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RESISTÊNCIA DE GENÓTIPOS DE MILHO A *Dichelops melacanthus* (DALLAS) (HEMIPTERA: PENTATOMIDAE) E CARACTERIZAÇÃO DE INJÚRIAS DE PERCEVEJOS EM PLÂNTULAS DE MILHO

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RESISTANCE OF CORN GENOTYPES TO *Dichelops melacanthus* (DALLAS) (HEMIPTERA: PENTATOMIDAE) AND INJURY CHARACTERIZATION OF STINK BUGS ON CORN SEEDLINGS

Dissertation presented to School of Agriculture from UNESP - Campus of Botucatu, to obtain the degree of Doctor in Agronomy: Crop Protection

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Botucatu, 28 de maio de 2019.

To my beloved parents, Fátima and
Sérgio, that have always encouraged the
search for knowledge, and for my
brothers, Vitor, Vinicius and Guilherme

I dedicate

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RESUMO

Percevejos fitófagos são insetos-praga que atacam diversas culturas de importância agrícola. Estes insetos, no geral apresentam preferência pela parte reprodutiva de leguminosas, porém, devido a mudanças no sistema produtivo brasileiro, têm sido comumente observados alimentando-se de plântulas de milho (*Zea mays* L.), especialmente quando este sucede a soja [*Glycine max* (L.) Merr.] no campo, causando danos significativos e variados. O percevejo-barriga-verde, *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae), é considerado praga-chave de período inicial na cultura do milho no Brasil. A espécie *Euschistus heros* (F.) (Hemiptera: Pentatomidae) é a espécie mais abundante no Brasil e também pode se alimentar de plântulas de milho. O percevejo-marrom-marmorizado, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) foi introduzido nos Estados Unidos da América na década de 1990 e tem se dispersado rapidamente pelo país, trazendo prejuízos de até 100% em culturas de importância econômica. Embora percevejos adultos tenham seu potencial de danos reconhecido, pouco se sabe sobre os prejuízos relacionados aos demais estágios de desenvolvimento. Formas de controle alternativas ao método químico, e que causem menos impacto ao ambiente e ao homem são altamente desejáveis. A resistência de plantas é uma tradicional ferramenta do Manejo Integrado de Pragas (MIP) e tem como principais vantagens a eficiência, menor impacto ambiental e a compatibilidade com outros métodos de controle. Diante do exposto, o presente trabalho teve como objetivos: *i)* caracterizar antixenose e/ou antibiose de genótipos de milho a *D. melacanthus*; *ii)* determinar os danos causados por todas as fases de vida de *D. melacanthus* e *E. heros* em plântulas de milho; e *iii)* determinar os danos causados por diferentes fases de *H. halys* em plântulas de milho-doce nos Estados Unidos. Observou-se que, após 24 horas, *os* genótipos IAC 8046, SCS 156 Colarado e IAC 8390 foram menos infestados por *D. melacanthus*, indicando ocorrência de antixenose. Os genótipos IAC 8390 e JM 2M60 afetaram parâmetros biológicos do percevejo barriga-verde, revelando a ocorrência de antibiose. Os genótipos indicados acima podem ser utilizados em programas de melhoramento visando a resistência de plantas. Ninfas de quinto instar de *D. melacanthus* foram as que mais afetaram negativamente o peso fresco e diâmetro de caule das plântulas de milho. O quarto instar de *E. heros* foi o que mais afetou negativamente peso fresco e seco, altura e diâmetro de caule das plântulas de milho.

Plântulas de milho-doce servem como alimento ao percevejo *H. halys*, sendo os danos mais significativos quando as plantas são infestadas por ninfas de quarto instar. Em função do potencial de danos demonstrado, as fases jovens de percevejos devem ser consideradas para determinação de nível de dano econômico em milho, refletindo na tomada de decisão para controle desses insetos.

Palavras-chave: *Zea mays* L.; ninfas; resistência de plantas a insetos; potencial de danos.

ABSTRACT

Phytophagous stink bugs are insect pests that attack several crops of agricultural importance. These insects generally have a preference for the reproductive parts of leguminous plants, but due to changes in the Brazilian agricultural system, they have been commonly observed feeding on corn seedlings (*Zea mays* L.), especially when it succeeds soybean [*Glycine max* (L.) Merr.] in the field, causing significant and varied damage to the corn. The green-belly stink bug, *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae), is considered a key pest of early-stage corn in Brazil. The neotropical brown stink bug *Euschistus heros* (F.) (Hemiptera: Pentatomidae) is the most abundant stink bug in Brazil and feeds on corn seedlings as well. *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) was introduced in the United States of America in the 1990's and has quickly spread throughout the country, bringing yield losses of up to 100% in crops of economic importance. Thus, alternatives to chemical control which cause less impact to the environment and humans, are highly desirable. Plant resistance is a traditional Integrated Pest Management (IPM) tool and has advantages such as being efficient, having a lower environmental impact, and good compatibility with other control methods. Thus, the present work has the following objectives: i) to characterize antixenosis and/or antibiosis of corn genotypes to *D. melacanthus*; ii) to determine injury caused by different life stages of *D. melacanthus* and *E. heros* in corn seedlings iii) to determine the injury caused by different stages of *H. halys* in sweet corn seedlings in the United States. We observed that after 24 h *D. melacanthus* infested genotypes IAC 8046, SCS 156 Colorado and IAC 8390 significantly less than the others indicating antixenosis. The genotypes IAC 8390 and JM 2M60 affected biological parameters of the green-belly stink bug, revealing the occurrence of antibiosis. The genotypes indicated above may be used in breeding programs for plant resistance to stink bugs. Injuries caused by *D. melacanthus* caused greater injury to corn seedlings than injury from *E. heros*. Injury from fifth instars of *D. melacanthus* caused the shortest height, fresh weight, and stalk diameter in corn seedlings compared to other *D. melacanthus* life stages tested. Fourth instars of *E. heros* caused the smallest fresh and dry weight, shortest plant height, and smallest stalk diameter on corn seedlings compared to other *E. heros* life stages tested. *H. halys* fed on sweet corn seedlings and the most significant damage occured when plants were infested by fourth instars. Due to the injury potential demonstrated, the immature stages of stink bugs should be considered when determining the level of economic damage in corn, which is then reflected in decision-making about control of these insects.

Keywords: *Zea mays* L.; nymphs; host-plant resistance; injury potential.

SUMÁRIO

GENERAL INTRODUCTION

Corn crop (*Zea mays* L.) has become the most important cereal worldwide in the last decades. This change is mostly because of advances in the area of genetic engineering, and the release of cultivars adapted to all regions that produce this grain (CRUZ et al., 2019)

Better management of pests and pathogens has also contributed to the growth of crop yields in the latest harvests (CHAVAS; MITCHELL, 2018). Corn is a staple food for many people, and for animal feed as well (VALADARES FILHO et al., 2010). It is used in the production of other kinds of human food such as sweeteners (with a high amount of fructose), oils, maize syrup, and starches (VELJKOVIĆA et al., 2018). In parallel, industries have been increasing the use of corn as a raw material in the manufacture of products such as lysine, biodegradable items, isoglucose, ethanol, and others (BELLEMARE, 2015, VELJKOVIĆA et al., 2018).

In Brazil, corn has transformed in order to meet consumers demand and to increase Brazilian growers' profits. The increase in production is probably due to a greater acceptance of second harvests, as well as one crop succeeding the other in the fields, e.g., cultivation of cotton, wheat and soybean (WAQUIL et al., 2004). In addition, technological innovations regarding soil management, harvests, and storage and processing, contributed to the establishment of corn as the most important cultivated cereal worldwide (CRUZ et al., 2019).

Corn is cultivated in almost all regions of Brazil. The consumption of corn products in the past harvests was around 1,036 billion tons, while the expected consumption of the 2018/2019 harvest exceeds 1,087 billion tons. The increase in corn used as animal feed (13,41% increase), is one the main reasons for the increase in corn products consumption (CONAB, 2019).

In the United States of America (U.S.), corn is the most cultivated crop, occupying 30% of the cultivated area in the U.S., followed by soybean, with 24% (FAUSTI, 2015). In the past 2 decades, corn has been occupying lands that previously were used to cultivate soybean and wheat (WALLANDER et al., 2011). These change towards greater homogeneity in agricultural production systems also occurred in other regions of the U.S. and Canada (WIENS et al., 2011). The simplification of the landscape does not only imply a reduction of plant diversity, but also a decrease in ecosystem biodiversity as well (PURTAUF et al., 2005, MEEHAN et al., 2011).

However, the scenario of corn productivity for the future is uncertain, since recent increases are attributed, mainly, to *Bt* genotypes (FAUSTIN et al., 2012; SHI et al., 2013). In the world there are approximately 290 events developed to be resistant to insect pests, approved for more than 20 countries, resulting in 101 million hectares of transgenic corn planted worldwide (CTNBio, 2018). It is estimated that soybean, corn and cotton represent about 100 million hectares and 251 events together, but most of the approved events are for corn crop (ISAAA, 2017; BROOKES; BARFOOT ; 2018). Currently, the area of transgenic crops cultivated in Brazilian fields corresponds to 36 million hectares, making Brazil the country with the highest adoption of this technology (SILVA et al., 2018). In the U.S., *Bt* corn cultivation increased in 2014, on average, 80% in the Corn Belt, combining with the rapid expansion of ethanol production (CAI; STIEGERT, 2014, ISAAA, 2017; VELJKOVIĆA et al., 2018).

In spite of the increase in corn production in Brazil and U.S., this crop goes through intense phytosanitary problems, for example, insects attack corn in all the developmental stages, making control methods necessary, in order to avoid yield losses (CRUZ et al., 2012; DUARTE et al., 2015). Among insects that feed on corn, the complex of piercing sucking stink bugs, usually seen in soybean, have an important role as pests of early stages of corn (ÁVILA; PANIZZI, 1995; PANIZZI et al., 2015; SORIA et al., 2009; WAQUIL; OLIVEIRA, 2009). Stink bugs benefited from the massive adoption of no-till systems, since they have the habit of using debris as shelter (CHOCOROSQUI; PANIZZI, 2004). In addition, broad-spectrum insecticides used to target the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) are not used as frequently because of the wide adoption of *Bt* technology (NETTO et al., 2015; CRUZ et al., 2016). Among species that constitute the Brazilian stink bug complex, the neotropical brown stink bug *Euschistus heros* (F.) (Hemiptera: Pentatomidae) and the green belly stink bug *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae) are common and important species in the Brazilian soybean-corn production system (CHOCOROSQUI; PANIZZI, 2004; BUENO et al., 2015, CORRÊA-FERREIRA; SOSA-GÓMEZ, 2017, LUCINI; PANIZZI, 2018). *Dichelops melacanthus* is considered the stink bug species that causes the highest damage to corn plants, and has the potential to be up to three times more aggressive than other phytophagous Pentatomidae (ROZA-GOMES et al. 2011).

The green belly stink bug is usually found feeding on the stalk of corn seedlings, in an upside position, close to the soil (ÁVILA; PANIZZI, 1995; PANIZZI; LUCINI, 2018). Attacked plants show symptoms such as: holes on the leaves, that, as plant grows, becomes necrotic areas; plant height reduction; tillering; damages to the whorl and even plant death (ÁVILA; PANIZZI 1995; CHOCOROSQUI et al., 2004). Vegetative corn presents the most critical stage to stink bugs attack until V5 (5 completely expanded leaves) (RITCHIE; HANWAY, 1966), or while the stalk is less than 0,8 cm in diameter (CHIARADIA 2012).

The neotropical brown stink bug is the second species that has been frequently mentioned as causing damage in the early stages of corn crops. Due to its diapause ability, this species can remain in the field, surviving for a long time, even without the presence of soybean plants, which are their main host (HOFFMANN-CAMPO et al., 2012; PANIZZI et al., 2012; SOARES et al., 2018). However, there is little information about the interaction of these species with corn seedlings.

The brown marmorated stink bug (BMSB) *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) is an invasive species in the U.S. since 1996, being properly identified only in 2001 (HOEBEKE; CARTER, 2003). This species is native to Japan, Korea, China and Taiwan, but has been spreading to countries outside of Asia, including Switzerland, Germany, France, Italy, Greece, Russia, Chile, Canada and others (XU et al. 2014; LESKEY; NIELSEN, 2017). Currently, *H. halys* has spread to more than 40 American states (LESKEY; NIELSEN, 2017). BMSB is considered highly polyphagous, feeding in several hosts of economic importance (RICE et al., 2014). Corn is one of the crops target by *H. halys*, with population peaks during the R2-R4 growth stages, characterized as milk grains (VENUGOPAL et al 2015). When this insect feeds on developing grains, they can cause reduction in quality, directly impacting the final product (RICE et al., 2014; VENUGOPAL et al., 2014; CISSEL et al., 2015). Native species of stink bugs in the U.S., such as *Euschistus variolarius* (Palisot de Beauvois) and *E. servus* (Say) (Hemiptera: Pentatomidae) have been found feeding in corn seedlings and present a potential threat (TOWNSEND; SEDLACEK, 1986; SEDLACEK; TOWNSEND, 1988; SAPPINGTON et al., 2018). It is not known how the emerging problem of *H. halys* in the U.S. will affect corn in early stage. To our knowledge, no studies have been performed to test the effect of *H. halys* on early growth stages of corns.

In general, the main strategies used in Brazil to control phytophagous stink bugs on corn are seed treatments. Synthetic insecticides are also common, specifically the use of neonicotinoids, oxine methylcarbamate (thiodicarb) pyrazole (fipronil) and avermectin (abamectin) (DOUGLAS; TOOKER; 2015; MILOSAVLJEVIĆ et al., 2019). These insecticides are considered persistent, offering long-term protection for seeds and seedlings. However, these compounds have also received great attention because of their potential effects on non-target organisms, such as pollinators and natural enemies, as well as for concerns regarding insect resistance (AFIFI et al., 2014; FURLAN, KREUTZWEISER, 2015; ALFORD; KRUPKE, 2017, CAMARGO et al., 2019).

The consequences from abusing chemical insecticides make it necessary to search for new, efficient, and environmentally safe control strategies. The use of resistant genotypes is a traditional Integrated Pest Management (IPM) tool, if this technology is persistent and compatible with other control techniques (PAINTER, 1951).

Host plant resistance to insects can be classified into three categories: antixenosis, antibiosis and tolerance (PAINTER, 1951, BALDIN et al., 2019). Plants that express antixenosis are used less as food, oviposition sites or shelter. In general, this happens because the plant possesses allelochemicals and/or morphological characteristics that affect insect behavior during its choice for a host (Painter 1951; Smith 2005). Antibiosis occurs when plants adversely affect insects' biological parameters, such as changes to development period, reduction in size and weight, lower fertility and fecundity rates and high mortality rates in early stages (PANDA; KHUSH, 1995; SMITH, 2005). A genotype is considered tolerant to an insect when it does not affect the insects behavior or biological parameters, and when under the same level of insect infestation, the plant is less damaged than others cultivars (SMITH, 2005).

Although there are studies that describe the damage potential of *D. melacanthus* on plants of Poaceae family, including corn (CHOCOROSQUI; PANIZZI, 2004; SMANIOTTO; PANIZZI, 2015), to date there are no studies that assessed behavior and biological performance of *D. melacanthus* on a wide range of corn germplasm (*Bt* and non-*Bt* genotypes), aiming to characterize resistance categories.

The economic damage level of *D. melacanthus* in corn seedlings can vary depending on the corn producing region, but in general it is around 0,8 insects per m^2. However, these levels are usually based on only adult insects (DUARTE et al., 2015). As mentioned, no studies have reported the economic damage levels for *E. heros* in Brazil, and *H. halys* in U.S. in the early stages of corn. Little is known about the role that stink bug nymphs can have in the injury caused to corn seedlings. Since nymphs from third to fifth instars of *D. melacanthus* are commonly found finishing their development in corn seedlings which were planted following soybean, this information should be considered (CÔRREA-FERREIRA; SOSA-GÓMEZ, 2017).

It is known that injury caused by stink bugs in corn seedlings can vary according to the number of insects and plant development stage (NETTO et al., 2016). Stink bugs feeding on early stage corn can cause irreversible injury, since it occurs in a short period after plant emergence (SAPPINGTON et al., 2018). In this sense, it is indispensable to investigate if a recently invasive polyphagous species, such as *H. halys*, would demonstrate the same feeding behavior as native species, and the amount of injury that it can cause, in order to develop management strategies.

Thus, the specific goals of our study were: a) to characterize the possible expression of antixenosis and/or antibiosis in 16 corn genotypes (*Bt* and non-*Bt*) to *D. melacanthus*; b) to assess injuries caused by development stages of *D. melacanthus* and *E. heros* to corn seedlings; C) to assess injuries caused by different stages of *H. halys* to sweet corn seedlings.

In order to complete these objectives, the dissertation is divided in three chapters, which were written according to the Journal of Economic Entomology's guidelines.

CHAPTER 1

Characterization of antixenosis and/or antibiosis in corn genotypes to *Dichelops melacanthus* (Hemiptera: Pentatomidae)

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ABSTRACT

Stink bugs are considered damaging pests to important crops worldwide. Several species are associated to leguminous crops, usually feeding on their reproductive stage. In Brazil, the green-belly stink bug, *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae), has become a key pest to the early stages of corn crops, especially when corn succeeds soybean in the field. Injuries caused by this species vary from holes on new leaves to plant death in severe attacks. The main control techniques for this pest are seed treatments and insecticide spraying. Although both methods show some efficiency, factors such as resistance to insecticides and differences in the susceptibility among stink bugs population to insecticides, leads to the search for other control strategies. Thus, tools that are efficient and pose a low risk to the environment and humans are highly desirable. In this sense, natural plant resistance could be a valuable stink bug management tool, being compatible with other integrated pest management (IPM) methods. This study evaluated the resistance of 16 corn genotypes to *D. melacanthus* in order to characterize antixenosis and/or antibiosis expression. Antixenosis was assessed through preference multi-choice tests with 5^{th} instar nymphs. Antibiosis was assessed using 60 2^{nd} instar nymphs that were confined on seedlings of 13 selected genotypes. Seedlings of IAC 8046, IAC 8390 and SCS 156 Colorado corn genotypes were less infested by *D. melacanthus* 24 h after bugs were released indicating antixenosis expression. Genotypes IAC 8390 and JM 2M60 negatively affected some biological parameters of the green-belly stink bug, indicating the occurrence of antibiosis. These corn genotypes can be useful to breeding programs focusing on corn resistance to stink bugs species.

Keywords: *Zea mays* L.; green-belly stink bug; host plant resistance.

Introduction

Phytophagous stink bugs, (Hemiptera: Pentatomidae) are known to be pests of many important crops, feeding on several structures of their host plants, but giving preference to reproductive structures such as seeds and fruits (PANIZZI 1997; MCPHERSON; MCPHERSON 2000). Changes in Brazilian agricultural practices, such as the massive adoption of no-tillage and the increase of continuous cropping (corn and soybean), have favored stink bugs that spend part of their life in the soil and under debris (CHOCOROSQUI; PANIZZI 2004; Panizzi 2015). The presence of the green-belly stink bug, *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae) has increased in Brazilian and Argentinian fields, being found damaging wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.) seedlings, and becoming a key pest in these two crops (ÁVILA; PANIZZI 1995; CHOCOROSQUI; PANIZZI 2004; MANFREDI-COIMBRA et al. 2005; PANIZZI et al. 2007; PANIZZI 2015). Both species of the green-belly stink bugs, *D. melacanthus* and *D. furcatus* (F.), are neotropical species and pose a potential risk of invasion to the United States of America (PANIZZI 2015). Thus, they have been receiving the attention of researchers around the world.

Additionally, the preference of Brazilian growers to plant *Bt* hybrids may have contributed to the increased damage to corn seedlings by *D. melacanthus*, since this technology leads to fewer applications of insecticides previously used to control the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). Management of *S. frugiperda* would also control non-target pests, as stink bugs (NETTO et al. 2015; CRUZ et al. 2016).

Dichelops melacanthus is usually found feeding on the stem of corn seedlings, in an upside down position close to the soil (ÁVILA; PANIZZI 1995; PANIZZI; LUCINI 2018). Attacked plants show symptoms such as wilting and feeding marks (holes on the new leaves inside the whorl). As the plant grows, the holes become necrotic areas in transversal direction, and height reduction, tillering and whorl rolling (when the new leaves do not open, rolling one to the other) is commonly observed. In severe attacks, *D. melacanthus* can also cause plant death (ÁVILA; PANIZZI 1995; CHOCOROSQUI 2001, ROZA-GOMES et al. 2011). Corn seedlings are most susceptible to *D. melacanthus* feeding until V5 stage – when plants have five completely expanded leaves (RITCHIE; HANWAY 1966), or while the stem is smaller than 0.8 cm in diameter (CHIARADIA 2012). Although there is no information regarding yield losses because of *D. melacanthus* feeding on corn seedlings, when infesting no-tillage wheat plants it can cause up to 34% reduction on the number of seed heads and 31% on seed yield (CHOCOROSQUI; PANIZZI 2004).

Current management tools for *D. melacanthus* include seed treatment with neonicotinoids and spraying with several chemical insecticides (ÁVILA; PANIZZI 1995; MARTINS et al. 2009; ÁVILA; DUARTE 2012). Although synthetic insecticides have some efficiency, overuse, especially of broad-spectrum compounds, have been favoring outbreaks of pest resistance to insecticides (TEMPLE et al. 2009; BAUR et al. 2010; SOSA-GÓMEZ; SILVA 2010). In addition, there are significant differences in insecticide susceptibility among stink bugs populations (BASSO et al. 2016).

Thus, the use of resistant genotypes could be considered a valuable strategy for the management of green-belly stink bugs in crop systems. Resistant genotypes present several advantages, such as maintaining the pest population under the economic injury level and being more persistent and compatible with other control methods (PAINTER 1951). Resistance can be divided in three categories: antixenosis, antibiosis and tolerance (PANDA; KHUSH 1995). Plants that express antixenosis are used by pests less as food, oviposition sites or shelter than non-resistant plants. In general, this happens because the plant possess allelochemicals and/or morphological characteristics that affect insect behavior during its choice for a host (PAINTER 1951; SMITH 2005). Antibiosis occurs when plants adversely effect insects' biological parameters, such as changes to development period, reduction in size and weight, lower fertility and fecundity rates and high mortality rates (PANDA; KHUSH 1995, SMITH 2005). A plant genotype is considered tolerant to an insect when it does not affect its behavior and biological parameters, but instead is less damaged than others cultivars under the same level of insect infestation (SMITH 2005).

Although there are studies that describes damage potential of *D. melacanthus* on plants of Poaceae family, including corn (CHOCOROSQUI; PANIZZI 2004; SMANIOTTO; PANIZZI 2015), to date there are no studies that assessed behavior and biological performance of *D. melacanthus* on a wide range of corn germplasm (*Bt* and non *Bt* genotypes).

Thus, considering the economic importance of the green-belly stink bug to corn crops, and the need for tools that are less damaging to the environment, humans and non-target insects, this study was carried out to assess possible resistance to *D. melacanthus* of corn genotypes, aiming to characterize antixenosis and antibiosis expression.

Material and Methods

Green-belly stink bug stock colony

A stock colony of *D. melacanthus* was started with egg masses provided by DuPont Company (Paulínia, São Paulo, Brazil) and maintained under laboratory conditions (26 ± 2º C, 65 ± 10% RH, and 14 h photophase), similar to the methodology described by Canassa et al. (2017). Insects were placed in arenas made of plastic containers (40 × 22 × 14 cm) closed with lids, containing a central cut covered with organdy fabric, to allow proper ventilation. The lower surface of cages was covered with filter paper in the same size, for excrement absorption.

Separate arenas were used to keep nymphs and adults, and each arena contained around 50 bugs. They were fed a natural diet, consisting of pods from common beans (*Phaseolus vulgaris* L.), small portions of raw peanuts (*Arachis hypogaea* L.) and sunflower seeds (*Helianthus annuus* L.) replacing food twice a week. Cotton moistened with distilled water was placed inside cages, over Petri dishes (4 cm diameter) meeting bugs water needs and humidity maintenance.

Additionally, dry cotton disks were placed in each corner of adult cages, serving as shelter and oviposition sites. Eggs were collected daily and placed inside a Petri dish (8.5 cm diameter) lined with filter paper on the bottom and a green bean pod was added to provide food for the hatched 1st instar nymphs. Then, a new cage was set up with 2nd instar nymphs as previously described.

Multi-choice test

To assess the preference of *D. melacanthus* to 16 corn genotypes (Table 1), we performed a free choice test, using a methodology similar to that which was used for *Aphys glycines* Matsumura (Hemiptera: Aphididae) (DIAZ-MONTANO et al. 2006, BALDIN et al. 2016).

Corn seeds were planted in a Styrofoam tray (128 cells), filled with commercial substrate Tropstrato® (Vida Verde Ltda, Mogi-Mirim, São Paulo, Brazil), until they reached V3 stage. After that, seedlings were transplanted equidistantly, in circle, in a round container (60 cm diameter × 25 cm height) filled with soil, sand, manure and substrate, in the ratio of 1:1:1:1 (v/v/v/v). Plants were irrigated as need. In the center of the container, 16 5th instar stink bugs (24 h old, 6 h starved) were released. The number of insects per plant was counted 3 h, 6 h and 24 h after nymphs were released.

Antibiosis test

Biological performance of *D. melacanthus* confined on 13 selected corn genotypes (Table 1) was assessed in partially controlled greenhouse climate conditions, (25.1 ºC, 72% RH, and natural light). Corn seedlings were infested with 2nd instar nymphs, because *D. melacanthus* presents high mortality rates in the first immature stage (CHOCOROSQUI; PANIZZI 2004). The three most infested genotypes in the multiple-choice test were excluded from this experiment, since they were considered highly susceptible to *D. melacanthus* nymphs. Corn plants in the stages between V1-V5 were infested with stink bugs because those stages are known to be susceptible to the green-belly stink bug attack (CHIARADIA et al. 2016).

Corn seeds were sowed in 11 L plastic pots, filled with the same substrate as described previously. Sowing occurred every seven days, to ensure the presence of V5 plants for the entirety of the insect’s life. Each pot had between 10 to 15 seedlings, and the old ones were removed when necessary. This procedure was adopted to avoid having to move the bugs onto new plant, reducing potential stress.

Four pots per genotype were used, and each one of them was infested with 15 stink bugs (60 nymphs per genotype, in total). After nymphs were released, a metallic cage (35 cm diameter × 55 cm height) was affixed around the pots and covered with organdy fabric and closed with an elastic piece on the bottom to avoid stink bug escape. Each pot with plants and bugs was considered one replication (four per genotype) in a completely randomized design. The experiment was assessed daily, to evaluate duration of each nymphal stage (N2, N3, N4 and N5), development period (egg-adult), weight of 5th instars nymph (at 24 h post ecdysis), weight of adults (at 24 h post ecdysis), nymphal mortality and adult longevity.

Statistical analysis

A general linear model (GLM) (NELDER; WEDDERBURN 1972) with quasi-Poisson distribution was used for the analysis of number of insects attracted to different corn genotypes. The goodness-of-fit was determined using a half-normal probability plot with a simulated envelope (DEMETRIO; HINDE 1997, HINDE; DEMETRIO 1998). When a significant difference was observed between treatments, multiple comparisons (Tukey's test, $p<0.05$) were performed using the glht function of the multicomp package with adjustment of p values. The analyses were performed using the software "R", version 2.15.1 (THE R DEVELOPMENT CORE TEAM 2012). Biological parameters had normality verified by Shapiro-Wilk test and homogeneity, by Levene test. The significance of treatment effects was determined using a LSD test to compare means. For the analysis, was used the statistical package PROC MIXED-SAS 9.2 (SAS Instititute 2001).

Results

Antixenosis

Regarding the preference of *D. melacanthus* nymphs for different corn genotypes, no differences were observed at 3 ($F = 1.15$; df = 15, 304; $P = 0.3124$) and

6 hours (F = 0.55; df = 15, 304; P = 0.9126) of insect release. However, 24 h after the release, IAC 8046 (0.00 nymphs), SCS 156 Colorado (0.10 nymphs), and IAC 8390 (0.15 nymphs) genotypes were the least infested by *D. melacanthus* 5th instars (F = 3.09; df = 15, 304; P = 0.0001). Conversely, seedlings of 30F 53 VYHR (0.80 nymphs), 30F 53 (0.80 nymphs) and 30F 53 YHR (0.55 nymphs) genotypes were the most infested (Table 2).

Antibiosis

Corn genotype did not significantly affect 2nd instar development time (Table 3). Development time of *D. melacanthus* nymphs was significantly affected by different corn genotypes in 3rd, 4th and 5th instars. The genotypes SCS 155 Catarina (8.58 days), IAC 8390 (8.32 days) and IAC 8046 (8.01 days) induced the longest development duration on 3rd instar (F = 3.06; df = 12, 36; P = 0.0046) whereas SCS 154 Fortuna required the shortest period in this stage (5.84 days). Fourth instars fed on IAC 8390 (9.02 days) and IAC 8077 (8.43 days) showed the longest developmental duration in this stage (F = 2.53; df = 12, 36; P = 0.0157), differing from most other genotypes, mainly SCS 154 Fortuna (6.10 days), SCS 156 Colorado (6.63 days), 2 B 433 PW (6.81 days), 2 B 512 PW (6.83 days) and JM 2M 80 (6.89 days). The IAC 8390 genotype (15.88 days) extended the developmental period of 5th instars (F = 2.15; df = 12, 36; P = 0.0393), differing of most genotypes mainly SCS 154 Fortuna and 30F 53 HR (9.85 and 10.02 days, respectively).

The total nymphal period was significantly longer (F = 3.01; df = 12, 36; P = 0.0064) when the green-belly stink bug fed on IAC 8390 seedlings (41.72 days). The shortest duration was when nymphs fed on SCS 154 Fortuna (Table 3).

We observed a wide variation of nymphal viability among corn genotypes, ranging from 17.27% to 82.22% (Figure 1). Higher rates of adult emergence were observed when nymphs were confined on plants of 2B 433 PW (61.11 %), 2B 512 PW (67.75 %) and 2B 610 PW (82.22 %). On the other hand, the genotypes IAC 8390 (17.27%) and JM 2M60 (25.00%) negatively affected stink bug survival.

Corn genotype did not significantly affect 5th instars development time (F = 0.93; df = 12, 37; P = 0.5306), adult weight (F = 1.82; df = 12, 19; P = 0.1181), or adult longevity (F = 0.74; df = 12, 34; P = 0.7061).

Discussion

Plants expressing antixenosis resistance to insects have morphological factors and/or chemical compounds that deter insects (PANDA; KHUSH 1995). Seedlings of all varieties used in these assays did not appear to differ in color or to have any pronounced morphological characteristic among them, which could indicate physical or morphological causes of resistance. It is possible volatile chemical substances may differ between genotypes, and since phytophagous insects can detect chemical stimulation during host selection, volatile chemical substances may affect if a genotype will be more or less colonized (BERNAYS; CHAPMAN 1994). Plants can produce several insecticidal compounds to defend themselves. Chemical compounds like saponine, glucoside, ethylene and jasmonate acid are known to be produced by corn plants and reduce insect attacks (GEYTER et al. 2007, YAN et al. 2012, MEIHLS et al. 2013, LOUIS et al. 2015). In our study, significant differences were observed among corn genotypes only 24 h after bugs were released, probably after they were able to interact with the diverse set of genotypes. Genotypes IAC 8046, SCS 156 Colorado and IAC 8390 were infested by *D. melacanthus* nymphs significantly less than other genotypes indicating the presence of antixenosis.

Corn genotype significantly affected some biological parameters of *D. melacanthus*, indicating antibiosis resistance, as observed by Painter (1951). Phytophagous stink bugs, in general, prefer and only reproduce when they feed on leguminous or Brassicaceae crops, although they can be found on several hosts (OLSON et al. 2011, SMANIOTTO; PANIZZI 2015). This means that on the majority of alternative hosts, such as corn, stink bugs are searching for nutrients, water or shelter. *Dichelops melacanthus* has been associated with 29 hosts, but reported to reproduce only on five of them, wheat being the only plant in the Poaceae family (SMANIOTTO; PANIZZI 2015). This information diverges from our study, because although that was not our goal (selection of hosts), we observed insects becoming adults and laying viable eggs while exclusively feeding on corn seedlings.

Phytophagous stink bugs depend on essential nutrients, such as proteins, lipids and carbohydrates. Although these insects are generalists and feed on stems, flowers, fruits and leaves, they can find all the essential nutrients in the endosperm of seeds (SLANSKY JR.; PANIZZI 1987, OLSON et al. 2011). Despite that fact, there are stink bugs that prefer to feed on stems instead of reproductive parts of plants, such as the brown-winged stink bug, *Edessa meditabunda* (F.) (Hemiptera: Pentatomidae), on soybean (SILVA et al. 2012). *Dichelops melacanthus* is reported to prefer soybean pods, and will feed on corn seedling in its absence (ÁVILA; PANIZZI 1995), although its nymphal viability was higher when fed on some genotypes of corn seedlings compared to feeding only on soybean pods (CANASSA et al. 2017).

IAC 8390 genotype prolonged *D. melacanthus* nymphal development, besides resulting in the lowest nymphal viability (< 20 %) and being one of the least infested genotypes 24 h after a multi-choice antixenosis test, indicating resistance (antibiosis and/or antixenosis) to the green belly stink bug. The low colonization of IAC 8046, SCS 156 Colorado, and IAC 8390 by *D. melacanthus* suggests that few stimuli to start feeding are present and/or the presence of unpalatable compounds, which can affect stink bugs, such as *Nezara viridula* (L.) (Hemiptera: Pentatomidae) in soybean genotypes (SOUZA et al. 2013). Less infested genotypes usually present feeding deterrents which inhibit feeding, suggesting antixenosis expression (SMITH 2005).

Antibiosis and antixenosis usually overlap, which means that genotypes with high level of antixenotic factors may also cause deleterious effects on insect biology, resulting in similar effects as plants that express antibiosis (PAINTER 1951; SMITH 2005). Because of this, it might be difficult to differentiate between categories, requiring specific examination of the insect consumption (SMITH 2005, BALDIN et al. 2014, MORANDO et al. 2017). For stink bugs, the electrical penetration graph (EPG) technique has been used (LUCINI; PANIZZI 2017, PANIZZI; LUCINI 2018) for feeding behavior characterization, and may be used in the future to distinguish plant resistance categories.

In experiments with *S. frugiperda*, IAC 8390 also caused reduction in larvae weight and caterpillars feeding on this genotype had less reproductive capacity in the adult stage due lower oviposition rates indicating antibiosis expression (MORAES et al. 2018). On the other hand, in resistance assessments with *Bt* and non-*Bt* corn and *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae), IAC 8390 did not significantly differ from other genotypes in antibiosis and antixenosis tests (LOPES 2014). IAC 8390 was considered resistant to the root lesion nematode *Pratylenchus brachyurus* Godfrey (NIKUMA et al. 2012) and along with IAC 8046 (the least colonized genotype in our study), to the southern rust *Puccinia polysora* Underw. (DUDIENAS et al. 2017).

The genotype JM 2M60 showed the second lowest rate for nymphal viability in our study. Plants producing secondary compounds, such as observed with soybean isoflavones over *Piezodorus guildinii* (West.) (Hemiptera: Pentatomidae) (SILVA et al. 2013), may influence in the biology of the insect, leading to antibiosis resistance. This is the first study to characterize JM 2M60 resistance to insects, and further studies should be carried out to examine chemical and/or morphological traits and the potential of JM 2M60 as a resistant corn genotype.

In the present study, we observed that 30F 53 HR led to faster development of *D. melacanthus*. Additionally, 30F 53 YHR, 30F 53 VYHR and 30F 53 genotypes were the most preferred 24 h after nymphal release, demonstrating susceptibility to the green-belly stink bug. The isoline, 30F 53, was considered susceptible to the fungus that causes the maize white spot *Cercospora zeae-maydis* Tehon and E. Y. Daniels, and the bacteria that causes the gray leaf spot *Pantoea ananatis* Serrano (DE BRITO et al. 2011). However, no studies have evaluated the relationship of these genotypes to insect feeding.

Based on the results obtained in our study, we verified that the genotypes IAC 8390 and JM 2M60 interfered negatively on biological parameters of *D. melacanthus*, indicating antibiosis and/or antixenosis expression. The genotypes IAC 8046, SCS 156 Colorado and IAC 8390 were the least colonized by *D. melacanthus* 5^{th} instars, indicating antixenosis expression.

This study is the first to access corn seedling resistance (antibiosis and/or antixenosis) to stink bugs, providing valuble information regarding this issue. Some of the genotypes tested in these experiments have never been tested for host plant resistance to insects, so it is not possible to compare our work to other results. Thus, further research aiming to assess corn seedling resistance to *D. melacanthus* is recommended. Our results indicate resistant plants could be an effective IPM tool for managing *D. melacanthus* on corn seedlings. This information can be also explored by breeding programs aiming to develop resistant corn genotypes to insects.

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Table 1. Corn genotypes, characteristics and sources used to test antixenosis and antibiosis to *Dichelops melacanthus*.

Genotype	Source	Characteristics/history of resistance
IAC 8077	IAC*	Non-*Bt* corn, commercial material (IAC 2014)
IAC 8390	IAC	Non-*Bt* corn, commercial material (IAC 2019)
IAC 8046	IAC	Non-*Bt* corn, commercial material (IAC 2014)
JM 2M80	IAC	Non-*Bt* corn, commercial material (Freitas et al. 2017)
JM 2M77	IAC	Non-*Bt* corn, commercial material (Freitas et al. 2017)
JM 2M60	IAC	Non-*Bt* corn, commercial material (Freitas et al. 2017)
2B433PW	IAC	*Bt* corn, resistant to lepidopteran insects and tolerant to herbicides (Freitas et al. 2017)
2B512PW	IAC	*Bt* corn, resistant to lepidopteran insects and tolerant to herbicides (Freitas et al. 2017)
2B610PW	IAC	*Bt* corn, resistant to lepidopteran insects and tolerant to herbicides (Freitas et al. 2017)
30F53	EPAGRI**	Non-*Bt* corn, isoline (Pioneer 2019)
30F53 HR	EPAGRI	*Bt* corn, resistant to lepidopteran insects and tolerant to herbicides (Pioneer 2019)
30F53 YHR	EPAGRI	*Bt* corn, resistant to lepidopteran insects and tolerant to herbicides (Pioneer 2019)
30F53 VYHR	EPAGRI	*Bt* corn, resistant to lepidopteran insects and tolerant to herbicides (Pioneer 2019)
SCS 155 Catarina	EPAGRI	Non-*Bt* corn, commercial material with open pollination (EPAGRI 2018)
SCS 154 Fortuna	EPAGRI	Non-*Bt* corn, commercial material with open pollination (EPAGRI 2018)
SCS 156 Colorado	EPAGRI	Non-*Bt* corn, commercial material with open pollination (EPAGRI 2018)

*IAC - Agronomic Institute of Campinas.
**EPAGRI - Research Center for Family Agriculture, Research and Rural Extension Company of Santa Catarina.

Table 2. Mean (±SE) number of 5th instar nymphs of *Dichelops melacanthus* on corn genotypes over time in multi-choice test.

Genotypes	Time (hours)		
	3ns	6ns	24*
IAC 8046	0.30 ± 0.16	0.25 ± 0.16	0.00 ± 0.00 e
SCS 156 Colorado	0.10 ± 0.08	0.25 ± 0.14	0.10 ± 0.08 de
IAC 8390	0.05 ± 0.06	0.25 ± 0.11	0.15 ± 0.09 cde
IAC 8077	0.35 ± 0.12	0.35 ± 0.15	0.25 ± 0.14 bcde
30F 53 HR	0.15 ± 0.09	0.30 ± 0.12	0.25 ± 0.14 bcde
JM 2M 80	0.15 ± 0.09	0.30 ± 0.14	0.25 ± 0.11 bcde
JM 2M 77	0.10 ± 0.08	0.30 ± 0.14	0.30 ± 0.14 bcde
JM 2M 60	0.15 ± 0.09	0.20 ± 0.13	0.30 ± 0.14 bcde
2B 512 PW	0.05 ± 0.06	0.10 ± 0.08	0.35 ± 0.15 bcde
2B 610 PW	0.20 ± 0.10	0.20 ± 0.10	0.35 ± 0.12 bcde
2B 433 PW	0.15 ± 0.09	0.40 ± 0.19	0.45 ± 0.17 abcd
SCS 154 Fortuna	0.20 ± 0.10	0.45 ± 0.21	0.50 ± 0.21 abc
SCS 155 Catarina	0.15 ± 0.09	0.20 ± 0.10	0.50 ± 0.19 abc
30F 53 YHR	0.25 ± 0.14	0.40 ± 0.15	0.55 ± 0.17 ab
30F 53 ISOLINE	0.00 ± 0.00	0.35 ± 0.12	0.80 ± 0.25 a
30F 53 VYHR	0.10 ± 0.08	0.35 ± 0.12	0.80 ± 0.17 a
P	0.1529	0.8805	0.0001

ns non-significant.
* Means followed by different letters in a column indicate a significant difference among treatments (GLM with Gaussian distribution followed by a post hoc Tukey test, (p<0.05).

Table 3. Mean (± SE) values of nymphal development and total duration (days) of *Dichelops melacanthus* on corn genotypes in antibiosis and/or antixenosis test.

Genotypes	Instar duration (days)				Nymphal period (days)
	2^{nd}	3^{rd*}	4^{th*}	5^{th*}	$(2^{nd}$-$5^{th})^{*}$
IAC 8390	7.99 ± 0.75	8.32 ± 1.07 ab	9.02 ± 0.49 a	15.88 ± 2.54 a	41.72 ± 1.80 a
IAC 8077	6.48 ± 0.81	6.58 ± 0.17 defg	8.43 ± 0.60 ab	13.42 ± 1.57 ab	33.81 ± 2.28 b
SCS 155 Catarina	8.49 ± 1.91	8.58 ± 0.33 a	7.48 ± 0.52 bcd	10.86 ± 0.80 bc	33.40 ± 2.07 b
JM 2M 80	7.64 ± 0.49	7.86 ± 0.52 abcde	6.89 ± 0.68 cd	12.61 ± 0.49 abc	32.87 ± 1.00 b
2B 610 PW	6.13 ± 1.12	6.85 ± 0.29 bcdefg	6.96 ± 0.10 bcd	11.76 ± 1.27 bc	32.77 ± 1.76 b
JM 2M 60	7.13 ± 0.75	6.68 ± 0.29 cdefg	8.08 ± 0.33 abc	10.75 ± 0.89 bc	32.04 ± 1.04 b
IAC 8046	7.48 ± 0.52	8.01 ± 0.31 abc	7.45 ± 0.32 bcd	10.45 ± 0.89 bc	32.20 ± 2.31 bc
2B 433 PW	6.00 ± 0.55	7.38 ± 0.60 abcdef	6.81 ± 0.89 cd	10.52 ± 1.20 bc	31.94 ± 1.62 bc
JM 2M 77	6.78 ± 0.93	7.98 ± 0.58 abcd	7.53 ± 0.56 bc	10.71 ± 1.10 bc	31.64 ± 1.09 bc
30F 53 HR	6.29 ± 1.02	7.93 ± 0.54 abcd	7.88 ± 0.70 abc	10.02 ± 0.78 c	30.63 ± 2.91 bc
2B 512 PW	6.94 ± 0.48	6.45 ± 0.30 efg	6.83 ± 0.31 cd	10.87 ± 0.41 bc	29.99 ± 1.25 bc
SCS 156 Colorado	7.16 ± 0.56	6.31 ± 0.44 fg	6.63 ± 0.06 cd	10.10 ± 0.48 bc	29.85 ± 0.50 bc
SCS 154 Fortuna	6.19 ± 0.64	5.84 ± 0.28 g	6.10 ± 0.26 d	9.85 ± 0.38 c	27.37 ± 1.36 c
P	0.7534	0.0046	0.0157	0.0393	0.0064

[ns] non-significant.
[*] Different letter indicates significant difference between treatments by the LSD test (α = 0,05).

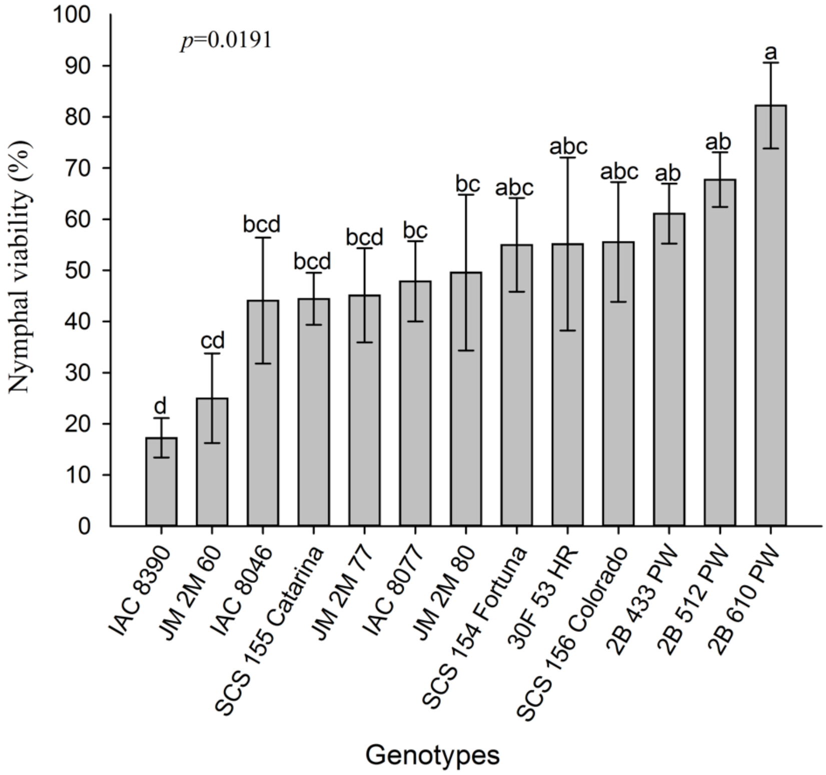

Figure 1. Nymphal viability (%) (± SE) of *Dichelops melacanthus* on corn genotypes, in antibiosis and/or antixenosis test. Different letters indicate significant difference between treatments by the LSD test (α = 0,05).

Chapter 2

Understanding the potential for injury from different life stages of *Dichelops melacanthus* and *Euschistus heros* (Hemiptera: Pentatomidae) on corn seedlings

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ABSTRACT

Changes in Brazilian agricultural practices in corn crop, such as expansion of the use of no-till farming and *Bt* plants, favors stink bugs, which feed on corn seedlings, especially when corn succeeds soybean in the field. *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae), is already considered a key pest in corn, and *Euschistus heros* (F.) (Hemiptera: Pentatomidae) appears to be a potential threat to corn. Injuries caused by both stink bug species can vary, from small holes in the leaves to drying the whorl and killing the plant. Threshold levels (TL) for management are usually calculated using the damage that adult insects cause. However, little is known about the impact of stink bug nymphs on corn seedlings. Thus, this work evaluated the effects of infestation with different nymphal instars on corn plant weight (fresh and dry), height and stalk diameter. We also classified the level of injuries to the plants using a rating scale. For the experiments, corn seedlings in the V3 stage were infested with two insects of either *D. melacanthus* or *E. heros* in the 2nd, 3rd, 4th, 5th or adult stage, in a greenhouse. Plants without infestation were used as control. Both species of stink bugs feeding on corn seedlings affected all the assessed parameters. The injuries caused by *D. melacanthus* were greater than injuries caused by *E. heros* and 5th instars of *D. melacanthus* caused the greatest damage among all experiments performed. The most damage life stage of both nymph species reduced plant height and fresh weight more than 40% and 70%, respectively, when compared to control. These results are important to help in the development of a more accurate TL for stink bug management in early vegetative corn.

Keywords: *Zea mays* L., green belly stink bug, neotropical brown stink bug, nymphal injury.

Introduction

In the last decades, corn (*Zea mays* L.) has increased in production, becoming the most important cereal cultivated worldwide (CRUZ et al., 2019). This growth happened mostly because of advances in management techniques and genetic engineering (CHAVAS; MITCHELL, 2018; CRUZ et al., 2019). The largest corn producer in the world is the United States of America, followed by China and Brazil (USDA, 2019). In Brazil, the 2018/2019 harvest is expected to produce 96 million tons, which would be the second largest in history (CONAB, 2019; USDA, 2019).

In spite of the production increase observed in the past years, corn has intense phytosanitary problems, specifically insect pests attacking all the development stages of this crop and demanding control methods in order to avoid yield losses (CRUZ et al., 2012; DUARTE et al., 2015).

The population of phytophagous insects in corn increased as the corn production in the past years increased (CHOCOROSQUI; PANIZZI, 2004; HUSCH et al., 2018). It is possible that the wide cultivation of corn in the off-season, complemented by the increase of no-tillage systems, has provided favorable conditions for the increase in phytophagous insects populations found in corn fields (CHOCOROSQUI; PANIZZI, 2004; BRUSTOLIN et al., 2017). Among phytophagous insects that feed on corn, stink bugs of the Pentatomidae family have been found in the early vegetative stage of corn (CHIARADIA et al., 2012, SMANIOTTO; PANIZZI, 2015).

The green belly stink bug, *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae) is one of the main species associated with corn seedlings (CHOCOROSQUI; PANIZZI, 2004; CRUZ et al., 2016), being predominant in the region of Brazilian Cerrado (BRUSTOLIN et al., 2011; 2017). This species was recorded for the first time feeding on corn seedlings in 1993, in Mato Grosso do Sul state, Brazil (ÁVILA; PANIZZI, 1995). The neotropical brown stink bug, *Euschistus heros* (Fabr.) (Hemiptera: Pentatomidae), is another species that has been mentioned as causing damage in the early stages of corn plants. Due to its diapause ability, *E. heros* can remain in the field, surviving for a long time, even in the absence of soybean plants, their main host (HOFFMANN-CAMPO et al., 2012; PANIZZI et al., 2012; SOARES et al., 2018).

Problems with Pentatomidae insects in corn production are possibly caused by the presence of multiple crops and intense agricultural landscapes which provide continuous sources of food for insects. Due to this, stink bugs can exchange their feeding habits. An example of this is the presence of *E. heros* feeding on and/or sheltering in vegetative structures of corn, especially when corn succeeds soybean in the field. Piercing-sucking insects are known to cause damage both by sucking sap and by injecting degrading enzymes into the plant cell-wall, such as pectinases, that may affect the normal development of corn plants (LUCINI; PANIZZI, 2018). *D. melacanthus* is found in greater numbers in corn than *E. heros*, and consequently, causes greater damage than *E. heros* (CHOCOROSQUI; PANIZZI, 2004; ROZA-GOMES et al., 2011; TORRES et al., 2013).

When field corn is attacked by stink bugs in the early stages, plants can present holes transversely to the veins of leaves. Stink bugs can also cause deformation and twisted leaves in the whorl. In more severe attacks, plant can emit tillers and even die as a result of insect feeding. Generally, seedlings with these symptoms grow more slowly and have to compete for light with other plants in normal growth. In many cases, these plants become unproductive or produce small ears, drastically reducing yield. The damage from stink bugs, such as *D. melacanthus* and *E. heros*, in the early stages of corn is considered a menace for corn growers, since the damage caused, in many cases, cannot be reversed (ROZA-GOMES et al., 2011).

The threshold level (TL) considered to control *D. melacanthus* in corn is 0,8 insects per m^2 (DUARTE et al., 2015), whereas for *E. heros*, this level has not yet been estimated. However, studies to determine threshold levels are usually conducted evaluating only injury from adult insects, and very little is known about the role that nymphs can have in the injury caused to corn seedlings. Since nymphs from the 3^{rd} to 5^{th} instar of *D. melacanthus* are often the first colonizers of corn seedlings that succeed soybean in the field this information should be considered (CÔRREA-FERREIRA; SOSA-GOMES, 2017). In addition, to date there are no studies comparing the damage caused by nymphs of *D. melacanthus* and *E. heros*. Thus, larger studies to elucidate the potential for damage from immature stages of stink bugs on corn are needed and may provide more accurate information to be considered in IPM systems involving stink bugs in corn.

In the face of the presented scenario, the objective of this study was to evaluate the injury caused by 2^{nd} to 5^{th} instars and adults of *D. melacanthus* and *E. heros* on young corn plants under greenhouse conditions.

Materials and Methods

Stink bug stock colony

Egg masses of the green belly and neotropical brown stink bugs were provided by DuPont Company (Paulínia, São Paulo, Brazil) and used to start stock colonies at the Crop Protection Department of Sao Paulo State University – UNESP, Botucatu, São Paulo, Brazil. Insects were separated by species and reared under laboratory conditions (26 ± 2 ºC, RH = 65 ± 10%, and photoperiod = 14L:10D h) following the methodology described by Canassa et al. (2017). Stink bugs nymphs and adults were separated and kept in rearing cages, made with plastic jars (19 × 22 × 10 cm), covered with a lid, in which a cut was made in the center, covered with organdy fabric, to allow for proper ventilation. Filter paper the same size of the cage was used to cover the bottom, aiming to facilitate excrement absorption.

Insects received a mixed natural diet composed of raw peanuts grains (*Arachis hypogaea* L.), sunflower seed (*Helianthus annuus* L.), fruits of privet (*Ligustrum lucidam* T.), fresh common beans (*Phaseolus vulgaris* L.) and portions of cotton moistened with distilled water to meet the bugs water needs and humidity inside cages. Food, cotton, and filter paper were replaced twice a week.

Portions of raw cotton cloth were positioned near the lateral corners of the cages, serving as shelter and oviposition sites for stink bugs adults. Eggs of *D. melacanthus* and *E. heros* were removed daily and placed in new cages containing only a portion of moist cotton. After eggs hatched, nymphs received the same food described previously.

Greenhouse experiments

Corn seeds of ‘SCS 155 Catarina’ genotype, provided by the Research Center for Family Agriculture, Research and Rural Extension Company of Santa Catarina (EPAGRI – Chapecó, Santa Catarina, Brazil), were sown in 3 L plastic pots, filled with soil, sand, manure and substrate, in the ratio of 1:1:1:1 (v/v/v/v). The SCS 155 Catarina genotype was selected since it was found to be susceptible in previous tests. Environmental conditions in the greenhouse were partially controlled (averages of 25,09ºC e 71,52 RH) and plants were irrigated as need.

For these experiments we used corn seedlings at the V3 stage (i.e., plant with three expanded leaves) (RITCHIE; HANWAY, 1966), which happened, on average, 7 days after plants emerged. This stage was chosen because stink bugs are reported to cause damage to corn seedlings from the V1 to V5 stage (CHIARADIA et al., 2012). Thus, 2 insects of *D. melacanthus* or *E. heros* in each life stage (treatments), were confined to one V3 stage corn seedling for 7 days. Insects went through 24 h of starvation before being placed on plants. First instars were not used because of high mortality reported in this stage (CHOCOROSQUI; PANIZZI, 2004). To avoid escape of the nymphs, we placed a layer of petroleum jelly on the border of the pots where nymphs were confined. For adults, pots were covered with organdy fabric, which was fixed with 4 wood stakes, and closed in at the end with an elastic piece.

We performed 6 replications testing 6 treatments for each stink bug species. Treatments included: control, plants not infested with stink bugs; plants infested with 2nd instars; plants infested with 3rd instars; plants infested with 4th instars; plants infested with 5th instars; and plants infested with adults. Bioassays were checked daily and dead or molting bugs were replaced, so there was always two bugs of the proper development stage for each treatment.

Evaluations were performed at 30 days after plant emergence (15 days after insect removal). The parameters evaluated were: diameter of the stalk (cm), plant height (cm), fresh and dry weight (g). Plants were dried using a 60ºC oven until the weight became constant. A rating scale was used to measure the injury caused by the stink bugs: 0 – plant without injury; 1 – leaves with holes and no impact on the plant size; 2 – plants with slight injury to the whorl; 3 – severe injury to the whorl or tillering; 4 – plants with a dry or dead whorl (ROZA- GOMES et al., 2011).

Data analysis

All analyses were conducted in R version 3.5.1 (R CORE TEAM, 2018) and RStudio Desktop version 1.1.463 (RSTUDIO TEAM, 2016). Data were analyzed with a generalized linear model with treatment as an explanatory variable followed by an ANOVA (R package, command: stats, anova; CHAMBERS; HASTIE, 1992). Means were compared using Tukey's test at $P < 0.05$ (agricolae, HSD.test; MENDIBURU, 2019) for significant effects. For *D. melacanthus*, the variables fresh and dry weights and rating scale were Box-Cox transformed (yλ = [((y + 1)λ - 1) / λ]; λ = -0.1414, -0.1414 and -0.5858, respectively) to follow assumptions of normality (Jarque-Bera: JB = 1.03, P = 0.508, JB = 2.3, P = 0.146 and JB = 2.79, P = 0.118, respectively) and heteroscedasticity (Levene's test: $F_{5;30} = 0.98$, P = 0.445, $F_{5;30} = 1.96$, P = 0.113 and $F_{5;30} = 1.05$; P = 0.404, respectively). For *E. heros*, the variables fresh and dry weights and rating scale were also Box-Cox transformed (λ = 0.0202, -0.1818 and 1.7, respectively; MASS, boxcox; VENABLES; RIPLEY, 2002) to follow assumptions of normality (Jarque-Bera: JB = 1.37, P = 0.337, JB = 1.7, P = 0.264 and JB = 2.41, P = 0.141, respectively; (JARQUE; BERA 1987) and heteroscedasticity (Levene's test: $F_{5;30} = 1.03$, P =

0.418, $F_{5;30}$ = 1.26, P = 0.306 and $F_{5;30}$ = 1.90; P = 0.124, respectively; car, leveneTest; FOX; WEISBERG, 2011).

Results

Dichelops melacanthus

All parameters evaluated in this experiment were significantly different (P < 0,05) among treatments (Table 1). Plants infested with 5th instar and adult *D. melacanthus* had the lowest fresh weight (F = 9.489; df = 5,30; P < 0.001), differing from the control and plants infested with 2nd instars (Table 1). In comparison to the control, these two insect stages caused wet weight reductions exceeding 70% (Fig. 1). A similar pattern was found in the dry weight and plant height, in which 5th instars and adult treatments of the green belly stink bug resulted in lower averages (F = 7.469; df = 5,30; P < 0.001; F = 7.203; df = 5,30; P < 0.001), with reductions higher than 70% and around 50%, respectively (Fig. 1). The lowest average diameter of plants occurred when plants were infested with 5th instars (F = 9.149; df = 5,30; P < 0.001), compared to the control, 2nd and 4th instars (Table 1) reduced average plant diameter by 50% (Fig. 1). Plants infested with insects from the 3rd instar to adults (1.2 – 2.5) were rated significantly higher on the rating scale (F = 25.56; df = 5,30; P < 0.001) on treatments (Fig. 2).

Euschistus heros

Similar to the green belly stink bug, *E. heros* life stages also significantly affected all measured parameters (Table 2). Plants infested with 4th instar *E. heros* had the smallest fresh and dry weight (F = 7.997; df = 5,30; P < 0.001; F = 6.528; df = 5,30; P < 0.001, respectively) (Table 2), causing a reduction around 75% in comparison with the control (Fig. 3). Plants infested with 5th instars had intermediate weight averages. Regarding plant height (Table 2), the 4th instar and adult neotropical brown stink bug showed lower averages when comparing with the control and 2nd instars (F = 7.235; df = 5,30; P < 0.001), and caused, respectively, 42% and 31% reductions in this parameter when compared with the control (Fig. 3). The lowest plant diameter average was found in plants infested with 4th instars (F = 6.095; df = 5,30; P < 0.001), comparatively to the treatments control, 2nd instars and adults (Table 2), which reduction was more than 45% when comparing with the control (Fig. 3). The average injury rating was significantly higher (F = 5.479; df = 5,30; P = 0.001) on treatments infested with 3rd and 4th instars and adults (1.0 – 1.33) compared to the control (Fig. 4).

Discussion

The green belly stink bug is the most common species feeding on corn seedlings in Brazil and the neotropical brown stink bug is the most predominant stink bug in soybean fields but is also reported to feed on vegetative corn (ROZA-GOMES et al., 2011; PANIZZI, 2015; SOUZA et al., 2015). Both species of stink bugs are described as causing injury to the young phase of corn (ÁVILA; PANIZZI; 1995; ROZA-GOMES et al., 2011; CHOCOROSQUI; PANIZZI, 2004). Our results show these species are capable of causing a great deal of damage and demonstrates that control strategies are highly necessary when these stink bug species infest corn seedlings in order to avoid yield losses. Although *E. heros* is more predominant and harmful to soybean, either because of its larger population in soybean or because it feeds on vegetative and reproductive phases of soybean (CORRÊA-FERREIRA, 2002), infestation of this species in corn seedlings does occur often, mainly on no-till systems when corn succeeds soybean in the field (CHOCOROSQUI; PANIZZI, 2004). Thus, knowing its capacity to damage corn seedlings is important information for growers.

Movements of these stink bugs among different crops are not aleatory and/or directional. Insects that feed on cultivated plants disperse and colonizes crops according to the seasonal resources that are available, which means that if their preferred food source is absence, insects will search for another host that provides a favorable development condition (TILLMAN, 2010; 2011; VENUGOPAL et al., 2014).

It is known that the intensity of the injury caused by stink bugs is directly related to stink bug populational level and host development stage (NI et al., 2010; ROZA-GOMES et al., 2011, SCOPEL et al., 2016; BABU; REISIG, 2018). In general, the major injury caused by the neotropical brown and the green belly stink bugs is in vegetative corn that is less than 30 days from emergence, or while the stalk is less than 0.8 cm in diameter, meaning that while corn is in this critical development stage (until V5, five expanded leaves) the injury caused by stink bugs can lead to plant death (COPATTI; OLIVEIRA, 2011, CHIARADIA et al., 2016).

Our study showed significant differences in all assessed parameters for plants fed on by *D. melacanthus.* The control and nymphs from the 2nd to the 4th instar presented the lowest level of injuries in comparison with 5th instars. In addition, for plants fed on by *D. melacanthus* 5th instars, we observed the highest damage of all assessments in both experiments, even surpassing all stages of *E. heros* (Fig. 5). Fifth instar green belly stink bugs caused reductions of more than 75% in fresh weight and almost 50% in plant height and stalk diameter in comparison with the control. During the exposure time tested, corn seedlings infested with *E. heros* showed the highest injury level when fed on by 4th instars, with reductions of around 70% for fresh and dry weight, 40% for plant height and 50% for stalk diameter in comparison with the control. Adults of both species caused greater grades on the rating scale than 2nd and 3rd instars. Experiments with soybean evaluated the depth that stink bugs can insert their stylets during a single feeding (around 70 min); *E. heros* reached the maximum deep of 0.8 mm, while *D. melacanthus* reached 0.5 mm (DEPIERI; PANIZZI, 2011). These authors observed higher injuries from *E. heros* than *D. melacanthus*, probably related to the depth that stylets reached by the first species. It is known that soybean is the preferred host for *E. heros* but *D. melacanthus* does not have high success when feeding on soybean (HOFFMANN-CAMPO et al., 2012; CANASSA et al., 2017). On the other hand, *D. melacanthus* food and salivary canals are around 15% bigger than *E. heros*, which can explain the greater damage caused by the green belly stink bug (DEPIERI; PANIZZI, 2010). Roza-Gomes et al. (2011) also observed *D. melacanthus* causing more injury to corn seedlings than *E. heros* in the same environmental conditions.

We observed that both species and all life stages affected plant health 30 days after plant emergence, when assessments were performed. However, *D. melacanthus* showed higher average injury ratings than *E. heros*, which reinforces that the green belly stink bug causes more injury to corn in its young stage than the neotropical brown stink bug (ÁVILA; PANIZZI, 1995; BABU; REISIG, 2018). Another study, that aimed to assess injury evaluation of the same two stink bug species in corn plants, concluded that *D. melacanthus* caused more damage as well, regardless the density of insects accessed (1, 2 or 3 adults/plant) or phenological stage of plants. In addition, when evaluating the highest infestation level, the green belly stink bug was able to cause severe damage to the whorl. Conversely, it was found effects from the neotropical brown stink bug were not so severe (COPATTI; OLIVEIRA, 2011).

Most of studies about threshold levels (TL) measure damage from only adult stink bugs, ignoring the fact that it is not the only life stage that can cause damage. The best known TL for *D. melacanthus* for Brazilian growers recommends taking action to control when two adult stink bugs m^{-2} are found in the first crop (summer corn), and one adult stink bug m^{-2} for the second crop ("safrinha corn"), but can change depending on the region of the country, going down to 0.8 stink bugs m^{-2} and 0.6 stink bugs m^{-2} (BIANCO, 2005; CHOCOROSQUI; PANIZZI 2004; DUARTE et al., 2015). For *E. heros*, it is recommended to start control techniques in soybean for grain production when two stink bugs m^{-2} are found, and one stink bug/m^{-2} for seed production since the neotropical brown stink bug can cause severe physiological damage to soybean (CORRÊA-FERREIRA; ROGGIA, 2013). To our knowledge, no TL for *E. heros* in early stage corn has been determined.

Considering our results, it is evident that for both species later stages of nymphs (*D. melacanthus* 5th instar and *E. heros* 4th instar) cause more injury to corn seedlings than the adults and younger nymphs. In general, Pentatomidae are known to feed from the egg shells right after they hatch, staying aggregated until their first molt, after which they will look for a food source (TAYLOR et al., 2014). *Euschistus heros* nymphal duration was extended when feeding on cotton since cotton is not its preferred food source (AZAMBUJA et al., 2013), and corn is not either. Stink bugs feeding on inadequate hosts have the tendency of having longer nymphal stages in order to accumulate enough nutritional resources to reach the adult phase (PANIZZI; SILVA, 2009). The stink bugs in the present study may have had to feed more in order to accumulate adequate nutrients to reach adulthood. In addition, development comes with an increase in size, which includes the insects' mouth parts, and, consequently, the amount of plant material ingested (BROWNIE, 1975; VALLES et al., 1996), which explains later instars causing more damage than earlier instars. The fact that stink bug adults caused less damage than late instars could be due to concentration on reproduction rather than feeding, although we did not perform experiments to confirm that. Additionally, in piecing-sucking insects, it is important to consider the role of toxic saliva on the injuries caused to the plant host, which can vary from one species to the other, as well as the size of mouth parts in each stage of each species. Thus, more studies to investigate those hypotheses should be carried out.

The present study contains novel information regarding the role of stink bug developmental stages in the injury to corn seedlings. Although our study demonstrated that *D. melacanthus* presents higher injury potential to corn seedlings than *E. heros*, this species should not be neglected in management programs, since growing populations have been observed in Brazil, in part because its strong adaptation to the soybean-corn system. These results are useful to Integrated Pest Management (IPM) plans and can be considered when determining the TL of stink bugs in early stage corn.

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Table 1. Mean (±SE) fresh and dry weight, height, diameter and rating scale in corn seedlings infested with different stages of *D. melacanthus* under greenhouse conditions.

	Fresh weight[1]	Dry weight[1]	Height	Diameter
	(g)	(g)	(cm)	(cm)
Control	19.10 ± 2.83 a	2.55 ± 0.51 a	68.65 ± 4.99 a	1.10 ± 0.06 a
2nd instar	21.31 ± 4.58 a	3.05 ± 0.77 a	60.67 ± 5.39 a	1.02 ± 0.08 ab
3rd instar	11.71 ± 2.01 ab	1.48 ± 0.32 ab	56.67 ± 4.10 ab	0.77 ± 0.06 bcd
4th instar	11.92 ± 3.94 ab	1.37 ± 0.42 ab	53.48 ± 7.38 ab	0.88 ± 0.08 abc
5th instar	4.22 ± 0.67 c	0.45 ± 0.19 b	35.88 ± 5.13 b	0.55 ± 0.04 d
Adult	5.83 ± 0.75 bc	0.65 ± 0.08 b	34.98 ± 1.60 b	0.70 ± 0.08 cd
P	<0.001	<0.001	<0.001	<0.001

Mean followed by the same letter do not differ statistically by a Tukey's test ($p<0.05$).
[1]BoxCox transformed (lambda=-0.1414) -fresh and dry weight was used for analysis, untransformed means and errors are being shown.

Table 2. Mean (±SE) fresh and dry weight, height, diameter and rating scale in corn seedlings infested with with different stages of *E. heros* under greenhouse conditions.

Treatments	Fresh weight[1]	Dry weight[1]	Height	Diameter
	(g)	(g)	(cm)	(cm)
Control	19.10 ± 2.83 a	2.55 ± 0.51 a	68.65 ± 4.99 a	1.10 ± 0.06 a
2nd instar	19.78 ± 5.55 a	2.68 ± 0.78 a	66.90 ± 5.83 a	0.90 ± 0.12 a
3nd instar	12.59 ± 1.66 a	1.68 ± 0.26 a	58.47 ± 4.24 ab	0.82 ± 0.05 ab
4th instar	5.29 ± 1.06 b	0.61 ± 0.11 b	39.88 ± 3.71 c	0.58 ± 0.07 b
5th instar	10.23 ± 0.99 ab	1.27 ± 0.16 ab	51.68 ± 2.50 abc	0.87 ± 0.04 ab
Adult	12.73 ± 0.98 a	1.58 ± 0.15 a	47.47 ± 2.91 bc	0.93 ± 0.03 a
P	<0.001	<0.001	<0.001	=0.001

Mean followed by the same letter do not differ statistically by Tukey’s test (p<0.05).
[1] BoxCox transformed fresh (lambda=0.0202) and dry weight (lambda=-0.1818) was used for analysis, original means and errors are being shown.

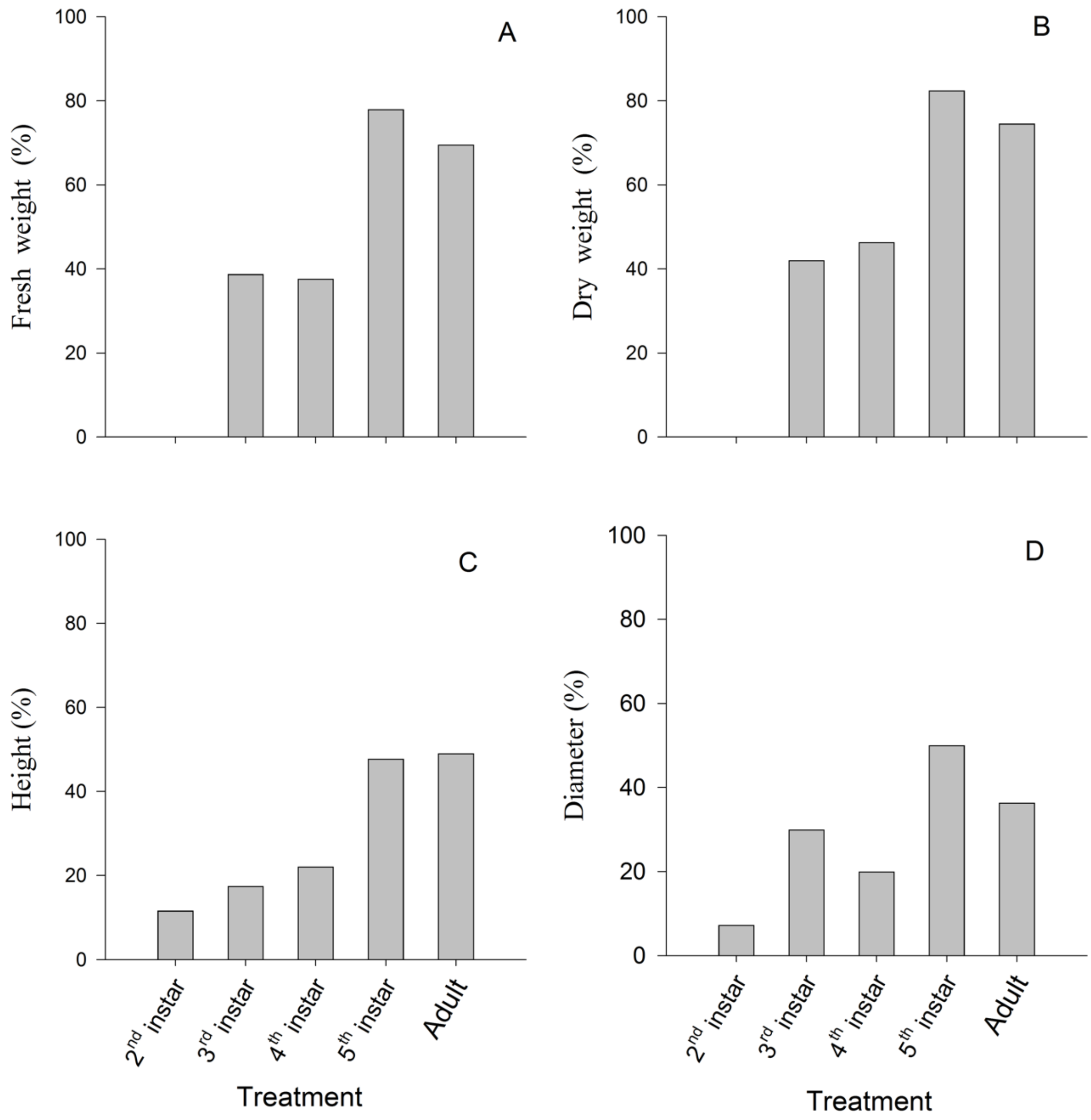

Figure 1. Percentage reduction of A) fresh weight, B) dry weight, C) height, and D) plant diameter in comparison to an uninfested control on corn seedlings infested *D. melacanthus* 30 days after plant emergence.

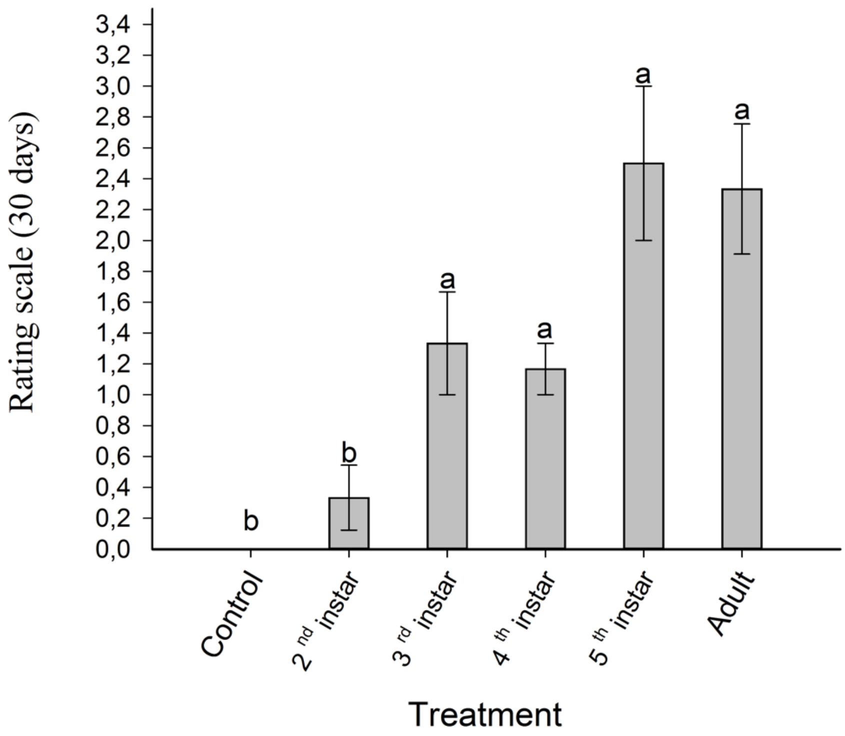

Figure 2. Mean (±SE) injury rating of corn plants infested with *D. melacanthus* assessed 30 days after corn emergence.

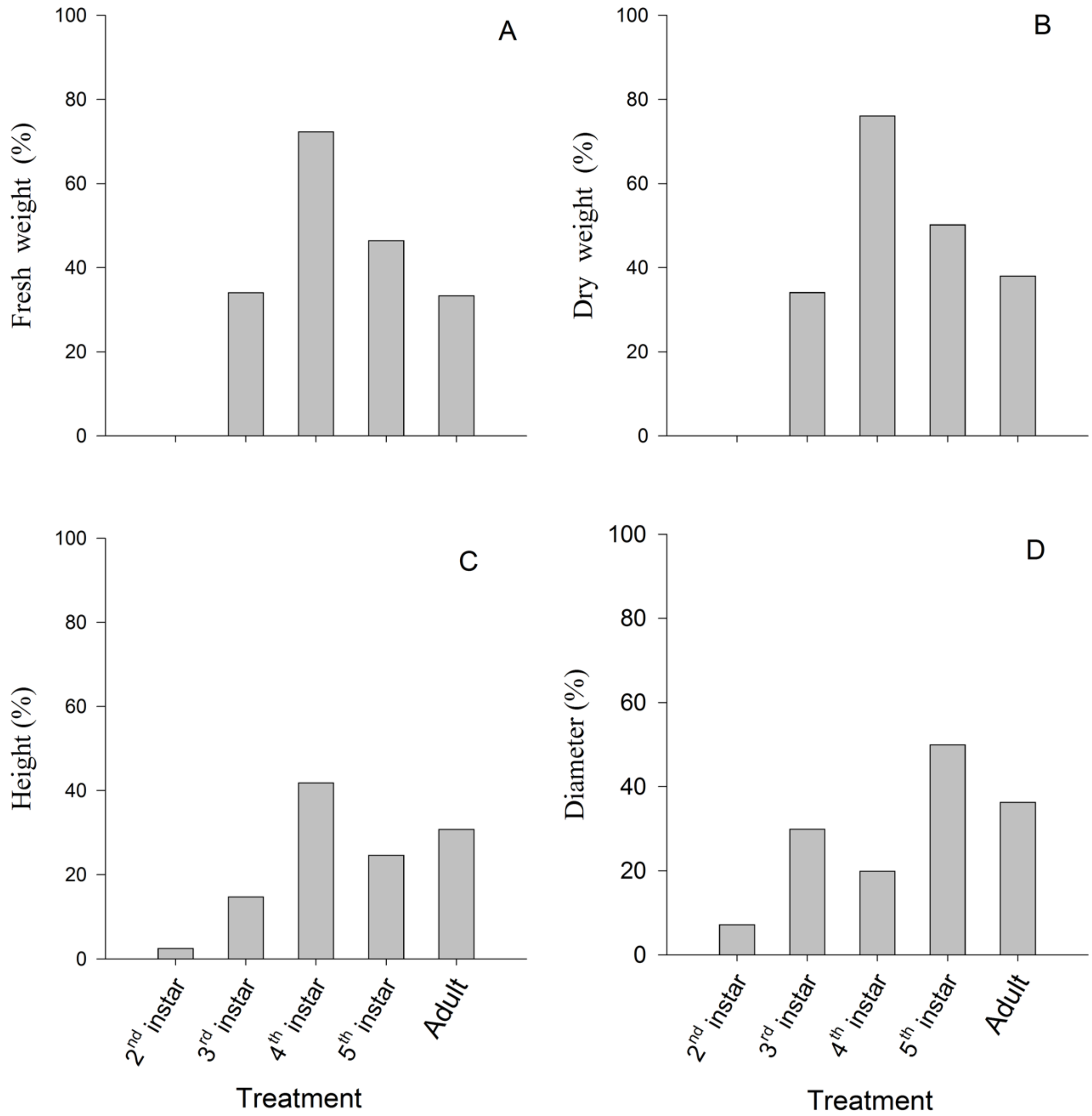

Figure 3. Reduction (%) of A) fresh weight, B) dry weight, C) height, and D) plant diamter in comparison to an uninfested control on corn seedlings infested with *E. heros* 30 days after plant emergence.

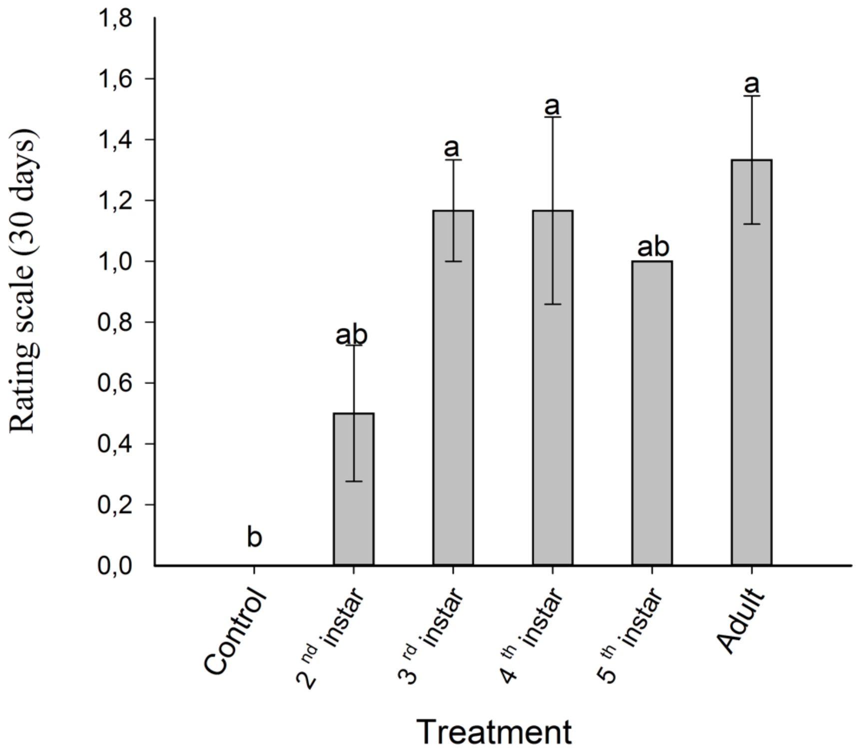

Figure 4. Mean (±SE) injury rating of corn plants infested with *E. heros* assessed 30 days after corn emergence.

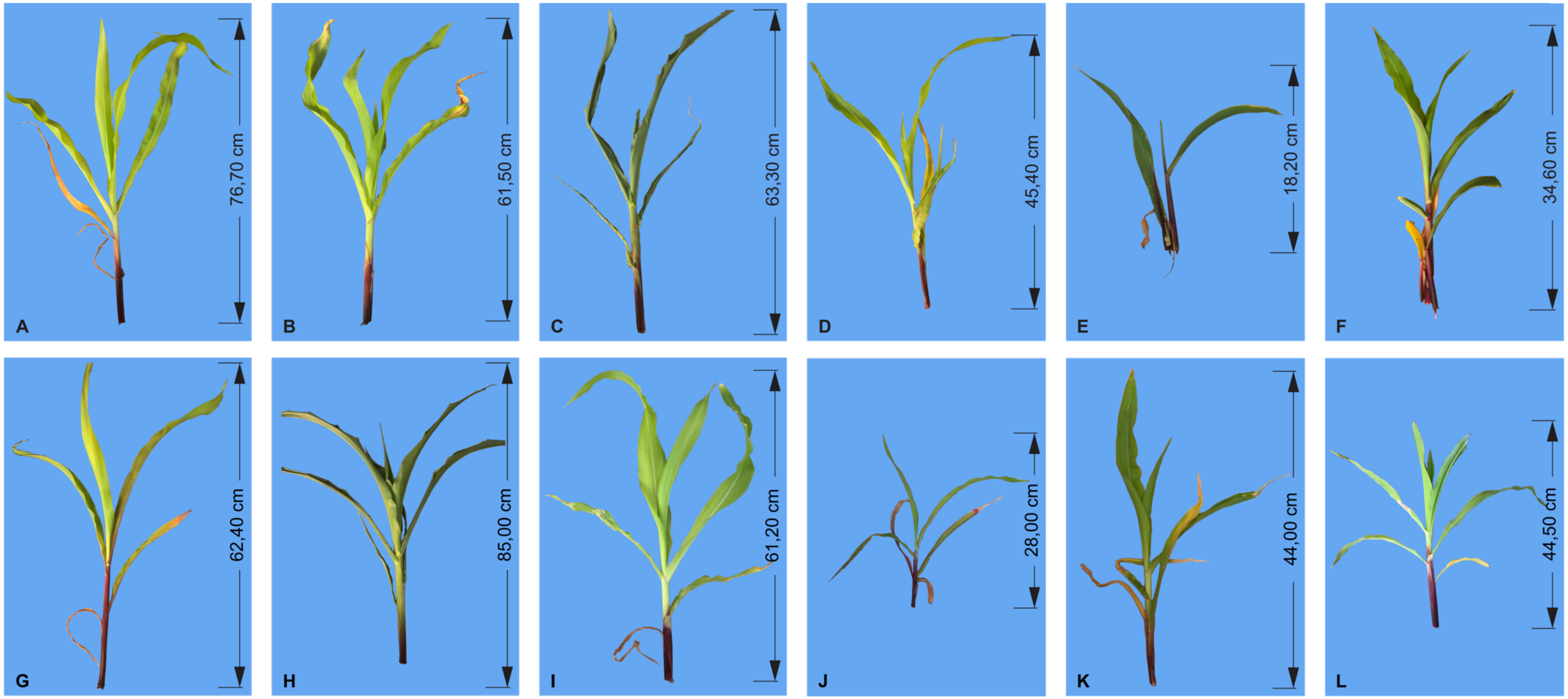

Figure 5. Visual comparison of control (A and G) and injuries on corn seedlings provoked by different stages of *D. melacanthus* (B to F, 2nd instars, 3rd instars, 4th instars, 5th instars and adults, respectively) and *E. heros* (H to L, 2nd instars, 3rd instars, 4th instars, 5th instars and adults, respectively), 30 days after plant emergence.

Chapter 3

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) as a potential risk for early season corn

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ABSTRACT

Stink bugs are important insect pests worldwide causing damage up to 100% on crops of economic importance. The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive species in the United States that represents a great threat for crops of economic importance such as soybean and corn. Due to the lack of information about its damage to corn seedlings, this study was carried out with the objective to provide information about BMSB damage to sweet corn seedlings. In the field experiment, caged sweet corn seedlings were exposed to sexed BMSB adults at the densities of zero, one or two insects plant-1 for seven days. The second experiment was conducted with caged sweet corn seedlings in greenhouse exposed to zero or two non-sexed BMSB at different stages (second to fifth instars and adult) plant-1 for 14 days. In both experiments, we evaluated plant fresh and dry weights, plant height, stalk diameter and plant injury using a rating scale (zero to five). In the field experiment, the rating scale was greater in plants exposed to the insects.

Fresh and dry weights, height and diameter of greenhouse seedlings were lower in plants fed by fourth stage nymphs. This stage also had the greater rating scale. In general, our results indicate that *H. halys* can feed in sweet corn seedlings, and that fourth instars cause more damage to it. Rating scale adopted here can be used by growers and/or technicians for an early identification of *H. halys* occurrence and assess its damage on field.

Key words: Brown marmorated stink bug, nymphal injury, *Zea mays* L.

Introduction

Corn, *Zea mays* L., both field and sweet corn, are important crops to the United States. Field corn is considered the most important grain crop of the country, with expected production of 397,000 thousand tons for 2018, the second highest on record for the United States (USDA, 2018). Sweet corn is harvested in all 50 states of the United States, both as fresh and processed products. It is the second largest processing crop in terms of production and value, concentrated in the upper Midwest and Pacific Northwest of the United States (USDA, 2012).

Cultivated plants are more vulnerable to stressors immediately after germination (BARDNER; FLETCHER, 1974), and the impact of early season insect feeding on seedlings may significantly reduce yield (SAPPINGTON et al., 2018). Injuries from piercing-sucking insects may be conspicuous due to the injection of toxic saliva and mechanical injury (BARDNER; FLETCHER, 1974). Among the piercing-sucking insects that feed on seedling corn, stink bugs (Heteroptera: Pentatomidae) have been noticed in larger numbers in corn field fields since the 1980's, possibly due to increased adoption of minimum tillage practices. The presence of stink bugs on seedling corn creates a management challenge, because there is little time to intervene between insect attack and irreversible damage (SAPPINGTON et al., 2018).

Stink bugs are important insect pests worldwide, causing severe damage to crops of economic importance such as sweet and field corn (KUHAR et al., 2012, LESKEY et al., 2012a, KOCH et al., 2017, LUCINI; PANIZZI, 2017, SAPPINGTON et al., 2018). Stink bug species that occurs in the U.S., such as *Euschistus variolarius* (Palisot de Beauvois), *E. servus* (Say), *Nezara viridula* (L.) and *Chinavia hilaris* (Say)

(Hemiptera: Pentatomidae), have been reported feeding on developing ears and kernels of corn and also on corn seedlings (COTTRELL; TILLMAN, 2015; KOCH et al., 2017; NEGRÓN; RILEY, 1987; NI et al., 2010; REISIG, 2011; TOWNSEND; SEDLACEK, 1986). Nymphs and adults feeding on corn seedlings cause linear feeding holes with yellow necrotic halos leading to plant shortening, tillering, wilting of whorl leaf and even plant death (ANNAN; BERGMAN, 1988; TOWNSEND; SEDLACEK, 1986). Later in the season, *N. viridula*, for instance, can destroy or cause abortion of young ears (NEGRÓN; RILEY, 1987). Also, corn in the reproductive stages fed on by *E. servus* adults have been reported with significant yield and grain quality losses (NI et al., 2010).

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is a polyphagous insect native from Japan, Taiwan, China and Korea (HOEBEKE; CARTER, 2003). This species is considered invasive in North America and was first detected in Allentown, PA, in 1996 although it was only properly identified in 2001 (HOEBEKE; CARTER, 2003). *Halyomorpha halys* is rapidly spreading through other states, and became a serious agricultural pest in the Mid-Atlantic Region of the United States, feeding on hosts of economic importance (RICE et al., 2014).

The BMSB prefer to feed on reproductive parts of their host plants (MCPHERSON; MCPHERSON, 2000). Among host crops targeted by the BMSB, sweet corn has been suggested as being a strongly preferred, especially in reproductive stage, causing kernel injury up to 100% when at high population densities (CISSEL et al., 2015; KUHAR et al., 2012; LESKEY et al., 2012b). In field corn, the highest population occur during ear formation, and the BMSB feeds on developing kernels (RICE et al., 2014). However, it is not known if *H. halys* is capable of feeding on vegetative growth stages of crop plants. Thus, this study was carried out with the objectives to: 1. provide information about the potential of the BMSB to cause injury to sweet corn seedlings and 2. compare impacts of feeding by different BMSB life stages on corn seedlings.

Material and Methods

BMSB stock colony

Insects used in these experiments were sourced from a colony maintained at the University of Minnesota. A stock colony of *H. halys* was started with egg masses collected from Wyoming, MN, and maintained under laboratory conditions (26 ± 2 ºC and photoperiod = 14 h), with similar methodology as described by Cira et al. (2017). Nymphs and adults were kept in mesh cages of 38 × 38 × 61 cm and 61 × 61 × 91 cm (BioQuip, Rancho Dominguez, CA), respectively. In the cages, insects were fed with a natural diet, consisting of green pods of common beans (*Phaseolus vulgaris* L.) and baby carrots (*Daucus carota* L. subsp. *sativus* L.) replaced three times per week, sunflower (*Helianthus annuus* L.) and soybean [*Glycine max* (L.) Merr.] seeds replaced twice per week. One pot with 13 common bean plants was included in the cages and watered twice a week to help meet humidity requirements inside cages and provide shelter and oviposition sites for the insects. Egg masses were collected every weekday from inside the cages and placed inside Petri dishes (4-cm diameter) lined with filter paper at the bottom. After developing to second instars, nymphs were transferred to cages and reared using the methodology described above.

Field experiment

To evaluate if *H. halys* adults can feed on and injure corn seedlings under field conditions, an experiment was conducted in an experimental field at the Agricultural Experiment Station of the University of Minnesota, Saint Paul, Minnesota between August and September of 2018. Corn seed used was “Allure” Sweet Corn Sh2xSE variety. Seed was sown in small plots of four seeds arranged in a 2×2 grid (four seed total) with 5 cm spacing between plants. Plots were arranged in a completely randomized design with a 3×2 factorial treatment structure with three levels of *H. halys* density (zero, one or two stink bug adults per plant) and *H. halys* sex (female or male), and with eight replications of each treatment. Plots were spaced 0.5 m apart from one another and each plot was surrounded by a cage. The cage frames were made with three pieces of PVC pipe (60 cm long) evenly spaced around the outside of each plot and pounded vertically into the soil leaving 50 cm of pipe above ground. The cage frames were covered with a No-See-Um mesh that was buried in the soil around the base of the cages.

Halyomorpha halys used on this assay were adults at 10-day old. Prior to experimentation, adults were moved from the original colony cage to a new one with no food or potted bean plants to starve them for 24 hours. One week after plant emergence, when plants were at V3 corn growth stage (RITCHIE; HANWAY, 1966), *H. halys* adults were released inside the cages. The cages were assessed daily to monitor survival of *H. halys*, with dead individuals replaced (insects from the same generation) to maintain constant densities of *H. halys* adults over time. At seven days after infestation, *H. halys* were removed from all cages and cages were closed again to protect plants from other herbivores.

At 23 days after plant infestation (30 days after plant emergence), each plant was rated for injury using a rating scale modified from Roza-Gomes et al. (2011), with 0 = plant without injury; 1 = leaves with holes and no impact on the plant size; 2 = leaves with holes and impact on the plant size; 3 = plant whorl slightly injured; 4 = severe injury to the whorl or tillering; 5 = plant with dry or dead whorl (Fig. 1). At this time, corn plants in each cage were also evaluated for: above ground fresh weight, plant height (ground to upper boundary of the highest leaf), and stalk diameter. In addition, dry weight of above ground plant material was evaluated after plants dried for 5 days in an oven at 60 ºC. For each cage (experimental unit), the measured response variables were averaged across the plants in the cage for use in the analyses. In three cages, one plant died for unknown reasons, so cage-level averages from those cages were calculated from three plants.

Greenhouse experiment

To more thoroughly investigate *H. halys* injury to corn seedlings, an experiment was conducted in a greenhouse at the Plant Growth Facility of the University of Minnesota, Saint Paul, Minnesota, during October and November of 2018. In particular, the experiment aimed to assess the injury caused to corn seedlings by second to fifth instars and adults. First instars were not assessed because they generally do not feed on host plants and stay aggregated on the egg mass until their first molt (LESKEY et al., 2012a). Corn seed used was ‘Allure’ Sweet Corn Sh2xSE variety. Three seeds were sown per 3-L plastic pot and only one seedling per pot remained after thinning. Each pot was surrounded by a metal cage frame (galvanized tomato cage, Gilbert and Bennett, 137.16 cm long) covered with a No-See-Um mesh. Cages, which were considered experimental units, were arranged in a completely randomized design with six treatments (four nymphal stages, adult and control) and with eight replications. Non-infested plants were used as control.

One week after plant emergence, when seedlings were at V3 stage, two *H. halys* from each stage (recently molted), were released inside the cages. Prior to experimentation, insects were starved for 24 hours, as previously described. Non-sexed adults were used because effects of sex or its interaction with treatment were not observed in the previous experiment. The cages were assessed daily, with dead or molting individuals replaced to maintain constant densities of each stage of *H. halys* over time. At 14 days after infestation, *H. halys* were removed from all cages and cages were closed again to protect plants from other herbivores. We increased the time the stink bugs stayed in the cages from the field to the greenhouse in order to more thoroughly investigate the potential injury of *H. halys* to sweet corn seedlings.

At 23 days after plant infestation (30 days after plant emergence), each plant was rated for injury using the rating scale previously described. At this time, corn plants were also evaluated for above ground fresh and dry weight, plant height and stalk diameter as in the previous experiment.

Statistical Analyses

All analyses were conducted in R version 3.5.1 (R CORE TEAM, 2018) and RStudio Desktop version 1.1.463 (RSTUDIO TEAM, 2016). Data from the field experiment were analyzed using an unbalanced two-way analysis of variance (Anova) (R package, command: car, Anova; FOX; WEISBERG, 2011) using a general linear model (stats, lm; WILKINSON; ROGERS, 1973). Treatment, sex and their interaction were included as explanatory variables in the linear model. For the greenhouse experiment, data were analyzed using Anova (stats, anova; CHAMBERS; HASTIE, 1992) using a general linear model with treatment as an explanatory variable. Means were compared using Tukey's test at $P < 0.05$ (agricolae, HSD.test; MENDIBURU, 2019) for significant effects. The variables height and rating scale for the greenhouse experiment were Box-Cox transformed $y\lambda = [((y + 1)\lambda - 1) / \lambda]$; $\lambda = 3$ and 0.04242, respectively; Mass, boxcox (VENABLES; RIPLEY, 2002) to follow assumptions of normality (Jarque-Bera: JB = 1.26, P = 0.394, and JB = 1.06, P = 0.476, respectively (JARQUE; BERA, 1987) and heteroscedasticity (Levene's test: $F_{5;42} = 0.93$, P =

0.472, and $F_{5;42} = 2.18$; $P = 0.074$, respectively; car, leveneTest; FOX; WEISBERG, 2011).

Results

Field experiment

Effects of *H. halys* density, sex and their interaction were not significant for plant height ($F_{5;16} = 1.41$, $P = 0.274$), stem diameter ($F_{5;16} = 2.38$, $P = 0.085$), and fresh ($F_{5;16} = 1.77$, $P = 0.176$) and dry ($F_{5;16} = 1.81$, $P = 0.168$) weights of plants (Fig. 2). For plant injury ratings at 23 days after infestation, effects of *H. halys* sex ($F = 0.84$, df = 1, $P = 0.362$) and its interaction with *H. halys* density $F = 1.94$, df = 2, $P = 0.151$) were not significant, but there was a significant effect of *H. halys* density on injury ratings ($F = 40.87$, df = 2, $P < 0.001$). Injury ratings from cages with 1 or 2 bugs per plant were similar to one another (1.1 – 1.36), and significantly higher than the control cages (Fig. 3).

Greenhouse experiment

A significant difference among treatments was observed for all evaluated characteristics (fresh weight: $F_{5;42} = 4.31$, $P = 0.003$; dry weight: $F_{5;42} = 3.76$, $P = 0.006$; height: $F_{5;42} = 7.40$, $P < 0.001$; stem diameter: $F_{5;42} = 3.61$, $P = 0.008$; injury rating: $F_{5;42} = 9.29$, $P < 0.001$). Fresh weight of corn seedlings was significantly lower for plants exposed to fourth instars compared to second and third instars, adults and the control (Fig. 4A). Dry weight of plants exposed to fifth instars was similar to all other exposed to on by fourth instars compared to third instars, adults and the control (Fig. 4B). Dry weights of plants exposed to second and fifth instars differed from all other treatments (Fig. 4B). Plants exposed to fourth instars had significantly lower plant height than all other treatments (Fig. 4C). Plant heights of the other treatments did not differ (Fig. 4C). Stem diameter of corn seedlings was significantly lower for plants exposed to fourth instars compared the control (Fig. 4D). Stem diameter of the other life stages did not differ from the control or fourth instars (Fig. 4D). At 23 days after infestation, injury ratings were significantly higher for all infested plants compared to the control. Among infested plants, injury ratings for plants infested with fourth instars (> 2.5) was greater than the rating for plants infested with third instars (Fig. 5).

Discussion

Sweet corn is a highly consumed vegetable of great economic importance in the U.S. (KHAN et al., 2018) and emerging pests such as *H. halys* threaten this crop by causing direct damage to the ears and kernels (CISSEL et al., 2015; KUHAR et al., 2012; LESKEY et al., 2012b). As native stink bugs are also a menace to corn at the vegetative growth stage, knowing their impact on plants can be helpful for growers to prevent yield losses. Despite the concern of *H. halys* on reproductive corn, it was not known if this species could cause damage to corn seedlings. To our knowledge, this is the first study that assessed the potential impact of *H. halys* on corn seedlings.

The *H. halys* injury observed here did not differ significantly between female or male individuals, an effect already described for other stink bug species (APRIYANTO; TOWNSEND; SEDLACEK, 1989; SIMMONS; YEARGAN, 1988). With the exception of the injury rating scale, all evaluated variables from the field-grown plants exposed to *H. halys* showed response similar to the control. This may have occurred because we used just adults during this experiment. As showed in greenhouse plants exposed to different stages of *H. halys*, adults caused similar damage to the control for all variables except for the injury rating scale, which supports the results obtained on the field. Mean injury ratings of infested plants in the field experiment were around 2, which means it is an injury caused by stink bugs feeding on leaves, without severe damage extending to the whorl. Injuries from stink bug feeding that did not affect plant height and weight were also observed in *E. heros* (F)., *N. viridula* and *Dichelops furcatus* (F.) (Hemiptera: Pentatomidae) feeding on corn seedlings (ROZA- GOMES et al., 2011). This may have happened because stink bugs causes more damage when they feed on the stalk, near to the soil, in an upside down position (APRIYANTO; TOWNSEND; SEDLACEK, 1989; PANIZZI; LUCINI, 2018), a preferred feeding behavior observed in some stink bugs species (ÁVILA; PANIZZI, 1995; PANIZZI; MACHADO NETO, 1992).

In the present experiments, we generally observed *H. halys* feeding on leaf surfaces and not on the stem near the soil surface. In spite of stink bugs being found feeding on vegetative structures of host plants (ÁVILA; PANIZZI, 1995; CHOCOROSQUI; PANIZZI, 2004; SAPPINGTON et al., 2018; TOWNSEND;

SEDLACEK, 1986), they usually prefer to feed on reproductive parts, especially immature seeds (OLSON et al., 2011). This may be due to the fact that the seed contents meet essential nutrients needed for the insect development (LUCINI; PANIZZI, 2017). In the absence of a more suitable food source, stink bugs can feed of leaves and stems in order to maintain their development (PANIZZI; SILVA, 2012). Thus, despite the likely preference of *H. halys* for reproductive parts of corn, it was able to feed on seedling sweet corn.

Irreversible damage caused by stink bug feeding near seedling emergence can occur in a short amount of time (SAPPINGTON et al., 2018). Thus, despite the fact that the adult stage caused low levels of injury to corn seedlings when compared to other stages, the injury rating scale can be a valuable tool to verify early infestations, as shown in our study. However, it is important to highlight that this early identification does not exclude the importance of sampling the field to help guide management decisions (KOCH et al., 2017; WEBER et al., 2017). In this sense, studies to develop sampling plans for management decision-making for *H. halys* and other stink bugs in sweet corn may be desirable.

In the present work, we observed variability in plant injury according to the stink bug developmental stage. In general, differences among insect stages regarding insect feeding behavior varies because of their ecological role (COOK; NEAL, 1999). Smaller insects are usually less mobile (COOK; NEAL, 1999) than later developmental stages, then the later tend to cause more damage to plants as already described for *E. servus* and *E. variolarius* (NI et al., 2010; SEDLACEK; TOWNSEND, 1988). This is to be expected because nutritional requirements usually increases with insect growth (BARTON BROWNE, 1995; GORDON, 1998). Development often comes with an increase in size, including larger sized mouth parts, which represents greater potential mechanical injury and food intake (BARTON BROWNE, 1995; VALLES; STRONG; KOEHLER, 1996). However, here the fourth instar of *H. halys* caused higher damage to sweet corn seedlings instead of fifth instars or adults. Acebes-Doria et al. (2016), evaluating *H. halys* damage to apple and peach, observed that adults cause more injuries than nymphs. Despite the fact that they did not evaluate injuries caused by each stage separately, fourth and fifth instars caused more injury than second and third instars, which generally matches responses in our study. The peculiarity of fourth instars causing higher levels of injury observed in this work may be related to reduced feeding frequency or duration by later insect stages (BERNAYS, 2001; RAUBENHEIMER; BARTON BROWNE, 2000). This change as insects develop is due to an increase in food intake through a higher rate of food ingestion as insects grow (BARTON BROWNE; RAUBENHEIMER, 2003). Other potential explanations may include differences in salivary amount or activity, but these were not evaluated in the present study. Further investigation is needed to better understand why fourth instars caused greater levels of injury to corn seedlings.

In general, our results indicate that *H. halys* is a potential pest of sweet corn seedlings and scouting for this insect may be necessary in early plant stages. In addition, the adapted injury rating scale used in this study could be adopted for early detection of *H. halys* infestations and for assessment of its injury in the field. Nevertheless, further observations are need under open field conditions to determine conditions conducive for *H. halys* feeding on sweet corn seedlings.

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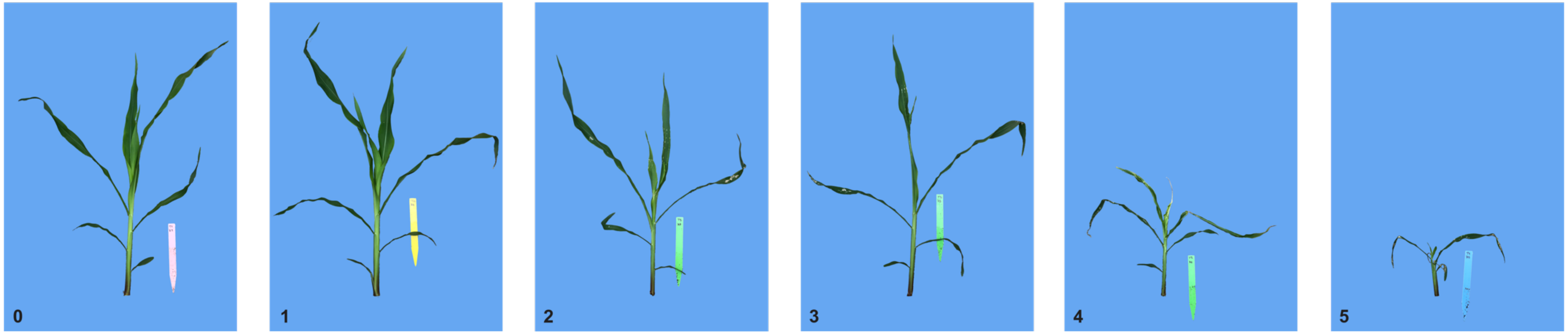

Figure 1. Injury rating scale demonstrative, being: 0 = plant without injury; 1 = leaves with holes and no impact on the plant size; 2 = leaves with holes and impact on the plant size; 3 = plant whorl slightly injured; 4 = severe injury to the whorl or tillering; and 5 = plant with dry or dead whorl. Stakes used for comparison are 15 cm x 1.5 cm.

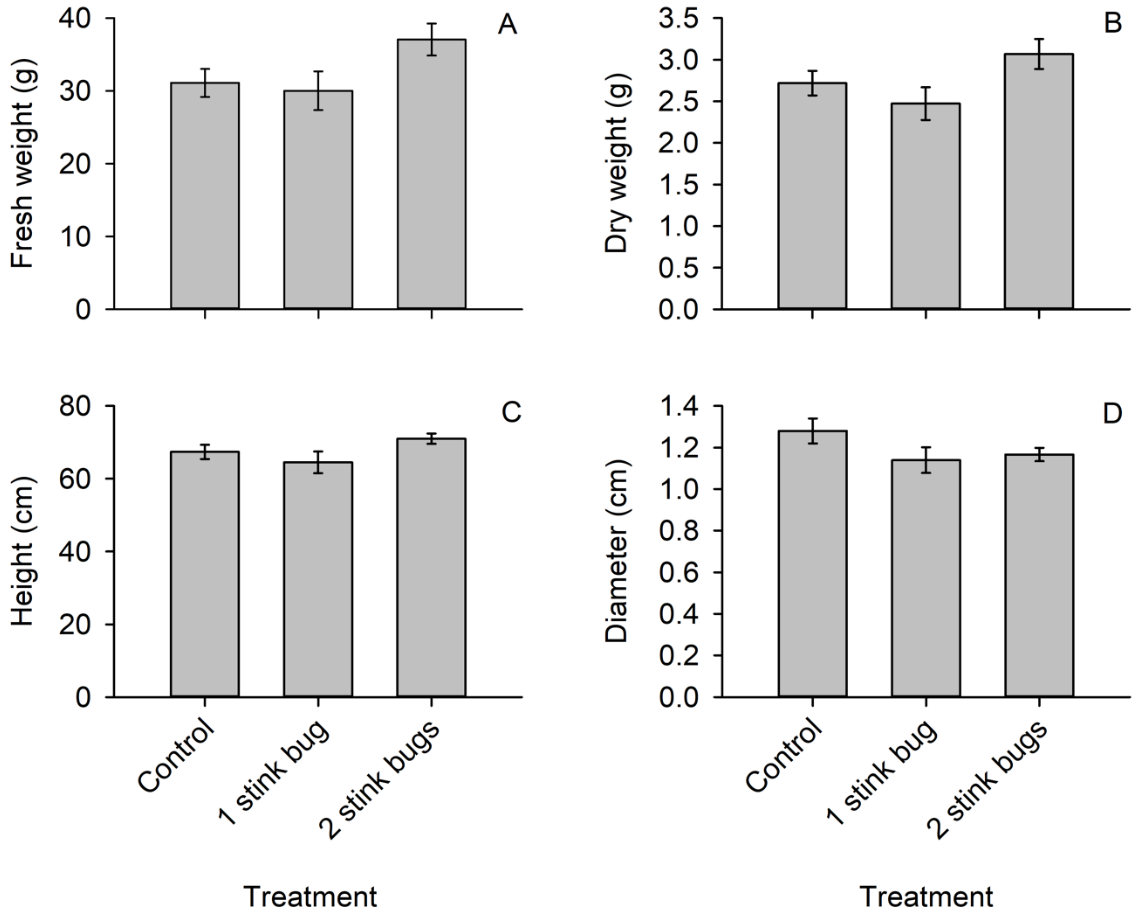

Figure 2. Mean (± SE) fresh and dry weights, height and stem diameter for corn seedlings exposed to *Halyomorpha halys* at densities of zero (i.e., control), one or two insects per plant. Plants were infested one week after emergence and insects were maintained on plants for seven days. Means did not differ significantly among treatments (Tukey's test, $P > 0.05$).

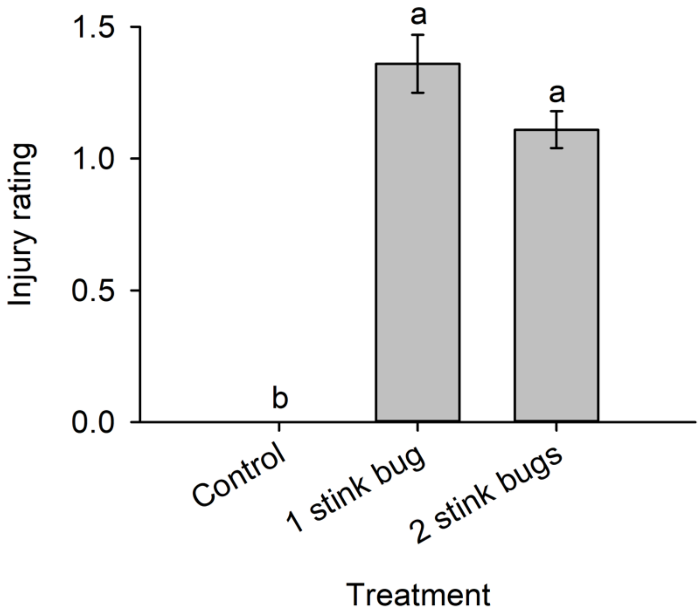

Figure 3. Mean injury rating (± SE) at 23 days after infestation for corn seedlings exposed to *Halyomorpha halys* at densities of zero (i.e., control), one or two insects per plant. The injury rating scale was: 0 = plant without injury; 1 = leaves with holes and no impact on the plant size; 2 = leaves with holes and impact on the plant size; 3 = plant whorl slightly injured; 4 = severe injury to the whorl or tillering; and 5 = plant with dry or dead whorl. Plants were infested one week after emergence and insects were maintained on plants for seven days. Different letters indicate significant differences among treatments (Tukey's test, $P < 0.05$).

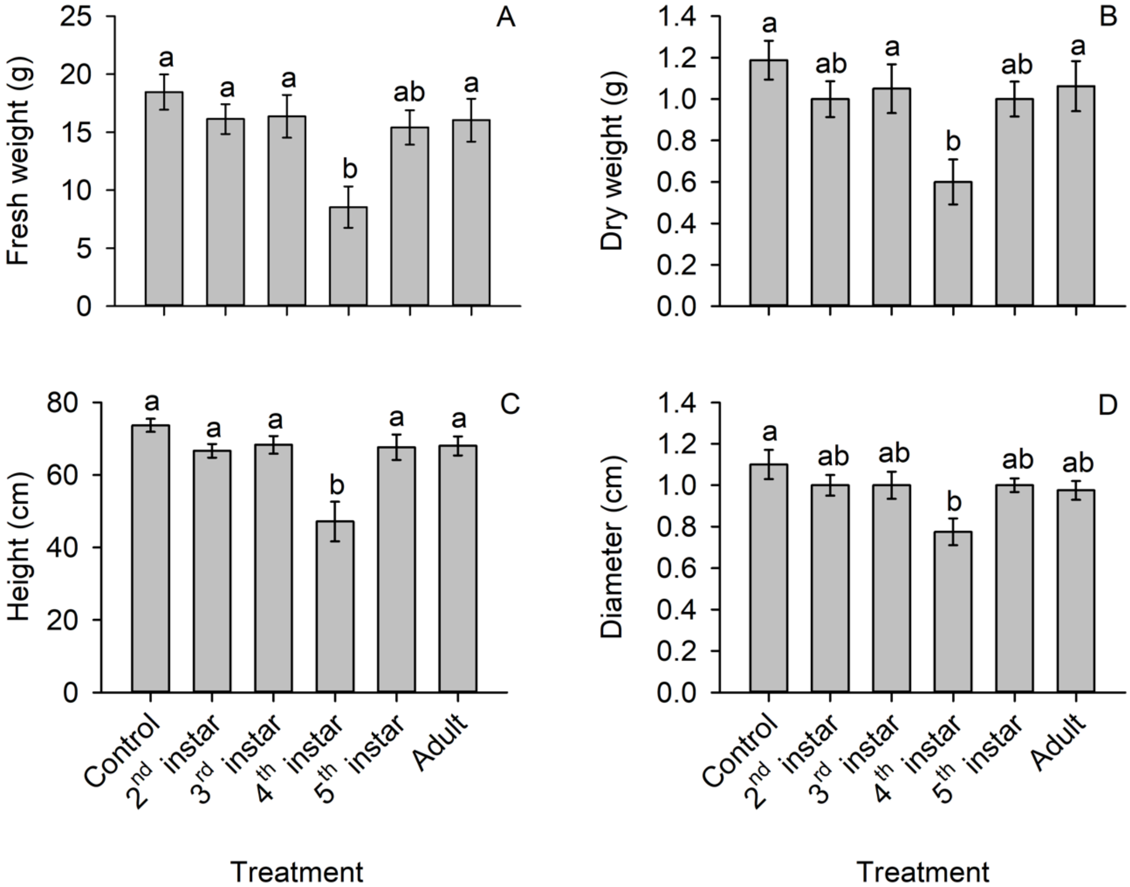

Figure 4. Mean (± SE) fresh and dry weights, height and stem diameter for corn seedlings exposed to second, third, fourth and fifth instars and adults of *Halyomorpha halys* at densities of two insects per plant or zero insects (i.e., control). Plants were infested one week after emergence and insects were maintained on plants for 14 days. Different letters indicate significant differences among treatments (Tukey's test, $p<0.05$).

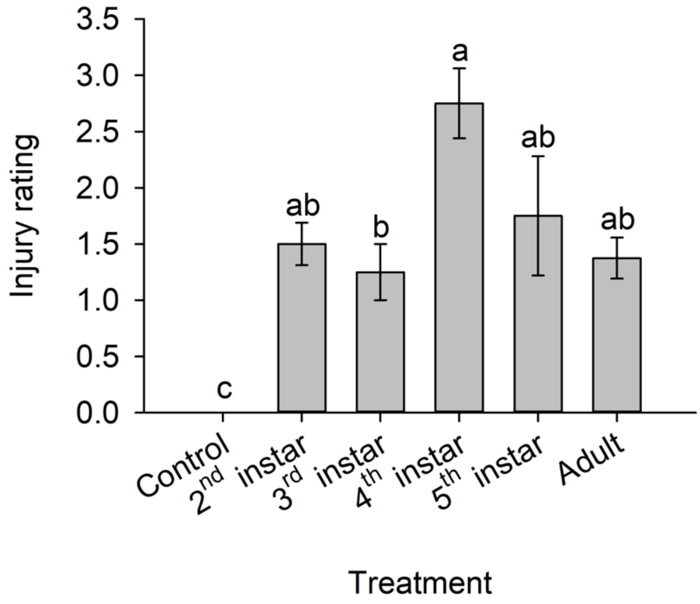

Figure 5. Mean injury rating (± SE) at 23 days after infestation for corn seedlings exposed to second, third, fourth and fifth instars and adults of *Halyomorpha halys* at densities of two insects per plant or zero insects (i.e., control). The injury rating scale was: 0 = plant without injury; 1 = leaves with holes and no impact on the plant size; 2 = leaves with holes and impact on the plant size; 3 = plant whorl slightly injured; 4 = severe injury to the whorl or tillering; 5 = plant with dry or dead whorl. Plants were infested one week after emergence and insects were maintained on plants for 14 days. Different letters indicate significant differences among treatments (Tukey's test, $P < 0.05$).

FINAL CONSIDERATIONS

Dichelops melacanthus is a stink bug that represents a great menace to Brazilian corn growers, because it is the most abundant on that crop. *Euschistus heros* is a species also seen feeding on corn, and deserves Brazilian growers attention because of the high population found on soybean fields. *Dichelops melacanthus* was reported feeding on corn seedlings for the first time in 1995, and since then, has been considered a key pest to corn on its vegetative stage. The damages of stink bugs feeding on corn seedlings vary from holes on the leaves and can lead to plant death, in more severe attacks, provoking yield lossess. Its management is made, mostly, with the use of treated seeds and synthetic insecticides. It is known that this chemicals have some efficiency, but the abusive use, especially with large-spectrum compounds, have been favoring outbreaks of pest resistance to insecticides. In this sense, other management tools, that are efficient and environmentally safe are desirable. The use of resistante genotypes is an interisting strategy among Integrated Pest Management (IPM) tools, because is persistent and compatible with other control techniques.

The threshold levels used to iniciate stink bugs control on corn early stage is usually based on the number of adults in the area, and the role that nymphs may have is unknown.

Stink bugs are increasing its importance worldwide. The brown marmorated stink bug (BMSB), *Halyomorpha halys*, have its origin in Asia, and have been spreading in world basis, to several countries in Europe and more than 40 states of the United States. Due to its high polyphagy, represents a threat to economic important crops, such is corn, the most produced cereal in that country. *Halyomorpha halys* have been reported as an important pest to the reprodutive stage of corn, but its role in the vegetative stage of corn is unknown.

The data obtained in the experiments with *D. melacanthus* shows that the genotypes IAC 8046, SCS 156 Colorado and IAC 8390 were less colonized by the green belly stink bug 24 h after releasing, indicating the occurrence of antixenosis. Genotypes IAC 8390 and JM 2M60 affected negatively its biology, indicating resistance for antibiosis.

Dichelops melacanthus causes more injuries to corn seedlings than *E. heros*, although both species caused significant injuries in all evaluated parameters. The most damage phase of the first species is in the 5th instar, and for the second species, the 4th instar.

To our knowledge, this is the first time that *H. halys* is reported feeding on corn seedlings. We also observed fourth instars causing more injuries than the other development stages.

CONCLUSIONS

- The genotypes IAC 8046, SCS 156 Colorado and IAC 8390 may have the occurrence of antixenosis to *Dichelops melacanthus*. Genotypes IAC 8390 and JM 2M60 may be resistant for antibiosis to *D. melacanthus*.
- *Dichelops melacanthus* causes more injuries to corn seedlings than *E. heros*.
- *Halyomorpha halys* can feed and cause injury on sweet corn seedlings.

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