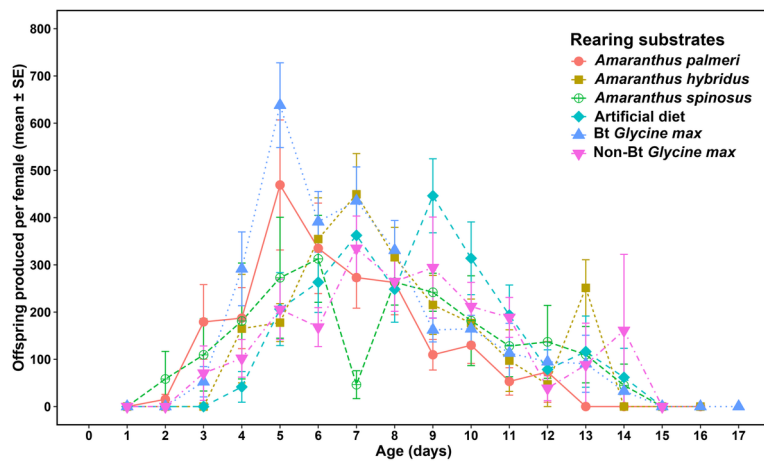
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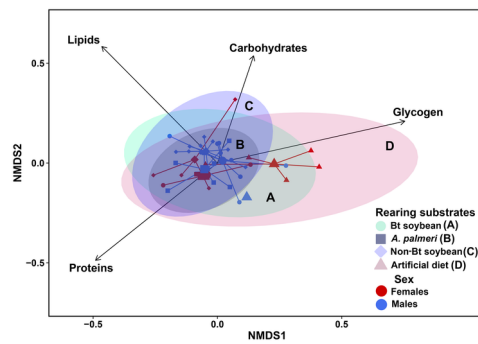
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**GRAPHICAL ABSTRACT**

# All for one? The role of genetically engineered soybean and *Amaranthus* weeds on the population growth of *Spodoptera cosmioides* (Lepidoptera: Noctuidae)

Paula G Páez Jerez, Jorge G Hill, Eliseu J G Pereira, Pilar Medina Pereyra, Maria T Vera

Development and fitness of the black armyworm was better when larvae fed both on non-Bt and Cry1Ac Bt soybeans varieties and on the exotic weed *A. palmeri*. Development on *A. hybridus* and *A. spinosus* resulted in decreased larval survival and population growth. *Amaranthus viridis* was an unsuitable food resource.
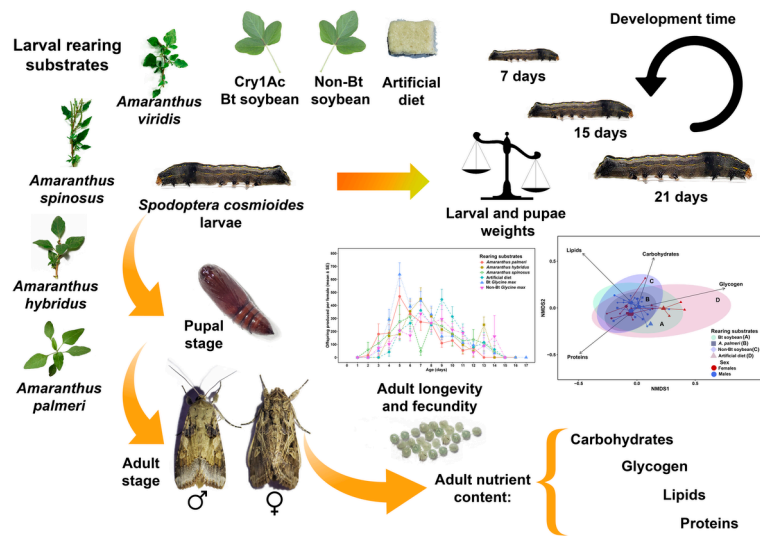
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**Table 1**. Duration in days (mean ± SE) of egg, larval and pupal stages, adult longevity and life span for each rearing substrate of *S. cosmioides*.

| Life cycle stage | *Amaranthus palmeri* (n) | | *Amaranthus hybridus* (n) | | *Amaranthus spinosus* (n) | | Artificial diet (n) | | Cry1Ac Bt *Glycine max* (n) | | Non-Bt *Glycine max* (n) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Egg [†] | 4.22 ± 0.1 (45) | a | 4.33 ± 0.16 (15) | a | 4.92 ± 0.24 (13) | a | 4.29 ± 0.1 (58) | a | 4.16 ± 0.07 (100) | a | 4.02 ± 0.08 (46) | a |
| Larval stage | 25.37 ± 0.26 (73) | a | 29.58 ± 0.29 (24) | b | 22.69 ± 0.25 (26) | a | 25.57 ± 0.27 (80) | a | 29.52 ± 0.30 (79) | b | 29.51 ± 0.34 (80) | b |
| Pupa | 13.62 ± 0.25 (42) | a | 14.38 ± 0.49 (13) | a | 13.58 ± 0.56 (12) | a | 14.55 ± 0.31 (40) | a | 13 ± 0.17 (43) | a | 12.51 ± 0.29 (41) | a |
| Adult longevity [‡] | ♀ 9.1 ± 0.83 (18) *<br>♂ 7.28 ± 0.61 (21) | a | ♀ 12.1 ± 0.63 (9) *<br>♂ 9.7 ± 1.85 (3) | ab | ♀ 11 ± 1.39 (6) *<br>♂ 9.66 ± 0.88 (3) | ab | ♀ 10.6 ± 0.75 (19) *<br>♂ 9.4 ± 0.76(15) | ab | ♀ 11.6 ± 0.49 (20) *<br>♂ 9.35 ± 0.77 (20) | b | ♀ 9.95 ± 0.61 (21) *<br>♂ 7.85 ± 0.51 (20) | ab |
| Life span | 49.61 ± 0.61 (39) | a | 58.72 ± 0.96 (12) | b | 50.46 ± 1.11 (9) | ab | 52.36 ± 0.57 (34) | ab | 55.57 ± 0.53 (40) | b | 52.9 ± 0.52 (41) | ab |

Values followed by different letters within a row are statistically significant (P ≤ 0.05) according to all pairwise multiple comparisons on least square means using a Holm-Sidak correction method.

† Duration of embryogenesis (egg stage) was obtained from the data recorded from the egg masses collected from the adults when reproductive parameters were determined.

**Table 2**. Larval and pupal weight (mean ± SE) *of S. cosmioides* larvae reared on seven substrates.

| Weight (mg) | | *Amaranthus palmeri* (n) | *Amaranthus hybridus* (n) | *Amaranthus spinosus* (n) | *Amaranthus viridis* (n) | Artificial diet (n) | Cry1Ac Bt *Glycine max* (n) | Non-Bt *Glycine max* (n) |
|---|---|---|---|---|---|---|---|---|
| Larva | 7 days | 10.67 ± 0.41 (97) d | 6.69 ± 0.34 (96) c | 9.19 ± 0.45 (98) d | 2.7 ± 0.16 (98) a | 4.16 ± 0.25 (100) b | 3.04 ± 0.17 (98) a | 6.82 ± 0.30(96) c |
| | 15 days | 281.43 ± 11.34 (93) d | 156.02 ± 6.56 (96) c | 531.98 ± 19.11 (79) e | 13.05 ± 0.9 (83) a | 256.52 ± 0.1547 (98) d | 95.92 ± 5.39 (97) b | 120.24 ± 5.35 (92) b |
| | 21 days | 1744.62 ± 44 (58) d | 1003.35 ± 36.96 (77) c | 1407.07 ± 52.68 (12) cd | 21.7 ± 1.97 (61) a | 1294.52 ± 59.04 (69) cd | 653.91 ± 22.47 (92) b | 594.16 ± 20.67 (90) b |
| Pupa | | 362.46± 4.99 (73) c | 328.95 ± 11.61 (24) b | 358.29 ± 10.66 (25) bc | - | 541.21 ± 5.99 (80) d | 377.17 ± 4.42 (79) c | 280.29 ± 4.68 (80) a |

Values followed by different letters within a row are statistically significant ($P \le 0.05$) according to all pairwise multiple comparisons on least square means using a Holm-Sidak correction method.

**Table 3**. Population growth parameters (means ± SE) of *S. cosmioides* reared on six rearing substrates.

| Rearing substrate | Population growth parameter | | | | | |
|---|---|---|---|---|---|---|
| | GRR | RO | T | $r_m$ | λ | DT |
| *Amaranthus palmeri* | 1750.90 ± 268.22 b | 223.90 ± 4.49 d | 45.51 ± 0.90 cd | 0.119 ± 0.0001 ab | 1.127 ± 0.0002 ab | 5.83 ± 0.006 de |
| *Amaranthus hybridus* | 2007.22±176.99 ab | 151.67 ± 2.95 e | 51.47 ± 0.99 a | 0.098 ± 0.0001 e | 1.103 ± 0.0001 e | 7.10 ± 0.007 a |
| *Amaranthus spinosus* | 1442.43 ± 355.84 b | 101.78 ± 1.64 f | 44.11 ± 0.69 d | 0.105 ± 0,0005 d | 1.111 ± 0.0001 d | 6.61 ± 0.006 b |
| Artificial diet | 1866.05 ± 281.14 b | 317.68 ± 6.16 b | 48.60 ± 0.93 bc | 0.120 ± 0.0001 a | 1.128 ± 0.0002 a | 5.78 ± 0.007 e |
| Cry1Ac Bt soybean | 2571.45 ± 179.05 a | 372.71 ± 6.84 a | 51.17 ± 0.93 a | 0.116 ± 0.0001 b | 1.123 ± 0.00013 b | 5.99 ± 0.09 d |
| Non-Bt soybean | 1575.7 ± 161.64 b | 238.25 ± 4.12 c | 49.39 ± 0.85 ab | 0.111 ± 0.0001 c | 1.118 ± 0.0001 c | 6.25 ± 0.006 c |

Values within the same column followed by different letters indicate significant statistic differences (Kruskal-Wallis test P ≤ 0.05) between larval rearing substrates.

GRR = gross reproductive rate; RO = net reproductive rate (females per female per generation); T = mean generation time (days); rm =intrinsic rate of increase (per day); λ = finite rate of increase (per day) and DT = doubling time (days)

**Table 4**. Nutrient content in μg (mean ± SE) of *S. cosmioides* adults obtained from different larval rearing substrates.

| Larval rearing substraes | Carbohydrates | | Glycogen (n) | | | | Lipids (n) | | | | Proteins (n) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Females (n) | Males (n) | Females (n) | Males (n) | LRS | FxM | Females (n) | Males (n) | LRS | FxM | Females (n) | Males (n) | LRS | FxM |
| Artificial diet | 5.54 ± 1.04 (7) b | 3.90 ± 1.01 (6) ab | 2.24 ± 0.34 (7) | 2.88 ± 1.68 (3) | c | | 2.92 ± 0.91 (6) | 4.12 ± 1.14 (4) | a | | 17.53 ± 3.10 (9) | 14.10 ± 1.44 (6) | a | |
| *Amaranthus palmeri* | 1.74 ± 0.46 (3) a | 4.16 ± 0.89 (9) ab | 0.61 ± 0.18 (3) | 0.83 ± 0.09 (9) | a | n.s. | 3.33 ± 0.12 (2) | 3.51 ± 0.25 (9) | a | n.s. | 21.34 ± 0.38 (3) | 19.87 ± 1.90 (9) | ab | n.s. |
| *Glycine max* | 3.33 ± 0.84 (2) ab | 6.29 ± 0.77 (10) b | 1.14 ± 0.88 (2) | 1.42 ± 0.21 (10) | b | | 3.17 ± 0.38 (2) | 4.59 ± 0.59 (10) | ab | | 20.39 ± 0.55 (2) | 20.17 ± 1.50 (10) | ab | |
| Non-Bt *Glycine max* | 3.41 ± 0.29 (6) ab | 7.21 ± 0.68 (9) b | 0.71 ± 0.09 (6) | 1.22 ± 0.13 (9) | ab | | 5.07 ± 1.47 (4) | 5.98 ± 0.60 (9) | b | | 29.44 ± 6.96 (6) | 20.73 ± 0.88 (9) | b | |

Values followed by different letters differ significantly (P ≤ 0.05) between substrates and sex.

LRS (larval rearing substrates): Different letters indicate significant differences according to post-hoc multicomparison tests (Sidak adjust) at P ≤ 0.05 between larval rearing substrates.

FxM (females vs. males): n.s. indicates a lack of statistical significance (P > 0.05) for the same nutrient between adults reared from different larval rearing substrates.