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Cínthia Gabriela Garlet

# GENÉTICA DA RESISTÊNCIA DE *Spodoptera frugiperda* (J. E. SMITH, 1797) A CLORPIRIFÓS E RESISTÊNCIA CRUZADA COM OUTROS INSETICIDAS

Santa Maria, RS
2022

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-graduação em Agronomia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Agronomia**.

Orientador: Prof. Dr. Oderlei Bernardi

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**DEDICATÓRIA**

*À minha mãe Anilda Inácia da Silva e minhas irmãs Adriana Garlet e Jaqueline Garlet pelo exemplo, amor, incentivo e apoio incondicional em minha formação pessoal e profissional.*

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*“Não haverá borboletas se a vida não passar por longas e silenciosas metamorfoses”*

*Rubem Alves*

*“Feliz aquele que transfere o que sabe e aprende o que ensina”*

*Cora Coralina*

## RESUMO

### GENÉTICA DA RESISTÊNCIA DE *Spodoptera frugiperda* (J. E. SMITH, 1797) A CLORPIRIFÓS E RESISTÊNCIA CRUZADA COM OUTROS INSETICIDAS

AUTORA: Cínthia Gabriela Garlet
ORIENTADOR: Oderlei Bernardi

A lagarta-do-cartucho, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae), é uma espécie polífaga de relevância global devido aos danos que causa às culturas agrícolas e sua capacidade em evoluir para resistência a táticas de controle. Neste estudo, selecionou-se um genótipo de *S. frugiperda* resistente ao inseticida clorpirifós (inibidor da enzima acetilcolinesterase), a partir de uma população de campo (coletada em área com histórico de falhas de controle com clorpirifós). Após a seleção, foram realizados estudos de caracterização da herança da resistência, estimativa do custo adaptativo, avaliação da resistência cruzada com outros modos de ação e dos mecanismos de resistência usando sinergistas. No estudo de herança da resistência, curvas de dose-resposta foram obtidas em bioensaios de aplicação tópica de clorpirifós com os genótipos resistente (Clorp-R), suscetível (Sus) e progênie $F_1$ dos cruzamentos recíprocos (heterozigotos). Para a avaliação da resistência cruzada, os genótipos Clorp-R e Sus foram expostos a acefato, tiodicarbe, metomil, clorfenapir, flubendiamida, metoxifenozida, espinetoram e teflubenzuron. Para avaliar os possíveis mecanismos de resistência, lagartas do genótipo Clorp-R foram previamente expostas aos sinergistas butóxido de piperonila (PBO), dietil maleato (DEM) e *S,S,S*-tribultiltrifosforotritioato (DEF) e, posteriormente, receberam doses de clorpirifós. O custo adaptativo da resistência foi estimado a partir da comparação dos parâmetros biológicos dos genótipos Clorp-R, Sus e heterozigotos em folhas de algodão, milho, soja e aveia. Os valores de $DL_{50}$ de clorpirifós para os genótipos Clorp-R e Sus foram 24,26 e 0,023 μg i.a./larva, respectivamente, indicando uma razão de resistência >1050 vezes. Os valores de $DL_{50}$ de clorpirifós para os heterozigotos foram 3,34 e 4,00 μg i.a./larva, sugerindo que a herança da resistência é autossômica. Detectou-se também que a resistência de *S. frugiperda* a clorpirifós é influenciada por poucos genes, com um número mínimo de segregações de 1,74 e 1,88. Em plantas e folhas de milho pulverizadas com clorpirifós, os genótipos Clorp-R e heterozigotos apresentaram sobrevivência >95% e >52%, respectivamente, enquanto o genótipo Sus não sobreviveu, indicando que a resistência é incompletamente dominante na dose de bula de clorpirifós. O genótipo Clorp-R apresentou resistência cruzada ao acefato, mas baixa resistência cruzada a tiodicarbe, metomil, clorfenapir, flubendiamida, metoxifenozida, espinetoram e teflubenzuron. O estudo com os sinergistas indicou que a resistência a clorpirifós no genótipo resistente de *S. frugiperda* selecionado para este estudo teve pouca influência de mecanismos metabólicos. Constatou-se também que o genótipo Clorp-R apresentou custo adaptativo da resistência a clorpirifós em todos as plantas hospedeiras avaliadas. Em resumo, a herança da resistência a clorpirifós em *S. frugiperda* é autossômica, incompletamente dominante, poligênica e associada a custo adaptativo. Baixa resistência cruzada entre clorpirifós e inseticidas com outros modos de ação ocorre em *S. frugiperda*. Portanto, a realização de rotação de modos de ação é essencial para retardar a evolução da resistência de *S. frugiperda* a clorpirifós e outros inseticidas.



**Palavras-chave:** Lagarta-do-cartucho. Inibidor da acetilcolinesterase. Herança da Resistência. Manejo da Resistência de Insetos.

## ABSTRACT

### GENETICS OF RESISTANCE OF *Spodoptera frugiperda* (J. E. SMITH, 1797) TO CHLORPYRIFOS AND CROSS-RESISTANCE WITH OTHER INSECTICIDES

AUTHOR: Cínthia Gabriela Garlet
ADVISOR: Oderlei Bernardi

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae), is a polyphagous species of global relevance due to the damage it inflicts to agricultural crops and its ability to evolve resistance to control tactics. In this study, a genotype of *S. frugiperda* resistant to chlorpyrifos (acetylcholinesterase inhibitor), was selected from a field population (collected in an area with a history of control failures by chlorpyrifos). After selection, studies were carried out to characterize the inheritance of resistance, estimate fitness costs, evaluate cross-resistance with other modes of action and mechanisms of resistance using synergists. In the resistance inheritance study, dose-response curves were obtained applying chlorpyrifos in topical bioassays in the resistant (Clorp-R), susceptible (Sus) and $F_1$ progeny from reciprocal crosses (heterozygotes). To assess cross-resistance, the Clorp-R and Sus genotypes were exposed to acephate, thiodicarb, methomyl, chlorfenapyr, flubendiamide, methoxyfenozide, spinetoram and teflubenzuron. To evaluate mechanisms of resistance, larvae from Clorp-R genotype were previously exposed to synergists piperonyl butoxide (PBO), diethyl maleate (DEM) and *S,S,S*-tributyltriphosphorotrithioate (DEF), and then received doses of chlorpyrifos. The fitness costs of resistance were estimated by comparing the biological parameters of the Clorp-R, Sus and heterozygotes in leaves of cotton, maize, soybean, and oats. The $LD_{50}$ values of chlorpyrifos for the Clorp-R and Sus genotypes were 24,26 and 0,023 µg a.i./larva, respectively, representing a resistance ratio >1050-fold. The $LD_{50}$ values of chlorpyrifos for the heterozygotes were 3,34 and 4,00 µg a.i./larva, suggesting that resistance is autosomally inherited. The chlorpyrifos resistance in FAW was influenced by few genes, with the minimum number of segregations being 1.74 and 1.88. On chlorpyrifos-sprayed plants and leaves, Clorp-R and heterozygotes genotypes showed >95% and >52% survival, respectively, whereas the Sus genotype had no survival, indicating that the resistance is incompletely dominant at the field rate of chlorpyrifos. The Clorp-R genotype presented some cross-resistance to acephate, but low cross-resistance to thiodicarb, methomyl, chlorfenapyr, flubendiamide, methoxyfenozide, spinetoram, and teflubenzuron. The synergists did not have relevant effects on the Clorp-R genotype, suggesting a minor role for metabolic resistance. It was also found that the Clorp-R genotype showed fitness costs of the resistance in all host plants evaluated. In summary, the inheritance of resistance to chlorpyrifos in *S. frugiperda* is autosomal, incompletely dominant, polygenic and associated with fitness costs. Low cross-resistance between chlorpyrifos and insecticides with different modes of action occurs in *S. frugiperda*. Therefore, performing the rotation of modes of action is a strategy to delay the evolution of *S. frugiperda* resistance to chlorpyrifos and other insecticides.



**Keywords:** Fall Armyworm. Acetylcholinesterase Inhibitor. Heritability. Insect Resistance Management.

## LISTA DE FIGURAS

### ARTIGO 1



### ARTIGO 2

## LISTA DE TABELAS

### ARTIGO 1



### ARTIGO 2

**SUMÁRIO**

## 1 INTRODUÇÃO

A lagarta-do-cartucho, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae), é uma espécie nativa do Hemisfério Ocidental, tendo como principal hospedeiro o milho (*Zea mays* L.) (POGUE, 2002). *Spodoptera frugiperda* também invadiu os continentes africano, asiático e, mais recentemente, a Oceania (GOERGEN et al., 2016; WANG et al., 2019; CRDC 2020). Na América do Sul, *S. frugiperda* é considerada a espécie mais destrutiva do milho (JUÁREZ et al., 2012), entretanto possui mais de 350 plantas hospedeiras, para as quais também causa perdas econômicas significativas quando da infestação (MONTEZANO et al., 2018; OVERTON et al., 2021). Além da polifagia, o sucesso dessa espécie como praga se deve a outras características bioecológicas, tais como: elevada capacidade reprodutiva (NAGOSHI et al. 2015), várias gerações por ano (BUSATO et al., 2005; FITT et al., 2006; FARIAS et al., 2014) e capacidade de dispersão ao longo da faixa de distribuição de suas plantas hospedeiras (NAGOSHI et al., 2020).

No Brasil, *S. frugiperda* têm sido manejada usando basicamente duas táticas de controle: plantas geneticamente modificadas que expressam proteínas inseticidas da bactéria *Bacillus thuringiensis* Berliner (Bt) e inseticidas químicos e/ou biológicos (BURTET et al., 2017; MURARO et al., 2019; MOSCARDINI et al., 2020). O cultivo contínuo de plantas Bt associado à baixa adoção de estratégias de Manejo da Resistência de Insetos (MRI) contribuiu para a evolução da resistência a várias proteínas Bt expressas em milho (FARIAS et al., 2014; BERNARDI, et al., 2015; SANTOS-AMAYA et al., 2015; BERNARDI et al., 2016; OMOTO et al., 2016). Da mesma forma, o uso generalizado de inseticidas contra *S. frugiperda* ocasionou a seleção de populações resistentes a diferentes grupos químicos, incluindo carbamatos, organofosforados, espinosinas, diamidas, avermectinas e benzoiluréias (DIEZ-RODRÍGUEZ & OMOTO, 2001; CARVALHO et al., 2013; NASCIMENTO et al., 2016; OKUMA et al., 2018; BOLZAN et al., 2019; LIRA et al., 2020; MURARO et al., 2021; NASCIMENTO et al., 2021). Além disso, mecanismos de resistência múltipla a proteínas Bt e inseticidas foram relatados em populações de *S. frugiperda* do Brasil (BOAVENTURA et al., 2020).

A resistência é uma consequência de processos evolutivos básicos, em que alguns indivíduos de uma população de determinada espécie-praga são capazes de sobreviver a exposição inicial a um agente de controle concebido para matá-los e passar essa característica para seus descendentes (GEORGHIOU, 1983). O *Insecticide Resistance Action Committe* (IRAC) definiu resistência como uma mudança hereditária na suscetibilidade de uma população da praga que se reflete na falha repetida de um produto em atingir o nível de controle esperado,

quando utilizado de acordo com a recomendação do rótulo para determinada espécie de inseto-praga.

*Spodoptera frugiperda* é uma espécie com grande capacidade de evoluir para resistência à inseticidas, como é demostrando pelo elevado número de casos em todo o mundo (APRD, 2021). Diante disso, é importante entender, para cada caso, as características genéticas que favorecem a evolução da resistência para assim refinar os programas de MRI (TABASHNIK, 1989; FFRENCH-CONSTANT et al., 2004). Portanto, o conhecimento do padrão de herança, da resistência cruzada, dos mecanismos de resistência e do custo adaptativo da resistência é essencial para subsidiar estratégias proativas de MRI para prolongar a vida útil de plantas Bt e inseticidas utilizados no manejo de insetos (GEORGHIOU & TAYLOR, 1977; ROUSH & MCKENZIE, 1987; GOULD et al., 2018; HAWKINS et al., 2019; PU et al., 2020).

Os inseticidas organofosforados têm sido amplamente utilizados, em todo o mundo, no controle de *S. frugiperda* desde meados do século XX (SIEGFRIED & SCHARF, 2001). Dentre eles, o inibidor da enzima acetilcolinesterase clorpirifós é uma das principais moléculas inseticidas que é utilizada na América do Sul desde a década de 1990 para controle de *S. frugiperda*, mas desde o início dos anos 2000, estudos demostraram uma baixa suscetibilidade de populações brasileiras de *S. frugiperda* ao inseticida (MICHEREFF FILHO et al., 2002; BARROS et al., 2005; CARVALHO et al., 2013). No Rio Grande do Sul, durante a safra de inverno de 2019, relatos de baixa eficácia de clorpirifós no controle de *S. frugiperda*, mesmo após três pulverizações, foram reportados em área de produção de aveia, ocupada anteriormente com a cultura da soja. A partir das lagartas coletadas em área de aveia com histórico de falhas de controle com o uso de clorpirifós, estabeleceu-se, em laboratório, uma população resistente de *S. frugiperda* para a caracterização das bases genéticas da resistência. Diante disso, os estudos apresentados nesta dissertação têm como objetivo:

1) Caracterizar a herança da resistência de *S. frugiperda* a clorpirifós, avaliar a resistência cruzada com outros inseticidas e os mecanismos de resistência mediante o uso dos sinergistas butóxido de piperonila (PBO), dietil maleato (DEM) e *S,S,S*-tribultiltrifosforotritioato (DEF).

2) Estimar o custo adaptativo da resistência de *S. frugiperda* a clorpirifós em diferentes plantas hospedeiras (algodão, milho, soja e aveia).

**2 ARTIGO 1**

# Field-evolved resistance to chlorpyrifos by *Spodoptera frugiperda* (Lepidoptera: Noctuidae): inheritance mode, cross-resistance patterns, and synergism

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## ABSTRACT

**BACKGROUND:** Fall armyworm (FAW), *Spodoptera frugiperda* (Smith), is an economically important pest worldwide. In this study, we selected a genotype of FAW resistant to chlorpyrifos from a field-collected population, characterized the genetic basis of resistance, evaluated cross-resistance and mechanisms of resistance using synergists.

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**RESULTS:** The $LD_{50}$ values of chlorpyrifos for the resistant (Clorp-R) and susceptible (Sus) FAW genotypes were 24.26 and 0.023 μg $larva^{-1}$, respectively, representing a resistance ratio >1,050-fold. The $LD_{50}$ values of chlorpyrifos against heterozygotes were 3.34 and 4.00 μg $larva^{-1}$, suggesting that resistance is autosomally inherited. The chlorpyrifos resistance in FAW was influenced by few genes, with the minimum numbers of segregations being 1.74 and 1.88. On chlorpyrifos-sprayed plants and leaves, Clorp-R and heterozygotes genotypes showed >95% and >52% survival, respectively, whereas the Sus genotype had no survival, indicating that the resistance is incompletely dominant at the field rate of chlorpyrifos. The Clorp-R genotype presented some cross-resistance to acephate, but low cross-resistance to thiodicarb, methomyl, chlorfenapyr, flubendiamide, methoxyfenozide, spinetoram, and teflubenzuron. The synergists piperonyl butoxide, diethyl maleate and *S*,*S*,*S*-tributyl phosphorotrithiotate did not have relevant effects on the Clorp-R genotype, suggesting a minor role for metabolic resistance.

**CONCLUSIONS:** The inheritance of chlorpyrifos resistance in FAW was characterized as autosomal, incompletely dominant and polygenic, with metabolic resistance playing a small role in the detoxification of chlorpyrifos. Low cross-resistance between chlorpyrifos and other Mode of Action (MoA) insecticides occurs in FAW, highlighting the importance of considering the rotation of MoA as a strategy to delay resistance.

**Keywords:** fall armyworm; acetylcholinesterase inhibitor; inheritance pattern; resistance management

## 1 INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* (Smith), is an important polyphagous pest that attacks several agricultural crops worldwide.[1,2] For long time, chemical insecticides have been the principal control tactic for managing FAW; however, the deployment of genetically engineered plants expressing insecticidal proteins from *Bacillus thuringiensis* (Bt) Berliner providing resistance against FAW have revolutionized its management.[3] In South American countries, during the 2019/2020 maize season, nearly 30 million hectares were cultivated with Bt maize (~20 million hectares in Brazil), representing more than 80% of the total area occupied by this crop.[4] This high adoption of Bt maize reduced the use of insecticides against FAW.[5]

The wide adoption of Bt maize and low compliance with refuge favors the evolution of resistance to Bt proteins by FAW.[6–8] Field evolved resistance in FAW increased the applications of older and new insecticides against this pest in maize fields in Brazil and other South American countries.[3,5] In the past few decades, chemical companies registered new insecticides,[9,10] but older insecticides remain widely used against several pest species, mainly for their low price.[11–13] Among the oldest insecticides commercially available, organophosphates are still one of the major groups used for controlling FAW in various crops.[14,15]

Organophosphates act on the nervous system killing insects by inhibiting the acetylcholinesterase enzyme.[16–18] The organophosphate chlorpyrifos has widely used against FAW in South America since 1990s, but from the early 2000s studies have stated a low susceptibility of FAW to this mode of action in Brazil.[14,19,20] Resistance to chlorpyrifos was reported in populations of FAW in the United States, Puerto Rico, México, and China.[21–23] Furthermore, the resistance of FAW was also documented for other organophosphates such as acephate in Puerto Rico[24] and to diazinon, dichlorvos, malathion, and methyl parathion in the United States.[21]

The FAW is a pest with high capacity to develop resistance to pesticides, as demonstrated by the wide range of cases worldwide.[25] For each case, it is important to understand the genetic characteristics that favor the resistance evolution to improve resistance management programs.[26,27] Therefore, the knowledge of the inheritance mode, cross-resistance patterns, and mechanisms of resistance is essential to design or modelling proactive and more effective resistance management strategies to prolong the lifetime of chemical insecticides.[28–32]

The genetic basis of FAW resistance was studied for lambda-cyhalothrin,[33,34] methomyl,[34] lufenuron,[35] spinosad,[36] chlorantraniliprole,[37] spinetoram,[38] teflubenzuron,[39] and emamectin benzoate.[40] So far, no studies about inheritance patterns of chlorpyrifos resistance in FAW have been published. Given this knowledge gap, we selected a genotype of FAW resistant to chlorpyrifos, reported the genetic basis of resistance, and evaluated cross-resistance patterns to other insecticides and the effects of synergists on the resistance.

## 2 MATERIAL AND METHODS

### 2.1 Collection and rearing of insects

A population of FAW reared in the laboratory for eight years without insecticide exposure was used as a source of susceptible insects (hereafter Sus). A field population of FAW was collected from a grain production farm in oats (*Avena sativa* L.) in Tupanciretã, Rio Grande do Sul, Brazil (18°37’25” S and 52°54’13” W) in July of 2019. In this area, three applications of chlorpyrifos (Lorsban®, 480 BR, Dow AgroSciences Industrial Ltda., Santo Amaro, SP, Brazil) were performed against FAW, but control failures were detected. From this area, a total of 54 surviving FAW larvae were collected, transported to the laboratory, and in subsequent generations selected for resistance to chlorpyrifos. Larvae from the susceptible and the field-collected population were maintained under laboratory conditions at 25 ± 2°C,

65 ± 5% RH, 14:10 h (L:D) photoperiod and reared on an artificial diet based on white beans, wheat germ, and yeast.[41]

### 2.2 Selection and toxicological bioassays

The field-collected population of FAW was selected for resistance to chlorpyrifos during 14 consecutive generations using two methods: diet-overlay and topical bioassays. During the $F_1$ generation, larvae were maintained on an artificial diet.[41] From $F_2$ to $F_8$, early L3 larvae were exposed to selection pressure at a single dose of 3,200 ppm of chlorpyrifos (Lorsban® 480 BR) diluted in water and applied over the diet surface (equivalent to 51 μg active ingredient (a.i.) $cm^{-2}$) as described by Garlet *et al.*[42] From $F_9$ to $F_{15}$, the selection process was performed in topical bioassays using the technical grade chlorpyrifos (98% purity, Sigma Aldrich, São Paulo, SP, Brazil) diluted in acetone (99.5% purity; Sigma Aldrich, São Paulo, SP, Brazil) at a single dose of 10 μg chlorpyrifos $larva^{-1}$. This concentration was applied to the dorsal thoracic region of early L3 larvae (1 μL $larva^{-1}$) using a hand microapplicator (Burkard Manufacturing, Rickmansworth, England). Later, larvae were placed in 24-well acrylic plates (Costar®, São Paulo, SP, Brazil) containing artificial diet[41] and maintained at 25 ± 2ºC, 65 ± 5% RH, 14:10 h (L:D) photoperiod. After 48 h post-exposure, surviving larvae were transferred to artificial diet[41] without insecticide to complete its larval development and to establish the chlorpyrifos-resistant FAW colony (hereafter Clorp-R). Larvae from $F_{16}$ to $F_{18}$ generations of the Clorp-R genotype were used to perform the studies here presented.

### 2.3 Characterization of resistance to chlorpyrifos in FAW

Early L3 larvae from Clorp-R and Sus genotypes were exposed from six to seven logarithmically spaced doses of technical grade chlorpyrifos (0.0056 to 100 μg a.i. $larva^{-1}$) in topical bioassays as previously described. The experimental design was completely

randomized with four to five replicates of 24 larvae replicate$^{-1}$, totaling 96–120 larvae tested dose$^{-1}$. Mortality was assessed after 48 h post-exposure. Mortality data of both genotypes were used to estimate $LD_{50}$ values (lethal dose required to kill 50% of larvae tested) and respective 95% confidence intervals (CIs) using Probit analysis[43] with the PROC PROBIT procedure in SAS® 9.1.[44] Tests for parallelism and equality were performed to compare the angular and linear coefficients of regression lines of both genotypes as described by Robertson *et al*.[43] The resistance ratio was calculated by dividing the $LD_{50}$ of the Clorp-R genotype by the correspondent value of the Sus genotype.

**2.4 Genetic of resistance to chlorpyrifos in FAW**

Virgin adults from Clorp-R and Sus genotypes were crossed to obtain progenies from two reciprocal crosses (Clorp-R♀ × Sus♂ and Clorp-R♂ × Sus♀). The adults (25 pairs cross$^{-1}$) were maintained in PVC cages (23-cm in height × 10-cm in diameter) lined with white paper for oviposition and fed with a 10% honey solution. The $F_1$ larvae were reared on an artificial diet[41] until the early L3 stage, and then exposed to toxicological bioassays as previously described. Five doses of technical grade chlorpyrifos, spaced on a logarithmic scale, between 1 to 10 μg a.i. larva$^{-1}$, were tested in both progenies. The bioassay procedure, experimental design and estimation of LD values were performed as described above.

Concentration-mortality data from heterozygotes ($F_1$), Sus and Clorp-R genotypes were also used to estimate the dominance level ($D_{ML}$) of chlorpyrifos resistance following the equation [1] proposed by Bourguet *et al*.[45]:

[1] $$D_{ML} = (M_{RS} - M_{SS})/(M_{RR} - M_{SS})$$

where $M_{RS}$, $M_{SS}$ and $M_{RR}$ are the mortalities of the heterozygotes ($F_1$), Sus and Clorp-R genotypes, respectively, in different doses of chlorpyrifos. $D_{ML}$ values range from 0 (completely recessive resistance) to 1 (completely dominant resistance).

Additionally, the degree of dominance (*D*) was also calculated by the equation [2] proposed by Stone[46]:

[2] $$D = (2X_F - X_R - X_S)/(X_R - X_S)$$

where $X_F$, $X_R$ and $X_S$ are logarithms ($\log_{10}$) of the $LD_{50}$ estimated for the heterozygotes ($F_1$), Clorp-R, and Sus genotypes, respectively. Values of *D* range from –1 to 1: if $D = 1$, it shows complete dominance; if $0 < D < 1$ it shows incomplete dominance; if $-1 < D < 0$ it shows incomplete recessivity; and $D = -1$ indicates complete recessivity.

Four backcrosses (25 pairs backcross$^{-1}$) between $F_1$ progenies (heterozygotes) and the Sus genotype (parental phenotypically more distinct from heterozygotes) were also performed to estimate the number of genes controlling chlorpyrifos resistance as suggested by Tsukamoto[47] and Roush and Daly.[48] For this study, early L3 larvae from backcrosses were subjected to nine doses of technical grade chlorpyrifos (0.056 to 5.6 µg a.i. larva$^{-1}$) in three replicates of 24 larvae dose$^{-1}$, following the bioassay method previously described. Chi-square analyses of backcrosses were used to test the hypothesis of monogenic inheritance using equation [3] proposed by Sokal and Rohlf[49]:

[3] $$\chi 2 = (Ni - pni)^2)/pqni$$

where $Ni$ is the mortality observed in the backcrossed larvae, and $p$ is the expected mortality calculated from the Mendelian model [equation 4] suggested by Georghiou[50], *ni* is the number of larvae tested and $q = 1 - p$:

[4] $$p = (a + b)/2$$

where $a$ represents the mortality in the parental genotype Sus, and $b$ is the mortality of the heterozygous genotype. Significant differences among observed and expected mortalities would reject the hypothesis of monogenic inheritance.

The minimum number of effective genes influencing the chlorpyrifos resistance in FAW was calculated using Lande's[51] method [equation 5]:

[5] $$n_E = (X_{RR} - X_{SS})^2/(8\sigma_S^2)$$

where $X_{RR}$ and $X_{SS}$ are the $\log_{10}$ of the $LD_{50}$ of the Clorp-R and Sus genotypes, respectively, and where $\sigma_S^2$ estimated as equation [6][51]:

[6] $$\sigma_s^2 = \sigma_{B1}^2 + \sigma_{B2}^2 - [\sigma_{F1}^2 + 0.5\sigma_{RR}^2 + 0.5\sigma_{SS}^2]$$

where $\sigma_{B1}^2$ , $\sigma_{B2}^2$ , $\sigma_{F1}^2$ , $\sigma_{RR}^2$ and $\sigma_{SS}^2$ are the phenotypic variances of the backcrosses, heterozygotes, resistant and susceptible genotypes, respectively. Variances were estimated by the inverse of the slope squared.

**2.5 Functional dominance to chlorpyrifos in FAW**

In this study, non-Bt maize (30F35, DuPont Pioneer, Santa Rosa, RS, Brazil) were sown in 5-L plastic pots (2 seeds/pot) containing soil and composted plant material at a 2:1 ratio in a greenhouse. At the $V_{3–4}$ growth stage, plants were sprayed with the field dose of chlorpyrifos (Lorsban®, 480 BR) at a rate of 600 mL $ha^{-1}$ diluted in 150 L of water. Unsprayed plants were used as a control treatment. After spraying, each maize plant had its whorls infested with a single early L3 larva from Clorp-R, Sus, or heterozygotes (one larva $plant^{-1}$). To prevent larvae mobility, each infested plant was covered with a sheer fabric supported by an aluminum structure. The experimental design was completely randomized, with 100 early L3 larvae tested insect $genotype^{-1}$ (50 on plants treated with chlorpyrifos and 50 on untreated plants). Each pot containing two maize plants was considered one replicate, totalizing 25 replicates insect $genotype^{-1}$ and treatment (sprayed and unsprayed). Maize leaves were also removed from sprayed and unsprayed plants and, in the laboratory, cut into 15 $cm^2$ pieces and placed over a gelled mixture of 2.5% agar-water in 50 mL plastic pots (one piece $pot^{-1}$). Each pot was infested with a single early L3 larva from Clorp-R, Sus, or heterozygotes genotypes. From 40 to 60 larvae $genotype^{-1}$ $treatment^{-1}$ were tested (each pot was considered a replicate).

Larval survival was evaluated after three days. Data were subjected to nonparametric analysis using the Kruskal-Wallis test with the PROC NPAR1WAY procedure in SAS® 9.1.[44]

### 2.6 Cross-resistance between chlorpyrifos and other insecticides in FAW

Toxicological bioassays were performed with early L3 larvae of Clorp-R and Sus genotypes exposed to different modes of action insecticides. Using topical bioassays as previously described, cross-resistance patterns were evaluated to acephate (99.5% purity; Sigma Aldrich, São Paulo, SP, Brazil), methomyl (98% purity; Sigma Aldrich, São Paulo, SP, Brazil), and thiodicarb (99.8% purity; Sigma Aldrich, São Paulo, SP, Brazil). Concentration-response bioassays were also carried out using diet-overlay bioassays as described by Garlet *et al*.[42] In this bioassay method the insecticides chlorfenapyr (Pirate® 240 g a.i. $L^{-1}$, BASF SA, São Paulo, SP, Brazil), flubendiamide (Belt® 480 g a.i. $L^{-1}$, Bayer CropScience Ltda., São Paulo, SP, Brazil), methoxyfenozide (Intrepid® 50 g a.i. $L^{-1}$, Dow AgroSciences Industrial Ltda., Santo Amaro, SP, Brazil), spinetoram (Exalt® 120 g a.i. $L^{-1}$, Dow AgroSciences Industrial Ltda., Santo Amaro, SP, Brazil), and teflubenzuron (Nomolt® 150 g a.i. $L^{-1}$, BASF S.A., São Paulo, SP, Brazil) were tested. For both genotypes, three to five replicates of 24 larvae $replicate^{-1}$, totaling 72–120 larvae tested $dose^{-1}$. Mortality data were subjected to Probit analysis[43] and parallelism and equality tests as earlier described.

### 2.7 Synergist bioassays

The lethality of chlorpyrifos against Clorp-R and Sus genotypes were evaluated in the presence of three synergists: piperonyl butoxide (PBO), an inhibitor of cytochrome P450s; diethyl maleate (DEM), an inhibitor of glutathione S-transferases; and *S*,*S*,*S*-tributyl phosphorotrithiotate (DEF), an inhibitor of esterases. Non-lethal doses of PBO (0.1 μg $larva^{-1}$), DEM (1 μg $larva^{-1}$), and DEF (0.32 μg $larva^{-1}$) were stablished in preliminary bioassays.

Two hours prior to chlorpyrifos exposure, PBO, DEM and DEF were diluted in acetone at the doses mentioned above and applied onto the pronotum of early L3 larvae from Clorp-R and Sus genotypes using a hand microapplicator (1 μL larva$^{-1}$). Then, six doses of technical grade chlorpyrifos (0.0032 to 56 μg a.i. larva$^{-1}$) were applied topically as earlier described. Acetone alone was used as a control treatment. Three to four replicates of 24 larvae dose$^{-1}$ of chlorpyrifos (with or without synergist) were used, totaling 72–96 larvae tested dose$^{-1}$. Mortality was evaluated after 48 h post-exposure. Data were subjected to Probit analysis[43] to estimate the $LD_{50}$ values and 95% CIs as earlier described. Synergistic ratios were calculated by dividing the $LD_{50}$ of the genotype treated with insecticide alone by the $LD_{50}$ of the genotype treated with synergist + insecticide. We also calculated resistance ratios by dividing the $LD_{50}$ value of the Clorp-R genotype by the corresponding $LD_{50}$ value of the Sus genotype in each combination of chlorpyrifos + synergist.

## 3 RESULTS

### 3.1 Selection and characterization of resistance to chlorpyrifos in FAW

The field-collected population of FAW in the first eight generations of selection presented survivorship of up to 30% when exposed to 3,200 ppm of a commercial formulation of chlorpyrifos applied on the diet surface. From $F_9$ to $F_{15}$, larval survivorship ranged from 33% to 38% in topical bioassays using 10 μg chlorpyrifos larva$^{-1}$ (equivalent to 10,000 ppm larva$^{-1}$), whereas the mortality of the susceptible genotype (Sus) was 100% in all bioassays. These results revealed that the field population of FAW used to establish the chlorpyrifos-resistant colony (Clorp-R) displayed a significant reduction in susceptibility to chlorpyrifos.

The $LD_{50}$ (95% CI) values of chlorpyrifos against the Clorp-R genotype at $F_{16}$ generation was 24.26 (17.90–32.07) μg a.i. larva$^{-1}$, whereas for the Sus genotype was 0.023 (0.020–

0.027) μg a.i. larva$^{-1}$, indicating a resistance ratio of 1,054.78-fold (Table 1). Both genotypes showed non-significant chi-square values in the goodness-of-fit test ($P > 0.05$), indicating that concentration-mortality data adjusted the Probit model (Table 1). Clorp-R differed from the Sus genotype by the equality test ($\chi2 = 354.01$; $df = 2$; $P < 0.001$) but was similar according to the parallelism test ($\chi2 = 1.03$; $df = 1$; $P = 0.310$), indicating that mortality curves had distinct intercepts, but similar slopes.

### 3.2 Genetic of resistance to chlorpyrifos in FAW

The $F_1$ progenies from reciprocal crosses (Clorp-R♀ × Sus♂ and Clorp-R♂ × Sus♀) presented similar susceptibility to chlorpyrifos with $LD_{50}$ values (95% CI) of 3.34 (2.86–3.85) and 4.00 (3.54–4.53) μg a.i. larva$^{-1}$, respectively (Table 1). Similar susceptibility was also indicated by equality ($\chi2 = 4.50$; $df = 2$; $P = 0.105$) and parallelism ($\chi2 = 0.05$; $df = 1$; $P = 0.820$) tests, that demonstrated that the heterozygotes had similar mortality curves. The resistance ratios for heterozygotes were 145.22 and 173.91-fold, based on the Sus genotype. The overlapping of the 95% CIs of both heterozygotes suggested an autosomal inheritance of resistance to chlorpyrifos (Table 1).

The degrees of dominance ($D_{ML}$) calculated by the Bourguet *et al.*[45] method indicated an incompletely recessive resistance ($D_{ML} < 0.47$) at the highest doses tested (5.6 and 10 μg a.i. larva$^{-1}$) (Fig. 1). However, at lower doses than 3.2 μg a.i. larva$^{-1}$, the inheritance was an incompletely dominant trait ($D_{ML} > 0.58$) (Fig. 1). By Stone's[46] method, the dominance levels were 0.42 and 0.48 for the $F_1$ progenies from Clorp-R♀ × Sus♂ and Clorp-R♂ × Sus♀, respectively. These values also indicated that the resistance to chlorpyrifos in FAW is an incompletely dominant trait.

Chi-square analyses of backcrosses revealed significant differences between observed and expected mortalities in all doses of chlorpyrifos tested (Table 2). Therefore, the direct

hypothesis test for the monogenic effect was rejected, characterizing the resistance to chlorpyrifos as a polygenic trait. According to Lande's[51] method, the minimum number of independently segregating loci with equal or additive contribution were 1.74 and 1.88, which supports the conclusion that resistance to chlorpyrifos in FAW is associated with multiple genes.

### 3.3 Functional dominance to chlorpyrifos in FAW

Significant differences in larval survival were detected in FAW genotypes exposed to non-Bt maize treated with the field dose of chlorpyrifos (whole plants: $\chi2 = 64.26$; $df = 3$; $P < 0.0001$ and excised leaves: $\chi2 = 108.55$; $df = 3$; $P < 0.0001$). Clorp-R larvae on whole plant and excised leaves treated with chlorpyrifos showed higher survival (>95%) than the heterozygotes (52% to 60%) and Sus (no survival) genotypes (Fig. 2). By contrast, Clorp-R, Sus and heterozygotes had a similar survival (from 87.5% to 98%) on untreated maize (whole plants: $\chi2 = 3.06$; $df = 3$; $P = 0.3819$ and excised leaves: $\chi2 = 3.11$; $df = 3$; $P = 0.3753$) (Fig. 2). These results suggested that resistance to chlorpyrifos is an incompletely dominant trait under field conditions ($D_{ML} > 0.52$ according to Bourguet *et al.*[45]).

### 3.4 Cross-resistance between chlorpyrifos and other insecticides in FAW

The Clorp-R genotype presented a resistance ratio of 28.74-fold to acephate, relative to the Sus genotype, indicating the presence of cross-resistance (Table 3). For this insecticide, the Clorp-R genotype differed from the Sus genotype by the equality test ($\chi2 = 284$; $df = 2$; $P < 0.001$), showing that mortality curves had distinct intercepts, but curves had similar slopes according to parallelism test ($\chi2 = 3.68$; $df = 1$; $P = 0.055$). Contrary to previous results, the Clorp-R genotype showed low cross-resistance to thiodicarb, methomyl, chlorfenapyr, flubendiamide, methoxyfenozide, spinetoram, and teflubenzuron, with a resistance ratio

<4.48-fold, relative to the Sus genotype (Table 3). Mortality curves of Clorp-R and Sus genotypes exposed to flubendiamide and spinetoram had distinct intercepts and slopes according to equality (flubendiamide: $\chi2 = 81.54$; $df = 2$; $P < 0.001$ and spinetoram: $\chi2 = 128$; $df = 2$; $P < 0.001$) and parallelism (flubendiamide: $\chi2 = 6.14$; $df = 1$; $P = 0.013$ and spinetoram: 15.01; $df = 1$; $P < 0.001$) tests. Mortality curves of Clorp-R and Sus genotypes also had distinct intercepts, as indicated by the equality test, when exposed to thiodicarb ($\chi2 = 17.06$; $df = 2$; $P < 0.001$), methomyl ($\chi2 = 134$; $df = 2$; $P < 0.001$), chlorfenapyr ($\chi2 = 58.13$; $df = 2$; $P < 0.001$), methoxyfenozide ($\chi2 = 145$; $df = 2$; $P < 0.001$), and teflubenzuron ($\chi2 = 29.74$; $df = 2$; $P < 0.001$). However, the parallelism test demonstrates that Clorp-R and Sus genotypes presented similar slopes for thiodicarb ($\chi2 = 1.14$; $df = 1$; $P = 0.285$), methomyl ($\chi2 = 0.01$; $df = 1$; $P = 0.941$), chlorfenapyr ($\chi2 = 0.25$; $df = 1$; $P = 0.620$), methoxyfenozide ($\chi2 = 0.09$; $df = 1$; $P = 0.760$), and teflubenzuron ($\chi2 = 0.04$; $df = 1$; $P = 0.843$).

### 3.5 Synergist effects on FAW

The $LD_{50}$ values of chlorpyrifos did not differ (based on the overlap of 95% CIs) for Sus larvae pre-exposed or without exposure to the synergists PBO, DEM and DEF (Table 4). The Clorp-R genotype pre-exposed to the synergist PBO showed significant differences (based on the overlap of 95% CIs) from the other treatments (Table 4). However, all synergists slightly increased the toxicity of chlorpyrifos by 1.28 to 2.13-fold for the Sus and Clorp-R genotypes. The resistance ratio of the Clorp-R genotype to chlorpyrifos was 712.5-, 962.78- and >1,064-fold when previously treated with PBO, DEM and DEF, respectively, whereas it was >1,050-fold for larvae treated with synergists only (Table 4). These results suggested a small contribution of metabolic resistance to chlorpyrifos in the selected FAW genotype.

## 4 DISCUSSION

The FAW genotype collected from oat field with chlorpyrifos control failures history presented a resistance ratio of >1,050-fold after 16 generations of selection. The resistance in the chlorpyrifos-resistant FAW genotype was confirmed by its high larval survival on whole maize plants and excised leaves sprayed with the field dose of chlorpyrifos, demonstrating that this phenotype presents a genetically based decrease in susceptibility to this mode of action caused by exposure to the pesticide in the field. The resistance ratio to chlorpyrifos estimated for the selected FAW genotype was greater than that reported in field populations of FAW from United States (25-fold),[21] Mexico (20-fold) and Puerto Rico (47-fold),[22] but near to that found in FAW populations from China (615- to 1,068-fold).[23]

The inheritance patterns of chlorpyrifos resistance in the selected FAW genotype were characterized as autosomal, incompletely dominant, and polygenic. The high survival of heterozygotes in the field rate of chlorpyrifos also confirmed that the resistance is an incompletely dominant trait in the field. Similar inheritance patterns of resistance to chlorpyrifos have been reported in *Blattella germanica* (L.),[52] *Tetranychus urticae* (Koch),[53] and *Plutella xylostella* (L.).[54] In contrast, the resistance to chlorpyrifos in *Culex pipiens* (L.) and *Apolygus lucorum* (Meyer-Dür) was controlled by a single major gene,[55,56] whereas in *Phenacoccus solenopsis* (Tinsley) was characterized as an incompletely recessive trait.[57] Autosomal and polygenic resistance was also found in FAW resistant to methomyl,[34] lufenuron,[35] spinosad,[36] spinetoram,[38] teflubenzuron,[39] and emamectin benzoate.[40] Contrary to our results, FAW showed incompletely recessive resistance to previous insecticides, with the exception of emamectin benzoate (incompletely dominant inheritance), and monogenic resistance to lambda-cyhalothrin[33] and chlorantraniliprole.[37]

The selected FAW genotype had low cross-resistance to other mode-of-action insecticides. These findings suggested that the mechanisms that confer resistance to chlorpyrifos in FAW are not the same as that affecting the toxicity to thiodicarb, methomyl, chlorfenapyr, flubendiamide, spinetoram, methoxyfenozide, and teflubenzuron. However, the chlorpyrifos-resistant FAW genotype presented cross-resistance to acephate, an acetylcholinesterase inhibitor commonly used against sucking pests. Cross-resistance between inhibitors of the acetylcholinesterase enzyme in FAW was also verified for diazinon, malathion, methyl parathion, and thiodicarb.[22,58] The presence of cross-resistance among organophosphates can be explained by the similarity between the chemical structures,[18] binding sites,[59] and resistance mechanisms including overexpression of multiple detoxification enzymes and target site insensitivity mutations.[60–62]

The synergism studies showed that PBO, DEM and DEF had low effects on the lethality of chlorpyrifos for the selected FAW genotype. These results suggested that enzyme families such as cytochrome P450s, glutathione S-transferases, and esterases play a minor role in chlorpyrifos detoxification. Previous biochemical and molecular studies have shown that insensitivity of target-sites is a mechanism of resistance to organophosphates in FAW.[20,24,60–62] However, the metabolic mechanism cannot be excluded because overexpressions of glutathione S-transferases, cytochrome P450s and carboxylesterases were also found in FAW, conferring resistance to chlorpyrifos.[20,58] Both target-site and metabolic mechanisms also underlie the resistance to chlorpyrifos in *Laodelphax striatellus* (Fallen),[63] and *Liriomyza sativae* (Blanchard).[64] Nevertheless, in *Chilo suppressalis* (Walker),[65] *Culex quinquefasciatus* (Say),[66] and *A. lucorum*[56] the resistance to chlorpyrifos was not associated with metabolic resistance, whereas in *T. urticae, P. solenopsis*, *Bemisia tabaci* Gennadius, and *Spodoptera exigua* (Hübner) was related with detoxification enzymes.[53,57,67,68] Further biochemical and

molecular studies are necessary to better understand the mechanism of chlorpyrifos resistance in the selected FAW genotype.

From a resistance management viewpoint, our findings highlight the importance of considering the rotation of insecticides with different modes of action to delay or prevent the resistance evolution to chlorpyrifos in the field population of FAW. Alternatively, non-insecticide management strategies such as Bt plants, cultural control, egg parasitoids and baculovirus-based biopesticides are also important for the sustainability of all control strategies used against FAW, where there are intensive agricultural systems. We hope that the genetic basis of FAW resistance to chlorpyrifos reported here provides new insights for design resistance management programs for this notorious pest of global relevance.

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**Table 1.** Responses of Clorp-R, Sus and heterozygotes genotypes of FAW to chlorpyrifos.

| FAW genotype | *n* | Fit of probit lines | | | $LD_{50}$ (95% CI)[b] | $RR_{50}$[c] |
|---|---|---|---|---|---|---|
| | | Slope ± SE | $\chi 2$ (*df*[a]) | *P* | | |
| Clorp-R | 864 | 1.86 ± 0.20 | 9.63 (5) | 0.09 | 24.26 (17.90–32.07) | 1,054.78 |
| Clorp-R♀ × Sus♂ | 600 | 2.57 ± 0.26 | 2.47 (3) | 0.48 | 3.34 (2.86– 3.85) | 145.22 |
| Clorp-R♂ × Sus♀ | 624 | 2.49 ± 0.23 | 3.60 (3) | 0.31 | 4.00 (3.54–4.53) | 173.91 |
| Sus | 744 | 2.01 ± 0.17 | 3.35 (4) | 0.50 | 0.023 (0.020–0.027) | - |

[a]*df* = degrees of freedom.

[b]$LD_{50}$ (µg a.i. $larva^{-1}$) and 95% confidence interval (95% CI).

[c]Resistance Ratio (RR) = $LD_{50}$ of Clorp-R or heterozygotes genotypes/$LD_{50}$ of Sus genotype.

**Table 2.** Direct test of monogenic inheritance for resistance to chlorpyrifos in FAW by comparing expected and observed percent mortality of the backcrosses between the $F_1$ progeny of the reciprocal crosses ($H_1$ = Clorp-R♀ × Sus♂ and $H_2$ = Clorp-R♂ × Sus♀) and Sus genotypes.

| Concentration (µg a.i. larva$^{-1}$) | $H_1$♀ × Sus♂ | | | $H_1$♂ × Sus♀ | | | $H_2$♀ × Sus♂ | | | $H_2$♂ × Sus♀ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Obs[a] | Exp[b] | χ2 | Obs[a] | Exp[b] | χ2 | Obs[a] | Exp[b] | χ2 | Obs[a] | Exp[b] | χ2 |
| 0.056 | 9.7 | 34.4 | 37.4* | 8.3 | 34.4 | 37.5* | 13.9 | 34.4 | 37.3* | 12.5 | 34.4 | 37.3* |
| 0.10 | 15.3 | 44.3 | 56.6* | 19.4 | 44.3 | 56.5* | 22.2 | 44.3 | 56.4* | 20.8 | 44.3 | 56.5* |
| 0.18 | 29.2 | 50.0 | 70.8* | 27.8 | 50.0 | 70.9* | 30.6 | 50.0 | 70.8* | 23.6 | 50.0 | 71.1* |
| 0.32 | 33.3 | 50.0 | 70.7* | 54.2 | 50.0 | 69.8* | 45.8 | 50.0 | 70.2* | 54.2 | 50.0 | 69.8* |
| 0.56 | 43.1 | 50.0 | 70.3* | 68.1 | 50.0 | 69.3* | 54.2 | 50.0 | 69.8* | 63.9 | 50.0 | 69.5* |
| 1.0 | 63.9 | 55.2 | 85.9* | 70.8 | 55.2 | 85.6* | 69.4 | 54.2 | 82.1* | 75.0 | 54.2 | 81.8* |
| 1.8 | 70.8 | 65.6 | 133.4* | 75.0 | 65.6 | 133.1* | 72.2 | 58.3 | 97.4* | 88.9 | 58.3 | 96.6* |
| 3.2 | 76.4 | 71.8 | 177.7* | 90.3 | 71.8 | 176.7* | 75.0 | 71.6 | 176.0* | 90.3 | 71.6 | 174.9* |
| 5.6 | 84.7 | 85.4 | 410.2* | 91.7 | 85.4 | 409.2* | 84.7 | 79.1 | 263.8* | 93.1 | 79.1 | 263.1* |

[a]Observed mortality.

[b]Expected mortality, based on Mendelian inheritance.

*Percent mortalities differed significantly at $P < 0.05$.

**Table 3.** Susceptibility of Clorp-R and Sus genotypes of FAW to a different mode-of-action insecticides.

| FAW genotype | *n* | Fit of probit lines | | | $LD_{50}$ (95% CI) [b] | $RR_{50}$ [c] |
|---|---|---|---|---|---|---|
| | | Slope ± SE | $\chi 2$ (*df*[a]) | *P* | | |
| **Acephate** – *Inhibitor of acetilcholinesterase* | | | | | | |
| Clorp-R | 765 | 1.91 ± 0.22 | 3.69 (5) | 0.59 | 9.77 (7.93–11.68) | 28.74 |
| Sus | 672 | 2.17 ± 0.21 | 1.97 (4) | 0.74 | 0.34 (0.29–0.39) | - |
| **Thiodicarb** – *Inhibitor of acetilcholinesterase* | | | | | | |
| Clorp-R | 576 | 1.59 ± 0.21 | 2.34 (5) | 0.80 | 0.50 (0.37–0.66) | 1.92 |
| Sus | 504 | 1.36 ± 0.13 | 4.35 (4) | 0.36 | 0.26 (0.20–0.34) | - |
| **Methomyl** – *Inhibitor of acetilcholinesterase* | | | | | | |
| Clorp-R | 644 | 2.33 ±0.18 | 5.46 (4) | 0.24 | 0.12 (0.11–0.14) | 4.00 |
| Sus | 598 | 2.32 ± 0.23 | 2.25 (3) | 0.52 | 0.03 (0.03–0.04) | - |
| **Chlorfenapyr** – *Uncoupler of mitochondrial oxidative phosphorylation* | | | | | | |
| Clorp-R | 623 | 3.49 ± 0.36 | 1.51 (4) | 0.82 | 0.20 (0.17–0.22) | 1.82 |
| Sus | 552 | 3.72 ± 0.43 | 5.34 (4) | 0.25 | 0.11 (0.09–0.12) | - |
| **Flubendiamide** – *Ryanodine receptor modulator* | | | | | | |
| Clorp-R | 744 | 1.51 ± 0.11 | 9.13 (5) | 0.10 | 0.40 (0.33–0.49) | 3.08 |
| Sus | 648 | 2.00 ± 0.19 | 3.86 (4) | 0.42 | 0.13 (0.11–0.15) | - |
| **Methoxyfenozide** – *Ecdysone receptor agonist* | | | | | | |
| Clorp-R | 768 | 2.21 ± 0.18 | 3.29 (4) | 0.51 | 1.03 (0.87–1.20) | 4.48 |
| Sus | 624 | 2.21 ± 0.20 | 3.06 (4) | 0.55 | 0.23 (0.19–0.27) | - |
| **Spinetoram** – *Nicotinic acetylcholine receptor allosteric modulator* | | | | | | |
| Clorp-R | 576 | 2.70 ± 0.25 | 3.66 (3) | 0.30 | 0.048 (0.042–0.055) | 4.36 |
| Sus | 432 | 2.83 ± 0.33 | 4.09 (3) | 0.25 | 0.011 (0.010–0.013) | - |
| **Teflubenzuron** – *Inhibitor of chitin biosynthesis* | | | | | | |
| Clorp-R | 666 | 2.28 ± 0.20 | 2.24 (4) | 0.69 | 0.023 (0.020–0.027) | 1.92 |
| Sus | 672 | 1.69 ± 0.28 | 7.83 (4) | 0.10 | 0.012 (0.006–0.019) | - |

[a] *df* = degrees of freedom.

[b] $LD_{50}$ (µg a.i. $larva^{-1}$) and 95% confidence interval (95% CI).

[c] Resistance Ratio (RR) = $LD_{50}$ of tested insecticide on Clorp-R genotype/$LD_{50}$ of tested insecticide on Sus genotype.

**Table 4.** Toxicity of chlorpyrifos with and without PBO, DEM or DEF to Clorp-R and Sus genotypes of FAW.

| FAW genotype | Treatment | Fit of probit lines | | | $LD_{50}$ (95% CI) [b] | SR[c] | RR[d] |
|---|---|---|---|---|---|---|---|
| | | Slope ± SE | $\chi 2$ (*df*[a]) | *P* | | | |
| Clorp-R | Chlorpyrifos | 1.86 ± 0.20 | 9.63 (5) | 0.09 | 24.26 (17.90–32.07) | - | 1,054.78 |
| | Chlorpyrifos + PBO | 2.56 ± 0.27 | 4.29 (4) | 0.37 | 11.40 (9.40–13.40) | 2.13 | 712.5 |
| | Chlorpyrifos + DEM | 1.78 ± 0.18 | 5.76 (4) | 0.22 | 17.33 (14.40–20.96) | 1.41 | 962.78 |
| | Chlorpyrifos + DEF | 1.75 ± 0.19 | 7.17 (4) | 0.13 | 17.03 (13.88–20.86) | 1.42 | 1,064.38 |
| Sus | Chlorpyrifos | 2.01 ± 0.17 | 3.35 (4) | 0.50 | 0.023 (0.020–0.027) | - | - |
| | Chlorpyrifos + PBO | 1.93 ± 0.21 | 7.38 (4) | 0.12 | 0.016 (0.014–0.020) | 1.44 | - |
| | Chlorpyrifos + DEM | 2.01 ± 0.20 | 2.38 (4) | 0.67 | 0.018 (0.015–0.022) | 1.28 | - |
| | Chlorpyrifos + DEF | 2.00 ± 0.21 | 6.26 (4) | 0.18 | 0.016 (0.013–0.020) | 1.44 | - |

[a]*df* = degrees of freedom.

[b]$LD_{50}$ (µg a.i. $larva^{-1}$) and 95% confidence interval (95% CI).

[c]Synergistic Ratio (SR) = $LD_{50}$ of chlorpyrifos without synergist/$LD_{50}$ of chlorpyrifos + synergist.

[d]Resistance Ratio (RR) = $LD_{50}$ of Clorp-R genotype/$LD_{50}$ of Sus genotype in each combination of chlorpyrifos + synergist.

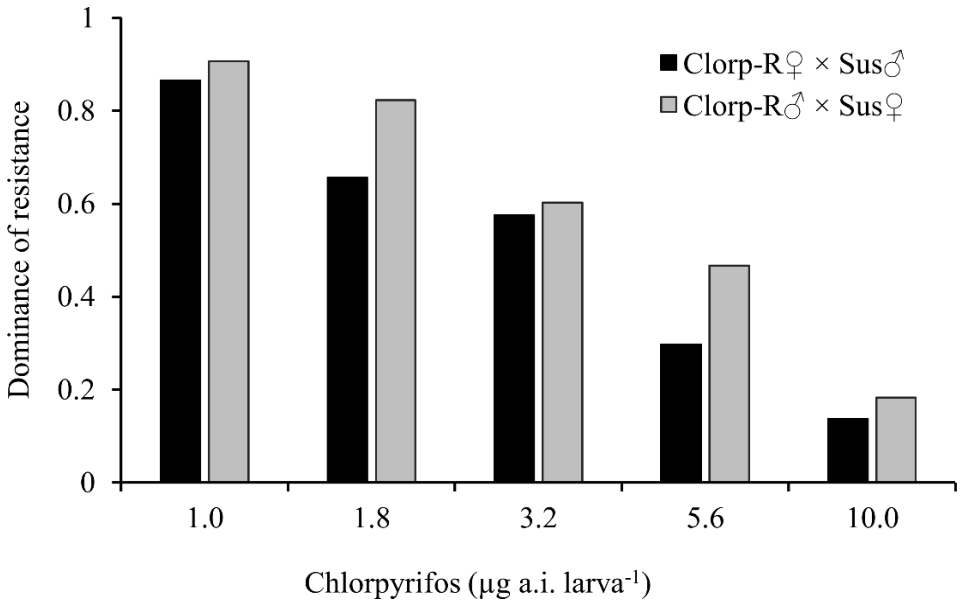


**Figure 1.** Effective dominance of chlorpyrifos resistance in FAW.

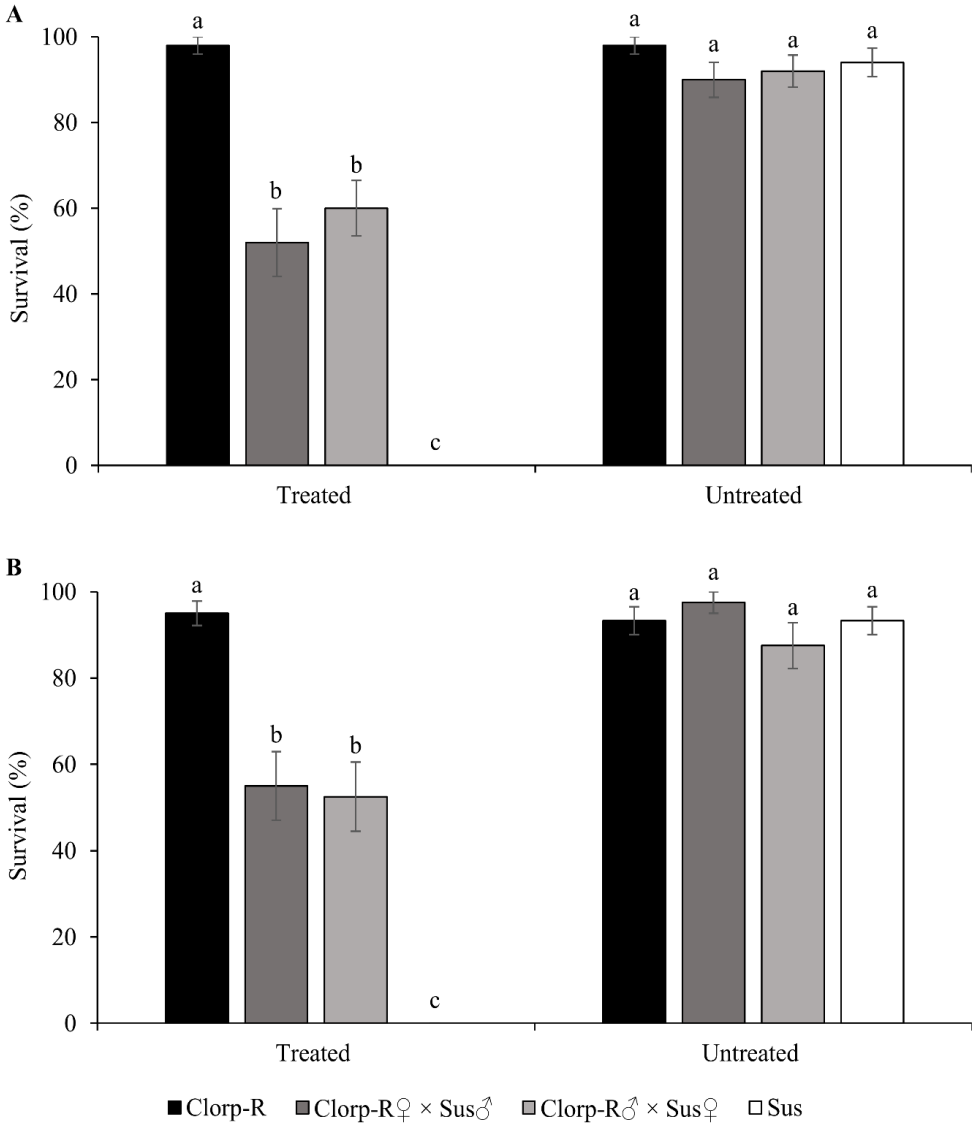


**Figure 2.** Survival (± SE) of early L3 larvae of FAW genotypes in whole plants (A) and excised leaves (B) of non-Bt maize treated and untreated with the field dose of chlorpyrifos. Group of bars (± SE) with the same letters are not significantly different ($P > 0.05$).

**3 ARTIGO 2**

# Fitness cost of chlorpyrifos resistance in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on different host plants

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**Abstract —** *Spodoptera frugiperda* (J. E. Smith, 1797) is a polyphagous pest of global relevance due to the damage it inflicts on agricultural crops. In South American countries, this species is one of the principal pests of maize and cotton. Currently, *S. frugiperda* is also emerging as an important pest of soybeans and winter cereals in Brazil. Chemical control is one the main control tactics against *S. frugiperda*, even though resistance against numerous modes of action insecticides has been reported. To support insect resistance management (IRM) programs, we evaluated the fitness costs of resistance of *S. frugiperda* to the acetylcholinesterase inhibitor chlorpyrifos. Fitness costs were quantified by comparing biological parameters of chlorpyrifos-resistant and -susceptible *S. frugiperda* and their $F_1$

hybrids (heterozygotes) on non-Bt cotton, non-Bt maize, non-Bt soybean, and oats. The results revealed that the chlorpyrifos-resistant genotype showed lower pupa-to-adult and egg-to-adult survivorship and reduced larval weights on oats; longer neonate-to-pupa and egg-to-adult developmental periods, and lower pupal weights and fecundity on maize; lower pupal weights on soybean; and reduced fecundity on cotton compared with the chlorpyrifos-susceptible genotype. Fitness costs also affected fertility life table parameters of the resistant genotype, increasing the mean length of a generation on cotton and maize and reducing the potential for population growth on all hosts. These findings suggest fitness costs at the individual and population levels of chlorpyrifos resistance in *S. frugiperda*, indicating that removal of the selective agent from the environment would result in reduced resistance and opportunities for the restoration of susceptibility.

**Keywords:** fall armyworm, insecticide resistance, acetylcholinesterase inhibitor, population growth, resistance management

## Introduction

*Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) is a native and pest species from tropical regions of the Western Hemisphere (Pogue, 2002). Recently, this species has also invaded Africa, Asia, and Oceania (Goergen et al. 2016, Wang et al. 2019, CRDC 2020). In South America, *S. frugiperda* is the most destructive pest of maize (*Zea mays* L.) (Juárez et al. 2012, Montezano et al. 2018) and also causes economic losses for cotton (*Gossypium hirsutum* L.) (Martinelli et al. 2006), soybean (*Glycine max* (L.) Merrill) (Machado et al. 2020), rice (*Oryza sativa* L.) (Busato et al. 2005), sorghum (*Sorghum bicolor* L. Moench) (Oliveira et al. 2019), wheat (*Triticum aestivum* L.) and oats (*Avena sativa* L.)

(Silva et al. 2017). Biological characteristics of *S. frugiperda*, such as polyphagia (Montezano et al. 2018), long-distance migration (Nagoshi et al. 2015, 2019, 2020), short generation time (Busato et al. 2005), and a high fecundity and fertility (Nagoshi et al. 2015), are responsible for its success as an insect pest. These aspects, combined with the current Brazilian crop production system, allow rapid population increases and damage to cultivated crops throughout the seasons (Machado et al. 2020).

In Brazil, *S. frugiperda* is managed using two main approaches: cultivation of *Bacillus thuringiensis* Berliner (Bt) plant technologies and chemical insecticides (Burtet et al. 2017, Muraro et al. 2019, Moscardini et al. 2020). The frequent adoption of Bt maize (>86% of total maize area) and cotton (>84% of total cotton area) and low compliance of refuge areas has contributed to the evolution of resistance of *S. frugiperda* to Bt toxins expressed in maize and cotton technologies (Farias et al. 2014, Bernardi et al. 2015, Santos-Amaya et al. 2015, Horikoshi et al. 2016a, Omoto et al. 2016). Control failures by Bt maize technologies against *S. frugiperda* has increased the use of insecticides; currently, up to four sprays per maize season are required to control this insect pest (Burtet et al. 2017).

The widespread use of insecticides against *S. frugiperda* has exposed its populations to selection pressure for resistance. Among the insecticides applied to control *S. frugiperda*, the acetylcholinesterase inhibitor chlorpyrifos has been used since the 1990s in Brazil (Agrofit 1996). According to previous reports, the susceptibility of Brazilian populations of *S. frugiperda* to chlorpyrifos has decreased, and inheritance of resistance has been reported to lambda-cyhalothrin, spinetoram, lufenuron, spinetoram, spinosad, and chlorantraniliprole (Diez-Rodríguez et al. 2001, Michereff Filho et al. 2002, Barros et al. 2005, Carvalho et al. 2013, Nascimento et al. 2016, Okuma et al. 2018, Bolzan et al. 2019, Lira et al. 2020). Multiple or cross-resistance mechanisms to chemical insecticides and Bt toxins has also been found in *S. frugiperda* in Brazil (Boaventura et al. 2020).

The frequent use of chlorpyrifos and other organophosphates against insect pests in Brazil has also favored the evolution of resistance to this mode of action by *Tetranychus urticae* Koch (Acari: Tetranychidae), *Leucoptera coffeella* (Guérin-Mèneville & Perrottet) (Lepidoptera: Lyonetiidae), *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae), and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) (Guedes et al. 1996, Nauem et al. 2001, Fragoso et al. 2002, Ribeiro et al. 2003). Previous studies also reported resistance to chlorpyrifos in *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) in Pakistan, and *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and *Laodelphax striatella* (Fallén) (Hemiptera: Delphacidae) in China (Zhang et al. 2015, Ejaz et al. 2017, Wang et al. 2017).

The evolution of resistance is determined by the greater adaptation of resistant genotypes in relation to susceptible ones in the presence of selection pressure (Carrière et al. 1994). Resistant insects often have development and reproduction disadvantage relative to susceptible ones in the absence of the selective agent (Roush and McKenzie 1987). For example, the resistance of *S. frugiperda* to spinosad was related to reduced survivorship of immature stages and a low reproductive rate (Okuma et al. 2018). By contrast, the absence of fitness costs to major Bt toxins (Cry1F, Cry1A.105, Cry2Ab2, and Vip3A) expressed in maize was reported in *S. frugiperda* (Jakka et al. 2014, Vélez et al. 2014, Horikoshi et al. 2016b, Niu et al. 2017, Chen et al. 2019). In other *Spodoptera* species, the fitness costs of resistance to tebufenozide, imidacloprid, emamectin benzoate, profenofos, and methoxyfenozide included reduced survivorship, pupal weight, and reproductive rate (Jia et al. 2009, Abbas et al. 2012, 2014, Zaka et al. 2014, Rehan and Freed 2015). Low pupal weight and survivorship as well as reduced reproductive performance were also associated with the resistance of *P. solenopsis*, *P. xylostella*, and *L. striatella* to chlorpyrifos (Zhang et al. 2015, Ejaz et al. 2017, Wang et al. 2017).

The fitness cost is an important component of the resistance evolution process; therefore, understanding fitness costs associated with resistant genotypes will help to predict restoration of susceptibility once selection pressure is removed and the design of better insect resistance management (IRM) programs. Furthermore, evaluation of the interactions between host plants and fitness cost can be used to improve IRM strategies for polyphagous species as *S. frugiperda*. On this basis, the objective of this study was to evaluate the fitness costs of chlorpyrifos resistance in *S. frugiperda* developing on cotton, maize, soybean, and oats.

## Material and methods

**Insect sources.** The chlorpyrifos-resistant genotype (Clorp-R) was selected from a field population collected during the winter of 2019 in oats in Tupanciretã, RS, Brazil (18°37'25" S and 52°54'13" W). In this location, even after three applications of chlorpyrifos (Lorsban® 480 BR, Dow AgroSciences Industrial Ltda., Santo Amaro, SP, Brazil) against *S. frugiperda*, control failures were detected. A total of 54 surviving larvae were collected and then transported to the laboratory. During the $F_1$ generation, larvae were maintained on an artificial diet based on white beans, wheat germ, and yeast (adapted from Greene et al. 1976), and in the subsequent seven generations ($F_2$ to $F_8$), third-instar larvae (~1 cm in length) were exposed to selection using commercial chlorpyrifos (Lorsban® 480 BR) diluted in distilled water and the surfactant Triton™ X-100 (Sigma Aldrich, São Paulo, SP, Brazil) at 0.1% was added to spread the solution over the diet surface. During selection, a single dose of 3,200 ppm of chlorpyrifos was applied in the diet surface (30 µl/well) in 24-well acrylic plates (Costar®, São Paulo, SP, Brazil) — equivalent to 51 µg active ingredient (a.i.)/$cm^2$ of diet. After 48 h, survivors were transferred to artificial diet without insecticide. This dose was defined in preliminary bioassays and causes complete mortality of susceptible and

heterozygous genotypes. The $LC_{50}$ of chlorpyrifos against third-instar larvae of the Clorp-R genotype from the $F_9$ generation was estimated to be 42.18 [95% CI (36.20–49.50)] µg a.i/$cm^2$ (*n* = 1,008; Slope (± SE) = 2.32 (± 0.14); $\chi 2$ = 4.97; *df* = 4) — larvae of this generation were used in fitness cost studies. A susceptible genotype (Sus) of *S. frugiperda*, which had been maintained in the laboratory since 2012, free from exposure to insecticides, was used as a source of susceptible insects. The $LC_{50}$ of chlorpyrifos against the Sus genotype was 0.07 [95% CI (0.06–0.09)] µg a.i./$cm^2$ (*n* = 1,152; Slope (± SE) = 2.68 (± 0.18); $\chi 2$ = 9.28; *df* = 5). This indicates that the Clorp-R genotype presented a resistance ratio of 602.6-fold relative to the Sus genotype. For heterozygotes evaluation, reciprocal crosses between resistant and susceptible genotypes (Clorp-R♀ × Sus♂ and Clorp-R♂ × Sus♀) were performed.

**Plant sources.** Seeds of host plants were sown in 5-L plastic pots containing two parts soil and one part composted plant material and maintained in a greenhouse. The following host plants (varieties) were used: non-Bt cotton (FMT701, Fundação Mato Grosso, Nova Mutum, MT, Brazil), non-Bt maize (30F35, DuPont Pioneer, Santa Rosa, RS, Brazil), non-Bt soybean (ICS 1032 RR, Sementes Ponteio, Cruz Alta, RS, Brazil), and oats (AF1340, Fundação Pró-Sementes, Passo Fundo, RS, Brazil).

**Assessing the fitness costs of chlorpyrifos resistance in *S. frugiperda*.** To perform fitness cost studies, neonates (<24 h old) from Clorp-R, Sus, and $F_1$ hybrids of the resistant and susceptible genotypes (heterozygotes) were fed on leaves of four host plants (cotton, maize, soybean, and oats) excised from the upper third part of greenhouse-grown plants from 30 to 60 d after emergence. In the laboratory, leaves were cut into pieces and placed on a gelled 2% agar-water mixture in 50 ml plastic cups. Then, a single neonate was placed on each cup. Leaves were replaced every 24 h until pupation. Cups were sealed and maintained in a room at 25 ± 2°C, 60 ± 10% RH, and a 14:10 h light:dark photoperiod. The experimental design

was completely randomized with 13 to 15 replicates of 10 larvae/insect genotype/host plant. For larval and pupal weights were considered only the first 10 replicates/treatment, due to the large number of insects to be weighed. The following life history traits were evaluated: survivorship and developmental time of the neonate-to-pupa, pupa-to-adult, and egg-to-adult (total cycle) periods; larval weight at 12 d; pupal weight 24 h after pupal formation; sex ratio; number of eggs per female (fecundity); and egg hatch rate (fertility). Survivorship and developmental time were determined by daily observations. The number of eggs per female was assessed daily from 7 to 20 pairs kept in PVC cages (23-cm in height × 10-cm in diameter) internally coated with a paper towel and closed at the top with a sheer fabric (one pair/cage). The egg hatch rate was calculated from 50 to 200 eggs of the 2nd or 3rd oviposition of each pair being neonates counted daily.

**Data analysis.** The fitness cost parameters of *S. frugiperda* genotypes developed on maize, cotton, soybean, and oats were subjected to two-way analysis of variance (ANOVA) using the PROC GLM procedure with insect genotype and host plant as the two main factors. Treatment means were compared by the least-square means statement (LSMEANS statement) using a Tukey-Kramer adjustment at $P<0.05$ in SAS® 9.1 (SAS Institute 2002). Survivorship, development, and reproduction data were used to estimate fertility life table parameters, including mean length of a generation ($T$), net reproductive rate ($R_o$; average number of female offspring that would be born to a cohort of females), and intrinsic rate of population increase ($r_m$; daily production of females per parental female). Fertility life table parameters were estimated by the jackknife technique using the "*lifetable.sas*" protocol developed by Maia et al. (2000) in SAS® 9.1 (SAS Institute 2002). The relative fitness cost was calculated using the equation proposed by Cao and Han (2006): Relative fitness cost = ($R_o$ Clorp-R or

heterozygotes)/($R_o$ Sus), where $R_o$ is the net reproductive rate parameter from the fertility life table.

## Results

**Survivorship of *S. frugiperda* genotypes on different host plants.** There was a significant insect genotype × host plant interaction ($F$ = 4.21; $df$ = 9, 222; $P$<0.0001) and host plant ($F$ = 17.68; $df$ = 3, 222; $P$<0.0001) effect on neonate-to-pupa survivorship. In contrast, the main effect of insect genotype was not significant ($F$ = 2.28; $df$ = 3, 222; $P$ = 0.0805). The neonate-to-pupa survivorship of Clorp-R genotype was lower on cotton (70% survival), than on soybean (89.3% survival) but did not differ from survival on maize and oats (Fig. 1). In general, the heterozygote presented higher survival on maize and soybean (>84.7%) compared to those fed on cotton and oats. The Sus genotype showed similar neonate-to-pupa survival on all host plants (Fig. 1).

A significant interaction between the *S. frugiperda* genotype and host plant ($F$ = 5.21; $df$ = 9, 222; $P$<0.0001) was detected for pupa-to-adult survivorship. The main effects of insect genotype ($F$ = 19.64; $df$ = 3, 222; $P$<0.0001) and host plant ($F$ = 9.01; $df$ = 3, 222; $P$<0.0001) on this variable were also significant. The Clorp-R genotype showed lower pupa-to-adult survivorship (50.2%) on oats relative to the Sus and heterozygous genotypes (69.5% and 91.1%, respectively), which differed significantly from each other (Fig. 1). In contrast, *S. frugiperda* genotypes showed similar pupa-to-adult survivorship on cotton, maize, and soybean. However, the Clorp-R and Sus genotypes presented lower pupa-to-adult survivorship on oats (<69.5%) in comparison with their development on other hosts (77.3%–88.9%) (Fig. 1).

Egg-to-adult survivorship was also significantly affected by the insect genotype × host plant interaction ($F$ = 2.36; $df$ = 9, 222; $P$ = 0.0144), insect genotype ($F$ = 27.18; $df$ = 3, 222; $P$<0.0001), and host plant ($F$ = 47.09; $df$ = 3, 222; $P$<0.0001). The Clorp-R genotype showed lower egg-to-adult survivorship on oats (28.6%) than the heterozygous and Sus genotypes (46.2%–55.0%, respectively) (Fig. 1). In contrast, Clorp-R and Sus genotypes had similar egg-to-adult survivorship on cotton, maize, and soybean. In general, the heterozygous had higher egg-to-adult survivorship on maize (81.9%) and soybean (73.7%) compared to cotton (56.8%) and oats (53.0%).

**Duration of developmental stages of *S. frugiperda* genotypes on different host plants.** The effects of the insect genotype × host plant interaction, insect genotype, and host plant on the duration of the neonate-to-pupa period were all significant ($F$ = 8.23; $df$ = 9, 222; $P$<0.0001 for interaction; $F$ = 118.55; $df$ = 3, 222; $P$<0.0001 for insect genotype; $F$ = 849.92; $df$ = 3, 222; $P$<0.0001 for host plant). The duration of neonate-to-pupa period of the Clorp-R was ∼4 d longer than that of Sus and heterozygous genotypes on cotton, maize, and oats (Fig. 2). Overall, the duration of neonate-to-pupa period of all *S. frugiperda* genotypes was longer on cotton than on other host plants (Fig. 2).

There were statistically significant effects of the insect genotype × host plant interaction ($F$ = 6.61; $df$ = 9, 222; $P$<0.0001) and host plant ($F$ = 4.83; $df$ = 3, 222; $P$ = 0.0028) on the duration of the pupa-to-adult period. In contrast, there was no effect of insect genotype ($F$ = 1.44; $df$ = 3, 222; $P$ = 0.2324). The Clorp-R♂ × Sus♀ genotype had a longer pupa-to-adult period on cotton and soybean than on maize, whereas the pupa-to-adult period of the Sus genotype was 2 d longer on oats than on other hosts (Fig. 2).

The effect of the insect genotype × host plant interaction on the duration of the egg-to-adult period was significant ($F$ = 6.99; $df$ = 9, 222; $P$<0.0001). The main effects of the insect

genotype ($F$ = 76.06; $df$ = 3, 222; $P$<0.0001) and host plant ($F$ = 497.09; $df$ = 3, 222; $P$<0.0001) were also significant. The duration of the egg-to-adult period of the Clorp-R genotype was ~2 d longer than that of the Sus genotype only on maize (Fig. 2). In contrast, the duration of the egg-to-adult period of the Clorp-R, Sus, and the heterozygous genotypes was similar when developing on the same host. Across host plants and *S. frugiperda* genotypes, there was a shorter egg-to-adult period on maize, soybean and oats than on cotton (Fig. 2).

**Larval and pupal weights of *S. frugiperda* genotypes on different host plants.** The main effects of the insect genotype × host plant interaction, insect genotype, and host plant on larval weight were all significant ($F$ = 15.09; $df$ = 9, 144; $P$<0.0001 for interaction; $F$ = 113.20; $df$ = 3, 144; $P$<0.0001 for insect genotype; $F$ = 404.14; $df$ = 3, 144; $P$<0.0001 for host plant). The Clorp-R genotype had a lower larval weight (138.8 mg/larva) on oats, but a similar larval weight to the Sus genotype on maize, cotton, and soybean (Fig. 3). In general, the larval weight of heterozygotes was higher than that of the other genotypes on all host plants. Larvae weighed less on cotton, (20.3–63.0 mg/larva) than those fed on the other three food sources (>131 mg/larva) (Fig. 3).

Significant main effects of the insect genotype × host plant interaction ($F$ = 14.27; $df$ = 9, 144; $P$<0.0001), insect genotype ($F$ = 19.81; $df$ = 3, 144; $P$<0.0001), and host plant ($F$ = 46.22; $df$ = 3, 144; $P$<0.0001) on pupal weights were detected. The Clorp-R genotype produced significantly lighter pupae on maize and soybean (131.1 and 156.4 mg/pupa, respectively) than the Sus genotype (>157.9 mg/pupa), whereas on oats, pupae of the Clorp-R genotype were heavier (172.0 mg/pupa) than those of the Sus genotype (Fig. 3). The pupal weights of the heterozygotes were similar on cotton, maize and soybean to those of the Clorp-R and Sus genotypes. Regarding host plants, the Clorp-R and heterozygote genotypes

presented lower pupal weights on maize than on other host plants. In contrast, the Sus genotype had a lower pupal weight on oats than on cotton, maize, and soybean (Fig. 3).

**Sex ratio, fecundity, and fertility of *S. frugiperda* genotypes on different host plants.** The effects of the insect genotype × host plant interaction, insect genotype, and host plant on the sex ratio were all non-significant ($F = 0.36$; $df = 9, 222$; $P = 0.9519$) for interaction, $F = 0.67$; $df = 3, 222$; $P = 0.5743$ for insect genotype; and $F = 0.64$; $df = 3, 222$; $P = 0.5876$ for host plant). The sex ratio of the four insect genotypes on the four host plants ranged from 0.48 to 0.60 (Fig. 4).

The number of eggs per female (fecundity) was affected by the insect genotype × host plant interaction ($F = 3.68$; $df = 9, 203$; $P = 0.0003$), insect genotype ($F = 22.70$; $df = 3, 203$; $P<0.0001$), and host plant ($F = 7.93$; $df = 3, 203$; $P<0.0001$). The Clorp-R genotype generated females that laid fewer eggs when fed on cotton and maize (309 and 303 eggs/female, respectively) than the Sus genotype (>850 eggs/female) (Fig. 4). In contrast, the Clorp-R and Sus genotypes produced similar numbers of eggs on soybean and oats. In relation to host plants, the fecundity of heterozygotes that fed on oats was higher (1321 eggs/female) than that fed on cotton (586 eggs/female) and maize (905 eggs/female), but did not differ from that fed on soybean (1016 eggs/female) (Fig. 4).

The main effects of the insect genotype × host plant interaction ($F = 2.08$; $df = 9, 177$; $P = 0.0336$), insect genotype ($F = 6.64$; $df = 3, 177$; $P = 0.0003$), and host plant ($F = 4.45$; $df = 3, 177$; $P = 0.0048$) on the egg hatch rate (fertility) were significant. Females of the Clorp-R, Sus, and heterozygous genotypes had a similar egg hatch rate, ranging from 81.3% to 94.8%, when they developed on soybean and oats. In contrast, the egg hatch rate of the Sus genotype was lower on cotton (73.8%) and maize (84.4%) compared to Clorp-R (>86.5%) plus Clorp-R♂ × Sus♀ (>92%), and heterozygotes (89.2%) (Fig. 4). Across host plants, the fertility of

the Sus genotype was lower on cotton (73.8%) than on soybean and oats (>88.9%), whereas the egg hatch rate of the other insect genotypes was similar on all host plants (Fig. 4).

**Life history traits of *S. frugiperda* genotypes on different host plants.** According to the estimated life table parameters (Table 1), the Clorp-R genotype had a high mean generation length ($T$) and low population growth ($R_o$ and $r_m$) on all host plants. These findings indicated that Clorp-R females obtained from cotton and maize generated fewer than 79 females/female/generation ($R_o$), in a generation time of up to 51 d, whereas the Sus genotype on the same hosts generated more than 251 females/female/generation in less than 50 d, revealing that resistant females originated 69% and 73% fewer females than Sus females, respectively. On soybean and oats, Clorp-R females also generated 54% and 52% fewer females/female than Sus, respectively. The mean generation time of heterozygotes was up to 6 d shorter on cotton, maize, and oats than of the Clorp-R and Sus genotypes, but all genotypes had similar generation times on soybean (Table 1). In general, the heterozygotes and Sus genotype had similar net reproductive rates on cotton, maize, and soybean, but the heterozygotes usually produced more females than the Clorp-R genotype on all host plants. The Clorp-R genotype also presented a 14% and 27% lower population increase ($r_m$) than the Sus genotype and heterozygotes, respectively, on all host plants (Table 1). In contrast, heterozygotes presented a higher rate of population increase than the Sus genotype on maize and oats, but similar rates on cotton and soybean.

Regarding generation time and population growth, the mean length of a generation of the Clorp-R genotype increased by approximately 10 d and reduced the intrinsic rate of population increased by up to 25% on cotton compared with other food sources, whereas a similar net reproductive rate was recorded on all host plants (Table 1). The generation length and rate of population increase of heterozygotes was shorter and higher, respectively, on

maize and oats, followed by soybean and cotton. Heterozygous females generated fewer females (<197 females/female/generation) on cotton than on other host plants (>309 females/female/generation). The Sus genotype had a shorter mean generation length on maize than on other host plants but generated a similar number of females (251–290 females/female) on cotton, maize, and soybean (Table 1).

The relative fitness cost (based on $R_o$ values) of the Clorp-R genotype ranged from 0.27 to 0.49 across the four host plants, indicating substantial fitness costs of chlorpyrifos resistance in *S. frugiperda* at the population level. In contrast, the relative fitness of the heterozygotes was >1.06, indicating that heterozygotes had life history traits similar to or better than the Sus genotype on all host plants, revealing that the relative fitness costs are recessive traits.

## Discussion

This study evaluated the magnitude of the fitness cost of chlorpyrifos resistance in *S. frugiperda* on different host plants. Our findings indicated fitness costs in the chlorpyrifos-resistant genotype developing on cotton, maize, soybean, and oats. At the individual level, the chlorpyrifos-resistant genotype showed lower pupa-to-adult and egg-to-adult survivorship and reduced larval weight on oats; longer neonate-to-pupa and egg-to-adult developmental periods, and lower pupal weight and fecundity on maize; lower pupal weight on soybean; and reduced fecundity on cotton when compared to the chlorpyrifos-susceptible genotype. When life history traits were compiled at the population level, the chlorpyrifos-resistant genotype also had a longer mean generation on cotton and maize and reduced potential for population increases on all host plants relative to the susceptible genotype. In contrast, heterozygotes presented life history traits similar to or better than those of the chlorpyrifos-susceptible

genotype on all host plants, indicating a lack of relevant fitness costs of heterozygotes at the individual and population levels and the recessive inheritance of these traits.

The association of fitness costs and insecticide resistance in *S. frugiperda* and other *Spodoptera* species has been reported previously. Using an artificial diet, Okuma et al. (2018) determined the fitness costs in a laboratory-selected spinosad-resistant colony of *S. frugiperda*, which had reduced survivorship to adulthood and a lower reproductive rate than a susceptible colony. Similar to our results, the fitness costs of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) resistance to tebufenozide measured on an artificial diet included reduced larval survival and pupal weight, prolongation of the larval and pupal stages, and effects on fecundity (Jia et al. 2009). The fitness costs of resistance to imidacloprid (Abbas et al. 2012), emamectin benzoate (Zaka et al. 2014), profenofos (Abbas et al. 2014), and methoxyfenozide (Rehan and Freed 2015) in *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) quantified on an artificial diet also negatively impacted the larval survivorship, duration of larval and pupal stages, pupal weight, number of adults, fecundity, and fertility. As in our study, the resistance of *P. xylostella*, *P. solenopsis*, and *L. striatella* to chlorpyrifos was linked to relevant fitness costs that reduced pupal survival, pupal and adult weights, and reproductive performance (Zhang et al. 2015, Ejaz et al. 2017, Wang et al. 2017).

The magnitude of fitness costs may be influenced by ecological conditions (e.g., different host plants and weather) and allelochemicals but seems to be greater in low-quality host plants and stressful environments (Carrière et al. 2001, Janmaat and Myers 2005, Gassmann et al. 2009, Raymond et al. 2011). Previous studies showed greater expression of fitness costs when *S. frugiperda* resistant to Cry1F and *Helicoverpa armigera* (Hübner) (Lepidoptera Noctuidae) resistant to Cry1Ac developed on cotton compared with other host plants, including maize, soybean, sorghum, and pigeon pea (*Cajanus cajan* (L.) Millsp.) (Bird and Akhurst 2006, Jakka et al. 2014). This can be attributed to the presence of gossypol, a

phenolic aldehyde, which affects the digestive process, as verified in *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) (Carrière et al. 2004). Biological studies comparing life history traits of *S. frugiperda* on different host plants also indicated worse development on cotton compared to maize, soybean, millet, wheat, and oats (Barros et al. 2010, Silva et al. 2017). However, contrary to our expectation, the development of the chlorpyrifos-resistant *S. frugiperda* genotype on cotton did not substantially increase the expression of fitness costs compared to other hosts. Consistent with our results, Chen et al. (2019) reported the absence of relevant fitness costs in a laboratory-selected *S. frugiperda* genotype resistant to Vip3A reared on cotton, suggesting that, in some cases, host-plant phytochemicals do not affect the magnitude of fitness costs.

The resistance of *S. frugiperda* to chlorpyrifos was related with overexpression of glutathione S-transferases, cytochrome P450s and carboxylesterases and target-site mechanisms (Carvalho et al. 2013). According to Xiao et al. (2020) and Zhou et al. (2020), detoxification-related genes are widely expressed in all developmental stages of *S. frugiperda*, conferring the ability of this pest to detoxify insecticides and also plant secondary compounds that are toxic to the insects, as cyclic hydroxamic acids in maize and cereals, gossypol in cotton, and phenolic acids and isoflavonoids in soybean (Carrière et al. 2004, Stipanovic et al. 2006, Kojima et al. 2010, Balmer et al. 2013, Peruca et al. 2018). These widespread expression of detoxification enzymes in *S. frugiperda* have facilitated its genetic adaptation to several host plants, enabling its rapid expansion worldwide. The overproducing of detoxification enzymes by *S. frugiperda* may explain the lack of substantial fitness costs of the chlorpyrifos-resistant genotype developed on cotton in our study. Therefore, the fitness costs of resistance in polyphagous species do not always increase on plants that produce defensive chemical contents, and should therefore be studied for each host plant.

From an IRM perspective, host plants that magnify the fitness costs of resistance could delay the evolution of resistance more effectively. Life history traits of chlorpyrifos-resistant *S. frugiperda* indicated longer neonate-to-pupa and egg-to-adult developmental periods (on maize) and a longer mean generation time (on both cotton and maize), which may increase the exposure of larvae to entomopathogens, parasitoids, predators, and the weather in the field and further impact on the population growth rate. In the central-west region of Brazil, where maize, soybean, and cotton are planted in proximity in the field and are major hosts for *S. frugiperda*, the use of good agricultural practices, such as distinct control tactics and rotation of modes of action insecticides, is essential to reduce the frequency of resistance to chlorpyrifos and to provide opportunities for the restoration of susceptibility. In contrast, in southern Brazil, maize and soybean are cultivated during the summer and cereals (including oats) during the winter. In this region, low temperatures reduce *S. frugiperda* infestations in winter cereals and, consequently, exposure to insecticides. However, the interaction of field environment conditions, host plants, natural enemies, and good agricultural practices can increase the fitness costs of chlorpyrifos resistance in *S. frugiperda* at the individual and population levels, providing the opportunity for restoring susceptibility.

In conclusion, our findings demonstrate the magnitude of the fitness costs of chlorpyrifos resistance in *S. frugiperda* on different host plants. To the best of our knowledge, this is the first documentation of patterns of fitness costs in *S. frugiperda* selected for resistance to chlorpyrifos. Our results show some recessive fitness costs in a chlorpyrifos-resistant *S. frugiperda* genotype at the individual and population level on all host plants evaluated. These selective disadvantages of the resistant genotype might be sufficiently large to be useful in field conditions. These fitness costs can be further explored by rotating insecticides with different modes of action and the use of other control tactics against *S. frugiperda*, which could intensify the reduction in the frequency of resistance alleles. Thus, diversifying

cropping landscapes and integrating multiple control tactics could prolong the lifetime of chemical insecticides, where the resistance of *S. frugiperda* to insecticides is already widespread.

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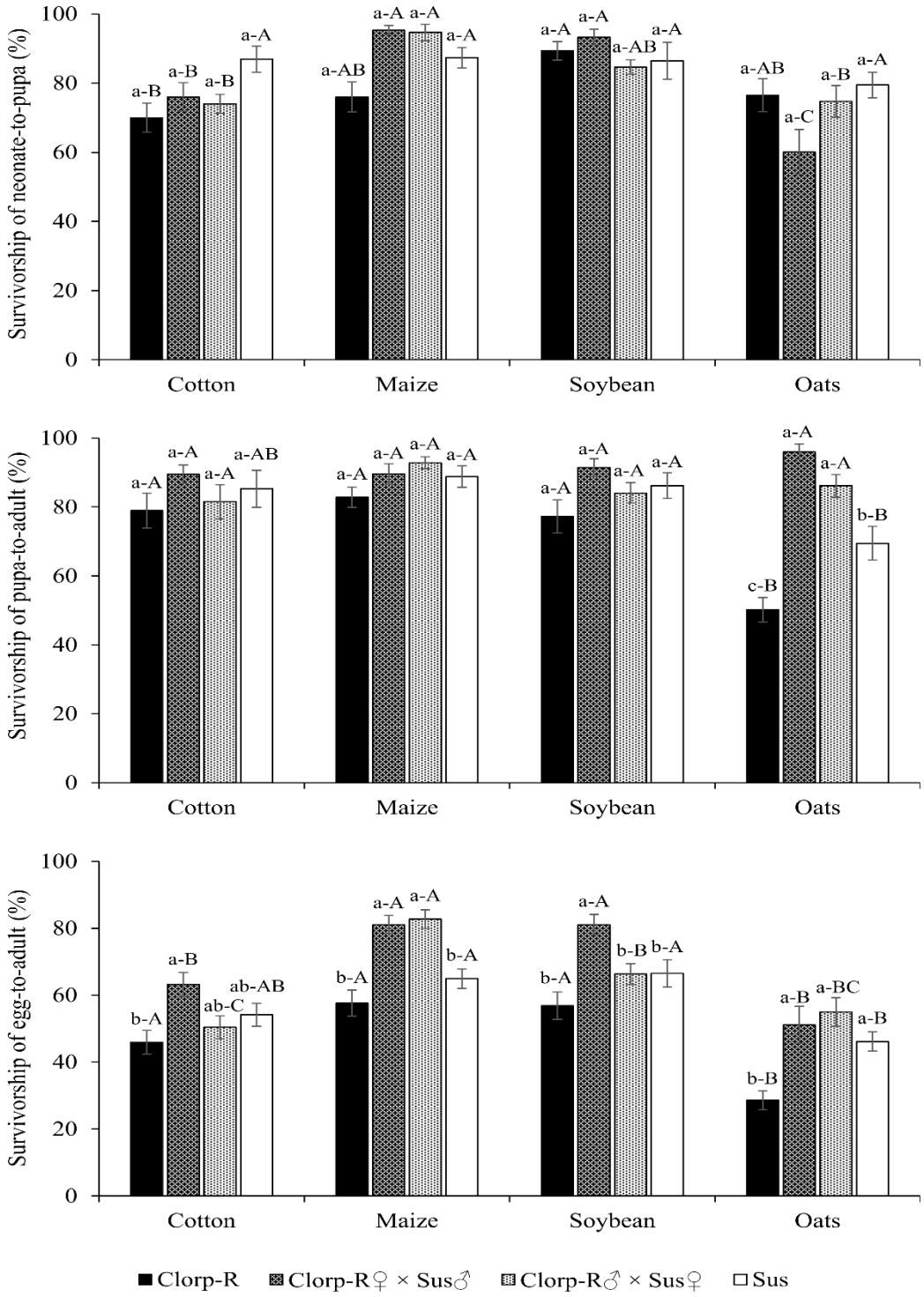


**Figure 1.** Survivorship of the neonate-to-pupa, pupa-to-adult, and egg-to-adult periods of chlorpyrifos-resistant (Clorp-R) and -susceptible (Sus) *S. frugiperda* and their $F_1$ hybrids (heterozygotes) fed on leaves of different host plants. Bars (± SE) with the same lowercase letter in each host plant or uppercase letter in distinct hosts are not significant different as determined by the LSMEANS statement using the Tukey-Kramer test at $P < 0.05$.

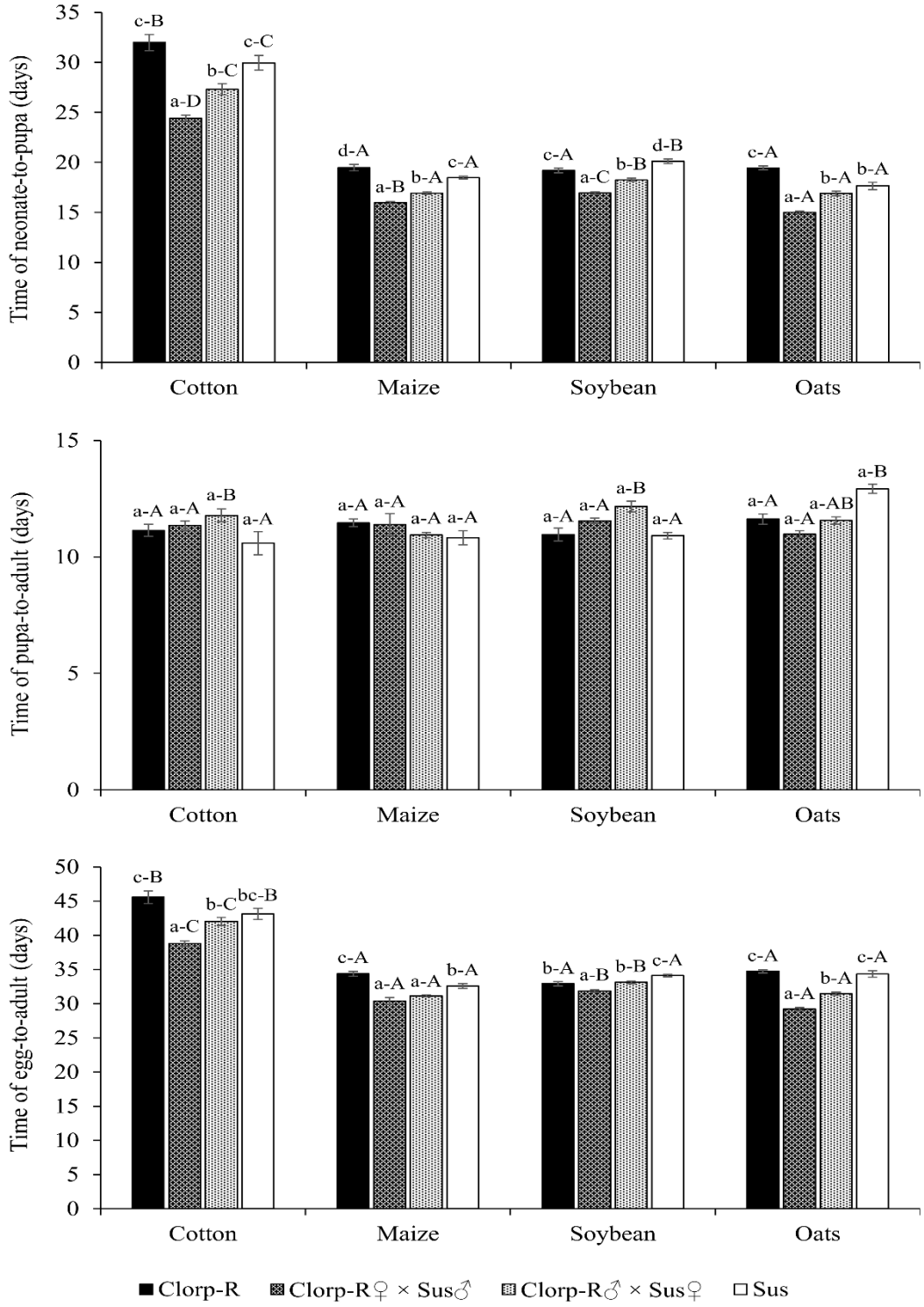


**Figure 2.** Developmental times of the neonate-to-pupa, pupa-to-adult, and egg-to-adult periods of chlorpyrifos-resistant (Clorp-R) and -susceptible (Sus) *S. frugiperda* and their $F_1$ hybrids (heterozygotes) fed on leaves of different host plants. Bars (± SE) with the same lowercase letter in each host plant or uppercase letter in distinct hosts are not significant different as determined by the LSMEANS statement using the Tukey-Kramer test at $P < 0.05$.

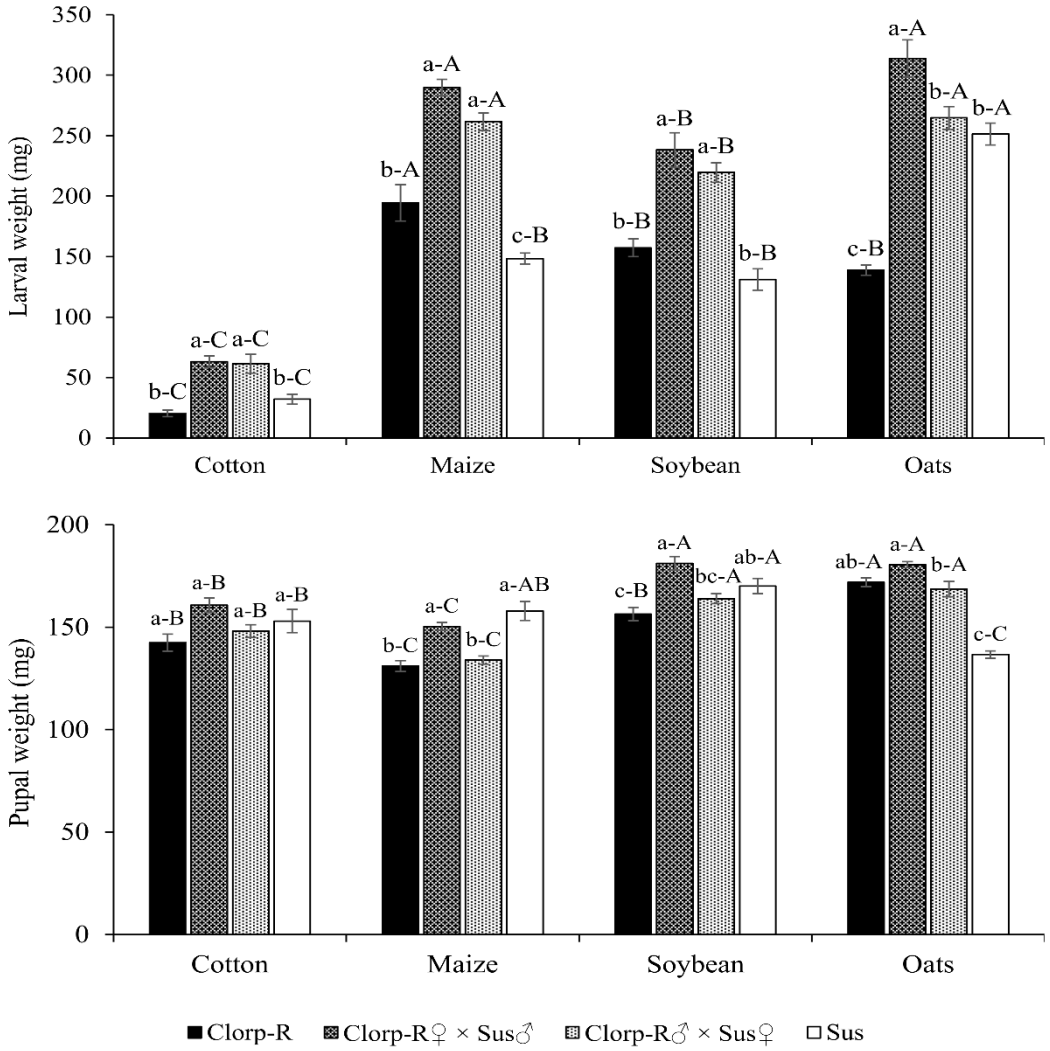


**Figure 3.** Larval and pupal weights of chlorpyrifos-resistant (Clorp-R) and -susceptible (Sus) *S. frugiperda* and their F1 hybrids (heterozygotes) fed on leaves of different host plants. Bars (± SE) with the same lowercase letter in each host plant or uppercase letter in distinct hosts are not significant different as determined by the LSMEANS statement using the Tukey-Kramer test at $P < 0.05$.

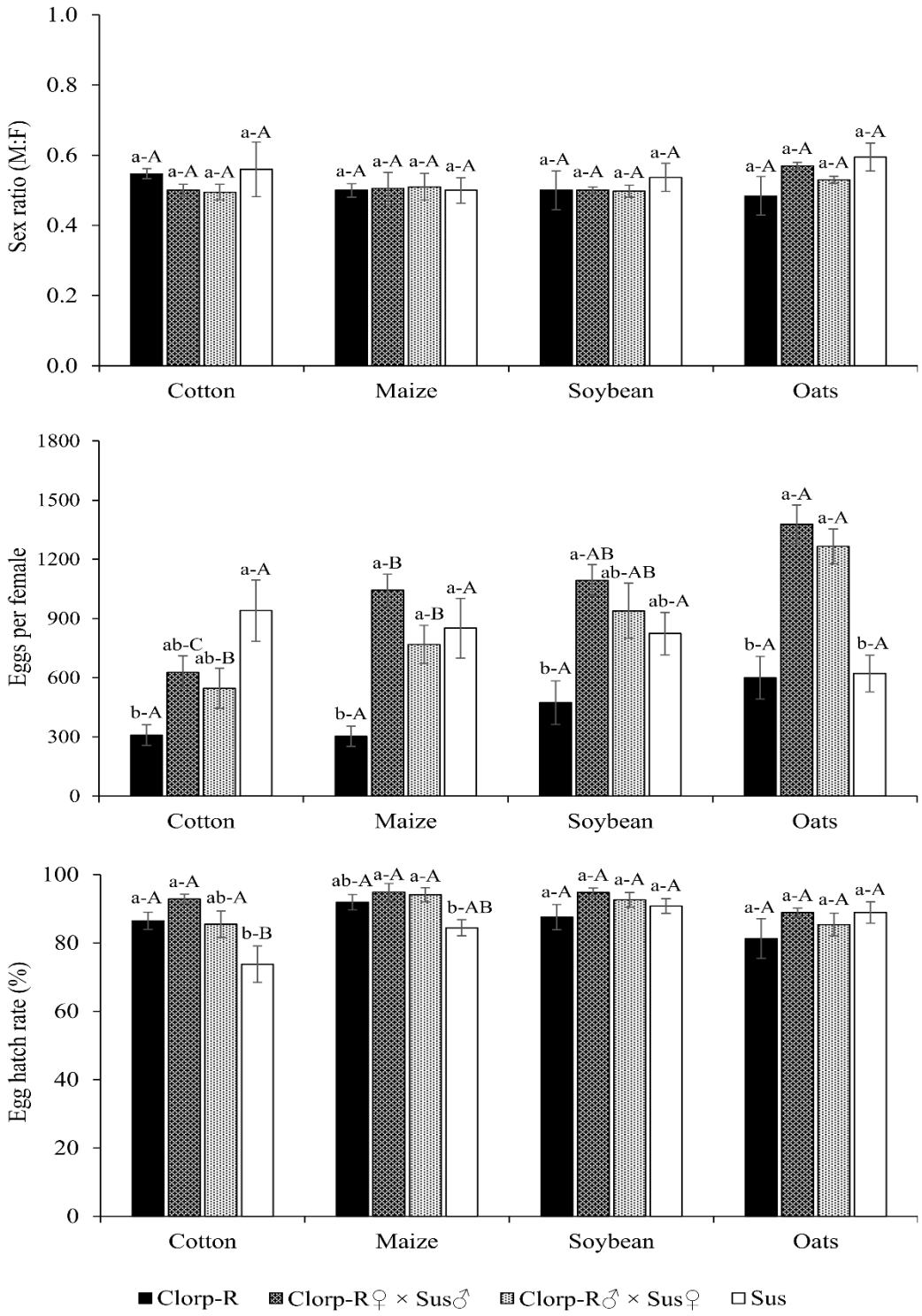


**Figure 4.** Sex ratio, fecundity (eggs per female), and fertility (egg hatch rate) of chlorpyrifos-resistant (Clorp-R) and -susceptible (Sus) *S. frugiperda* and their $F_1$ hybrids (heterozygotes) fed on leaves of different host plants. Bars (± SE) with the same lowercase letter in each host plant or uppercase letter in distinct hosts are not significant different as determined by the LSMEANS statement using the Tukey-Kramer test at $P < 0.05$.

**Table 1.** Fertility life table parameters of chlorpyrifos-resistant (Clorp-R) and -susceptible (Sus) *S. frugiperda* and their $F_1$ hybrids (heterozygotes) fed on leaves of different host plants.

| *S. frugiperda* genotype | Fertility life table parameter[a,b] | | |
|---|---|---|---|
| | *T* (days) | $R_o$ (♀/♀) | $r_m$ (♀/♀*day) |
| **Cotton** | | | |
| Clorp-R | 51.01 ± 0.22 dB | 78.51 ± 13.34 cA | 0.09 ± 0.003 cB |
| Clorp-R♀ × Sus♂ | 42.78 ± 0.12 aC | 197.58 ± 26.07 abB | 0.12 ± 0.002 aC |
| Clorp-R♂ × Sus♀ | 48.17 ± 0.35 bC | 136.48 ± 25.33 bcB | 0.10 ± 0.004 bC |
| Sus | 49.67 ± 0.23 cC | 251.78 ± 46.88 aA | 0.11 ± 0.003 bC |
| **Maize** | | | |
| Clorp-R | 40.00 ± 0.31 dA | 75.53 ± 16.98 cA | 0.11 ± 0.006 dA |
| Clorp-R♀ × Sus♂ | 35.27 ± 0.16 aA | 422.25 ± 33.25 aA | 0.17 ± 0.002 aA |
| Clorp-R♂ × Sus♀ | 35.96 ± 0.17 bA | 325.36 ± 41.06 abA | 0.16 ± 0.003 bA |
| Sus | 38.23 ± 0.16 cA | 276.42 ± 49.29 bA | 0.15 ± 0.005 cA |
| **Soybean** | | | |
| Clorp-R | 39.87 ± 0.45 bA | 135.07 ± 31.32 cA | 0.12 ± 0.007 cA |
| Clorp-R♀ × Sus♂ | 37.77 ± 0.24 aB | 444.89 ± 31.80 aA | 0.16 ± 0.002 aB |
| Clorp-R♂ × Sus♀ | 38.93 ± 0.35 bB | 309.81 ± 46.31 bA | 0.15 ± 0.004 bB |
| Sus | 39.64 ± 0.28 bB | 290.74 ± 36.70 bA | 0.14 ± 0.003 bA |
| **Oats** | | | |
| Clorp-R | 40.83 ± 0.19 cA | 83.61 ± 15.01 cA | 0.11 ± 0.005 dA |
| Clorp-R♀ × Sus♂ | 35.11 ± 0.23 aA | 400.52 ± 28.39 aA | 0.17 ± 0.002 aA |
| Clorp-R♂ × Sus♀ | 36.44 ± 0.21 bA | 368.70 ± 25.91 aA | 0.16 ± 0.002 bA |
| Sus | 40.06 ± 0.17 cB | 171.42 ± 25.46 bB | 0.13 ± 0.004 cB |

[a]*T* = mean length of a generation (days); $R_o$ = net reproductive rate (females per female per generation); $r_m$ = intrinsic rate of population increase (per day).

[b]Means followed by the same lowercase letter within of each host and same uppercase letter in a same genotype across host plants are not significantly different (*t*-tests for pairwise group comparisons, $P<0.05$).

## 4 DISCUSSÃO

Os resultados desse estudo indicaram que as falhas de controle de *S. frugiperda* após pulverizações sequenciais de clorpirifós em área de cultivo de aveia em Tupanciretã, RS, durante a safra de inverno de 2019 se devem à resistência. Após coleta de uma população de *S. frugiperda* e sua seleção para resistência a clorpirifós, verificou-se que o genótipo resistente apresentou uma razão de resistência maior que 1050 vezes em relação a um genótipo suscetível de referência. Ainda, as lagartas resistentes, quando expostas a alimentação em plantas de milho pulverizadas com a dose de bula de clorpirifós em casa-de-vegetação ou alimentadas com folhas de milho tratadas apresentaram sobrevivência larval superior a 95%. Portanto, trata-se de uma redução de base genética na suscetibilidade de *S. frugiperda* a clorpirifós.

A herança da resistência de *S. frugiperda* a clorpirifós foi caracterizada como autossômica, ou seja, os dois parentais transmitem a característica aos seus descendentes. Além disso, a resistência foi caracterizada como poligênica (influenciada por mais de um gene) e incompletamente dominante, que indica que uma fração dos indivíduos de genótipo heterozigoto se comportam fenotipicamente como resistentes, quando expostos a dose de bula de clorpirifós em condições de campo.

Também se constatou uma baixa resistência cruzada entre clorpirifós e inseticidas com outros modos de ação em *S. frugiperda*. Isso indica que, a rotação de inseticidas com modos de ação distintos, é uma estratégia que deve ser implementada para evitar e/ou retardar a evolução da resistência de *S. frugiperda* a inseticidas, bem como, contornar o problema de resistência a clorpirifós. Para o genótipo resistente a clorpirifós também se detectou que houve pouca influência de mecanismos metabólicos na resistência, pois os sinergistas PBO, DEM e DEF não alteraram de forma significativa a resposta dos resistentes quando expostos ao clorpirifós. Mesmo assim, a resistência metabólica ainda não pode ser excluída, pois em estudo prévio foi demonstrada superexpressão das enzimas glutationa *S*-transferases, citocromo P450s e carboxilesterases em *S. frugiperda* selecionada em laboratório para resistência a clorpirifós (CARVALHO et al., 2013).

Também se detectou a presença de custo adaptativo da resistência a clorpirifós em *S. frugiperda* quando alimentada com folhas de algodão, milho, soja e aveia. O genótipo resistente apresentou menor sobrevivência de pupa a adulto e ciclo total, além de peso larval reduzido em aveia; períodos mais longos de desenvolvimento larval e ciclo total, menor peso de pupas e fecundidade em milho; menor peso de pupas na soja; e redução da fecundidade no algodão, quando comparados aos insetos de genótipo suscetível. Em nível populacional, os resistentes

apresentaram menor capacidade de aumento populacional em comparação aos suscetíveis. Do ponto de vista prático, a presença de custo adaptativo indica que a remoção do agente de seleção (neste caso o inseticida) do ambiente pode reduzir a resistência e possibilitar o restabelecimento da suscetibilidade.

No contexto do manejo da resistência de insetos, os resultados deste estudo reforçam a importância da rotação de inseticidas com modo de ação distintos para evitar que populações de *S. frugiperda* evoluam para resistência a clorpirifós em outros locais. Além disso, o uso integrado de outras estratégias de manejo de *S. frugiperda* como plantas Bt, parasitoides de ovos e biopesticidas à base de baculovírus são igualmente importantes para retardar a evolução da resistência, especialmente num sistema agrícola intensivo como o do Brasil. Os resultados aqui reportados fornecem importante informações para programas de manejo integrado e da resistência desta espécie-praga de relevância global.

## 5 CONCLUSÕES

A herança da resistência de *S. frugiperda* a clorpirifós é autossômica, incompletamente dominante e poligênica, com pouca influência da resistência metabólica na desintoxicação do inseticida.

Há baixa resistência cruzada entre clorpirifós e inseticidas com outros modos de ação em *S. frugiperda*, o que demonstra a importância da rotação de modos de ação como estratégia de manejo da resistência.

A resistência de *S. frugiperda* a clorpirifós está associada à custo adaptativo, indicando que uma redução no uso do inseticida pode favorecer o restabelecimento da suscetibilidade.

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