



Helicoverpa armigera Harm 1 Haplotype Predominates in the Heliiothinae (Lepidoptera: Noctuidae) Complex Infesting Tomato Crops in Brazil

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Abstract

Tomato (*Solanum lycopersicum* L.) is a natural host for the *Helicoverpa-Chloridea* (Lepidoptera: Noctuidae: Heliiothinae) pest complex. The species *Helicoverpa armigera* (Hübner) was responsible for significant yield losses in several crops after its detection in Brazil. The morphology of its larval stage resembles common Heliiothinae species, making pest control decisions difficult. The overall lack of studies on the Heliiothinae associated with tomatoes in Brazil and the establishment of *H. armigera* in the country plus their recent outbreaks supported our investigation about the relative importance of the insects from the *Helicoverpa-Chloridea* complex in this vegetable crop. A nationwide survey was carried out across fresh-market and processing tomato fields. Molecular analyses targeting a segment of the mitochondrial *cytochrome oxidase* subunit I (*mtCOI*) gene and their sequence analyses indicated the presence of a pest complex, comprising the introduced species *H. armigera* and the indigenous species, *Helicoverpa zea* (Boddie), and *Chloridea virescens* (Fabricius). The Harm 1 haplotype of *H. armigera* was identified as the predominant Heliiothinae pest infesting fresh-market tomatoes. The New World species *Chloridea subflexa* (Guenée) as well as the exotic Solanaceae-specific species *Helicoverpa assulta* (Guenée) were not found in our survey. Additional larvae surveys in processing tomato fields during 2013/2014 in Central Brazil also indicated *H. armigera* as the most abundant Heliiothinae species (95%) together with *H. zea* (4.75%) and *C. virescens* (0.25%). The occurrence of distinct *Helicoverpa* species (which are potentially capable of interbreeding) indicates that novel crop management strategies will be necessary in order to minimize damages caused by this pest complex in tomatoes.

Keywords Geographical distribution · invasive pests · lepidopteran insect · Solanaceae · genetic diversity

Introduction

The Heliiothinae subfamily (Lepidoptera: Noctuidae) is composed by more than 300 moth species, including a large number of crop-damaging pests such as the members of the *Helicoverpa-Chloridea* complex (Cho et al. 2008). The highly polyphagous *Chloridea virescens* (Fabricius) (former *Heliothis virescens*) is widely distributed in the New World, being a major pest of tobacco and other field crops (Fitt 1989; Brito et al. 2001). *Chloridea subflexa* (Guenée) (former *Heliothis subflexa*) is another economically important polyphagous pest endemic to the New World (Souza et al. 2015). On the other hand, the majority of the 18 species ascribed within the cosmopolitan genus *Helicoverpa* are oligophagous and they are considered minor agricultural pests (Hardwick 1965; Mitter et al. 1993). However, this genus also encompasses

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polyphagous and economically important lepidopteran species able to cause severe damage in a wide range of field and vegetable crops (Daly and Gregg 1985; Mitter et al. 1993; Behere et al. 2008). The four most important *Helicoverpa* pests are the Old World bollworm *H. armigera* (Hübner), *H. assulta* (Guenée), and *H. punctigera* (Wallengren), as well as the New World bollworm *H. zea* (Boddie). *Helicoverpa armigera* is currently the predominant species in the Old World region, whereas *H. zea* is the predominant pest species in New World region (Behere et al. 2007). However, this scenario has been changing abruptly after the incursion of *H. armigera* lineages into Brazil and other South American countries, which has been associated with severe damages in field crops such as cotton, sorghum, maize, soybean, beans, and citrus (Czepak et al. 2013; Specht et al. 2013; Tay et al. 2013; Bueno et al. 2014; Leite et al. 2014; Mastrangelo et al. 2014; Murúa et al. 2014; Arneodo et al. 2015). Due to recent invasion, at least from 2008 (Sosa-Gómez et al. 2016), studies on the relative incidence of *H. armigera* in major crops have been done (e.g., Leite et al. 2014; Jones et al. 2019; Dourado et al. 2020); however, host range of *H. armigera* populations needs more intensive investigations in the Neotropics.

A range of species belonging to the *Helicoverpa-Chloridea* complex can also affect the cultivated tomato (*Solanum lycopersicum* L.) as reported in distinct global regions (Fitt 1989; Leite et al. 2014; Kriticos et al. 2015). Tomato is described as a natural host of *C. virescens* (Brito et al. 2001), *H. zea*, *H. armigera*, and *H. assulta* (Kennedy 2003; Behere et al. 2007). *Chloridea subflexa* has been also reported in association with tomato plants (Souza et al. 2015). *Helicoverpa gelotopoeon* (Dyar) (which occurs endemically in South America) is found mainly in association with maize, cotton, and Fabaceae crops, although it has been also reported causing fruit losses in tomato (Cork and Lobos 2003). *Helicoverpa armigera*, *H. zea*, and *H. gelotopoeon* are morphologically similar and they can only be reliably distinguished through either dissection of adult genitalia (Pogue 2004) or via molecular markers (Arneodo et al. 2015). This resemblance makes pest control decisions difficult since, according to the Brazilian law (SISLEGIS 2020), the insecticide dosage recommendation is made based upon the target species.

Helicoverpa zea and *C. virescens* have been considered secondary pests in tomato crops in Brazil (Souza and Reis 2003; Pratisoli et al. 2006; Fornazier et al. 2010). However, more recent reports based solely upon morphological analysis are also indicating the presence of the invasive *H. armigera* in association with fresh-market tomato fruit damage in Southeast Brazil (Pratisoli et al. 2015). The polyphagous nature of *H. armigera* and the intensification

of lepidopteran outbreaks in tomato fields (coinciding with its recent detection) prompt us to investigate the relative importance of this pest in major fresh-market and processing tomato-producing areas of Brazil. The limited number of reliable morphological characteristics employed to discriminate *Helicoverpa* species makes the molecular analyses of informative genomic regions an alternative to conventional taxonomic differentiation allowing for the establishment of effective strategy to carry out broad diversity studies at infraspecific and intraspecific (haplotype) levels, irrespective of the pest life stage (Behere et al. 2007). In fact, biochemical and molecular marker systems are now being used in large-scale surveys as well as population diversity studies within the genus *Helicoverpa* (Nibouche et al. 1998; Behere et al. 2007, 2013). Segments of the mitochondrial genome corresponding to the genes coding for the mitochondrial cytochrome oxidase subunit I (*mtCOI*) and cytochrome *b* (Cyt *b*) have been employed as universal molecular marker systems for taxonomic delimitation of a wide range of insect species (Simon et al. 1994). It includes the ones belonging to Lepidoptera (Noctuidae) genera for which a growing global sequence database is available (Behere et al. 2007, 2008; Li et al. 2011; Tay et al. 2013, 2017).

In this context, the main objective of the present work was to carry out a survey of the *mtCOI* gene diversity across members of the *Helicoverpa-Chloridea* (Lepidoptera: Noctuidae: Heliothinae) complex associated with fruit damage in distinct tomato production systems (fresh-market vs. processing) covering distinct ecological and geographical regions of Brazil. In fact, there is an overall lack of extensive studies on the genetic variability of Heliothinae species infesting tomato crops in the country. This lack of information on the current levels of insect diversity and relative importance of the distinct Heliothinae species infesting tomatoes is a major limiting factor for establishing effective pest management programs.

Materials and methods

Collection of tomato fruit worm and DNA extraction

A total of 66 samples (= individual larvae) was collected directly from damaged fruits in fresh-market and processing tomato fields during the 2012/2013/2014 seasons (Table 1 and Table 1S). These samples were obtained in a large range of microclimate conditions encompassing 14 districts of Goiás State and Federal District (Central Brazil), Espírito Santo and São Paulo States (Southeast Brazil), and Santa Catarina State (South Brazil). DNA of these samples (named by the code BRH-Brazilian Heliothinae collection) was extracted either from fresh samples or from samples preserved in 100% ethanol and stored at -20°C . Total genomic DNA

Table 1 Brazilian Heliiothinae larvae collected from damaged tomato (*Solanum lycopersicum* L.) fruits in field surveys carried out in distinct crop producing systems and geographic regions of Brazil, across 2012 to 2014 crop seasons. The genomic information via sequencing of the *mtCOI*-derived PCR amplicons (511 bp) was used for identification of *Chloridea virescens* specimens as well as for haplotype determination of *Helicoverpa armigera* and *H. zea* samples

Collection site ^a	Tomato production system	Species/haplotype ^b		
		<i>H. armigera</i>	<i>H. zea</i>	<i>C. virescens</i>
Brazlândia-DF	Fresh market	3 (H1)	0	0
Gama-DF	Fresh market	11 (H1)	0	1
Planaltina-DF	Fresh market	8 (H1)	0	0
Rajadinha-DF	Fresh market	1 (H1)	0	0
Cristalina-GO	Processing	15 (H1)	5 (H5) 2 (H8) 1 (H10)	1
Formosa-GO	Fresh market	5 (H1)	0	0
Hidrolândia-GO	Fresh market	2 (H1)	0	0
Morrinhos-GO	Processing	3 (H1)	0	0
Castelo-ES	Fresh market	1 (H1)	0	0
Domingos Martins-ES	Fresh market	1 (H1)	0	0
Venda Nova-ES	Fresh market	2 (H1)	0	0
Sto Antônio da Posse-SP	Fresh market	1 (H1)	0	0
Caçador-SC	Fresh market	1 (H1)	0	0
Urubici-SC	Fresh market	2 (H1)	0	0

^a The acronyms of the Brazilian states are as follows: the Federal District (DF), Espírito Santo (ES), Goiás (GO), Santa Catarina (SC), and São Paulo (SP)

^b Total number of specimens. Haplotype numbers/codes of *H. armigera* and *H. zea* are shown in parenthesis. See Table 1S for a detailed listing of specimens

was extracted from the whole sample using a slightly modified protocol (pH = 8.0 and supplemented with sodium sulfite) with the CTAB 2× buffer plus organic solvents (Boiteux et al. 1999). The DNA concentration and quality (A260/A280 ratio) were measured with a NanoDrop® Lite spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) using 1 µL of each sample. DNA integrity was assessed by agarose (1.5%) gel electrophoresis.

PCR primers and amplification profiles

PCR assays were carried out using the primer pair COI-F01 (5'-TTA TTT CAC ATC AGC TAC TAT-3') and COI-R01 (5'-CTT TAT AAA TGG GGT TTA AAT-3') with the following reaction conditions: 94°C for 4 min (one cycle); 1 min each of 94°C, 50°C, and 72°C (35 cycles), followed by a final extension cycle of 72°C for 5 min (Li et al. 2011). PCR amplification of individual DNA samples was carried out in a total reaction volume of 25 µL, and containing 25 ng of genomic DNA, 0.2 µM each of the forward and reverse primers, 0.2 mM of dNTPs, 1× PCR buffer (Promega, Madison, Wisconsin, USA), 1.5 mM MgCl₂, and one unit of *Taq* DNA polymerase (Promega). Amplicons of 680 bp of the *mtCOI* gene were obtained and subsequently trimmed to its highly informative 511-bp region. This polymorphic region can discriminate the representative haplotypes of *H. zea* and *H. armigera* as well as other *Helicoverpa* species and other genera of the Heliiothinae (Lepidoptera: Noctuidae) associated

with tomato crop throughout the world (Hajibabaei et al. 2006; Behere et al. 2007, 2008; Li et al. 2011; Albernaz et al. 2012). Single amplicons were agarose gel-purified using the PureLink® (Invitrogen, San Diego, California, USA) purification kit prior to the sequencing reaction.

Sanger dideoxy sequencing and phylogenetic analyses

As previously mentioned, our diversity analysis included only the partial *mtCOI* (511 bp) gene segment rich in genetically informative single-nucleotide polymorphisms (SNPs) (Li et al. 2011). The sequencing reaction was done using the protocol of the BigDye® terminator cycle sequencing version 3.1 using the same PCR primer pair. DNA sequencing was carried out in an automatic ABI Prism sequencer model 3100 (Applied Biosystems, Foster City, California, USA). Forward and reverse sequences were obtained for all samples, allowing for unequivocal contig assembling. Sequences that differed by one or more SNPs were considered different haplotypes, while the ones exhibiting identical sequence at informative nucleotide positions were considered same haplotypes. A subset of samples was sequenced (at least twice) using amplicons from distinct PCR assays aiming to confirm the haplotype classification. Sequences were assembled and the quality of the trace files was screened with the SeqMan program (Lasergene package; DNASTar, Madison, Wisconsin, USA) (Allex 1999). Phylogenetic analyses were performed

by comparing the obtained sequences with sequences of the network for the global *mtDNA* COI haplotype database (Behere et al. 2007; Cho et al. 2008; Li et al. 2011) as well as sequences available at the GenBank (<http://www.ncbi.nlm.nih.gov/>) and International Barcode of Life (iBOL) (<http://www.ibol.org/>). GenBank accessions of the following reference sequences were employed for the final phylogenetic tree construction: *H. armigera* haplotype 1 (EF116226), *H. zea* haplotype 5 (EF116263), *H. zea* haplotype 8 (EF116266), *H. zea* haplotype 10 (EF116268), *H. assulta* haplotypes 1 (EF116270) and 2 (EF116271), *H. punctigera* haplotypes 1 (EF116272) and 2 (EF116273), and *C. virescens* (JN799038). The *mtCOI* gene segment information (511 bp from the sequence between nucleotides 981 and 1492) was extracted from the whole *C. subflexa* mitochondrial genome (KT598688) and included in the alignments. Sequences were aligned using the Clustal W multiple sequence alignment program under the default settings of the Geneious 11 (<https://www.geneious.com>). Alignments were inspected visually and edited manually by deleting unaligned border sequences. A phylogenetic tree was constructed using maximum likelihood via PAUP* version 4.0 (Swofford 2002). The HKY (Hasegawa-Kishino-Yano) evolutionary model (Hasegawa et al. 1985) of nucleotide substitution was selected by the software MODELTEST (version 3.7). Tree searching was carried out using Heuristic search strategy with simple stepwise sequence addition method. The confidence levels of the inferred relationships were determined via bootstrap analysis with 1,000 replications. The analysis was carried out using *Spodoptera exigua* (HQ857475) and *S. eridania* (HQ177329) as the out-groups.

Identification of the prevalent Heliiothinae species in processing tomato fields in Central Brazil

In order to identify the predominant Heliiothinae species present in association with damaged fruits in tomato fields for industrial processing, adults were monitored with light traps and collections of individual larvae attacking the fruits. The study was carried out between June 2013 and July 2014, in five tomato crops irrigated by central pivot, located in the Cristalina County (Goiás State, Central Brazil). In each tomato field, a light trap of the “Luiz de Queiroz” model (Biocontrole Ltda., São Paulo-SP, Brazil) was set up, with a fluorescent lamp of black light, powered by battery. The captured moths were deposited in a cylindrical polyethylene flask (20 cm in diameter and 30 cm in height), containing absolute alcohol, which was attached to the bottom of the trap. The trap was set up outside the fields, at about 3 m from the border and at a height of 1.50 m. It was turned on at sunset and turned off at dawn of the following day, considering five consecutive nights during the new moon phase of each month in

order to exclude the effect of the moon phases on light-trap efficiency (Kishimoto-Yamada and Itioka 2015). The moths were removed daily from the collecting flask and transferred to another container with absolute alcohol, in order to preserve them. The experimental data were recorded as the monthly proportion of individuals belonging to the Heliiothinae species that were captured in the traps. For that, the daily collection data were organized individually within the five sampled tomato fields. In order to determine the levels of larvae infestation within each field, eight sampling points (3.0 × 3.0 m plots) at 20-m intervals were randomly established. At each point, ten plants were randomly inspected (equivalent to 2 m of cultivation row), at 7-day intervals after the installation of the trap. All larvae found, regardless of size and species, were collected and maintained on a standard artificial diet for noctuids (Greene et al. 1976; Montezano et al. 2013, 2014), up to the stage that allows for morphological identification. The experimental data were expressed in number of larvae per 10 plants, corresponding to the average population density of four weekly evaluations per month. The identification of the adult moths was carried out based on the reference collection of Embrapa Cerrados (Brasília, DF, Brazil). The discrimination between *H. armigera* and *H. zea* adults from these light trap surveys was made through morphological characteristics of the genitalia of the moths (Hardwick 1965; Pogue 2004). Differences in the proportions of Heliiothinae species captured by light traps were assessed performing a chi-square (χ^2) test for the hypothesis of equal proportions. In addition, pairwise comparisons regarding *H. armigera* and *H. zea* were performed with 1 d.f., after Yates’s correction for continuity (Zar 2010). All analyses were done using the statistical package (CHISQ option, PROC FREQ) available in SAS software (SAS Institute 2001). A sample of fruit-damaging Heliiothinae insects were also collected in these field assays and characterized via partial *mtCOI* gene sequence as previously described (only 12 specimens collected in this survey were included in Supplementary Table 1S).

Results

Phylogenetic analyses of *mtCOI* gene segments of Heliiothinae samples associated with tomato fruit damage

Total DNA was extracted from 66 Heliiothinae samples (37 collected in fresh-market and 29 collected in processing tomato fields) that were collected from damaged fruits in distinct agro-ecological and macro-geographic regions of Brazil in processing and fresh-market tomato fields during the

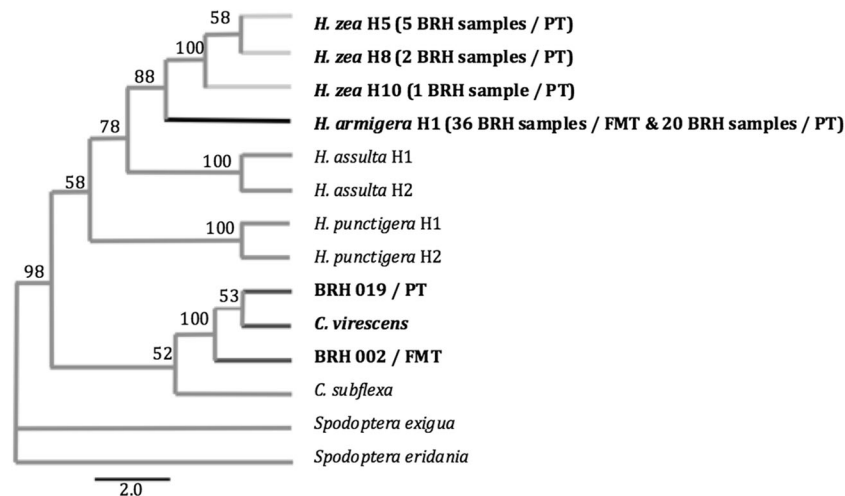


Fig. 1 Maximum likelihood-based phylogenetic analysis displaying the genetic relationships among 66 samples of the Brazilian Heliothinae collection (BRH) collected in association with damaged fresh-market tomato (FMT: $n = 37$) and processing tomato (PT: $n = 29$) fruits in Brazil. Clusters were defined based upon the nucleotide identities for a segment of 511 bp of the mitochondrial cytochrome *C oxidase* subunit I (*mtCOI*) gene. The tree was constructed using as reference sequences *Helicoverpa armigera* haplotype 1 (EF116226), *H. zea* haplotype 5 (EF116263), *H. zea* haplotype 8 (EF116266), *H. zea* haplotype 10 (EF116268), *H. assulta* haplotypes 1 (EF116270) and 2 (EF116271), *H. punctigera* haplotypes 1 (EF116272) and 2 (EF116273), *Chloridea virescens*

2012/2013/2014 seasons (Table 1 and Table 1S). The sequence analyses of *mtCOI* gene segments with genetically informative SNPs allowed the identification of a complex of Heliothinae species associated with damaged tomato fruit. The phylogenetic analysis using the *mtCOI* gene information indicated a monophyletic group with *H. armigera* haplotype 1 (Harm 1) supported by a bootstrap value of 88% (Fig. 1). No nucleotide diversity was observed among these 56 *H. armigera* samples from either fresh-market (FMT; $n = 36$) or processing tomatoes (PT; $n = 20$). All *H. armigera* sequences from our survey displayed 100% identity with one Harm 1 specimen formerly described in Thailand (GenBank EU768935). *Chloridea virescens* (2 out of 66 samples) and three haplotypes of *H. zea* (eight out of 66 samples) were also identified in our survey (Table 1). In summary, it was observed during the survey period a predominance of *H. armigera* haplotype Harm 1 (84.85% of the samples) across distinct Brazilian production regions, occurring both fresh-market (under open-field tutored/trellised and drip-irrigated conditions) and processing tomato fields (under center-pivot irrigation). *Helicoverpa zea* corresponded to 12.12% of the samples and they were found exclusively in processing tomato fields conducted at complex agricultural landscapes (i.e., landscape composed also with corn, cotton, and soybean fields), whereas *C. virescens* was found in 3.03% of the samples in both fresh-market and processing tomato fields (Table 1). The exotic Solanaceae-specific pest, *H. assulta*, was not found infesting tomatoes in our survey.

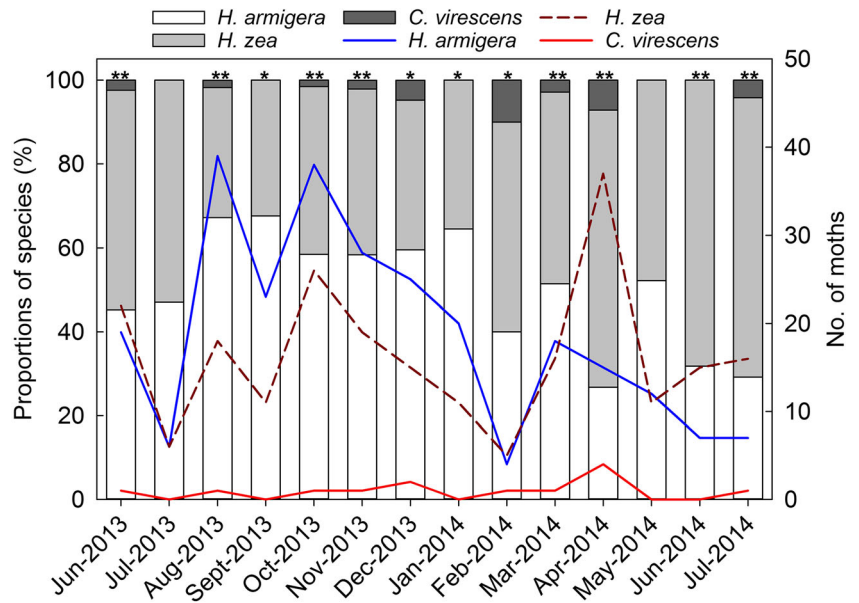
(JN799038), and *C. subflexa* (obtained from KT598688). Branch lengths are measured in number of substitutions per site. The numbers next to the nodes of the tree branches are the bootstrap support values (1,000 bootstrap trials). Sequences of *mtCOI* gene of the tomato-infesting species *H. gelatopoeon* were not available and they were not included in the analysis. *Spodoptera exigua* (HQ857475) and *S. eridania* (HQ177329) served as out-groups. No nucleotide diversity was observed among the 56 *H. armigera* samples from either fresh-market tomato (FMT: $n = 36$) or processing tomatoes (PT: $n = 20$), being all classified as Harm 1 haplotype. See Supplementary Table 1S for a detailed listing of specimens

Likewise, the *C. subflexa*, which is also endemic to the New World (Souza et al. 2015), was not found in the present study. Sequences of *mtCOI* gene of the South American species *H. gelatopoeon* (Murúa et al. 2014, 2016) are not yet available at public databases. Hence, *H. gelatopoeon* was not included in our study.

Identification of the prevalent Heliothinae species in processing tomato fields in Central Brazil

A total of 507 Heliothinae moths was collected between 2013 and 2014 in the processing tomato fields that were monitored with light traps in Cristalina, Goiás State (Fig. 2). *Helicoverpa armigera* was the most abundant species, with 263 individuals, corresponding to 51.85% of the total moths. The second most common species was *H. zea*, with 231 moths captured (45.56%). In contrast, *C. virescens* was less abundant, with only 13 individuals (2.56%). The overall proportions of captured moth varied across the species (Fig. 2, $\chi^2 = 1003.90$, $df = 3$, $p < 0.0001$). Furthermore, the proportions of the species rated by month showed also differences among species in 12 out of 14 months (Fig. 2, significance indicated by asterisks). In most of the cases, this variation was a result of lower presence of *C. virescens* and its complete absence in some month (July and September 2013 and January and May 2014). The predominant species, *H. armigera* and *H. zea*, had similar proportions (χ^2 test, $p > 0.05$) during four months (lines of total number of moths collected in Fig. 2 touching each other; July 2013, and

Fig. 2 Relative percentage of occurrence (stacked bars) and overall moths captured (lines) of three Heliiothinae species (viz. *Helicoverpa armigera*, *H. zea*, and *Chloridea virescens*) during five subsequent nights each month in surveys with light traps installed in processing tomato fields between June 2013 and July 2014, Cristalina, Goiás State, Central Brazil. Note: y-scales differ as function of the variables. Bars with asterisks indicate significant differences across species by chi-square test (* $p < 0.05$, ** $p < 0.01$). The period between November 2013 and January 2014 corresponds to the interseason period of processing tomatoes cultivation in the region

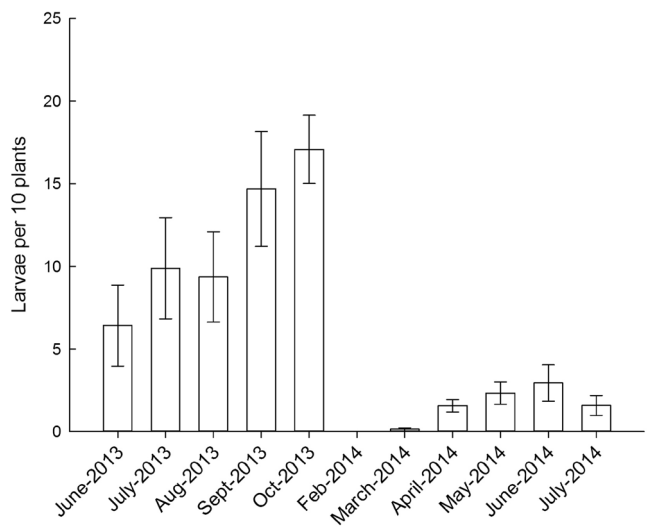


February, March, and May 2014). Otherwise, *H. armigera* moths were significantly more abundant in three months (January, August, and September 2014), while *H. zea* was prevalent in the other three months (April, June, and July 2014). The decrease in *H. armigera* and *H. zea* populations across the off-season of processing tomato (November 2013 until late January 2014) followed a similar pattern. However, *H. armigera* was the prevalent species as tomato fruit-damaging insect, corresponding to 95% of the collected larvae ($n = 413$), all of which were classified as Harm 1 haplotype. The remaining samples of larvae consisted of *H. zea* (4.5%) and *C. virescens* (0.5%). The infestation of *H. armigera* larvae was higher in 2013 compared to 2014, with the highest population densities between June and October (Fig. 3).

Discussion

Invasive species are recognized as a major threat to biodiversity as they can cause environmental disturbances and dislodge naturally endemic species (Lee 2002; Pimentel et al. 2005; Jones et al. 2019). In fact, some of the most serious arthropod pests in new agricultural frontiers of Neotropical regions are invasive species. For most of the invasive pests, chemical pesticides are the prevalent pest management methods (Achaleke and Brévault 2010; Vivan et al. 2017), contributing to increase production costs besides their intrinsic environmental risks and negative side effects on non-target organisms and human health (Pimentel et al. 2005).

Fig. 3 Density of *Helicoverpa armigera* larvae per 10 tomato plants assessed from processing tomato fields carried out between June 2013 and July 2014 in Cristalina, Goiás State, Central Brazil. Mean values (\pm SE) represent the average of 10 samples per month from five tomato fields. Note: The period between November 2013 and January 2014 corresponds to the interseason period for processing tomatoes cultivation in the region, hence, without survey



The polyphagous species *C. virescens*, *H. zea*, and *H. gelotopoeon* have been considered the major pests within the *Helicoverpa-Chloridea* complex in the Neotropics (Cork and Lobos 2003; Specht et al. 2013; Murúa et al. 2014, 2016; Arneodo et al. 2015). The recent invasion of *H. armigera* lineages into the New World has changed this scenario, being responsible for severe yield losses in field crops such as soybean, cotton, sorghum, and corn (Kriticos et al. 2015; Pomari-Fernandes et al. 2015). *Helicoverpa armigera* currently the major pest of tomatoes in Mediterranean countries years has become a major pest in both greenhouse and open-field tomato crops in this area (Torres-Vila et al. 2002). Tomatoes are also natural hosts of a complex *Helicoverpa* species, including the New World species *H. zea* and *H. gelotopoeon* (Biezanko et al. 1974; Cork and Lobos 2003; Kennedy 2003; Behere et al. 2007). *Chloridea subflexa* has been also reported in association with tomatoes and other Solanaceae species such as tobacco and *Physalis* (Souza et al. 2015). However, we did not detect this Heliethinae species in our survey.

The exclusive presence of the *H. armigera* Harm 1 haplotype in association with damaged tomato fruits in Brazil is somehow surprising. Previous studies have demonstrated the presence of a wide array of *H. armigera* haplotypes infesting soybean, maize, cotton, beans, and vegetables fields in South America (Czepak et al. 2013; Specht et al. 2013; Tay et al. 2013, 2017; Leite et al. 2014; Mastrangelo et al. 2014; Murúa et al. 2014, 2016; Arneodo et al. 2015; Tembrock et al. 2019). At the present moment, the sole presence of *H. armigera* haplotype Harm 1 infesting fruits of tomatoes remains elusive and subsequent surveys will be necessary to verify if this scenario will change in the near future and most notably if this haplotype Harm 1 will persist as the dominant tomato fruit-infesting *H. armigera* haplotype.

Helicoverpa zea also clustered into a monophyletic group with high bootstrap value. These results demonstrated that the genomic region corresponding to a segment of the *mtCOI* gene can be employed as a precise discrimination tool of *H. armigera* and *H. zea* samples. Contrary to the scenario observed for *H. armigera*, nucleotide diversity was observed among the tomato fruit-infesting *H. zea* samples. The pairwise comparison of the *H. zea* sequences allowed us to identify the haplotype 5 in five samples, haplotype 8 (in two samples), and haplotype 10 (in one sample). These *H. zea* samples were found infesting only processing tomatoes in Cristalina, Goiás State (Central Brazil). In this region, it is quite common the cultivation of large fields cultivated with Bt and non-Bt hybrids throughout the year, which are established simultaneously and/or alongside and even sharing the same center-pivot with processing tomatoes (Sousa et al. 2020).

The nucleotide differences among *H. armigera* and the three *H. zea* haplotypes reported infesting tomato fruits in Brazil ranged from 18 (haplotypes 5 and 10) to 21 nucleotides

(haplotype 8), which is in agreement with the genetic divergence expected for most Lepidoptera species (Hajibabaei et al. 2006). According to Behere et al. (2007), these relatively high levels of genetic similarity between *H. armigera* and *H. zea* are probably due to their recent evolutionary divergence, which occurred approximately 1.5 million years ago.

Two BRH samples also grouped with *C. virescens* into a monophyletic group with high bootstrap value. However, the original *C. virescens* specimen used as the reference sequence (GenBank JN799038) and the two BRH samples that clustered together with *C. virescens* did not show complete nucleotide identity, with genetic differences ranging from one SNP to three SNPs. *Chloridea virescens* (together with *H. zea*) is considered one of the key pests of the heliothines in the New World (Cunningham and Zalucki 2014). This pest can infest a wide array of species within the families Fabaceae, Malvaceae, Asteraceae, Solanaceae, Convolvulaceae, and Scrophulariaceae (Cunningham and Zalucki 2014). *Chloridea virescens* is a major pest in cotton and soybean-producing areas in Brazil, displaying a wide range of haplotypes based upon diversity of a distinct set of *mtDNA* markers (*coxI*, *coxII*, and *nad6*) (Albernaz et al. 2012). In our survey, we were not able to characterize the corresponding haplotypes described by Albernaz et al. (2012). In fact, the currently available *mtCOI* database does not allow the identification of haplotypes for *C. virescens*. However, the two *C. virescens* samples obtained here displayed polymorphisms in the *mtCOI* gene segment (Fig. 1), indicating that distinct haplotypes for this gene are present in the insects infesting tomatoes. The detection of the polyphagous *C. virescens* also infesting processing tomatoes in Central Brazil might represent a potential pest management problem especially in relation to the establishment of more effective crop rotation systems. This geographical region is now one of the major producing areas of cotton (Hoffmann et al. 2020) and soybean (Godoy et al. 2015) crops in Brazil, which are severely affected by *C. virescens* (Bergvinson 2005; Albernaz et al. 2012; Karpinski et al. 2014).

It was also found that moths of *H. armigera*, *H. zea*, and *C. virescens* can occur simultaneously in the agricultural landscape of the Cristalina-GO region, but in distinct capture frequencies throughout the tomato crop seasons and years (Fig. 2). Although all three species are stated as polyphagous species, the host availability can be the primary force driving their dynamics. Studies indicate that *H. armigera* populations are mainly associated with cotton and soybean crops and *H. zea* with corn (Leite et al. 2014; Sosa-Gómez et al. 2016; Dourado et al. 2020). In the area of the study, Cristalina (GO), large irrigated processing tomato fields are cultivated from February to October. Within this cropping period, tomato fields share

the landscape with soy fields (October–March), cotton (November–June), and corn (almost the whole year under irrigation). During the dry winter season (June–September) occurs a significant reduction in the presence of cotton and soybean crops; hence, tomato fields become a major crop-sink for these moths. On the other hand, during the tomato crop interseason (November–January), corn and soybean become the major hosts for these moths. Decreasing in abundance of *H. armigera* relative to *H. zea* during 2013 and 2014, as well as the prevalence of *H. zea*, in 2014, was also observed by Specht et al. (2021) through a survey carried out in the Distrito Federal, nearly 140 km from Cristalina (GO). This increase in proportion of *H. zea* relative to *H. armigera* can be also linked to the increase in cultivated area of the preferential host of *H. zea* (area with corn increased 13%) and reduction of area cultivated with soybean (11.2% reduction between 2013 and 2014) for Cristalina County (BDE-Goiás 2020). Besides that, a large portion of this area with corn is also cultivated with sweet corn (non-Bt), which is more susceptible to *H. zea*.

Even though the moth assembly sampled with light traps is relatively diverse in the agricultural landscape (Fig. 2), *H. armigera* predominated as fruit-boring pest in processing tomato fields. The relative prevalence of each Heliiothinae species in this complex agricultural landscape is influenced by the simultaneous presence of other host crops as well as by the pest management system deployed by the farmers (Bergvinson 2005; Dourado et al. 2020; Specht et al. 2021). In corn fields, it is common to find simultaneous infestations of *H. zea* and *H. armigera*, whereas *H. armigera* and *C. virescens* are often found in association in soybean and cotton fields (Pomari-Fernandes et al. 2015; Bentivenha et al. 2016; Dourado et al. 2020). Two factors could explain, at least in part, the prevalence of *H. armigera* larvae in processing tomato fields. Firstly, the suppression of other Heliiothinae species that are more susceptible to insecticides, especially in the larval stage, given the increase in dosages and the frequency of spraying to control fruit-damaging pests. Second, the greater adaptive capacity of *H. armigera* to the dynamics of events in the agricultural system (e.g., adoption of genetically modified corn, soybean, and cotton cultivars with *Bt* technology) and changes in the climatic patterns, including prolonged droughts and summer season with high temperatures (Pomari-Fernandes et al. 2015; Specht et al. 2021).

Even though reproductive isolation between *H. armigera* and *H. zea* has been reported due to the presence of copula-preventing mechanisms (Hardwick 1965), there is the possibility of compatibility and potential production of fertile hybrids in studies using laboratory strains of these species (Laster and Hardee 1995;

Laster and Sheng 1995). The potential presence of *H. armigera* and *H. zea* hybridization events have been suggested in a subset of Brazilian specimens collected under natural conditions (Anderson et al. 2016, 2018; Leite et al. 2017; Lopes et al. 2017; Cordeiro et al. 2020). Therefore, more detailed studies should be carried out with tomato populations aiming to analyze possible introgression events between these two insects.

A growing number of studies on Heliiothinae species have been conducted in commodity crops such as soybeans, cotton, and corn (Albernaz et al. 2012; Czepak et al. 2013; Specht et al. 2013; Tay et al. 2013, 2017; Leite et al. 2014, 2017; Mastrangelo et al. 2014; Bentivenha et al. 2016; Gonçalves et al. 2019). However, to our knowledge, this is the first survey of the diversity of Heliiothinae species associated with major fresh-market and processing tomato areas of Brazil. The presence of a complex *Helicoverpa* species and haplotypes (which are capable of interbreeding to produce fertile offspring) will demand the establishment of fast species diagnostic tools in order to facilitate making control decisions and novel crop management strategies, in order to minimize tomato damage caused by these highly aggressive pest species. In this scenario, the control measures for *H. armigera* infesting tomato crops may include the establishment of new cultural practices, and effective insecticide spray programs based on economic survey (Sousa et al. 2021) and threshold recently defined (Sousa et al. 2020) as well as the employment of biological control (i.e., egg parasitoid *Trichogramma* spp., *Baculovirus*, and *Bacillus thuringiensis*-based products) and cultivars with genetic resistance/tolerance to this pest complex.

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Author Contribution MMF, MENF, and LSB conceived the study. KFASS, AS, MMF, and MENF contributed material preparation and data

collection. MENF, LSB, and JBT analyzed data and conducted statistical analyses. MMF, LSB, MENF, and JBT wrote the manuscript with input from all authors. LSB and MENF contributed funding acquisition and resources. All authors reviewed the MS and approved the final manuscript.

Declarations

Ethical Approval All applicable international, national, and institutional guidelines for the research were considered in the present work.

Conflict of Interest The authors declare no competing interests.

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