UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”

FACULDADE DE CIÊNCIAS AGRONÔMICAS

CAMPUS DE BOTUCATU

ESTUDOS DE DISPERSÃO DE LAGARTAS EM MILHO E SOJA VISANDO AO MANEJO DE PRAGAS NO BRASIL E EUA

LUIZ EDUARDO DA ROCHA PANNUTI

Tese apresentadaàFaculdade de Ciências AgronômicasdaUNESP – Campus de Botucatu, para obtençãodotítulodeDoutorem Agronomia(Proteção de Plantas)

BOTUCATU – SP

Julho 2015

UNIVERSIDADE ESTADUAL PAULISTA ―JÚLIO DE MESQUITA FILHO”

FACULDADE DE CIÊNCIAS AGRONÔMICAS

CAMPUS DE BOTUCATU

ESTUDOS DE DISPERSÃO DE LAGARTAS EM MILHO E SOJA VISANDO AO MANEJO DE PRAGAS NO BRASIL E EUA

LUIZ EDUARDO DA ROCHA PANNUTI

Orientador: Prof. Dr. Edson Luiz Lopes Baldin

Coorientador: Prof. Dr. Thomas Elliot Hunt

Coorientadora: Dr. Silvana Vieira de Paula-Moraes

Tese apresentadaàFaculdade de Ciências Agronômicasda UNESP – Campus de Botucatu, para obtenção do títulodeDoutorem Agronomia (Proteção de Plantas)

BOTUCATU – SP

Julho 2015

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉCNICA DE AQUISIÇÃO E TRATAMENTO DA INFORMAÇÃO – SERVIÇO TÉCNICO DE BIBLIOTECA E DOCUMENTAÇÃO - UNESP – FCA – LAGEADO- BOTUCATU (SP)

Pannuti, Luiz Eduardo da Rocha, 1985-
P194e Estudos de dispersão de lagartas em milho e soja visando ao manejo de pragas no Brasil e EUA = Studies of larval dispersion in corn and soybean aiming pest manage ment in Brazil and the U.S. / Luiz Eduardo da Rocha Pannuti. – Botucatu : [s.n.], 2015
xi, 112 f. : grafs., tabs.

Tese (Doutorado) - Universidade Estadual Paulista, Faculdade de Ciências Agronômicas, Botucatu, 2015
Orientador: Edson Luiz Lopes Baldin
Coorientador: Thomas Elliot Hunt
Coorientador: Silvana Vieira de Paula-Moraes
Inclui bibliografia

1. Milho – Doenças e Pragas. 2. Soja – Doenças e pragas. 3. Lagarta. 4. Pragas agrícolas - Controle integrado. 4. Relação inseto-planta. I. Baldin, Edson Luiz Lopes. II. Hunt, Thomas Elliot. III. Paula-Moraes, Silvana Vieira de. IV. Universidade Estadual Paulista “Júlio de Mesquita Filho” (Campus de Botucatu). Faculdade de Ciências Agronômicas de Botucatu. V. Título.

SÃO PAULO STATE UNIVERSITY “JÚLIO DE MESQUITA FILHO”

COLLEGE OF AGRONOMIC SCIENCES

CAMPUS OF BOTUCATU

STUDIES OF LARVAL DISPERSION IN CORN AND SOYBEAN AIMING PEST MANAGEMENT IN BRAZIL AND THE U.S.

LUIZ EDUARDO DA ROCHA PANNUTI

Advisor: Prof. Dr. Edson Luiz Lopes Baldin

Co-advisor: Prof. Dr. Thomas Elliot Hunt

Co-advisor: Dr. Silvana Vieira de Paula-Moraes

Dissertation presented to College of Agronomic Sciences from UNESP – Campus of Botucatu, to obtain the degree of Doctor in Agronomy (Crop Protection)

BOTUCATU – SP

July 2015

unesp **UNIVERSIDADE ESTADUAL PAULISTA**
CAMPUS DE BOTUCATU
FACULDADE DE CIENCIAS AGRONOMICAS DE BOTUCATU

CERTIFICADO DE APROVAÇÃO

TÍTULO: "ESTUDOS DE DISPERSÃO DE LAGARTAS EM MILHO E SOJA VISANDO AO MANEJO DE PRAGAS NO BRASIL E EUA"

AUTOR: LUIZ EDUARDO DA ROCHA PANNUTI
ORIENTADOR: Prof. Dr. EDSON LUIZ LOPES BALDIN
CO-ORIENTADOR: Prof. Dr. THOMAS ELLIOTT HUNT
CO-ORIENTADORA: Profa. Dra. SILVANA VIEIRA DE PAULA-MORAES

Aprovado como parte das exigências para obtenção do Título de DOUTOR EM AGRONOMIA (PROTEÇÃO DE PLANTAS), pela Comissão Examinadora:

Prof. Dr. EDSON LUIZ LOPES BALDIN
Dep de Proteção Vegetal / Faculdade de Ciencias Agronomicas de Botucatu

Prof. Dr. CARLOS FREDERICO WILCKEN
Dep de Proteção Vegetal / Faculdade de Ciencias Agronomicas de Botucatu

Prof. Dr. JOSÉ DJAIR VENDRAMIM
Departamento de Entomologia, Fitopatologia e Zoologia / Escola Superior de Agricultura "Luiz de Queiroz" - Usp

Prof. Dr. EFRAIN DE SANTANA SOUZA
Faculdade de Ciências Sociais e Agrárias de Itapeva

Prof. Dr. ARLINDO LEAL BOICA JUNIOR
Departamento de Fitossanidade / Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal

Data da realização: 08 de julho de 2015.

"I offer this dissertation to my family due to their support, companionship, and affection all long."

I dedicate

To my parents Mário Flávio Pannuti and Regina Célia da Rocha Pannuti,
for the encouragement, confidence, and unconditional love.

To my siblings Márcia Ione da Rocha Pannuti and Fernando da Rocha Pannuti,
for the companionship and friendship in life.

ACKNOWLEDGEMENTS

To God for giving me the gift of life.

To Professor Dr. Edson Luiz Lopes Baldin for his guidance, support, confidence, and friendship above all.

To Dr. Silvana Vieira de Paula-Moraes for her support, teachings, and full participation in my dissertation.

To Professor Dr. Thomas Elliot Hunt for the opportunity and confidence to have received me at the University of Nebraska, NE(USA). I think this period was a turning-point in my professional life.

To the College of Agronomic Sciences Campus of Botucatu for all the opportunities for improving myself professionally.

To all professors and employees from the Plant Protection Program and Department of Crop Protection (UNESP - Botucatu). I amindebted to Dr. Carlos Gilberto Raetano for supporting me as the Program representative in the last months of my dissertation.

To the University of Nebraska - Lincoln, Northeast Research and Extension Center Haskell Agricultural Laboratory - Concord, NE (USA),and all professors for the opportunity and support to develop part of my research, providing me excellent research facilities.

To the Coordination for the Improvement of Higher Level -or Education-Personnel (CAPES) for the financial support of my doctoral program and grant to study in the USA.

To my girlfriend Ana Paula Rodrigues de Andrade for her companionship and cumplicity, sharing all the good and bad moments during this period of time.

To all my friends from LARESPI for helping me in the development of my dissertation and moments of diversion.

To my friends from ZA fraternity and all others who helped me on this journey somehow.

To all those who directly or indireclty contributed to accomplish this work, my sincere thanks.

TABLES OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

ESTUDOS DE DISPERSÃO DE LAGARTAS EM MILHO E SOJA VISANDO AO MANEJO DE PRAGAS NO BRASIL E EUA. Botucatu, 2015. 112p. Tese (Doutorado em Agronomia/Proteção de Plantas) – Faculdade de Ciências Agronômicas. Universidade Estadual Paulista (UNESP).

Autor: LUIZ EDUARDO DA ROCHA PANNUTI

Orientador: EDSON LUIZ LOPES BALDIN

Co-Orientador: THOMAS ELLIOT HUNT

Co-Orientador: SILVANA VIEIRA DE PAULA-MORAES

2. RESUMO

O milho (*Zea mays* L.) é uma das culturas mais cultivadas em todo o mundo. No entanto, sua produtividade está constantemente ameaçada por diversos fatores como o ataque de insetos-praga. Entre eles, se destacam os insetos da família Noctuidae, como *Spodoptera frugiperda*(J.E. Smith) (Lepidoptera: Noctuidae), *Striacosta albicosta*(Smith) (Lepidoptera: Noctuidae) e *Helicoverpa armigera*(Hubner) (Lepidoptera: Noctuidae). Alterações no sistema de cultivo como plantios safrinha e sucessão de culturas hospedeiras, aliadas à ampla utilização de milho transgênico *Bt* vêm demandando maior informações relacionadas a diversos aspectos comportamentais desses insetos, os quais podem influenciar diretamente importantes componentes na implementação de um manejo integrado (MIP) e manejo de resistência (MRI) dessas pragas. Alguns desses estudosnão são facilmente estudadosem condições de campo,assim, exigindo a utilização de técnicas de marcação de indivíduos. Nesse sentido, o trabalho teve como objetivo avaliar o movimento larval de espécies de noctuídeos na planta e entre plantas de milho, bem como desenvolver técnicas de marcação de lagartas de ínstares iniciais para sua aplicação em estudos comportamentais desses insetos. Inicialmente, foi investigado o movimento larval de *S. frugiperda* na planta de milho em condições de campo (EUA e Brasil) e casa de vegetação (Brasil), bem como o comportamento alimentar da lagarta em estágios reprodutivos de milho em laboratório. Em outro estudo, foi caracterizada a dispersão de lagartas de *S. albicosta* e *S. frugiperda* em campos de milho (EUA). Por fim, foram investigadas novas técnicas de marcação de lagartas de ínstares iniciais de *H. armigera* e

suas aplicações em um estudo comportamental do inseto em casa de vegetação, usando plantas de soja como modelo. Os resultados demonstraram que lagartas de *S. frugiperda* preferem a região da espiga como sítio de alimentação. Essa escolha é feita por lagartas de primeiro ínstar e está relacionada com preferência alimentar. Folhas de milho não são adequadas para o desenvolvimento de ínstares iniciais de lagartas de *S. frugiperda*, no entanto, o estigma e os grãos de milho têm papel importante na sobrevivência e desenvolvimento das lagartasem estágios reprodutivos de milho. No segundo experimento, *S. albicosta* apresentou sobrevivência larval variável durante os anos, mas possibilitou avaliar a dispersão da planta infestada. Da mesma maneira, a baixa sobreviência larval de *S. frugiperda* em 2013 não comprometeu a avaliação de dispersão larval. Ambas as espécies se dispersam, em campos de milho, governadas por informações sensoriais não direcionadas e, também apresentam distribuição agregada e simétrica. No entanto, lagartas de *S. frugiperda* permanecem mais próximas do ponto de liberação do que *S. albicosta.*Essas espéciesapresentam considerável movimento entre plantas em milho. Esses resultados podem ajudar determinar, com maior acurácia, importantes componentes de um MIP como planos de amostragem e níveis de dano econômico, bem como influenciar na implementação de estratégias de refúgio e mistura de sementes em um MRI dessas pragas.Em laboratório, os corantes Sudan Azul ou Sudan Vermelho 7B podem ser incorporados em dieta aritifical de *H. armigera* a 200 ppm para obter insetos marcados sem efeitos deletérios na biologia e comportamento. Da mesma forma, corante vermelho de Luminous Powder na dieta a 600 ppm foi mais eficiente na obtenção de lagartas marcadas sem efeitos deletérios na sua biologia e comportamento. Esse é o primeiro relato do uso de Luminous Powder em dieta contendo o corante sobre aspectos biológicos de *H. armigera.* Lagartas criadas em dietas com Sudan Vermelho 7B a 200 ppm e Luminous Powder a 600 ppm, até segundo ínstar, não apresentaram traços visuais dos corantes em ínstares posteriores, quando transferidas para dieta normal. Ambos os corantes marcaram externamente lagartas de *H. armigera* com sucesso. A marcação externa durou um ínstar larval sem efeitos deletérios no inseto, na qual pode ser útil para estudos de curta duração. Em casa de vegetação, a marcação externa através do polvilhamento do corante vermelho de Luminous Powder permitiu verificar que lagartas de segundo ínstar de *H. armigera* não apresentam variação no movimento larval independentemente do período do dia (manhã, tarde e noite). Após algumas horas, a maioria das lagartas estabelece seus sítios de alimentação e permanece se alimentando em folhas de soja, não justificando aplicações noturnas de inseticidas para o controle do inseto.

Palavras-chave: movimento larval, dispersão larval, movimento entre plantas, *Striacosta albicosta*, *Spodoptera frugiperda*, *Helicoverpa armigera*, marcação de lagartas.

STUDIES OF LARVAL DISPERSION IN CORN AND SOYBEAN AIMING PEST MANAGEMENT IN BRAZIL AND THE U.S. Botucatu, 2015.112p.Dissertation (Degree of Doctor in Agronomy/Crop Protection) – College of Agronomic Sciences. São Paulo State University (UNESP).

Author: LUIZ EDUARDO DA ROCHA PANNUTI

Adviser: EDSON LUIZ LOPES BALDIN

Co-Adviser: THOMAS ELLIOT HUNT

Co-Adviser: SILVANA VIEIRA DE PAULA-MORAES

1. SUMMARY

Corn (*Zea mays* L.) is one of the most cultivated crops worldwide. However, its productivity is constantly threatened by several factors such as the attack of insect pests. Among them stand out the species from Noctuidae family, including the fall armyworm *Spodoptera frugiperda*(J.E. Smith) (Lepidoptera: Noctuidae), the western bean cutworm *Striacosta albicosta*(Smith) (Lepidoptera: Noctuidae), and the old world cotton bollworm *Helicoverpa armigera*(Hubner) (Lepidoptera: Noctuidae). Alterations in crop systems such as "off-season" plantations and succession of host crops, combined with the extensive use of transgenic *Bt* corn, have been demanding more information related to various behavioral aspects of these insects, which can directly influence important components in the implementation of integrated pest management (IPM) and resistance management (IRM) of these pests. Some of these behavioral aspectsare not easy to observe in field conditions, and require the use of marking techniques for individuals. In this sense, this work aimed to evaluate the larval movement of noctuidae species in reproductive corn stages, as well as develop marking techniques for early instars and test their application in behavioral studies of these insects. Initially, the on-plant larval movement of the fall armyworm was investigated in field (US and Brazil), and greenhouse conditions (Brazil), as well as the feeding behavior of the larvae on different corn tissues in laboratory. In another study, the dispersion of the western bean cutworm and fall armyworm larvae was characterized in corn fields (USA). In the last step, new marking techniques of early larval instars were investigated for*H. armigera*, and their application were tested in a behavioral study at greenhouse, using soybean plants as model. Overall results indicated that fall armyworm larvae prefer the ear zone as a feeding site regardless of the reproductive corn stage. The feeding site choice is done by first-instar larvae and is directly related to feeding preference. Corn leaves were not suitable for early instars development, and silk and kernel tissues played a positive role in survival and development of the fall armyworm larvae in reproductive stage corn. In the second study, larval survival of western bean cutworm presented high variety throughout the years, but it was possible to evaluate the dispersion from infested plant. Although fall armyworm larval survival was considered low in 2013, it did not compromise the larval dispersion evaluation. Western bean cutworm and fall armyworm larvae disperse governed by non-directional sensory information with aggregated and symmetrical distribution in field corn, but fall armyworm remained nearby the release point more than western bean cutworm. Both species present considerable plant-to-plant movement in corn. These results may help in determining more accurate important IPM components such as scouting and economic thresholds, as well as influence the implementation of refuge and seed mixture strategiesin IRM. In laboratory, Sudan Blue or Sudan Red 7B dyes can be incorporated in *H. armigera* artificial diet at 200 ppm to obtain marked insects without major effects on its biology or behavior. Likewise, Red Luminous Powder dye into diet at 600 ppm was more efficient in obtaining marked larvae without deleterious effects on its biology or behavior. This is the first report of the use of Luminous Powder dye diet on biological aspects of *H. armigera*. Larvae reared on Sudan Red 7B dye diet at 200 ppm and Luminous Powder Red dye diet at 600 ppm until second instar did not show visual dye traces at posterior instars when transferred to regular diet. Both dyes marked*H. armigera* externally with success by dusting procedure. The external marking lasted one larval instar without deleterious effects which can be useful for short-term studies. In greenhouse, the dusting marking with Luminous Powder red dye allowed verifying thatsecond-instar larvae of *H. armigera*do not show variation on larval movement regardless the period of the day (morning, afternoon and evening). After few hours, most of larvae establish their feeding site and remain feeding on soybean leaves, not justifying overnight insecticide sprayings to control the insect.

Keywords:larval movement, larval dispersal, plant-to-plant movement, *Striacosta albicosta*, *Spodoptera frugiperda*, *Helicoverpa armigera*, larval marking tools.

3. GENERAL INTRODUCTION

Corn (*Zea mays* L.) is the most important feed crop and food cultivated worldwide with a total global production of 988 million metric tons in 2014. The United States is the world's largest corn producer, with 36% of corn production globally. Brazil also have significant production and appear as the third (USDA, 2015). Until recently, many advances in breeding and management have allowed farmers increase in corn yields (ORT& LONG, 2014). On the other hand, this productivity is constantly threatened by several factors such as the attack of insect pests. Damages caused by major corn pests can cause significant losses depending on several factors such as the type of corn,cultivar, and time when the attack occurs (CRUZ& TURPIN, 1983).

Many arthropods are associated with corn production, from sowing to harvesting (HUANG, 2015). Among them, several Lepidoptera species deserve close attention due to their significant damages in corn, including some species from Noctuidae family, such as the fall armyworm *Spodoptera frugiperda*(J.E. Smith) (Lepidoptera: Noctuidae), the western bean cutworm *Striacosta albicosta*(Smith) (Lepidoptera: Noctuidae), and the old world cotton bollworm *Helicoverpa armigera*(Hubner) (Lepidoptera: Noctuidae).

Fall armyworm is considered a major pest in the Tropics, rangingfrom Argentina to North America (LUGINBILL, 1928;CLARK et al., 2007;

ADAMCZYK et al., 2008; FARIAS et al., 2008). This insect has already been reported infesting more than 80 plant species, including important agricultural crops such as cotton, soybean, and corn (LUGINBILL, 1928; POGUE, 2002; CAPINERA, 2008). In Brazil, the fall armywormis considered a key pest in corn causing serious economic damages (CRUZ& TURPIN, 1983; CRUZ et al., 1999; DIEZ-RODRIGUEZ& OMOTO, 2001; CARVALHO et al., 2013). In the United States, it has been described as an important yield-and-quality-limiting pest in Southern cornfields (BUNTIN et al., 2004; CHILCUTT et al., 2007; HARDKE et al., 2011).Fall armyworm larvae can cause damage to seedlings, vegetative and reproductive stage of corn plants (ILSI, 1998). Nevertheless, the attack of this pest is typically related to foliar consumption and indirect damage to grain production due to photosynthetic area reduction (CRUZ& TURPIN, 1983; PITRE& HOGG, 1983; BUNTIN, 1986; CAPINERA, 2000; VILARINHO et al., 2011). Sporadically, the larvae are reportedto penetrate in the ear and feed on kernels, whichcan cause greater damage to corn because of the injury to reproductive plant components (BUNTIN, 1986; CAPINERA, 2000).

Western bean cutworm is reported as an important Lepidoptera pest of corn (*Zea mays* L.) in the U.S. Corn Belt and parts of Canada (O'ROURKE& HUTCHISON 2000; DIFONZO&HAMMOND, 2008; MICHEL et al., 2010; TOOKER& FLEISCHER, 2010; PAULA-MORAES et al., 2012). After eggs hatching, the larvae feed on different corn tissues, and at later instars, they colonize the ear and feed on kernels (APPEL et al., 1993, SEYMOUR et al., 2004; PAULA-MORAES et al., 2013).Since its description, the range and distribution of the western bean cutworm has expanded, mostly in the last decade (MICHEL et al., 2010). Because of its recent range expansion, there is an increased demand for the development of management tools for this pest in corn.

Helicoverpa armigera is an extremely polyphagous insect capable of feeding over 100 plant species, including several economically important crops such as cotton, soybean, corn, and vegetables. Its high incidence, considerable potential of damage, and difficult control make this pest extremely important. Damage to plants is caused by larvae that feed on vegetative and reproductive organs of the plant (ZALUCKI et al., 1986). *Helicoverpa armigera*isconsidered to be seed, fruit, and flower feeders in preference to leaf feeders(PERKINS et al., 2008).As such, tend to damage economically-important plant components. Recently, the occurrence of this pest was detected in Brazil crop plantations (EMBRAPA, 2013). Since then, it has caused great concern among farmers (CZEPAK et al., 2013). World crop losses caused by *H. armigera* larvae in different cultures is estimated in 5 billion dollars year (LAMMERS& MACLEOD, 2007). In Brazil, the insect seems to have found favorable conditions for its fast proliferation, causing proportions of losses has never reported before (AVILA et al, 2013; CZEPAK et al, 2013).

Various factors in Brazil such as succession host crops, "off-season" corn and sorghum plantation, and nearby areas with different planting phenology have been changing fall armyworm occurrence scenery in corn (NAGOSHI, 2009; BARROS et al., 2010). Due to these changes in corn cultivation, the insect has been found plentiful food supply and its damage is constantly reported in corn reproductive stages. Such behavior tends to inflict higher damage to corn by attacking reproductive plant components, and could also limit the use of important control strategies such as biological and conventional control through pesticides spraying, as the larvae may be protected in the ear region.

In the US, although fall armyworm is constantly reported to attack late corn plantations in the Northern region, very limited information on this behavior is available in the literature. Therefore, a better understanding of fall armyworm infestation in reproductive corn stages, and more information on its mixed feeding behavior are still necessary. This information not only have considerable value in the elaboration and implementation of pest management programs, but also for ecological interactions.

Nevertheless, alterations in crop systems combined with the broad acceptance of the use of transgenic *Bt* corn (SHELTON et al., 2002; BROOKS& BARFOOT, 2005; MURPHY et al., 2010), have been demanding more information on various behavioral aspects of several pest insects. One of the less understood aspects in insects' behavior is related to the extent of larval dispersal.

Larval dispersal and survival can directly influence the accuracy of scouting methods and determine more precisely economic thresholds (OSTLIE& PEDIGO, 1987; ROSS& OSTLIE, 1990; PAULA-MORAES et al., 2013). Economic threshold, economic injury level, and a sampling plan represent important steps of an integrated pest management (IPM) program (KOGAN, 1998). In addition, these behavioral aspects are the major concern of the fast adoption of transgenic *Bt* crops due to the selection of resistant insect populations to *Bt* toxins (GOULD, 1998; GUSE et al., 2002; TABASHNIK et al., 2003; BATES et al., 2005; ONSTAD, 2008; TABASHNIK et al., 2008; TABASHNIK et al., 2009; HEAD& GREENPLATE, 2012).

Implementation of refuge areas is one of the most important components of the insect resistance management (IRM) plan (ALSTAD& ANDOW, 1995; MACINTOSH, 2009). However, these areas are constantly considered to have different forms and distance from the *Bt* areas because of the insect dispersal. Refuges where non-*Bt* and*Bt* plants are nearby, the movement of these pests between them can accelerate the selection of insect resistance (GOLDSTEIN et al., 2010). Finding the appropriate refuge design will directly depend on the behavior of each target pest for each crop.

Designing studies capable of representing these situations are not easily performed. Probabilistic models have been developed in order to simulate these situations (ONSTAD& GOULD, 1998; PECK et al., 1999; DAVIS& ONSTAD, 2000; IVES et al., 2011; CARROL et al., 2012). However, many aspects of insects necessary for a higher accuracy of these models are still unclear and depend upon assumptions, such as the within and plant-to-plant larval movement (ONSTAD, 2006). Therefore, information on larval behavior of important pests in corn, including western bean cutworm and fall armyworm is critical to improve pest management strategies and manage resistance, as well as provide reliable data to support modeling programs to these insects more precisely.

This larval movement is related the adaptive mechanism by which insects seek resources, including food (BELL, 1990; PRICE, 1997). Locating a suitable feeding site is essential for the development, growth, , and maintenance of the insect (BELL, 1990). Generally, early instars of Lepidoptera larvae largely determines where feeding sites become established and deserve great attention for their management (ZALUCKI et al., 2002; PERKINS et al., 2008). However, early instars movement are difficult to handle and observe, (ZALUCKI et al., 2002), especially in tropical countries such as Brazil, where the natural infestations of several insect populationscause many methodological problems.

Factors as nocturnal behavior (EPPO, 1981) and high pressure of natural infestations of the same or another morphologically identical species can create confounding effects in field experiments under normal conditions. For example, *H. armigera* was recently detected in Brazil (EMBRPA, 2013), and should be target of many studies henceforth. However, its similarity with the corn earworm *Helicoverpa zea* Boddie (Lepidoptera: noctuidae) has been making its identification and management a challenge for researchers and farmers.

Therefore, many studies of insects require marking techniques. A wide variety of markers can be used in such studies to assess dispersal, feeding behavior, insect population dynamics, and other ecological interactions (HAGLER& JACKSON, 2001). An ideal marker must not affect the insect's normal biology, persist, be easily applied, safe, and cost-effective (AKEY, 1991; HAGLER& JACKSON, 2001).

Dye is considered an ideal marking material, because it is usually non-toxic, inexpensive, and easily applied (HAGLER& JACKSON, 2001; QURESHI et al., 2004). Coleoptera, Isoptera, Diptera, Lepidoptera, and Hymenoptera have been successfully marked with dyes (OSTLIE et al., 1984; HUNT et al., 2000; QURESHI et al. 2004; VILARINHO et al., 2006; ZHAO et al., 2008; VILARINHO et al., 2011). They can be applied externally and internally to mark multiple life stages (AKEY, 1991).

Dusting procedure is the most popular method in use to mark larvae and adults externally. However, dusts can be ineffectiveness or show negative effects on insects whether used incorrectly. Several problems have been reported such as the difficulty in getting lasting adherence, and alteration in biological aspects of the insects (AKEY, 1991), requiring a better use of this marking technique.

Dyes dissolved in oil and incorporated into artificial diet have been used to mark many insects, especially Lepidoptera and Diptera (QURESHI et al., 2004; VILARINHO et al., 2006). However, different dyes in different amounts show variable marking effects and efficiencies on the development of the same or different insect species (HENDRICKS, 1971; QURESHI et al., 2004; VILARINHO et al., 2006). Therefore, marking efficiency of dyes and their effects on the biology of the species should be investigated.

In addition, a great deal of marking techniques has focused on marking adults for area-wide pest-management programs, resistance-management programs, and sterile insect release programs (QURESHI et al., 2004; VILARINHO et al., 2006). Although many studies reported marking effects on the larvae (VILARINHO et al., 2006; ZHAO et al., 2008), very few or none of them focused on marking early larval instars. Considering the difficulty of handling behavioral studies of early instars larvae and their recognized importance in management strategies, alternative methods to mark early larval instars are necessary.

Facing of the previous considerations, the study aimed to investigate behavioral aspects of three different species from Noctuidae family, as well as design techniques to allow behavioral studies of early instars in conditions that require marking individual.

The specific objectives were: a) investigate the on-plant movement of fall armyworm larvae and feeding behavior on reproductive corn stages, as well as the larval performance on different corn tissues; b) investigate the larval dispersion of the western bean cutworm and fall armyworm larvae in corn fields; c) investigate the efficiency of new marking techniques for early larval instars of *H. armigera*, and test their application in behavioral study.

The dissertation was divided into three chapters in order to achieve these objectives. The first was entitled "On-Plant movement and feeding behavior of fall armyworm on reproductive corn stages", according to the Environmental Entomology's guidelines; the second was entitled "Plant-to-plant movement of the western bean cutworm and fall armyworm in corn" written according to the Journal of AppliedEntomology's guidelines; and the third was entitled "Designing marking techniques for early larval instars of *H. armigera* and their application for behavioral studies, using soybean asmodel", written according to the Journal of Economic Entomology's guidelines.

CHAPTER I - On-plant movement and feeding behavior of fall armyworm on reproductive corn stages

Journal: Environmental Entomology

Abstract

Spodoptera frugiperda(J.E. Smith)(fall armyworm)is considered one of the most destructive and economically important pests of corn throughout Americas. Although this pest has been extensively studied, much research in corn has been directed at its management on early corn stages and less is known about its larval movement and feeding behavior on late reproductive corn stages. Various factors in modern agriculture, such as succession planting of host crops, nearby areas with different host crop phenology, expansive areas with *Bt* transgeniccorn, and increasing reports of insect resistance to *Bt* toxins require information related to fall armyworm larval movement and behavior on all stages of corn. Thus, the objective of our study was to evaluate on-plant movement and feeding behavior of fall armyworm larvae on corn in reproductive stage corn. Studies were conducted in the field at Concord, NE (USA) and the field, greenhouse, and laboratory at Botucatu, State of Sao Paulo (Brazil). For the on-plant larval movement studies under field conditions, treatment design was a factorial with two corn stages (silking, or R1 and milk, or R3) and four corn plant zones (tassel, above ear, ear zone, and below ear) in a randomized complete block design with four replications. Another on-plant movement study was conducted in the greenhouse in a completely randomized design with five replications following the above factorial arrangement. The effects of different corn tissues on larval survival and development were also investigated in the laboratory in a completely randomized design with 40 replications. Treatments were based on different corn tissues (exposed tassel, closed tassel, silk, kernel, leaf), two feeding sequence scenarios (closed tassel-leaf-silk-kernel and leaf-silk-kernel), and a diet (positive control). Results demonstrated that fall armyworm larvae prefer the ear zone as a feeding site regardless of reproductive corn stage. Overall results indicate that feeding site choice is done by first-instar larvae and is directly related to feeding preference. Corn leaves were not suitable for early instar development, and silk and kernel tissues played a positive role in survival and development of fall armyworm larvae in reproductive stage corn.

Keywordslarval movement, larval dispersal, *Spodoptera frugiperda*, feeding behavior

Introduction

The fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is considered a major pest of tropical-subtropical origin in the western hemisphere ranging from Argentina to North America (Luginbill 1928, Clark et al. 2007, Adamczyk et al. 2008, Farias et al. 2008). It has been reported infesting more than 80 plant species, including important agricultural crops such as cotton, soybean, and corn (Luginbill 1928, Pogue 2002, Capinera 2008).

In Brazil, the fall armywormis considered the most destructive and economically important pest in corn (Cruz and Turpin 1983, Cruz et al. 1999, Diez-Rodriguez and Omoto 2001, Carvalho et al. 2013, Huang et al. 2014). In the United States it has been described as an important yield-limiting pest in Southern cornfields (Buntin et al. 2004, Chilcutt et al. 2007, Hardke et al. 2011). This species does not diapause over winter, being vulnerable to low winter temperatures in other US regions, only surviving yr-round in the subtropical climates from the Southern regions of Florida and Texas (Sparks 1979, Buntin 1986, Mitchell et al. 1991). Therefore, *S. frugiperda* populations migrate back and reinvade corn crops in cooler regions of North American, including Canada, during the summer mo (Mitchell et al. 1991, Nagoshi et al. 2012). Although fall armywormcan attack all corn stages (Flanders et al. 2007, Knutson 2009), its injury is typically related to foliar consumption and indirect damage to grain production due to photosynthetic area reduction (Cruz and Turpin 1983, Pitre and Hogg 1983, Buntin 1986, Melo and Silva 1987, Capinera 2000, Vilarinho et al. 2011). Consequently, much *S. frugiperda* research in corn has been directed towards its management on early corn stages, generally when the whorl region is still present, where most fall armyworm larvae are found feeding on the developing leaves (Cruz andTurpin 1983, Harrison 1986, Melo and Silva 1987, Bokonon-Ganta et al. 2003, Siebert et al. 2008).

Although fall armyworm has also been reported to behave similarly to corn ear worm [*Helicoverpa zea* (Boddie), Lepidoptera: Noctuidae], penetrating into the ear and feeding on kernels (Buntin 1986, Capinera 2000, Vilarinho et al. 2011), a better understanding about fall armyworm infestation in reproductive corn stages is still necessary, as well as why the larvae "choose" that feeding site. Such behavior tends to inflict greater damage to corn because of injury to reproductive plant components. It could also limit the use of important control strategies, such as biological and conventional control through pesticide spraying, because the larvae may be protected in the ear.

In this context, knowledge of larval movement is critical to effectively apply pest management strategies (Ross and Ostlie 1990, Spangler and Calvin 2001, Paula-Moraes et al. 2012). Movement of early instar Lepidoptera within host plants largely determines where feeding sites become established (Zalucki et al. 2002, Perkins et al. 2008). Describing this movement could help in applying these strategies efficiently, since during their exploration, larvae are vulnerable to predators, parasitoids, pathogens, and other pest control strategies (Zalucki et al. 2002, Johnson et al. 2007, Perkins et al. 2008).

Considering that fall armyworm reinvades the northern US Northern during summer moevery yr, usually finding corn in reproductive stages, information on larval movement during these stages is important to this country. In Brazil, factors that affect fall armyworm incidence such as succession of host crops, "off-season" corn and sorghum production, and nearby areas with corn at different phenology have all been increasing (Nagoshi 2009, Barros et al. 2010), which further necessitates a better understanding of this insects behavior. This information is not only important for understanding ecological interactions, but has also valuable to design and implement pest management programs.

Another important reason to better understand larval behavior arises with the advent of transgenic corn that expresses *Bt* toxins. Information on pest movement, as well as larval feeding behavior, is important due to the mobility of the insect, which could impacts in larval exposure to lethal and sublethal concentrations of Bt toxins in different plant tissues (Paula-Moraes et al. 2012), thus influencing survival and the selection of insect resistance. This information will also help in designing strategies to manage resistance (Gould 1998, Dirie et al. 2000). Especially in the case of Brazil, where unexpected survival and consequently resistance of *S. frugiperda* on *Cry*1F and *Cry*1Ab) corn have been reported on several regions (Farias et al. 2014, Huang et al. 2014, Niu et al. 2014, Monnerat et al. 2015).

Therefore, this study investigated the on-plant movement of fall armyworm larvae at two different corn stages under field and greenhouse conditions, as well as the feeding behavior on reproductive corn stages. We also evaluated the larval performance on different corn tissues in the laboratory.

Material and Methods

On-Plant larval movement

Larval survival, development, and dispersion on the corn plant were characterized in the field at the University of Nebraska Northeast Research and Extension Center Haskell Agricultural Laboratory, Concord, NE, USA, during 2013, and also in the greenhouse and field at Sao Paulo State University, Department of Crop Protection, Botucatu, SP, Brazil, during the season of 2014/2015. For the studies, two non-transformed corn hybrids Channel 208-71R and Pioneer 30F35 were used in the USA (field) and Brazil (greenhouse and field), respectively.

The treatment design was a 2 by 4 factorial for all studies, which corresponds to two corn stages (silking or R1 and milk stage or R3) (Ritchie et al., 1993), and four different plant zones (tassel, above ear, ear zone, and below ear).

Three experiments were conducted in field conditions, one in the US and two in Brazil. For all experiments, the experimental areas had two corn stages established using different planting dates. The two corn stages were randomly assigned in a randomized complete block design. There were four plots per corn stage with eight in total. In Concord - NE (US), each experimental plot consisted of 8 rows by 10 meters, with 15-cm plant spacing and 0.76-m rows. In Botucatu - SP (Brazil), each experimental plot was consisted of 6 rows by 12 meters, with 15-cm plant spacing and 0.45-m rows. In the second area in Brazil, ten corn plants of each plot in the field were caged from emergence in order to assure plants remained free of natural infestations and natural enemies. These plants were assessed to determine the on-plant movement. The cages were 1.00 m wide, 1.30 m long, and 2.50 m high. The support was made of PVC pipe (3/4") and the entire structure was covered with white insect screen (mesh 16).

In the US, the plots were irrigated as required with an overhead lateral irrigation system. In Brazil, all plots (except for the field cages) were sprayed with a non-persistent insecticide deltametrine Decis® 25 EC (200 ml ha^{-1}) every seven d until the end of the whorl stage in order to ensure no lepidopteran natural infestations prior to ear formation.

Another on-plant movement trial was conducted in the greenhouse in Brazil. Seeds of conventional Pioneer 30F35 hybrid corn were planted in 8 L plastic pots, containing fertilized soil, according to standard cultural recommendations. The study was conducted as a completely randomized design with 5 replications. Irrigation and standard management practices were used as required to ensure optimum growth until the reproductive corn stages.

Artificial infestation with egg masses was conducted in all studies. In the US, the egg masses were purchased from a stock colony in a commercial laboratory, Benzon Research, Carlisle, PA. In Brazil, the egg masses were collected from a research colony at the Department of Crop Protection, College of Agronomic Sciences, UNESP, Botucatu - SP (Brazil). The insects were maintained under controlled conditions (T = 25 ± 2ºC; R.H. = 60 ± 10%; 14:10-L:D), and reared according to Parra (2001). The oviposition paper sheets containing egg masses were cut and maintained in growth chamber at temperature of 25°C until the eggs reached the ideal stage.

The number of eggs were counted using microscope stereoscope (Nikon - Stereo Zoom Microscope SMZ 645). In the US, 100 eggs were used to infest each plant in the filed. In Brazil, 200 eggs per plant were used because of the high number of natural enemies observed and heavy rains that usually occur during the rainfall season that could jeopardize larval survival and recovery. Eggs were selected for uniformity and infested when darkening head capsules became visible through the egg chorion (backhead stage), indicating imminent eclosion.

An artificial infestation method was developed and selected egg masses were transferred into an “envelope” (6 cm in length x 4 cm high) made of organdy tissue with an opening on top, in order to prevent desiccation or excessive moisture. One single “envelope” per plant was stapled on the upper leaf surface, at the end of the first leaf completely extended above the primary ear (above ear zone). The leaf's selection simulated natural oviposition by *S. frugiperda* female moths in reproductive stage corn (preliminary field observations). Ten plants in the central row of each plot were infested in the US and Brazil studies.

Prior to infestation, the plants were inspected for the presence of natural infestations. In the US, no natural infestation of fall armyworm was detected in the plots during the study. In Brazil, undamaged plants by insects were chosen for the study.

To determine the larval movement, the evaluations were performed based on the methods described by Paula-Moraes et al. (2012), where the corn plant was divided in five plant zones (tassel, above ear, primary ear, secondary ear, and below ear). However, in the present study we decided to join primary and secondary ear zones in one zone called "ear zone", since many of the corn plants used in our studies had only one ear each.

Plant sampling was carried out at 3, 6, 10, 13 and 16 d after infestation (DAI) (eggs hatched within 24 hours of infestation), totalizing five evaluations. One plant was evaluated in each plot, on each sampling day. Plants that did not present live fall armyworm larvae from the eggs masses were discarded, and other plants were sampled. Each corn plant zone was inspected for the presence of larvae (destructive sampling).

Larval movement on the corn plant was evaluated based on the number of recovered larvae in each plant zone. Larval survival was calculated by dividing the number of larvae found by the estimate of the number of eggs infested per plant. The recovered larvae were transferred to 6 ml translucent vials filled with 100 % ethyl alcohol for later measurement of head capsule width. Instar classification was based on Pitre and Hogg (1983).

The data were separately analyzed by cultivation system (USA field, Brazil field, field cages and greenhouse), and tested for normality of the residuals and homogeneity of variance (PROC GLIMMIX PLOT = RESIDUAL PANEL) (SAS Institute 2009). The distribution with the best fit was lognormal, which was implemented via the DISTRIBUTION option in PROC GLIMMIX (SAS Institute 2009). The relationships and interactions between number of the larvae in different plant zones and corn stages were examined. The Dunnett procedure was performed to detect differences from the control (plant zone where the egg mass infestation was done) to the other plant zones (Paula-Moraes et al. 2012).

Larval feeding

Fall armyworm larval survival and development were evaluated under laboratory conditions (T = 25 ± 2ºC; R.H. = 60 ± 10%; 14:10-L:D) during the season of 2014/2015. Different corn tissues were tested from the same corn hybrid Pioneer 30F35 used in the field studies in Brazil. The different tissues were removed from the field when needed and cleaned with alcohol (70%) to avoid contamination and remove foreign material.

Egg masses were obtained from the research colony as previously described for the on-plant study. The eggs were kept in a growth chamber at 25°C until hatching. Fall armywormneonates (< 24h age) were randomly selected and transferred using a fine paintbrush no. 2/0 into vials (100 ml) containing a moistened filter paper in the bottom and sealed with plastic lids. Each vial contained one neonate and a specific amount of each different corn tissue. Corn tissues were replaced every day. The vials were kept in the laboratory at 25 ± 2 ºC, 60 ± 10% RH and 14: 10 h light: dark photoperiod.

The study was conducted as a completely randomized design with 40 replications. Each vial represented one replication. One diet (positive control), five different corn tissues and two feeding scenarios were evaluated: Diet for fall armyworm according to Parra (2001), exposed tassel (OT), closed tassel (T), silk (S), kernels (K), leaves (L), L-S-K (leaves, silk and kernel) and T-L-S-K (closed tassel, leaves, silk, and kernels). The exposed tassel was collected in tassel stage, when it was already exposed in the air above the other parts, but not completely dried. The closed tassel was collected during pretassel stage, when it was totally green enclosed in the whorl leaves. Silk was removed from the ear during silk stage, when the structures were still green. Kernels were used in milk stage, and the leaves were collected from the superior part of the corn plants (above ear zone). The feeding scenarios were chosen based on fall armyworm damages and possible movement on corn plants. In the scenarios, the source of food was changed at five d after larval exposure to the tissue until the last material, which remained until pupation stage or the end of the assays.

Considering the operational capacity, larval survival was evaluated at 3, 5, 8, 10, 13, 15, 17, and 20 DAI. After this period, larval survival was checked daily until pupation by recording the number of live larvae. Larval development was evaluated daily by comparing the larval time period. Pupal weight was also evaluated and conducted on the second day after pupation using a scale (Model Marte AY220, Shimadzu, Kyoto, Japan) to 0.0001g. Data were analyzed using generalized mixed model (Proc Glimmix, SAS Institute 2009) to detect differences between means. When appropriate, means were separated using Fisher's least significant differences procedures ($\alpha = 0.05$).

Results

On-Plant larval movement

The overall larval recovery in all experiments was low when considering the number of eggs infested, regardless the location or cultivation system. Exception for USA - field, a decrease in the number of recovered larvae was also observed from the first to the last sampling date. The percentage of larval recovery was lower than 10% for all sampling dates, except for the first sampling date in the greenhouse study, with a larval recovery of 12.25% (245 recovered individuals) (Table 1).

All larvae recovered on the first sampling dates were classified as first instar (Table

2), and on the second date all larvae were second instar. In general, on the third, fourth, and fifth sampling dates, most of the larvae were classified as third, fourth, and fifth instars, respectively (Table 2). In the greenhouse, some of the recovered larvae had already reached the fourth instar on the third sampling date, and most of the larvae were fifth instar on the fourth and fifth sampling date. There were no plants evaluated on the fifth sampling dates in the field experiments in Brazil (Brazil - field and field cages). Some plants presented no fall armyworm infestation on the earlier sampling dates, and had to be discarded. Thus, there were no infested plants for the last sampling date (Table 2).

There was no significant corn stage effect for mean number of fall armyworm larvae for any sampling date for all experiments (Table 3). Likewise, there was no significant interaction between corn stage and plant zone (Table 3). There was a significant plant zone effect on all sampling dates for all experiments ($P < 0.05$), except for the first sampling date in Brazil for caged corn plants (Table 3). There were no plants evaluated for the fifth sampling date in the field areas in Brazil (Brazil - field and field cages) due to the lack of infested plants (Table 3).

No larvae were found in the tassel zone regardless the sampling date or corn stage (Table 4). In general, most of the larvae were recovered in the above ear and ear zone with a higher concentration in the ear zone on the first and second sampling dates. From the third sampling date, almost all of the infested larvae were recovered in the ear zone in all experiments (Table 4).

For the US and Brazil field areas (excluding field cage study), the mean numberof recovered larvae in the ear zone was significantly higher (P = 0.0005) when compared to the other plant zones on the first sampling date, including the above ear zone where the egg masses were placed (Table 5). However, the mean number of larvae recovered in the ear zone was the same as in the above ear zone for the experiment in the greenhouse, and also did not differ from the same zone in the cage experiment for the same sampling date. Overall, a significantly higher concentration of larvae in the ear zone was observed in all experiments from the second to the last sampling dates (Table 5).

Larval feeding

Larval survival was significantly higher for insects reared on diet (85.0 %) (positive control) (P <0.0001) (Table 6). When comparing the larvae reared on the different corn tissues and feeding scenarios, closed tassel and silk had the highest larval survival (57.5% each). The lowest percentages were observed for larvae feeding on leaf (10.0%) and exposed tassel (0.0%) (Table 6). The survival rates in other corn tissues and sequence of feeding scenarios varied from 40.0% (T-L-S-K) to 25.0% (kernel and L-S-K).

The fastest development was observed with larvae reared on kernels (16.5 d) differing from the other corn tissues, sequence of feeding scenarios, and diet (20.2 d) (P <0.0001) (Table 6.). Besides causing high mortality rates, leaf tissue (24.0 d) also delayed the larval development in comparison to the other treatments (P <0.0001).

Larvae reared on closed tassel resulted in the lowest pupal weight (P <0.0001). Although closed tassel and silk provided highest larval survivals among the corn tissues, they resulted in the lowest pupal weights. There was no difference between the T-L-S-K and L-S-K sequence of feeding scenarios, kernel, and diet (positive control) for pupal weight (Table 6).

All larvae confined on exposed tassel died by 8 DAI (Figure 1). Likewise, more than 80% of the larvae feeding on leaf did not survive by the same period. Larvae reared on diet had the lowest mortality at all evaluated days and did not reach 20 % of mortality until the end of the trial. No larvae died after 20 DAI.

Discussion

According to Zalucki et al. (2002), mortality in the early larval stages of Lepidoptera is commonly high and can reach around 40% for the egg stage and 50% for the first instar. This lepidopteran mortality pattern might explain the low percentage of larval recovery (3.50 to 12.50%) observed in our study, especially for the first sampling of first instars (Table 1). These results also ensure the efficiency of our infestation method.

Nevertheless, the causes of mortality are not always clear, partly because of the small size of first instars. In addition, several factors such as exposure tonatural enemies and weather effects including rainfall and high/low temperatures can also influence this mortality (Zalucki et al., 2002).Although more studies are needed to better define the causes of mortality, theoretically, the stability of the environment artificially created in our study seems to have influenced the number of recovered larvae in Brazil (Table 1). On the first sampling date, the percentages varied from 4.63% in the field area, 6.5% in the field cages, to 12.5% in greenhouse (Table 1). In general, this same pattern was observed for all evaluations in the experiments. In Brazil field areas, we observed a high number of natural enemies, especially *Doru* spp. (Dermaptera: Forficulidae), and heavy rains throughout the evaluation period, which may have impacted the fall armyworm larval survival. On the other hand, the lack of biotic and abiotic factors that compromise FAW larval survival may have provided a higher larval recovery in the US study, since the severe winter temperatures of the US Northern region reduces fall armyworm infestation and, consequently, the number of its natural enemies.Also, the absence of competitors and natural enemies is favorable for the insect's population increase.

Except for USA - field, a decrease in the number of recovered larvae was observed from the first to the last sampling date (Table 1). Fall armyworm is known to have cannibalistic behavior. Despite laying egg masses comprised of hundreds of eggs, only one or very few larvae can be found per plant due to its behavior (Sparks, 1979; Vilarinho et al., 2011). Although other factors such as environmental effects and an array of potential natural enemies could have contributed to this decrease, relatively high mortality was also observed for the greenhouse and field cage studies (Table 1), which reinforces the possible influence of cannibalism at a shared feeding site (Chapman et al., 2000).

The results in our on-plant studies did not indicate an effect of corn stage or an interaction between corn stage and plant zones on the distribution of the larvae (Table 3). On the other hand, our data indicate significant on-plant movement with a concentration at the ear zone starting from the first sampling date, independent of the reproductive corn stage evaluated (Table 4). Early-instar movement within host plants is discussed in the literature as the behavior that largely determines where the feeding site becomes established (Zalucki et al., 2002; Johnson and Zalucki, 2007; Perkins et al., 2008). However, plant injury caused by fall armyworm larvae is typically related to foliar consumption and sporadically to damage of reproductive parts such as ear structures in corn (Cruz & Turpin, 1983; Pitre & Hogg, 1983; Buntin, 1986; Melo & Silva, 1987; Capinera, 2000; Vilarinho et al., 2011). In contrast, our findings indicate a strong preference of fall armyworm larvae to the ear zone in corn reproductive stage instead of leaf consumption. It is important to emphasize that high leaf consumption is usually reported when corn plants are still in vegetative stage, and to date little is known about fall armyworm larval preference in corn reproductive stages.

In Brazil studies, on the first sampling dates, we observed a high number of recovered larvae on the leaf where the egg masses were stapled (Table 4). According to the literature (Zalucki et al. 1986, 2002; Johnson et al. 2007), during the exploration for a suitable feeding site, if larvae probe the substrate and recognize the host or plant part is unsuitable, then exploration is likely to continue (Chang et al., 1985; Hochberg, 1987;

Varela and Bernays, 1988; Terry et al., 1989; Khan et al., 1996; Foster and Howard, 1999; Zalucki et al., 2002). It may explain the high number of recovered larvae on the infested leaf in the studies in Brazil, because the egg masses used to infest these plants were not completely homogeneous at blackhead stage. Thus, some of the larvae may have just hatched. This observation may be reinforced by the head capsule width of the recovered larvae on the first sampling dates (Table 2), where it was observed that the shorter head capsule width of recovered larvae, the higher number of recovered larvae on the infested leaf (above ear) (Table 4).

Once the larvae are on a suitable host they will usually settle and establish a feeding site (Chang et al., 1985; Hochberg, 1987; Varela and Bernays, 1988; Therry et al., 1989; Khan et al., 1996; Foster and Howard, 1999; Zalucki et al., 2002). Overall, the highest concentration of larvae was observed in the ear zone from the second to the last evaluation, independent of the evaluated corn stages (Table 4). Thus, after the exploration the larvae established their feeding site in the ear zone, more specifically in the silk tissue, where most of the recovered larvae were found until they were able to reach the kernels.

Larval movement within plants is reported to be influenced by several factors both abiotic and biotic, age, predators, light, including plant architecture, and phototaxis (upward and outward movement) (Madge, 1964; Alonso and Herrera, 1996; Johnson and Zalucki, 2007; Johnson et al., 2007). Although these mechanisms by which larvae locate suitable hosts or part of hosts are not clear, some of them can be discarded in our studies such as predators and upward movement. We did not observe any natural enemies in the US field area and greenhouse study, but it is important to say that it was not used any especific method for natural enemies detection. Also, although the infestation was done above the ear zone, larvae moved downward to find a suitable feeding site.

Considering the overall results from the field study we presume that this behavior would be in part because of feeding preference. After hatching, the neonates stayed on the infested leaf for a short period and then moved to a suitable feeding site (corn ear). Indeed, we observed one of the lowest larval survival on leaves in the feeding study (Table 6). Five d after infestation, there was 67.5% mortality, and at eight DAI, approximately 82.50% of larvae had already died (Figure 1). Although fall armyworm can be considered a folivore insect, their larvae may also have a mixed feeding habit. According to Scriber and Slansky, (1981), and also Bernays and Chapman (1994), for these folivores leaf age and quality are critical factors that affect establishment, growth, and survival of neonates. Generally, some factors change in a leaf as it ages such as water availability, toughness, nitrogen and another quality factors, which may result in high neonate mortality, even if the same leaves are suitable for older instars (Cockfield and Mahr, 1993). These changes in leaf quality, during reproductive stage of corn plant probably influenced the neonates' choice in our field study. In addition, the tassel stage (exposed tassel) available for the larvae during the field studies appeared to be unsuitable for fall armyworm larvae, and did not allow development (Table 6). Five d after infestation, there was 87.5% mortality, and 100% of the larvae were dead after 8 DAI (Figure 1). It may explain why no larvae were found in the tassel zone in the on-plant studies (Table 4), and that they did not move upward in corn reproductive stages.

Fall armyworm larvae have been observed feeding on tassel structure in cornfield by many researchers. However, damage to this structure was always observed when it was enclosed in whorl leaves. For this reason, the decision was to include this structure in the feeding study even if it (tassel enclosed in whorl leaves or green tassel) was not available for the larvae in the corn stages evaluated. It was confirmed a high larval survival and fast development for larvae reared on this structure; however, the remaining pupae had the lowest weight when compared to those reared on the other corn tissues. Likewise, larvae reared on silk tissue had similar results (Table 6). Considering the results, the decision was to test the source of tissues based on possible feeding scenarios. For L-S-K (leaf-silk-kernel), five d of leaf tissue seemed to be excessive for fall armyworm neonates, which resulted in approximately 55% of mortality (Figure 1). For the T-L-S-K (tassel-leaf-silk-kernel) feeding scenario, larval survival had intermediate value (Table 6). Nevertheless, all larvae that fed on kernels had higher pupal weight (Table 6). Kernels seems to have some positive effect on fall armyworm development, since larvae reared only on this structure exhibited significant faster development when compared to larvae reared on the other tissues and scenarios (Table 6).

Based on our results we conclude that fall armyworm larvae have a preference for ear tissue over leaf tissue in reproductive stage corn, and its feeding site choice seems to be done by first instar. It suggests that corn leaves are not suitable for early instar development on reproductive stage corn. It is also possible that silk and kernel tissues play a role in larval survival and development of fall armyworm larvae. Silk seems to provide shelter and an ideal microclimate for larval development, and kernels seem to have some positive nutritional quality for larval development. However, further studies focused on new scenarios, chemical composition of corn tissues and times of larval exposure to the tissues are necessary to better understand their nutritional requirements.

Considering the implementation for pest management programs, there is a short gap between fall armyworm egg hatch to larval establishment in the ear for reproductive stage corn. This establishment on corn reproductive structure would probably provide shelter to the larvae and, consequently, reduce the efficiency of some control strategies such as insecticide spraying. Insecticide application, when necessary, should be done before larvae colonize the ear and during the larvae exploration, when they would be exposed to insecticidal control.

Knowledge about larval on-plant movement and feeding behavior is also necessary for insect resistance management strategies. There is a potential variability of Bt toxin expression in different corn tissues (Nguyen and Jehle 2007, Székács et al., 2010) and this should be focus of future studies, especially for insects that can have plant stage-specific feeding differences or a mixed feeding behavior, where insects could possibly be exposed to lethal or sublethal doses of toxins (Paula-Moraes et al., 2012).

In the case of fall armyworm, the results presented in this work indicated that the larvae preferred the ear zone as feeding site. Such feeding preference may expose the larvae to a lower rate of toxin concentration in corn grains (Nguyen and Jehle 2007, Székács et al., 2010; Burkness et al., 2011). Likewise, the larvae may be exposed to intermediate levels of toxins in refuge with cross-pollinated corn ears. Both scenarios can accelerate the selection of resistant insect populations (Chilcutt and Tabashnik, 2004; Ives et al., 2011; Razze and Mason, 2012). In temperate areas, such as in the corn belt (USA), selected populations of FAW do not overwinter, but the pressure of selection can be aggravated in tropical areas, such as Brazil. Fall armyworm can reach up to 12 generations a yr (Capinera, 2000) which occurs also during the reproductive stage of the corn. These factors require great attention in IRM henceforth.

Acknowledgements

We thank Logan Dana for his technical support.We also thank to Coordination for the Improvement of Higher Education Personnel (CAPES) for a doctoral scholarship granted to the first author. To the National Council for Scientific and Technological Development – CNPq for the productivity in research fellowship granted to the second author.

References Cited

Adamczyk, J. J. Jr, S. Greenberg,J. S. Armstrong,W. J. Mullins,L. B. Braxton,R. B. Lassiter, and M. W. Siebert. 2008. Evaluations of Bollgard®, Bollgard II®, and Widestrike® technologies against beet and fall armyworm larvae (Lepidoptera: Noctuidae). Fla. Entomol. 91: 531–536.

Alonso, C., and C. M. Herrera. 1996. Variation in herbivory within and among plants of *Daphne laureola* (Thymelaeaceae): correlation with plant size and architecture. J. Ecol. 84: 495–502.

Barros, E. M.,J. B. Torres, and A. F. Bueno. 2010. Oviposition, development, and reproduction of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) fed on different hosts of economic importance. Neotrop. Entomol. 39: 996-1001.

Bernays, E.A., andR. F. Chapman. 1994. Host-plant selection by phytophagous Insects. Chapman & Hall, New York, USA.

Bokonon-Ganta, A. H., J. S. Bernal,P. V. Pietrantonio, and M. Setamou. 2003. Survivorshipand development of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera:Noctuidae), on conventional and transgenic maize cultivars expressing *Bacillusthuringiensis* Cry9C and Cry1A(b) endotoxins. Int.J. Pest Manage. 49: 169-175.

Buntin, G. D. 1986. A review of plant response to fall armyworm, *Spodoptera frugiperda* (J. E. Smith), injury in selected field and forage crops. Fla. Entomol. 69: 549-559.

Buntin, G. D., J. N. All, R. D. Lee, and D. M. Wilson. 2004. Plant-incorporated *Bacillus thuringiensis* resistance for control of fall armyworm and corn earworm (Lepidoptera: Noctuidae) in corn. J. Econ. Entomol. 97: 1603-1611.

Burkness, E. C., P. K. O'Rourke, and W. D. Hutchison. 2011. Cross pollination of non-transgenic sweet corn ears with transgenic Bt sweet corn: efficacy against Lepidopteranpests and implications for resistance management. J. Econ. Entomol. 104:

1476-1479.

Capinera, J.L. 2000. Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Insecta: Lepidoptera: Noctuidae). The University of Florida, Institute of Food and Agricultural Sciences. (UF/IFAS), Gainesville, FL.

Capinera, J. L. 2008. Encyclopedia of entomology. 2nd ed. Springer, Dordrecht, The Netherlands.

Carvalho, R.A.,C. Omoto,L. M. Field, M. S. Williamson, and C. Bass. 2013. Investigating the molecular mechanisms of organophosphate and pyrethroid resistance in the fall armyworm *Spodoptera frugiperda*. PLoS One, v.8, n.4, p.e62268.

Chang, N. T., B. R. Wiseman, R. E. Lynch, and D. H. Habeck. 1985. Influence of nitrogen fertilizer on the resistance of selected grasses to fall armyworm (Spodoptera frugiperda) larvae. J. Agric. Entomol. 2: 137–46.

Chapman, J. W., T. Williams,A. M. Martinez, J. Cisneros, P. Caballero, R. D.Cave, and D. Goulson. 2000. Does cannibalism in *Spodopterafrugiperda* (Lepidoptera: Noctuidae) reduce the risk of predation?Behav. Ecol. Sociobiol.48: 321–327.

Chilcutt, C. F., and B. E. Tabashnik. 2004. Contamination of refuges by *Bacillus thuringiensis* toxin genes from transgenic maize. Proc. Natl. Acad. Sci. U.S.A.: 101: 7526-7529.

Chilcutt, C. F., G. N. Odvody,J. C. Correa, and J. Remmers. 2007. Effects of *Bacillus thuringiensis* transgenic corn on corn earworm and fall armyworm (Lepidoptera: Noctuidae) densities. J. Econ. Entomol.100: 327-334.

Clark, P.L., J. Molina-Ochoa, S. Martinelli, S. R. Skoda, D. J. Isenhour, D. J. Lee, J. T. Krumm, andJ. E. Foster. 2007. Population variation of the fall armyworm, *Spodoptera frugiperda*, in the Western Hemisphere. J. Insect Sci. 7: 5.

Cockfield, S. D., and D. L. Mahr. 1993. Consequences of feeding site selection on growth and survival of young blackheaded fireworm (Lepidoptera: Tortricidae). Environ. Entomol. 22: 607–612.

Cruz, I., and F. T. Turpin. 1983. Yield impact of larval infestation of the fall armyworm *Spodoptera frugiperda* (J. E. Smith) to mid-whorl growth stage of corn. J. Econ.Entomol. 76: 1052- 1054.

Cruz, I., M. L. C. Figueiredo, A. C. Oliveira, and C. A. Vasconcelos. 1999. Damage of *Spodoptera frugiperda* (Smith) in different maize genotypes cultivated in soil under three levels of aluminium saturation. International J. Pest Manage. 45: 293-296.

Cruz, I. 2008. Manejo de pragas, pp. 303-362. *In*J. C. Cruz, J. C., D. Karam, M. A. Monteiro, and P. C. Magalhães (eds.), A cultura do milho. Brasília, Embrapa Informação Tecnológica, Brasília, Brasil.

Diez-Rodrigues, G. I., and C. Omoto. 2001. Inheritance of lambda-cyhalothrin resistance in *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). Neotrop. Entomol. 30: 311–316.

Dirie, A. M., M. B. Cohen, and F. Gould. 2000. Larval dispersal and survival of *Scirpophaga incertulas* (Lepidoptera: Pyralidae) and *Chilo suppressalis* (Lepidoptera: Crambidae) on cry1Ab-transformed and Non-transgenic rice. Environ. Entomol. 29: 972 978.

Farias, P.R. S., J. C. Barbosa, A. C. Busoli, W. L. Overal, V. S. Miranda, and S. M. Ribeiro. 2008. Spatial analysis of the distribution of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and losses in maize crop productivity using geostatistics. Neotrop. Entomol. 37: 321–327.

Farias, J. R., D. A. Andow, R. J. Horikoshi, R. J. Sorgatto, P. Fresia, A. C. Santos, and C. Omoto. 2014. Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. Crop Prot. 64: 150-158.

Flanders, K.L., D. M. Ball, andP. P. Cobb. 2007. Management of fall armyworm in pastures and hayfields. Alabama Cooperative Extension System. Alabama A&M and Auburn Universities, Auburn, USA.

Foster, S. P., and A. J. Howard. 1999. Adult female and neonate larval plant preferences of the generalist herbivore, *Epiphyas postvittana*. Entomol. Exp. Appl. 2: 53–62.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu. Rev. Entomol. 43: 701-726.

Hardke, J. T., B. R. Leonard, F. Huang, F., and R. E. Jackson. 2011. Damage and survivorship of fall armyworm (Lepidoptera: Noctuidae) ontransgenic field corn expressing *Bacillus thuringiensis* Cry proteins. Crop Prot. 30: 168-172.

Harrison, F. P. 1986. Oviposition and subsequent infestations of corn by fall armyworm.Fla Entomol. 69: 588-592.

Hochberg, M. E. 1987. The within-plant distribution and feeding behaviour of *Heliothis armigera* Hubner (Lep., Noctuidae) on greenhouse tomatoes. J. Appl. Entomol. 104: 256–61.

Huang, F., J. A. Qureshi, R. L. Meagher, D. D. Reisig, G. P. Head, andD. A. Andow. 2014. Cry1F Resistance in Fall Armyworm *Spodoptera frugiperda*: Single Gene versus Pyramided Bt Maize. PLoS One 9: e112958.

Ives, A. R., P. R. Glaum, N. L. Ziebarth, and D. A. Andow. 2011. The evolution of resistance to two-toxin pyramided transgenic crops. Ecol. Appl.21: 503-515.

Jarrod, T., B. Hardke, L. Rogers, F. Huang, and R. E. Jackson. 2011. Damage and survivorship of fall armyworm (Lepidoptera: Noctuidae) on transgenic field corn expressing *Bacillus thuringiensis* Cry proteins. Crop Prot. 30: 168-172.

Johnson, M. L., S. Armitage, B. C. G. Scholz, D. J. Merritt, B. W. Cribb, and M. P.Zalucki. 2007. Predator presence moves *Helicoverpa armigera* larvae to distraction. J. Insect Behav. 20: 1-18.

Johnson, M. L., and M. P. Zalucki. 2007. Feeding and foraging behaviour of a generalist caterpillar: are third instars just bigger versions of firsts? Bull. Entomol. Res. 97: 81–88.

Khan, Z. R., M. L. P. Abenes, and N. J. Fernandez. 1996. Suitability of graminaceous weed species as host plants for rice leaffolders, *Cnaphalocrocis medinalis* and *Marasmia patnalis*. Crop Prot. 15: 121–37.

Knutson, A. 2009. The fall armyworm – pest of pasture, hayfields and small grains. AgriLife Extension. Texas A&M System, Dallas, TX.

Luginbill, P. 1928. The fall armyworm. U.S. Dept. Agric. Tech. Bull 34: 92.

Madge, D. S. 1964. The light reactions and feeding activity of the larvae of the cutworm Tryphaena pronuba L. (Lepidoptera: Noctuidae). Part II. Field investigations. Entomol. Exp. Appl. 7: 105–114.

Melo, M.,and R. F. P. Silva. 1987. Influência de três cultivares de milho no desenvolvimento de *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae). Anais da Sociedade Entomológica do Brasil16: 37-49.

Mitchell, E. R., J. N. Mcneil, J. K. Westbrook, J. F. Silvain, B. Lalanne-Cassou, R. B. Chalfant, S. D. Pair, V. H. Waddill, A. Sotomayor-Rios, and F. I. Proshold. 1991. Seasonal periodicity of fall armyworm (Lepidoptera: Noctuidae) in the Caribbean basin and northward to Canada. J. Entomol. Sci. 26: 39-50.

Monnerat, R., E. Martins, C. Macedo, P. Queiroz, L. Praça, C. M. Soares, H. Moreira, I. Grisi, J. Silva, M. Soberon, and A. Bravo. 2015. Evidence of field-evolved resistance of *Spodoptera frugiperda* to Bt corn expressing Cry1F in Brazil that is still sensitive to modified Bt toxins. Plos One10: e0119544.

Nagoshi, R. N. 2009. Can the amount of corn acreage predict fall armyworm (Lepidoptera: Noctuidae) infestation levels in nearby cotton? J. Econ. Entomol. 102: 210-218.

Nagoshi, R. N., R. L. Meagher, and M. Hay-Roe. 2012. Inferring the annual migration patterns of fall armyworm (Lepidoptera: Noctuidae) in the United States from mitochondrial haplotypes. Ecol. Evol. 2: 1458–1467.

Nguyen, H. T., and J. A. Jehle. 2007. Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810. J. Plant Dis. Prot. 114: 82-87.

Niu, Y., F. Yang, V. Dangal, and F. Huang. 2014. Larval survival and plant injury of Cry1F-susceptible, -resistant, and heterozygous fall armyworm (Lepidoptera: Noctuidae) on non-Bt and Bt corn containing single or pyramided genes. Crop Prot. 59: 22-28

Parra, J.R. P. 2001. Técnicas de criação de insetos para programas de controle biológico . FEALQ, Piracicaba, Brasil.

Paula-Moraes, S. V.,T. E. Hunt, R. J. Wright, G. L. Hein, andE. E. Blankenship. 2012. On-Plant movement and feeding of western bean cutworm (Lepidoptera: Noctuidae) early instars on Corn. Environ. Entomol.41: 1494-1500.

Perkins, L. E., B. W. Cribb, J. Hanan, E. Glaze, C. Beveridge, and M. P. Zalucki. 2008. Where to from here? The mechanisms enabling the movement of first instar caterpillars on whole plants using *Helicoverpa armigera* (Hubner) Arthropod-Plant Interactions2: 197–207.

Pitre, H. N., and D. B. Hogg. 1983. Development of the fall armyworm on cotton, soybean and corn. J. Ga. Entomol. Soc. 18: 187-194.

Pogue, G. M. 2002. A world revision of the genus Spodoptera Guenée (Lepidoptera: Noctuidae). Mem Am Entomol Soc 43: 1-202.

Razze, J. M., and C. E. Mason. 2012. Dispersal behavior of neonate European corn borer (Lepidoptera: Noctuidae) on Bt corn. J. Econ. Entomol. 105: 1214-1223.

Ritchie, S. E., J. J. Hanway, and G. O. Benson. 1993. How a corn plant develops. Iowa Coop. Ext. Serv. Spec. Rep. 48.

Ross, S. E., and K. R. Ostlie. 1990. Dispersal and survival of early instars of European corn borer (Lepidoptera: Pyralidae) in field corn. J. Econ. Entomol. .83: 831-836.

SAS Institute. 2009. SAS user's guide: statistics, version. 9.1.3. SAS Institute, Cary, NC.

Scriber, J. M., and F. J. Slansky. 1981. The nutritional ecology of immature insects. Annu. Rev. Entomol. 26: 183–211.

Siebert, M. W., K. V. Tindall, B. R. Leonard, J. W. Van Duyn, and J. M. Babcock. 2008. Evaluation of corn hybrids expressing Cry1F (Herculex I insect protection) against fall armyworm (Lepidoptera: Noctuidae) in the Southern United States. J. Entomol. Sci. 43: 41-51.

Spangler, S. M., and D. D. Calvin. 2001. Vertical distribution of European corn borer (Lepidoptera: Crambidae) egg masses on sweet corn. Environ. Entomol. 30: 274-279.

Sparks, A. N. 1979. A review of the biology of the fall armyworm. Fla Entomol. 62: 82–87.

Székács, A., E. Lauber, J. Juracsek, and B. Darvas. 2010. Cry1Ab toxin production of Mon810 transgenic maize. Environ. Toxicol. Chem. 29: 182-190.

Terry, I., J. R. J. Bradley, and J. W. Van Duyn. 1989. Establishment of early instar *Heliothis zea* on soybeans. Entomol. Exp. Appl. 51: 233–40.

Varela, L. G., and E. A. Bernays. 1988. Behavior of newly hatched potato tuber moth larvae, *Phthorimaea operculella* Zell. (Lepidoptera: Gelechiidae), in relation to their host plants. J. Insect Behav. 1: 261– 76.

Vilarinho, E. C., O. A. Fernandes, T. E. Hunt, and D. F. Caixeta. 2011. Movement of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) adults in maize in Brazil. Fla Entomol. 94: 480-488.

Zalucki, M. P., G. Daglish, S. Firempong, and P. Twine. 1986. The biology and ecology of *Heliothis armigera* (Hubner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae) in Australia: what do we know? Aust. J. Zool. 34: 779–814.

Zalucki, M. P., A. R. Clarke, and S. B. Malcom. 2002. Ecology and behavior of first instar larval Lepidoptera. Annu. Rev. Entomol. 47: 361-393.

Table 1. Overal fall armyworm larval recovery and total number of recovered larvae from corn plants

Larval recovery[a]	Sampling date				
	First	Second	Third	Fourth	Fifth
USA - Field[b]					
Overall recovery (%)	3.50	6.63	7.00	5.50	2.25
Number of recovered larvae	28	53	56	44	18
Brazil - Field[c,d]					
Overall recovery (%)	4.63	1.81	0.88	0.69	---
Number of recovered larvae	74	29	14	11	---
Brazil - Field cages[c,d]					
Overall recovery (%)	6.5	2.19	1.06	0.94	---
Number of recovered larvae	104	35	17	15	---
Brazil - Greenhouse[c]					
Overall recovery (%)	12.25	5.60	1.75	0.90	0.95
Number of recovered larvae	245	112	35	18	19

Sampling dates: First= 3DAI (days after infestation); Second= 6 DAI; Third= 10 DAI; Fouth= 13 DAI; Fifth = 16 DAI.

[a]Larval recovery was calculated by dividing the number of larvae found by the estimate of the number of eggs infested per plant.

[b] Infested with 100 eggs per plant.

[c] Infested with 200 eggs per plant.

[d]There were no plants evaluated on fifth sampling dates.

Table 2. Average diameter of fall armyworm head capsule and instar estimate

Sampling date	Average diameter of head capsule (mm)	Estimate instar
USA - Field		
First	0.35	First
Second	0.46	Second
Third	0.77	Third
Fourth	1.35	Fourth
Fifth	2.29	Fifth
Brazil - Greenhouse		
First	0.30	First
Second	0.64	Second
Third	1.26	Third/Fourth
Fourth	2.01	Fourth/Fifth
Fifth	2.10	Fifth
Brazil - Field		
First	0.35	First
Second	0.59	Second
Third	0.89	Third
Fourth	1.70	Fourth
Fifth	---	---
Brazil - Field cages		
First	0.32	First
Second	0.67	Second
Third	1.09	Third
Fourth	1.68	Fourth
Fifth	---	---

Sampling dates: First= 3DAI (days after infestation); Second= 6 DAI; Third= 10 DAI; Fouth= 13 DAI; Fifth = 16 DAI.

Head capsule widths are approximately 0.35, 0.45, 0.75, 1.3, 2.0, and 2.6 mm, respectively, for instars 1-6 (Pitre & Hogg, 1983).

Table 3. Corn stage and plant zone effects on mean number of fall armyworm larvae recovered

Effect	DF	Sampling date				
		First	Second	Third	Fourth	Fifth
USA - Field[a]				*P value*		
Corn stage	1	0.6593	0.7005	0.3179	0.3305	1.0000
Plant zone	3	0.0006	0.0046	<0.0001	<0.0001	<0.0001
Corn stage x Plant zone interaction	3	0.5019	0.8489	0.5917	0.8077	1.0000
Brazil - Greenhouse[a,b]				*P value*		
Corn stage	1	0.0830	0.1256	0.2789	1.0000	0.5189
Plant zone	3	0.0002	<0.0001	0.0003	0.0036	0.0148
Corn stage x Plant zone interaction	3	0.1051	0.6244	0.0695	0.8764	0.7344
Brazil - Field[a,b]				*P value*		
Corn stage	1	0.1623	0.0694	1.0000	0.2042	---
Plant zone	3	<0.0001	<0.0001	<0.0001	<0.0001	---
Corn stage x Plant zone interaction	3	0.2943	0.2512	0.7077	0.6502	---
Brazil - Field cages[a,b]				*P value*		
Corn stage	1	0.9211	0.1942	0.1393	0.3215	---
Plant zone	3	0.2594	0.0003	<0.0001	<0.0001	---
Corn stage x Plant zone interaction	3	0.2083	0.6179	0.5306	0.7989	---

Sampling dates: First= 3DAI (days after infestation); Second= 6 DAI; Third= 10 DAI; Fouth= 13 DAI; Fifth = 16 DAI.

[a] Infestation of egg mass on leaf above primary ear (above ear zone).

[b] There were no plants evaluated on the fifth sampling dates.

Table 4. Mean number (+SE) of fall armyworm larvae recovered in each plant zone

Plant zone	Mean no. of larvae per plant zone				
	Sampling date				
	First	Second	Third	Fourth	Fifth
USA - Field					
Tassel	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Above ear	0.63 (0.23)	0.13 (0.15)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ear zone	2.88 (0.81)	6.50 (1.57)	8.25 (0.56)	5.50 (0.82)	2.25 (0.25)
Below ear	0.00 (0.00)	0.00 (0.00)	0.13 (0.24)	0.00 (0.00)	0.00 (0.00)
Brazil - Greenhouse					
Tassel	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Above ear	11.90 (2.32)	3.30 (0.51)	0.20 (0.14)	0.00 (0.00)	0.00 (0.00)
Ear zone	11.90 (1.75)	6.70 (0.51)	2.90 (0.37)	1.70 (0.34)	1.80 (0.29)
Below ear	0.70 (1.75)	1.20 (0.51)	0.40 (0.19)	0.10 (0.17)	0.10 (0.10)
Brazil - Field					
Tassel	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	---
Above ear	2.63 (0.44)	0.25 (0.18)	0.13 (0.13)	0.00 (0.00)	---
Ear zone	6.25 (0.69)	3.38 (0.50)	1.63 (0.26)	1.38 (0.18)	---
Below ear	0.38 (0.21)	0.00 (0.00)	0.00 (0.00)	0.00 (0.10)	---
Brazil - Field cages					
Tassel	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	---
Above ear	4.25 (1.18)	0.50 (0.19)	0.00 (0.00)	0.00 (0.00)	---
Ear zone	8.13 (1.98)	3.88 (0.62)	2.13 (0.48)	1.88 (0.23)	---
Below ear	0.63 (0.26)	0.00 (0.00)	0.00 (0.00)	0.00 (0.10)	---

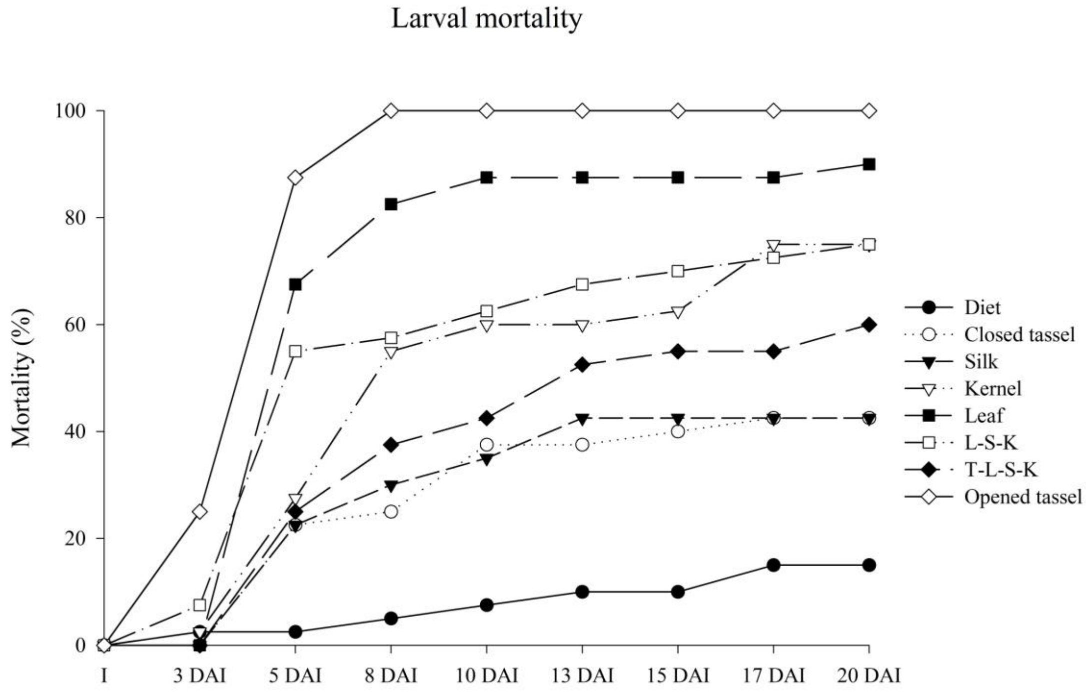
Sampling dates: First= 3DAI (days after infestation); Second= 6 DAI; Third= 10 DAI; Fouth= 13 DAI; Fifth = 16 DAI.

[a] Mean number of larvae per plant zone based on the real number of larvae recovered.

Table 5. Comparison of mean number of fall armyworm larvae recovered in each plant zone

Plant zone	Comparison of the mean no. of larvae per plant zone									
	Sampling date (days after infestation)									
	First (3 DAI)		Second (6 DAI)		Third (10 DAI)		Fourth (13 DAI)		Fifth (16 DAI)	
USA - Field	Mean[a]	*P value*[b]	Mean[a]	*P value*[b]	Mean[a]	*P value*[b]	Mean[a]	*P value*[b]	Mean[a]	*P value*[b]
Tassel	0.63 (0.23)	0.0315	0.13 (0.15)	0.4771	0.00 (0.00)	1.0000	0.00 (0.00)	1.0000	0.00 (0.00)	1.0000
Above ear	0.63 (0.23)	reference	0.13 (0.15)	reference	0.00 (0.00)	reference	0.00 (0.00)	reference	0.00 (0.00)	reference
Ear zone	- 2.25 (0.56)	0.0005	- 6.38 (0.94)	<0.0001	- 8.25 (0.56)	<0.0001	- 5.50 (0.43)	<0.0001	- 2.25 (0.14)	<0.0001
Below ear	0.63 (0.23)	0.0315	0.13 (0.15)	0.4771	-0.13 (0.24)	0.6097	0.00 (0.00)	1.0000	0.00 (0.00)	1.0000
Brazil - Greenhouse										
Tassel	11.90 (2.90)	0.0024	3.30 (0.72)	<0.0001	0.20 (0.19)	0.3067	0.00 (0.24)	1.0000	0.00 (0.11)	1.0000
Above ear	11.90 (2.90)	reference	3.30 (0.72)	reference	0.20 (0.19)	reference	0.00 (0.00)	reference	0.00 (0.00)	reference
Ear zone	0.00 (2.90)	1.0000	- 3.40 (0.72)	<0.0001	- 2.70 (0.39)	0.0009	- 1.70 (0.38)	0.0038	- 1.80 (0.41)	0.0104
Below ear	11.20 (2.90)	0.0035	2.10 (0.72)	0.0064	- 0.20 (0.23)	0.4093	- 0.10 (0.24)	0.6826	- 0.10 (0.13)	0.4462
Brazil - Field										
Tassel	2.63 (0.44)	<0.0001	0.25 (0.18)	0.2125	0.13 (0.13)	0.3752	0.00 (0.00)	1.0000		
Above ear	2.63 (0.44)	reference	0.25 (0.18)	reference	0.13 (0.13)	reference	0.00 (0.00)	reference		---
Ear zone	- 3.63 (0.82)	0.0005	- 3.13 (0.32)	<0.0001	- 1.50 (0.21)	<0.0001	- 1.38 (0.10)	<0.0001		
Below ear	2.25 (0.49)	0.0003	0.25 (0.18)	0.2125	0.13 (0.13)	0.3752	- 0.00 (0.00)	1.0000		
Brazil - Field cages										
Tassel	4.25 (30.28)	0.9112	0.50 (0.19)	0.0317	0.00 (0.00)	1.0000	0.00 (0.00)	1.0000		
Above ear	4.25 (30.28)	reference	0.50 (0.19)	reference	0.00 (0.00)	reference	0.00 (0.00)	reference		---
Ear zone	- 3.88 (31.83)	0.9176	- 3.38 (0.65)	<0.0001	- 2.13 (0.25)	<0.0001	- 1.88 (0.12)	<0.0001		
Below ear	3.63 (30.28)	0.9242	0.50 (0.19)	0.0317	- 0.00 (0.00)	1.0000	- 0.00 (0.00)	1.0000		

[a]LSMeans (± SEM). [b]Mean comparison based on Dunnett test. The reference is the plant zone where the egg mass infestation was done.



I = Infestation; DAI = d after infestation; L-S-K = Leaf - Silk - Kernel; T-L-S-K = Closed tassel - Leaf - Silk - Kernel.

For the feeding scenarios, the source of food was changed at 5 d after the larvae were exposed to the tissue. The last source of food remained until the end of the trial.

Fig.1. Fall armyworm larval mortality exposed to different corn tissues and artificial diet up to 20 days after infestation. Laboratory conditions (T = 25 ± 2ºC; R.H. = 60 ± 10%; 14:10-L:D).

Table 6. Effects of different corn tissues on fall armyworm larval mortality, larval development, and pupal weight. Laboratory conditions (T = 25 ± 2ºC; R.H. = 60 ± 10%; 14:10-L:D).

Corn tissue	Larval survival (%)[a]	Development (d)[b]	Pupal weight (g)[c]
Diet	85.0 (5.72) a	20.2 (0.53) b	0.24 (0.007) a
Closed tassel	57.5 (7.92) b	18.9 (0.26) c	0.15 (0.009) c
Silk	57.5 (7.92) b	19.7 (0.61) bc	0.20 (0.008) b
T-L-S-K	40.0 (7.84) bc	20.6 (0.25) b	0.24 (0.007) a
Kernel	25.0 (6.93) cd	16.5 (0.45) d	0.24 (0.005) a
L-S-K	25.0 (6.93) cd	20.1 (0.35) bc	0.25 (0.010) a
Leaf	10.0 (4.80) de	24.0 (0.82) a	0.22 (0.009) ab
Exposed tassel	0.0 (0.00) e	---	---
P value	<0.0001	<0.0001	<0.0001

[a] Percentage of larval survival. LSMean (± SEM). The means with the same letter are not significantly different, $P \leq 0.05$.

[b] Larval development. LSMean (± SEM). The means with the same letter are not significantly different, $P \leq 0.05$.

[c] Pupal weight. LSMean (± SEM). The means with the same letter are not significantly different, $P \leq 0.05$.

CHAPTER II –Plant-to-plant movement of western bean cutworm and fall armyworm in corn

Journal: Journal of Applied Entomology

Abstract

Western bean cutworm [*Striacosta albicosta* (Smith)] (Lepidoptera: Noctuidae) and fall armyworm [*Spodoptera frugiperda* (J.E. Smith)] (Lepidoptera: Noctuidae) are considered important pests of corn. However, some aspects in their bioecology are unclear and critical to apply pest management strategies successfully, such as larval dispersal, which can directly influence the accuracy to apply pest management strategies and manage resistance. Thus, this study aimed to investigate the plant-to-plant movement of western bean cutworm and fall armyworm larvae in field corn. Studies were conducted in field corn at Concord, NE (USA). Experiments on western bean cutworm larval movement and survival were accomplished in 2008, 2009, and 2010. A main study with western bean cutworm was performed in 2012 in a randomized complete block with nine replications. Another fall armyworm larval movement study was performed in 2013 in a randomized complete block design with eight replications. The plant-to-plant movement and larval survival of both species were measured in single variety plots with non-toxic corn plants to the insects. Larval survival was evaluated based on the number of eggs infested and the number of larvae recovered. Overall results indicate that larval survival of western bean cutworm presented high variety throughout the years, but it was possible to evaluate the dispersion from infested plant. Although thefall armywormoccurredinlow infestationduring2013, it did not compromise the larval dispersion assessment. Larvae of both species dispersed governed by non-directional sensory information, and presented aggregated and symmetrical distribution in field corn, but fall armyworm remained nearby the release point more than western bean cutworm. Western bean cutworm and fall armyworm presented expressive plant-to-plant movement in corn. These results may help in determining more accurate important integrated pest managements (IPM) components such as scouting and economic thresholds, as well as influence the implementation of refuge and seed mixture strategies in integrated resistance management (IRM).

Keywords:larval dispersal, larval movement, *Striacosta albicosta*, *Spodoptera frugiperda*, *Zea mays*.

Introduction

Dispersal is defined as the movement away from a densely place that results in the spreading of part of the original individuals (Price 1997). This behavior is related an adaptive mechanism by which insects seek resources and thereby acquire resources such food, mates, and refuge (Bell 1990; Price 1997). Thus, these resources are absolutely essential for the establishment, growth, and development of the insect (Bell 1990).

An inherent tendency to disperse seems to be present to some rate in all species of arthropods (Andrewartha and Birch 1954). Most Lepidoptera disperse as winged adults, but many groups can also disperse as larvae. Larval dispersal commonly occurs by crawling or ballooning. Ballooning is the movement which larvae use silk to hang off of the plant structures to come in contact with another structure or taken by wind. This behavior is recorded for several lepidopteran families, including Noctuidae. As caterpillars grow, ballooning becomes impossible due to their increased weight, and dispersal mostly occurs by walking. However, walking movement may happen any moment when the parts of the host plant are unsuitable for larval establishment (Zalucki et al. 2002).

Environmental factors may also influence the rate and success of dispersal (Bell1990; Tikkanen et al. 1999; Zalucki et al. 2002). However, it is unclear how the insects perceive their environment (Baker 1978; Harris and O'Miller 1982). According to Bell (1990), the types of information used by insects are governed by nondirectional or directional sensory information, cognized from the external environment, and internally derived kinesthetic or stored genetic information.

Western bean cutworm [*Striacosta albicosta*(Smith)] (Lepidoptera: Noctuidae) is considered an important Lepidoptera pest of corn (*Zea mays* L.) in the U.S. Corn Belt and parts of Canada (O'Rourke and Hutchison 2000; DiFonzo and Hammond 2008; Michel et al. 2010; Tooker and Fleischer 2010; Paula-Moraes et al. 2012). Likewise, fall armyworm [*Spodoptera frugiperda*(J.E. Smith)] (Lepidoptera: Noctuidae) is considered an important yield-limiting pest in the U.S and America cornfields (Buntin et al. 2004; Chilcutt et al. 2007; Farias et al. 2008; Hardke et al. 2011). Although commonly related as important pests in corn, some aspects on their behavior are still unclear, but considered critical to apply management strategies. One of the less understood aspects is related to the extent of larval dispersal.

Larval dispersal can directly influence the accuracy of scouting methods to apply pest management strategies successfully (Ross and Ostlie 1990). Scouting practices have been often developed without considering the dispersion of larvae among plants. Knowledge of western bean cutworm and fall armyworm behaviors and the resulting dispersion in field corn would contribute with the development of a more effective sampling method. In addition, knowledge of larval survival patterns would determine more precisely economic thresholds (Ostlie and Pedigo 1987; Ross and Ostlie 1990).

Another important reason to deeper understand such behavior arises with the broad acceptance of the use of transgenic *Bt* corn (Shelton et al. 2002; Brooks and Barfoot 2005; Murphy et al. 2010). Its fast adoption can jeopardize the long-term durability of the technology (Tabashnik et al. 2008; Tabashnik et al. 2013). The major concern is the quick selection of resistant insect populations to *Bt* toxins, since these insects are constantly exposed to them. In fact, this scenario produces a high and constant selection pressure for insect resistance (Gould 1998; Guse et al. 2002; Tabashnik et al. 2003; Bates et al. 2005; Onstad 2008; Tabashnik et al. 2009; Head and Greenplate 2012).

Adopting of refuge areas is one of the main recommended components of an insect resistance management plan (IRM) to *Bt* toxins (Alstad and Andow 1995; Macintosh 2009). These areas are constantly considered to have different forms and distance from the *Bt* areas. The refuge must be no more than 0.8km away from the *Bt* cornfield.The percentage of refuge area is variable.Depending on the trait and region must make up 50% in cotton-growing areas and 20% of a grower's corn in the Corn Belt (EPA 1998). In recent years, the pyramiding of Bt traits allowed the reduction of some refuges from 20% to 10% or 5%, depending on the trait (Difonzo 2015). The design of the refuge area can take many forms: in blocks, stripes, a separate field, or border around *Bt* cornfields (Cullen et al. 2008). In those refuges where *Bt* and non-*Bt* plants are in proximity, the movement of these pests between *Bt* and non-*Bt* corn (refuge) can accelerate the selection of resistant insects (Goldstein et al. 2010).

Although the importance of adopting refuge areas is a common knowledge, there is a concern that growers may not comply with refuge requirements due toadditional effort associated with lower returns on refuge crops compared with transgenic (Mallet and Porter 1992; Hurley et al. 2006; Murphy et al. 2010; Wangila et al. 2013).

Seed mixture or refuge "in-the-bag" (RIB strategy) has been appointed as an alternative to the low adoption of refuge areas by growers (Gould 1996; Wangila et al. 2013). Recently, pyramided corn in seed mixture was approved for some traits in the US ―Corn Belt" region (Difonzo 2015). This recommendation was based on the commercial disposable of traits which have more than one Bt protein (Tabashnik et al 2008) which target pest, such as corn earworm and fall armyworm that do not overwintering in the region. In this way, the risk of resistance selection decreases.

Finding the appropriate refuge design will directly depend on the behavior of each target pest for each crop. Considering larval movement, species that have higher capacity of dispersion would not be recommended for the use of seed mixtures (Davis and Onstad 2000). Insects that tend to disperse within rows would be better fitted in structured refuges than integrated refuges, since the insects would likely meet the same type of plant in block or strip refuges (Petzold-Maxwell et al. 2013). According to Davis and Onstad (2000), and Siegfried and Hellmich (2012), the european corn borer, for example, is considered a good candidate for structured refuges in corn, since most of larvae usually diperse within the infested row.

Due to the complexity in designing studies capable of representing these situations, probabilistic models have been developed and reported in the literature (Onstad and Gould 1998; Peck et al. 1999; Davis and Onstad 2000; Ives et al. 2011; Carrol et al. 2012). However, many aspects necessary for a higher accuracy of these models are still unclear and depend upon assumptions, such as the insect's larval movement (Onstad 2006). In the case of western bean cutworm and fall armyworm, information on larval movement are relative limited and may support the development of models accurately.

In short, information on larval behavior of western bean cutworm and fall armyworm is critical to improve pest management strategies, manage resistance, as well as provide reliable data to support modeling programs to these insects more precisely. Thus, this study aimed to investigate the plant-to-plantmovement of western bean cutworm and fall armyworm larvae in field corn.

Material and Methods

Larval movement of western bean cutworm

The study of larval movement of western bean cutworm was conducted in field corn at University of Nebraska Northeast Research and Extension Center Haskell Agricultural Laboratory, Concord, NE, USA. Field experiments were conducted during 2008, 2009, 2010, and 2012. A corn hybrid (DKC 61-72 RR) expressing *Bacillus thuringiensis* (Bt) protein Cry1Ab (YieldGard, Monsanto, St Louis, MO) that is not toxic to western bean cutworm (Catangui and Berg 2006) was used to minimize the confounding effect of european corn borer. The corn stage evaluated was posttassel (silking) (Ritchie et al. 1993). All fields were under center-pivot irrigation and conventional agronomic practices were followed for the region.

Exploratory trials of western bean cutworm larval movement

Prior to setting the main experiment of western bean cutworm larval movement, several trials on smaller scale were performed in order to better understand the movement pattern of the insect.

Three consecutive trials were evaluated in 2008, 2009, and 2010. In 2008, two plots with five rows of 17 plants each (3.04 by 2.88 m) were evaluated. In 2009, there were two plots with three rows of 21 plants per row (1.52 by 3.6 m). In 2010, three more plots with five rows of nine plants each (3.04 by 1.44 m) were performed. In all years, row spacing was 0.76 m and average plant spacing within rows of 0.18 m.Each experimental plot had approximately 50 m^2.

Artificial infestation was conducted in all exploratory trials by using egg masses collected from commercial cornfields.The "egg mass sandwich infestation" methodology was used as described by Paula-Moraes et al. (2013). An overall mean number of 50 eggs were observed per plant. The central plant from the central row of each plot was infested.

Main experiment of western bean cutworm larval movement

Based on the movement pattern observed in the exploratory trials, a main experiment in larger scale was performed in 2012. For this study, nine plots with 13 rows of 41 plants (9.12 by 6 m) were evaluated. Row spacing was 0.76 m and average plant spacing within rows of 0.15 m.

The "wild moths" methodology described by Paula-Moraes et al. (2013) were used to infest the plots for this year. Due to the low larval survival of western bean cutworm observed in preliminary tests, we decided to keep two egg masses per plant in this study. An overall mean number of 170 eggs were observed per plant, and infested plants were identified with flagging tape. The central plant from the central row of each plot was infested.

For the exploratory and main experiments of western bean cutworm, all experimental plots were randomly arranged in a randomized complete block design. A five-row border of corn plants was maintained at the edges of each plot to separate and avoid interaction between plots. Plants were inspected for the presence of western bean cutworm egg masses prior to artificial infestation. No natural infestation was observed in the experimental plots during all years.

For all experimental plots,destructive sampling was carried out and larval presence was recorded on all corn ears in all plants. Injured ear was counted as larval presence. Western bean cutworm larval dispersion, and distance from the release point was evaluated approximately 20 days after infestation (DAI). The number of recovered larvae was correlated with the number of infested eggs, and percentage of larval survival was calculated. Position of the larval presence was recorded in each plot. Plants were oriented as North (N) - South (S) in the same row related the infested plant, and East (E) - West (W) between rows. Larvae position was designed as 0 for the infested plant. The cornfields followed the same N-S orientation.

Based on the position of the recovered larvae, the maximum distance covered by the larvae, potential of maximum distance available, and number of larvae were evaluated for: different quadrants [Northeast (NE), Southeast (SE), Northwest (NW), and Southwest (SW)], two orientations (N and S), movement across rows (E and W axes), and movement within infested row (N and S axes). Distance was calculated by Euclidean distance, which distance between plants or rows is the length of the line segment connecting them (ordinary distance). The Euclidean distance of the farthest detected larvae in each quadrant calculates maximum distance. The potential of maximum distance available was calculated through an index of the maximum distance covered by the farthest detected larvae related the maximum distance they could cover in each quadrant. This index varies from 0 (no movement) to 1.00 (100% of covered distance).

Larval movement of fall armyworm

Larval movement of fall armyworm was performed at the University of Nebraska Northeast Research and Extension Center Haskell Agricultural Laboratory, Concord, Nebraska, in 2013. A conventional corn hybrid (Channel 208-71R) was used for this experiment. All plots were under center-pivot irrigation and conventional agronomic practices were followed for the region. The corn stage evaluated was R1 (silking) (Ritchie et al. 1993). The reproductive corn stage was used in order to simulate late infestation of fall armyworm in the Northern US regions during summer months. This species does not overwinter at North, since is vulnerable to severe winter temperatures in these regions. The infesting populationsare from the subtropical climates of the Southern regions of Florida and Texas that migrate back and reinvade the crops in the Northern US regions (Mitchell et al. 1991).

Row spacing was 0.76 cm and plant space within rows of 0.15 cm. Each plot consisted in 16 rows of 30 plants per row (11.4 by 4.5 m). Each experimental plot had approximately 22 m^2. There were a total of 8 plots randomly assigned in a randomized complete block design. A five-row border of corn plants was maintained at the edges of each plot to separate and avoid interaction between plots. Plants were inspected for the presence of natural infestations. No natural infestation of fall armyworm was detected in all experimental plots.

Artificial infestation was conducted by using egg masses purchased from a stock colony in a commercial laboratory Benzon Research, Carlisle, PA, USA. The oviposition paper sheets containing egg masses were cut and maintained in growth chamber at temperature of 25°C until darkening head capsules became visible through the egg chorion (blackhead stage). The number of eggs were counted using microscope stereoscope (Nikon - Stereo Zoom Microscope SMZ 645). An overall mean number of 200 eggs were used to infest each plant. Eggs were selected for uniformity and transferred into an―envelope” (6 cm in length x 4 cm high) made of organdy tissue in order to prevent desiccation or excessive moisture (new developed method of infestation). The "envelope" had an opening on top allowing the larvae output. One ―envelope” was stapled per plant.

For infestation period of time (blackhead stage), marking and position of infested plants, and egg mass position on the plant, the same methodology was followed as described for western bean cutworm larval movement study.

Destructive sampling was carried out and larval presence was recorded on all corn tissues in all plants. Fall armyworm larval movement was only considered based on the larval presence. Fall armyworm larval dispersion and distance from the release points were evaluated 14 days after infestation (DAI) in order to reach late instars already established in a feeding site. Larval survival was also calculated by the correlation of number of recovered larvae and number of infested eggs. Position of the fall armyworm larvae was recorded in each plot. Plants and field orientation, as well as evaluated parameters followed the same methodology described for western bean cutworm study.

Statistical analysis

The data were separately analyzed by species and years. Results were tested for normality and homogeneity of variance. The normal distribution was assumed. Data were analyzed using generalized mixed model (Proc Glimmix, SAS Institute 2009) to detect differences between means. When appropriate, means separated using Tukey's test significant differences procedures ($\alpha = 0.05$). The potential of maximum distance did not meet the normality and the data were transformed using arcsine square root transformation (SAS Institute 2009).

Results

Larval survivorship of western bean cutworm and fall armyworm in field corn

Larval survival was extremely low and few western bean cutworm larvae (up to five individuals) were observed in all experimental plots in 2008 and 2009 (Table 1). For 2010 and 2012, larval survival of western bean cutworm increased considerably, with a percentage of 23.31% and 17.49%, respectively. The percentage of western bean cutworm larval survival varied from 6.12% (season of 2008) to 23.31% (season of 2010). For the fall armyworm, larval survival was 5.06% in 2013 (Table 1).

Exploratory trials of western bean cutworm larval movement

In 2008, only two larvae were recovered in both axes (across and within rows). In 2009, no larvae were found in the axes related the infested plant (across and within rows) and did not allow statistical comparisons (Table 2).

There were no significant differences on larval presence among the different quadrants (NE, SE, NW, and SW), N and S orientations, across rows (E and W axes), and within infested row (N and S axes) for all years (Table 2).

No significant differences were observed on maximum distance between the quadrants (NE, SE, NW, and SW) in all experiments, except for 2010 (P= 0.0336) (Table 3). In 2010, larvae positioned at NE quadrant (2.48 m) covered in average a maximum distance significantly higher than the larvae found at NW quadrant (0.61). The SW (2.19 m) and SE (2.13 m) quadrants showed intermediate values of maximum distance and did not differ from NE and NW quadrants. There were no significant differences between N and S orientations or axes (across rows and within row) regardless the cultivation year (Table 3). In all years, one larva covered the maximum distance available in the plot for at least one replication.

Regardless of the year, western bean cutworm larvae moved similarly to all quadrants (NE, SE, SW, and NW), N and S orientations, or axes (across rows and within row), and no statistical differences on potential index were observed. Considering the quadrants (NE, NW, SE, and SW), the larvae covered 50% of the potential of distance available in maximum in 2008 (SE quadrant) and 2009 (NE quadrant) (Table 4). In 2010, the larvae moved farther at all quadrants highlighting NE quadrant, where the larvae covered in average 98% of the potential of distance available (Table 4). For N and S orientations, the larvae covered almost the potential of available distance in 2010 and showed the highest values among all experiments, with an index value of 0.98 and 0.97 at N and S orientations, respectively (Table 4).

Main experiment of western bean cutworm larval movement

There was no significant differences on number of larvae among the different quadrants (NE, SE, NW, and SW), orientations (N and S), within infested row, and across rows related the infested plant (Table 5). The larval frequency varied from 31.4% (SW) to 15.7 % (NW) between the quadrants. Considering N and S orientations, within row (N and S axes), and across rows (W and E axes), larval frequency was similar and did not vary more than 12% between the compared positions (Table 5).

Regarding the average of maximum distance covered by the western bean cutworm larvae in the different quadrants, the highest value was observed for SE position(3.54 m), but did not differ from the others (Table 5). Considering the potential of distance available, there was no significant difference between the quadrants, and the larvae did not overtake in average more than 50% of the potential at all quadrants (Table 5).

The western bean cutworm larvae moved similarly to N and S orientations and no significant differences on maximum distance was observed between the sides. Likewise, there was no statistical difference on potential of maximum distance available at both orientations (Table 5).

Considering the within infested row (N and S axes) and across rows (E and W axes) related the infested plant, there were also no statistical differences between the compared positions (Table 5).

The pattern of western bean cutworm dispersion is presented in Figure 1. Most of recovered larvae were observed on the originally plant infested or moved into the neighboring plants within the same row and neighboring rows. A number of 84 out of 263 larvae were recovered within the infested row and 198 out of 263 in an area of approximately 9 m^2 (radius of 1.7m) around the release points. The maximum distance of larval detection in cornfield was 6.8 m (NE quadrant) from the release point (Fig. 1).

Larval movement of fall armyworm

There were no statistical differences on number of fall armyworm larvae between the different quadrants (NE, SE, NW, and SW), N and S orientations, across rows (E and W axes), and within infested row (N and S axes) (Table 6). The larval frequency varied from 45.4% (SW quadrant) to 15.2 % (NE and SE quadrants). Comparing N and S orientations, the larvae distributed almost similarly with frequencies of 54.2% and 45.8 %, respectively. Within the infested row (N and S axes), 73% of the larvae moved to North side. Considering the movement of larvae across rows (W and E axes), 56.8 % of the larvae was recovered on the Western axis in relation to the infested plant (Table 6).

No significant differences were observed on the variable maximum of distance between the different quadrants, N and S orientations, and across rows. For the evaluation within the infested row (N and S axes), the larvae recovered on North axis (0.68 m) covered in average a maximum distance significantly higher than the larvae recovered on South axis (0.17 m) (P = 0.0048) (Table 6). Likewise, no significant differences were observed on potential of maximum distance available for quadrants, N and S orientations, and across rows; but the larvae recovered on North (0.45 m) side within the infested row (N and S axes) showed a potential significantly higher than the larvae on South (0.11 m) side related the infested plant ($P < 0.05$) (Table 6).

Figure 2 shows the pattern of fall armyworm dispersion in field corn. Most of recovered fall armyworm larvae were observed on the originally plant infested or moved into the neighboring plants within the same row and neighboring rows. No larvae were recovered 3 or 4 rows far from the infested row. A number of 41 out of 81 larvae were recovered within the infested row and 74 out of 81 in an area of approximately 3.8 m^2 (radius of 1.1m) around the release points. The maximum distance of larval detection in corn field was 1.9 m (SE quadrant) from the release point (Fig. 2).

Discussion

Information on larval movement of western bean cutworm and fall armyworm is very limited and one of the challenges is the difficulty to design such studies and the analysis of the data. The larval recovery of the insects is an important factor to be considered, which is commonly low (Zalucki et a., 2002). According to the same authors, low larval survival occurs mainly because of the high rate of early instars mortality of Lepidoptera species. High mortality of western bean cutworm has already been reported in the literature (Paula-Moraes et al. 2013), and accordingto these authors, the insect presents high rate of egg survival, but only a few neonates can survive to maturity. Some abiotic and biotic factors may increase these mortality rates, such as predators, pathogens, parasitoids, or morphological and chemical factors from resistant genotypes (Zalucki et al. 2002). For the exploratory trials of western bean cutworm in 2008 and 2009, the percentage of larval survival was considered low. On the other hand, a high number of larvae were recovered in 2010. Paula-Moraes et al. (2013) also reported a variability of larval survival in cornfields during three years across three regions of Nebraska. According to the authors, temperature and humidity during eggs hatching may have a high influence on larval survival.

Low larval survival could jeopardize the evaluations of larval dispersal. Therefore, the number of eggs per plot for the following experiments was higher in comparison to the exploratory trials. Thus, a reasonable number of larvae were recovered for western bean cutworm providing a better perspective about this insect' dispersion in field corn. For the western bean cutworm experiment, a number of 263 larvae were recovered in the experimental plots with an overall percentage of larval survival of 17.49%. However, the larval survival (5.06%) was considered low for fall armyworm in 2013 regardless the high infestation. According to Zalucki et al. (2002), the range of mortality also is reported to be quite variable depending on the species. Therefore, further studies focused on density of eggs are necessary to improve the larval survival of fall armyworm. Knowledge of larval survival is not only necessary to design larval behavior studies, but also critical to be incorporated in important factors in pest management strategies such as economic thresholds (Paula-Moraes et al. 2013).

Based on the results obtained in the exploratory trials of western bean cutworm, the larvae were observed to move similarly to all directions and reached the farthest plant in almost all plot sizes independent of the cultivation year. In order to achieve a more realistic western bean cutworm larval dispersion in commercial field corn, the plot size was extended to all directions in 2012, since the larvae presented a non-directional movement. Therefore, a better outlook of the insect spatial dispersion in cornfield was provided. However, such dimension still had to be possible to evaluate with accuracy. Considering the results, the new plot size was representative to characterize the western bean cutworm dispersion in 2012. Although few western bean cutworm larvae from infested plant reached distant points in the plots, an important factor that confirmed the successful design was the calculation of the potential of maximum distance available for the larvae. Most of larvae did not overtake 50% of the potential at all quadrants or axes related the infested plant. Considering the results obtained in this study, the field areas of fall armyworm study were assembled. Likewise, the potential index value did not reach more than 0.50 (50% of the potential) for any direction.

Western bean cutworm dispersed and infested similarly all directions in all years. For fall armyworm, the larvae showed a higher movement to the North side within the infested row. However, no differences were observed when considering general N and S orientations. In an overview, it seems that fall armyworm also had a nondirectional movement and this northern movement within row occurred due to a random reason influenced by any environmental factor unknown. Insect's movement between or within plants (resources) is commonly related to an active mechanism by which insects seek resources (Bell 1990). There are several possible types of information described in the literature to be used by insects to guide search orientation. Between many factors, this sensory information can be directional (Baker 1978; Harris and O'Miller 1982) or nondirectional (Barrows 1975; Bell 1990). As such, the movement patterns of both insects' larvae seemed likely to be governed by nondirectional clues.

Many positions of refuge related the *Bt* crop field has already been considered because of the target insect's behavior. Based on the results for western bean cutworm and fall armyworm, the structured refuge's placement position does not interfere in its effectiveness, but probably its distance from the *Bt* crop field when considering larval movement.

Western bean cutworm larvae presented symmetrical an aggregated spatial dispersion in cornfield, which most of the recovered larvae were observed on the originally plant infested or moved into the neighboring plants within the same row and neighboring rows. Likewise, fall armyworm also presented such dispersion. In comparison, western bean cutworm larvae seemed to have dispersed farther from the release points than fall armyworm larvae. For western bean cutworm, about32% of larvae were recovered within the infested row and 75.3% in aradius of 1.7m encompassing the release points. Conversely, Blickenstaff (1983) observed a larval dispersal of *Loxagrotis* (*Striacosta*) *albicosta* more readily in the row than across rows in common beans. Characterization of plant-to-plant larval movement of insects (plant-to-plant movement) must be considered individually for each crop and cultivation system. Depending on several factors the larvae may be able to disperse differently, or more rapidly and successfully. For example, movement within rows is more likely to happen where leaves are in contact than across rows (Blickenstaff 1983).

For fall armyworm, almost 50% of the larvae were recovered within the infested row and 91.4% of larvae in aradius (1.1m) considerably smaller. A similar pattern has been reported for fall armyworm on cotton where larvae remain predominantly withinaverage distances of 1 to 2.4 plants from the originally infested plant, not dispersingmore than five plants from the site of release (Ali et al. 1990). Ross and Ostlie (1990) reported that about 50%of the european corn borer [*Ostrinia nubilalis* Hubner (Lepidoptera: Crambidae)] larvae infesting whorl stage corn remained onthe infested plant and 90% remained within theinfested row.

Besides having significant value in ecology understanding, larval movement of each insect can influence several factors on its management, such as the scouting method. Western bean cutworm and fall armyworm presented symmetrical an aggregated spatial dispersion on corn plants, and such behavior should be considered in further sampling methods.

Considering refuge strategies, western bean cutworm presented expressive larval dispersion between rows in comparison to the other species in literature (Blickenstaff 1983; Ali et al. 1990; Ross and Ostlie 1990; Davis and Onstad 2000; Siegfried and Hellmich 2012), once larvae were found 6.8 m from the release point. Although fall armyworm seems to move less than western bean cutworm, the insect also presented expressive larval dispersion between rows. Therefore, based on the results in this study, structured refuges may be more appropriate than integrated refuges (RIB strategy) for both species. According to Mallet and Porter (1992), insects with low plant-to-plant movement are considered the best candidates for successful implementation of aRIB strategy. Insects that have higher plant-to-plant movement seeking resources would be worst candidatesfor the use of seed mixtures (Davis and Onstad 2000). In addition, the present results corroborate with Onstad et al. (2011), who presume that each species must be considered independently and should not expect a one-size-fits-all IRM plan to be ideally suited to all pest species.

Based on the results, larval survival of western bean cutworm presented high variety throughout the years. Fall armyworm larval survival was low in 2013. Western bean cutworm and fall armyworm larvae dispersed in cornfield governed by nondirectional sensory information. Both species presented aggregated and symmetrical spatial distribution (or dispersion) in cornfield, but fall armyworm remained nearby the release point more than western bean cutworm. Western bean cutworm and fall armyworm presented considered plant-to-plant movement in corn.

Different crop systems, such as the adoption of *Bt* crops, demand more information on insect's behavior. Larval feeding behavior and plant-to-plant movement can also influence how to design pest management strategies and resistance management and must be regarded. Paula-Moraes et al. (2012) characterized the on-plant movement and feeding behavior of western bean cutworm in corn. Likewise, the movement within plant and feeding requirements of fall armyworm was investigated in the previous chapter in this dissertation. However, information on plant-to-plant movement of theses pests was relative limited and still necessary to apply management strategies successfully. Due to the complexity of designing these studies, many modeling programs have been developed based on assumptions of larval dispersal.

Although further studies are encouraged to better understanding the plant-to-plant movement of western bean cutworm and fall armyworm, the present findings give great contribution in designing larval dispersal studies, as well as provide more accurate information to be used in management strategies and resistance management for these insects.

Acknowledgments

We thank to Coordination for the Improvement of Higher Education Personnel (CAPES) for a doctoral scholarship granted to the first author. To the National Council for Scientific and Technological Development – CNPq for the productivity in research fellowship granted to the fourth author.

References

Ali A, Luttrell RG, Pitre HN, 1990. Feeding sites and distribution of fall armyworm (Lepidoptera: Noctuidae) larvae on cotton. Environ. Entomol. 19, 1060-1067.

Alstad DN, Andow DA, 1995. Managing the evolution of insect resistance to transgenic plants. Science. 268, 1894-1896.

Andrewartha HG, Birch LC, 1954. The distribution and abundance of animals. Univ. Chicago Press, Chicago.

Baker RR, 1978. The Evolutionary Ecology of Animal Migration. Hodder & Stoughton, London.

Barrows EM, 1975. Individually distinctive odors in an invertebrate. Behav. BioI.15, 57-64

Bates SL, Zhao JZ, Roush RT, Shelton AM, 2005. Insect resistance management in GM crops: past, present and future. Nat. Biotechnol. 23, 57-62.

Bell WJ, 1990. Searching behavior patterns in insects. Annu. Rev. Entomol. 35, 447-67.

Blickenstaff CC, 1983. Dispersal of western bean cutworm larvae from egg masses as measured by damage to beans. Environ. Entomol. 12, 902-904.

Brooks G, Barfoot P, 2005. GM crops: the global economic and environmental impact: the first nine years 1996-2004. AgBioForum. 8, 187-196.

Buntin GD, All JN, Lee RD, Wilson DM, 2004. Plant-incorporated *Bacillus thuringiensis* resistance for control of fall armyworm and corn earworm (Lepidoptera: Noctuidae) in corn. J. Econ. Entomol. 97, 1603-1611.

Carrol MW, Head G, Caprio M, 2012. When and where a seed mix refuge makes sense for managing insect resistance to *Bt* plants. Crop Prot. 38, 74-79.

Catangui MA, Berg RK, 2006. Western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), as a potential pest of transgenic Cry 1Ab *Bacillus thuringiensis* corn hybrids in South Dakota. Environ. Entomol. 35, 1439-1452.

Chilcutt CF, Odvody GN, Correa JC, Remmers J, 2007. Effects of *Bacillus thuringiensis* transgenic corn on corn earworm and fall armyworm (Lepidoptera: Noctuidae) densities. J. Econ. Entomol. 100, 327-334.

Cullen E, Proost R, Volenberg D, 2008. Insect Resistance Management and Refuge Requirements for Bt Corn. University of Wisconsin-Extension Cooperative Extension Publishing, Madison.

Davis PM, Onstad DW, 2000. Seed mixtures as resistance management strategy for European corn borers (Lepidoptera: Crambidae) infesting transgenic corn expressing Cry1Ab protein. J Econ Entomol. 93, 937–984.

DiFonzo CD, Hammond R, 2008. Range expansion of western bean cutworm, *Striacosta albicosta* (Noctuidae), into Michigan and Ohio. Crop Manage. doi:10.1094/ CM-2008-0519-01-B..

Difonzo CD, 2015. Handy Bt trait table. International Service for the Acquisition of Agri-biotech Applications. URL http://www.isaaa.org/gmapprovaldatabase.

(EPA) Environmental Protection Agency, 1998. FIFRA scientific advisory panel, subpanel on *Bacillus thuringiensis* (B.t.) plant pesticides and resistance management. Environmental Protection Agency, Washington DC.

Farias PRS, Barbosa JC, Busoli AC, Overal WL, Miranda VS, Ribeiro SM, 2008. Spatial analysis of the distribution of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and losses in maize crop productivity using geostatistics. Neotrop. Entomol. 37, 321–327.

Goldstein JA, Mason CE, Pesek J, 2010. Dispersal and movement behavior of neonate european corn borer (Lepidoptera: Crambidae) on non-bt and transgenic Bt corn. J. Econ. Entomol. 103, 331-339.

Gould F, 1996. Deploying pesticidal engineered crops in developing countries. In: Biotechnology and Integrated Pest Management. Ed. by Persley GJ, CABI, Wallingford, UK, 264-293.

Gould F, 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Ann. Rev. Entomol. 43, 701-726.

Guse CA, Onstad DW, Buschman LL, Porter P, Higgins RA, 2002. Modeling the development of resistance by stalk-boring (Lepidoptera: Crambidae) in areas with irrigated transgenic corn. Environ. Entomol. 31, 676-685.

Hardke JT, Leonard BR, Huang F, Jackson RE, 2011. Damage and survivorship of fall armyworm (Lepidoptera: Noctuidae) ontransgenic field corn expressing *Bacillus thuringiensis* Cry proteins. Crop Prot. 30, 168-172.

Harris M, O'MillerLR, 1982. Synergism of visual and chemical stimuli in the oviposition behavior of *Delia antiqua* (Meigen) (Diptera: Anthomyiidae). Proc. 5th Int. Symp. Insect-Plant Relationships. Pudoc, Wageningen.

Head GP, Greenplate J, 2012. The design and implementation of insect resistance management programs for Bt crops. GM Crops and Food: Biotechnol. Agric. and the Food Chain 3, 144-153.

Hurley TM, Kangrock I, Ostlie K, 2006. Estimating the benefits of Bt corn and cost of insect resistance management ex ante. J. Agr. Resour. Econ.31, 355-375.

Ives AR, Glaum PR, Ziebarth NL, Andow DA, 2011. The evolution of resistance to two-toxin pyramid transgenic crops. Ecol. Appl. 21, 503–515.

Macintosh SC, 2009. Managing the risk of insect resistance to transgenic insect control traits: practical approaches in local environments. Pest Manage. Sci. 66,100-106.

Mallet J, Porter P, 1992. Preventing insect adaptation to insect resistant crops: are seed mixtures or refugia the best strategy? Proc. R. Soc. Lond. B. 250, 165-169.

Michel AP, Krupke CH, Baute TS, DiFonzo CD, 2010. Ecology and management of the western bean cutworm (Lepidoptera: Noctuidae) in corn and dry beans. J. Integr. Pest Manage. 1, 1-10.

Mitchell ER, Mcneil JN, Westbrook JK, Silvain JF, Lalanne-Cassou B, Chalfant RB, Pair SD, Waddill VH, Sotomayor-Rios A, Proshold FI, 1991. Seasonal periodicity of fall armyworm (Lepidoptera: Noctuidae) in the Caribbean basin and northward to Canada. J. Entomol. Sci. 26, 39-50.

Murphy AF, Ginzel MD, Krupke CH, 2010. Evaluating corn rootworm (Coleoptera: Chrysomelidae) emergence and root damage in a seed mix refuge. J. Econ. Entomol.103, 147-157.

Onstad DW, Gould F, 1998. Modeling the dynamics of adaptation to transgenic maize by European corn borer (Lepidoptera: Pyralidae). J. Econ. Entomol.91. 585-593.

Onstad DW, 2006. Modeling larval survival and movement to evaluate seed mixtures of transgenic corn for control of Western corn rootworm (Coleoptera: Chrysomelidae) J. Econ. Entomol. 99, 1407-1414.

Onstad DW, 2008. Insect resistance management: biology, economics and prediction. Academic, San Diego.

Onstad DW, Mitchell PD, Hurley TM, Lundgren JG, Porter RP, Krupke CH, Spencer JL, Difonzo CD, Baute TS, Hellmich RL, Buschman LL, Hutchison WD, Tooker JF, 2011. Seeds of Change: Corn Seed Mixtures for Resistance Management and Integrated Pest Management. J. Econ. Entomol. 104, 343-352.

O'Rourke PK, Hutchison WD, 2000. First report of the western bean cutworm *Richia albicosta* (Smith) (Lepidoptera: Noctuidae), in Minnesota corn. J. Agric. Urban Entomol. 17, 213-217.

Ostlie K, Pedigo L, 1987. Incorporating pest survivorship into economic thresholds. Bull. Entomol. Soc. Am. 33, 98-102.

Paula-Moraes SV, Hunt TE, Wright RJ, Hein GL, Blankenship EE, 2012. On-Plant movement and feeding of bean cutworm (Lepidoptera: Noctuidae) early instars on Corn. Environ. Entomol. 41, 1494-1500.

Paula-Moraes S, Hunt TE, Wright RJ, Hein GL, Blankenship EE, 2013. Western bean cutworm survival and the development of economic injury levels and ecnonomic thresholds in field corn. J. Econ. Entomol. 106, 1274-1285.

Peck S, Gould F, Ellner S, 1999. Spread of resistance in spatially extended regions of transgenic cotton: implications for management of *Heliothis virescens* (Lepidoptera: Noctuidae). J. Econ. Entomol. 92, 1-16.

Petzold-Maxwell JL, Alves AP, Estes RE, Gray ME, Meinke LJ, Shields EJ, Thompson SD, Tinsley NA, Gassmann AJ, 2013, Applying an Integrated Refuge to manage Western corn rootworm (Coleoptera: Chrysomelidae): Effects on survival, fitness, and selection pressure. J. Econ. Entomol. 106, 2195-2207.

Price PW, 1997. Insect Ecology. Third edition. John Wiley & Sons, New York. 874p.

Ritchie SE, Hanway JJ, Benson GO, 1993. How a corn plant develops. University of Iowa Cooperative Extension Service- Special Reportn.48, Ames, Iowa.

Ross SE, Ostlie KR, 1990. Dispersal and survival of early instars of European corn borer (Lepidoptera: Pyralidae) in field corn. J. Econ. Entomol. 83, 831-836.

SAS Institute, 2009. SAS user's guide: statistics, version. 9.1.3. SAS Institute, Cary, NC.

Shelton AM, Zhao JZ, Roush RT, 2002. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. Annu. Rev. Entomol. 47, 845-881.

Siegfried BD, Hellmich RL, 2012. Understanding successful resistance management: the European corn borer and Bt corn in the United States. GM Crops Food3,184-193.

Tabashnik BE, Carrière Y, Dennehy TJ, Morin S, Sisterson MS, Roush RT, Shelton AM, Zhao JZ, 2003. Insect Resistance to transgenic Bt crops: lessons from the laboratory and field. J. Econ. Entomol. 96, 1031-1038.

Tabashnik BE, Gassmann AJ, Crowder DW, Carrière Y, 2008. Insect resistance to Bt crops: evidence versus theory. Nat. Biotechnol. 26, 199–202.

Tabashnik BE, Van Rensburg JBJ, Carrière Y, 2009. Field-evolved insect resistance to Bt crops: definition, theory, and data. J. Econ. Entomol. 102, 211-225.

Tabashnik BE, Brévault T, Carrière Y, 2013. Insect resistance to Bt crops: lessons from the first billion acres. Nat. Biotechnol. 31, 510-521.

Tikkanen OP, Carr TG, Roininen H, 1999. Factors influencing the distribution of a generalist spring-feeding moth, *Operophtera brumata* (Lepidoptera: Geometridae) on host plants. Environ. Entomol. 28, 461- 469.

Tooker JF, Fleischer SJ, 2010. First report of western bean cutworm (*Striacosta albicosta*) in Pennsylvania. Crop Manage. doi: http://dx.doi.org/10.1094/CM-2010-0616-01- RS.

Wangila DS, Leonard BR, Ghimire MN, Bai Y, Zhang L, Yang Y, Emfinger KD, Head GP, Yang F, Niu Y, Huang F, 2013. Occurrence and larval movement of *Diatraea saccharalis* (Lepidoptera: Crambidae) in seed mixes of non-Bt and Bt pyramid corn. Pest Manage. Sci. 69, 1163–1172.

Zalucki MP, Clarke AR, Malcom SB, 2002. Ecology and behavior of first instar larval Lepidoptera. Annu. Rev. Entomol. 47, 361-393.

Table 1 Western bean cutworm and fall armyworm larval survival in cornfield. Concord, NE, USA,from 2008 to 2013

Year	% larval survival[1]	Number of larvae
Western bean cutworm		
2008	6.12	6
2009	6.94	5
2010	23.31	107
2012	17.49	263
Fall armyworm		
2013	5.06	81

[1] Larval survival percentage based on total number of eggs infested.

Table 2 Mean number (±SE) of western bean cutworm larvae in different positions related the infested plant in cornfield. Concord, NE, USA, from 2008 to 2010

	Concord 2008	Concord 2009	Concord 2010
	---------------------- WBC recovered larvae/damage ----------------------		
Position	Mean[1]	Mean[1]	Mean[1]
NE	0.50 (0.50) a	1.00 (1.00) a	5.00 (1.00) a
NW	0.00 (0.00) a	0.50 (0.50) a	1.67 (1.67) a
SE	0.50 (0.50) a	0.50 (0.50) a	3.67 (1.76) a
SW	0.00 (0.00) a	0.00 (0.00) a	2.67 (0.88) a
P value	0.6151	0.7344	0.4190
N	1.50 (0.50) a	1.00 (1.00) a	11.67 (3.67) a
S	0.50 (0.50) a	1.00 (0.00) a	11.67 (2.91) a
P value	0.2929	1.0000	1.0000
Within rows			
N	1.00 (1.00) a	0.00	5.00 (1.00) a
S	0.00 (0.00) a	0.00	5.33 (0.33) a
P value	0.4226	---	0.7676
Across rows			
W	0.00	0.00	0.33 (0.33) a
E	0.00	0.00	0.33 (0.33) a
P value	---	---	1.0000

[1] Mean number of western bean cutworm larvae. Tukey (± SEM). The means with the same letter are not significantly different, P ≥ 0.05.

Table 3 Mean value (±SE) of maximum distance covered by western bean cutworm larvae in different positions related the infested plant in cornfield. Concord, NE, USA, from 2008 to 2010

	Concord 2008	Concord 2009	Concord 2010
	---------- Maximum distance (m) ----------		
Position	Mean[1]	Mean[1]	Mean[1]
NE	1.10 (1.10) a	1.27 (1.27) a	2.48 (0.06) a
NW	0.00 (0.00) a	0.00 (0.00) a	0.61 (0.61) b
SE	1.48 (1.48) a	0.92 (0.92) a	2.13 (0.41) ab
SW	0.00 (0.00) a	0.74 (0.74) a	2.19 (0.20) ab
P value	0.6083	0.7718	0.03336
N	1.45 (0.74) a	1.27 (1.27) a	2.48 (0.06) a
S	1.48 (1.48) a	1.65 (0.18) a	2.30 (0.24) a
P value	0.9893	0.7950	0.5094
Within rows			
N	0.36 (0.36) a	0.00 (0.00) a	1.19 (0.26) a
S	0.00 (0.00) a	0.00 (0.00) a	1.66 (0.06) a
P value	0.4226	---	0.1495
Across rows			
W	0.00 (0.00) a	0.00 (0.00) a	0.25 (0.25) a
E	0.00 (0.00) a	0.00 (0.00) a	0.25 (0.25) a
P value	---	---	1.0000

[1]Maximum distance covered by western bean cutworm larvae. Tukey (± SEM). The means with the same letter are not significantly different, P ≥ 0.05.

Table 4 Potential (±SE) of maximum distance covered by western bean cutworm larvae in different postions related the infested plant in cornfield. Concord, NE, USA, from 2008 to 2010

	Concord 2008	Concord 2009	Concord 2010
	---------- Potential of maximum distance ---------		
Position	Index value[1]	Index value[1]	Index value[1]
NE	0.37 (0.37) a	0.50 (0.50) a	0.98 (0.02) a
NW	0.00 (0.00) a	0.00 (0.00) a	0.24 (0.24) a
SE	0.50 (0.50) a	0.36 (0.36) a	0.84 (0.16) a
SW	0.00 (0.00) a	0.29 (0.29) a	0.86 (0.08) a
P value	0.4226	0.7620	0.1236
N	0.62 (0.12) a	0.50 (0.50) a	0.98 (0.02) a
S	0.50 (0.50) a	0.65 (0.07) a	0.97 (0.03) a
P value	0.8882	0.8635	0.6663
Within rows			
N	0.25 (0.25) a	0.00	0.67 (0.15) a
S	0.00 (0.00) a	0.00	0.93 (0.03) a
P value	0.4226	---	0.1236
Across rows			
W	0.00	0.00	0.33 (0.33) a
E	0.00	0.00	0.33 (0.33) a
P value	----	----	1.0000

[1] Potential of maximum distance covered by western bean cutworm larvae. Tukey (± SEM). The means with the same letter are not significantly different, P $\geq$ 0.05. Potential of maximum distance was calculated through an index of the maximum distance covered by the farthest larvae related the maximum distance they could cover in each quadrant. This index varies from 0 (no movement) to 1.00 (100% of covered distance).

Table 5 Mean value (±SE) of recovered larvae, frequency, maximum distance, and potential of maximum distance available for western bean cutworm larvae in different positions related the infested plant in cornfield. Concord, NE,USA, 2012

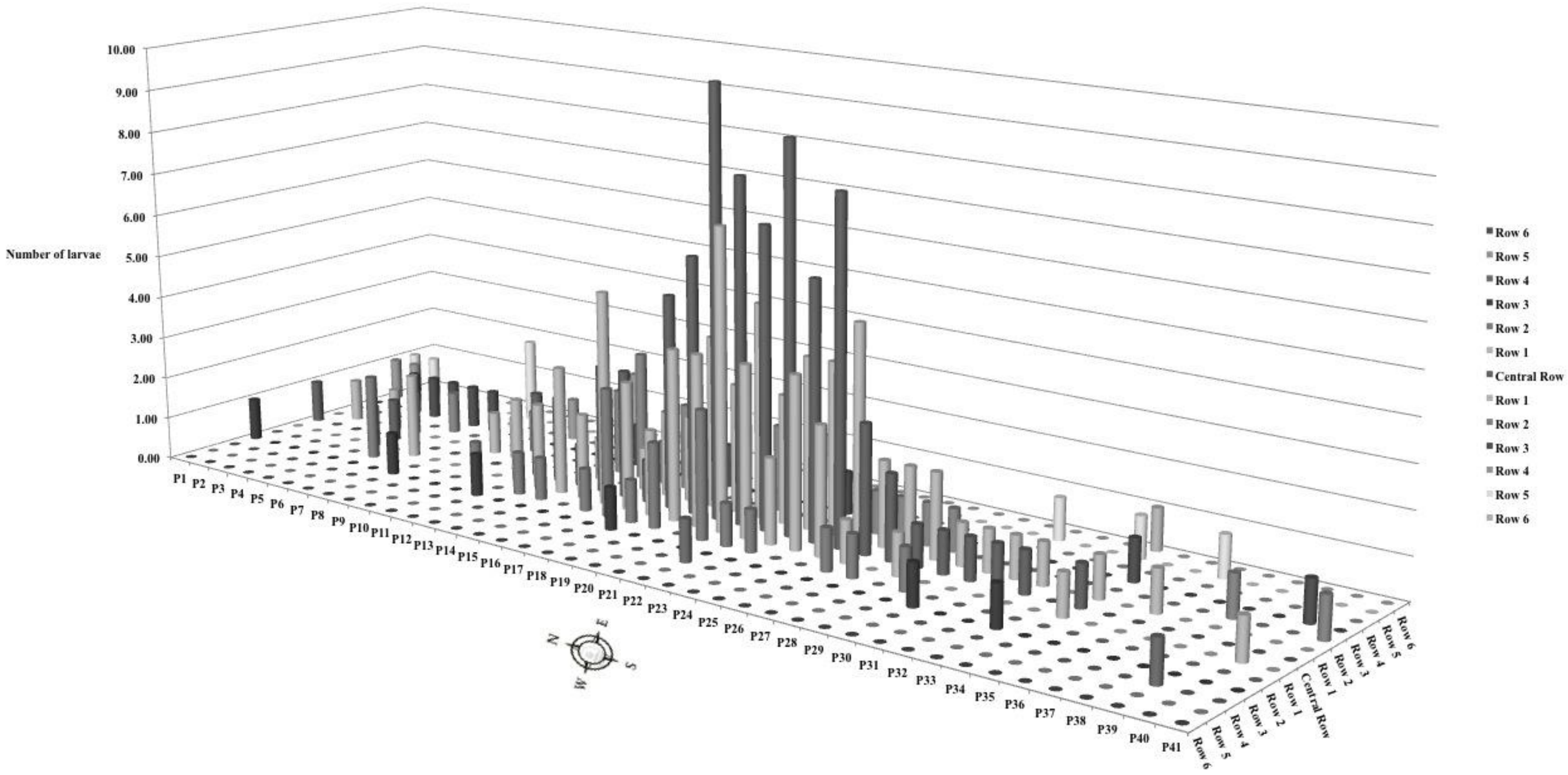
	Concord 2012			
	---Recovered larvae/damage---		------ Maximum distance ------	
Position	Mean[1]	Frequency (%)[2]	Mean (m)[3]	Index value[4]
NE	3.67 (1.12) a	23.6	2.96 (0.60) a	0.39 (0.08) a
NW	2.44 (0.84) a	15.7	1.41 (0.46) a	0.18 (0.06) a
SE	4.56 (1.21) a	29.3	3.54 (0.84) a	0.46 (0.11) a
SW	4.89 (2.03) a	31.4	1.86 (0.48) a	0.24 (0.06) a
P value	0.5994	---	0.0727	0.1608
N	10.78 (2.58) a	44.3	3.29 (0.53) a	0.47 (0.07) a
S	13.56 (3.63) a	55.7	3.71 (0.81) a	0.57 (0.12) a
P value	0.6664	---	0.9048	0.8421
Within rows				
N	4.67 (1.18) a	53.2	0.91 (0.18) a	0.39 (0.06) a
S	4.11 (0.90) a	46.8	1.19 (0.33) a	0.30 (0.11) a
P value	0.7133	---	0.4226	0.4226
Across rows				
W	0.78 (0.28) a	53.8	0.51 (0.33) a	0.11 (0.04) a
E	0.67 (0.24) a	46.2	0.68 (0.32) a	0.15 (0.07) a
P value	0.7643	----	0.6525	0.7860

[1] Number of western bean cutworm recovered larvae. Tukey (± SEM). The means with the same letter are not significantly different, P $\geq$ 0.05.

[2] Frequency (%) of western bean cutworm larvae in each position.

[3]Maximum distance covered by western bean cutworm larvae. Tukey (± SEM). The means with the same letter are not significantly different, P $\geq$ 0.05.

[4] Potential of maximum distance covered by western bean cutworm larvae. Tukey (± SEM). The means with the same letter are not significantly different, P $\geq$ 0.05. Potential of maximum distance was calculated through an index of the maximum distance covered by the farthest larvae related the maximum distance they could cover in each quadrant. This index varies from 0 (no movement) to 1.00 (100% of covered distance).



Distance of 0.76 m between rows and 0.15 m between plants.

Fig. 1 Pattern of western bean cutworm larval spatial distribution in corn field. Concord, NE,USA, 2012

Table 6 Mean value (±SE) of recovered larvae, frequency, maximum distance, and potential of maximum distance available for fall armyworm larvae in different positions related the infested plant in cornfield. Concord, NE, USA, 2013

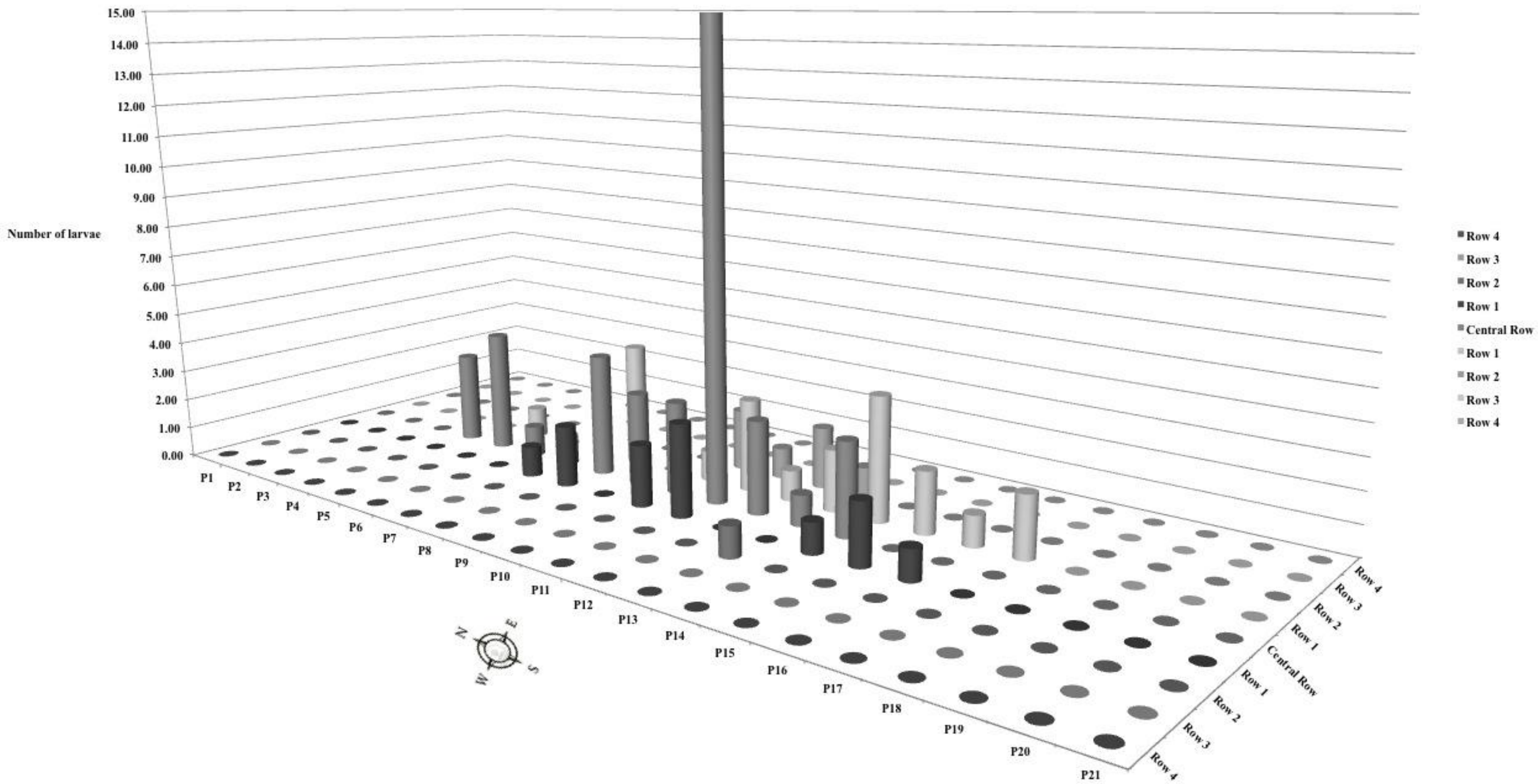
	Concord 2013			
	-----Recovered larvae -----		------ Maximum distance ------	
Position	Mean[1]	Frequency (%)[2]	Mean (m)[3]	Index value[4]
NE	0.63 (0.32) a	15.2	0.44 (0.22) a	0.10 (0.05) a
NW	1.00 (0.33) a	24.2	0.89 (0.28) a	0.20 (0.06) a
SE	0.63 (0.32) a	15.2	0.57 (0.28) a	0.13 (0.06) a
SW	1.88 (0.40) a	45.4	1.34 (0.21) a	0.30 (0.05) a
P value	0.0511	---	0.0658	0.0878
N	4.00 (1.24) a	54.2	1.00 (0.25) a	0.45 (0.09) a
S	3.38 (0.68) a	45.8	1.40 (0.21) a	0.31 (0.05) a
P value	0.6651	---	0.2905	0.1955
Within rows				
N	2.38 (0.73) a	73	0.68 (0.13) a	0.45 (0.09) a
S	0.88 (0.35) a	27	0.17 (0.07) b	0.11 (0.05) b
P value	0.0853	---	0.0048	0.0031
Across rows				
W	0.50 (0.19) a	56.8	0.48 (0.20) a	0.16 (0.07) a
E	0.38 (0.26) a	43.2	0.19 (0.12) a	0.06 (0.04) a
P value	0.7054	---	0.2462	0.2740

[1] Mean number of fall armyworm recovered larvae. Tukey (± SEM). The means with the same letter are not significantly different, $P \geq 0.05$.

[2] Frequency (%) of fall armyworm larvae in each position.

[3] Maximum distance covered by fall armyworm larvae. Tukey (± SEM). The means with the same letter are not significantly different, $P \geq 0.05$.

[4] Potential of maximum distance covered by fall armyworm larvae. Tukey (± SEM). The means with the same letter are not significantly different, $P \geq 0.05$. Potential of maximum distance was calculated through an index of the maximum distance covered by the farthest larvae related the maximum distance they could cover in each quadrant. This index varies from 0 (no movement) to 1.00 (100% of covered distance).



Distance of 0.76 m between rows and 0.178 m between plants.

Fig. 2 Pattern of fall armyworm spatial distribution in corn field. Concord, NE, USA, 2013

CHAPTER III - Designing marking techniques for early larval instars of *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) and their application for behavioral studies using soybean as model

Revista: Journal of Economic Entomology

Abstract

Larval studies focusing on early instars have important implications and may help for choosing the most efficient pest management strategy. However, such research is highly limited mainly because of the difficulty for handling and observing so tiny insects. Many of these studies require the marking individual. Up to date, a great deal of marking researches has been focused on adults and studies designing alternative methods to mark early instars larvae are needed. This work aimed to investigate the efficiency of marking approaches on early instars larvae of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) and their application in its behavioral study using soybean as model. Five consecutive trials were performed in laboratory (Temp.: 25 ± 2 ºC, RH: 60 ± 10%; 14:10-L:D) in order to identify the best option for marking *H. armigera* larvae, besides a behavioral study with larvae marked by dusting using soybean plants in greenhouse conditions. In laboratory, the rate of marked larvae and effects of Luminous Powder (BioQuip, Rancho Dominguez, CA) and Sudan (Sigma-Aldrich Corporation, St. Louis, MO) (200, 400, 600 ppm) dye diets on biological performance of *H. armigera* larvae were evaluated. The persistence of Red Luminous powder (600 ppm) dye diet and Sudan Red 7B (200 ppm) dye diet in the larvae were observed. The efficiency of these markers was also assessed on eggs by spraying dye solutions (1g of dye/10 mL of corn oil), and externally on larvae by dusting. Based on laboratory results, Red Luminous Powder dye was selected for a behavioral second-instar larvae study in greenhouse. In laboratory, Sudan Blue or Sudan Red 7B can be incorporated in old world cotton bollworm artificial diet at 200 ppm to obtain marked insects without major effects on their biology or behavior. Likewise, Red Luminous Powder dye into diet at 600 ppm was more efficient in obtaining marked larvae without deleterious effects on their biology or behavior. This is the first report of the use of Luminous Powder dye diet on biological aspects of *H. armigera*. Larvae reared on Sudan Red 7B dye diet at 200 ppm and Luminous Powder Red dye diet at 600 ppm until second instar did not show visual dye traces at posterior instars when transferred to regular diet. Both dyes marked old world cotton bollworm externally with success by dusting procedure. The external marking lasted one larval instar without deleterious effects, which can be useful for short-term studies. In greenhouse, the dusting marking allowed verifying that second-instar larvae of *H. armigera* did not show variation on larval movement regardless the period of the day (morning, afternoon and evening). After few hours, most of larvae established their feeding site and remained feeding on soybean leaves, not justifying overnight insecticide sprayings to control the insect.

Keywordslarval marking tools, dye,*Helicoverpa armigera*.

Introduction

Among its several important aspects, movement of early Lepidoptera instars larvae largely determines where feeding sites become established and deserve great attention (Zalucki et al. 2002, Perkins et al. 2008). During their reconnaissance phase, larvae are vulnerable to predators, parasitoids, and pathogens (Zalucki et al. 1986, 2002, Johnson et al. 2007, Perkins et al. 2008), as well as the use of control strategies. Finding this gap is critical to apply pest management strategies (Ross and Ostlie 1990, Spangler and Calvin 2001, Paula-Moraes et al. 2012). Depending on the feeding site, many Lepidoptera species can find shelter, which could limit the use of important control strategies such as biological and conventional control through pesticides spraying.

Recently, larval dispersal of Lepidoptera species has also become important for resistance management in transgenic crops, because the mobility of the species impact on larval exposure to lethal and sublethal concentrations of *Bt* toxins. In addition, larval feeding behavior, on-plant movement, and plant-to-plant movement can lead how to design strategies to manage resistance (Gould 1998, Dirie et al. 2000, Shelton et al. 2002, Paula-Moraes et al. 2012).

Although having decisive and recognized importance for the successful of insect's management, larval studies focusing on early instars are rare in literature, probably due to difficulty to handle and observe initial stages (Zalucki et al. 2002). In addition, these behaviors can be nocturnal for many species (EPPO 1981), which makes difficult to observe in normal conditions, especially in field areas. Studying these specific behavioral aspects becomes even more difficult in tropical countries where the pressure of natural infestations of insect populations generally creates confounding effects.

Therefore, many investigations of insect behavioral studies require the marking individual. A wide variety of marking techinques have been used for insect studies (Akey 1991, Southwood and Henderson 2000, Hagler and Jackson 2001), ranging from technological and expensive methods, likeradar or molecular markers, to less costly approaches such as paints ordyes (Warner and Bierzychudek 2009). Among the desirable characteristics, markers should be easily applied and recognized, require minimal manipulation, persist in a long term, and do not offer deleterious effects to the recipient (Gangwere et al. 1964, Bartlett 1982, Akey 1991).

Dye is considered an ideal marking material, because it is inexpensive, non-toxic, and easily applied and identifiable (Hagler and Jackson 2001, Qureshi et al. 2004, Zhao et al. 2008). Several species of Coleoptera, Lepidoptera, Diptera, Isoptera, and Hymenoptera have been successfully marked with dyes (Ostlie et al. 1984, Hunt et al. 2000, Qureshi et al. 2004, Vilarinho et al. 2006, Zhao et al. 2008, Vilarinho et al. 2011).

Dyes have been applied internally and externally to mark multiple insect's life stages. External marking by dusting dye is the most popular method in use to mark larvae and adults. However, concerns using dusts have been reported such as the difficulty in getting lasting adherence, and disturbances in biological aspects of the insects (Akey 1991), which require deeper studies using this marking technique. Dyes incorporated into larval diet have been used to mark insects internally, mainly for sterile insect release programs, are-wide pest management programs, and resistance-management programs (Reynolds et al. 1997, Shimoji et al. 1999, Qureshi et al. 2004, Stephens et al. 2008, Vilarinho et al. 2011). Although, different dyes in different concentrations can show variable efficiencies and effects on the development of different species (Hendricks 1971, Qureshi et al. 2004, Vilarinho et al. 2006). As such, preliminary evaluations are needed to verify the efficacy of each marker dye and identify suitable insect species (Qureshi et al. 2004). In addition, a great deal of researches using dye has been focused on marking adults (Reynolds et al. 1997, Shimoji et al. 1999, Qureshi et al. 2004, Stephens et al. 2008, Vilarinho et al. 2011), but few or none focused on early larval instars.

Egg marking would be a good approach to mark early instars larvae. The use of marked F_1 offspring from adults fed on dye diets has been reported in the literature (Qureshi et al. 2004, Zhao et al. 2008). However, the results are often inconsistent. Zhao et al. (2008) observed no marking effect on F_1 offspring from marked adults of cotton bollworm using Sudan dye diet. In addition, dye diets can also negatively effect the pupation or adult's emergence (Zhao et al. 2008).

Facing of the previous considerations, investigations on alternative methods for marking early larval instars should be encouraged. Designing such techniques has important implications in our understanding of larval behavior, and is critical to obtain success from pest management strategies.

Among several harmful pests worldwide, *Helicoverpa armigera* (Lepidoptera: Noctuidae) has great importance for several economically important crops (Perkins et al. 2008). This species was first reported in Brazil (Embrapa 2013), causing economic impact in several crops, such as cotton, soybean, corn, dry beans and tomato (Czepak et al. 2013, Specht et al. 2013) and should be target of many studies from now.

Considering *H. armigera* potential of damaging economically important crops, the difficulty to assess early larval instars and its critical importance in pest management strategies, this study aimed to investigate the efficiency of new marking approaches on eggs and early instars larvae of *H. armigera*. A study at greenhouse was also conducted in order to evaluate the effect of the method in the behavior of *H. armigera*.

Material and Methods

The bioassays were carried out in laboratory (Temp.: 25 ± 2 ºC, RH: 60 ± 10%; 14:10-L:D) and greenhouse conditions in 2015 at Sao Paulo State University, Department of Crop Protection, Botucatu, SP, Brazil. A stock rearing of *H. armigera* (Parra 2001) was maintained at same environmental conditionsin order to provide sufficient insects for the bioassays. Preparation of solutions and artificial diet were similar for the following bioassays. We used the bean-based artificial diet for *H. armigera* proposed by Parra (2001). Two different oil-soluble brand dyes were used to mark the insects: Luminous Powder (blue and red) purchased from BioQuip Products (Rancho Dominguez, CA), and Sudan (blue and red 7B) purchased from Sigma-Aldrich Corporation (St. Louis, MO). The following evaluations on marked old world cotton bollworm were performed: (a) effects of Luminous Powder and Sudan dyes on biology of *H. armigera*; (b) marking efficiency of Luminous Powder and Sudan dyes on *H. armigera*; (c) persistence of Luminous Powder and Sudan dye diets on *H. armigera*; (d)effects of Luminous Powder and Sudan dye solutions on eggs of *H. armigera*;(e)persistenceof dusting with markers on second-instar larvae of *H. armigera*; (f) behavioral aspects of second-instar larvae of *H. armigera*using soybean**.**

(a) Effects of Luminous Powder and Sudan Dyes on biology of *H. armigera*

The dyes were previously diluted in corn oil (1 g of dye/10 ml of oil) for posterior incorporation into the artificial diet to obtain the specific concentrations (e.g. 0.2 ml of the dye solution was incorporated into 1 liter of diet for a final concentration of 200 ppm) (Vilarinho et al. 2006, Vilarinho et al. 2011). The diets (with and without dye) were prepared and placed into 100-ml plastic cups (approximately 15 ml per cup). After cooling, the cups were kept closed by using a plastic lid.

Forty old world cotton bollwormfirst-instar larvae were individualized in the cups per treatment along with the diet. Each cup represented one replication (40 in a total) in a completely randomized design. The treatments were control (only diet), corn oil enriched diet (corn oil control), and oil-soluble-dye enriched diets (200, 400, and 600 ppm concentrations). Each diet was prepared separately. The biological parameters evaluated were: larval survival, larval period, pupal weight, and percentage of pupal deformation. Insects were daily assessed. Pupal weight was determined by using a scale 0.0001g (Model AY220 Marte, Shimadtzu, Kyoto, Japan).

Considering that the dyes and concentrations might have negative effects on the biology and behavior of *H. armigera*, the decision was to consider the data from each dye separately in order to find the optimal concentration for the following bioassays using dye incorporated into diet.

(b) Marking efficiency of Luminous Powder and Sudan dyes on*H. armigera*

Thirty old world cotton bollwormfirst-instars larvae were individualized in 100-ml plastic cups following the same methodology described for the bioassay (a). The study was arranged in a completely randomized design with 30 replications and each replication was composed of one cup containing one larva. The same dye colors and concentrations were again added to artificial diet. The treatments were composed of the oil-soluble-dye enriched diets (200, 400, and 600 ppm concentrations) for both Luminous and Sudan dyes. The larvae were kept feeding on the treatments until they reached the fifth instar. Then, the larvae were frozen (-5°C) for further evaluations. Two different marking observations were performed: internal by squeezing the larvae using a pestle of porcelain, and external by visualization under light (normal for Sudan and UV for Luminous Powder). UV light procedure was performed by using a UV flashlight (Ultra Fire WF-501B, Latarka, Guangdong, China). The percentage of marked insects was determined for each treatment. The dyes were also evaluated separately as described for the bioassay (a). Based on this and previous results the concentrations of each dye was defined to be incorporated into diet for the following bioassays.

(c) Persistence of Luminous Powder and Sudan dye diets on *H. armigera*

Red Luminous powder dye diet at 600 ppm and Red 7B Sudan dye diet at 200 ppm were used for this bioassay. Ninety first-instars larvae of old world cotton bollworm were kept feeding on each oil-soluble-dye enriched diet in a 1-L container. When they turned second instar (up to 12 hours in second instar), larvae were individualized in 100-ml plastic cups with the regular diet (without dye). Thirty individualized larvae of each dye concentration were evaluated at the three posterior instars (third, fourth, and fifth instars).

There were 30 replications randomly assigned as a completely randomized design and each replication was composed of one cup with one larva. The treatments were: Red Luminous Powder (600 ppm concentration) and Sudan Red 7B (200 ppm) oil-soluble-dye enriched diets. The percentage of marked insects was determined. The same procedures for marking visualizations were employed as described for the bioassay (b).

(d) Effects of Luminous Powder and Sudan dye solutions on eggs of *H. armigera*

Pieces of paper sheets containing egg masses were collected from the stock rearing and the number of eggs was assessed under microscope stereoscope (Stereo Zoom Microscope SMZ 645, Nikon, Tokyo, Japan). One hundred eggs were evaluated per treatment with four replications (400 in total). The study was conducted in a completely randomized design. Each dye (Red Luminous Powder and Sudan Red 7B) was separately diluted in corn oil in a proportion of 1 g of dye per 10 ml of corn oil (Vilarinho et al. 2006, Vilarinho et al. 2011). The solutions were sprayed on the paper sheets containing the eggs, until full coverage of the area. The spraying was conducted by using an airbrush (Airbrush 684040, Lee Tools, Santo André, Brazil) coupled in an air compressor (Pratic Air CSA 8.2/25, Schulz, Joinville, Brazil), in order to produce small droplets. After drying (30 min in average), the paper sheets where then attached on the plastic lid of 700-ml plastic cups containing the regular diet and kept closed until the evaluations. The treatments were control (without spraying), corn oil, Red Luminous Powder solution, and Sudan Red 7B solution. The percentage of hatched larvae and the number of marked neonates were evaluated. The evaluations were performed two days after eggs hatching. The marking visualizations were performed as described for the bioassay (b).

(e) Persistenceof dusting with markers on second-instar larvae of *H. armigera*

First-instar larvae of *H. armigera* were monitored until the second instar (< 6h). Then, the dusting was performed. A number of five second-instar larvae were used per treatment with 10 replications (50 individuals in total). The study was conducted in a completely randomized design. Before splitting in five-larvae per replication, the 50 larvae (individuals) from each treatment were placed in a 1-L container for subsequent dusting. After dusting, larvae were separated (5 by 5) in 100-ml plastic cups containing 15 ml of regular diet and closed with plastic lid. Each cup represented one replication. Red Luminous Powder and Sudan Red 7B dyes were used for external labeling of the larvae. An amount of 0.5g of each dye was filtered and dusted on the fifty larvae. The powders were pre-filtered with a nylon fabric (200 mesh), and applied on the larvae in a sufficient amount to not clog the insect spiracles. Treatments were control (larvae without dusting), Sudan Red 7B, and Red Luminous Powder. The number of marked larvae and instar period were evaluated every 12 h until all the larvae lose the external marking. The external marking visualization was checked as described previously.

(f) Behavioral aspects of second-instar larvae of *H. armigera*using soybean

The experiment was conducted under greenhouse conditions (Temp.: 25 ± 4 ºC, RH: 60 ± 10%; natural light), and soybean plants were used as model to investigate the behavioral study of *H. armigera*.Soybean was chosen as model because of the large amount of leaves presented in the plant, as well as the second-instar larvae would probably not conceal in the reproductive organs, which would allow a better judgement of the marking method's efficiency.Soybean "Conquista" genotype was cultivated in 5-L pots containing autoclaved substrate (soil, sand, and organic matter at ratio of 1:1:1). The substrate was fertilized as recommended for the crop (Mascarenhas and Tanaka 1997). The plants were individualized into cages (45 cm diameter x 65 cm) when reached the R4-R5 reproductive stage (Fehr and Caviness 1977), and subsequently infested with 15 second-instar larvae of *H. armigera*. The cages were kept covered with voile fabric during all experiment period.

Prior to infestation, the larvae were externally marked with Red Luminous Powder dye following the dusting methodology described for bioassay (d). There were five replications and each one was composed of one plant with 15 larvae. The behavioral evaluations were performed in morning, afternoon and evening. The experiment was randomly assigned in a complete block design. Each plant composed one block. The evaluated parameters were number of observed larvae, larval movement (behavior), and feeding site choice. To verify these behaviors, the efficiency of the marking effects on the number of larvae had to be tested during the different periods of the day. Therefore, observing the number of larvae up to 48h after infestation compared the efficiency of the dusting marking. The number of larvae was calculated by averaging the values of the first two days, since these days allowed nocturnal evaluations and no larva missed the marking up to this period. The larval behavior was divided in two categories: static (feeding or resting) and dispersing (crawling or ballooning). Feeding site choice was divided in two categories: leaves and pods consumption. The treatment design was a 2 by 3 factorial, which corresponds to two larval behavior (static or dispersing behavior for larval movement variable; and leaves or pods consumption for feeding site choice variable), and three different periods of the day (morning, afternoon, and evening).

The evaluations were performed until all the larvae lose the external marking. At this time, all larvae were recovered for posterior instar classification based on the measurement of head capsule width (Butler 1976). Morning, afternoon, and evening evaluations were conducted at 6-7 a.m., 2-3 p.m., and 8-9 p.m., respectively. Morning and afternoon evaluations were conducted under natural light, and evening evaluations under UV light as described for the bioassay (b).

Statistical analyses

Results were tested for normality and homogeneity of variance. The normal distribution was assumed. Data were analyzed using generalized mixed model (Proc Glimmix, SAS Institute 2009) to detect differences between means. When appropriate, means separated using Fisher's least significant differences procedures ($\alpha = 0.05$).

Results

Effects of Luminous Powder and Sudan Dyes on biology of *H. armigera*

The addition of Luminous Powder dye at 200, 400, 600 ppm concentrations and/or corn oil did not interfere significantly on larval survival, pupal weight, and pupal deformation (Table 1).There were no differences among treatments for larval period between the control insects, and larvae fed diet containing blue (200 and 400 ppm) or red (600 ppm) dye diets. However larval periods with the blue (600 ppm) and red (200 and 400 ppm) dye diets, and corn oil control were significantly higher ($P = 0.0052$).

When the larvae were fed on diets containing Sudan dye, differences were observed in larval survival among treatments and controls ($P<0.0001$) (Table 2). The larval survival for insects fed on diets containing blue dye (200 ppm), red (200 and 400 ppm) dye, and corn oil did not differ from the control insects, however, the blue (400 and 600 ppm) and red (600 ppm) dye diets increased the larval mortality. Likewise, the larval period showed differences among treatments and controls ($P <0.0001$). The larval period for insects fed on diet containing blue (400 ppm) and red (400 and 600 ppm) dyes was significant higher when compared to the control insects. Insects that fed on diet containing blue dye at 200 ppm showed the lowest larval period differing from the other treatments, except from the control insects. The addition of corn oil into the diet did not cause significant effect on larval period when compared to the control insects. There were no differences between treatments and controls for pupal weight and deformation rates (Table 2).

Marking efficiency of Luminous Powder and Sudan dyes on *H. armigera*

For the Luminous Powder dyes, there were no differences among the treatments for internal marking (Table 3). The percentage of insects showing internal marking ranged from 90 % (red at 600 ppm) to 72.50 % (blue at 200 and 600 ppm). However, the larvae that fed on diets with the red dye color (200, 400, and 600 ppm) showed 100 % of external marking under UV light, differing from the blue dye color at 200 and 400 ppm (P = 0.0002) (Table 3).

The percentage of larvae externally and internally marked was 100 % on the diets containing Sudan Blue and Red 7B at 200, 400, and 600 ppm (Table 3).

Persistence of Luminous Powder and Sudan dye diets on *H. armigera*

Regardless of the visualization methods used for this evaluation, no visual dyes' traces were detected at posterior instars on the larvae that fed on Red Luminous Powder dye diet at 600 ppm and Sudan Red 7B dye diet at 200 ppm until the second instar.

Effects of Luminous Powder and Sudan Dye solutions on eggs of *H. armigera*

The number of larvae recovered ranged from 63.20 (Sudan dye solution) to 73.00 (control), however there were no differences between the dye solutions and controls for the number of hatched larvae (Table 4).

Persistenceof dusting with markers on second-instar larvae of*H. armigera*

There were no significant differences between the treatments for the percentage of larvae externally marked by dusting procedure in all evaluations (Table 5). The percentage was 100 % for the Red Luminous Powder and Sudan Red 7B dyes up to 24h after dusting. Although some larvae lost the external marking at 36 and 48h, the percentage remained high with 90 % (Sudan dye) and 82 % (Sudan dye), respectively. On the other hand, most of larvae lost the external marking from 60h after dusting with 10% and 6% of marked larvae for Luminous Powder Red and Sudan Red 7B dyes, respectively. At 72h after dusting, no larvae showed visual external marking (Table 5). The second instar period of *H. armigera*varied from 57.36 hours (Sudan Red 7B dye) to 59.52 hours (Red Luminous Powder) (Table 5), however there were no differences between the treatments.

Behavioral aspects of second-instar larvae of *H. armigera*using soybean

The plants were infested during the morning on the first sampling date, and most of larvae were observed to lose their external marking on the evening evaluation of the third sampling day. Thus, data of morning period on the first sampling day, and evening period on the third sampling day are not presented in tables.

The number of larvae observed on the plants was similar to all periods of the first two days and did not present differences between periods (Table 6). We observed 13 out of 15 larvae in average on each soybean plant at the first two days of evaluation, independent of the period.

There was a significant larval behavior effect on all sampling days ($P < 0.05$), where most of larvae were found resting/feeding (static behavior) regardless the period of the day (Table 7). On the first sampling day, there was a significant interaction between larval behavior and period of the day ($P < 0.0001$). The mean number of observed larvae feeding/resting (static behavior) at night (14.25) was higher when compared to the afternoon period (9.80) on this sampling day. On the other hand, the mean number of observed larvae crawling/ballooning (dispersing behavior) at afternoon (3.80) was higher than the evening period (0.75) for the same sampling day (Table 7).

There was no significant period effect on mean number of *H. armigera* larvae on the second and third sampling days regarding the larval behavior. Likewise, there was no significant interaction between larval behavior and period of the day for the same sampling days (Table 7).

Regarding the feeding site choice by *H. armigera* larvae on soybean plants, there was a significant feeding site effect on all sampling days ($P < 0.05$), where most of larvae were found feeding on leaves independent of the day's period (Table 8).

There was no significant interaction between feeding site and period of the day for the first sampling day (Table 8). However, there was a significant period effect on mean number of *H. armigera* larvae for the same sampling day ($P = 0.0180$), where the mean number of larvae feeding on leaf structures was significantly higher at night when compared to the afternoon period (Table 8). There was no significant period effect on mean number of *H. armigera* larvae on the second and third sampling days when comparing the feeding sites. Conversely, there was no significant interaction between feeding site and period of the day for the same sampling days (Table 8).

Most of the recovered larvae on the fourth sampling day were in third instar with an average of 0.76 mm of head capsule width (Table 8).

Discussion

Prior to designing some marking techniques in this study, the effects of these dyes incorporated into artificial diet on the biology of *H. armigera* were investigated. Among the desirable features, markers must have no deleterious biological affects to the recipient (Gangwere et al. 1964, Bartlet 1982, Akey 1991). Considering the results in the first experiment, the addition of the Luminous Powder dye into the diet up to 600 ppm concentration had no deleterious effects on larval survival, pupal weight or deformation rate (Table 1). Likewise, the larvae were observed feeding on the diet up to 24h after infestation, presenting no avoidance to the treatments (data not shown). However, the larval period showed some variation between the colors and concentrations (Table 1). Among the treatments, Blue Luminous Powder (200 and 400 ppm) and Red Luminous Powder (600 ppm) did not cause negative effect on the larval period when compared to the control insects. Considering the insignificant effects of these treatments on the biology of *H. armigera* larvae, these treatments obtained the ideal features to be used in the following bioassays with Luminous Powder dye diet to this point. Although fluorescent dyes have been used in many forms aiming to mark different insect species in the literature (Simmons et al. 2011, Mei et al. 2012), this is the first report of the use of Luminous Powder dye into artificial diet on biological aspects of *H. armigera*.

Regarding the use of Sudan dye diets, both Blue and Red 7B incorporated into the diet at 400 and 600 ppm caused some negative effects on larval mortality and/or larval period of *H. armigera* (Table 2). However, the same dyes at 200 ppm had minimal or insignificant effect on the biological aspects of *H. armigera* when incorporated into diet. These results contrasts from Zhao et al. (2008) which reported significant reduction on the pupation of the same speciesfed on diets containing Sudan Red 7B dye (100, 200, and 500 ppm). Sudan dyes have been extensively reported as a good marker for many species (Qureshi et al. 2004, Vilarinho et al. 2006, Zhao et al. 2008, Vilarinho et al. 2011). However, the dyes and concentrations can show variable marking efficiencies and effects on the development of different insect species (Hendricks 1971). Vilarinho et al. (2006) reported high mortality of fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) larvae with Sudan Red 7B (400 ppm) and Solvent Blue 35 (400 and 600 ppm) dyes added to artificial diet. Previous studies with Sudan Blue 670 and Sudan Red Red 7B dye diets also reported negative effects on the larval period of *Ostrinia* *nubilalis* (Hubner) (Lepidoptera: Crambidae) (Ostlie et al. 1984, Hunt et al. 2000), recommending adye concentration lower than 600 ppm (Ostlie et al. 1984).

These negative effects of dye metabolism at higher concentrations seem to be accumulative in the insect as they kept feeding on the diet normally. At least, the larvae also did not show avoidance to the treatments up to 24h after exposure to these dye diets. Also, most of the larvae died during pre-pupation stage (data not shown).

For Sudan dyes, concentrations at 400 and 600 ppm into diet did not meet the markers' criteria stated before. These negative effects on the insect' biology could jeopardize the results on the following bioassays. As 200 ppm did not affect the mortality and larval period of the insect, this concentration was considered for Sudan dye diet to this point.

Another important factor, if not the major, is the efficiency of these dyes on labeling the insects. Dyes should be easily recognized and also persist in a long term (Gangwere et al. 1964, Bartlet 1982). In this work, all insects fed on diets containing Sudan Blue and Red 7B, regardless the concentration, were easily recognized showing evident blue and red markings, respectively. These markings were clearly observed on the ventral part of the larvae. In general, the larvae showed increasing pigmentation with the increase of dye concentration. Likewise, the internal content of the insects was also marked with the dyes independent of the concentration used. Zhao et al. (2008) obtained similar efficiency on marking adults of *H. armigera*with the same Sudan Blue and Red 7B at 200 and 500 ppm concentrations.

Comparing the Sudan dye colors, the larvae marked with the blue color often showed a light blue coloration or some times not easily recognized due to the natural color variation of *H. armigera* on larval stages, which could create confounding effects on marking visualization.

Although both colors of Luminous Powder dye showed high rate of insects marked externally and internally, the red color seemed to be more easily recognized. Of the two appropriate methods of marking evaluation for this dye, the external marking was considered more efficient for *H. armigera* larvae. The colors were mainly detected in the exposed intersegmental membranes of the abdomen under UV light.

Considering the negative effects on the biology and marking visualization, Sudan Red 7B dye at 200 ppm and Luminous Powder dye at 600 ppm into diet were chosen for the dye persistence experiment.

The dyes and concentrations did not show any persistence at posterior instars when *H. armigera* larvae were kept feeding on the dye diets until second instar (up to 12h). Regardless the visualization methods used for this evaluation, no visual dyes' traces were detected at posterior instars (third, fourth and fifth). Sudan Red 7B dye was expected to have higher persistence, as after exposure and feeding on the dye, the larvae quickly acquired the same coloration. However, the larvae rapidly lost the marking in the same way after transferring to regular artificial diet on the second instar larvae. When the larvae reached third instar, no marking effects were observed in the larvae. According to Zalucki et al. (2002), younger instars tend to grow faster, consume more and digest their food better, but they tend to convert digested food less efficiently. However, the amount of diet consumed by first-instar larvae seemed to have been rapidly metabolized by the larvae, and was not enough to keep them marked at posterior instars.

Although feeding more proportionally, consumption of Lepidoptera species is minimal during the first instar and increases considerably over the instars (Liu et al. 2004, Naseri et al. 2010). Nevertheless, it is important to remind that each dye and concentration has different persistence effects as previously stated. Similarly, detection can be a more limiting parameter for this technique than the dye persistence by itself (Akey 1991). Thus, the results indicate that this first-instar-feeding methodology was inefficient in marking *H. armigera* with Sudan Red 7B at 200 ppm and Luminous Powder Red at 600 ppm dye diets. However, further detailed investigations using different visualization methods and time of larval exposure to these dyes are necessary to test the persistence in insects.

Regarding the eggs marking by spraying with dye solutions, neonates were expected to ingest the dye, as many of Lepidoptera species stay feeding on the remains of hatched eggs (Sparks 1979, Michel et al. 2010, Brandenburg and Freeman 2012). Another hypothesis was that the larvae could "contaminate" with the dyes somehow by crawling over them. However, the spraying should have no influence on eggs hatching. Although no negative effects on eggs hatching was observed, the methodology was not efficient in marking neonates, which no larvae presented visual traces of the dyes using both visualization methods at two days after eggs hatching (Table 4).

A high rate of larvae marked externally by dusting with Luminous Powder Red and Sudan Red 7B dyes was observed up to 48h . After this period, the larvae started losing the external marking due to molting. These results corroborate the previous studies in the literature (Stern and Mueller 1968, Akey 1991), which dusts are usually restricted to one life stage. In case of Lepidoptera species, it lasts only one larval instar. This fact can be verified through the average of instar period for both dye treatments and control. Although lasting one instar stage, the dusting method used did not show negative effect on second-instar larvae of *H. armigera*, as well as the particles got successfully adhered to the larvae, allowing short-term studies with marked larvae. Several concerns for using dusting marking are discussed in the literature such as toxicity and retention (Gangwere et al. 1964). According to Stern and Mueller (1968), the particle size is important in getting dusts to adhere, and can show negative effectson behavioral activities if the particles are coated too heavily on the tegument of small or delicate arthropods (Akey 1991).

Considering the results in the bioassays, the dusting marking was chosen to develop a behavioral study with *H. armigera* second-instar larvae on soybean plants in greenhouse. The Red Luminous Powder dye was used in this experiment, as it is a fluorescent dye and could allow performing nocturnal observations.

As verified in the fifth experiment, most of larvae lost the external marking due to molting, and second instar period lasted less then 60h. Thus, no visual observation was performed during the night on the third sampling day. Likewise, the larvae were collected on the fourth sampling day, and most of them were classified as third instar. The number of larvae observed were similar to all periods of the day, which allowed comparisons of the larval behavior between the periods.

Twelve hours after infestation, most of *H. armigera* larvae were already static (feeding or resting) on the different tissues of the plants. According to Zalucki et al. (2002), after hatching, neonates shelter for a short period and then enter a 'pre-feeding movement phase'. In this study, the infestation occurred when the larvae turned second instar. Therefore, the assumption is that the larvae did not have this short period of shelter and rapidly entered in movement to find a suitable feeding site. This period of time seems to be less than 24 h, since almost all the larvae were already established in a feeding site on the evening evaluation (24h after infestation). Nevertheless, a significant number of larvae were still dispersing by crawling or ballooning at 12h after infestation. According to Zalucki et al. (2002), if the host or plant part is unsuitable, then exploration within andbetween plants is likely to continue. For example, neonates of fall armyworm were reported to be able to survive starvation periods of 20 to 35 hours, allowing about a day to select a suitable feeding site (Morrill and Greene 1973).

Hence, few larvae were still crawling on the plant or ballooning on the other sampling days, but most of them stayed in the same feeding site nearby or far from the release point, regardless the period of the day. Considering the results, it can be inferred that after infestation, second instar larvae start dispersing on the plant for few hours until finding a suitable feeding site. After this exploration, most of larvae remain established regardless the period of the day.

Many researchers and farmers have been considering the possibility of spraying pesticides during the night, as many of Lepidoptera species are reported to have nocturnal activities, including *H. armigera* (EPPO 1981). Therefore, the larvae were expected to move more overnight, which they would be exposed to these control strategies, which was not verified in this study. For early instars larvae of *H. armigera*, it seems to have a short gap until the larval establishment. Thus, if the feeding site provides shelter to the larvae, the efficiency of these control strategies would be reduced considerably.

Old world cotton bollworm larvae are known to be fruit, seed, and flower feeders in preference to leaf feeders(Wilson and Waite 1982, Green et al. 2002, Rajapakse and Walter 2007). Besides inflicting higher damage by attacking reproductive plant components, such behavior could limit the use of the control strategies as the structures usually provide shelter. However, for this study, most of larvae were observed feeding on leaves on all sampling days independent the period (Table 8). Possibly, the second instar larvae were unable to drill the pods and feed on them properly due to their small size. It is valid to remind that the insect's behavior may be different in other hosts. The results in this study can be inferred for soybean, which was used as model. According to Liu et al. (2004), *H. armigera*initially start feeding on tender leaves on whole plants, but eventually their movements on plants primarily take them to the reproductive organs, which was observed in the present study. Although there is no variance in larval movement during the period of the day, the larvae of old world cotton bollworm at second instar remained exposed on soybean leaves.

Based on the results, Sudan Blue or Sudan Red 7B can be incorporated in old world cotton bollworm artificial diet at 200 ppm to obtain marked insects without major effects on their biology or behavior. Likewise, Luminous Powder Red dye into diet at 600 ppm was more efficient in obtaining marked larvae without deleterious effects on their biology or behavior. Larvae reared on Sudan Red 7B dye diet at 200 ppm and Red Luminous Powder dye diet at 600 ppm until second instar did not show visual dye traces at posterior instars when transferred to regular diet. Both dyes marked *H. armigera* externally with success by dusting procedure. The external marking lasted one larval instar without deleterious effects, which can be useful for short-term studies. Moreover, the results in this study can be useful in designing new marking techniques aiming to label early instars larvae. Designing such techniques can provide a better understanding of their behavior, which are often not studied due to the difficulty to handle and observe, but critical to apply pest management strategies.

In greenhouse, when using second instar larvae marked externally by Red Luminous Powder dye, it was possible to observe that larvae of *H. armigera* did not show variation on larval movement regardless the period of the day. After few hours, most of larvae established their feeding site and remained feeding on soybean leaves, not justifying overnight sprayings.

Acknowledgements

We thank to Coordination for the Improvement of Higher Education Personnel (CAPES) for a doctoral scholarship granted to the first author. To the National Council for Scientific and Technological Development – CNPq for the productivity in research fellowship granted to the second author.

References

Akey, D. H. 1991. A review of marking techniques in arthropods and an introduction to elemental marking. Suppl. Southwest. Entomol. 14: 1–8.

Bartlett, A. C. 1982. Genetic markers, discovery and use in insect population dynamic studies and control programs, pp.461-465. *In* Sterile insect techniqueand radiation in insect control. International Atomic Energy Agency, Vienna.

Brandenburg, R. L., and C. P. Freeman. 2012. Handbook of turfgrass insect pests. 2 ed, Entomological Society of America.

Butler Jr., G. D. 1976. Bollworm: development in relation to temperature and larval food. Environ. Entomol. 5: 520-522.

Czepak, C., K. C. Albernaz, L. M. Vivan, H. O. Guimarães, and T. Carvalhais. 2013. Primeiro registro de ocorrência de *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) no Brasil. Pesquisa Agropecuária Tropical 43: 110-113.

Dirie, A. M., M. B. Cohen, and F. Gould. 2000. Larval dispersal and survival of *Scirpophaga incertulas* (Lepidoptera: Pyralidae) and *Chilo suppressalis* (Lepidoptera: Crambidae) on cry1Ab-transformed and non-transgenic rice. Environ. Entomol. .29: 972-978.

(EMBRAPA) Brazilian Corporation of Agricultural Research. 2013. Nota técnica sobre resultado do trabalho inicial de levantamento da lagarta do gênero *Helicoverpa*—detecção da espécie *Helicoverpa armigera* no Brasil. EMBRAPA Cerrados and EMBRAPA Soja, Planaltina, Brazil.

(EPPO)European and Mediterranean Plant Protection Organization. 1981. Data sheets on quarantine organisms nº 110: *Helicoverpa armigera*. EPPO, Paris, France.

Fehr, W. R., C. E. Caviness. 1977. Stages of soybean development. Iowa State University Cooperative Extension Service Special Rep. 80. Iowa State University, Ames, IA.

Gangwere, S. K., W. Chavin, and F. C. Evans. 1964. Methods of marking insects, with especial reference to Orthoptera (Sens. Lat.). Ann. Entomol. Soc. Am. 57: 662-669.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu. Rev. Entomol. 43: 701-726.

Green, P. W. C., P. C. Stevenson, M. S. J. Simmonds, and H. C. Sharma. 2002. Can larvae of the pod-borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae), select between wild and cultivated pigeonpea *Cajanus* sp. (Fabaceae)? Bull. Entomol. Res. 92: 45–51.

Hagler, J.R., and C. G. Jackson. 2001. Methods for marking insects: current techniques and future prospects. Annu. Rev. Entomol. 46: 511–543.

Hendricks, D. E., M. P. Leal, S. H. Robinson, and N. S. Hernandez. 1971. Oil-soluble black dye in larval diet marks adults and eggs of tobacco budworm and pink bollworm. J. Econ. Entomol. 64: 1399-1401.

Hunt, T. E., R. L. Hellmich, J. M. Dyer, L. H. Higley, and J. F. Witkowski. 2000. Oil-soluble dyes for marking European corn borer (Lepidoptera: Crambidae). J. Entomol. Sci. 35: 338-341.

Johnson, M. L., S. Armitage, B. C. G. Scholz, D. J. Merritt, B. W. Cribb, and M. P. Zalucki. 2007. Predator presence moves *Helicoverpa armigera* larvae to distraction. J. Insect Behav. 20: 1-18.

Liu, Z.,D. Li, P. Y. Gong, and K. J. Wu. 2004. Life table studies of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), on different host plants. Environ. Entomol. 33: 1570-1576.

Mascarenhas, H. A. A., and R. T. Tanaka. 1997. Soja, pp.202-203*In*B. Van Raij, H. Cantarella, J. A. Quaggio, and A. M. C. Furlani (eds.), Recomendações de adubação e calagem para o Estado de São Paulo. 2. ed. rev. IAC, Campinas, Brazil.

Mei, L., Z. G. Guan, H. J. Zhou, J. Lv, Z. R. Zhu,, J. A. Cheng, F. J. Chen, C. Lofstedt, S. Svanberg, and G. Somesfalean. 2012. Agricultural pest monitoring using fluorescent lidar techniques feasibility study. Appl. Phys. B106: 733-740.

Michel, A. P., C. H. Krupke, T. S. Baute, C. D. Difonzo. 2010. Ecology and management of the western bean cutworm (Lepidoptera:Noctuidae) in corn and dry beans. J.Int.Pest Manage. 1: 1-10.

Morrill, W. L., and G. L. Greene. 1973. Distribution of fall armyworm larvae. 2. Influence of biology and behavior of larvae on selection of feeding sites. Environ.Entomol. 2: 415-418.

Naseri, B., Y. Fathipour, S. Moharramipour, and V. Hosseininaveh. 2010. Nutritional

Indices of the Cotton Bollworm, *Helicoverpa armigera*, on 13 Soybean Varieties. J. Insect Sci. 10: 151.

Ostlie, K. R., L. G. Higley, L. V. Kaster, and W. B. Showers. 1984. European corn borer (Lepidoptera: Pyralidae) development, larval survival and adult vigor on meridic diets containing marker dyes. J. Econ. Entomol. 77: 118-120.

Parra, J.R. P. 2001. Técnicas de criação de inseto s para programas de controle biológico . FEALQ, Piracicaba, Brazil.

Paula-Moraes, S. V., T. E. Hunt, R. J. Wright, G. L. Hein, and E. E. Blankenship. 2012. On-plant movement and feeding of Western bean cutworm (Lepidoptera: Noctuidae) early instars on Corn. Environ. Entomol. 41: 1494-1500.

Perkins, L.E., B. W. Cribb, J. Hanan, E. Glaze, C. Beveridge, M. P. Zalucki. 2008. Where to from here? The mechanisms enabling the movement of first instar caterpillars on whole plants using *Helicoverpa armigera* (Hubner) Arthropod-Plant Interactions 2: 197–207.

Qureshi, J.A., L. L. Buschman, J. E. Throne, and S. B. Ramaswamy. 2004. Oil-soluble dyes incorporated in meridic diet of *Diatraea grandiosella* (Lepidoptera: Crambidae) as marker for adult dispersal studies. J. Econ. Entomol. 97: 836–845.

Rajapakse, C. N. K., and G. H. Walter. 2007. Polyphagy and primary host plants: oviposition preference versus larval performance in the lepidopteran pest *Helicoverpa armigera*. Arthropod–Plant Interactions 1: 17–26.

Reynolds, D. R., J. R. Riley, R. J. Armes, N. J. Cooter, M. R. Tucker, and J. Calvin. 1997. Techniques for quantifying insect migration, pp.111-145.*In*D. R. Dent, M. P. Walton (eds.), Methods in ecological and agricultural entomology. CAB Int., Cambridge, United Kingdom.

Ross, S. E., and K. R. Ostlie. 1990. Dispersal and survival of early instars of European corn borer (Lepidoptera: Pyralidae) in field corn. J. Econ. Entomol. 83: 831-836.

SAS Institute. 2009. SAS user's guide: statistics, version. 9.1.3. SAS Institute, Cary, NC.

Shelton, A. M., J. Z. Zhao, R. T. Roush. 2002. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. Annu. Rev. Entomol. 47: 845-881.

Shimoji, Y., M. Sugiyama, and T. Kohama. 1999. Marking of the West Indian sweet potato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae) with Calco oil red dye I. Effect of dye added to the artificial larval diet on development of the weevils, yield of adults and long lasting internal coloration in the adult. Jap. Soc.Appl. Entomol. Zool.34: 231-234.

Simmons, G. S., A. R. McKemey, N. I. Morrison, S. O'Connell, B. E. Tabashnik, J. Claus, G. Fu, G. Tang, M. Sledge, and A. S. Walker. 2011. Field performance of a genetically engineered strain of pink bollworm. PLoS One 6: e24110.

Southwood, T.R. E., and P. A. Henderson. 2000. Ecological Methods, 3rd edn. Blackwell Science, Oxford, UK.

Spangler, S. M., and D. D. Calvin. 2001. Vertical distribution of European corn borer (Lepidoptera: Crambidae) egg masses on sweet corn. Environ. Entomol. 30: 274-279.

Sparks, A. N. 1979. A review of the biology of the fall armyworm. Fla. Entomol. 62: 82–87.

Specht, A., D. R. Sosa-Gomez, S. V. Paula-Moraes, S. A. C. Yano. 2013. Morphological and molecular identification of *Helicoverpa armigera* (Lepidoptera: Noctuidae) and expansion of its occurrence record in Brazil. Pesquisa Agropecuaria Brasileira 48: 689–692.

Stephens, A. E. A., A. M. Barrington, V. A. Bush, N. M. Flatcher, V. J. Mitchell, D.

M. Suckling. 2008. Evaluation of dyes for marking painted apple moths (*Teia anartoides* Walker, Lep. Lymantriidae) used in a sterile insect release program. Aust. J. Entomol. 47: 131-136.

Stern, V. M., and A. Mueller. 1968. Techniques of marking insects with micronized fluorescent dust with special emphasis on marking millions of *Lygus hesperus* for dispersal studies. J. Econ. Entomol. 61: 1232-1237.

Vilarinho, E. C., O. A. Fernandes, C. Omoto, and T. E. Hunt. 2006. Oil-soluble dyes for marking *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Econ. Entomol. 99: 2110-2115.

Vilarinho, E.C., O. A. Fernandes, T. E. Hunt, and D. F. Caixeta. 2011. Movement of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) adults in maize in Brazil. Fla. Entomol. 94: 480-488.

Warner, K. A., and P. Bierzychudek. 2009. Does marking with fluorescent powders affect the survival or development of larval *Vanessa cardui*? Entomol. Exp. Appl. 131: 320-324.

Wilson, L.T., G. K. Waite. 1982. Feeding pattern of Australian *Heliothis* on cotton. Environ. Entomol. 11: 297–300.

Zalucki, M. P., A. R. Clarke, and S. B. Malcom. 2002. Ecology and behavior of first instar larval Lepidoptera. Annu. Rev. Entomol. 47: 361-393.

Zhao, X. C., K. M. Wu, and Y. Y. Guo. 2008. oil-soluble dyes in larval diet used for marking *Helicoverpa armigera*. Entomol. Exp. Appl. 126: 256-260.

Table 1. Larval survival, larval period, pupal weight, and pupal deformation (±SE) of old world cotton bollwormfed on Luminous Powder dye diet at 200, 400, and 600 ppm concentrations. Laboratory conditions (T = 25 ± 2ºC; R.H. = 60 ± 10%; 14:10-L:D)

Treatment	Larval survival (%)[a]	Larval period (d)[a]	Pupal weight (g)[a]	Pupal deformation (%)[a]
Blue dye				
200 ppm	86.21 ± 6.41 a	22.00 ± 0.53 bc	0.381 ± 0.008 a	0.00 ± 0.00 a
400 ppm	86.67 ± 6.31 a	22.46 ± 0.70 bc	0.367 ± 0.008 a	15.38 ± 7.22 a
600 ppm	76.67 ± 7.85 a	25.61 ± 0.88 a	0.380 ± 0.008 a	13.04 ± 7.18 a
Red dye				
200 ppm	75.00 ± 8.05 a	23.90 ± 0.84 ab	0.379 ± 0.007 a	14.29 ± 7.82 a
400 ppm	86.67 ± 6.31 a	24.69 ± 0.84 a	0.366 ± 0.015 a	7.69 ± 5.33 a
600 ppm	82.76 ± 7.02 a	23.50 ± 0.61 abc	0.369 ± 0.010 a	12.50 ± 6.90 a
C. oil control	86.67 ± 6.31 a	23.81 ± 0.80 ab	0.374 ± 0.012 a	3.85 ± 3.85 a
Control	93.33 ± 4.63 a	21.68 ± 0.44 c	0.365 ± 0.009 a	7.14 ± 4.96 a
P	0.6047	0.0052	0.8970	0.5152

C. oil control = Corn oil control.

[a]LSMean (± SEM). The means with the same letter are not significantly different, $P \leq 0.05$.

Table 2. Larval survival, larval period, pupal weight, and pupal deformation (±SE) of old world cotton bollworm fed on Sudan dye diet at 200, 400, and 600 ppm concentrations. Laboratory conditions (T = 25 ± 2ºC; R.H. = 60 ± 10%; 14:10-L:D)

Treatment	Larval survival (%)[a]	Larval period (d)[a]	Pupal weight (g)[a]	Pupal deformation (%)[a]
Blue dye				
200 ppm	72.50 ± 7.15 ab	19.89 ± 0.27 e	0.395 ± 0.006 a	6.90 ± 4.79 a
400 ppm	60.00 ± 7.84 bc	22.62 ± 0.53 abc	0.387 ± 0.006 a	12.50 ± 6.90 a
600 ppm	45.00 ± 7.97 cd	22.33 ± 0.45 bcd	0.407 ± 0.007 a	16.67 ± 9.04 a
Red dye				
200 ppm	70.00 ± 7.34 ab	21.43 ± 0.57 cd	0.405 ± 0.007 a	3.57 ± 3.57 a
400 ppm	62.50 ± 7.75 abc	23.89 ± 0.68 a	0.399 ± 0.010 a	8.00 ± 5.54 a
600 ppm	37.50 ± 7.75 d	23.70 ± 1.05 ab	0.399 ± 0.017 a	6.67 ± 6.67 a
C. oil control	80.00 ± 6.41 ab	21.41 ± 0.40 cd	0.396 ± 0.005 a	6.25 ± 4.35 a
Control	82.50 ± 6.08 a	20.86 ± 0.38 de	0.385 ± 0.009 a	6.06 ± 4.22 a
P	<0.0001	<0.0001	0.5640	0.8275

C. oil control = Corn oil control.

[a]LSMean (± SEM). The means with the same letter are not significantly different, $P \leq 0.05$.

Table 3. Percentage (±SE) of old world cotton bollworm fifth-instar larvae marked by feeding on diets containing Luminous Powder and Sudan dyes at 200, 400, and 600 ppm concentrations. Laboratory conditions (T = 25 ± 2ºC; R.H. = 60 ± 10%; 14:10-L:D)

	Luminous Powder		Sudan	
Concentration	Internal marking[a,b]	External marking[a,c]	Internal marking[a,b]	External marking[a,c]
Blue dye				
200 ppm	72.50 ± 7.15 a	80.00 ± 6.41 c	100.00 ± 0.00	100.00 ± 0.00
400 ppm	75.00 ± 6.93 a	90.00 ± 4.80 b	100.00 ± 0.00	100.00 ± 0.00
600 ppm	72.50 ± 7.15 a	95.00 ± 3.49 ab	100.00 ± 0.00	100.00 ± 0.00
Red dye				
200 ppm	82.50 ± 6.08 a	100.00 ± 0.00 a	100.00 ± 0.00	100.00 ± 0.00
400 ppm	82.50 ± 6.08 a	100.00 ± 0.00 a	100.00 ± 0.00	100.00 ± 0.00
600 ppm	90.00 ± 4.80 a	100.00 ± 0.00 a	100.00 ± 0.00	100.00 ± 0.00
P	0.0588	0.0002	---	---

[a]LSMean (± SEM). The means with the same letter are not significantly different, P ≤ 0.05. Marking evaluation of larvae proceeded at fifth instar.

[b]Marking evaluation was performed by carefully squeezing the larvae.

c Marking evaluation was performed under UV light for Luminous Powder dye, and under normal light for Sudan dye.

Table 4. Number of hatched larvae and percentage (±SE) of old world cotton bollworm neonates marked by spraying egg masses with Luminous Powder and Sudan Red 7B dye solutions. Laboratory conditions (T = 25 ± 2ºC; R.H. = 60 ± 10%; 14:10-L:D).

	Eggs marking	
Treatment	Number of larvae[a]	Marked neonates (%)
Luminous Powder	69.20 ± 3.11 a	0.00 ± 0.00
Sudan	63.20 ± 4.10 a	0.00 ± 0.00
Corn oil control	70.20 ± 2.47 a	---
Control	73.00 ± 2.46 a	---
P	0.8806	---

[a]LSMean (± SEM). The means with the same letter are not significantly different, $P \leq 0.05$.

Table 5. Instar period and percentage (±SE) of old world cotton bollworm second-instar larvae marked by dusting procedure with Red Luminous Powder and Sudan Red 7B dyes at different times after dusting. Laboratory conditions (T = 25 ± 2ºC; R.H. = 60 ± 10%; 14:10-L:D)

Treatment	Dusting duration						Instar Period[1]
	12h[a]	24h[a]	36h[a]	48h[a]	60h[a]	72h	
	--------------- Number of larvae ---------------						Hours
L. Powder	100.00	100.00	96.00 + 2.67 a	92.00 + 4.42 a	10.00 + 3.33 a	0.00	59.52 ± 0.90 a
Sudan	100.00	100.00	90.00 + 4.47 a	82.00 + 6.29 a	6.00 + 3.06 a	0.00	57.36 ± 1.20 a
Control	---	---	---	---	---	---	57.60 ± 1.14 a
P	---	---	0.2643	0.2098	0.3880	---	0.3094

[a]LSMean (± SEM). The means with the same letter are not significantly different, $P \leq 0.05$.

Table 6. Mean number (±SE) of old world cotton bollworm larvae observed on soybean plants at three different positions of the day. Botucatu, SP, Brazil.

Period of evaluation	Number of larvae[a]
Morning	13.00 ± 0.82 a
Afternoon	13.00 ± 0.55 a
Evening	12.33 ± 0.91 a
P	0.7817

Periods of evaluation = Morning: 6-7 a.m.; Afternoon: 2-3 p.m.; and Evening: 8-9 p.m. Mean number of larvae from Day 1 and 2.

[a]LSMean (± SEM). The means with the same letter are not significantly different, $P \leq 0.05$.

Table 7. Mean number (±SE) of old world cotton bollworm larvae on soybean plants divided in two behavioral aspects at different periods of the day. Botucatu, SP, Brazil.

	Larval behavior		
Evaluation period	Static[a]		Dispersing[a]
Day 1	-------------- Number of larvae ---------------		
Morning	---		---
Afternoon	9.80 ± 0.49 bA		3.80 ± 0.49 aB
Evening	14.25 ± 0.48 aA		0.75 ± 0.48 bB
P period		0.1856	
P behavior		<0.0001	
P interaction		<0.0001	
Day 2			
Morning	10.25 ± 0.85 aA		2.75 ± 0.25 aB
Afternoon	10.00 ± 0.71 aA		2.50 ± 0.50 aB
Evening	9.25 ± 0.48 aA		1.25 ± 0.75 aB
P period		0.1398	
P behavior		<0.0001	
P interaction		0.8991	
Day 3			
Morning	8.25 ± 0.48 aA		0.25 ± 0.25 aB
Afternoon	8.00 ± 0.71 aA		0.25 ± 0.25 aB
Evening	---		---
P period		0.8347	
P behavior		<0.0001	
P interaction		0.8347	

Periods of evaluation = Morning: 6-7 a.m.; Afternoon: 2-3 p.m.; and Evening: 8-9 p.m. Static behavior represents the larvae that were observed feeding or resting on the plants. Dispersing behavior represents the larvae that were observed ballooning or crawling on the plants.

[a]LSMean (± SEM). The means with the same letter are not significantly different, $P \leq 0.05$.

Table 8. Mean number (±SE) of old world cotton bollworm larvae feeding on soybean tissues at different periods of time, average diameter of head capsule and estimate instar. Botucatu, SP, Brazil.

	Feeding site	
Evaluation period	Pods[a]	Leaves[a]
Day 1	--------------- Number of larvae ---------------	
Morning	---	---
Afternoon	0.80 ± 0.58 aB	9.00 ± 0.84 bA
Evening	2.75 ± 0.85 aB	11.50 ± 0.87 aA
P period	0.0180	
P Feeding site	<0.0001	
P interaction	0.7341	
Day 2	--------------- Number of larvae ---------------	
Morning	1.00 ± 0.71 aB	9.25 ± 0.95 aA
Afternoon	2.00 ± 0.91 aB	8.00 ± 0.71 aA
Evening	1.50 ± 0.65 aB	7.00 ± 1.08 aA
P period	0.8067	
P Feeding site	<0.0001	
P interaction	0.1752	
Day 3	--------------- Number of larvae ---------------	
Morning	1.25 ± 0.25 aB	7.00 ± 0.71 aA
Afternoon	1.25 ± 0.48 aB	6.75 ± 1.11 aA
Evening	---	---
P period	0.8486	
P Feeding site	<0.0001	
P interaction	0.8486	
Day 4	Head capsule width (mm)	Estimated instar[b]
Larvae	0.76	Third

Periods of evaluation = Morning: 6-7 a.m.; Afternoon: 2-3 p.m.; and Evening: 8-9 p.m.

[a]LSMean (± SEM). The means with the same letter are not significantly different, $P \leq 0.05$.

[b] Head capsule widths are about 0.29, 0.47, 0.77, 1.30, 2.12, and 3.10 mm, respectively, for instars 1-6 (Butler, 1976).

4.FINAL CONSIDERATIONS

Aboveground species from Noctuidae family deserve great attention due to their recognized potential of damage in corn. Understanding their biology and behavioral aspects is important to implement integrated pest management (IPM)andinsect resistance management (IRM) successfully.Larval dispersal on-plant and plant-to-plant can influence in how to design these strategies. Notwithstanding, early larval instars of Lepidoptera species are directly related to these behavioral aspects. Due to the difficulties to observe and handle these tiny insects, especially in tropical countries, it is believed that marking techniques may help in conducting these studies.

Results in this study indicated that fall armyworm larvae prefer ear zone as feeding site, and this choice is done by first instar larvae. Considering the implementation of IPM strategy, there is a short gap until larval establishment in the corn ear, which this structure would provide shelter for the larvae, reducing the efficiency of important control strategies such as the chemical through pesticides spraying or biological using predators, parasitoids or pathogens.This feeding choice is also characterized as insect's feeding preference, once the corn leaves are not suitable for the fall armyworm larval development, and silk and kernels play a positive rolein the survival and development of the insect.This feeding choice may also influence in the IRM, since the larvaewould likely be exposed to lower rates or sub-lethal doses of the toxin generally exposed to lower rates or sub-lethal doses of the toxin generallyfound in corn grains (kernels). In tropical countries, this scenery would be as many times aggravated as the number of the insect's generations a year, requiring constantly monitoring of insect's resistance selection.

In the second study, results demonstrated that thewestern bean cutworm and fall armywormdispersedgoverned by non directional clueswithaggregated and symmetrical distribution in corn field.Although the characterization of this dispersion has great value in sampling plans to be incorporated in an IPM, these results may also help in the development and implementation of refuge areas for IRM of these insects.Likewise, both species presented considerable plant-to-plant movementin corn field, which must be considered in the implementation of seed mixture strategy.

Regarding the insect marking techniques performed in this study, Sudan Blueor Sudan Red 7B were incorporated into *H. armigera*dietsuitably to obtain marked larvae without negative effects on the insect's biology and behavior. Likewise, the red Luminous Powder dye diet also obtained*H. armigera* marked successfully.However, the methodology of the dyes' persistence used here did not achieve positive results, probably requiring adjustments in visualization methods and exposure time of the larvae to the treatments.Among the marking techniques proposed, the external marking by dusting was more reliable and useful for behavioral studies of early larval instars of *H. armigera*, although still for a short period of time.Conversely, the results of the marking techniques in this study may help in developing new techniques for early larval instars marking focused on behavioral studies to improve IPM and IRM strategies.

For now, the behavioral study of *H. armigera* at greenhouse using dusting marking with Luminous Powder dye allowed verifying that second-instar larvae do not show variation on larval movement regardless the period of the day (morning, afternoon and evening) in soybean plants, however they remain exposed on the leaves.These results, for example, may helpin decision-making for the use of important control strategies such as chemical control through pesticides spraying.

5. CONCLUSIONS

-Fall armyworm (*S. frugiperda*) larvae prefer the ear zone as a feeding site in reproductive corn stage;

-The feeding site choice is done by first-instar larvae due to feeding preference.

- Corn leaves are not suitable for early instar development, and silk and kernel tissues play a positive role in survival and development of fall armyworm larvae in reproductive corn stage.

- Western bean cutworm (*S. albicosta*) and fall armyworm larvae disperse in field corn governed by nondirectional sensory information, and present aggregated and symmetrical distribution;

- Fall armyworm remain nearby the release point more than western bean cutworm, andboth species present considerable plant-to-plant movement in corn.

- Sudan Blue or Sudan Red 7B can be incorporated in *H. armigera*artificial diet at 200 ppm to obtain marked insects without major effects on their biology or behavior;

- Red Luminous Powder dye into diet at 600 ppm is more efficient in obtaining marked larvae without deleterious effects on the insect'sbiologyandbehavior.

- Sudan and Luminous Powder dyes mark *H. armigera* externally with success by dusting procedure;

- The external marking last one larval instar without deleterious effects, and can be useful for short-term studies;

- Second-instar larvae of *H. armigera* do not show variation on larval movement regardless the period of the day (morning, afternoon and evening);

-*H. armigera*second-instar larvae prefer soybean leaves as feeding site.

6.REFERENCES

ADAMCZYK JR, J. J.; GREENBERG, S.; ARMSTRONG, J. S.; MULLINS, W. J.; BRAXTON, L. B.; LASSITER, R. B.; SIEBERT, M. W. Evaluations of Bollgard®, Bollgard II®, and Widestrike® technologies against beet and fall armyworm larvae (Lepidoptera: Noctuidae). **Florida Entomologist**, v.91, p.531–536, 2008.

AKEY, D. H. A review of marking techniques in arthropods and an introduction to elemental marking. **Supplement to the Southwestern Entomologist**, v.14, p.1–8, 1991.

ALSTAD, D. N.; ANDOW, D. A. Managing the evolution of insect resistance to transgenic plants. **Science**, v. 268, p.1894-1896, 1995.

APPEL, L. L.; WRIGHT, R. J.; CAMPBELL, J. B. Economic injury levels for western bean cutworm, *Loxagrotis albicosta* (Smith) (Lepidoptera: Noctuidae), larvae in field corn. **Journal of the Kansas Entomolical Society**,v.66,p.434-438, 1993.

ÁVILA, C. J.; VIVAN, L. M.; TOMQUELSKI, G. V. **Ocorrência, aspectos biológicos, danos e estratégias de manejo de *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) nos sistemas de produção agrícolas.** Dourado: Embrapa. 12 p. (Circular Técnica, 23), 2013.

BARROS, E. M.; TORRES, J. B.; BUENO, A. F. Oviposition, development, and reproduction of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) fed on different hosts of economic importance. **Neotropical Entomology**, v.39, n.6, p.996-1001, 2010.

BATES, S. L.; ZHAO, J. Z.; ROUSH, R. T.; Shelton, A. M. Insect resistance managementin GM crops: past, present and future. **Nature Biotechnology**, v.23, p.57-62, 2005.

BELL, W. J. Searching behavior patterns in insects. **Annual Review of Entomology**, v.35, p.447-67, 1990.

BROOKS, G.; BARFOOT, P. GM crops: the global economic and environmental impact: the first nine years 1996-2004. **AgBioForum**, v.8, p.187-196, 2005.

BUNTIN, G. D.A review of plant response to fall armyworm, *Spodoptera frugiperda* (J. E. Smith), injury in selected field and forage crops. **Florida Entomologist**,v.69, p.549-559,1986.

BUNTIN, G. D.; ALL, J. N.; LEE, R. D.; WILSON, D. M. Plant-incorporated *Bacillus thuringiensis* resistance for control of fall armyworm and corn earworm (Lepidoptera: Noctuidae) in corn. **Journal of Economic Entomology**, v.97, p.1603-1611, 2004.

CAPINERA, J. L. **Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Insecta: Lepidoptera: Noctuidae).**Gainesville: The University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS), 2000.

CAPINERA, J. L. **Encyclopedia of entomology**. 2nd ed., v.1-4. Springer, Dordrecht, The Netherlands. 2008. 4346 p.

CARROL, M. W.; HEAD, G.; CAPRIO, M. When and where a seed mix refuge makes sense for managing insect resistance to Bt plants. **Crop Protection**, v.38, p.74-79, 2012.

CARVALHO, R. A.; OMOTO, C.; FIELD, L. M.; WILLIAMSON, M. S.; BASS, C. Investigating the molecular mechanisms of organophosphate and pyrethroid resistance in the fall armyworm *Spodoptera frugiperda*. **Plos One**, v.8, n.4, 2013.

CHILCUTT, C. F.; TABASHNIK, B. E. Contamination of refuges by *Bacillus thuringiensis* toxin genes from transgenic maize. **Proceedings of the National Academy of Sciences of the United States of America**. v.101, p.7526-7529, 2004.

CHILCUTT, C. F.; ODVODY, G. N.; CORREA, J. C.; REMMERS, J. Effects of *Bacillus thuringiensis* transgenic corn on corn earworm and fall armyworm (Lepidoptera: Noctuidae) densities. **Journal of Economic Entomology**, v.100, p.327-334, 2007.

CLARK, P. L.; MOLINA-OCHOA, J.; MARTINELLI, S.; SKODA, S. R.; ISENHOUR, D. J.; LEE, D. J.; KRUMM, J. T.; FOSTER, J. E. Population variation of the fall armyworm, *Spodoptera frugiperda*, in the Western Hemisphere. **Journal of Insect Science**, p.10, 2007.

CRUZ, I.; TURPIN, F. T. Yield impact of larval infestation of the fall armyworm *Spodoptera frugiperda* (J. E. Smith) to mid-whorl growth stage of corn. **Journal of Economic Entomology**, College Park, v. 76, p. 1052- 1054, 1983.

CRUZ, I.; FIGUEIREDO, M. L. C.; OLIVEIRA, A. C.; VASCONCELOS, C. A.

Damage of *Spodoptera frugiperda* (Smith) in different maize genotypes cultivated in soil under three levels of aluminium saturation. International **Journal of Pest Management**, London, v. 45, p. 293-296, 1999.

CZEPAK, C., ALBERNAZ, K. C.; VIVAN, L..M.; GUIMARÃES, H. O.; CARVALHAIS, T. Primeiro registro de ocorrência de *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) no Brasil. **Pesquisa agropecuária Tropical**, v. 43, p. 110-113, 2013.

DAVIS, P.M.; ONSTAD, D.W. Seed mixtures as resistance management strategy for European corn borers (Lepidoptera: Crambidae) infesting transgenic corn expressing Cry1Ab protein. **Journal of Economic Entomology**, v.93, p.937–984, 2000.

DIEZ-RODRIGUES, G. I.; OMOTO, C. Inheritance of lambda-cyhalothrin resistance in *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). **Neotropical Entomology**, v.30, p.311–316, 2001.

DIFONZO, C. D., HAMMOND, R. Range expansion of western bean cutworm, Striacosta albicosta (Noctuidae), into Michigan and Ohio. **Crop Management**, 2008.

EMBRAPA. **Nota técnica sobre resultado do trabalho inicial de levantamento da lagarta do gênero *Helicoverpa*—detecção da espécie *Helicoverpa armigera* no Brasil**. Planaltina: EMBRAPA CERRADOS e EMBRAPA SOJA; 2013. 2 p.

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION (EPPO). **Data sheets on quarantine organisms no 110: *Helicoverpa armigera*.** Paris: EPPO, 1981.

FARIAS, P. R. S.; BARBOSA, J. C.; BUSOLI, A. C.; OVERAL, W. L.; MIRANDA, V. S.; RIBEIRO, S. M. Spatial analysis of the distribution of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and losses in maize crop productivity using geostatistics. **Neotropical Entomology**, v.37, p.321–327, 2008.

GOLDSTEIN, J. A.; MASON, C. E.; PESEK, J. Dispersal and movement behavior of neonate european corn borer (Lepidoptera: Crambidae) on non-bt and transgenic Bt corn. **Journal of Economic Entomology**, v.103, n.2, 2010.

GOULD, F. Deploying pesticidal engineered crops in developing countries. In: PERSLEY, G. J. (Ed.), **Biotechnology and Integrated Pest Management**. CABI, Wallingford, UK, p. 264-293, 1996.

GOULD, F. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. **Annual Review of Entomology**, v.43, p.701-726, 1998.

GUSE, C. A.; ONSTAD, D. W.; BUSCHMAN, L. L.; PORTER, P.; HIGGINS, R. A. Modeling the development of resistance by stalk-boring (Lepidoptera: Crambidae) in areas with irrigated transgenic corn. **Environmental Entomology**, v.31, p.676-685, 2002.

HAGLER, J. R.; JACKSON, C. G. Methods for marking insects: current techniques and future prospects. **Annual Review of Entomology**, v.46, p.511–543, 2001.

HARDKE, J. T.; LEONARD, B. R.; HUANG, F.; JACKSON, R. E. Damage and survivorship of fall armyworm (Lepidoptera: Noctuidae) ontransgenic field corn expressing *Bacillus thuringiensis* Cry proteins. **Crop Protection**, v.30, p.168-172, 2011.

HARRISON, F. P. Oviposition and subsequent infestations of corn by fall armyworm. **Florida Entomologist**, v.69, p.588-592, 1986.

HEAD, G. P.; GREENPLATE, J. The design and implementation of insect resistance management programs for Bt crops.**GM Crops and Food: Biotechnology in Agriculture and the Food Chain**, v.3, n.3, p.144-153, 2012.

HENDRICKS, D. E.; LEAL, M. P.; ROBINSON, S. H.; HERNANDEZ, N. S. Oil-soluble black dye in larval diet marks adults and eggs of tobacco budworm and pink bollworm. **Journal of Economic Entomology**, v.64, p.1399-1401, 1971.

HUANG, F. Resistance management for Bt maize and above-ground Lepidopteran targets in the USA: From single gene to pyramided traits. In: SOBERÓN, M.; GAO, Y.; BRAVO, A.**Bt resistance**. CAB International, p.173-185, 2015.

HUNT, T. E.; HELLMICH, R. L.; DYER, J. M.; HIGLEY, L. H.; WITKOWSKI, J. F. Oil-soluble dyes for marking European corn borer (Lepidoptera: Crambidae). **Journal of Entomological Science**, v.35, p.338-341, 2000.

International Life Sciences Institute. 1998. **An evaluation of insect resistance management in Bt field corn: a Science-based framework for risk assessment and risk management**. Health and Environmental SciencesIns., USA. 78 p.

IVES, A. R.; GLAUM, P. R.; ZIEBARTH, N. L.; ANDOW, D. A. The evolution of resistance to two-toxin pyramid transgenic crops. **Ecological Applications**, v.21, n.2, p. 503–515, 2011.

KOGAN, M. Integrated pest management: historical perspectives and contemporary developments. **Annual Review of Entomology**, v.43, p.243–270, 1998.

LUGINBILL, P. **The fall armyworm**. U S Department of Agriculture Technical Bulletin 34. 1928. 92 p.

LAMMERS, J. W.; MACLEOD, A. **Report of a pest risk analysis: Helicoverpa armigera (Hubner, 1808)**. Disponível em: <http://www.fera.defra.gov.uk/plants/planthealth/pestsdiseases/documents/helicoverpa.pdf> Acesso em: 20 de maio de 2013, 2007.

MACINTOSH, S. C. Managing the risk of insect resistance to transgenic insect control traits: practical approaches in local environments. **Pest Management Science**, v.66, p.100-106, 2009.

MICHEL, A. P.; KRUPKE, C. H.; BAUTE, T. S.; DIFONZO, C. D. Ecology and management of the western bean cutworm (Lepidoptera: Noctuidae) in corn and dry beans. **Journal of Integrated Pest Management**, v.1, p.1-10, 2010.

MURPHY, A. F.; GINZEL, M. D.; KRUPKE, C. H. Evaluating corn rootworm (Coleoptera: Chrysomelidae) emergence and root damage in a seed mix refuge. **Journal of Economic Entomology**, v.103, n.1, p.147-157, 2010.

NAGOSHI, R. N. Can the amount of corn acreage predict fall armyworm (Lepidoptera: Noctuidae) infestation levels in nearby cotton? **Journal of Economic Entomology**, v.102, p.210-218, 2009.

O'ROURKE, P. K., HUTCHISON, W. D. First report of the western bean cutworm *Richia albicosta* (Smith) (Lepidoptera: Noctuidae), in Minnesota corn. **Journal of Agricultural and Urban Entomology**, v.17, p.213-217, 2000.

ONSTAD, D. W.; GOULD, F. Modeling the dynamics of adaptation to transgenic maize by European corn borer (Lepidoptera: Pyralidae). **Journal of Economic Entomology**, v.91, p.585-593, 1998.

ONSTAD, D. W. Modeling larval survival and movement to evaluate seed mixtures of transgenic corn for control of Western corn rootworm (Coleoptera: Chrysomelidae).**Journal of Economic Entomology**, v.99, p.1407-1414, 2006.

ONSTAD, D. W. **Insect resistance management: biology, economics and prediction**. Academic, San Diego, CA, 2008.

ORT, D. R.; LONG, S. P. Limits on yields in the Corn Belt. **Science**, v.344, p.484, 2014.

OSTLIE, K. R.; HIGLEY, L. G.; KASTER, L. V.; SHOWERS, W. B. European corn borer (Lepidoptera: Pyralidae) development, larval survival and adult vigor on meridic diets containing marker dyes. **Journal of Economic Entomology**, v.77, p.118-120, 1984.

OSTLIE, K.; PEDIGO, L. Incorporating pest survivorship into economic thresholds. **Bulletin of Entomological Society of America**, v.33, p.98-102, 1987.

PAULA-MORAES, S. V.; HUNT, T. E.; WRIGHT, R. J.; HEIN, G. L.; BLANKENSHIP, E. E. On-Plant movement and feeding of bean cutworm (Lepidoptera: Noctuidae) early instars on Corn. **Environmental Entomology**, v.41, n.6, p.1494-1500, 2012.

PAULA-MORAES, S.; HUNT, T. E.; WRIGHT, R. J.; HEIN, G. L.; BLANKENSHIP, E. E. Western bean cutworm survival and the development of economic injury levels and ecnonomic thresholds in field corn. **Journal of Economic Entomology**, v. 106, n.3, v.1274-1285, 2013.

PECK, S.; GOULD, F.; ELLNER, S. Spread of resistance in spatially extended regions of transgenic cotton: implications for management of *Heliothis virescens* (Lepidoptera: Noctuidae). **Journal of Economic Entomology**, v.92, p.1-16, 1999.

PERKINS, L. E.; CRIBB, B. W.; HANAN, J.; GLAZE, E.; BEVERIDGE, C.; ZALUCKI, M. P. Where to from here? The mechanisms enabling the movement of first instar caterpillars on whole plants using *Helicoverpa armigera* (Hubner) **Arthropod-Plant Interactions**, v.2, p.197–207, 2008.

PITRE, H. N.; HOGG, D.B. Development of the fall armyworm on cotton, soybean and corn. **Journal of the Georgia Entomological Society**, v.18, p.187-194, 1983.

POGUE, G. M. A world revision of the genus Spodoptera Guenée (Lepidoptera: Noctuidae). **Memoirs of the American Entomological Society**, v.43, p.1-202, 2002.

PRICE, P. W. **Insect Ecology**. Third edition. John Wiley & Sons, New York, 1997. 874p.

QURESHI, J.A.; BUSCHMAN, L.L.; THRONE, J.E.; RAMASWAMY, S.B. Oil-soluble dyes incorporated in meridic diet of Diatraea grandiosella (Lepidoptera: Crambidae) as marker for adult dispersal studies. **Journal of Economic Entomology**, v.97, p.836-845, 2004.

ROSS, S. E.; OSTLIE, K. R. Dispersal and survival of early instars of European corn borer (Lepidoptera: Pyralidae) in field corn. **Journal of Economic Entomology**, v.83, p.831-836, 1990.

SEYMOUR, R. C.; HEIN, G. L.; WRIGHT R. J.; CAMPBELL, J. B. **Western bean cutworm in corn and dry bean**s. Neb- Guide G1359. Nebraska Cooperative Extension, Lincoln, NE, 2004.

SHELTON, A. M., ZHAO, J. Z.; ROUSH, R. T. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. **Annual Review of Entomology**, v.47, p.845-881, 2002.

SOUTHWOOD, T. R. E.; HENDERSON, P. A. **Ecological Methods**, 3rd edn. Blackwell Science, Oxford, UK, 2000.

TABASHNIK, B. E.; CARRIÈRE, Y.; DENNEHY, T. J.; MORIN, S.; Sisterson, M. S.; Roush, R. T.; Shelton, A. M.; Zhao, J. Z. Insect Resistance to transgenic Bt crops: lessons from the laboratory and field. **Journal of Economic Entomology**, v.96, p.1031-1038, 2003.

TABASHNIK, B. E.; GASSMANN, A. J.; CROWDER, D. W.; CARRIÈRE, Y. Insect resistance to Bt crops: evidence versus theory. **Nature Biotechnology**, v.26, p.199–202, 2008.

TABASHNIK, B. E.; VAN RENSBURG, J. B. J.; CARRIÈRE, Y. Field-evolved insect resistance to Bt crops: definition, theory, and data. **Journal of Economic Entomology**, v.102, p.211-225, 2009.

TOOKER, J. F., FLEISCHER, S. J. First report of western bean cutworm (Striacosta albicosta) in Pennsylvania. **Crop Management**, 2010.

USDA - United States Department of Agriculture. **World Agricultural Supply and Demand Estimates, 2015**. Acesso em: 11 de Maio de 2015. Disponível em: http://usda.mannlib.cornell.edu.

VILARINHO, E. C., FERNANDES, O. A.; OMOTO, C.; HUNT, T. E. Oil-soluble dyes for marking *Spodoptera frugiperda* (Lepidoptera: Noctuidae). **Journal of Economic Entomology**, v.99, p.2110-2115, 2006.

VILARINHO, E. C.; FERNANDES, O. A.; HUNT, T. E.; CAIXETA, D. F. Movement of Spodoptera frugiperda (Lepidoptera: Noctuidae) adults in maize in Brazil. **Florida Entomologist**, v.94, p.480-488, 2011.

ZALUCKI, M. P. et al. The biology and ecology of *Helicoverpa armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae) in Australia: what do we know? **Australian Journal of Zoology**, v. 34, p. 779-814, 1986.

ZALUCKI, M. P.; CLARKE, A. R.; MALCOM, S. B. Ecology and behavior of first instar larval Lepidoptera. **Annual Review of Entomology**, v.47, p.361-393, 2002.

ZHAO, X. C.; WU, K. M.; GUO, Y. Y. Oil-soluble dyes in larval diet used for marking *Helicoverpa armigera*. **Entomologia Experimentalis et Applicata**, v.126, p.256-260, 2008.