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RESISTÊNCIA DE GENÓTIPOS DE SOJA A *Helicoverpa armigera* (Hübner) E SIMULAÇÃO DE DANOS DE *Helicoverpa zea* (Boddie) EM ESTRUTURAS REPRODUTIVAS DA SOJA

Botucatu

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RESISTANCE OF SOYBEAN GENOTYPES TO *Helicoverpa armigera* (Hübner) AND SIMULATION OF *Helicoverpa zea* (Boddie) DAMAGE IN REPRODUCTIVE STRUCTURES OF SOYBEAN

Thesis presented to School of Agricultural Science from UNESP – Campus of Botucatu, to obtain the degree of Doctor in Agronomy: Crop Protection

Advisor: Prof. Dr. Edson L. Lopes Baldin

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RESUMO

A soja, [*Glycine max* (L.) Merrill], é cultivada em vários países e é considerada um dos principais cultivos agrícolas em todo o mundo. Embora apresente grande potencial produtivo, muitas espécies de insetos-praga estão comumente associadas à cultura, causando danos significativos e comprometendo sua produtividade. Dentre os insetos que podem afetar negativamente o rendimento da soja, destacam-se *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) e *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae). O uso de inseticidas sintéticos, associado às culturas geneticamente modificadas são os métodos de controle mais utilizados para a manutenção das populações dessas pragas a campo. No entanto, a adoção abusiva dessas práticas pode ocasionar prejuízos ao meio ambiente e à saúde do trabalhador, além de selecionar populações resistentes dos insetos, no campo. O uso de resistência varietal no controle de pragas pode ser uma tática valiosa para o Manejo Integrado de Pragas (MIP) da soja, podendo ser empregado em conjunto com outras táticas de controle. Ainda dentro dos preceitos do MIP, o conhecimento quanto ao potencial de danos do inseto sobre as plantas e seu impacto sobre as produtividades vem sendo periodicamente reavaliado. Dessa forma, a simulação da ação dos insetos sobre as plantas também pode auxiliar na validação e determinação dos níveis de ação (NA) e, assim, favorecer a racionalização do uso de inseticidas químicos. Com base no exposto, o presente estudo teve como objetivos caracterizar possíveis categorias de resistência (antixenose e/ou antibiose) de 22 genótipos de soja sobre *H. armigera* em laboratório e avaliar o impacto da remoção artificial de flores e vagens na maturação e rendimento da soja, simulando o ataque de *H. zea* a campo. Inicialmente, foi realizado um teste preliminar de performance, empregando-se como alimento, folhas e vagens dos genótipos, visando selecionar aqueles mais promissores quanto à resistência. Na sequência, os genótipos selecionados foram novamente avaliados por meio de ensaios de confinamento (folhas), onde além de parâmetros biológicos, avaliou-se o consumo foliar, visando distinguir antixenose e antibiose. Em ensaio a campo, avaliou-se o efeito da remoção artificial de flores e vagens, em diferentes níveis de remoção e diferentes fases fenológicas da soja, sobre a maturação e produtividade da soja nos EUA, sob condições desfavoráveis de umidade. Em laboratório, PI 227687, PI 274453, PI 274454, PI 229358, PI 171451, ‘IAC 17’ e ‘IAC 19’ expressaram

resistência do tipo antibiose e/ou antixenose sobre *H. armigera*, apresentando-se como genótipos viáveis para programas de melhoramento genético focando em resistência de soja a lagartas desfolhadoras. A campo observou-se que as plantas de soja são capazes de compensar os danos causados por *H. zea*, quando as flores e/ou vagens são removidas no estádio inicial de desenvolvimento reprodutivo (R2-R4), quando em condições desfavoráveis (sem irrigação). Os dados também indicaram que a planta de soja é mais sensível à perda de estruturas reprodutiva durante a fase R5, período em que o ataque reduz significativamente a produtividade das plantas.

Palavras-chave: Antibiose. Antixenose. Resistência de Plantas a Insetos. Noctuidae. Dano artificial.

ABSTRACT

Soybean, [*Glycine max* (L.) Merrill], is cultivated around several countries and it is considered one of the major agricultural crops worldwide. Although it presents great productive potential, many pest insects are commonly associated to its crop, causing significant damages and implicating on its yield. Among the insects which interfere negatively on soybean yield, it is highlighted *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) and *Helicoverpa zea* (Boddie, 1850) (Lepidoptera: Noctuidae). The use of synthetic insecticide associated with genetically modified crops, are the most used control method to the maintenance of this pest population in the field. The abusive use of this practice can damage the environment and the laborer's health, moreover, contribute to the field evolved resistance of insects. The use of varietal resistance on pest control might be a rich tool for the Integrated Pest Management (IPM) on soybean, that could be used along to other control methods. Still in the precept of IPM, the knowledge about the potential of the insect damages on the plants and their impact on the yield has been periodically re-evaluated. Thus, the simulation of the insect action on the plants might also help on the validation and on the determination of the action threshold (AT) and, support the rationalization of the chemical insecticide utilization. Based on the exposed, the present study had as objectives characterize possible resistance categories (antixenosis and/or antibiosis) of 22 soybean genotypes on *H. armigera* in laboratory and evaluate the impact of the artificial removal of fruiting during the soybean maturation and yield, simulating *H. zea* attack on it, under field conditions. Initially, a preliminary performance experiment was performed using the leaves and pods of the genotypes, in order to select the most promising ones for resistance. On the sequence, the genotypes selected were evaluated again, by the confinement test (leaves), where beyond the biological parameters, it was evaluated the foliar intake, looking forward to distinguishing antixenosis and antibiosis. In field experiment, it was tested the effects of the artificial removal of the pods and flowers, in different levels of the removal and different phenological phase of the soybean, on the maturation and the yield of the crop in the USA, under unfavorable humidity conditions. In the laboratory, the genotypes PI 227687, PI 274453, PI 274454, PI 229358, PI 171451, 'IAC 17' and 'IAC 19' expressed antibiosis and/or antioxenosis resistance against *H. armigera*, presenting themselves as viable in breeding programs, focusing on soybeans resistance to

lepidopterans. On field, it was observed that the soybean plants can compensate the damages caused by the *H. zea,* when the flowers and pods are removed during the initial growth stage of the reproductive development (R2-R4), in unfavorable conditions (no irrigation). The data also showed that soybean more sensitive to the loss of the reproductive structure during the R5 growth stage, when the attack decreases significantly the productivity of the plants.

Keywords: Antibiosis. Antixenosis. Host plant resistance. Noctuidae. Artificial damage.

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GENERAL INTRODUCTION

Soybean, [*Glycine max* (L.) Merrill] is an herbaceous plant which belongs to the dicotyledon group, Rosaceae Order, Leguminosae family and *Glycine* genus (LIU, 1997; THOMAS; COSTA, 2010). The development of soybean consists in two growth stages, the vegetative (the period of seedling emergency, the opening of the first leaves) and the reproductive (the period of flowering until the end of the crop development cycle). Genetic factors and environmental conditions influence the duration of these stages. The classification is done based on the observation of the leaves, flowers and pods and seeds development, which are at the knot of the main stem of the plant. The crop presents characteristics of high plasticity, it means, after plant evolution it has got the ability to fit in favorable environmental condition and management, by the morphology modification and the yield components. It is an annual cycle plant, with 90 to 160 days of duration and it is classified into maturation groups (FEHR et al., 1971; MIRANDA et al., 1998; KOMATSU, 2010; NOGUEIRA et al., 2013).

The soybean crop is of great interests into the worldwide agriculture due to the high protein level of the grains and its high productive potential, beyond the multiplicity of application in human and animal's food (MAUAD et al., 2010). Soybean is the main oleaginous cultivated in Brazil and it is considered the main exportation commoditie of Brazil (CONAB, 2018 a). The estimated harvest for 2018/2019 indicates that Brazilian production could reach around 119 million tons, with an average yield of 3.394 kg/ha, which would represent a new record for the crop. This productivity is the result of the combination of favorable environmental conditions, such as rainfall and temperature, and the use of a good technological package (CONAB, 2018 b). In relation to world production, the estimated made for the 2018/2019 harvest places Brazil as the biggest worldwide soybean producer, followed by the United States, with an estimated production of 116,48 million of tons, and posteriorly, Argentina with a 55,5 mi of tons. Together, these three countries are responsible by 81% of the worldwide harvest of soybean (USDA, 2018).

The productivity of soybean is constantly threatened by several factors, like the insects-pest infestation, which is considered one of the most important ones (FORMENTINI et al., 2015). Among several harmful insects commonly associated

to the soybean, some species of Lepidoptera are responsible to cause great economic loss, especially the ones included on the Noctuidae family, for exemple *Helicoverpa zea* (Boddie) and *H. armigera* (Hübner) (STACKE et al., 2018; CARNEIRO et al., 2018; SUITS et al., 2017; MOSCARDI et al., 2012; ÁVILA et al., 2013). Both species reduce the yield and the quality of the soybean by feeding their leaves and the pods (PANIZZI et al., 2012; FORMENTINI et al., 2015).

The species *H. zea* and *H. armigera* are very similar morphologically between each other (HARDWICK, 1965; TAY et al., 2013; POGUE et al., 2014). *Helicoverpa zea*, also known as corn earworm (REISIG et al., 2017), is a polyphagous pest and it attack more than 100 species of plants which has economic importance. In Brazil, the insect occurs all the year (PINTO et al., 2004), however, in the United States, this lepidopteran must cause damages on cotton, soybean, sorghum and tomato, and it is considered one of the main corn pests (ICAC RECORDER, 2011; BARBOSA et al., 2016). The damage caused by *H. zea* on soybean has increased in the south of the United States, during the last years (MUSSER et al., 2011; MUSSER et al.; 2013).

Helicoverpa zea has up to six generations per year in USA, but in the first one, the bollworm usually eats wild host (SHARMA, 2005; PAN et al., 2015) and the follow generations migrates to corn, which is considered the main host. The preference for corn is related to the maturation stage of the plant, and the insect usually appears since the flowering growth stage (JOHNSON et al., 1975; ADAMS et al., 2016). When the corn starts the senescence process, the adults migrate to the neighbour crop, as cotton and soybean, and the third and the fourth generation occur in these hosts, considerable damages. The last generation occurs on the plants without economic relevance (HARTSTACK et al., 1973; ADAMS et al., 2016). The *H. zea* caterpillars feed on several plant part, including leaves, corn grains, cotton bolls, and soybean seeds (FITT, 1989; SUITS et al., 2017).

The *H. zea* adults have the yellow-brown color with a dark transversal strip, containing disperse spots on wings. The female can lay around 1.000 eggs during your life and it prefers to oviposit on the stile-stigma of female flower, but it may lay its eggs in any part of the plant. The caterpillars present color that varies from light green or pink to brown, and it moves to the ground to become pupae (BARROS et al., 2011). On soybean, the caterpillars feed the seedlings, causing

defoliation. In the reproductive stage they attack the pods, feeding the grain, and causing direct damages (DEGRANDE; VIVAN, 2007; SOSA-GÓMEZ et al., 2014). The chemical control, the use of genetically modified varieties or the combination of the two methods are management practices used to maintain the population of pests below the economic injury level (OLMSTEAD et al., 2016). However, this species is considered the more tolerant to the insecticide than the others, with frequent unsuccess reports by using this control method (CUI et al., 2018; ADAMS et al., 2016; PAN et al., 2016; McCAFFERY, 1998). By the selection of resistant individuals, the frequent and inadequate use of genetically modified varieties must also result into low efficiency (PIETRANTONIO et al., 2007; REISIG; KURTZ, 2018), indicating the necessity of rationalization of the use of the existent tools, making them more efficient (OLMSTEAD et al.; 2016).

Also known as Old World bollworm in other countries, *Helicoverpa armigera* belongs to Heliothinae subfamily complex and it is spread around the world, and being present in Europe, Africa, Asia, Oceania and South and Central America, in tropical and tempered climate areas (GUO, 1997; GUOQING et al., 2001; LAMMERS; MACLEOD, 2007; OEPP, 2008; CZEPAK et al., 2013; MÚRUA et al., 2014). The fast dissemination of the species around South and Central America suggests that the colonization in North America is a question of time (KRITICOS et al., 2015). In 2015, a male moth was identified in the USA, which alerted the country (HYDEN; BRAMBILA, 2015).

In the middle of 2013 was detected the presence of *H. armigera* in Brazil, in states of Goiás, Distrito Federal, Mato Grosso and Paraná (CZEPAK et al., 2013; EMBRAPA, 2013; SPECHT et al., 2013). Some researchers reported the presence of this species since 2008 in the country, by the collected samples in Paraná, in 2011 in Rio Grande do Sul and in 2012 in Amapá (SOSA-GÓMEZ et al., 2016). After this detection, there have been a fast dissemination of the insect around South America, and this species was reported afterwards in Argentina (MURÚA et al., 2014), Paraguay (PARAGUAY, 2014) and Uruguay (ARNEMANN et al., 2016).

It is about a polyphagous pest which is able to infest a variety crop plants including cotton, sorghum, soybean, tomato and sunflower (REED, 1965; FITT, 1989; CZEPAK et al., 2013; TAY et al., 2013). It has a complete biological development, passing by eggs, larvae, pupae and adult phase (ALI et al., 2009).

The female oviposite during the nocturnal period and it must oviposit around 3.000 eggs (REED, 1965; ZALUCKI, 1991; NASERI et al., 2011). During the larval period, it passes from five to six instars, anyways the sixth instar depends on the genetic factors, climate conditions and food quality (ARAÚJO, 1990). The caterpillars present different colors varying from the yellow-brown to the green, presenting brown strips on the sideview of the thorax, on the abdomen and the head (ALI et al., 2009). The caterpillars feed on different parts of the host plant and in every stage of plant development, although they present more preference to the reproductive structures (LIMA et al., 2006; LAMMERS; MACLEOD, 2007; PERKINS et al., 2008). The pre-pupa phase consists the moment when the caterpillar stops eating and moves to the ground, due to find humidity conditions and ideal temperature. When it arrives to the ground, the caterpillar makes the cocoon, and it becomes into pupae (ALI et al., 2009; ÁVILA et al., 2013).

The adults present nocturnal habits and sexual dimorphism, it might be differentiated by color and size of the wings. The oviposition usually occurs in the nocturnal period, and the female prefer the surface with hairiness or reproductive structures. This choice might be influenced by physical and chemical impulses (ZALUCKI, 1991; CUNNINGHAM et al., 1998, 1999; CUNNINGHAM; ZALUCKI, 2014). If the host and the environmental conditions are favorable, the females might be copulated once or more and lay more than 4.000 eggs during their cycle of life (HARDWICK, 1965; HOU; CHENG, 1999; ALI et al., 2009; NASERI et al., 2011; BAKER; TANN, 2013; ÁVILA et al., 2013). The moths present great mobility between the host plants and great dispersion ability; it can reach 1.000 km in nocturnal flights (PEDGLEY, 1985).

The ability of developing in a wide range of host permits *H. armigera* not to interrupt its development being able to present more than 10 generation each agricultural year (ZALUCKI et al., 1986). The characteristics such as high mobility, polyphagia and high index of reproduction favor the survival on inappropriate habitats, well succeeded colonization and the exploration of several agricultural systems (FITT, 1989). These adaptative characteristics grant the insect the pest “status” with a great potential to cause damages in Brazilian crops (FITT, 1989; CRUZ et al., 2013). In addition, the large incidence of individuals in the commercial crops and the resistance to the active ingredients used, as well as *Bt* plants, increase its importance as agricultural pest (ZALUCKI et al., 1986; MORAL

GARCIA, 2006; ZHANG et al., 2012; CRUZ et al., 2013; TABASHNIK et al., 2013; CHAKROUN et al., 2016; DOURADO et al., 2016).

In Brazil, the intensive productive system characterized by consecutive harvest, mainly in Brazilian Cerrado areas, where it is cultivated specially cotton, corn and soybean, might favor the settlement of *H. armigera* population, which needs to find ideal environmental condition due to the unbroken food supply (JALLOW; ZALUCKI, 2003; CRUZ et al., 2013; PAIVA; YAMAMOTO, 2014; REIGADA et al., 2016; PESSOA et al., 2016). The continuous food, associated to the excessive pesticide application and the use of inappropriate action of control, might favor the occurrence of populational pest outbreaks, intensifying its attack and causing losses to be even greater (CZEPAK et al., 2013; EMBRAPA, 2013).

Different methods of control have been used to minimize the attacks and the soybean damage caused by these two-noctuid species. The synthetic insecticide spraying, including products of the organophosphates, pyrethroids, diamides, spinosinas, clorfenapir and growth regulator groups, have been the main used tools by farmers (WANG et al., 2009; RUI-MIN et al., 2013; BIRD, 2016) but the intense use of few active ingredients have selected resistant individuals to the main products and also to the plants which express *Bt* protein (MAHON et al., 2007; TABASHNIK et al., 2013; CHAKROUN et al., 2016; SANTOS et al., 2016), requiring studies of development of other management strategies (FATHIPOUR; SEDARATIAN, 2013; LEITE et al., 2014; KUSS et al., 2016; PERINI et al., 2016).

With the objective of reducing the use of insecticide and their possible undesirable effects, it has increased the number of researches with other recommended methods of the Integrated Pest Management (IPM) (FATHIPOUR; SEDARATIAN, 2013). The use of resistant genotypes is considered an alternative method to the chemical one, with a recognized efficiency (SMITH, 2005; NASERI et al., 2009; NASERI et al., 2010; SOLEIMANNEJAD et al., 2010; FATHIPOUR; NASERI, 2011). The varietal resistance might be a valuable strategy on the arthropod-pests control, once it is not harmful to the environment and it offers more advantages to the farmers (LI et al., 2004). Furthermore, in the case of conventional breeding, it presents less risk on the selection of resistant individuals (SMITH, 2005), and it is a technique which integrates harmoniously with IPM programs (McAUSLANE, 1996).

Resistant plant has inheritable characteristic, that are present in their genetic constitution, which permits a cultivar or species to be less damaged than another by a pest insect, under similar conditions of infestation (SMITH, 2005). There are three categories of resistance: antixenosis, antibiosis and tolerance (SMITH, 2005), which can be expressed isolation or in combination (PAINTER, 1951). The expression of resistance might involve either behavioral or biological changes of the insects as well as reaction of the own plant in the presence of the arthropod (LARA, 1991; VENDRAMIM; GUZZO, 2009).

Antixenosis occurs when, for a reason, the plant is less selected by the insect as food, shelter and/or oviposition in comparation to others. This category might be caused by the presence of chemical or morphological factors such as volatile compounds, color and morphology of the plants. In relation to food, it is characterized when the food intake volume is comparatively reduced or by the few numbers of insects colonizing a plant (KOGAN, 1975; PANDA, 1979; LARA, 1991; VENDRAMIM; GUZZO, 2009; KLOTH et al., 2012). On antibiosis, the insect feeds on the plant normally; however, because of the group of characteristics of the plants, its development is negatively affected. High index of mortality of the immature phase, size and weight reduction of individuals, low emergency of adults, beyond the impact on longevity, oviposition, fertility and extension of the cycle are common effects of plants that express antibiosis (KOGAN, 1975; PANDA, 1979; AUCLAIR, 1989; LARA, 1991). Tolerant plant is one that is less damaged than others, without adverse effects on the behavior or biology of the insects. This plant has the ability to respond against the arthropod-pest infestation by compensation or reparation mechanism of the damaged areas without significant yield loss (KOGAN, 1975; PANDA, 1979; LARA, 1991; KLOTH et al., 2012).

The complex insect-plant interaction can modify, to a higher or lower intensity, the expression of resistance and the plants have developed, through selection, some mechanism or factors of protection, which interfere in their use by insects. We can mention, as well, physical, chemical and morphological factors (SMITH, 2005; VENDRAMIM; GUZZO; 2009). The physical causes of resistance are mainly related to the color of the substratum and the light, and it can influence on the host selection, on the food preference and on the insect's oviposition (LARA, 1991; SMITH, 2005; VENDRAMIM; GUZZO, 2009). The chemical causes

are represented by chemical compounds which acts on the behavior or on the metabolism of the insect. The effects on the metabolism are resulted by the intake to the compounds or the substances (toxic, metabolites and enzymatic inhibitors), which might affect its biology (antibiosis resistance) (LARA, 1991; VENDRAMIM; NISHIKAWA, 2001). The morphological causes comprehend all the structural characteristics of the plant that acts negatively on the insect and it affect locomotion, mating and host selection to feed and oviposition. These characteristics care basically classified as structural factors related to dimension and to the disposition of the plant structures and to the epidermis factors, which includes thickness, hardness, texture, cerosity and the hairiness (trichomes) (LARA, 1991; VENDRAMIM; NISHIKAWA, 2001).

The knowledge about the relation between the pest infestation and the crop yield are important aspects to stablish of a succeeded integrated management program (QUINTELA; BARRAGOSSI, 2001). The simulation of insect damage on the plants permit to verify the impacts on the crop yield, similar to the one which occurs in the reality. The artificial removal of the structure in plants of economic importance permits to estimate how much damage the crop can withstand in specific stage of its development, once the reaction of the plant to the artificial defoliation is more similar to that caused when the plant is attacked by an insect-pest (GAZZONI, 1974; RIBEIRO; COSTA, 2000). This practice also permits to quantify the loss of yield on different levels of structural removal (JUNIOR et al., 2010). This knowledge might help in the validation of the economic threshold and economic injury level (EIL) established for these pests on soybean crop. Furthermore, this information can contribute to the reduction of expenses with the use of chemical control, as well as reduce the intensity of the use of these practices, minimizing the selection pressure on insect population (FAZOLIN; ESTRELA, 2003; JUNIOR et al., 2010).

Soybean is able to withstand specific levels of foliar reduction area and reproductive structures without affecting the grain yield. The tolerance of soybean to defoliation might be influenced by several factors, such as the intensity of damages, the phenological phase of the plant development and the environmental conditions (PEDIGO et al., 1986; HAILE et al., 1998). Several works have reported effects of the damages simulation in different phenological phases (from the vegetative phase to the last reproductive ones) and the different levels of removal

(0 to 100%) in relation to the yield crop (SALVADORI; CORSEUIL, 1979; PIKCLE; CAVINESS, 1984; PETERSON et al., 1998; CAMPELO; SEDIYAMA, 1999; BARROS et al., 2002; PELUZIO et al., 2002; COSTA et al., 2004; PELUZIO et al., 2004; FONTOURA et al., 2006; BUENO et al., 2010; BAHRY et al., 2013; SOUZA et al., 2014). Based on the above and considering the lack of studies which bring the relation to the resistant soybean genotypes to *H. armigera* in Brazil and a necessity to push the knowledge of the impact of the *H. zea* infestation on the soybean reproductive structure (flowers and pods) in the USA, the present study was developed under laboratory and field conditions.

The specific objectives were: a) characterize the possible expression of antibiosis and/or antixenosis in 22 soybean genotypes on *H. armigera*; b) to evaluate the impact of artificial removal of flowers and pods on soybean maturation and yield, simulating the natural attack of *H. zea*, in different reproductive growth stage and under abiotic conditions of stress, that could limit the crop productive potential.

The dissertation was divided onto two chapters in order to reach these objectives. The first was entitled “Assessing soybean genotypes for resistance to *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)” and the second was entitled “Impact of fruiting structure loss at various growth stages on soybean simulating corn earworm damage (Lepidoptera: Noctuidae) under non-irrigated conditions”, both written according to the Journal of Economic Entomology’s guidelines.

CHAPTER 1

Assessing soybean genotypes for resistance to the *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

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ABSTRACT

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is an important pest able to colonize several species of cultivated plants, including soybean [*Glycine max* (L.) Merrill]. The main method to manage the insect, is the utilization of chemical control and adoption of *Bt* plants. However, alternative and less harmful strategies to the environment and humans should be investigated and adopted, proposing a sustainable management of insects. The host plant resistance represents a potentially valuable method to insect control. The objective of this study was to characterize categories of resistance (antixenosis and/or antibiosis) of 22 soybean genotypes on *H. armigera* in laboratory. In order to select the most promising genotypes for resistance, a preliminary test was performed using leaves and pods of the genotypes as food. Then, the selected genotypes were evaluated by confinement tests using soybean leaves. In addition, biological parameters and leaf intake were also assessed. The genotypes PI 229358, PI 227687, PI 274453

and PI 274454 expressed resistance by prolonging the development larval period, causing high larvae mortality and low weight of fifth instar larvae. 'IAC 17' and PI 171451 caused high mortality of larvae. 'IAC 19' exhibited high larval mortality, low leaf intake, and low weight of fifth instar larvae. The genotypes PI 227687, PI 274453, PI 274454, PI 229358, PI 171451, 'IAC 17' and 'IAC 19', expressed resistance against *H. armigera*. The expression of antixenosis, was characterized in genotypes PI 227687, PI 274454 and 'IAC 19'. These genotypes might be utilized in breeding programs focusing on soybean resistance to lepidopterans.

Keywords: old-world bollworm, host plant resistance, antibiosis, antixenosis.

Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the most important agricultural products in the world (Conab 2019). It has been considered the main source of protein and oil for human and animal nutrition (Ainsworth et al. 2012, Kim et al. 2014). The productivity of soybean plant is severe damaged by pest insects, which can cause significant losses in the yield (Hoffman-Campo et al. 2012, Oliveira et al. 2014, Wille et al. 2017). Among the pests associated with soybean production, the old-world bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is one of the most economically important (Rogers and Brier 2010, Stacke et al. 2018).

Helicoverpa armigera is recognized as an important agricultural pest all over the world (Fitt 1989, Cunningham and Zalucki 2014). Also known as "Old World Bollworm" (Lammers and Macleod 2007). The insect damages several cultivated plants, including cotton, sorghum, tobacco, wheat and soybean (Tay et al. 2013, Kriticos et al. 2015). The wide range of hosts and high level of damage caused by the insect has elevated this species as one of the most devastating agricultural pests (Cunningham and Zalucki 2014).

The species presents a wide geographic distribution and economic damage has been reported from countries in Europe, Asia, Africa, Australia and South and Central America (Zalucki et al. 1986, Guo 1987, Specht et al. 2013, EFSA 2014, Kriticos et al. 2015). In the crop season 2012/2013, *H. armigera* was officially detected in Brazil (Specht et al. 2013 a, 2013b, Tay et al. 2013), with possible

introduction in 2008 (Sosa-Gomez et al. 2016). The lepidopteran presents a potential danger to the North America continent, especially because of products and people traffic on the agricultural barrier (Kriticos et al. 2015).

The larvae are able to cause damage in both vegetative and reproductive stages of plants (Cunningham et al. 1999). It feeds on leaves, stems, shoots, inflorescence, fruits and pods, with variable economic loss (Fitt 1989, Tay et al. 2013, Murua et al. 2014). Even in adverse conditions, *H. armigera* undergoes several generations each year, completing its life cycle in approximately six weeks (Pogue 2004, Nunes et al. 2017). The ability of dispersion, adaptation to different hosts (Pedgley 1985, Fitt 1989, Zalucki et al. 1994) and high rates of reproduction makes this species a key-pest in several crops (Cunningham and Zalucki 2014).

Currently, the use of synthetic insecticides and *Bt* plants are the most used methods in the management of *H. armigera* in different parts of the world (Wu 2007, Gonza and Avilla 2010, Carneiro et al. 2014, Downes et al. 2017, Limin et al. 2018). Neverthless, the abusive and inadequate use of these products can cause undesirable effects to non-target organism and to the environment and can lead to pests' outbreaks, human toxicity, and selects for resistant insects to active ingredients commonly used, such as pirethroides, phosphates and neonicotinoids (Vieira et al. 2011, Fathipour and Sedaratian 2013). Therefore, it is necessary to incorporate the use of less aggressive control methods which can reduce the risks caused by excessive use of agrochemicals (Fathipour and Sedaratian 2013), but it also must show good efficiency and be compatible with Integrated Pest Management (IPM) (Dourado et al. 2016, Bird 2017, Durigan et al. 2017, Pan et al. 2017).

The use of resistant genotypes is a valuable control strategy (Smith and Clement, 2012), when it enables the reduction of insect populations below the level of economic damages (Painter 1951, Panda and Khush 1995, Smith 2005). Resistant plants might negatively influence the biology and/or the behavior of the pest-insects by changing the ability of the plants to resist high population of certain insects without yield reduction (Painter 1951, Smith and Clement 2012, Canassa et al. 2017). Resistance can be divided in three different categories: antibiosis, antixenosis and tolerance (Painter 1951). Plants, which express antibiosis affect negatively the biology of the insects that try to colonize it by interfering in their growth and development, reproduction, and survival. Modification in the insect

size, weight, longevity, and high levels of deformity are also common effects in plants, which carry this category of resistance. Antixenosis affects the behavior of the insect and colonization of the host by interfering with chemical and physiological and morphological factors. Tolerance is the ability of plants to resist or recover from an injury caused by the attack of pests, not affecting their biology or the behavior (Painter 1951, Panda and Khush 1995, Smith 2005, Baldin et al. 2019). Commonly it overlaps expressions at antibiosis and antixenosis (Smith 2005). Plants exhibiting high levels of antixenosis may also cause opposite effects on insect development, provoking similar effects of plants that express antibiosis. Because of this, might be difficult to differentiate both categories, demanding specific examination of the insect consumption (Smith 2005, Baldin et al. 2015, Morando et al. 2017).

Despite many studies describing the performance of *H. armigera* in different soybean varieties (Naseri et al. 2009, Naseri et al. 2010, Fathipour and Naseri 2011), there are few studies characterizing categories of genotype resistance of soybean against the insect. Additionally, as it is a newly introduced pest in Brazil, it is important to fully understand the interaction of the insect with soybean genotypes in local conditions. Thus, the objective of this study was to characterize the possible expression of antibiosis and/or antixenosis of 22 soybean genotypes, which belong to different soybean groups of maturation, against *H. armigera*, in laboratory conditions.

Material and Methods

The genotypes used was maintained in a greenhouse and the tests was performed under laboratory conditions in the years 2017 and 2018.

Stock rearing of *Helicoverpa armigera*

A stock rearing of *H. armigera* (provided and identified by Dr. Alexandre Specht, Embrapa Cerrados, Brasília, DF, Brazil) was maintained under laboratory conditions (T: 25 ± 2ºC, photoperiod of 12:12 h, R.H.: 65-10%) to provide sufficient insects for the bioassays. The initial insects were collected from citrus in São Paulo, corn in Distrito Federal and cotton in Bahia. In order to reinvigorate

breeding, insects collected in São Paulo were introduced until enough number of insects were reached to start the bioassays. The rearing methodology was based on Kao's protocol (1995) and Jah et al. (2012).

For molecular characterization, DNA from males and females was extracted according protocol of Rogers and Bendich (1988). The amplification products were analyzed by polyacrylamide gel electroforesis (12.5%) at 20V/cm for 8 h in Tris-glycine buffer. The UV light was used to visualize the bands, which were photographed and scanned using a transilluminator (model L-Pix Ex, Loccus Biotecnologia R, Cotia, São Paulo, Brazil) coupled to a computer. The gels were treated with ethidium bromide (10 mg per mL -1). A 100 bp DNA marker (GeneRuler, Thermoscientific R, São Paulo, São Paulo, Brazil) was used to estimate the sizes of the fragments. The bands produced were coded and arranged in an array that was used to construct a dendrogram of genetic distance using the Neighbor Joining procedure (Saitou and Nei 1987) with the software Darwin 5, version 5.5.155 (Silva et al. 2018).

Moths were divided in pairs and were conditioned in PVC cages (20 cm in diameter and 30 cm of height) covered with *voil* fabric. The lower portion was supported in a plastic round container (28 cm in diameter) covered with filter paper. Adults were fed with portions of humid cotton with honey solution (10%) and water. Cages were lined with brown paper, where eggs were deposited.

Eggs were collected daily and moved to plastic containers (500 ml) containing 50g of bean-based artificial diet described by Parra (2001) and Greene et al. (1976) with adaptations. After hatching, neonate-larvae were individualized in 50 ml plastic containers with 5 g of artificial diet and kept until pupae formation. Pupae were sexed (Kirkpatrick 1961) and divided in pairs and placed in transparent acrylic containers (11,5 x 11,5 x 3,5 cm) containing moist vermiculite. All insects remained in this environment until the emergence of the adults, restarting the cycle of the rearing.

Screening bioassay

Initial testing consisted of one bioassay with 22 soybean genotypes (Table 1) distributed into different maturation groups: early, semi-early and late. The objective of this bioassay was to select the genotypes with better resistance

potential for subsequent bioassays and to choose the best structure of plant for the characterization of resistance.

Polyethylene vases (2 L) containing soil, sand, manure, and substratum, in ratios of 1:1:1:1 were kept in greenhouse under controlled condition (T: 25 ± 2ºC, photoperiod of 12:12 h, R.H.: 65-10%). There were three plants per vase, free from insect infestation. All plants received recommended fertility for the crop (Malavolta 2006, Camara 2015). Other necessary cultural practices (irrigation, thinning, cleaning, etc) were also provided. Leaves and pods from phenological growth stages V5 and R6, respectively (Fehr and Caviness 1977), and from medium part of the plant were used for testing.

For the bioassay, thirty caterpillars were added individually to Petri dishes (10 cm in diameter) containing leaf discs or pods from each genotype onto moist filter paper. Petri dishes were arranged in a completely randomized design for each rep. In order to reduce stress by manipulation, caterpillars with 48 h old were used.

The bioassay was kept in laboratory under previously described environmental conditions. Larval duration, viability, and pupal weight of the individuals for each genotype was recorded. During the bioassay, the frass were removed, the filter paper was replaced, and plant parts were replaced as necessary based on feeding.

Performance of *H. armigera* in selected genotypes

Based on the screening test, ten best performing genotypes for resistance to H. armigera biological performance were chosen for further testing. The selection of genotypes were based on high levels of resistance expressed in the bioassay with leaves (‘IAC19’); absence of previous studies (‘ANTA 82’, ‘TMG 4185’ and ‘TMG 132’); resistance for other Lepidoptera species (‘IAC 17’); or an established history of resistance verified in other insect orders, with possible relation to the leaves and pods bioassays (PI 171451, PI 229358, PI 227687, PI 274453 and PI 274454). The genotypes ‘Conquista’ and ‘Coodetec 208’ were included as susceptible commercial materials (Silva et al. 2012). Because H. armigera have the habit of feeding the internal part of the pods, leaves were chosen for the bioassay since internal pod feeding could potentially affect the

performance during the test. Eighty newly hatched larvae (48h old) were individualized on Petri dishes (8 x 2cm) containing leaf discs (6.93 cm^2) from the chosen genotypes under moist filter paper. Each dish contained one neonate larvae. Petri dishes were arranged in a completely randomized design. Bioassay methods were used as described in the preliminary bioassay.

Evaluation of biological parameters were performed daily. Duration of each larval instar, larval period; fifth instar larvae weight (24 h old); larval viability; duration of pre-pupal and pupal period; pupal weight (24 h old); pupal viability; index of pupal deformity; cycle (egg – adult); duration of pre-oviposition and oviposition period, and total number of eggs and viable eggs per female were recorded. Fifth instar larvae weight and the pupae were obtained with analytical weighting scale (AL-500, Marte Científica®, São Paulo, SP, Brasil).

In order to better describe the antibiosis and antixenosis categories, food consumption of the insects during the larval period was also evaluated. Leaf discs were replaced daily, and non-consumed food was measured on a leaf area meter (LI-COR® Model LI-3100, LI-COR, Inc, Lincoln, NE, USA), making it possible to know the consumption (cm^2) of each individual during the larval period.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA). Normality and homogeneity of the data were verified by Shapiro-Wilk and Levene tests, respectively (Winer et al. 1991). When the differences had been verified ($p \leq 0.05$), the results were compared by Fisher Protected LSD (PROC MIXED, SAS Institute 2009).

Table 1. Soybean genotypes used, grouped according to phenology, genealogy and resistance history

Maturity	Genotype	Genealogy	Resistance history
Early	'IAC 17'	D72-9601 x 'IAC 8'	Antibiosis to *Anticarsia gemmatalis* and *Bemisia tabaci* biotype B (Fugi et al. 2005, Valle and Lourenção 2002)
	'IAC 23'	BR-6 X IAC-83-23	Resistant to insects (Miranda et al. 2003)
	PI 171451	Tóquio, Japan	Resistant to *Helicoverpa zea*, *Chloridea virescens* and stink bugs (Hatchett et al. 1976, Silva et al. 2013)
	PI 229358	Japan	Resistant to *A. gemmatalis* and stink bugs (Fugi et al. 2005, Silva et al 2012)
	D75-10169	'Govan' x (F4 'Bragg' x PI 229358)	Multiple insect resistance in its genealogy (Silva et al. 2013); Resistant to *Bemisia tabaci* biotype B (Valle and Lourenção 2002)
	Coodetec 208	OC-4 x Williams 20	Commercial susceptible (Silva et al. 2012)
	'TMG 4182'	Tropical Melhoramento e Genética	Not previously investigated
	'TMG 1179' RR	Tropical Melhoramento e Genética	Not previously investigated
Semi-early	'IAC 24'	IAC 80-1177 X IAC 83-288	Resistant to *A. gemmatalis* and *B. tabaci* biotype B (Valle and Lourenção 2002, Fugi et al. 2005)
	IAC 74-2832	'Hill' x PI 274454	Resistant to *A. gemmatalis* and *Piezodorus guildinii* (Hoffmann-Campo et al. 1994)
	'IAC 100'	'IAC 12' x IAC 78-2318	Antibiosis to *Spodoptera cosmioides* and *P. guildinii* (Silva et al. 2012, Boiça Júnior et al. 2015)

	IAC 78-2318	D 72-9601-1 x IAC 73-227	Resistant to stink bug and *Crocidosema aporema* (Lourenção et al. 1987, 1989; Silva et al. 2013)
	PI 227687	Okinawa, Japan	Resistant to stink bugs and *S. cosmioides* (Silva et al. 2013, Boiça Júnior et al. 2015)
	'TMG 4185'	Tropical Melhoramento e Genética	Not previously investigated
	'TMG 132'	Tropical Melhoramento e Genética	Not previously investigated
	'ANTA 82'	Tropical Melhoramento e Genética	Not previously investigated
Late	PI 274453	Japan	Resistant to stink bugs (Lourenção et al. 1989, Silva et al. 2014)
	'IAC 19'	D72-9601-1 x 'IAC 8'	Antixenosis to *B. tabaci* biotype B (Valle e Lourenção, 2002), antibiosis to *P. guildinii* (Silva et al. 2013)
	L1-1-01	BR-6 x 'IAC 100'	Antibiosis to *P. guildini* (Silva et al. 2013)
	PI 274454	Okinawa, Japan	Resistant to stink bugs and *Omiodes indicata* (Lourenção et al. 1989, Silva et al. 2013)
	'Conquista'	Lo 76-4484 x 'Numbaíra'	Commercial susceptible (Silva et al. 2012)
	'TMG 133' RR	Tropical Melhoramento e Genética	Not previously investigated

Results

Screening bioassay

In the preliminary test, when larvae were fed with pods, the genotypes PI 171451, PI 229358, PI 227687, 'TMG 4185', PI 274453 and PI 274454 did not permit the larvae to complete the larval phase; the other genotypes did not differ among each other, with larval duration ranging from 16.0 to 20.8 days (F = 2.69; df = 15.90; P > 0.0001; Table 2). For pupae weight, 'TMG 1179 (0.156 g), 'ANTA 82' (0.192 g), 'IAC 17' (0.213 g) and IAC 742832 (0.221 g) had the lowest weight, while the genotypes D75-10169 (0.370 g), 'IAC 23' (0.330 g) and 'TMG 132' (0.339 g) had the heaviest pupal weight (F = 3.87; df = 14.72; P < 0.0001). For larval viability, the genotypes 'TMG 132', 'TMG 1179', 'TMG 4182', IAC 24', 'IAC 23', 'IAC 17', D75-10169, IAC 74-2832 and ANTA 82 had the lowest mean (3.3 – 16.7%), whereas the 'IAC 19' (56.7%), L1-1-01 (53.3%) and 'Coodetec 208' (46.7%) had the highest mean (F = 4.04; df = 15.464; P > 0.0001).

When leaves were used as food source, the genotype PI 274454 (32.0 days) prolonged the larval phase while the genotypes 'Coodetec 208' (19.9 days), 'IAC 24' (19.8 days), 'IAC 17' (19.7 days), 'ANTA 82' (19.6 days), 'IAC 23' (19.5 days), 'TMG 1179' (19.1 days), and 'TMG 4182' (18.9 days) had lower duration of the larval period (F = 10.05; df = 19.25; P > 0.0001; Table 2). The genotypes IAC 78-2318 (0.113 g), 'IAC 100' (0.124 g), 'TMG 132' (0.125 g), 'ANTA 82' (0.124 g), PI 227687 (0.134 g) and 'TMG 4185' (0.130 g) had the lowest mean weights among genotypes, while 'IAC 17' (0.188 g), 'Coodetec 208' (0.197 g), L1-1-01 (0.195 g), 'TMG 4182' (0.207 g), 'IAC 23' (0.215 g) and 'TMG 133' (0.221 g) resulted in the highest weight for *H. armigera* pupae (F = 10.66; df = 19.58; P > 0.0001). PI 274453 and 'IAC 19' did not permit the larvae to complete the larval stage, resulting in 0% of larval viability. The genotypes PI 274454 (3.0%), L1-1-01 (13.3%), 'TMG 132' (17.0%), 'IAC 17' (23.0%), 'IAC 100' (23.3%), 'TMG 1179' (23.3%) and IAC 74-2832 (27.0%) had the lowest larval viability (F = 10.66; df = 19.58; P > 0.0001). The genotypes 'TMG 4182' (90.0%), 'IAC 23' (90.0%), 'IAC 24' (77.0%), 'Coodetec 208' (70.0%), 'TMG 4185' (70.0%) e 'ANTA 82' (70.0%) were the most favorable for larval development (Table 2). Based on the results of the initial test, genotypes with potential resistance were

selected: ‘IAC 17’, PI 171451, PI 229358, ‘TMG 4185’, ‘TMG 132’, ‘ANTA 82’, PI 227687, ‘IAC 19’, PI 274453 and PI 274454. It was also selected the susceptible commercial genotypes ‘Coodetec 208’ and ‘Conquista’.

Performance of *H. armigera* in selected genotypes

The mean duration of first instars of *H. armigera* was significantly greater when the insects fed on leaves of PI 227687 (3.6 days), ‘ANTA 82’ (3.5 days), PI 229358 (3.4 days) and ‘Coodetec 208’ (3.4 days), compared when fed on ‘Conquista’ (2.6 days), ‘IAC 19’ (3.0 days), ‘TMG 4185’ (3.1 days), PI 171451 (3.1 days) and PI 274453 (3.1 days) (F = 8.15; df = 11.94; P > 0.0001; Table 3). In the second instar, PI 227687 (7.1 days), ‘IAC 19’ (6.8 days) and PI 274453 (6.1 days) induced prolonged development period, differing from the other genotypes, and mainly from ‘IAC 17’ (4.0 days), PI 274454 (3.9 days), ‘Conquista’ (3.8 days) and ‘TMG 4185’ (3.4 days) (F = 4.68; df = 11.83; P > 0.0001).

In the third instar, genotypes PI 229358 (6.6 days), PI 274453 (5.9 days) and ‘IAC 19’ (5.5 days) caused the longest duration, while ‘Conquista’, ‘IAC 17’, ‘Coodetec 208’, ‘TMG 132’ and ‘TMG 4185’ allowed shorter periods of larval development (< 4.0 days) (F = 4.08; df = 11.61; P > 0.0001). The prolonged insect development period for the fourth instar were verified in PI 274454, PI 274453, PI 229358, ‘IAC 19’, and ‘IAC 17’ (4.2 – 5.8 days) differing from ‘TMG’s’, which promoted the shortest periods of duration (≤ 3.3 days) (F = 1.86; df = 11.47; P > 0.0001). In the fifth instar, PI 274453 (8.0 days) presented the longest duration, followed by PI 229358 (6.1 days) and PI 227687 (6.0 days), differing from most of the other genotypes (3.2 – 5.3 days) (F = 17.88; df = 11.88; P > 0.0001). In the sixth instar, PI 274453 (5.0 days) and PI 229358 (4.0 days) continued to induce the longest duration, differing from the other genotypes (exception for PI 274454) (F = 4.19; df = 10.17; P > 0.0001). The larval period was greatest when larvae were confined to the genotypes PI 274453 (30.0 days), PI 229358 (28.6 days) and PI 274454 (25.6 days), compared to other genotypes (F = 14.34; df = 10.26; P > 0.0001). In PI 227687, the insects did not complete the larval phase (Table 3).

Mortality of first instar larvae was detected on ‘ANTA 82’ (2.3%), PI 229358 (1.3%) and PI 227687 (1.2%) (Figure 1). The highest mortality index in

the second instar (23.9 – 47.5%) was detected in PI 227687, PI 274453 and 'IAC 19'. During the third instar, the highest mortality occurred in PI 274453 (44.3%) e PI 227687 (40.0%). 'Conquista', PI 229358, 'TMG 132' and 'IAC 19' genotypes caused the highest mortality (21.1 – 31.4%) of the fourth instar larvae. The highest mortality of fifth instar larvae were in PI 274454 (58.3%), while in the sixth instar 'ANTA 82', 'TMG 132', 'TMG 4185' and PI 171451 were the genotypes which caused more mortality (32.2 – 44.1%). The genotypes PI 227687, PI 274453, PI 229358, PI 274454, 'IAC 19', 'IAC 17' and PI 171451 provided the lowest percentage of larval viability of *H. armigera* (< 30.0%). The genotype PI 227687 caused complete mortality in all larvae tested (Figure 2). 'TMG's and 'Conquista' were more conducive for successful larval development (55.0 – 70.0%).

PI 227687 (7.3 cm²), 'IAC 19' (8.2 cm²), PI 274453 (8.3 cm²) and 'IAC 17' (8.5 cm²) were the least consumed genotypes for the larvae in the second instar (F = 61.36; df = 11.83; P > 0.0001; Table 4). The genotypes PI 227687, PI 274453, 'IAC 19', PI 229358 and PI 274454 presented the lowest mean foliar intake for the third (7.6 - 10.2 cm²) (F = 67.92; df = 11.66; P > 0.0001) and fourth instar, together with 'ANTA 82' and PI 171451 (fourth instar) (7.3 – 9.8 cm²) (F = 50.51; df = 11.55; P > 0.0001). In the fifth instar, PI 227687, 'IAC 19', 'TMG 4185', PI 171451, 'ANTA 82 and PI 274454 had the lowest foliar intake indexes (3.4 – 7.1 cm²) (F = 12.09; df = 11.37; P > 0.0001). PI 274454, 'TMG 4185' and PI 171451 were the least consumed by sixth instar larvae (3.7 – 4.4 cm²), while PI 229358, 'Conquista', 'IAC 17' and 'ANTA 82' presented the highest mean (5.9 – 7.3 cm²) (F = 14.29; df = 10.26; P > 0.0001). The genotype PI 227687 was not consumed on the sixth instar. The genotype 'Conquista' showed the highest mean of foliar intake in all larval instars of *H. armigera*, with 12.9, 17.4, 17.1, 9.8 and 6.6 cm² for the second, third, fourth, fifth, and sixth instar, respectively.

The genotypes 'IAC 19' (0.135 g), PI 227687 (0.135 g), PI 229358 (0.129 g), PI 274454 (0.140 g), and PI 274453 (0.158 g) provided the lowest mean weight of the fifth instar larvae (F = 12.59; df = 11.48; P > 0.0001; Table 5). The highest weight in the fifth instar were measured on 'Conquista' (0.224 g) and 'TMG 4185' (0.225 g), differing from the other genotypes. For pupae weight, PI 274453 (0.021 g) revealed the lowest mean; 'Conquista' (0.140 g), 'TMG 132' (0.139 g), 'TMG 4185' (0.141 g) and 'IAC 17' (0.153 g) showed the highest mean

of pupal weight (*F* = 5.08; df = 10.25; *P* > 0.0001). The larvae feeding on genotype PI 227687 did not reach the pupal phase.

The genotype PI 229358 (3.3 days) had the highest mean duration in the pre-pupal phase, differing from ‘Coodetec 208’ (2.5 days), ‘TMG 4185’ (2.4 days) and PI 274454 (2.4 days) which presented the shortest duration (*F* = 2.41; df = 10.26; *P* = 0.0095; Table 6). ‘TMG 4185’ (14.1 days) had the longest pupal phase, differing from PI 171451 (12.0 days), ‘IAC 17’ (13.1 days) and ‘TMG 132’ (13.5 days), with the shortest duration (*F* = 2.62; df = 6.76; *P* = 0.0229). The genotype PI 171451 (9.5%) provided the lowest viability of *H. armigera* pupae, while ‘TMG 4185’, ‘IAC 17’ e ‘TMG 132’ (51.8 – 34.0%) were the most appropriate (*F* = 3.02; df = 10.26; *P* = 0.0013). The larvae confined to PI 274453, ‘IAC 19’, PI 229358, PI 274454 and PI 227687 did not reach the pupal stage. In relation to larvae-to-adult period, there was no significant difference between genotypes (*F* = 0.71; df = 6.76; *P* = 0.6381; Table 6), and the same occurred to the pre-oviposition (*F* = 1.13; df = 5.90; *P* = 0.4085) and oviposition period (*F* = 1.57; df = 5.12; *P* = 0.2414), and number of eggs per female (*F* = 1.18; df = 5.40; *P* = 0.3366; Table 7).

Table 2. Mean (± SE) larval period (days), weight of pupae (mg) and larval viability (%) of *H. armigera* when fed with pods and leaves of 22 soybean genotypes in the laboratory

Genotype[a]	Pod [b,c]			Leaf [b,c]		
	Larval stage (days)	Weight of pupae (g)	Larval Viability (%)	Larval stage (days)	Weight of pupae (g)	Larval Viability (%)
PI 274453 (L)	--	--	--	--	--	--
PI 171451 (E)	--	--	--	21.1 ± 0,60def (*n* = 13)	0.176 ± 0.01b (*n* = 13)	43.3 ± 7.95def (*n* = 13)
PI 229358 (E)	--	--	--	25.8 ± 0.68b (*n* = 10)	0.161 ± 0.01bc (*n* = 10)	33.3 ± 7.95efgh (*n* = 10)
PI 227687 (SE)	--	--	--	22.6 ± 0.54cd (*n* = 16)	0.134 ± 0.01cdef (*n* = 16)	53.3 ± 7.95cde (*n* = 16)
'TMG 4185' (SE)	--	--	--	21.1 ± 0.47de (*n* = 21)	0.130 ± 0.01cdef (*n* = 21)	70.0 ± 7.95abc (*n* = 21)
PI 274454 (L)	--	--	--	32.0 ± 2.17a (*n* = 1)	0.141 ± 0.03bcdef (*n* = 1)	3.0 ± 7.95i (*n* = 1)
'TMG 132' (SE)	16.0 ± 2.35a (*n* = 1)	0.339 ± 0.01ab (*n* = 1)	3.3 ± 7.64f (*n* = 1)	22.6 ± 0.97cd (*n* = 5)	0.125 ± 0.01cdef (*n* = 5)	17.0 ± 7.95ghi (*n* = 5)
'Conquista' (L)	16.5 ± 0.83a (*n* = 8)	0.256 ± 0.02bc (*n* = 8)	26.7 ± 7.64cde (*n* = 8)	21.8 ± 0.52de (*n* = 17)	0.145 ± 0.01bcde (*n* = 17)	57.0 ± 7.95bcd (*n* = 17)
IAC 78-2318 (SE)	16.5 ± 0.83a (*n* = 8)	0.276 ± 0.01bc (*n* = 8)	26.7 ± 7.64cde (*n* = 8)	24.4 ± 0.68bc (*n* = 10)	0.113 ± 0.01f (*n* = 10)	33.3 ± 7.95efgh (*n* = 10)

D75-10169 (E)	16.8 ± 1.05a	0.370 ± 0.03a	16.7 ± 7.64def	22.6 ± 0.54cd	0.179 ± 0.01b	53.3 ± 7.95cde
	(*n* = 5)	(*n* = 5)	(*n* = 5)	(*n* = 16)	(*n* = 16)	(*n* = 16)
‘IAC 19’ (L)	18.0 ± 0.71a	0.303 ± 0.01bc	56.7 ± 7.64a	--[d]	--	--
	(*n* = 17)	(*n* = 17)	(*n* = 17)			
‘IAC 23’ (E)	19.0 ± 1.05a	0.330 ± 0.07ab	16.7 ± 7.64def	19.5 ± 0.42fg	0.215 ± 0.01a	90.0 ± 7.95a
	(*n* = 5)	(*n* = 5)	(*n* = 5)	(*n* = 27)	(*n* = 27)	(*n* = 27)
‘IAC 24’ (SE)	19.0 ± 1.35a	0.278 ± 0.06bc	10.0 ± 7.64ef	19.8 ± 0.45efg	0.158 ± 0.01bcd	77.0 ± 7.95ab
	(*n* = 3)	(*n* = 3)	(*n* = 3)	(*n* = 23)	(*n* = 23)	(*n* = 23)
‘TMG 133’ (L)	19.0 ± 0.78a	0.240 ± 0.02bc	33.3 ± 7.64bcd	22.3 ± 0.65cd	0.221 ± 0.01a	37.0 ± 7.95defg
	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 11)	(*n* = 11)	(*n* = 11)
‘TMG 1179’ (E)	19.0 ± 1.05a	0.156 ± 0.07d	16.7 ± 7.64def	19.1 ± 0.82fg	0.155 ± 0.01bcd	23.3 ± 7.95fghi
	(*n* = 5)	(*n* = 5)	(*n* = 5)	(*n* = 7)	(*n* = 7)	(*n* = 7)
‘TMG 4182’ (E)	19.5 ± 1.17a	0.237 ± 0.04c	13.3 ± 7.64def	18.9 ± 0.42g	0.207 ± 0.01ab	90.0 ± 7.95a
	(*n* = 4)	(*n* = 4)	(*n* = 4)	(*n* = 27)	(*n* = 27)	(*n* = 27)
IAC 74-2832 (SE)	19.6 ± 1.05a	0.221 ± 0.01cd	16.7 ± 7.64def	21.6 ± 0.77de	0.158 ± 0.01bcd	27.0 ± 7.95fghi
	(*n* = 5)	(*n* = 5)	(*n* = 5)	(*n* = 8)	(*n* = 8)	(*n* = 8)
‘ANTA 82’ (SE)	19.7 ± 0.96a	0.192 ± 0.02cd	20.0 ± 7.64def	19.6 ± 0.47fg	0.124 ± 0.01ef	70.0 ± 7.95abc
	(*n* = 6)	(*n* = 6)	(*n* = 6)	(*n* = 21)	(*n* = 21)	(*n* = 21)
‘IAC 17’ (E)	19.8 ± 0.88a	0.213 ± 0.01cd	23.3 ± 7.64def	19.7 ± 0.82efg	0.188 ± 0.01ab	23.0 ± 7.95fghi
	(*n* = 7)	(*n* = 7)	(*n* = 7)	(*n* = 7)	(*n* = 7)	(*n* = 7)

L1-1-01 (L)	20.1 ± 0.74a	0.247 ± 0.02bc	53.3 ± 7.64ab	22.0 ± 1.08cde	0.195 ± 0.01ab	13.3 ± 7.95hi
	(*n* = 16)	(*n* = 16)	(*n* = 16)	(*n* = 4)	(*n* = 4)	(*n* = 4)
'Coodetec 208' (E)	20.8 ± 0.78a	0.278 ± 0.01bc	46.7 ± 7.64abc	19.9 ± 0.47efg	0.197 ± 0.01ab	70.0 ± 7.95abc
	(*n* = 14)	(*n* = 14)	(*n* = 14)	(*n* = 21)	(*n* = 21)	(*n* = 21)
'IAC 100' (SE)	20.8 ± 0.74a	0.274 ± 0.01bc	33.3 ± 7.64bcd	22.1 ± 0.82cd	0.124 ± 0.01def	23.3 ± 7.95fghi
	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 7)	(*n* = 7)	(*n* = 7)
P	> 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a]E = early; SE = semi-early; L = late.

[b]Means followed by the same lowercase letter per column do not differ significantly by LSD test ($p > 0.05$).

[c]n = number of evaluated insects

[d]insects did not complete the larval stage.

Table 3. Mean (± SE) length of each larval instar and larval period of *H. armigera* in 12 soybean genotypes in the laboratory

Genotype[a]	Days [b,c]						
	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Larval period
PI 227687 (SE)	3.6 ± 0.08a (*n* = 80)	7.1 ± 0.40a (*n* = 42)	4.5 ± 0.74cde (*n* = 10)	4.7 ± 0.94bcd (*n* = 4)	6.0 ± 0.87ab (*n* = 4)	--	-- [d]
'ANTA 82' (SE)	3.5 ± 0.08ab (*n* = 79)	4.6 ± 0.13de (*n* = 78)	4.0 ± 0.11cd (*n* = 72)	3.6 ± 0.08d (*n* = 67)	3.3 ± 0.12d (*n* = 56)	3.1 ± 0.08b (*n* = 37)	21.9 ± 0.46b (*n* = 37)
PI 229358 (E)	3.4 ± 0.08abc (*n* = 79)	5.4 ± 0.22c (*n* = 71)	6.6 ± 0.32a (*n* = 56)	4.6 ± 0.20bc (*n* = 38)	6.1 ± 0.73a (*n* = 16)	4.0 ± 0.27a (*n* = 16)	28.6 ± 2.21a (*n* = 3)
'Coodetec 208' (E)	3.4 ± 0.08abc (*n* = 80)	4.2 ± 0.13efg (*n* = 76)	3.7 ± 0.08de (*n* = 64)	3.6 ± 0.08d (*n* = 55)	3.4 ± 0.09d (*n* = 38)	2.6 ± 0.12cd (*n* = 28)	20.9 ± 0.53bc (*n* = 28)
PI 274454 (L)	3.3 ± 0.08bcd (*n* = 80)	3.9 ± 0.11fg (*n* = 79)	4.4 ± 0.11c (*n* = 69)	4.9 ± 0.16a (*n* = 60)	5.3 ± 0.34bc (*n* = 18)	3.5 ± 0.19ab (*n* = 8)	25.6 ± 1.37a (*n* = 8)
'IAC 17' (E)	3.3 ± 0.08bcde (*n* = 80)	4.0 ± 0.12fg (*n* = 68)	3.9 ± 0.10cd (*n* = 53)	4.2 ± 0.20cd (*n* = 41)	3.6 ± 0.19cd (*n* = 25)	2.7 ± 0.29cd (*n* = 16)	20.8 ± 0.79bc (*n* = 16)
'TMG 132' (SE)	3.2 ± 0.08cdef (*n* = 80)	4.8 ± 0.15d (*n* = 77)	3.5 ± 0.11e (*n* = 75)	3.3 ± 0.07de (*n* = 68)	3.6 ± 0.14d (*n* = 60)	2.6 ± 0.09d (*n* = 47)	20.1 ± 0.41c (*n* = 47)
PI 171451 (E)	3.1 ± 0.08def (*n* = 80)	4.4 ± 0.12def (*n* = 72)	4.2 ± 0.14cd (*n* = 66)	3.9 ± 0.14d (*n* = 59)	4.4 ± 0.20bc (*n* = 40)	2.8 ± 0.09cd (*n* = 21)	23.0 ± 0.57b (*n* = 21)
PI 274453 (L)	3.1 ± 0.08def (*n* = 80)	6.1 ± 0.24b (*n* = 57)	5.9 ± 0.37b (*n* = 22)	5.8 ± 0.49a (*n* = 13)	8.0 ± 0.57a (*n* = 3)	5.0 ± 0.64a (*n* = 1)	30.0 ± 2.36a (*n* = 1)

‘TMG 4185’ (SE)	3.1 ± 0.08def	3.4 ± 0.12h	3.2 ± 0.06e	3.2 ± 0.06e	3.0 ± 0.06d	2.7 ± 0.11cd	18.2 ± 0.39d
	(*n* = 80)	(*n* = 79)	(*n* = 73)	(*n* = 70)	(*n* = 64)	(*n* = 56)	(*n* = 56)
‘IAC 19’ (L)	3.0 ± 0.08f	6.8 ± 0.23a	5.5 ± 0.24b	4.4 ± 0.28bc	3.2 ± 0.18d	2.9 ± 0.11bcd	23.1 ± 1.05b
	(*n* = 80)	(*n* = 63)	(*n* = 44)	(*n* = 29)	(*n* = 12)	(*n* = 9)	(*n* = 8)
‘Conquista’ (L)	2.6 ± 0.08g	3.8 ± 0.10g	3.8 ± 0.10de	3.9 ± 0.13d	3.5 ± 0.12d	3.0 ± 0.05bc	19.9 ± 0.41c
	(*n* = 80)	(*n* = 80)	(*n* = 72)	(*n* = 61)	(*n* = 50)	(*n* = 45)	(*n* = 45)
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a] E = early; SE = semi-early; L = late.

[b] Means followed by the same lowercase letter per column do not differ significantly by LSD test ($p > 0.05$).

[c] *n* = number of evaluated insects.

[d] insects did not turn into pupae.

Table 4. Mean (± SE) leaf intake per instar of *H. armigera* in 12 soybean genotypes in the laboratory

Genotype[a]	Foliar intake (cm²) [b,c]				
	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar
PI 227687 (SE)	7.3 ± 0.19g (*n* = 42)	7.6 ± 0.31g (*n* = 10)	8.6 ± 1.64de (*n* = 4)	3.4 ± 3.03b (*n* = 1)	--[d]
PI 171451 (E)	10.3 ± 0.21cd (*n* = 72)	13.2 ± 0.37de (*n* = 66)	9.8 ± 0.42d (*n* = 59)	7.1 ± 0.48b (*n* = 40)	4.4 ± 0.52c (*n* = 21)
'Coodetec 208' (E)	8.9 ± 0.23e (*n* = 76)	14.2 ± 0.28c (*n* = 64)	13.8 ± 0.18b (*n* = 58)	8.9 ± 0.36[a] (*n* = 38)	5.6 ± 0.20ab (*n* = 28)
PI 274453 (L)	8.3 ± 0.18f (*n* = 57)	9.4 ± 0.44f (*n* = 22)	8.7 ± 1.11de (*n* = 13)	10.5 ± 0.67[a] (*n* = 3)	4.7 ± 0.91abc (*n* = 1)
PI 274454 (L)	9.8 ± 0.26d (*n* = 79)	10.2 ± 0.23f (*n* = 69)	9.1 ± 0.34d (*n* = 60)	7.1 ± 0.70b (*n* = 18)	3.7 ± 0.48c (*n* = 8)
PI 229358 (E)	9.1 ± 0.22e (*n* = 71)	10.0 ± 0.35f (*n* = 56)	8.9 ± 0.44d (*n* = 38)	8.9 ± 0.53[a] (*n* = 16)	7.3 ± 0.69[a] (*n* = 3)
'TMG 132' (SE)	10.8 ± 0.19c (*n* = 77)	15.5 ± 0.32b (*n* = 75)	14.1 ± 0.31b (*n* = 68)	8.8 ± 0.29[a] (*n* = 60)	5.4 ± 0.17ab (*n* = 47)
'IAC 19' (L)	8.2 ± 0.30f (*n* = 63)	9.9 ± 0.63f (*n* = 44)	7.3 ± 0.65e (*n* = 29)	5.4 ± 0.77b (*n* = 12)	4.6 ± 0.47bc (*n* = 8)
'TMG 4185' (SE)	10.6 ± 0.26c (*n* = 79)	15.5 ± 0.23b (*n* = 73)	12.1 ± 0.32c (*n* = 70)	6.5 ± 0.37b (*n* = 64)	4.0 ± 0.18c (*n* = 56)
'IAC 17' (E)	8.5 ± 0. 22ef	12.4 ± 0.17e	13.3 ± 0.28b	10.5 ± 0.36[a]	6.3 ± 0.24[a]

	(*n* = 68)	(*n* = 52)	(*n* = 42)	(*n* = 25)	(*n* = 16)
‘ANTA 82’ (SE)	12.4 ± 0.17b	13.3 ± 0.23d	9.2 ± 0.46d	7.1 ± 0.21b	5.9 ± 0.19a
	(*n* = 78)	(*n* = 72)	(*n* = 67)	(*n* = 56)	(*n* = 37)
‘Conquista’ (L)	12.9 ± 0.15[a]	17.4 ± 0.21[a]	17.1 ± 0.45[a]	9.8 ± 0.10[a]	6.6 ± 0.19[a]
	(*n* = 80)	(*n* = 72)	(*n* = 61)	(*n* = 50)	(*n* = 45)
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a] E = early; SE = semi-early; L = late.

[b]Means followed by the same lowercase letter per column do not differ significantly by LSD test ($p > 0.05$).

[c]*n* = number of evaluated insects.

[d] insects did not have consumption.

Table 5. Means (± SE) of fifth instar and pupae weight of *H. armigera* in 12 soybean genotypes in the laboratory

Genotype[a]	Weight (g) [b,c]	
	Fifth instar	Pupae
PI 229358 (E)	0.129 ± 0.01d	0.105 ± 0.02d
	(*n* = 38)	(*n* = 3)
'IAC 19' (L)	0.135 ± 0.01d	0.122 ± 0.01bc
	(*n* = 29)	(*n* = 8)
PI 274454 (L)	0.140 ± 0.01cd	0.116 ± 0.01cd
	(*n* = 60)	(*n* = 8)
PI 227687 (SE)	0.134 ± 0.03d	--[d]
	(*n* = 4)	
'Coodetec 208' (E)	0.152 ± 0.01bcd	0.134 ± 0.01bc
	(*n* = 58)	(*n* = 28)
PI 274453 (L)	0.158 ± 0.02bcd	0.021 ± 0.03e
	(*n* = 13)	(*n* = 1)
PI 171451 (E)	0.160 ± 0.01bc	0.118 ± 0.01c
	(*n* = 59)	(*n* = 21)
'TMG 132' (SE)	0.174 ± 0.01b	0.139 ± 0.00ab
	(*n* = 68)	(*n* = 47)
'ANTA 82' (SE)	0.185 ± 0.01b	0.134 ± 0.00bc
	(*n* = 67)	(*n* = 37)
'IAC 17' (E)	0.178 ± 0.01b	0.153 ± 0.01a
	(*n* = 42)	(*n* = 16)
'Conquista' (L)	0.224 ± 0.01a	0.140 ± 0.00ab
	(*n* = 61)	(*n* = 45)
'TMG 4185' (SE)	0.225 ± 0.01a	0.141 ± 0.00ab
	(*n* = 70)	(*n* = 56)
P	< 0.0001	<0.0001

[a] E = early; SE = semi-early; L = late.

[b]Means followed by the same lowercase letter per column do not differ significantly by LSD test ($p > 0.05$).

[c]*n* = number of evaluated insects. / [d] insects did not turn into pupae.

Table 6. Mean (± SE) of the pre-pupae duration period, pupae stage, pupae viability (%), and larvae-to-adult cycle of *H. armigera* in 12 soybean genotypes in the laboratory

Genotype [a]	Days [b,c]			
	Pre-pupae stage	Pupae stage	Pupae viability (%)	Cycle (larvae-to adult)
PI 229358 (E)	3.3 ± 0.34a (*n* = 3)	--	--	--
PI 274453 (L)	3.0 ± 0.58ab (*n* = 1)	--	--	--
'IAC 19' (L)	2.9 ± 0.21ab (*n* = 9)	--	--	--
'ANTA 82' (SE)	2.8 ± 0.09ab (*n* = 37)	13.2 ± 0.49ab (*n* = 11)	29.7 ± 7.56ab (*n* = 11)	48.0 ± 1.83a (*n* = 11)
'IAC 17' (E)	2.7 ± 0.14ab (*n* = 16)	13.1 ± 0.56bc (*n* = 7)	43.7 ± 12.81a (*n* = 7)	51.8 ± 2.29a (*n* = 7)
'Conquista' (L)	2.7 ± 0.09ab (*n* = 45)	13.5 ± 0.56ab (*n* = 11)	24.4 ± 6.48ab (*n* = 11)	47.0 ± 1.83a (*n* = 11)
'TMG 132' (SE)	2.6 ± 0.08ab (*n* = 47)	13.5 ± 0.44bc (*n* = 16)	34.0 ± 6.99a (*n* = 16)	48.4 ± 1.51a (*n* = 16)
PI 171451 (E)	2.6 ± 0.13ab (*n* = 21)	12.0 ± 0.98c (*n* = 2)	9.5 ± 6.56b (*n* = 2)	51.0 ± 4.29a (*n* = 2)
'Coodetec 208' (E)	2.5 ± 0.11b (*n* = 28)	13.6 ± 0.69ab (*n* = 7)	25.0 ± 8.33ab (*n* = 7)	46.8 ± 2.29a (*n* = 7)
'TMG 4185' (SE)	2.4 ± 0.08b (*n* = 56)	14.1 ± 0.27a (*n* = 29)	51.8 ± 5.93a (*n* = 29)	49.4 ± 1.12a (*n* = 29)
PI 274454 (L)	2.4 ± 0.21b (*n* = 8)	--	--	--
PI 227687 (SE)	--[d]	--	-- [c]	--

[a] E = early; SE = semi-early; L = late.

[b]Means followed by the same lowercase letter per column do not differ significantly by LSD test ($p > 0.05$). [c]*n* = number of evaluated insects. / [d] insects did not turn into pupae.

Table 7. Mean (± SE) of the length pre-oviposition (days), oviposition period (days) and number of total eggs per female of *H. armigera* in 12 soybean genotypes in the laboratory

Genotype[a]	Pre-oviposition (days)[b,c]	Oviposition period (days)[b,c]	No. of eggs per female
'TMG 4185' (SE)	5.5 ± 1.21a (*n* = 6)	3.5 ± 0.90a (*n* = 6)	103.0 ± 62.3a
'Conquista' (L)	4.0 ± 2.10a (*n* = 2)	3.4 ± 0.98a (*n* = 2)	83.6 ± 39.3a
'ANTA 82' (SE)	2.0 ± 2.10a (*n* = 2)	6.0 ± 1.56a (*n* = 2)	224.2 ± 124.7a
'TMG 132' (SE)	1.0 ± 1.71a (*n* = 3)	3.0 ± 1.27a (*n* = 3)	53.5 ± 30.2a
'IAC 17' (E)	4.0*	1.0	0.33
'Coodetec 208' (E)	2.0*	8.0	335.5
'IAC 19' (L)	-- [d]	--	--
PI 274453 (L)	--	--	--
PI 227687 (SE)	--	--	--
PI 229358 (E)	--	--	--
PI 171451 (E)	--	--	--
PI 274454 (L)	--	--	--

[a] E = early; SE = semi-early; L = late.

[b]Means followed by the same lowercase letter per column do not differ significantly by LSD test ($p > 0.05$).

[c]*n* = number of evaluated insects.

*Means of one insect, data not included in statistical analysis

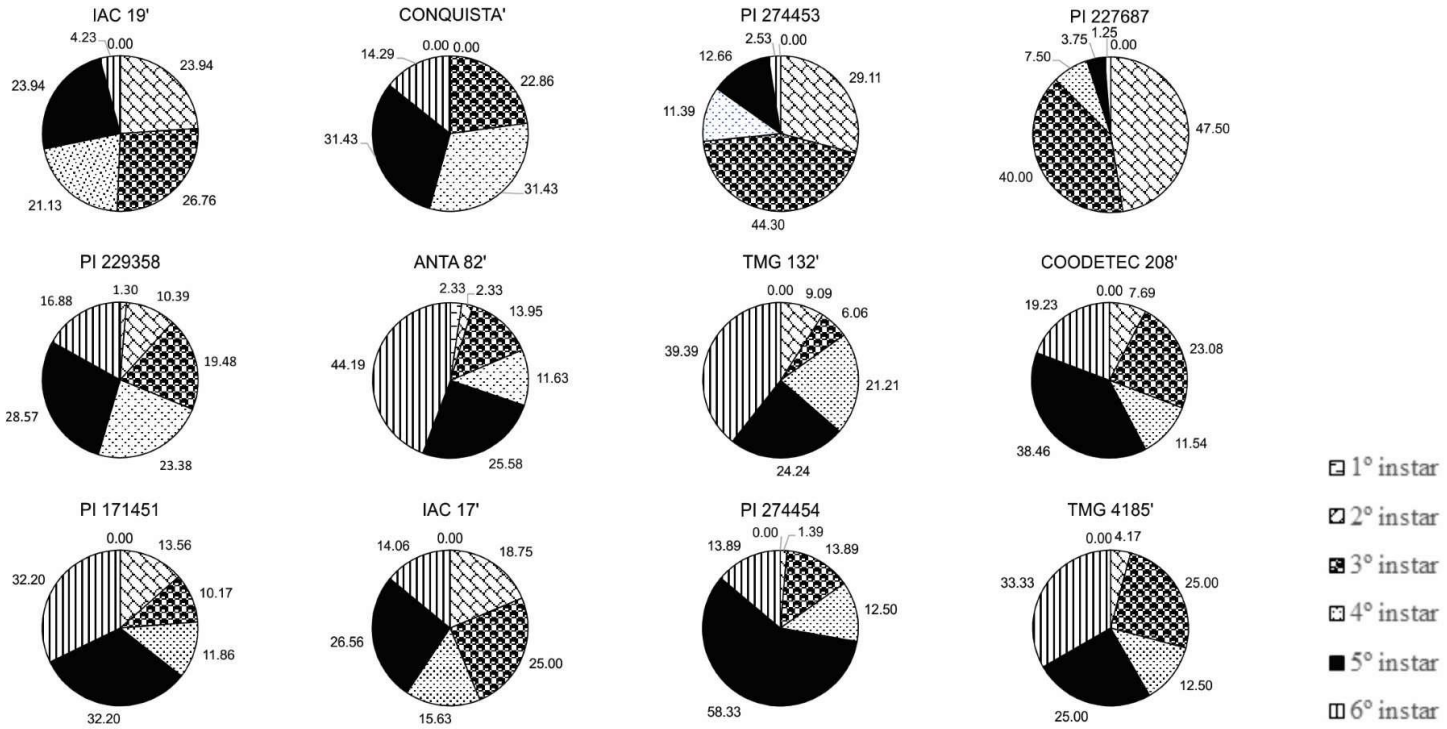

Fig 1. Mean larval mortality (%) of each instar of *H. armigera* in 12 soybean genotypes in the laboratory.

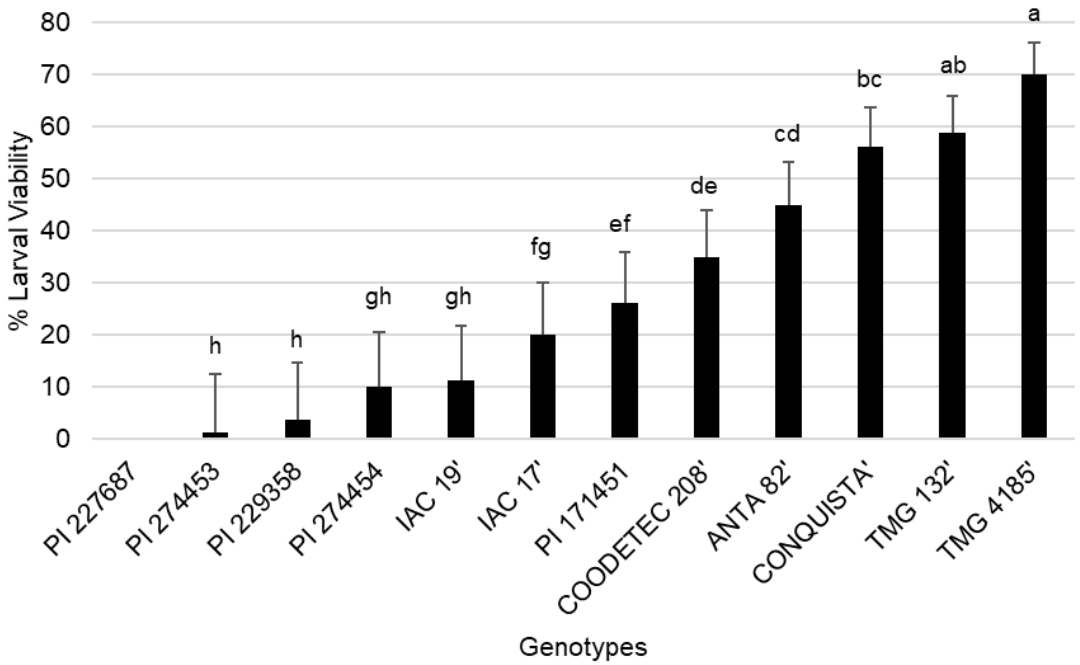

Fig 2. Mean (± SE) larval viability of *H. armigera* in 12 soybean genotypes in the laboratory. Means followed by the same lowercase letter per column do not differ significantly by LSD test ($p > 0.05$)

Discussion

Some soybean genotypes significantly affected several biological parameters of *H. armigera*, characterizing the expression of resistance by antibiosis and/or antixenosis at different levels (Smith 2005). In the screening bioassay test using pods, PI 171451, PI 229358, PI 227687, 'TMG 4185', PI 274453 and PI 274454 prevented the insects from developing into the pupal stage, causing 100% mortality of caterpillars and indicating expression of resistance. Although the mortality of initial phase of Lepidoptera is expected and it reaches around 40% in the embryonic phase and 50% in the first instar (Zalucki et al., 2002), the total mortality observed in the mentioned genotypes suggests the presence of antibiotic compounds or antixenosis in their pods. For genotypes in which the insects completed the immature phase, the low viability (< 20%) in 'TMG 132', 'IAC 24', 'TMG 4182', D75-10169, 'IAC 23', 'TMG 1179' and 'IAC

742832' can also be considered a strong indication for high levels of antibiosis and/or antixenosis.

As verified with pods, the leaves of PI 274453 also caused the mortality of all larvae. The same was observed with 'IAC 19'. This indicates a high level of resistance in these two genotypes and suggests that in PI 274453 this characteristic is more stable, since it manifests in the reproductive and vegetative structures of the plants. The genotypes PI 229358, IAC 782318 and PI 274454 caused the greatest prolongation of the larval phase (32.0 to 24.4 days), also indicating the expression of antibiosis and/or antixenosis. According to Painter (1951), prolongation of the immature phase may result from the intake of deleterious compounds, usually present in antibiotic bearing materials, and which inhibit the performance of the insect; however, genotypes with high levels of antioxenotic factors can also cause this effect, either by hampering the feeding of the insect or due to its nutritional improprieties. For the distinction between antibiosis and antixonosis resistance, it is suggesting the evaluation of the insect consumption (Baldin et al. 2019).

The low mean pupal weight (0.11 - 0.12 g) verified in IAC 78-2318, 'TMG 132', 'ANTA 82' and 'IAC 100' also suggest the occurrence of resistance. The lowest larval viability indexes (3.0-17.0%) verified in PI 274454, L1-1-01 and 'TMG 132' demonstrated high levels of antibiosis and/or antixonosis in these genotypes.

The average larval viability obtained from the individuals confined to pods (25.8%) was significantly lower than that observed from confinement in leaves (45.4%), suggesting that the expression of resistance is more effective in the reproductive structures of the genotypes. On the other hand, the necessity to periodically exchange food during the test with *H. armigera* larvae, including the removal of insects from the interior of the pods and their manipulation, may also have caused a considerable level of stress, increasing mortality rates. Therefore, in order to minimize the effect of non-plant related effects on immature mortality of the insect, it was decided to continue the subsequent phases of the study using only leaves as food substrate for *H. armigera*. We sought to select those genotypes with the greatest potential for resistance against the insect and greater availability of seeds representing the three maturation groups ('IAC 17', PI 171451, 'Coodetec 208', PI 229358, 'TMG 4185', 'TMG 132 ',' ANTA 82 ', PI

227687,' IAC 19 ', PI 274453 and PI 274454), besides the ‘Conquista’ genotype, considered a susceptible control in function of screening bioassay.

In the test with selected soybean genotypes, PI 227687 caused mortality of all larvae until to the sixth instar, whereas PI 229358 and PI 274454 were the ones that prolonged the larval period of the insect. These results were similar to the resistance expression verified with the genotypes in the preliminary test. Deleterious effects in the larval phase may be related to the presence of secondary compounds in soybean genotypes (Silva et al. 2005, Bentivenha et al. 2017), which can cause harmful consequences to the development insects. High mortality rates and prolongation of the life cycle characterize the expression of resistance by antibiosis and/or antixenosis (Lara 1991, Panda and Khush 1995, Smith 2005). In a similar study with *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae), genotypes PI 227687, PI 227682 and 'IAC 100' were attributed to antibiotic expression due to the prolongation that occurred in the larval phase of the insect when it was fed with leaves (Souza et al. 2014). In study involving the effect of cultivars and lineages of soybean leaves on the biology of *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae), it was verified that the pupae weight was significantly reduced in larvae that fed from 'Crockett' and ' IAC 17’ (Gazzoni and Tituda 1996). The lower weight of pupae may be associated with a low level of reserves acquired during the larval phase and may be related to the lower nutritional value of the food intake, a fact that may compromise the performance of the insect in the reproductive phase, reducing the number of copulations, oviposition, and fertility (Gazzoni and Tutida 1996). Also, with *A. gemmatalis*, another comparative study showed that 'IAC 100' and 'IAC 17' were the least suitable for development of the insect (Machado et al., 1999). In relation to *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) lower damage of caterpillars in genotypes PI 171451, PI 229358 and PI 227687 has been found, a fact attributed to the presence of antibiosis (Clark et al., 1972).

The lower larval viability verified in the genotypes PI 274453, PI 229358, PI 274454, PI 171451, 'IAC 19’ and ‘IAC 17' reinforces the presence of toxic compounds or high levels of antioxenotic factors, which makes the larval performance unfeasible (Smith 2005). The higher levels of mortality in the second and third instars observed in PI 227687, PI 274453 and 'IAC 19' also indicate that their adverse effects are more acute in comparison with the others genotypes

and promote rapid mortality of the individuals, as soon as they begin to feed. It is likely that joint expression of antixenosis and antibiosis will occur specifically for PI 227687, 'IAC 19' and PI 274454, since lower rates of consumption by larvae were found in these genotypes.

The genotype PI 227687 showed lower leaf intake in all instars, a common fact in the antixenosis. Materials carrying this category of resistance can produce phthalate compounds, which inhibit the feeding of the insect. These non-palatable compounds to insects might be responsible for the low consumption of these plants (Panda 1979, Panda and Khush 1995). Evaluated the consumption and growth rate of *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) caterpillars, fed with soybean leaves of PI 227687, researchers reported that caterpillars presented lower leaf intake, which affected their growth and increased their mortality. According to the authors, the low consumption in PI 227687 by caterpillars indicates the presence of deterrents or the lack of stimulant food compound, which decreases the feeding activity of caterpillars (Reynolds et al. 1984). With immature larvae of *S. eridania*, lower consumption of genotypes 'IAC 100', PI 227682, and PI 227687 was reported, a fact related to the presence of morphological and chemical factors in these genotypes (Souza et al., 2014). Fathipour and Naseri (2011) studied the effects of soybean cultivars on the development of *H. armigera* by evaluating nutritional indexes and physiology of digestive enzymes and verified that the genotypes 'L17' and 'Sahar' affected biological aspects of the insect, classified as antibiosis.

Here, the genotype PI 227687, known as a multiple resistance carrier against soybean insect pests (Kogan 1989, Lourenção et al 1989), promoted delay in the immature stage, besides causing 100% mortality of caterpillars. These results are similar to those found in study assessing the resistance of soybean genotypes to *Dichelops melacanthus* (Hemiptera: Pentatomidae), which reported that genotype PI 227687 did not allow nymphs to become adults (Canassa et al. 2017). Similar results were with *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidae), when caterpillars fed with leaves of PI 227687, not completed their larval stage (Boiça Junior et al. 2015). The possible reason for the low performance of the insect when confined to this genotype can be attributed to the chemical and/or morphological constitution of the material responsible for the manifestation of multiple resistance, which can be

characterized by high mortality, reduction of larval weight, and reduction of consumption (Boiça Junior et al., 2015). When analyzing leaf extracts of the genotypes 'IAC 100' and PI 227687 in the feeding of *A. gemmatalis*, Piubelli et al. (2005) identified and quantified the flavonoids rutin and genistin in materials, which play a role in protecting plants from pests.

The genotypes PI 229358, PI 274453 and PI 274454, considered resistant in this study, affect negatively the pest growth and development of the insect, besides high rates of mortality and prolongation of the cycle. These results are similar to those reported in research assessing biological aspects of *A. gemmatalis* in soybean genotypes, where it was observed that caterpillars fed with leaves of PI 229358, presented longer larval phase duration (Fugi et al. 2005). Studying the development and survival of *Piezodorus guildini* (Westwood) (Hemiptera: Pentatomidae) confined to pods of soybean genotypes, researches verified that PI 274453, PI 274454, and PI 229358 did not allow the emergence of adults (Bentivenha et al. 2017). When quantifying levels of genistin and rutin, the same authors observed high concentrations of the compounds in these genotypes, which could justify antibiosis resistance. The prolongation of the larval phase observed in the caterpillars fed with the PIs can be caused by the poor nutritional quality of the plant as well as by the presence of different substances that act in the plant defense routes against the herbivores (Tester 1977, Reynolds et al., 1984, Reynolds and Smith 1985, Hoffmann-Campo et al., 2001, Souza et al., 2014).

In relation to the weight of the fifth instar, the caterpillars fed in the genotypes PI 229358, 'IAC 19' and PI 227687 showed the lowest weights, confirming the resistance expression. Boiça Junior et al. (2015) verified lower weight of *S. cosmioides* caterpillars when fed with genotypes PI 227682, PI 227687 and 'IAC 100'. The caterpillars fed in PI 274453 and PI 229358 showed pupae with lower weights. Conversely, Fugi et al. (2005) evaluated the biological aspects of *A. gemmatalis* in leaves of genotypes PI 229358, 'IAC 17', 'IAC 24' and 'IAC PL-1' and did not find significant differences in pupal weight. However, the present results are similar to that by Beach and Todd (1988), who found lower pupal weight of *C. includens* when caterpillars were fed with leaves of genotype PI 229358. The lower weights of pupae obtained in caterpillars fed with these genotypes proves our suggestion of the lack of essential components for

nutrition or the presence of toxic compounds. The genotype PI 227687 did not allow the caterpillars to become pupae, which may be a consequence of the low consumption observed by these caterpillars in this genotype.

Differences between genotypes were observed when the pre-pupal phase (2.4 to 3.3 days) and pupal phase (12.0 to 14.1 days) were evaluated. Genotypes that provide the longest duration of the pupal stage usually have low nutritional quality and can influence of the development of the insect (Pereyra and Sanchez 2006). Regarding the pupal viability of *H. armigera*, ranging from 9.5 to 51.8%, the low index may be a consequence of nutritional improprieties of the resistant plants during the larval phase of the insect, which translate into deficiencies or quantitative and/or qualitative nutrient imbalances (Boiça Júnior et al., 2014). The caterpillars that were fed the genotypes 'IAC 19', PI 274453, PI 227687, PI 229358 and PI 274454 were unable to transform into pupae, and this is probably due to the presence of secondary compounds, or absence of feed stimulants (Piubelli et al., 2005, Lambert and Kilen 1984, Reynolds et al., 1984, Reynolds and Smith 1985, Hulburt et al., 2004).

Considering all the parameters evaluated and the deleterious effects on the biological performance of *H. armigera*, the expression of antibiosis in the genotypes PI 227687, PI 274454, PI 274453, PI 229358, 'IAC 19', 'IAC 17', and PI 171451 was characterized. Due to the low leaf intake by larvae in PI 227687, PI 274454 and 'IAC 19', the expression of antixenosis was also characterized in these genotypes. These genotypes are essential and should be present in soybean breeding programs because they have multiple resistance and, with the present results, are characterized as resistant to more than one pest, expanding the sources of resistance. Further investigations might be conducted to identify the mechanisms of resistance involved in these genotypes against *H. armigera*.

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CHAPTER 2

Impact of fruiting structure loss at various growth stages on soybean simulating corn earworm damage (Lepidoptera: Noctuidae)

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ABSTRACT

Soybean is considered one of the most valuable crops in the United States of America. Several insect pests are associated with soybeans, especially *Helicoverpa zea* Boddie. Artificial fruit removal is a method used to understand insect damage and to adjust action levels for control. Abiotic conditions such as moisture deficits can have adverse affects on yield, especially during the reproductive stages. The objective of this work was to evaluate five levels of fruit removal (0, 25, 50, 75 and 100%) at four stages (R2, R3, R4 and R5) and their impact on maturity and yield of soybean, in order to simulate the damage of *H. zea* in non-irrigated environments. There was a significant interaction between fruit removal timing and fruit removal level for percent of nonsenesced main stems and abscised leaves. For percent of nonsenesced main stems, at 100% fruit removal at the R2 growth stage there was a significantly higher percent of nonsenesced main stems and at 75% and 100% removal at R3 growth stage there were significantly less nonsenesced main stems. At 100% fruit removal at R2 growth stage, 25 and 50% removal at R4 growth stage, and 100% removal at R5 growth stage, soybean plants retained significantly more leaves. For soybean yield, there was no significant interaction between fruit removal timing and fruit removal level. There were no significant differences in soybean yields

at the R2, R3 and R4 growth stages. Plots that received fruit removal treatments at R5 significantly reduced soybean yield. The plots that received fruit removal of 100% significantly reduce yield. These data demonstrate that under adequate to optimal growing conditions recommendations for managing fruit feeding insects should be similar for non-irrigated soybean. However, these results may not apply to extremely late planted or severely moisture limited soybean.

Keywords: corn earworm, soybean, artificial removal

Introduction

Soybean [*Glycine max* (L.) Merrill] represents one of the world's main crops when considering the oil and protein content (Bellaloui et al. 2015). In terms of planted area and commodity value, soybean is considered one of the most valuable commoditeis in the Southern region of the United States of America (Adams et al. 2016). In 2017, the United States sowing area was more than 36 million hectares, production of about 120 million tons, and productivity of 3,299 kg/ha, making it the largest world producer of the grain. Brazil is considered the second largest producer with a production of more than 116 million tons, in a planted area of 35 million ha and productivity of 3,333 kg/ha in 2017 (USDA 2017, Conab 2018). Soybean is an important component in animal feed in the USA due to the high protein content present in the seed (Reisig et al. 2017). Most farmers in the state of Mississippi have adopted early soybean production, which includes early planting (beginning April to early May) and earlier maturing (maturity group IV and V) varieties with indeterminate growth habits (Heartherly and Spurlock 1999).

Several insect pests are associated with soybean which can feed at different stages of development and cause damage to leaves, stem, roots, and pods (Kogan 1981, Formentini et al. 2015, Catchot et al. 2018). *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), also known as "New World bollworm", is an important insect pest, being able to damage several crops of economic interest (Fitt 1989, Leite et al. 2014, Barbosa et al. 2016, Olmstead et al. 2016). Populations of *H. zea* are restricted to the American continent, distributed between the North and the South of the continent (Hardwick, 1965). Larvae of *H. zea* are polyphagous, having as hosts various cultivated plants such as cotton (*Gossypium hirsutum* L.), tomato (*Solanum*

lycopersicum L.) and soybean (*Glycine max* L.), however the preferred host for its development is maize (*Zea mays* L.) (Hardwick 1965, Fitt 1989, Johnson et al. 1975, Swenson et al. 2013, Olmstead et al. 2016).

Although soybean are not the main host, the damage caused by *H. zea* in the crop has increased over time, especially in crops in the Southern region of the United States (Kogan 1979, Musser et al. 2017, 2018, Kogan and Turnipseed 1987, Swenson et al. 2013, Adams et al. 2016). In relation to losses in yield and cost of control, *H. zea* has been considered one of the most economically important pest for soybean producing regions of the Southern U.S. (Musser et al. 2018, Adams et al. 2016). In 2016, damage caused by *H. zea* resulted in a loss of approxametely U$ 108 million, in contrast to the year 2017, damages increased to reach more than U$ 160 million of expenses divided between economic losses and cost of control (Musser et al. 2018). One of the causes for increased cost of control of *H. zea* may be due to the increase in area planted to corn over the years, which resulted in an increase in the number of moths that migrated to soybean and cotton crops (Jackson et al. 2008, Adams et al. 2015).

Historically, in Mississippi, *H. zea* is a reproductive stage pest of soybean (Catchot et al. 2014, Adams et al. 2015). Once maize plants enter the senescence phase, the adults of *H. zea* migrate to soybean and begin to oviposit, causing considerable damage (Johnson et al. 1975, Swenson et al. 2013, Musser et al. 2015, Adams et al. 2016). Infestations occur during the reproductive phase of soybean, between stages R1 and R3, and the caterpillar feeding results in late filling of grains, damaged grains, reduction of seed number per pod and abscission of pods, resulting in loss of yield (Johnson et al. 1975, Eckel et al. 1992, Swenson et al. 2013).

The impact of caterpillar feeding on soybean yield depends on the size of the caterpillars, the plant development stage, the time of damage, and the ability of the plant to compensate for damage (Thomas et al. 1974, Swenson et al. 2013). Soybean plants have the ability to compensate for these damaged reproductive tissues (McPherson and Moss 1989, Timsina et al. 2007, Reising et al. 2017), through the production of more pods or seeds of greater weight of the remaining reproductive tissues (McPherson and Moss 1989). Artificial removal of pods in the reproductive stages of soybean has been used to understand the damage caused by *H. zea* and to adjust action levels for control (Rowder 1987, Timsina et al. 2007, Stacke et al. 2018).

Beyond the pest damage, abiotic conditions such as moisture deficits may reduce soybean yield (Purcell and Specht 2004, Pathan et al. 2014, Aydinsakir 2018). Soybean is a very sensitive crop to drought and yield is highly affected especially when deficiency occurs in the reproductive period (Cox and Jolliff 1986, Oya et al. 2004, Pathan et al. 2004, Karam et al. 2005). In general, due to the high cost of technology and the lack of water availability, most of the soybean producing regions of the U.S. are dependent on natural rainfall, but uneven rainfall distribution can lead to varied productivities in the same field (Oya et al. 2004, Pathan et al. 2014). In situations where early soybean production systems are utilized, and environmental and watering conditions are favorable, it can lead to higher yield potential. Soybean plants cultivated under conditions of lower yield potential may not respond to fruit loss in the same way as those grown under conditions of higher yield potential (Ashley and Ethridge 1978, Purcell and Specht 2004, Karam et al. 2005, Zhao et al. 2018). The objective of this work was to evaluate the impact of flower and pod removal on the maturation and yield of the soybean plant, in order to simulate the damage of *H. zea* in different reproductive stages of development under non-irrigated conditions that may limit yield potential.

Material and Methods

Experiments were conducted during 2016 and 2017 at the Mississippi State Univerity R. R. Foil Plant and Soil Sciences Farm, Starkville, MS and during 2017 at Delta Research and Extension Center. During 2016 and 2017 experiments were conducted at the Delta Research and Extension Center on a Bosket very fine sandy loam soil (fine-loamy, mixed, active, thermic Mollic Hapludalfs) and a Tunica clay soil (clayey over loamy, smectitic over mixed, superactive, nonacid, thermic Vertic Epiaquepts). Methods were similar to Adams et al. (2015) with some modifications. The variety used belong to the maturation group (MG) IV (Asgrow 4632, Monsanto Company, St. Louis, MO). Soybeans were planted into raised conventional tilled beds with a 0.97 m row spacing in Starkville (305,915 seeds per ha) and 1.02 m row spacing in Stoneville (290,619 seeds per ha). Soybeans were planted in Stoneville, MS, on 5 April 2016 for silt loam trial and on 8 April 2016 for clay trial. During 2017, soybeans were planted in Stoneville on 2 May 2017 for silt loam and clay trial. Plot size during 2016 was 4 rows by 1.5 m and 4 rows by 3.05 m during 2017.

Treatments were imposed on the center 2 rows of each plot. Seed were treated with a comercial premix of imidacloprid, pyraclostrobin, metalaxyl and fluxapyroxad (Acceleron, Monsanto Company, St. Louis, MO) to minimize the impact of early season insect pests and seedlings disease. Weed and disease pests were managed according to Mississippi State University Extension Service recommendations. Additionaly, all experiments were conducted under non-irrigated conditions.

Insect pest populations were monitored weekly and insecticide were applied when published thresholds were reached to minimize the confounding insect damage. The experimental design was a randomized complete block with a complete factorial arrangement of treatments with four replications. Factor A was considered the development phase of the plant (reproductive growth stage) at the time of the removal of the flowers and pods (removal timing) and included the growth stages of R2, R3, R4 and R5 according to Fehr and Caviness (1977). Factor B, was the percentage of flowers and pods (0, 25, 50, 75 or 100%) removed at each of the previously mentioned growth stage. Once the soybean plots reached the determined development stage (R2, R3, R4 or R5), the artificial removal of the flowers and pods was performed by hand according to predetermined percentages. Plots were established for each growth stage – fruit removal level combination. During 2016 season soybean growth between the R3 and R5 growth stages was rapid and the R4 fruit removal timing was not included in the experiment.

Twenty five percent flower/pod removal was accomplished by removing one flower or pod and leaving the next three; 50% removal was accomplished by removing a flower or pod and leaving the next flower or pod, for 75% removal three flowers or pods out of every four were removed, and for 100% removal all flowers or pods on the plant were removed. Fruit removal treatments were initiated at the lowest node on the plant and proceeded to the terminal.

The impact of loss of flowers or pods on plant maturity was determined by visually estimating the percentage of abscised leaves in each plot at 141 days after planting when the untreated plot (no fruit removal) were 15 days from harvest during 2017, in Starkville and 136 days after planting in Stoneville. The percentage of non-senesced mainstems (mainstems that remained green) in each plot was determined at 161 and 159 DAP for the silt loam and clay soil experiments at Stoneville during 2016 and at 139 DAP for 2017, and at 150 DAP in Starkville during 2017. The total number of non-senesced main stems and the total number of plants on the center 2

rows of each plot were counted. These data were used to determine the percentage of non-senesced mainstems. Once plots had reached maturity and a harvestable moisture, each plot was machine-harvested with a Kincaid 8XP plot combined with weigh system and seed weights and moisture were determined. Seed yields were corrected to 13% moisture and converted to kg / ha.

Statistical analysis

All experiments were conducted in a rondomized complete block design with a factorial arrangement of treatements. Crop maturity and yield data were analyzed with generalized linear mixed model analysis of variance. Fruit removal timing, fruit removal level, and the interaction between removal timing and level of fruit removal were considered fixed effects. Control plots for each growth stage were identical (no fruiting structure removal), therefore data for the non-damaged plots were pooled by replication in an analysis for a randomized complete block design with incomplete factorial treatment arrangement. Siteyear and rep and siteyear nested in were designated as random effects (Blouin et al. 2011). Years and locations were considered environmental or random effects; this allowed inferences to be made over a range of environments (Carmer et al. 1989, Blouin et al. 2011). Fisher's protected LSD test ($P \leq 0.05$) was used for mean comparison. All the analyses were performed using the statistical software PROC GLIMMIX SAS (Institute Inc. 2011, Cary, NC).

Results

A significant interaction between fruit removal plant stage and fruit removal level was not observed for abscission of leaves (F= 3.61; df= 9; 168; P= 0.0004). Fruit removal below 100% at the R2 growth stage did not significantly impact natural leaf abscission compared with the nontreated plots. Whereas plots that received 100% fruit removal retained significantly more leaves (50.8 ± 6.2) than plots that incurred 25%, 50% and 75% of fruits removal at R2 (Table 1). There were no significant differences in natural leaf senescence and abscission between the nontreated control plots and plots that received fruit removal treatments at R3 growth stage. Plots that received fruit removal of 75% and 100% at R4 growth stage did not significantly impact natural leaf abscission compared with the nontreated plots. On

the other hand, removal of 25% and 50% of fruiting structures retained significantly more leaves (66.2 ± 6.7 and 66.6 ± 5.3, respectively) than the nontreated control plots. At R5 growth stage, there were no significant differences in natural leaf abscission between the nontreated control plots and plots that received fruit removal below 100%. Plots that received fruit removal of 100% retained significantly more leaves (65.0 ± 7.9) than the nontreated control plots (Table 1).

Table 1 Effect of fruit removal plant stage on mean percent of normally abscised leaves at during 2017

Growth stage	Removal level (%)	Mean percent abscised leaves (SEM)
Nontreated control	0	79.1 (1.5)a
R2	25	72.0 (1.9)abc
	50	78.7 (1.9)a
	75	77.0 (2.2)ab
	100	50.8 (6.2)d
R3	25	74.2 (5.8)abc
	50	77.9 (3.9)ab
	75	77.0 (2.8)ab
	100	80.0 (1.7)a
R4	25	66.2 (6.7) bc
	50	66.6 (5.3)bc
	75	70.0 (5.8)abc
	100	75.0 (4.1)abc
R5	25	80.0 (2.8)a
	50	77.9 (3.4)ab
	75	71.2 (4.8)abc
	100	65.0 (7.9)c

Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD test (α=0.05).

Results similar to those for percent of abscission of leaves was observed for natural senescence as measured by nonsenesced main stems. A significant interaction between fruit removal timing and fruit removal level was observed for percent of non-senesced main stems (F= 3.04; df= 9; 254.5; P= 0.0018). Percent of

nonsenesced main stems ranged from 70.2 (± 8.1) to 31.2 (± 5.7) %. Fruit removal up to 100% at the R2 growth stage did not significantly impact the percent of nonsenesced main stems compared with the nontreated plots. Whereas the removal of 100% of fruit resulted in significantly higher percent of nonsenesced main stems (70.2 ± 8.1) compared with all other fruit removal level and timing treatments (Table 2). At R3 through R5 growth stages no fruit removal levels impacted percent of nonsenesced main stems (Table 2).

Table 2 Effect of the interaction of fruit removal plant stage and fruit removal level on percent of nonsenesced main stems during 2016 and 2017

Growth stage	Removal level (%)	Mean percent nonsenesced main stems (SEM)
Nontreated control	-	46.8 (3.9) bcd
R2	25	48.5 (6.3) bc
	50	40.1 (7.1) bcd
	75	47.5 (6.7) bcd
	100	70.2 (8.1) a
R3	25	48.2 (8.2) bc
	50	47.6 (7.6) bc
	75	36.0 (6.7) cd
	100	31.3 (5.7) d
R4	25	45.6 (11.3) bcd
	50	46.8 (8.7) bcd
	75	55.9 (11.0) ab
	100	55.1 (7.1) ab
R5	25	42.9 (7.1) bcd
	50	41.4 (8.3) bcd
	75	45.4 (8.8) bcd
	100	53.9 (8.6) b

Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD test (α=0.05).

There was no significant interaction observed between fruit removal timing and fruit removal level for soybean yield (F = 0.88 df = 9; 250.6 P = 0.55). There were significant main effects of fruit removal timing (F = 5.13; df = 3; 63.39; P = <0.01) and

fruit removal level (F = 12.74; df = 3; 250.4; $P \leq 0.01$) for soybeans yield. There were no significant differences at soybean yields between the nontreated control plots and plots that received fruit removal treatments at the R2, R3 and R4 growth stages. Plots that received fruit removal treatments at R5 significantly reduced soybean yield compared with the nontreated control (Table 3).

Table 3 Effect of the fruit removal plant stage on soybean yield during 2016 and 2017 seasons

Growth stage	Mean yield in kg/ha (SEM)
Nontreated control	4227.5 (103.5) a
R2	3989.9 (105.4) a
R3	4035.1 (108.7) a
R4	3976.9 (99.5) a
R5	3644.4 (120.4) b

Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD test (α=0.05).

Fruit removal of 25% did no result in significant differences when compared with the nontreated plots (Table 4). The plots that received fruit removal of 50%, 75% and 100% had significantly lower yield when compared to the control, with 100% fruit removal resulting in the greatest yield reduction. Rainfall during late July through late August during both years at Stoneville was substantially higher than the 10-year average (Figure 1) and rainfall during the end of July and begining of August during 2017 at Starkville was higher than the other months but lower when compared with Stoneville during the same year (Figure 2). Although these studies did not receive irrigation, adequate moisture was available, and plants were likely able to compensate for fruit loss.

Table 4 Effect of fruit removal level on soybean yield during 2016 and 2017 seasons

Removal level (%)	Mean yield in kg/ha (SEM)
Nontreated control	4227.6 (103.5) a
25	4059.9 (99.5) ab
50	4008.9 (101.5) b
75	4010.6 (111.7) b
100	3566.9 (133.8) c

Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD test (α=0.05).

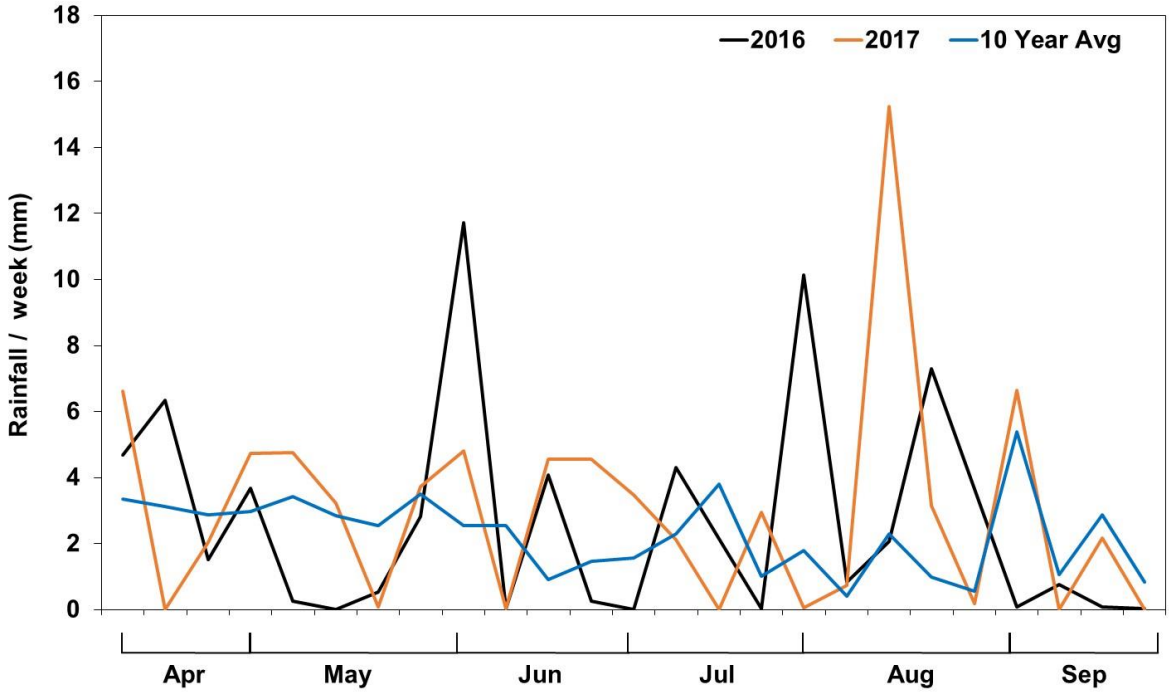

Fig. 1 Weekly rainfall totals from 1 Apr to 30 Sep 2016 and 2017, and 10 year average rainfall for Stoneville, MS

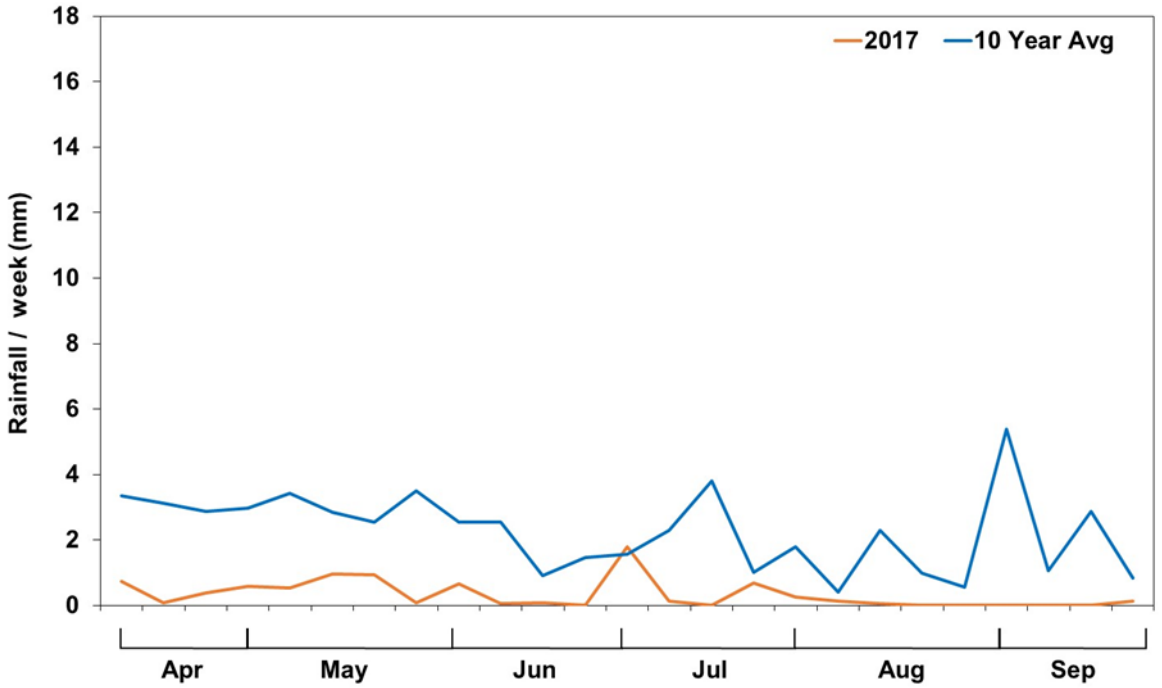

Fig. 2 Weekly rainfall totals from 5 May to 15 Nov 2017 average rainfall for Starkville, MS

Discussion

The timing of fruit initiation is important in soybean development. Any physiological stress during this period can have a impact on the final yield of the seeds, since the growth rate of the fruits affects the senescence time and the death of the plant (Meyer et al. 1981). In the current experiment, the interaction of fruit removal timing and fruit removal level on percent of nonsenesced main stems and on percent of normally abscised leaves was observed. When grain has reached a harvestable moisture content, the presence of nonsenesced leaves and main stems becomes a problem for farmers (Adams et al. 2015), which can potentially reduce yield and increase seed loss. A decline in photosynthesis and the loss of leaf protein characterize the senescence of soybean leaves. The mainly visual symptom of leaf yellowing is widely used as an index of plant and leaf senescence (Mondal et al. 1978, Wittenbach et al. 1980, Wittenbach 1982). The maturation of soybean and abscission of leaves are a natural process. Late planting of cultivars may lead to failures in natural senescence and retention of green leaves in response to stress to adverse conditions (Egli and Bruening 2006). The removal of 100% of the flowers and pods at the R2 growth stage, 75% and 100% at R3 growth stage and 100%

removal at R4 growth stage caused a higher percentage of green stems. McAlister and Krober (1958) when assessing the response of soybean cultivars to the removal of leaves and pods, they observed that plants with 40% and 80% of removal had lower percentages of maturation when compared to untreated plots. Delay in plant maturity allows some stressed plants to compensate for fruit loss (McPherson and Moss 1989). Hicks and Pendleton (1969) when evaluating the effect of floral bud removal on performance in indeterminate 'Wayne' soybeans, observed that plants that had 0 to 60 floral buds removed per plant at random over all nodes showed failures in the senescence of vegetative parts of soybean. The authors also report that the protein content increased, and the oil content decreased with increasing number of flower buds removed. Tayo (1977) when determinate the effects of different levels of fruit removal on soybean performance, concluded that the fruit removal led to lower accumulation of dry matter, higher leaf area and late maturation of plants as percentage of removal increased. The author also reports that the number of mature pods per plant was lower when all fruit were removed after three weeks of development. In an attempt to compensate for damage, plants may produce new flowers and pods within a short time, depending on the level of stress, leading to delayed maturity (McPherson and Moss 1989).

Depending on the level, fruit removal may affect yield by reducing the weight of the soybean seed (Hicks and Pendleton 1969, Tayo 1977). In this experiment, there was no significant interaction observed between fruit removal timing and fruit removal level for soybeans yield. When the removal levels were applied at the R5 growth stage, yield was significantly reduced relative to the control. Rocha et al. (1996) evaluated the effect of two levels of fruit removal (50% and 100%) at four growth stages (R4, R5, R5,5 and R6), and reported that both levels of fruit removal reduced the number of pods per plant compared to the control, being more pronounced when the pods were removed in the R5 and R5.5 stages. Adams et al. (2015) evaluated the impact of two levels of fruit removal (50% and 100%) on different stages of soybean development and they reported a significant reduction in yield and crop value when both removal levels were applied at the R5.5 stage of plant development, when compared to untreated plots. Evaluating the ability of soybeans to tolerate five pod removal levels (0, 10, 20, 30, 40 and 80%) at different growth stages of soybean, Smith and Bass (1972) found that when 80% of pods were removed at R5 stage, yield was significantly lower when compared to the

untreated plots. The 50%, 75% and 100% fruiting structure removal, regardless of growth stage, resulted in a mean yield reduction when compared to the control treatment. Rocha et al. (1996) reported that 100% pod removal at R6 did not allow the plants to compensate for damage, resulting in fewer pods per plant. Thomas et al. (1974) evaluated the influence of different levels of depodding on yield of soybeans; the authors observed that yield was reduced when high levels of removal (100%) were applied at the R5 growth stage. When levels of pod removal occurred in the early stages of development (R2, R3 and R4), yields were statistically equal to the control treatment, demonstrating the ability of plants to compensate damage when it occurs in the early growth stages. McAlister and Krober (1958) evaluated the response of 10, 20, 30, 40 and 80% removal pod of Hawkeye and Lincoln soybeans and reported that, during the initial reproductive stages soybean, produced almost the same number of pods when compared to plants without treatment. This may have occurred because soybean plants usually lose 30 to 85% of flower buds and this compensation of fruit loss occurs during the early reproductive stages without causing any delay in plant maturation (Swen 1933, Van Shaik and Probst 1958, Adams et al. 2015). Delayed leaf senescence is a compensatory response of the plant when damaged by an insect. When unfavorable conditions exist, soybean plants have the opportunity to recover from fruit or tissue loss, and this may influence yield recovery (Pedigo et al. 1986; Higley 1992, Peterson and Higley 1996, Haile et al. 1998). These data demonstrate that soybeans may be able to compensate for even severe levels of fruit loss early during the reproductive portion of the growing season if favorable growing conditions occur, such as adequate moisture. Adequate moisture is an important factor and can directly influence plant response to compensate for fruit loss. If average or below average rainfall had occurred during July and August, it is possible that more extreme results would have been observed. The data also illustrate that soybean is more sensitive to *H. zea* damage during the R5 growth stage when compared to earlier reproductive growth stages. Finally, these data demonstrate that under adequate to optimal growing conditions recommendations for managing fruit feeding insects should be similar for non-irrigated soybean. Also, soybean planted before and at the end of the optimal planting period responded similarly to fruit loss as soybean planted at an optimum time in other studies. However, these results may not apply to extremely late planted soybean.

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FINAL CONSIDERATIONS

The species *Helicoverpa zea* and *Helicoverpa armigera* are important insect-pest for significant crops in Brazil and in other parts of the world. In our country, *H. zea* presents great destructive capacity, mainly in corn crops. In the United States, this species has been causing great concern in the recent years, responsible for damage soybean crops, resulting in a great economic loss. Regarding *H. amigera*, until 2013, the species was considered a quarantine pest in Brazil. As soon as it was identified in our territory, showed a high destructive ability causing severe damage in the agricultural crops. Although there are still occasional reports of its presence on the North American continent, this species also presents great potential to colonize the agricultural crops of the United States in a short time.

Considering characteristic aspects of the species, such as polyphagous habit, high dispersibility, associated to the vast agricultural frontier and food supply in the fields of Brazil and the United States, additional information on alternative control methods and greater knowledge on the response of plant in the face of insect infestation, allow to recommend genotypes with higher levels of resistance to insect attack, as well as to define the best moment of control, reducing the unnecessary applications of synthetic insecticides. Knowledge of the relationship between pest infestation and crop yield is an important issue for the establishment of integrated management programs. The artificial removal of structures (fruit or vegetative) allows to estimate what levels of insect infestation the plants can compensate their damages, without the necessity to enter with a tactical control. In addition, the resistance of plants can also help to reduce the population level of the pest, besides being compatible with other control measures, collaborating to reduce insect infestation in the field.

The data obtained in the preliminary test of Chapter 1 with 22 soybean genotypes allowed the selection of materials with higher resistance potential on *H. armigera* (‘IAC 17', PI 171451, PI 229358,'TMG 4185','TMG 132','ANTA 82', PI 227687,'IAC 19', PI 274453 and PI 274454), which were again compared to susceptible commercial genotypes ('Coodetec 208' and 'Conquista'). After the evaluation of new biological parameters, the genotypes PI 227687, PI 274454, PI 274453, PI 229358, 'IAC 19', 'IAC 17', and PI 171451 confirmed to be the most

resistant, expressing antibiosis and / or antixenosis as resistance categories opposite *H. armigera*.

For the second study, when 100% of fruit (flowers and/or pods) were removed in the growth stage R2, 25% and 50% removal of the R4 growth stage and 100% removal of the R5 growth stage, the plants retained more leaves (lower percentage of normally abscised leaves). Regarding the percentage of nonsenesced main stems, when 100% of fruit (flowers and/or pods) were removed in the growth stage R2, it was resulted in the highest percentage of nonsenesced main stems. In relation to soybean yield, no interaction between the fruit removal level and growth stage was observed. No significant differences were observed for soybean yield when the removal occurred in the R2, R3 and R4 growth stage. Plots that received fruit removal at R5 and 50, 75 and 100% fruit removal significantly reduced productivity.

CONCLUSIONS

- The genotypes PI 227687, PI 274454, PI 274453, PI 229358, 'IAC 19', 'IAC 17' and PI 171451 expressed antibiosis with higher level in the first four genotypes.
- Due to the low leaf intake by larvae in PI 227687, PI 274454 and 'IAC 19', the expression of antixenosis is also characterized in these materials.

- When the fruit removal level occurred in the early reproductive growth stages, the plants are able to compensate the damages.
- Soybean is more sensitive flower and pod structure loss during the R5 growth stage when compared to early growth stage.

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