CAMILA OLIVEIRA SANTOS

TRANSGENIC Bt MAIZE, SEED TREATMENT AND FOLIAR INSECTICIDES AGAINST FALL ARMYWORM AND A STINK BUG

Dissertation presented to the Universidade Federal de Viçosa in partial fulfillment of requirements of the Graduate Program in Entomology for the degree of *Magister Scientiae*.

Advisor: Eliseu José Guedes Pereira

VIÇOSA - MINAS GERAIS
2020

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa

T
S237t
2020

Santos, Camila Oliveira, 1995-

Transgenic Bt maize, seed treatment and foliar insecticides against fall armyworm and a stink bug / Camila Oliveira Santos. – Viçosa, MG, 2020.
44f. : il. (algumas color.) ; 29 cm.

Orientador: Eliseu José Guedes Pereira.

Dissertação (mestrado) - Universidade Federal de Viçosa.
Referências bibliográficas: f.28-37.

1. Plantas - Doenças e pragas. 2. Plantas - Proteção. 3. *Spodoptera frugiperda*. 4. *Dichelops melacanthus*.

I. Universidade Federal de Viçosa. Departamento de Entomologia. Programa de Pós-Graduação em Entomologia. II. Título.

CDD 22 ed. 632.7

CAMILA OLIVEIRA SANTOS

TRANSGENIC Bt MAIZE, SEED TREATMENT AND FOLIAR INSECTICIDES AGAINST FALL ARMYWORM AND A STINK BUG

Dissertation presented to the Universidade Federal de Viçosa, in partial fulfillment requirements of the Graduate Program in Entomology for the degree of *Magister Scientiae*.

APPROVED: February 17, 2020.

Assent:

Camila Oliveira Santos

Camila Oliveira Santos
Author

Eliseu José Guedes Pereira
Advisor

To God, for the gift of life.

To my parents, Anisete and Jânio, my biggest loves.

I dedicate

ACKNOWLEDGEMENTS

To the Universidade Federal de Viçosa, for the opportunity to study at the most beautiful University in Brazil.

To the Graduate Program in Entomology, for the opportunity of pursuing the master's degree.

To CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico for the award of the scholarship.

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance code 001.

To my advisor Eliseu José Guedes Pereira, for welcoming me into his laboratory, for all the knowledge provided me well to for all the lessons learned I am very grateful.

To the laboratory of insect-plant interaction for all the partnership and learning in these two years of coexistence. I give special thanks to Clébson, Silvana, Adelson and Thadeu for all their help and partnership. To the interns: Morgana, Abrahim, Vinícius, Leidiana, Rodrigo, Letícia, Matheus, Igor and Evandro, without you guys I would not have made it.

To professor Eugênio and the doctoral student Javier, for availability and help in the rearing of stink bugs.

To the laboratory of integrated pest management, for the use of green houses to conduct the bioassays.

To all the friends I had the pleasure to meet in Viçosa: Paola, Silvana, Adelson, Viviana, Maribel, Vanessa, and Jose, you guys made everything happier.

To my boyfriend, partner and friend, Maikon, you were and are very important in this process, always giving me support when I need it.

Thank you very much!

“If I cannot do great things, I can do small things in a great way.”
(Martin Luther King)

ABSTRACT

SANTOS, Camila Oliveira, M.Sc., Universidade Federal de Viçosa, February, 2020. **Transgenic Bt maize, seed treatment and foliar insecticides against fall armyworm and a stink bug.** Advisor: Eliseu José Guedes Pereira.

Maize is one of the most important cereal crops in the world, and the high infestation by insect pests is one of the main yield-loss factors in the crop, especially when occurring in the early growth stages. In these, the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), and the green-belly stink bug (GBS), *Dichelops melacanthus* (Dalas, 1851) (Hemiptera: Pentatomidae), can be great a challenge, particularly if the maize crop is preceded by soybeans or grown using minimal tillage over the preceding-crop residues. In this dissertation, the integrated use of *Bacillus thuringiensis* (Bt) transgenic maize, seed treatment and foliar insecticides was tested for efficacy against these two insect species. Because some Bt-resistant FAWs can have altered response to stressors, the susceptibility of Bt-resistant populations of FAW to seed treatment and foliar insecticides was also determined. In bioassays of cumulative effects of control measures against FAW and GBS, the seed treatment using neonicotinoid (clothianidin) was effective against FAW and GBS; the diamide (chlorantraniliprole) was effective only for FAW until seven days after emergence in optimal edaphoclimatic conditions. After 21 days of emergence, the mortality effect of the seed treatment was no longer present and foliar insecticides may be needed to provide plant protection. In bioassays using field-rate sprayed maize plants, all foliar insecticides were efficacious (mortality > 80%) in controlling the Bt-resistant FAWs feeding on Cry1A.105+Cry2Ab Bt maize, except acephate. In contrast, acephate killed 100% of the stink bugs as did methomyl. Imidacloprid+beta-cyfluthrin also caused more than 80% mortality of GBS adults. The other insecticides (thiamethoxam+lambda-cyhalothrin, chlorantraniliprole and flubendiamide) had less than 80% mortality. Diamides as foliar insecticides or in seed treatment caused high mortality of FAWs, but not of GBSs. Regarding seed treatments, in concentration-mortality bioassays the larvae of a recently-isolated Bt-resistant FAW population showed resistance to diamides (43 fold for chlorantraniliprole and 17 fold for cyantraniliprole). In concentration-response bioassays with foliar insecticides, at least one FAW population resistant to Cry Bt toxins showed resistance to the insecticides tested (chlorantraniliprole, spinetoram, indoxacarb, flubendiamide, bifenthrin+carbosulfan, methomyl) when compared to the susceptible population; however, only to indoxacarb the larvae showed moderate-to-high resistance ratios. The LC_{90} values for the insecticides introduced more recently (e.g., chlorantraniliprole, spinetoram) were 300-900 fold lower than their field label rates, indicating a high likelihood of control success; for the insecticides introduced long ago (carbamates, pyrethroids) these values were only 10 fold, indicating a smaller safety margin for control success in the field. Therefore, the use of clothianidin to treat seeds of transgenic Bt maize may be a efficacious combination against FAWs and GBSs; if needed, foliar insecticides such as methomyl and imidacloprid+beta-cyfluthrin are likely to have high efficacy against both of these pest species and may be useful for integrated management in maize. These results are useful in decision-making and recommendations for integrated resistance management to important pest control technologies used against FAW and GBS, including Bt maize, seed treatment and foliar insecticides.

Keywords: *Spodoptera frugiperda*. *Dichelops melacanthus*. *Zea mays*. Resistance. Integrated pest management. Crop protection.

RESUMO

SANTOS, Camila Oliveira, M.Sc., Universidade Federal de Viçosa, fevereiro de 2020. **Milho transgênico Bt, tratamento de sementes e inseticidas foliares contra a lagarta-do-cartucho e um pentatomídeo.** Orientador: Eliseu José Guedes Pereira.

O milho é uma das culturas de cereais mais importantes do mundo, e alta infestação por insetos-praga é um dos principais fatores de perda de rendimento na lavoura, especialmente quando ocorre nos estágios iniciais de crescimento das plantas. Dentre essas pragas, a lagarta-do-cartucho, *Spodoptera frugiperda* (JE Smith, 1797) (Lepidoptera: Noctuidae) e o percevejo barriga verde, *Dichelops melacanthus* (Dalas, 1851) (Hemiptera: Pentatomidae) podem ser um grande desafio, particularmente se a safra de milho for precedida pela da soja ou cultivada em plantio direto sobre os resíduos da cultura anterior. Nesta pesquisa, o uso integrado de milho transgênico *Bacillus thuringiensis* (Bt), tratamento de sementes e inseticidas foliares foi testado quanto à eficácia contra essas duas espécies de insetos. Como algumas populações de *S. frugiperda* resistentes a Bt podem ter resposta alterada a certos estressores, também foi determinada a suscetibilidade de populações resistentes a Bt ao tratamento de sementes e inseticidas foliares. Nos bioensaios de efeitos cumulativos dos métodos de controle contra ambas as espécies, o tratamento de sementes usando neonicotinóide (clotianidina) é eficaz contra *S. frugiperda* e *D. melacanthus*; porém a diamida (clorantraniliprole) é eficaz apenas para *S. frugiperda* até sete dias após a emergência em condições edafoclimáticas ideais. Após 21 dias de emergência, o efeito da mortalidade do tratamento de sementes não estava mais presente e aplicações com inseticidas foliares podem ser necessárias para complementar a proteção das plantas. Em bioensaios usando plantas de milho pulverizadas com as concentrações de campo, todos os inseticidas foliares foram eficazes (mortalidade> 80%) no controle da população resistente de *S. frugiperda* alimentados com o milho Cry1A.105+Cry2Ab, exceto acefato. Por outro lado, o acefato matou 100% dos percevejos, assim como o metomil. O imidacloprido+beta-ciflutrina também causou mais de 80% de mortalidade em adultos do percevejo. Os demais inseticidas (tiametoxam+lambda-cialotrina, clorantraniliprole e flublendiamida) apresentaram mortalidade inferior a 80%. Diamidas como inseticidas foliares ou em tratamento de sementes causaram alta mortalidade em *S. frugiperda*, mas não em *D. melacanthus*. Em relação ao tratamento de sementes, em bioensaios de concentração-resposta, as larvas de uma população de *S. frugiperda* resistente a Bt recentemente isolada apresentou resistência as diamidas (43 vezes para clorantraniliprole e 17 vezes para ciantraniliprole). Nos bioensaios de concentração-resposta com inseticidas foliares, pelo menos uma população de *S. frugiperda* resistente as toxinas Bt mostrou resistência aos inseticidas testados (clorantraniliprole, espinetoram, indoxacarbe, flubendiamida, bifentrina+carbosulfan e metomil) quando comparados com a população suscetível, no entanto apenas para o indoxacarbe as larvas apresentaram razões de resistência de moderada a alta. Os valores de LC_{90} para os inseticidas introduzidos mais recentemente (clorantraniliprole, espinetoram) foram 300-900 vezes menores do que suas doses de campo, indicando uma alta probabilidade de sucesso no controle; para os inseticidas introduzidos há muito tempo (carbamatos, piretróides) esses valores foram 10 vezes menores, indicando uma margem de segurança menor para o sucesso de controle no campo. Portanto, o uso de clotianidina no tratamento de sementes de milho Bt pode ser uma combinação eficaz contra a lagarta-do-cartucho o percevejo barriga verde; se necessário, é provável que inseticidas foliares como metomil e imidacloprido+beta-ciflutrina tenham alta eficácia contra essas duas espécies-pragas e possam ser úteis para o manejo integrado no milho. Estes resultados auxiliarão na tomada de decisões e nas recomendações visando manejo integrado da resistência a importantes tecnologias de controle de pragas disponíveis contra *S. frugiperda* e *D. melacanthus*, incluindo milho Bt, tratamento de sementes e inseticidas foliares.

Palavras-chave: *Spodoptera frugiperda*. *Dichelops melacanthus*. *Zea mays.* Resistência. Manejo integrado de pragas. Proteção de plantas.

TABLE OF CONTENTS

1 Introduction

The annual worldwide demand in 2050 for cereals such as maize, rice and wheat, which are fundamental for world food security, is expected to reach about 3.3 billion tons (FAO, 2015). Maize is one of the most important cereals for human and animal consumption around the world, grown for grains and forage (FAO, 2015). Climate change adds new pressures on the current production model, including rising temperatures and a higher incidence of pests, diseases, droughts and floods. Among these challenges, the attack of pest insects is one of the most important problems that occur in maize (Oliveira et al., 2014), leading to losses ranging from 6 to 19% around the globe (Oerke, 2006).

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), is a polyphagous key pest indigenous throughout the Americas, that can attack all stages of maize development. In 2016 it was first reported in Africa (Cock et al., 2017; Goergen et al., 2016), and in May 2018 the pest was already in India (Sharanabasappa et al., 2018). At the end of 2018, FAW was first found in Yunnan Province, China (Guo et al., 2019, 2018) and has great potential to spread across the country (Wang et al., 2020). In addition, there is a high risk of FAW invading Japan and Korea soon (Ma et al., 2019). The use of transgenic plants, which express insecticidal protein genes from the bacterium *Bacillus thuringiensis* (i.e., Bt proteins) is the most used method for controlling FAW in maize. However, the intensive and large-scale use of this technology has selected resistant populations to Cry Bt toxins, including Cry1F, Cry1Ab e Cry1A.105+Cry2Ab (Bernardi et al., 2015; Santos-Amaya et al., 2015; Storer et al., 2010). This is a concern because of the risk of control failure and loss of efficacy of these technologies against FAW. Therefore, research on this topic is important for improving integrated pest and resistance management.

Another insect species that has gained importance in regions of succession soybean-maize and soybean-wheat is the green-belly stink bug (GBS), *Dichelops melacanthus* (Dalas, 1851) (Hemiptera: Pentatomidae). This stink bug is a polyphagous species first found in maize fields in the mid-1990s in central-western Brazil (Panizzi, 2015). The adults and nymphs remain in the straw and/or crop residues after the end of the soybean growing season (Smaniotto and Panizzi, 2015), especially under no-tillage cultivation, and attack maize fields in the early growth stage. The adoption of this system of crop succession is favorable for GBS population growth because of the continuous availability of host plants and survival conditions throughout the year (mild winter/dry season) (Cruz et al., 2016). Plant damage is a consequence of the insertion of the insect stylet in the newly developed tissues. In this process, toxic saliva is generally secreted, causing stunted growth and development of unproductive tillers. In certain situations, the stink bug attack can cause the death of the seedling, reducing the final stand of the crop, which is critical for high yield of maize and wheat. The green belly stink bug is considered one of the pentatomids with high risk to invade countries like the United States (Panizzi, 2015).

Although there are many technologies available for pest management in maize, the isolated use of some control measures such as Bt cultivars has already led to control failure and selection of resistant populations of FAW. In addition, Bt cultivars target only lepidopterans and/or coleopterans in maize while the pest complex is large, including hemipterans, such as leafhoppers and stink bugs. FAW has a longer history of exposure to synthetic insecticides than to Bt toxins and has shown increasingly cases of insecticide resistance since the late 1940s (Sparks and Nauen, 2015). Resistance of FAW populations to different classes of insecticides has already been described (Carvalho et al., 2013; Gutirrez-Moreno et al., 2018; Nascimento et al., 2015; Okuma et al., 2018; Ríos-Díez and Saldamando-Benjumea, 2011; Yu and Elzie McCord, 2007; Zhu et al., 2015).

The Cry1Ab Bt-maize technology was first introduced in 1996 in the United States, followed by Cry1F, Cry1A.105+Cry2Ab, and Vip3Aa Bt traits (ISAAA, 2020). After introducing the Cry1F maize, four years later a resistance case in FAW was reported to this toxin (Storer et al., 2010; Tabashnik and Carrière, 2017). A similar trend of Bt toxin deployment and FAW resistance development occurred in Brazil (Farias et al., 2016, 2014; Omoto et al., 2016). No published study on FAW resistance to Cry1A.105+Cry2Ab is available, but there are claims of pest managers and a report arguing that the technology has lost some of its original efficacy against FAW (Fatoretto et al., 2017). Indeed, the resistance to this Bt maize in a field-derived FAW population was shown to evolve rapidly, although incompletely, recessive and without cross-resistance to Vip3Aa Bt toxin (Santos-Amaya et al., 2015). Interestingly, the incomplete resistance to Cry1A.105+Cry2Ab Bt maize (i.e., when the fitness of the resistant individuals exposed to a pesticide is lower than the resistant individuals not exposed) (Tabashnik et al., 2014) may have interacted with its associated fitness costs (Santos-Amaya et al., 2020), (i.e., when in the absence of toxin the fitness of the resistant individuals is lower than that of the susceptible individuals), and helped maintain the control efficacy of FAW in the field. This is a likely scenario because under stress in an unsuitable host-crop (cotton) the resistant FAWs could not survive (Santos-Amaya et al., 2020).

In this context, it is possible that the Cry1A.105+Cry2Ab-resistant FAW will have altered susceptibility to certain insecticides. The insects may be more susceptible to more recently introduced compounds (e.g., some diamides and spinosyns) and less susceptible to compounds in the market over 30 years (e.g., some carbamates and pyrethroids), a hypothesis yet to be tested. The outcome of this research could indicate a favorable (if the resistant populations are more susceptible to insecticides), or unfavorable (if the resistance has some influence on the lower susceptibility of the pest) condition for pest/resistance management in regions with intense selection pressure on Bt maize and synthetic insecticides. Cross-resistance between Bt toxins and synthetic insecticides is unlikely because of their distinct mode of action (Casida and Durkin, 2013; Gulzar et al., 2012; Pardo-López et al., 2012; Sayyed et al., 2008), although there may be multiple resistance (Zhu et al., 2015). Therefore, testing resistant populations for their susceptibility to some insecticides will help understand whether resistance to Bt has any influence on insecticide susceptibility.

The judicious use of insecticides is important for integrated pest management. There have been increasing rates of adoption of insecticides for seed treatment (ST), which may help controlling GBS and FAW in the early stages of maize. In 2008, more than 80% of the world ST market was composed of neonicotinoid insecticides (Alford and Krupke, 2017; Jeschke et al., 2010). In Brazil, the ST was introduced since the 1970s and currently about 98% of soybean and hybrid maize seeds are treated with insecticides and fungicides (Seednews, 2016). In the United States, about 80% of maize seeds are planted treated with insecticide (Douglas and Tooker,

2015) and almost 50% of the total weight of insecticides applied is in seed treatment (Perry et al., 2016). With this application method, the amount of active ingredient used per unit area is argued to be reduced in relation to foliar applications (Jeschke et al., 2010). In addition, the application is considered safer for the operator and is likely to cause less impact on the environment compared to traditional spraying (Elbert et al., 2008), although some zoophytophagous insects may still be negatively affected by systemic ST insecticides (Gontijo et al., 2018, 2015, 2014; Moscardini et al., 2015; Resende-Silva et al., 2019a, 2019b; Seagraves and Lundgren, 2012). Currently, most commonly used compounds for ST are in the carbamate, neonicotinoid and diamide insecticidal classes. These insecticides are systemic (Yang et al., 2018b, 2018a; Zhang et al., 2019) and active against different target species, mostly leafminer lepidopterans and hemipterans (De Oliveira et al., 2008; Seagraves and Lundgren, 2012; Villegas et al., 2019). This scenario has changed since 1970's, when most ST insecticides were used only against soil insects attacking the seeds or the root system of plants. The market for ST insecticides has broadened and so has their crop protection spectrum.

The isolated use of these different pest management technologies, even showing good control efficacy in the short term, is unlikely to continue viable in the long term. Therefore, it is important to pursue the integration of different methods and tools to reduce the selection pressure in a single pest-management technology and maintain their longevity of efficacy/viability. This is one of core ideas in the well-known paradigm of the Integrated Pest Management - IPM (Kogan, 1998). Nevertheless, generally lacking are studies addressing the question on cost-effective combinations of control methods against the pest complex in a given crop, in our case, chewing and sucking insects in maize. Plenty of research have focused on the susceptibility of pest populations to particular technologies, but only few have examined their combined use to attain cost-effective and more-sustainable pest management. Therefore, the aim of this study was to determine the susceptibility of Bt-resistant fall armyworm populations to foliar insecticides and seed treatment and to test the potential efficacy of integrated pest management technologies, namely Cry1A.105+Cry2Ab Bt maize, seed treatment and foliar insecticides for controlling of *S. frugiperda* and *D. melacanthus*.

2 Material and methods

2.1 Insects

In the experiments, four populations of fall armyworm (FAW), *S. frugiperda*, were used, including the LabSS, a standard susceptible population obtained from the Embrapa Maize & Sorghum Research Center - Sete Lagoas, Minas Gerais, Brazil. These insects have been maintained for over 15 years out of exposure to any pesticide. The Bt-resistant populations, namely RHX11 and RPRO13, were previously characterized (Santos-Amaya et al., 2016, 2015). The RHX11 population is derived from collections of FAW from five states in Brazil in 2010/2011 (Santos-Amaya et al., 2016). The RPRO13 population was derived from a large collection of larvae present in TC1507 (Cry1F) Bt maize fields in Western Bahia in 2013. The fourth population, RPRO17, was isolated in 2017 from Bt maize fields planted to Bt maize event MON89034 (Cry1A.105 + Cry2Ab), in Cajuri, Minas Gerais (Orozco-Restrepo, 2019).

The populations were maintained using the rearing methodology of Kasten-Junior et al. (1978) with some modifications. Briefly, the moths are placed in PVC cages 40 cm high × 30 cm in diameter with sulfite paper on the inner walls for oviposition; cotton soaked in a solution of 10% sugar and 5% ascorbic acid is provided as food. Eggs are collected every other day for four days and stored in plastic bags until hatching. Batches of neonates are transferred to artificial diet in 500-mL plastic cups until the 2nd instar, and then individually placed in 16-well PVC trays (Advento do Brasil, Diadema, SP) until pupation.

The population of the green-belly stink bug (GBS), *D. melacanthus*, comes from Embrapa Soybean, Londrina, Paraná, Brazil. The population was maintained using the rearing methodology of Silva and Panizzi (2007). In the rearing of GBS, adults are kept in plastic cages lined with sulfite paper. The insects were fed green bean pods, (*Phaseolus vulgaris)*, a mix of soybeans (*Glycine max*), peanuts (*Arachis hypogaea*) and sunflower (*Helianthus annuus*). Cotton bolls were provided as an oviposition substrate. The eggs were collected every 3 days and placed in Petri dishes until hatching. The nymphs were maintained similarly to the adults, providing new food every 2 days. Both insect species were kept at the Federal University of Viçosa, in 27 ± 2°C, 70 ± 15% relative humidity and 14L: 10D photoperiod.

2.2 Insecticides

For bioassays involving seed-treatment insecticides, in the lethal effects in FAW and GBS and in the FAW susceptibility, the products used and their information are described in Table 1. Some characteristics and information of the foliar insecticides used in the bioassay involving interaction of several treatments against FAW and GBS are described in Table 2. For susceptibility of FAW populations to foliar insecticides, the products used, their concentrations and chemical groups are described in Table 3.

2.3 Potential of seed treatment for control of FAW and GBS

Seeds of maize plants expressing the Bt toxins Cry1A.105+Cry2Ab (maize hybrid BM709PRO2, Sementes Biomatrix, Patos de Minas, MG) were treated with chlorantraniliprole and/or clothianidin (Table 1). The first insecticide is recommended for FAW control and the other for GBS control, both in maize. The seeds were provided industrially-treated with clothianidin in addition to the treatment with microbiological contact nematicide (*Bacillus firmus* strain I-1582). To obtain untreated seeds, they were thoroughly washed in tap water for 120 s until leaving no visible seed coating. The insecticide preparation was thoroughly mixed with the seeds in a 100-mL plastic pot for 3 min. After homogenization, the seeds were placed in trays covered with paper towels to dry on the laboratory bench for about 60 minutes. The seeds were treated following the manufacturer protocol using the upper limit of the label rate recommended to control FAW and GBS in maize.

Plastic pots with a volume of 400 mL were filled with soil from horizon A of coffee plantation with organic matter = 8.73%; clay, silte and sand= 44, 16 e 40%, respectively (considered a clay soil). The organic substrate (MECPLANT, Mec Prec-Indústria e Comércio Ltda., Telêmaco Borba, PR) was added to the soil in the proportion 2: 1 and fertilized with N-P-K 4-14-8 (Fertipar Sudeste Adubos e Corretivos Agrícolas Ltda., Varginha, MG, Brasil). These treated seeds were sown in the pots (1 seed/pot) and kept in the greenhouse, daily irrigating the plants as needed to avoid leaching the insecticide. Groups of plants (30) of 7 days after emergence (V3-V4) were infested with two RPRO17 FAW larvae (L_3, 1 cm long) or one GBS adult (< 10 days old).

After infestation, the plants were covered with another 400-mL transparent plastic pot attached the plant pot using sticky tape, forming a chamber-like unit to avoid insect scape. After 96 h, insect mortality and foliar injury rating (Davis scale) were assessed to determine the potential control efficacy of Bt maize combined with seed treatment against the two target-insect species. The bioassays were conducted in the greenhouse from July to November 2019. Insects were considered dead if they did not move when gently touched using a tweezers.

2.4 Bt maize, seed treatment, and foliar insecticides against FAW and GBS

Cry1A.105+Cry2Ab Bt maize seeds were treated as described previously, but using the insecticides chlorantraniliprole + clothianidin. The seeds were planted in 2-l plastic pots using the same agronomic procedures previously described to grow the plants. To assess the cumulative effect of seed treatment plus foliar insecticides, the maize plants were sprayed with the following foliar insecticides (Table 2): flubendiamide, chlorantraniliprole, imidacloprid + beta-cyfluthrin, thiamethoxam + lambda-cyhalothrin, methomyl, acephate. The treatments were applied with a backpack sprayer (10.0 bar) with spray volume equivalent to 150 L/ha and nozzle like adjustable cone JD-12, following the manufacturer protocol and using the upper limit of the label rate to control FAW or GBS in maize.

The maize plants used were in the 21-day after emergence (V5- V6) were grown in 2-l pots using procedures previously developed in our laboratory (Pinto, 2019). After drying in the shade, the plants were placed in the greenhouse and infested with two RPRO17 FAW larvae (L_3, 1 cm long) or one GBS adult (< 10 days old) as previously described. The plants were covered using a plastic bag (like a pollination bag) to avoid insect escape. After 96 h, insect mortality and FAW leaf injury (Davis scale) were recorded to determine the potential efficacy of the foliar insecticides in interaction with MON89034 maize and seed treatment. Both insects were considered dead if they do not move with the touch of a tweezers.

2.5 FAW susceptibility to seed-treatment insecticides in maize

Larvae of the standard susceptible population (LabSS) or those resistant to Bt toxins Cry1A.105 and Cry2Ab (RPRO17 population) were let to feed on seedlings whose seeds were treated with synthetic insecticides. These included chlorantraniliprole, cyantraniliprole and imidacloprid + thiodicarb. Non-Bt maize seeds (hybrid BRS 3046, Embrapa germplasm, Priorizi Sementes, Goiânia, GO) were treated with serial dilutions of each insecticide (Table 1) totaling eight different concentrations. The seeds were treated individually using a micropipette and allow to dry for 60 min in the laboratory.

Using tweezers, the seeds were handled and sowed (1 single seed/pot) in plastic pots (400 mL) using the same soil and agronomic procedures previously described to grow the plants. Five days after emergence, 30 plants of each treatment (concentration) were infested. A single 3rd-instar larva was transferred to the whorl leaves. The plants were covered with another 400-mL transparent plastic pot attached the plant pot using sticky tape (forming a chamber-like unit). Mortality and foliar injury rating (Davis, 1992) was recorded after 96 h. The bioassay was repeated twice using one larvae per experimental unit. All the bioassays were conducted in the greenhouse from May to November 2019 at the Federal University of Viçosa, Minas Gerais, Brazil.

2.6 Susceptibility of FAW populations to foliar insecticides

We used the foliar-deep method recommended by Insecticide Resistance Action Committee (IRAC) for bioassays with some Lepidoptera. Whorl leaves of non-Bt maize were collected from plants in the V4-V9 growth stages, optimally grown in the field. The leaves were cut in 3-cm sections, immersed in the insecticide preparation for 5 s, and let dry in the laboratory for 60 min. The insecticides used were (Table 3): bifenthrin + carbosulfan, methomyl, flubendiamide, indoxacarb, chlorantraniliprole and spinetoram.

The treated-foliar sections were carefully placed in 16-well plastic trays (Advento do Brasil, Diadema, SP) using tweezers. A single 3rd instar FAW larva (< 1 cm) was transferred to each well. Larvae of all FAW populations described were bioassayed. The bioassay was repeated twice, totaling 200 larvae. After 96 h, mortality was recorded, considering dead the larvae that did not move when touched with a fine paintbrush. All the bioassays were conducted under the following laboratory conditions: 27±2°C, 70±15% relative humidity, and 14L: 10D photoperiod.

2.7 Statistical analysis

The data (i.e., mortality, leaf injury) for each insect species and plant stage were subjected to one-way variance analysis of variance and the means were separated using Tukey's honestly significant difference (HSD), at the 5% level of significance ($P < 0.05$). Homoscedasticity and normality were checked for the datasets and no transformation was needed. The analyses were run and graphs constructed using the SigmaPlot 12.5 software (Systat Software, San Jose, CA).

The data of the concentration-mortality bioassays were analyzed using the generalized linear probit model. The following estimates were obtained using the PoloPlus program (Robertson et al., 2007): lethal (LC_{50} and LC_{90}) concentrations and their respective confidence limits (95% CL) as well as slopes and their standard errors (Finney, 1971). The susceptibility was considered significantly different ($P < 0.05$) when 95% confidence intervals for the resistance ratios did not overlap the origin of the 95% confidence interval for the population compared. Each resistance ratio was determined using the standard susceptible strain (LabSS) or the Cry1F-resistant as reference in the comparisons (Robertson et al., 2007, 1995).

3 Results

3.1 Potential of seed treatment for control of FAW and GBS

Figure 1 shows the interaction of Cry1A.105+Cry2Ab Bt maize and seed treatment against Cry1A.105+Cry2Ab-resistant FAW larvae and GBS adults. After 7 days after emergence, the seed treatment with chlorantraniliprole, clothianidin, and chlorantraniliprole + clothianidin killed > 90% of the FAW larvae and protected the maize plants from the foliar injury (Figure 1a, b). Cry1A.105+Cry2Ab Bt maize plants caused less than 20% mortality of the Bt-resistant larvae, reaching the score 6 in the foliar injury rating (Figure 1a, b). In contrast, the Bt maize plants killed more than 80% of the standard susceptible larvae after 96 h feeding on the whorl leaves. Importantly, the natural mortality of standard susceptible larvae was less than 20% as indicated by their survival rates and foliar injury on the non-Bt isoline maize leaves (Figure 1a, b).

Regarding the stink bugs (Figure 1c), both seed treatments, with clothianidin and chlorantraniliprole + clothianidin, caused 100% mortality of GBS adults after 96 h feeding on the plants of 7 days after emergence. The mortality rate on control plants was less than 20% and was not different from the mortality caused by the chlorantraniliprole seed treatment, as expected.

3.2 Bt maize, seed treatment, and foliar insecticides against FAW and GBS

Figure 2 shows the interaction of Cry1A.105+Cry2Ab Bt maize, seed treatment with clothianidin + chlorantraniliprole, and foliar insecticides for control of Bt-resistant FAW larvae and GBS adults. Again, on Bt maize, the mortality rates of resistant FAW larvae and GBS adults were less than 20% (Figure 2a, c), thus allowing detecting the effect of the interacting factors in the bioassays. After 21 days after emergence, the seed treatment with chlorantraniliprole + clothianidin no longer was effective to control the fall armyworm larvae (45% mortality rate) nor the adults of the green-belly stink bug (60% mortality rate) (Figure 2a, c).

The foliar insecticides sprayed on the plants developed from seeds treated with clothianidin + chlorantraniliprole caused more than 80% mortality of the resistant fall armyworms (Figure 2a) protecting the plants (Figure 2b), except acephate, which caused 70% mortality of the larvae and allowed a score of 4 in the Davis injury rating (Figure 2a, b). In contrast, thiamethoxam + lambda-cyhalothrin did not reached 80% mortality of GBS, nor did the diamides chlorantraniliprole and flublendiamide (Figure 2c). Thus, applying diamides in maize fields is unlikely to help control GBS. However, the insecticides methomyl, acephate and imidacloprid + beta-cyfluthrin caused more than 80% mortality in GBS.

3.3 FAW susceptibility to seed-treatment insecticides in maize

Significant concentration-response of the insecticides was obtained (Figure 3), and the probit model fitted properly to the data as indicated by chi-square values associated with *P*-values greater than 0.05 (Table 4). The slopes of the concentration-mortality regression lines for the insecticides were not so different (Figure 3). Imidacloprid + thiodicarb had the lowest potency (highest LC_{50} values, c.a., 30 fold) among the insecticides. The LC_{50} values of imidacloprid + thiodicarb were not different for the resistant (RPRO17) and the susceptible (LabSS).

In contrast, the slope of the probit line was higher for the resistant larvae (Table 4), indicating that the variance in susceptibility to imidacloprid + thiodicarb in the resistant population was lower than in the susceptible population, which may be associated with selection for resistance to Cry1A.105+Cry2Ab maize. The resistant larvae showed resistance to chlorantraniliprole and cyantraniliprole of 42.9 and 17.4 fold, respectively. The most recently introduced diamide, cyantraniliprole, had the highest potency (lowest LC_{50} values) among the insecticides tested.

3.4 Susceptibility of FAW populations to foliar insecticides

Relative to the Cry1F-resistant population (RHX11), the larvae resistant to Cry1Ab.105+Cry2Ab maize (RPRO13 and RPRO17) showed resistance to four of the six insecticides (Table 5, last column). This was especially clear for the RPRO17 population, collected in 2017. The magnitude of the increased susceptibility was 2 fold for methomyl, 7 fold for chlorantraniliprole and indoxacarb, 14.5 fold for flubendiamide. Only for spinetoram and bifenthrin + carbosulfan, the Cry1Ab.105+Cry2Ab-resistant larvae were as susceptible as the Cry1F-resistant larvae. In addition, relative to the standard susceptible population, the levels of insecticide resistance were low (< 10 fold) among the resistant populations. Only for indoxacarb, the resistance levels reached 122 fold in the Cry1F-resistant population (RHX11) and 18-50 fold in the Cry1Ab.105+Cry2Ab-resistant populations.

Importantly, the LC_{90} values estimated (Table 6) were 6 to 1275 fold lower than label field rates of the insecticides, indicating that they may provide proper control efficacy in the field (~80%). This inference is possible because we used the foliar-dip method of bioassay, which represents the field conditions of fall armyworm exposure to the insecticides. Had we used topical treatment, diet-surface application or incorporation on artificial diet, none of these inferences were possible. The LC_{90} values for modern insecticides (chlorantraniliprole and spinetoram) were 316-908 fold lower than their field rate. For insecticides introduced in the 1990s and considered of average performance (indoxacarb and flubendiamide), the LC_{90} values varied 14-376 fold, while for those introduced more than 30 years ago (bifenthrin+carbosulfan and methomyl), the values were 6-19 fold (Table 6).

4 Discussion

The results of this research indicate that integrating plant resistance (Bt maize), seed treatment and foliar insecticides is technically viable to manage FAW and GBS. The use of seed treatment with systemic insecticides is a relatively recent practice used globally and with a high adoption rate in Brazil. The use of diamides and neonicotinoids in seed treatment broadens the range of protection, targeting both chewing and sucking insects. The combination of chlorantraniliprole + clothianidin may be a useful seed treatment both on-farm and industrially provided. Importantly, no altered toxicity to the insect or reduced plant vigor/germination was observed when both treatments were applied. Seed treatment can cause negative effects/phytotoxicity on the seed (Bittencourt et al., 2000; de Moraes Dan et al., 2010) and the seedling/plant (Taylor and Salanenka, 2012) or positive effects as for some neonicotinoids (Horii et al., 2007; Macedo et al., 2013; Macedo and Castro, 2011). This is a topic that deserves more research because of the scarcity of published reports, especially for systemic seed treatment against chewing insects on the plant aerial parts.

Integrating the use of foliar insecticides after 21 days of emergence may be a valuable tactic to control FAW and GBS in maize and avoid losses by plant mortality and severe damage. Our results indicate that insecticides released more than 30 years ago could still be a useful tool for pest and resistance management in maize. Insecticides such as methomyl showed high mortality of FAW and GBS while imidacloprid + beta-cyfluthrin and acephate showed good performance. Diamides (i.e., flubendiamide and chlorantraniliprole) showed high mortality of FAW and approximately 50% mortality GBS in a best-case scenario, although there may be negative and positive effects of diamides on sucking insects (Barry et al., 2015; Tuelher et al., 2017). The old insecticides (i.e., methomyl, acephate), however, may not be selective to non-target organisms (de Paiva et al., 2018; Pereira et al., 2009) and should be used judiciously, for example, exploiting ecological selectivity. Although diamides are selective in favor of non-target arthropods, mammals and aquatic organisms (Xu et al., 2016), not much is known about their sublethal effects, which may have implications for pest management (Guedes et al., 2016). The take home message is that old insecticides may help preserve the newer, fully-efficacious technologies for pest management of FAW and GBS at a lower cost to the grower.

Here, concentration-response was determined for the first time using the insecticides in seed treatment delivered by the plant as in the field. The FAW population resistant to Cry1A.105 + Cry2Ab Bt toxins showed resistance to the two diamides tested than the standard susceptible population. The history of using chlorantraniliprole and flubendiamide as foliar insecticides against FAW may have influenced these results. Different diamides can have cross-resistance (Bolzan et al., 2019; Sang et al., 2016; Silva et al., 2016), which may be related to shared binding sites of anthranilic and phthalic acid diamides in their receptor protein (Qi et al., 2014; Qi and Casida, 2013). This may help explain the significant resistance ratio for cyantraniliprole (17 fold) and chlorantraniliprole (43 fold). Interestingly, in the leaf-dip bioassays, in which there is contact and ingestion of the insecticide, there was no decreased susceptibility to chlorantraniliprole. This inconsistency is puzzling and may need further research to decipher whether the route of exposure to the insecticide and/or if during translocation to the leaves it is metabolized in a way that later affects its toxicokinetics/toxicodynamics in Bt-resistant and susceptible FAWs (Myung et al., 2014).

For the mixture imidacloprid + thiodicarb in seed treatment, there was no difference in susceptibility between the FAW populations. This may be due to the broader spectrum of action of the two active compounds, despite a generally low toxicity of imidacloprid (neonicotinoid) to chewing insects. Imidacloprid + thiodicarb were less potent than the diamides against FAW, requiring greater concentrations per seed to cause 50% FAW mortality. In addition, the LC_{50} value was close to the label rate recommended (3.5 mg i.a./seed). Therefore, its application on the seed must be homogeneous to minimize the risk of control failure. In a previous study in optimal edaphoclimatic conditions, the recommended label rate caused high mortality of 3rd and 5th instars of the standard susceptible FAW population (Pinto, 2019).

The difference in the potency of the ST insecticides may be linked to their physic-chemical properties, especially their affinity for lipids and solubility in water, which affect the absorption and translocation of the compounds in the plant (Myung et al., 2014). The values of octanol-water partition coefficient ($\log K_{ow}$) and water solubility for the insecticides used do not seem so different. Their respective values are as follows: thiodicarb, 1.62 and 19.1 mg/L; imidacloprid, 0.57 and 610 mg/L, chlorantraniliprole, 2.76 and 1.0 mg/L, cyantraniliprole, 2.02 and 14.2 mg/ (NCBI, 2019). $\log K_{ow}$ values < 0 indicate low lipophilicity, while high values (> 4) indicate high lipophilicity. Insecticides usually have $\log K_{ow}$ values ranging from -2 to 6 (Myung et al., 2014). Substances with very low water solubility have values < 1 mg/L (ChemSafetyPro, 2019). It is unknown whether the absorption (by both the seeds and the root system) is different when two or more active ingredients are applied together in seed treatment. The combination of neonicotinoids with diamides apparently resulted in different concentration patterns in the plant (Myung et al., 2014). Chlorantraniliprole had eight-fold higher concentrations in the roots, and thiamethoxam ($\log K_{ow} = -0.13$; ws = 4100 mg/L) had high acropetal translocation, protecting the apical portions (Lanka et al., 2013; Myung et al., 2014). In addition, other factors such as soil texture, its level of organic matter, and the amount of water available may play a role in determining the insecticide concentration reaching the site of insect feeding. The soil used in this study had high levels of organic matter (8.73%), which may affect sorption/desorption processes and may have interacted with the physical-chemical properties of the insecticides. Especially those with higher water solubility tend to be more affected as they are likely to be present in the soil solution in greater amount than compounds with lower water solubility. Further studies are needed to better understand how these factors interact. In addition, it will be useful to test other field populations of FAW and insect species to further check the potential of ST insecticides against chewing pests in the early growth stages of maize.

The hypothesis that Cry-resistant populations of FAW are more susceptible to foliar insecticides did not find support when they were compared to the standard susceptible population; the LC_{50} values found for the resistant populations were in most cases higher, indicating lower susceptibility. However, when compared to the Cry1F-resistant population (RHX11), the Cry1A.105+Cry2Ab-resistant populations (RPRO13, RPRO17) showed greater susceptibility to the insecticides tested. Thus, depending on the reference, the Bt-resistant populations are less or more susceptible to synthetic insecticides, and it is difficult to reach a consensual conclusion. Importantly, for the two diamides and indoxacarb, the Bt-resistant population collected in 2017 (RPRO17) was more susceptible than those from 2011 (RHX11) and 2013 (RPRO13), indicating that these insecticides can be used against Cry1A.105+Cry2Ab-resistant FAWs. So far, the efficacy of Cry1A.105+Cry2Ab maize against FAW have not declined so fast as in the case of Cry1F maize. Fitness costs and incomplete resistance in FAWs resistant to Cry1A.105+Cry2Ab was likely hindering the resistance development in the field (Santos-Amaya et al., 2015). Nevertheless, the more recently field-collected Cry1A.105+Cry2Ab-resistant FAWs may no longer carry fitness disadvantages (Orozco-Restrepo, 2019), indicating mitigation of the previously documented fitness costs (Coustau et al., 2000, Santos-Amaya et al., 2020). Therefore, the conditions that favored resistance management of FAW to Cry1A.105+Cry2Ab Bt maize may have changed and should be considered in order for the benefits of the new pyramided Cry1A.105+Cry2Ab+Vip3Aa Bt maize to be realized as expected.

It is unlikely that Bt resistance mechanisms lead to negative or positive cross-resistance to synthetic insecticides (Gulzar et al., 2012; Sayyed et al., 2008), even though Bt-resistant populations may show variable susceptibility (Muraro et al., 2019; Zhu et al., 2015) because of the history of insecticide use in the region from where the insects were collected. Here, most insecticides showed low resistance ratios (1-10 fold). Only indoxacarb showed resistance ratio values moderate to high (18-120 fold), as for other lepidopterans (Ghodki et al., 2009; Pang et al., 2012; Sayyed et al., 2008). Despite the non-altered susceptibility to spinetoram, a recently introduced spinosyn highly efficacious against FAW had already one case of resistance reported (Arthropod Pesticide Resistance Database, http://www.pesticideresistance.org) (Gutirrez-Moreno et al., 2018), in addition to several cases of resistance to spinosyn (spinosad) in lepidopterans (Campos et al., 2014; Okuma et al., 2018; Rehan and Freed, 2014; Rinkevich et al., 2010). Therefore, it is important the judicious use of spinetoram (and other insecticides), within an IPM framework and in rotation with compounds of different mode of action to avoid rapid selection of resistant FAWs.

The LC_{90} values for the insecticides were below the recommended field rate, showing that they one likely to reach acceptable control levels (ca. 80%) if properly applied. The calculated label rate ratio (LRR_{90}) values, i.e., how many times the estimated LC_{90} value of the insecticide is lower than its recommended field rate, indicate the level of safety (i.e., the likelihood of success) to achieve acceptable control efficacy if applying adequately the recommended field rate. The lower the LRR_{90} values the more optimal should be application conditions, such as timely when the pest is in the most susceptible stage and in favorable weather conditions (low wind speed and high relative humidity) to increase the likelihood of acceptable performance in the field. This inference is only possible because of the leaf-dip method of bioassay used, which represents the field conditions of fall armyworm exposure to the insecticides. As expected, the LC_{90} values of the insecticides more recently introduced (i.e., spinetoram, chlorantraniliprole) were in average 300-600 lower than their recommended field rates to control FAW larvae while the insecticides introduced 30-50 years ago (i.e., bifenthrin, carbosulfan, methomyl) had LC_{90} values only 6-15 lower than their recommended field rates for FAWs. Therefore, proper operational conditions should be in place when using these insecticides, including good spray coverage, sufficient field rates, and good timing of application in early insect developmental stages.

In summary, this research identified some options of seed treatment and low-cost foliar insecticides that may useful against FAW and GBS in an integrated system involving plant resistance and synthetic insecticides. Seed-treatment insecticides of at least two classes killed FAW larvae in a concentration-response manner although reduced susceptibility may be a concern because of their previous broadcast use. FAW larvae resistant to Cry1A.105+Cry2Ab Bt maize were less susceptible to foliar insecticides than a standard susceptible population although they were generally more susceptible than Cry1F-resistant FAWs. These results should assist in decision-making and recommendations for effective, integrated pest and resistance management. This effort is important to maintain the efficacy of currently available pest control technologies used against FAW and GBS, including Bt maize, seed treatment, and foliar insecticides.

5 Conclusions

The seed treatment using neonicotinoid (clothianidin) is effective against FAW (*S. frugiperda*) and GBS (*D. melacanthus)*; the diamide (chlorantraniliprole) is effective only for FAW until seven days after emergence in optimal edaphoclimatic conditions;

The foliar insecticides methomyl, flubendiamide, chlorantraniliprole, thiamethoxam+lambda-cyhalothrin, imidacloprid+beta-cyfluthrin are potentially efficacious in controlling Bt-resistant FAW. For GBS, the cholinesterase inhibitors acephate and methomyl are highly insecticidal followed by imidacloprid+beta-cyfluthrin;

For seed treatment against FAW larvae, chlorantraniliprole or cyantraniliprole are more potent insecticides than is thiodicarb+imidacloprid; and the FAW population resistant to Cry1A.105+Cry2Ab shows resistance to this diamides used in seed treatment (17-43 fold);

Even though certain foliar insecticides (chlorantraniliprole, spinetoram, indoxacarb, flubendiamide, bifenthrin+carbosulfan, methomyl) may be less toxic to FAW larvae of Cry-Bt-resistant populations than the susceptible population, only to indoxacarb the larvae have moderate to high resistance ratios, and the LC_{90} values of these insecticides are 10 to 800 fold lower than their recommended field rate. Therefore, all of the foliar insecticides tested may provide acceptable control efficacy of FAW larvae in the field if properly applied.

References

Alford, A., Krupke, C.H., 2017. Translocation of the neonicotinoid seed treatment clothianidin in maize. PLoS One 12, 1–19. https://doi.org/10.1371/journal.pone.0173836

Barry, J.D., Portillo, H.E., Annan, I.B., Cameron, R.A., Clagg, D.G., Dietrich, R.F., Watson, L.J., Leighty, R.M., Ryan, D.L., Mcmillan, J.A., Swain, R.S., Kaczmarczyk, R.A., 2015. Movement of cyantraniliprole in plants after foliar applications and its impact on the control of sucking and chewing insects. Pest Manag. Sci. 71, 395–403. https://doi.org/10.1002/ps.3816

Bernardi, D., Salmeron, E., Horikoshi, R.J., Bernardi, O., Dourado, M., Carvalho, R.A., Martinelli, S., Head, G.P., 2015. Cross-Resistance between Cry1 Proteins in Fall Armyworm (*Spodoptera frugiperda*) May Affect the Durability of Current Pyramided Bt Maize Hybrids in Brazil. PLoS One 1–15. https://doi.org/10.1371/journal.pone.0140130

Bittencourt, S.R.M., Fernandes, M.A., Ribeiro, M.C., Vieira, R.D., 2000. Desempenho de sementes de milho tratadas com inseticidas sistêmicos. Rev. Bras. Sementes 22, 86–93. https://doi.org/10.17801/0101-3122/rbs.v22n2p86-93

Bolzan, A., Padovez, F.E.O., Nascimento, A.R.B., Kaiser, I.S., Lira, E.C., Amaral, F.S.A., Kanno, R.H., Malaquias, J.B., Omoto, C., 2019. Selection and characterization of the inheritance of resistance of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to chlorantraniliprole and cross-resistance to other diamide insecticides. Pest Manag. Sci. 2019. https://doi.org/10.1002/ps.5376

Campos, M.R., Rodrigues, A.R.S., Silva, W.M., Silva, T.B.M., Silva, V.R.F., Guedes, R.N.C., Siqueira, H.A.A., 2014. Spinosad and the tomato borer *Tuta absoluta*: A bioinsecticide, an invasive pest threat, and high insecticide resistance. PLoS One 9. https://doi.org/10.1371/journal.pone.0103235

Carvalho, R.A., Omoto, C., Field, L.M., Williamson, M.S., Bass, C., 2013. Investigating the Molecular Mechanisms of Organophosphate and Pyrethroid Resistance in the Fall Armyworm *Spodoptera frugiperda*. PLoS One 8. https://doi.org/10.1371/journal.pone.0062268

Casida, J.E., Durkin, K.A., 2013. Neuroactive Insecticides: Targets, Selectivity, Resistance, and Secondary Effects. Annu. Rev. Entomol. 58, 99–117.

https://doi.org/10.1146/annurev-ento-120811-153645

Cock, M.J.W., Beseh, P.K., Buddie, A.G., Cafá, G., Crozier, J., 2017. Molecular methods to detect *Spodoptera frugiperda* in Ghana, and implications for monitoring the spread of invasive species in developing countries. Sci. Rep. 7, 1–10. https://doi.org/10.1038/s41598-017-04238-y

Coustau, C., Chevillon, C., Ffrench-Constant, R., 2000. Resistance to xenobiotics and parasites: Can we count the cost? Trends Ecol. Evol. https://doi.org/10.1016/S0169-5347(00)01929-7

Cruz, I., Bianco, R., Redoan, A.C.M., 2016. Potential risk of losses in maize caused by *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae) in Brazil. Rev. Bras. Milho e Sorgo 386–397.

Davis, F.M., 1992. Visual rating scales for screening whorl-stage corn for resistance to fall armyworm. Tech. Bull. - Mississippi Agric. For. Exp. Stn. 9.

de Moraes Dan, L.G., de Almeida Dan, H., de Lemos Barroso, A.L., de Lucca Braccini, A., 2010. Qualidade fisiológica de sementes de soja tratadas com inseticidas sob efeito do armazenamento. Rev. Bras. Sementes 32, 131–139.

De Oliveira, C.M., De Oliveira, E., Canuto, M., Cruz, I., 2008. Eficiência de inseticidas em tratamento de sementes de milho no controle da cigarrinha *Dalbulus maidis* (Hemiptera: Cicadellidae) em viveiro telado. Cienc. Rural 38, 231–235. https://doi.org/10.1590/S0103-84782008000100037

de Paiva, A.C.R., Beloti, V.H., Yamamoto, P.T., 2018. Sublethal effects of insecticides used in soybean on the parasitoid *Trichogramma pretiosum*. Ecotoxicology 27, 448–456. https://doi.org/10.1007/s10646-018-1909-5

Douglas, M.R., Tooker, J.F., 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in U.S. Field crops. Environ. Sci. Technol. 49, 5088–5097. https://doi.org/10.1021/es506141g

Elbert, A., Haas, M., Springer, B., Thielert, W., Nauen, R., 2008. Applied aspects of neonicotinoid uses in crop protection. Pest Manag. Sci. 64, 1099–1105. https://doi.org/10.1002/ps.1616

Farias, J.R., Andow, D.A., Horikoshi, R.J., Sorgatto, R.J., Fresia, P., dos Santos, A.C., Omoto, C., 2014. Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. Crop Prot. 64, 150–158. https://doi.org/10.1016/j.cropro.2014.06.019

Farias, J.R., Andow, D.A., Horikoshi, R.J., Sorgatto, R.J., Santos, A.C. dos, Omoto, C., 2016. Dominance of Cry1F resistance in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on TC1507 *Bt* maize in Brazil. Pest Manag. Sci. 72, 974–979. https://doi.org/10.1002/ps.4077

Fatoretto, J.C., Michel, A.P., Silva Filho, M.C., Silva, N., 2017. Adaptive Potential of Fall Armyworm (Lepidoptera: Noctuidae) Limits Bt Trait Durability in Brazil. J. Integr. Pest Manag. 8. https://doi.org/10.1093/jipm/pmx011

Finney, D.J., 1971. Probit Analysis, 3rd ed. Cambridge University Press, London, UK. https://doi.org/https://doi.org/10.1002/jps.2600600940

Ghodki, B.S., Thakare, S.M., Moharil, M.P., Rao, N.G.V., 2009. Genetics of Indoxacarb resistance in *Helicoverpa armigera* (Hubner). Entomol. Res. 39, 50–54. https://doi.org/10.1111/j.1748-5967.2008.00195.x

Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A., Tamò, M., 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One 11, 1–9. https://doi.org/10.1371/journal.pone.0165632

Gontijo, P.C., Abbade Neto, D.O., Oliveira, R.L., Michaud, J.P., Carvalho, G.A., 2018. Non-target impacts of soybean insecticidal seed treatments on the life history and behavior of *Podisus nigrispinus*, a predator of fall armyworm. Chemosphere 191, 342–349. https://doi.org/10.1016/j.chemosphere.2017.10.062

Gontijo, P.C., Moscardini, V.F., Michaud, J., Carvalho, G.A., 2015. Non-target effects of two sunflower seed treatments on *Orius insidiosus* (Hemiptera: Anthocoridae). Pest Manag. Sci. 71, 515–522. https://doi.org/10.1002/ps.3798

Gontijo, P.C., Moscardini, V.F., Michaud, J.P., Carvalho, G.A., 2014. Non-target effects of chlorantraniliprole and thiamethoxam on *Chrysoperla carnea* when employed as sunflower seed treatments. J. Pest Sci. (2004). 87, 711–719. https://doi.org/10.1007/s10340-014-0611-5

Guedes, R.N.C., Smagghe, G., Stark, J.D., Desneux, N., 2016. Pesticide-Induced Stress in Arthropod Pests for Optimized Integrated Pest Management Programs. Annu. Rev. Entomol. 61, 43–62. https://doi.org/10.1146/annurev-ento-010715-023646

Gulzar, A., Sayyed, A.H., Karimzadeh, J., Wright, D.J., 2012. Lack of cross resistance to non- Bt insecticides in a mode-1 , Cry1Ac-resistant population of *Plutella xylostella* (Lepidoptera : Plutellidae) 79, 29–35.

Guo, J.F., He, K.L., Wang, Z.Y., 2019. Biological characteristics, trend of fall armyworm *Spodoptera frugiperda*, and the strategy for management of the pest. Chin. J. Appl. Entomol. 56, 361–369.

Guo, J.F., Zhao, J.Z., He, K.L., Zhang, F., Wang, Z.Y., 2018. Potential invasion of the crop-devastating insect pest fall armyworm *Spodoptera frugiperda* to China. Plant Prot. 44, 1–10. https://doi.org/https://doi.org/ 16688 / j.zwbh.2018452

Gutirrez-Moreno, R., Mota-Sanchez, D., Blanco, C.A., Whalon, M.E., Terán-Santofimio, H., Rodriguez-Maciel, J.C., Difonzo, C., 2018. Field-Evolved Resistance of the Fall Armyworm (Lepidoptera: Noctuidae) to Synthetic Insecticides in Puerto Rico and Mexico. J. Econ. Entomol. 112, 792–802. https://doi.org/10.1093/jee/toy372

Horii, A., McCue, P., Shetty, K., 2007. Enhancement of seed vigour following insecticide and phenolic elicitor treatment. Bioresour. Technol. 98, 623–632. https://doi.org/10.1016/j.biortech.2006.02.028

Jeschke, P., Nauen, R., Schindler, M., Elbert, A., 2010. Overview of the Status and Global Strategy for Neonicotinoids. J. Agric. Food Chem. https://doi.org/dx.doi.org/10.1021/jf101303g

Kasten-Junior, P., Precetti, A.A.C.M., Parra, J.R., 1978. Comparative biological data of *Spodoptera frugiperda* (J.E. Smith, 1797) in two artificial diets and in a natural substrate. Rev. Agric. 53, v.68-78(1-2).

Kogan, M., 1998. Integrated Pest Management: Historical Perspectives and Contemporary Developments. Annu. Rev. Entomol. 43, 243–270. https://doi.org/10.1146/annurev.ento.43.1.243

Lanka, S.K., Ottea, J.A., Davis, J.A., Hernandez, A.B., Stout, M.J., 2013. Systemic effects of thiamethoxam and chlorantraniliprole seed treatments on adult *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae) in rice. Pest Manag. Sci. 69, 250–256. https://doi.org/10.1002/ps.3382

Ma, J., Wang, Y.P., Wu, M.F., Gao, B.Y., Liu, J., Lee, G.S., Otuka, A., Hu, G., 2019. High risk of the fall armyworm invading Japan and the Korean Peninsula via overseas migration. J. Appl. Entomol. 143, 911–920. https://doi.org/10.1111/jen.12679

Macedo, W.R., Araújo, D.K., Castro, P.R. de C. e., 2013. Unravelling the physiologic and metabolic action of thiamethoxam on rice plants. Pestic. Biochem. Physiol. 107, 244–249. https://doi.org/10.1016/j.pestbp.2013.08.001

Macedo, W.R., Castro, P.R. de C. e., 2011. Thiamethoxam: Molecule moderator of growth, metabolism and production of spring wheat. Pestic. Biochem. Physiol. 100, 299–304. https://doi.org/10.1016/j.pestbp.2011.05.003

Moscardini, V.F., Gontijo, P.C., Michaud, J.P., Carvalho, G.A., 2015. Sublethal effects of insecticide seed treatments on two nearctic lady beetles (Coleoptera: Coccinellidae). Ecotoxicology 24, 1152–1161. https://doi.org/10.1007/s10646-015-1462-4

Muraro, D.S., Garlet, C.G., Godoy, D.N., Cossa, G.E., Rodrigues Junior, G.L. do. S., Stacke, R.F., Medeiros, S.L.P., Guedes, J.V.C., Bernardi, O., 2019. Laboratory and field survival of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on Bt and non-Bt maize and its susceptibility to insecticides. Pest Manag. Sci. 75, 2202–2210. https://doi.org/10.1002/ps.5347

Myung, K., Satchivi, N.M., Kingston, C.K., 2014. Translocation of Agrochemicals in Plants. American Chemical Society. https://doi.org/10.1021/bk-2014-1171

Nascimento, A.R.B. do, Farias, J.R., Bernardi, D., Horikoshi, R.J., Omoto, C., 2015. Genetic basis of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) resistance to the chitin synthesis inhibitor lufenuron. Pest Manag. Sci. 72, 810–815. https://doi.org/10.1002/ps.4057

Oerke, E.C., 2006. Crop losses to pests. J. Agric. Sci. 144, 31–43. https://doi.org/10.1017/S0021859605005708

Okuma, D.M., Bernardi, D., Horikoshi, R.J., Bernardi, O., Silva, A.P., Omoto, C., 2018. Inheritance and fitness costs of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) resistance to spinosad in Brazil. Pest Manag. Sci. 74, 1441–1448. https://doi.org/10.1002/ps.4829

Oliveira, C.M., Auad, A.M., Mendes, S.M., Frizzas, M.R., 2014. Crop losses and the economic impact of insect pests on Brazilian agriculture. Crop Prot. 56, 50–54. https://doi.org/10.1016/j.cropro.2013.10.022

Omoto, C., Bernardi, O., Salmeron, E., Sorgatto, R.J., Dourado, P.M., Crivellari, A., Carvalho, R.A., Willse, A., Martinelli, S., Head, G.P., 2016. Field-evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. Pest Manag. Sci. 72, 1727–1736. https://doi.org/10.1002/ps.4201

Orozco-Restrepo, S.M., 2019. Resistance to Piramided Bt Corn Cry1A.105 + Cry2Ab in the Fall Armyworm: Field Situation, Inheritance and Stability (in Portuguese). Universidade Federal de Viçosa.

Pang, S., You, W., Duan, L., Song, X., Li, X., Wang, C., 2012. Resistance selection and mechanisms of oriental tobacco budworm (*Helicoverpa assulta* Guenee) to indoxacarb. Pestic. Biochem. Physiol. 103, 219–223. https://doi.org/10.1016/j.pestbp.2012.05.011

Panizzi, A.R., 2015. Stink Bugs Growing Problems with (Hemiptera: Heteroptera: Pentatomidae): Species Invasive to the U.S. and Potential Neotropical Invaders. Encycl. Entomol. 2120–2120. https://doi.org/10.1007/0-306-48380-7_4092

Pardo-López, L., Soberón, M., Bravo, A., 2012. *Bacillus thuringiensis,* insecticidal three-domain Cry toxins: Mode of action, insect resistance and consequences for crop protection. FEMS Microbiol. Rev. 37, 3–22. https://doi.org/10.1111/j.1574-6976.2012.00341.x

Pereira, J.L., Antunes, S.C., Castro, B.B., Marques, C.R., Gonçalves, A.M.M., Gonçalves, F., Pereira, R., 2009. Toxicity evaluation of three pesticides on non-target aquatic and soil organisms: Commercial formulation versus active ingredient. Ecotoxicology 18, 455–463. https://doi.org/10.1007/s10646-009-0300-y

Perry, E.D., Ciliberto, F., Hennessy, D.A., Moschini, G.C., 2016. Genetically engineered crops and pesticide use in U.S. maize and soybeans. Sci. Adv. 2, 1–9. https://doi.org/10.1126/sciadv.1600850

Pinto, B.S., 2019. Controle de *Spodoptera frugiperda* em milho por tratamento de semente. Universidade Federal de Viçosa.

Qi, S., Casida, J.E., 2013. Species differences in chlorantraniliprole and flubendiamide insecticide binding sites in the ryanodine receptor. Pestic. Biochem. Physiol. 107, 321–326. https://doi.org/10.1016/j.pestbp.2013.09.004

Qi, S., Lümmen, P., Nauen, R., Casida, J.E., 2014. Diamide insecticide target site specificity in the *Heliothis* and *Musca* ryanodine receptors relative to toxicity. J. Agric. Food Chem. 62, 4077–4082. https://doi.org/10.1021/jf501236h

Rehan, A., Freed, S., 2014. Selection, mechanism, cross resistance and stability of spinosad resistance in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Crop Prot. 56, 10–15. https://doi.org/10.1016/j.cropro.2013.10.013

Resende-Silva, G.A., Joseph, D.A., Guedes, R.N.C., Cutler, G.C., 2019a. Impact of Imidacloprid Soil Drenching on Survival, Longevity, and Reproduction of the Zoophytophagous Predator Podisus maculiventris (Hemiptera: Pentatomidae: Asopinae). J. Econ. Entomol. 113, 108–114. https://doi.org/10.1093/jee/toz283

Resende-Silva, G.A., Turchen, L.M., Guedes, R.N.C., Cutler, G.C., 2019b. Imidacloprid Soil Drenches Affect Weight and Functional Response of Spined Soldier Bug (Hemiptera: Pentatomidae). J. Econ. Entomol. 112, 558–564. https://doi.org/10.1093/jee/toy401

Rinkevich, F.D., Chen, M., Shelton, A.M., Scott, J.G., 2010. Transcripts of the nicotinic acetylcholine receptor subunit gene Pxyla6 with premature stop codons are associated with spinosad resistance in diamondback moth, *Plutella xylostella*. Invertebr. Neurosci. 10, 25–33. https://doi.org/10.1007/s10158-010-0102-1

Ríos-Díez, J.D., Saldamando-Benjumea, C.I., 2011. Susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Strains From Central Colombia to Two Insecticides, Methomyl and Lambda-Cyhalothrin: A Study of the Genetic Basis of Resistance. J. Econ. Entomol. 104, 1698–1705. https://doi.org/10.1603/ec11079

Robertson, J.L., Preisler, H.K., Ng, S.S., Hickle, L.A., Gelernter, W.D., 1995. Natural Variation: A Complicating Factor in Bioassays with Chemical and Microbial Pesticides. J. Econ. Entomol. 88, 1–10. https://doi.org/10.1093/jee/88.1.1

Robertson, J.L., Russell, R.M., Preisler, H.K., Savin, N.E., 2007. Bioassays with Arthropods, 2nd ed. CRC Press, Boca Raton.

Sang, S., Shu, B., Yi, X., Liu, J., Hu, M., Zhong, G., 2016. Cross-resistance and baseline susceptibility of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) to cyantraniliprole in the south of China. Pest Manag. Sci. 72, 922–928. https://doi.org/10.1002/ps.4068

Santos-Amaya, O.F., Rodrigues, J.V.C., Souza, T.C., Tavares, C.S., Campos, S.O., Guedes, R.N.C., Pereira, E.J.G., 2015. Resistance to dual-gene Bt maize in *Spodoptera frugiperda*: Selection, inheritance, and cross-resistance to other transgenic events. Sci. Rep. 5, 1–10. https://doi.org/10.1038/srep18243

Santos-Amaya, O.F., Tavares, C.S., Monteiro, H.M., Teixeira, T.P.M., Guedes, R.N.C., Alves, A.P., Pereira, E.J.G., 2016. Genetic basis of Cry1F resistance in two Brazilian populations of fall armyworm, *Spodoptera frugiperda*. Crop Prot. 81, 154–162. https://doi.org/10.1016/j.cropro.2015.12.014

Santos-Amaya, O.F., Tavares, C.S., Rodrigues, J.V.C., Santana, I. V., Queiroz, O.S., Oliveira, E.E., Guedes, R.N.C., Pereira, E.J.G., 2020. Strong fitness costs of insect resistance to dual-gene Bt corn are magnified on less-suitable host-crop cultivars.

Sayyed, A.H., Ahmad, M., Saleem, M.A., 2008. Cross-Resistance and Genetics of Resistance to Indoxacarb in *Spodoptera litura* (Lepidoptera: Noctuidae). J. Econ. Entomol. 101, 472–479. https://doi.org/10.1603/0022-0493(2008)101[472:cagort]2.0.co;2

Seagraves, M.P., Lundgren, J.G., 2012. Effects of neonicitinoid seed treatments on soybean aphid and its natural enemies. J. Pest Sci. (2004). 85, 125–132. https://doi.org/10.1007/s10340-011-0374-1

Sharanabasappa, M., K.C., R., A., Mahadeva, S.H.M., S., M.M., B., P.H., Kavita, H., Shivaray, N., S.T., P., Georg, G., 2018. First report of the fall armyworm, *Spodoptera frugiperda* (j E Smith) (Lepidoptera: Noctuidae), an alien invasive pest on maize in India. Pest Manag. Hortic. Ecosyst. 20, 50–54.

Silva, F.A.C., Panizzi, A.R., 2007. Cotton Balls as an Oviposition Substrate for Laboratory Rearing of Phytophagous Stink Bugs (Heteroptera: Pentatomidae). Ann. Entomol. Soc. Am. 100, 745–748. https://doi.org/10.1603/0013-8746(2007)100[745:cbaaos]2.0.co;2

Silva, J.E., Assis, C.P.O., Ribeiro, L.M.S., Siqueira, H.A.A., 2016. Field-Evolved resistance and cross-resistance of Brazilian *Tuta absoluta* (Lepidoptera: Gelechiidae) populations to diamide insecticides. J. Econ. Entomol. 109, 2190–2195. https://doi.org/10.1093/jee/tow161

Smaniotto, L.F., Panizzi, A.R., 2015. Interactions of Selected Species of Stink Bugs (Hemiptera: Heteroptera: Pentatomidae) from Leguminous Crops with Plants in the Neotropics. Florida Entomol. 98, 7–17. https://doi.org/10.1653/024.098.0103

Sparks, T.C., Nauen, R., 2015. IRAC: Mode of action classification and insecticide resistance management. Pestic. Biochem. Physiol. 121, 122–128. https://doi.org/10.1016/j.pestbp.2014.11.014

Storer, N.P., Babcock, J.M., Schlenz, M., Thompson, G.D., Bing, J.W., Huckaba, R.M., Storer, N.P., Babcock, J.M., Schlenz, M., Meade, T., Thompson, G.D., Bing, J.W., Huckaba, R.M., 2010. Discovery and Characterization of Field Resistance to Bt Maize : *Spodoptera frugiperda* (Lepidoptera : Noctuidae) in Puerto Rico. J. Econ. Entomol. 103, 1031–1038. https://doi.org/10.1603/EC10040

Tabashnik, B.E., Carrière, Y., 2017. Surge in insect resistance to transgenic crops and prospects for sustainability. Nat. Biotechnol. 35, 926–935. https://doi.org/10.1038/nbt.3974

Tabashnik, B.E., Mota-sanchez, D., Whalon, M.E., Hollingworth, R.M., Carrie, Y., 2014. Defining Terms for Proactive Management of Resistance to Bt Crops and Pesticides 496–507.

Taylor, A.G., Salanenka, Y.A., 2012. Seed treatments: Phytotoxicity amelioration and tracer uptake. Seed Sci. Res. 22, 86–90. https://doi.org/10.1017/S0960258511000389

Tuelher, E.S., da Silva, É.H., Freitas, H.L., Namorato, F.A., Serrão, J.E., Guedes, R.N.C., Oliveira, E.E., 2017. Chlorantraniliprole-mediated toxicity and changes in sexual fitness of the Neotropical brown stink bug *Euschistus heros*. J. Pest Sci. (2004). 90, 397–405. https://doi.org/10.1007/s10340-016-0777-0

Villegas, J.M., Wilson, B.E., Stout, M.J., 2019. Efficacy of reduced rates of chlorantraniliprole seed treatment on insect pests of irrigated drill-seeded rice. Pest Manag. Sci. https://doi.org/10.1002/ps.5437

Wang, R., Jiang, C., Guo, X., Chen, D., You, C., Zhang, Y., Wang, M., Li, Q., 2020. Potential distribution of *Spodoptera frugiperda* (J.E. Smith) in China and the major factors influencing distribution. Glob. Ecol. Conserv. 21. https://doi.org/10.1016/j.gecco.2019.e00865

Xu, C., Zhang, Z., Cui, K., Zhao, Y., Han, J., Liu, F., Mu, W., 2016. Effects of sublethal concentrations of cyantraniliprole on the development, fecundity and nutritional physiology of the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae). PLoS One 11. https://doi.org/10.1371/journal.pone.0156555

Yang, D., Avelar, S.A.G., Taylor, A.G., 2018a. Systemic seed treatment uptake during imbibition by corn and soybean. Crop Sci. 58, 2063–2070. https://doi.org/10.2135/cropsci2018.01.0004

Yang, D., Donovan, S., Black, B.C., Cheng, L., Taylor, A.G., 2018b. Relationships between compound lipophilicity on seed coat permeability and embryo uptake by soybean and corn. Seed Sci. Res. 28, 229–235. https://doi.org/10.1017/S096025851800017X

Yu, S.J., Elzie McCord, J., 2007. Lack of cross-resistance to indoxacarb in insecticide-resistant *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Plutella xylostella* (Lepidoptera: Yponomeutidae). Pest Manag. Sci. 63, 1100–

1106. https://doi.org/10.1002/ps

Zhang, Z., Xu, C., Ding, J., Zhao, Y., Lin, J., Liu, F., Mu, W., 2019. Cyantraniliprole seed treatment efficiency against *Agrotis ipsilon* (Lepidoptera: Noctuidae) and residue concentrations in corn plants and soil. Pest Manag. Sci. 75, 1464–1472. https://doi.org/10.1002/ps.5269

Zhu, Y.C., Blanco, C.A., Portilla, M., Adamczyk, J., Luttrell, R., Huang, F., 2015. Evidence of multiple/cross resistance to Bt and organophosphate insecticides in Puerto Rico population of the fall armyworm, *Spodoptera frugiperda*. Pestic. Biochem. Physiol. 122, 15–21. https://doi.org/10.1016/j.pestbp.2015.01.007

ChemSafetyPro, 2019. Water solubility. Available in: <https://www.chemsafetypro.com/Topics/CRA/Water_Solubility.html> Accessed on: 30 Jan.2020.

FAO, 2015. Food and Agriculture Organization of the United Nations. Available in: <http://www.fao.org/land-water/databases-and-software/crop-information/maize/en/> Accessed on: 20 jan. 2020.

ISAAA, 2020. International Service for the Acquisition of Agri-biotech Applications. Available in: <https://www.isaaa.org/gmapprovaldatabase/default.asp> Accessed on: 20 jan. 2020.

NCBI, Pubchem, 2019. National Center for Biotechnology Information. Available in: <https://pubchem.ncbi.nlm.nih.gov/> Accessed on: 11 Dec. 2019.

Seednews, 2016. Tratamento de sementes de soja como um processo industrial no Brasil. Available in: <https://seednews.com.br/edicoes/artigo/334-tratamento-de-sementes-de-soja-como-um-processo-industrial-no-brasil-edicao-janeiro-2016> Accessed on: 11 Dec. 2019.

Figures and tables

Table 1. Information about the seed-treatment insecticides used in the bioassays with FAW and GBS or in the characterization of susceptibility of fall armyworm populations.

Insecticide	Chemical class	Trade name	Label rate (c.p.)	Label rate a.i. ug/seed
Imidacloprid + Thiodicarb	Neonicotinoid + Carbamate	Cropstar	350 mL/60000 seeds	3500
Chlorantraniliprole	Diamide	Dermacor	72 mL/60000 seeds	750
Cyantraniliprole	Diamide	Fortenza	250 mL/100 kg seeds	500
Clothianidin	Neonicotinoid	Poncho	70mL/60000 seeds	700

Table 2. Some characteristics and information from the label of the insecticides used in study involving interaction of several treatments against fall armyworm and the green-belly stink bug.

Insecticide active ingredient (a.i.)	Chemical Class	Trade name	Formulation g/l or g/kg a.i.	Label information		FR* formulated product mL/l or g/l	FR a.i. mg/mL
				Field rate (FR) mL/ha	Spray volume l/ha		
Methomyl	Carbamate	Lannate	215	400	200	2.00	0.43
Flubendiamide	Diamide	Belt	480	100-150	100-300	1.50	0.72
Chlorantraniliprole	Diamide	Premio	200	100-125	150-250	0.83	0.17
Acephate	Organophosphate	Orthene	750	0.80 -1.00*	150-200	6.67	5.00
Thiamethoxam + Lambda-cyhalothrin	Neonicotinoid + Pyrethroid	Engeo Pleno	247 (141 + 106)	200 - 250	200	1.25	0.31
Imidacloprid + Beta-cyfluthrin	Neonicotinoid + Pyrethroid	Connect	112.50 (100 + 12.50)	750 - 1000	100-300	10.00	1.12

*The insecticide concentration that corresponds to the field rate (FR) was calculated by dividing the label rate (upper limit) by the spray volume (lower limit).

Table 3. Some characteristics and information from the label of the foliar insecticides used in experiment of susceptibility of fall armyworm populations.

Insecticide active ingredient (a.i.)	Chemical class	Trade name	Formulation g/l a.i.	Label information		FR* formulated product mL/l	FR a.i. g/l
				Field rate (FR) mL/ha	Spray volume l/ha		
Bifenthrin + carbosulfan	Pyrethroid + carbamate	Talisman	200 (50+150)	1000	250-350	4.00	0.80
Methomyl	Carbamate	Lannate	215	400	200	2.00	0.43
Flubendiamide	Diamide	Belt	480	100-150	100-300	1.50	0.72
Indoxacarb	Oxadiazine	Avatar	150	300-400	150-250	2.67	0.40
Chlorantraniliprole	Diamide	Premio	200	100-125	150-250	0.83	0.17
Spinetoram	Spinosyn	Exalt	120	50-100	300-400	0.33	0.04

*The insecticide concentration that corresponds to the field rate (FR) was calculated by dividing the label rate (upper limit) by the spray volume (lower limit).

Table 4. Characterization of the susceptibility of the MON89034-resistant fall armyworm population (RPRO17) to three seed-treatment insecticides.

Insecticide	Population	N	Slope ± EP	LC$_{50}$ (95% CL) mg/seed a.i.	χ^2	*P*	RR$_{50}$ (95% CL)[1]
Imidacloprid + Thiodicarb	LabSS	452	0.76 ± 0.12	1.37 (0.46 - 3.36)	17.70	0.13	-
	RPRO17	227	1.21 ± 0.23	1.34 (0.28 - 4.01)	8.04	0.15	1.00 (0.40 – 2.50)
Chlorantraniliprole	LabSS	236	0.57 ± 0.09	0.01 (0.00 - 0.11)	6.23	0.28	-
	RPRO17	617	0.43 ± 0.06	0.41 (0.14 - 1.55)	18.45	0.36	42.90 (9.00 – 204)
Cyantraniliprole	LabSS	228	0.70 ± 0.14	0.00 (0.00 - 0.01)	4.69	0.45	-
	RPRO17	207	0.51 ± 0.11	0.05 (0.02 - 0.16)	3.30	0.65	17.40 (3.80 – 80.30)

Concentration values are in mg/seed a.i., obtained by planting the seeds and exposing the larvae in the 7th day after plant emergence (V3-4) during 96 h.

[1] RR$_{50}$, resistance ratio and its 95% confidence limits using the standard susceptible population (LabSS) as the reference. It indicates how many times the larvae of the population RPRO17 are more resistant to the insecticide than those of the LabSS standard susceptible population.

Table 5. Susceptibility of Cry-resistant populations of fall armyworm to test was whether the RPRO populations had relatively higher susceptibility compared to the LabSS and RHX population.

Insecticide	Population	N	Slope ± EP	LC_{50} (95% CL)	χ^2	P	RR_{50} (95% CL)	SR_{50} (95% CL)
Chlorantraniliprole	RHX11	268	6.96 ± 1.93	0.17 (0.14 - 0.19)	5.8	0.88	4.5 (2.8 - 7.3)	-
(Premio)	RPRO13	265	2.31 ± 0.58	0.15 (0.07 - 0.20)	15.8	0.15	3.9 (2.3 - 6.7)	1.2 (0.9 - 1.6)
	RPRO17	227	0.99 ± 0.22	0.03 (0.01 - 0.05)	5.0	0.84	0.7 (0.2 - 1.9)	6.8 (2.6 - 17.5)
	LabSS	284	1.87 ± 0.28	0.04 (0.02 - 0.06)	7.9	0.79	-	4.5 (2.8 - 7.3)
Spinetoram	RHX11	160	7.01 ± 1.76	0.05 (0.04 - 0.06)	0.8	0.98	2.3 (1.7 - 3.1)	-
(Exalt)	RPRO13	160	5.64 ± 1.28	0.06 (0.05 - 0.07)	0.5	0.99	2.6 (2.0 - 3.4)	0.9 (0.7 - 1.1)
	RPRO17	160	3.87 ± 1.05	0.05 (0.03 - 0.06)	2.1	0.84	2.1 (1.5 - 2.9)	1.1 (0.8 - 1.4)
	LabSS	160	4.54 ± 1.41	0.02 (0.01 - 0.03)	2.4	0.79	-	2.3 (1.7 - 3.1)
Indoxacarb	RHX11	288	2.54 ± 0.44	1.15 (0.67 - 1.58)	4.7	0.97	122.4 (3.9 - 3864)	-
(Avatar)	RPRO13	263	1.67 ± 0.29	0.48 (0.20 - 0.79)	9.6	0.57	50.4 (1.5 - 1651)	2.4 (1.1 - 5.2)
	RPRO17	284	0.57 ± 0.14	0.17 (0.01 - 0.61)	11.2	0.52	17.6 (0.4 - 855)	6.9 (1.1 - 44.7)
	LabSS	288	0.62 ± 0.19	0.01 (0.00 - 0.08)	11.8	0.47	-	122.4 (3.9 - 3864)
Flubendiamide	RHX11	287	1.64 ± 0.35	3.42 (1.71 - 4.96)	10.2	0.60	9.8 (2.9 - 32.4)	-
(Belt)	RPRO13	284	0.99 ± 0.15	2.66 (0.88 - 5.35)	19.9	0.07	8.1 (2.4 - 27.4)	1.2 (0.6 - 2.4)
	RPRO17	251	0.79 ± 0.16	0.24 (0.03 - 0.71)	10.0	0.44	0.7 (0.1 - 4.1)	14.5 (3.2 - 66.0)
	LabSS	271	1.21 ± 0.24	0.35 (0.07 - 0.81)	10.7	0.47	-	9.8 (2.9 - 32.4)
Bifenthrin +	RHX11	216	3.10 ± 1.08	52.69 (20.13 - 78.31)	6.8	0.56	4.6 (2.6 - 8.0)	-
Carbosulfan	RPRO13	278	2.93 ± 0.73	31.37 (20.47 - 52.50)	7.9	0.79	2.7 (1.6 - 4.6)	1.7 (0.9 - 3.1)
(Talisman)	RPRO17	287	1.97 ± 0.28	26.07 (16.37 - 49.71)	19.9	0.07	3.2 (2.0 - 5.2)	1.4 (0.8 - 2.5)
	LabSS	288	2.27 ± 0.57	11.50 (7.86 - 15.92)	10.6	0.57	-	4.6 (2.6 - 8.0)
Methomyl	RHX11	140	4.88 ± 1.24	20.57 (14.65 - 28.00)	1.3	0.86	1.7 (1.2 - 2.6)	-
(Lannate)	RPRO13	160	2.54 ± 0.50	8.86 (6.90 - 11.27)	4.4	0.49	0.7 (0.5 - 1.1)	1.8 (1.2 - 2.7)
	RPRO17	160	2.28 ± 0.45	11.34 (8.82 - 15.38)	2.2	0.83	0.9 (0.6 - 1.4)	2.3 (1.6 - 3.4)
	LabSS	160	2.88 ± 0.59	11.94 (8.85 - 16.25)	4.3	0.51	-	1.7 (1.2 - 2.6)

Concentration values are in mg/l a.i. in water, obtained using the foliar-deep method in the bioassays.

[1] RR_{50}, resistance ratio and its 95% confidence limits using the standard susceptible population (LabSS) as the reference. It indicates how many times the populations RHX11, RPRO13, and RPRO17) are more resistant to the insecticide than the LabSS standard susceptible population.

[2] SR_{50}, susceptibility ratio and its 95% confidence limits using the Cry1F-resistant population (RHX11) as the reference. It indicates how many times the Cry1A.105/Cry2Ab-resistant populations (RPRO13 and RPRO17) are more susceptible to the insecticide than the Cry1F-resistant population (RHX11).

Table 6. Comparing the estimated LC_{90} values of the insecticides with their recommended field rates to assess the likelihood of attaining acceptable control efficacy (~80%) of Cry-Bt-resistant fall armyworms when properly spraying the label field rates.

Insecticide	Label rate a.i. (mg/l)	Population	LC_{90} (95% CL) mg/l	LRR_{90} (95% CL)[1]
Chlorantraniliprole	167	RHX11	0.30 (0.20 - 0.40)	635 (442 - 723)
(Premio)		RPRO13	0.50 (0.30 - 4.10)	316 (41 - 508)
		RPRO17	0.50 (0.20 - 2.30)	331 (72 - 729)
		LabSS	0.20 (0.10 - 0.30)	908 (580 - 1275)
Spinetoram	40	RHX11	0.10 (0.10 - 0.10)	513 (367 - 597)
(Exalt)		RPRO13	0.10 (0.10 - 0.20)	408 (260 - 494)
		RPRO17	0.10 (0.10 - 0.20)	396 (198 - 506)
		LabSS	0.00 (0.0 - 0.1)	930 (471 - 1176)
Indoxacarb	400	RHX11	3.70 (2.90 - 5.00)	109 (80 - 139)
(Avatar)		RPRO13	2.80 (1.80 - 4.70)	144 (85 - 219)
		RPRO17	24.30 (8.80 - 194.20)	16 (2 - 46)
		LabSS	1.10 (0.20 - 3.20)	376 (124 - 2116)
Flubendiamide	720	RHX11	20.70 (13.50 - 49.10)	35 (15 - 53)
(Belt)		RPRO13	52.40 (22.40 - 291.70)	14 (2 - 32)
		RPRO17	9.60 (4.20 - 29.20)	75 (25 - 173)
		LabSS	4.00 (2.10 - 8.20)	180 (87 - 350)
Bifenthrin +	800	RHX11	136.70 (89.90 - 622.50)	6 (1 - 9)
Carbosulfan		RPRO13	85.80 (51.60 - 289.30)	9 (3 - 16)
(Talisman)		RPRO17	117.0 (58.50 - 500.60)	7 (2 - 14)
		LabSS	42.20 (26.30 - 147.40)	19 (5 - 30)
Methomyl	430	RHX11	37.60 (27.70 - 73.80)	11 (6 - 16)
(Lannate)		RPRO13	28.30 (19.50 - 61.40)	15 (7 - 22)
		RPRO17	41.30 (26.20 - 108.20)	10 (4 - 16)
		LabSS	33.30 (22.70 - 73.10)	13 (6 - 19)

[1] LRR_{90}, label rate ratio at the LC_{90} and its 95% confidence limits using the label rate of the insecticide as a reference (LRR_{90} = label rate / LC_{90} value of the insecticide and its 95% confidence limits). It indicates how many times the estimated LC_{90} value of the insecticide is lower than its recommended field rate (i.e., the safety level of attaining acceptable control efficacy (~80%) properly spraying the label field rate of the insecticide).

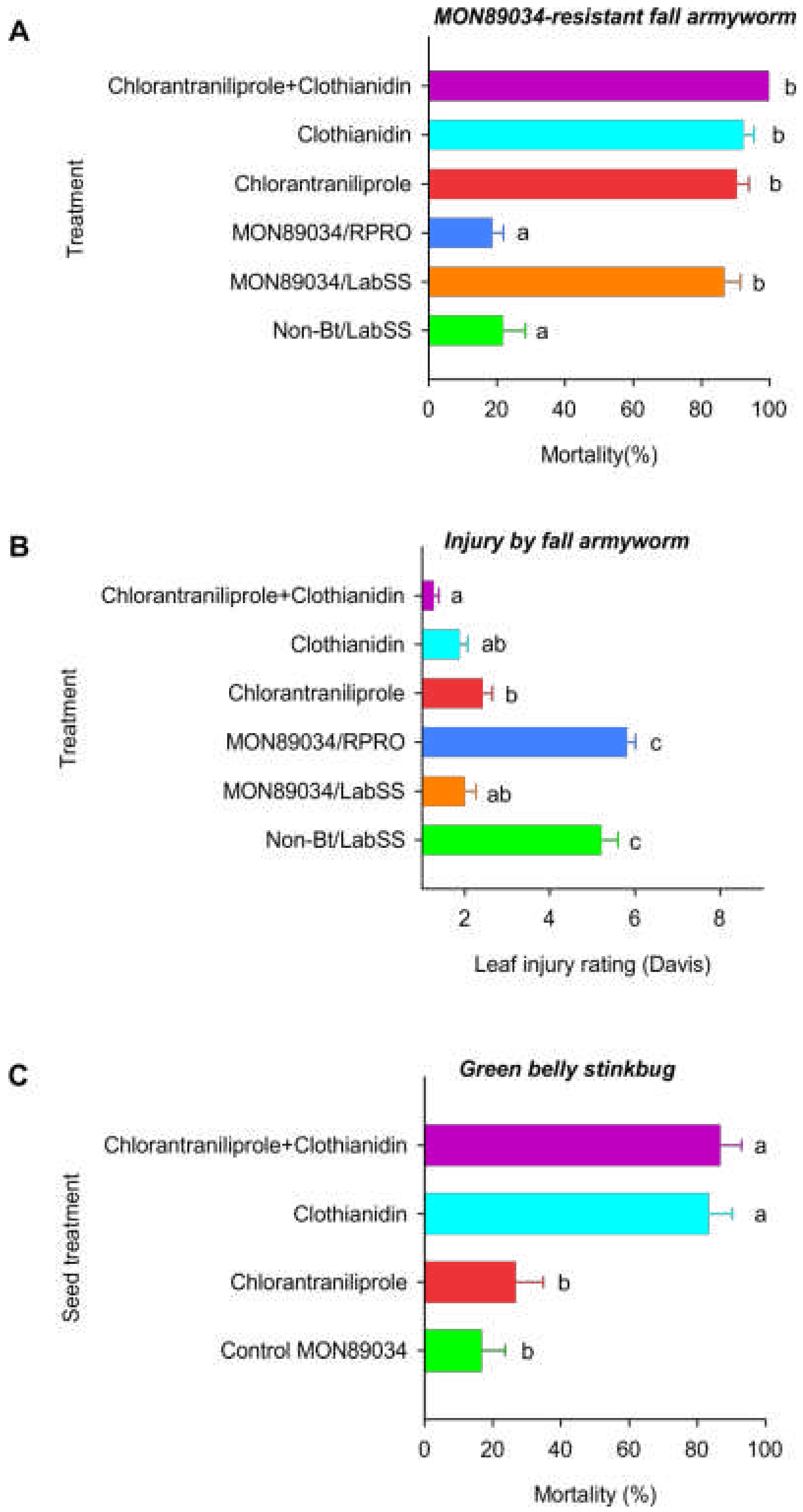

Figure 1. Interaction of MON89034 Bt maize (Cry1A.105+Cry2Ab) and seed treatment against MON89034-resistant fall armyworm larvae (L_3) and green-belly stink bug adults. Potted Bt or non-Bt maize plants (n = 30) deriving from seeds treated or untreated with chlorantraniliprole (Dermacor), clothianidin (Poncho), and chlorantraniliprole+clothianidin (Dermacor+Poncho) were optimally grown in the greenhouse. The infestation was in the 7th day after emergence using single individuals of the armyworm or the stink bug. Insect mortality and foliar injury rating were recorded after 96 h. (**A, B**) Performance of the resistant larvae and Bt-toxin expression of MON89034 maize. (**C**) Response of green-belly stink bugs to MON89034 maize plants deriving or not of insecticide-treated seeds. Means and standard errors followed by the same letter are not significantly different using Tukey's HSD (*P* > 0.05).

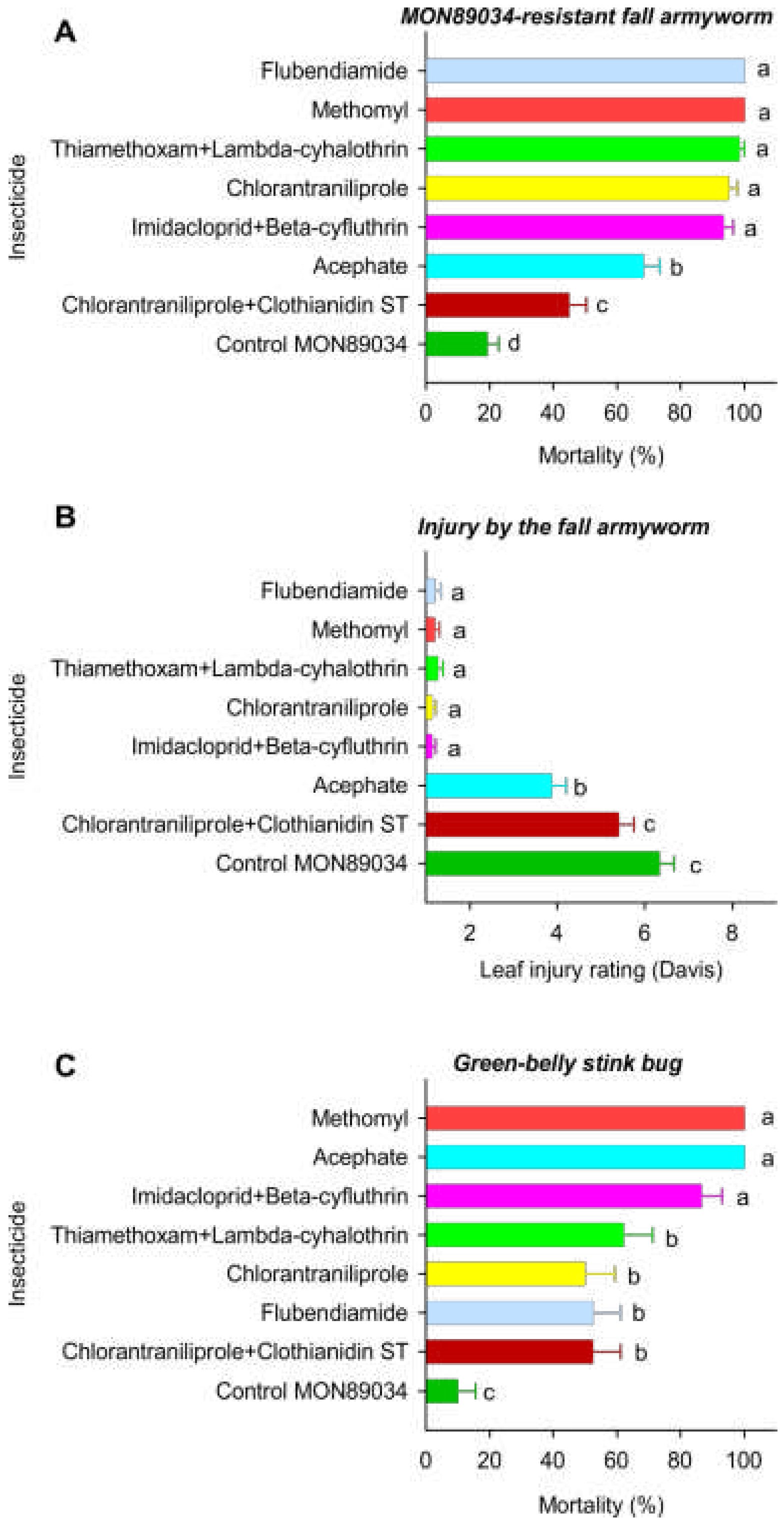

Figure 2. Interaction of MON89034 Bt maize (Cry1A.105+Cry2Ab), seed treatment (ST) with chlorantraniliprole + clothianidin, and foliar insecticides for control of MON89034-resistant fall armyworm larvae (L_3) and green-belly stink bug adults. Potted Bt or non-Bt maize plants (n = 30) deriving from seeds treated with clothianidin + chlorantraniliprole (Poncho + Dermacor) or untreated seeds were optimally grown in the greenhouse. The plants were infested on the 21th day after emergence with two individuals of the armyworm (L3) or with one stinkbug (adults). Insect mortality and foliar injury rating were recorded after 96 h. (**A**) Fall armyworm mortality. (**B**) Plant protection from the fall armyworm. (**C**) Stink bug mortality. Means and standard errors followed by the same letter are not significantly different using Tukey's HSD ($P > 0.05$).

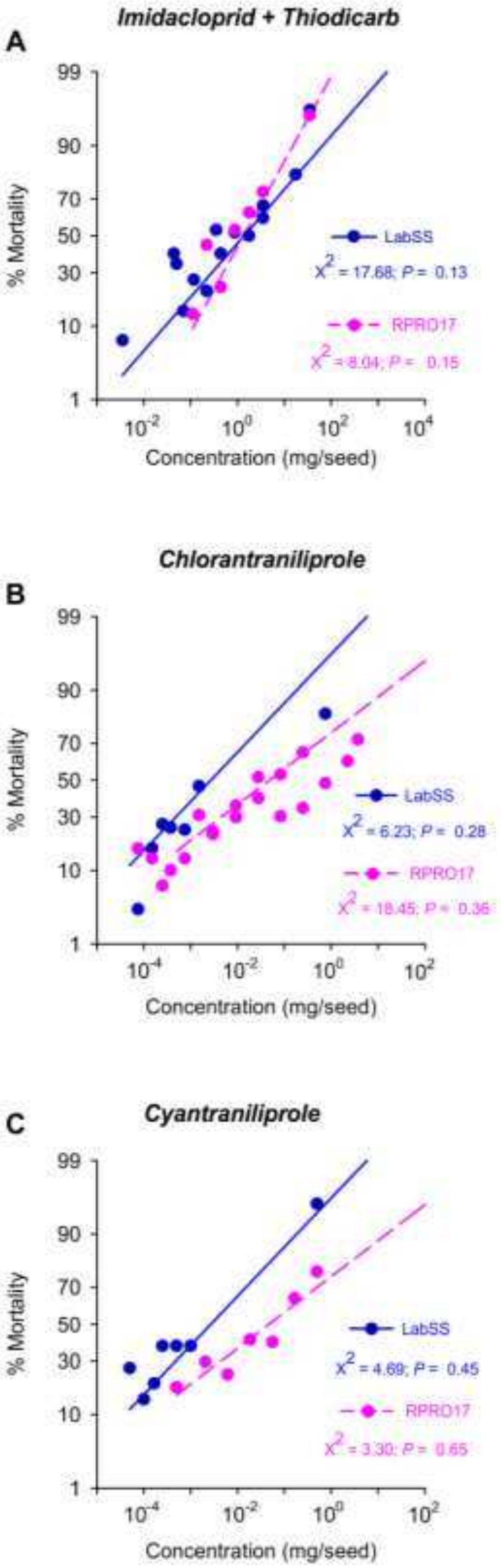

Figure 3. Graphical representation of the concentration-mortality curves for the seed-treatment insecticides. The circles represent the observed (experimental) mortality, and the lines represent the expected mortality given by the probit model fitted to the bioassay data.