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ZOOLOGY

Diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) and its natural enemies: Population fluctuations and influence of biotic and abiotic factors

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ABSTRACT. The annual worldwide costs associated with the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), are estimated to be between four and five billion dollars in yield losses and management costs. Studies aimed at better understanding this pest and its natural enemies are essential for the development of new control strategies to increase production and meet world demand for healthy foods produced in ways that protect the environment for future generations. The present study was aimed at (i) describing population fluctuations of *P. xylostella* in collard greens, (ii) identifying its larval parasitoids, and (iii) evaluating biotic and abiotic factors that affect the population dynamics of this pest and its parasitoids in commercially grown collard greens in the municipality of Tangará da Serra, Mato Grosso State, Brazil. Fluctuations in the population of *P. xylostella* were assessed from March 2016 to February 2017. During 12 months of sampling, 4,310 larvae and pupae were collected, of which 640 (15%) were parasitized by native natural enemies. Three species of larval parasitoids were observed: *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae), *Apanteles piceotrichosus* Blanchard and *Cotesia* sp. (Hymenoptera: Braconidae). Precipitation and release of the egg parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) were the factors that most influenced the population fluctuations of *P. xylostella*. Also, native parasitoids were affected by the use of non-selective insecticides. Our findings are a contribution to the understanding of interactions between this pest and its environment to improve control strategies and preserve biodiversity.

Keywords: *Oomyzus sokolowskii*; *Apanteles piceotrichosus*; *Cotesia* sp.; *Trichogramma pretiosum*; *Brassica oleracea* var. *acephala*.

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Introduction

The annual worldwide costs associated with the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), are estimated to be between four and five billion dollars in yield losses and management costs (Zalucki et al., 2012; Mason, 2022). This is the main pest of brassicaceous crops, which includes vegetables consumed worldwide, such as cabbage, broccoli, cauliflower, collard, radish, mustard, among others (Talekar & Shelton, 1993; Judd, Campbell, Kellog, Stevens, & Donoghue, 2009).

The production of these vegetables has increased in recent years due to changes in feeding habits, as consumers eat more vegetables and demand better quality products (Camargo Filho & Camargo, 2010). However, the increased demand for these vegetables and the search for healthy foods has been impacted by the occurrence of *P. xylostella*, because its control is mostly carried out with chemical insecticides (Andrews, Sanchez, & Cave, 1990; Beck & Cameron, 1992; Subramanian, Rabindra, & Sathiah, 2010).

These chemicals pose risks that impact the environment and their residues, especially in leafy greens, which are mostly consumed raw, can have adverse health effects and promote the development of resistant pest populations. To date, 862 cases of *P. xylostella* resistance to different chemical molecules have been reported (Köhler & Triebskorn, 2013; Heckel, 2012; Lamichhane, 2017; Arthropod Pesticide Resistance Database, 2018).

Therefore, new control strategies need to be evaluated in order to meet the world demand for healthy food produced in ways to protect the environment for future generations, given that discussions about the impacts of chemical insecticides on the environment have been carried out for over 50 years since Carson (1962).

Among control strategies, parasitoids are of special interest. They can keep pests at low population densities and are sometimes species-specific, thus not affecting other species (Sarfraz, Keddie, & Dosdall, 2005). For *P. xylostella*, over 135 species of parasitoids have been reported worldwide attacking different developmental stages. The most common species are six egg parasitoids (genera: *Trichogramma* and *Trichogrammatoidea*), 38 larval parasitoids (genera: *Diadegma*, *Microplitis*, *Cotesia*, *Oomyzus*, and *Diadromus*), and 13 pupal parasitoids (genus: *Diadromus*) (Lim, 1986; Talekar & Shelton, 1993).

The use of parasitoids, associated with the use of selective insecticides, can also promote effective pest control. In Brazil, there is a homepage that allows you to check which insecticides are "compatible", "moderately compatible" or "incompatible" with parasitoids, thus enabling the integration of pest management with different strategies (Koppert, 2024).

In order to develop new control strategies, knowledge on population dynamics of this pest and its natural enemies is needed as well as on the influence of biotic and abiotic factors, taking into consideration the numerous scenarios of collard production in different regions. Few studies have been conducted on *P. xylostella* in the state of Mato Grosso, located in west-central region of Brazil. The region's climate consists of two well-defined seasons, a dry period from May to September and a rainy period from October to April (Dallacort, Martins, Inoue, Freitas, & Coletti, 2011), which can also influence the natural biological control of *P. xylostella.* This region supports high levels of biodiversity, as it comprises the Amazon, Cerrado, and the Pantanal biomes. Thus, biodiversity is constantly threatened with the use of chemicals non-selective for pest control in the region, from small horticultural areas to large-scale farming.

Studies on the dynamics of *P. xylostella* and its natural parasitoids are essential to provide pest control alternatives to farmers. The present study was aimed at (i) describing population fluctuations of *P. xylostella*, (ii) identifying its larval parasitoids, and (iii) evaluating the biotic and abiotic factors that affect the population dynamics of this pest and its parasitoids in commercially grown collard greens in Tangará da Serra, Mato Grosso State, Brazil.

Material and methods

Study area and commercially produced collard greens

The study was carried out in a horticultural growing area in the municipality of Tangará da Serra, Mato Grosso State, Brazil, 20 km from the urban center (14°40'16" S, 57°17'26" W). The total area of the property was approximately seven hectares, where several vegetables were grown in conventional and hydroponic systems, such as lettuce (American, Curly, and Head), parsley, green onions, coriander, arugula, green beans, and collard greens, depending on local demand. The farm is surrounded by pasture and grass near a highway on one side and at the front, while on the other side and at the back is bordered by native vegetation.

Among leafy greens, collards (*Brassica oleracea* var. *acephala* (Brassicaceae) are cultivated. The population survey was carried out in plots of 50 x 45 m, divided into beds. The beds consisted of 1.10 m in width and 0.50 m between rows, where three plants are grown per row. The size of the cultivated area and plots vary depending on the demand of the regional market.

The cultivation of collard greens was carried out according to agricultural practices and empirical knowledge acquired by the farmer in the field, which will be mentioned in the results.

Sampling method of *Plutella xylostella* and its natural enemies

The population fluctuation of *P. xylostella* was evaluated from March 2016 to February 2017, with three samplings per month, totalizing 36. During the collections, all plots (50 x 45 m) with collard greens on the farm were sampled manually. In some months, there were two or three plots depending on local demand. Thirty plants were randomly evaluated in each plot; each plant was examined for the presence of larvae and pupae of *P. xylostella*.

The specimens found were placed in 145 ml plastic containers and taken to the Entomology Laboratory at the Center for Research, Studies and Agricultural-Environmental Development (CPEDA) of the *Universidade do Estado de Mato Grosso* (UNEMAT) in Tangará da Serra, Mato Grosso State, Brazil. The specimens were kept until the emergence of adults or parasitoids, according to the creation methodology of Massarolli et al. (2021).

Eulophidae specimens of the emerged parasitoid were identified by one of the authors (VAC) according to Graham (1991). Microgastrines (Hymenoptera: Braconidae) were identified at genus level using the key to New Word genera (Whitfield, 1997). The genus *Apanteles* was identified at species level using the key to neotropical species (Fernández-Triana et al., 2014), and by comparison with original descriptions (Blanchard, 1947).

The identification of genus *Cotesia* was first attempted using keys to species with agricultural importance (Sharkey, Whitfield, & Seltmann, 2005; Fagan-Jeffries & Austin, 2020), and by comparison with original descriptions of *Cotesia vestalis* (Haliday) (Haliday, 1834; Kurdjmov, 1912), the most common species of this genus attacking *P. xylostella*. However, the specimens obtained in this work clearly belong to a different species, which, to our knowledge, is not yet known, therefore, it will be presented in the result only at genus level.

Voucher specimens were deposited in the "Oscar Monte" Entomophagous Insect Collection (IB-CBE), *Instituto Biológico*, Campinas, São Paulo, Brazil (curator: Valmir A. Costa), under record IB-CBE-680.

Obtaining abiotic data

During collections, information on crop management were obtained from the farmer, such as pesticide use, chemical group, mode of action, and compatibility classification. The latter was determined with the aid of an application developed by Koppert® (https://www.koppert.com.br/centro-de-informacoes/aplicativo-de-compatibilidade-de-produtos/), in which products are classified as "compatible", "moderately compatible" or "incompatible" with parasitoids releases or the use of biological products (*Bacillus thuringiensis* var. *Kurstaki* (bacterium) and *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) (parasitoid).

Thermal amplitude (°C), relative humidity (%) (RH), and precipitation (mm) were obtained from UNEMAT weather station, located 12 km from the study area. The effect of climate factors on the occurrence of *P. xylostella* and its natural enemies was evaluated.

Statistical analysis

All analyzes were performed using the software R, version 3.4.1 (R Development Core Team, 2017). The data on *P. xylostella* and its natural enemies were compared with an analysis of variance (ANOVA at 5% significance), and the means were analyzed with the 5% Scott-Knott test (Scott & Knott, 1974), using the Skott-Knott package (Jelihovschi, Faria, & Allaman, 2014). To evaluate the influence of biotic and abiotic factors on pest fluctuation and its natural enemies, Generalized Linear Models (GLMs) (Nelder & Wedderburn, 1972) were used with a Gaussian distribution. Global models were built with GLMs and subjected to model selection.

The first global GLM model included the mean number of caterpillars per plant as the response variable, while the predictor variables were the mean parasitized caterpillars, mean thermal amplitude in a 10-day interval (°C), sum of precipitation in 10-day collection interval, chemical products used (compatible or incompatible), release of egg parasitoids (equivalent to 100 or 200 thousand per hectare) and season (dry or rainy). In the second global GLM model, the response variable was the mean parasitized caterpillars and the predictors were the same as those of the first global model, except mean parasitized caterpillars.

Both global models were selected according to the Akaike information criterion (Burnham & Anderson, 2002) using the dredge function of R's MuMIn package (Barton, 2016). The best Akaike models were chosen based on delta values (ΔAICc) < 4 (Burnham & Anderson, 2002). These models were considered equally parsimonious and the most robust that fit empirical reality (Burnham, Anderson, & Huyvaert, 2011). The parameters of the final model were defined by model averaging using the model.avg function of the MuMIn package. Thus, the mean of this model is calculated based on the sum of weights of all models in which the predictor variables were selected, providing values of the relative importance of each predictor to forecast the response variable (Burnham & Anderson, 2002; Lukacs, Burnham & Anderson, 2009).

Pre-diagnostic tests of normality, correlation between predictor variables, and linearity were performed. To verify normality, the Shapiro-Wilk test was carried out using the response variables and by graphically evaluating the fit of the residuals of the GLM models with quantile-quantile plots (QQ Plot) using quantiles predicted by the model and the set of data and histograms of the residuals. All predictor variables were subjected to Pearson correlation test using the chart Correlation function of Performance Analytics package (Peterson & Carl, 2018). Pearson coefficients greater than 0.70 or less than -0.70 were considered as a criterion and separation of variables with high multicollinearity, avoiding overlap and masking of the real linear contribution of each predictor variable to the prediction of the dependent variable (Dormann et al., 2013). To further assure multicollinearity control, the variance inflation factor (VIF) was also evaluated in GLMs using the vif function of the car package (Fox & Weisberg, 2011), considering any predictor with vif > 5 as high multicollinearity (Zuur, Ieno, & Elphick, 2010).

Additionally, pretests were carried out to examine linearity between dependent and predictor variables using Komolgorov-Smirnov and Cramér-Von-Mises tests (Lin, Wei, & Ying, 2002). The tests were performed with the cumres function of the gof package (Holst, 2014) to evaluate if any predictors had a nonlinear relationship with the response variable. Linearity tests for each model were performed by applying an LM model with the same dependent and predictor variables used in the GLMs and if p-values of the two tests were less than 0.05 for a predictor, then their relationship to the dependent was considered nonlinear.

Result and discussion

Plutella xylostella and its natural enemies in the study area

Throughout the sampling period, 4.310 *P. xylostella* larvae and pupae were collected. Of these, 640 (15%) were parasitized by the larval-pupal parasitoid *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae), and the larval parasitoids *Apanteles piceotrichosus* Blanchard and *Cotesia* sp. (Hymenoptera: Braconidae). The most abundant parasitoid was *O. sokolowskii*, reaching an average parasitism rate of 22.6 in October.

Thirty-eight species of larval and 13 pupal parasitoids of *P. xylostella* are known worldwide (Talekar & Shelton, 1993). In Brazil, Ferronatto and Becker (1984) reported the presence of three parasitoid species of *P. xylostella* in Rio Grande do Sul: *Tetrastichus (= Oomyzus) sokolowskii, A. piceotrichosus,* and *Spilochalcis* sp. (Hymenoptera: Chalcididae), with the former as the dominant species. In the state of Paraná, Marchioro and Foerster (2016) recorded *Diadegma leontiniae* (Brèthes) (Hymenoptera: Ichneumonidae), *A. piceotrichosus*, *Siphona* sp. (Diptera: Tachinidae), and *O. sokolowskii*. In that study, however, *A. piceotrichosus* was the most abundant species (47.5%), while *O. sokolowskii* was found in reduced number (1.3%). In the Federal District, Castelo Branco and Medeiros (2001) recorded in *P. xylostella, Diadegma* sp. and *Actia* sp. (Diptera: Tachinidae), *O. sokolowskii* and *Apanteles* sp.; being *Diadegma* sp. more abundant between April and August and *Apanteles* sp. between September and November. Guilloux, Monnerat, Castelo-Branco, Kirk, and Bordat (2003) registered seven species of larval parasitoids, *Diadegma leontiniae* (Brethes) being *Apanteles piceotrichosus* (Blanchard) the dominant. *Cotesia vestalis (=plutellae)* (Haliday) and *Actia* sp., were less abundant, showing the presence of the genus *Cotesia* in *P. xylostella* in central Brazil.

Although *C. vestalis* is frequently reported as one of the most common parasitoids on *P. xylostella* worldwide (Kfir, 1997; Shi, Liu, & Li, 2002; Bertolaccini, Sánchez, Arregui, Favaro, & Theiler, 2011), including some observations in Brazil (Guilloux et al., 2003; Silva-Torres, Pontes, Torres, & Barros, 2010), the specimens of *Cotesia* obtained in this study clearly belong to a different species. Therefore, this is a new record for a natural enemy of *P. xylostella* in Brazil, of a potential important species in the biological control of this pest. Further taxonomic studies are needed to explore its potential.

Relationship of *Plutella xylostella* and its parasitoids to biotic and abiotic factors

Analysis of GLMs revealed the relative importance of biotic and abiotic variables to the population density of *P. xylostella,* and abiotic variables to native parasitoids in the area. Significant variables are shown in Table 1 and the relative importance of each variable and the number of models in which they were included, in Table 2.

Influence of dry and rainy seasons on population density of *Plutella xylostella*

The population density of *P. xylostella* and the number of parasitized larvae/pupae did not vary significantly when comparing to dry and rainy seasons (Table 3), corroborating the GLM analysis (Tables 1 and 2). When parasitoid species were evaluated separately, *A. piceotrichosus* was more abundant during the rainy season, while *O. sokolowskii* was present throughout the year (Table 3). Climatic variables (temperature, RH, and precipitation) differed significantly when comparing to dry and rainy seasons (Table 3). This was the typical climatic variation for the region which consists of two well-defined seasons, a dry period from May to September and a rainy one from October to April (Dallacort et al., 2011).

Table 1. Analysis of variance of Generalized Linear Models (GLMs) testing the effects of biotic and abiotic variables on *Plutella xylostella* and their native natural enemies in Tangará da Serra, Mato Grosso State, Brazil (March 2016 to February 2017)[1].

(a) *P. xylostella* ~ native parasitoids + amplitude + precipitation + product + release + season			
Variable	Likelihood ratio	Degrees of freedom	*p*
Total Precipitation	3.849	1	0.049
Parasitoid egg release	4.173	1	0.041
Parasitized larvae	1.006	1	0.315
Chemical products	0.093	1	0.759
Thermal amplitude (°C)	0.086	1	0.768
Season (dry or rainy)	0.093	1	0.760
(b) Native Parasitoids ~ amplitude + precipitation + product + release + season			
Variable	Likelihood ratio	Degrees of freedom	*p*
Total Precipitation	0.483	1	0.486
Parasitoid egg release	1.457	1	0.227
Chemical products	4.472	1	0.034
Thermal amplitude (°C)	0.545	1	0.460
Season (dry or rainy)	0.809	1	0.368

[1]Only the best models (based on AICc values) are shown. Boldface indicates significant values.

Table 2. Relative importance and number of models that use each predictor variable to explain population fluctuations of *Plutella xylostella* and native natural enemies.

Response variable	Predictor variable	Relative importance %	Number of models
P. xylostella	Total precipitation	95	10
	Parasitoid egg release	80	8
	Parasitized larvae	28	4
	Chemical products	18	3
	Thermal amplitude (°C)	16	3
	Season (dry or rainy)	12	2
Native parasitoids	Chemical products	89	10
	Parasitoid egg release	48	5
	Season (dry or rainy)	32	4
	Thermal amplitude (°C)	22	3
	Total precipitation	14	3

Table 3. Mean (± standard deviation) of parasitized *Plutella xylostella* larvae and pupae collected from collard greens, mean temperature (° C), relative humidity (%), and total precipitation (mm) during the rainy and dry seasons (March 2016 to February 2017) in Tangará da Serra, Mato Grosso State, Brazil.

Parameter	Season[1]		*p*
	Dry	Rainy	
P. xylostella (larvae/pupae)	52.65 ± 45.25 a	38.90 ± 34.75 a	0.10
Parasitism (total)	5.80 ± 6.55 a	7.15 ± 14.10 a	0.60
Apanteles piceotrichosus	0.16 ± 0.48 a	0.69 ± 1.53 b	0.03
Oomyzus sokolowskii	5.62 ± 6.70 a	6.47 ± 14.08 a	0.73
Temperature (°C)	23.70 ± 3.70 a	24.95 ± 1.99 b	< 0.01
Relative humidity (%)	65.95 ± 14.60 a	79.15 ± 7.40 b	< 0.01
Total precipitation (mm)	121.18 a	1378.30 b	< 0.01

[1]Means followed by different letters are significantly different according to the analysis of variance at 5% ($p < 0.05$).

When evaluating the mean monthly number of caterpillars, the highest record was observed in July (112.5), at the peak of the dry season. The second highest mean was in October, at the end of the dry season and the beginning of the rainy period, when the mean number of caterpillars collected was 70.3 (Table 4).

The highest records of parasitized larvae and pupae were observed in October and November, with means of 22.90 and 16.00 parasitized individuals, respectively (Table 4). *A. piceotrichosus* was not recorded between March and July, at the end of the rainy season and the beginning of the dry period. The highest means of individuals parasitized by this species were observed from November to January, while for *O. sokolowskii*, the highest means were recorded from July to November (Table 4).

The absence of variation in the population density of *P. xylostella* and its natural enemies in the dry and rainy seasons may be associated with the fact that collard greens have been grown in the study area continuously for several years. This allows the pest population to be always abundant, unlike the observed in other studies, such as the reported in Brasilia, Distrito Federal, Brazil, where *P. xylostella* occurs throughout the year, but population density declines during the rainy season. This may be associated with egg removal and death of larvae and pupae through the rain (Castelo Branco & Gatehouse, 1997). Domiciano and Santos (1996) observed high densities of *P. xylostella* from early August to the end of September in canola crops in Paraná. Sow, Diarra, Arvanitakis, and Bordat (2013) observed that *P. xylostella* density varied significantly in cabbage crops during the two well-defined seasons in Senegal, with an abundance of 474.0 caterpillars in the dry period and 29.1 caterpillars in the rainy season. Ahmad and Ansari (2010) pointed out that during the rainy season in India, the density of *P. xylostella* in cauliflower tends to be lower as caterpillars fall from leaves or drown, corroborating the observed by other authors (Talekar & Shelton, 1993; Sivapragasam, Ito, & Saito, 1988; Kobori & Amano, 2003; Ayalew, Baumgärtner, Ogol, & Löhr, 2006).

Table 4. Monthly mean (± standard deviation) number of parasitized *Plutella xylostella* larvae and pupae, and the parasitoids *Apanteles piceotrichosus*. and *Oomyzus sokolowskii* collected from collard greens during the rainy and dry seasons (March 2016 to February 2017) in Tangará da Serra, Mato Grosso State, Brazil.

Month	Season	*P. xylostella* (Larva/pupa)	Parasitized	*Apanteles piceotrichosus*.	*Oomyzus sokolowskii*
March	Rainy	24.00 ± 14.70 c	0.45 ± 0.70 c	0.00 ± 0.00 b	0.45 ± 0.73 c
April	Rainy	43.30 ± 25.00 c	3.20 ± 4.10 c	0.00 ± 0.00 b	3.20 ± 4.13 c
May	Dry	33.80 ± 29.80 c	1.40 ± 1.50 c	0.00 ± 0.00 b	1.40 ± 1.51 c
June	Dry	39.00 ± 21.70 c	1.90 ± 1.60 c	0.45 ± 0.90 b	1.45 ± 1.33 c
July	Dry	112.5 ± 61.95 a	8.65 ± 9.60 b	0.00 ± 0.00 b	8.63 ± 9.58 b
August	Dry	41.00 ± 21.80 c	10.80 ± 6.25 b	0.17 ± 0.41 b	10.67 ± 6.31 b
September	Dry	36.35 ± 22.25 c	10.00 ± 6.00 b	0.17 ± 0.41 b	9.83 ± 5.78 b
October	Rainy	70.30 ± 59.60 b	22.90 ± 24.80 a	0.22 ± 0.44 b	22.67 ± 24.48 a
November	Rainy	46.65 ± 33.90 c	16.00 ± 19.60 a	2.33 ± 2.16 a	13.67 ± 19.05 b
December	Rainy	15.85 ± 20.10 c	5.15 ± 10.75 c	1.72 ± 2.98 a	3.43 ± 11.52 c
January	Rainy	24.40 ± 17.40 c	4.90 ± 3.75 c	1.38 ± 1.41 a	3.50 ± 4.47 c
February	Rainy	43.20 ± 28.90 c	0.25 ± 0.45 c	0.11 ± 0.33 b	0.11 ± 0.33 c
p		< 0.01	< 0.01	< 0.01	< 0.01

[1]Means followed by different letters are significantly different according to the Scott-Knott test at 5% ($p < 0.05$).

Marchioro and Foerster (2016), however, concluded that despite continuous and abundant plant availability throughout the year, *P. xylostella* occurred between June and November, and the highest abundance peaks were observed between August and September, when low temperatures and rainfall were recorded. This indicates a wide variation in the population dynamics of *P. xylostella* in different regions, validating that studies such as the present one provide essential information for the management of this pest.

A relationship between natural enemies and seasons was also observed in Senegal. *O. sokolowskii* was active throughout the year, but its population density varied significantly between dry and rainy seasons, while *Apanteles litae* Dixon (Hymenoptera: Braconidae) was most commonly found in the dry period (Sow et al., 2013).

Thermal amplitude and total precipitation

The best models showed that total precipitation was strongly correlated to the mean number of caterpillars per plant ($F = 1.72$, df = 1, gl = 32, $p = < 0.05$), while thermal amplitude had no significant influence ($F = 1.72$, df = 1, gl = 32, $p = > 0.05$), with a relative importance of 0.16 (Tables 1 and 2). Relative humidity was not included in the GLM analysis, as it presented high collinearity with thermal amplitude (-0.84).

The variation of climatic factors and the mean number of *P. xylostella* per plant for each sampling is shown in Figure 1 (A and B). In periods when the minimum temperature was low, ranging from 20 to 15 °C, and the maximum temperature did not exceed 30 °C, the pest population increased, reaching its peak in the second third of July, when the mean minimum temperature was 15.6 °C and the maximum, 26.1 °C (Figure 1A).

These temperatures are considered ideal for *P. xylostella* development, according to Marchioro and Foerster (2011). The optimal temperature reported by these authors for the development *P. xylostella* was 28.8 °C under laboratory conditions, while temperatures below 10 °C and above 30 °C negatively affect its survival and fecundity. Another study found that optimal temperatures for *P. xylostella* development in Africa were up to 33.5 °C, thus revealing that elevated temperatures may be favorable for the development of this pest (Ngowi et al., 2017). During the same period when temperatures were ideal, relative humidity was also low, between 40 and 60%, due to the absence of precipitation (Figure 1B), thus contributing to the development of the pest. Guo and Qin (2010), when evaluating the effect of different temperatures and relative humidity combined, observed that the emergence of *P. xylostella* adults is significantly lower at 90% RH at all temperatures evaluated and concluded that RH between 50 and 70% is ideal for the development of the diamondback moth.

For natural enemies collected in *P. xylostella* larvae and pupae, no significant differences were observed regarding thermal amplitude (F = 0.19, df = 1, gl = 34, p = > 0.05) and total precipitation (F = 1.49, df = 1, gl = 33, p = > 0.05) (Figure 1C and D).

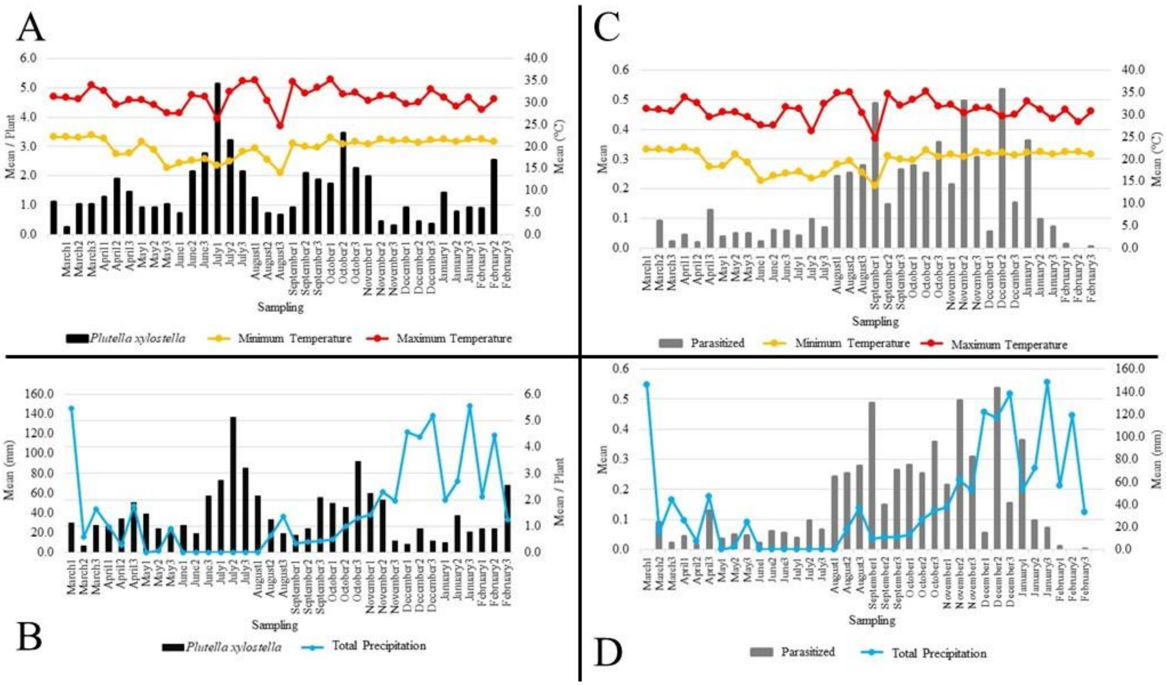

Figure 1. Mean number of *Plutella xylostella* and climatic factors (A) minimum temperature – maximum temperature = thermal amplitude (°C), (B) Total precipitation (mm), mean parasitism of *P. xylostella* and climatic factors (C) minimum temperature –/maximum temperature = thermal amplitude (°C), (D) Total precipitation (mm) recorded in each sampling in collard field from March 2016 to February 2017 in Tangará da Serra, Mato Grosso State, Brazil.

Sow et al. (2013) did not find a significant correlation between parasitoid population and precipitation, but temperature was negatively correlated with *A. litae*. No significant correlation was found between temperature and *O. sokolowskii*, in agreement with the observed in the present study.

Despite these results, temperature may affect the population growth of *O. sokolowiskii.* In the experiments of Talekar and Hu (1996) 50% of diamondback moth larvae were parasitized at 20 °C, while at 35 °C, parasitism rates reached 90%. This might explain why *O. sokolowiskii* was the most common species in the present study, as temperatures in the study area were high.

High temperature, absence of precipitation, and consequently low RH are typical for the study area. This is an essential information for the farmer to monitor the best conditions for the development of *P. xylostella* and where pest control is carried out with natural enemies, management strategies should be adopted to maintain the population below economic damage levels.

Pest management

Throughout the 12 months of sampling, the control of *P. xylostella* was carried out with releases of *T. pretiosum* and application of biological and synthetic insecticides. The products used during sampling are listed in Table 5, along with chemical compounds, mode of action, and compatibility classification.

Insecticide application did not affect population fluctuations in *P. xylostella* (F = 0.72, df = 1, gl = 31, p = > 0.05), as the relative importance of this variable was 18% in only three GLM models (Table 2). This result was unexpected, as the products are indicated for the control of the diamondback moth and the 15 products used comprise different chemical groups and modes of action (Table 5). This may be due to a possible resistance of P. *xylostella* to chemical compounds, as other 862 cases have been reported worldwide (Arthropod Pesticide Resistance Database, 2018).

Releases of different numbers of *T. pretiosum* significantly influenced the mean number of *P. xylostella* (F = 4.39, df = 1, gl = 30, p < 0.05), with 80% relative importance in 8 models (Table 2). This indicates that inundative releases of egg parasitoids are effective for the control of the diamondback moth, as also observed for other lepidopteran species (Parra, Zucchi, & Silveira Neto, 1987; Parra & Zucchi, 2004).

Table 5. List of products used to control *Plutella xylostella* in collard greens from March 2016 to February 2017 and their active ingredients, commercial name, chemical group, mode of action, recommendations, and compatibility.

Active Compound	Commercial	Chemical Group	Mode of Action	Recommendations[1]	Compatibility[2]
Acephate	Orthene®	Organophosphate	Contact, ingestion, systemic insecticide/acaricide	Crops: R, C, Br, CF Pests: Px, Tn, Bb, Mp	Incompatible
Bacillus thuringiensis	XenTari®	Biological	Biological ingestion insecticide	Crops: Br, R Pests: Px, Am	Compatible
Baculovirus	Diplomata®	Biological	Biological ingestion insecticide	All crops the crops where the biological target occurs (*Helicoverpa armigera*)	Compatible
Chlorantraniliprole	Premio®	Anthranilamide	Contact and ingestion insecticide	Crops: C, Br, CC, CB, CF Pests: Px, Tn.	Compatible
Chlorfenapyr	Pirate®	Pyrazole analog	Contact and ingestion insecticide/acaricide	Crops: R, C Pests: Px, Am, Bb, Mp	Incompatible
Cartap Hydrochloride	Cartap®	Bis (thiocarbamate	Contact and ingestion insecticide/fungicide	Crops: C Pests: Px, Am	Not Available
Cyantraniliprole	Benevia®	Anthranilamide	Contact and ingestion insecticide	Crops: R, C, Br, CC, CB, CF Pests: Px, Tn	Not Available
Diflubenzurum	Dimilin®	Benzoylurea	Chitin inhibitor insecticide	Not indicated for brassicaceous crops	Compatible
Spinosad	Tracer®	Spinosyn	Biological non-systemic insecticide	Crops: C, Br, CC, CB, CF Pests: Px, Am, Tn, Ai, Hp	Incompatible
Etofenprox	Safety	Diphenyl Ether	Contact insecticide	Not indicated for brassicaceous crops	Incompatible
Indoxacarbe	Rumo®	Oxadiazine	Ingestion insecticide	Crops: R, C, Br, CC, CB, CF Pests: Px, Tn, Hp	Compatible
Methomyl	Lannate®	oxime methyl carbamate	Systemic and contact insecticide	Crops: R, C, Br Pests: Am, Px, Bb	Incompatible
Novaluron	Rimon®	Benzoylurea	Physiological insecticide	Crops: R; Pests: Px	Compatible
Orange Essential Oil	Orobor®	Natural oil	Dispersing agent and foliar fertilizer	Crops: R, C, Br	Incompatible
Teflubenzurom	Nomolt®	Benzoylurea	Growth regulator insecticide, chitin inhibitor	Crops: C, Br, CC, CB Pests: Am, Tn, Hp	Compatible

[1]Crop recommended: R = cabbage; Br = Broccoli; C = Collard; CF = Cauliflower; CB = Brussels Sprouts; CC = Chinese cabbage; Pests: Px – Diamondback moth (*Plutella xylostella*); Am - Great southern white (*Ascia monuste orseis*); Tn - Cabbage looper (*Trichoplusia ni*); Ai - Black cutworm (*Agrotis ípsilon*); Hp - Cabbage budworm moth, (*Hellula phidilealis*); Bb - Cabbage aphid (*Brevicoryne brassicae*); Mp - Green peach aphid (*Myzus persicae*); [2]According to the applicative Koppert.

When analyzing the effect of pesticides on *O. sokolowskii* and *A. piceotrichosus*, the application of incompatible products affected their occurrence ($F = 15.07$, $df = 1$, $gl = 32$, $p < 0.001$), with a relative importance of 89% in 10 models (Table 2). This confirms that products classified as incompatible with *Trichogramma* are also incompatible with larval parasitoids.

Releases of *T. pretiosum* had no significant influence ($F = 1.88$, $df = 1$, $gl = 31$, $p > 0.05$) on native natural enemies. According to the models, their relative importance was 48% (Table 2), indicating that the population of native natural enemies were not affected by the release of egg parasitoids.

Figure 2 shows the mean number of *P. xylostella* (larvae and pupae) and the mean number of parasitized caterpillars in each sampling date, showing the effect of the chemical products used. Between March and July, when incompatible products were used, the highest means of *P. xylostella* per plant and low means of natural parasitoids were recorded.

Beginning in August, three weeks after compatible products began to be used, an increase in the number of natural parasitoids and a decrease in the number of *P. xylostella* were observed, a pattern repeated throughout the collections according to the management used. From the period when "compatible" products were used, both released (*T. pretiosum*) and native parasitoids were favored, with an increase in their population over the following months (Figure 2). Several authors reported that high levels of infestation by *P. xylostella* are the result of excessive insecticide applications, followed by the development of insecticide resistance and the ineffectiveness of natural enemies (Dennill & Pretorius, 1995; Shelton et al., 2000; Shelton, 2001; Ahmad & Ansari, 2010).

Our conclusions are that the precipitation is the climatic factor that most influenced population fluctuations of *P. xylostella* (95%), followed by the release of *T. pretiosum* egg parasitoids (80%). Native parasitoids in the area, however, did not contribute significantly to reduce pest population, but if maintained through the use of selective products, they might assist in the control of diamondback moth populations. On the other hand, the importance of chemical products, thermal amplitude, and season to *P. xylostella* population density were all under 20%.

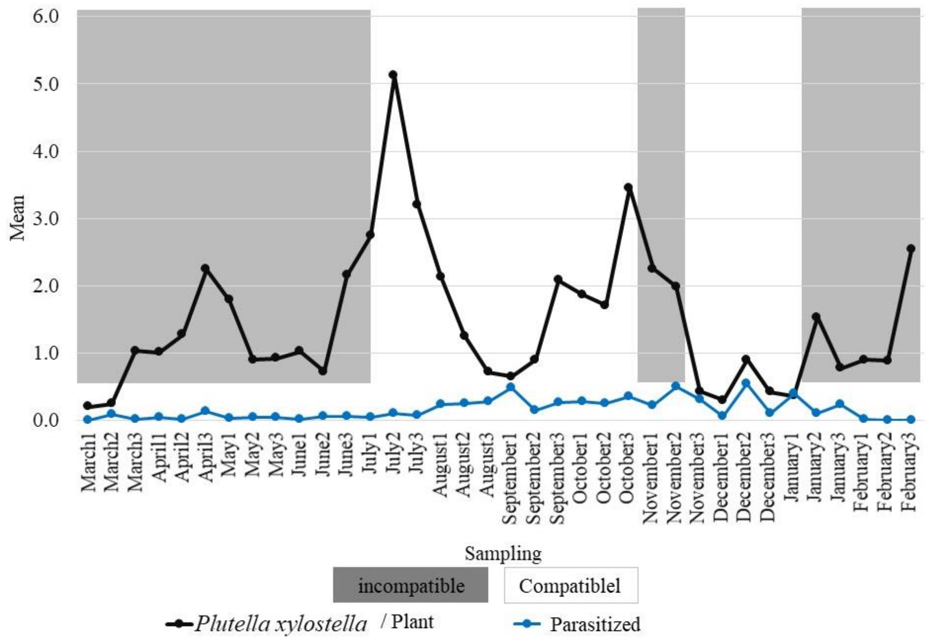

Figure 2. Mean number of larvae and pupae of *Plutella xylostella* per plant, mean number of parasitized larvae/pupae, and compatibility with the type of management used, in a collard field (March 2016 to February 2017) in Tangará da Serra, Mato Grosso State, Brazil.

Three species of larval parasitoids were registered, *O. sokolowskii*, *A. piceotrichosus* and *Cotesia* sp.; The native parasitoids were not affected by climate variables (thermal amplitude, precipitation, and season), but the use of "incompatible" products affected their density (89%), followed by *T. pretiosum* releases (48%).

Thus, deducing that the use of non-selective chemicals (broad spectrum) is inefficient and impairs the action of natural enemies and that, under the conditions studied (location, climate, types of products and community of natural enemies) the best recommendation would be the use of products compatible with the action of natural enemies, in addition to greater care in controlling the pest in the dry season.

Conclusion

Three species of larval parasitoids were observed: *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae), *Apanteles piceotrichosus* Blanchard and *Cotesia* sp. (Hymenoptera: Braconidae). Precipitation and release of the egg parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) were the factors that most influenced the population fluctuations of *P. xylostella*. Also, native parasitoids were affected by the use of non-selective insecticides. Our findings are a contribution to the understanding of interactions between this pest and its environment to improve control strategies and preserve biodiversity.

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