HÍGOR DE SOUZA RODRIGUES

ECOLOGICAL DYNAMICS OF STINK BUGS THAT ATTACK SOYBEAN AND CORN FIELDS IN THE NEOTROPICAL REGION

Thesis submitted to the Graduate Program in Entomology of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

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Co-advisers: Edson Hirose
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ABSTRACT

RODRIGUES, Hígor de Souza, D.Sc., Universidade Federal de Viçosa, August, 2021. **Ecological dynamics of stink bugs that attack soybean and corn fields in the Neotropical region.** Advisor: Eugenio Eduardo de Oliveira. Co-advisors: Edson Hirose and Gislaine Aparecida Carvalho.

Soybean and corn are the two most representative crops of Brazilian agribusiness. However, during the crop development cycle factors such as pest insect attack, especially the Neotropical brown stink bug, *Euschistus heros*, and the green belly stink bug, *Diceraeus melacanthus*, can reduce productivity and require care. Thus, tools to compose an integrated pest management (IPM) program become necessary to minimize the losses caused by these arthropods. Based on that, this thesis was constructed with the objectives of evaluating the competitive ability of *E. heros* and *D. melacanthus*, verifying the toxicity of different insecticide groups and the detoxification capacity of these insects under different temperatures and generating products (ie, maps of suitability) that indicate safer locations with greater risk of cultivation in the face of attack by these two pentatomids based on the current climate, thus generating preventive control tools for a bed bug MIP program. *Euschistus heros* was more competitive than *D. melacanthus* since the presence of its heterospecific did not impact the number of live insects. However, when the heterospecific was not present, *D. melacanthus* caused lower pod production and increased its growth rate. *Diceraeus melacanthus* was also the most tolerant species to imidacloprid compared to *E. heros* [RR_{50}=15.32 (4.9-48.1)], and was the species that had the most enzymatic detoxification when subjected to temperature changes (ie, 20 °C) with mortality at the LC_{50} reduced by 47.96%. Another result produced in this work was climate-based prediction models (i.e., ecological niche models). Model indicators were satisfactory for both species: for *E. heros*, the AUC (Area Under Curve) was 0.99 ± 0.01 and the TSS (True Skill Statistic) was 0.95 ± 0.03; for *D. melacanthus*, the AUC was 0.94 ± 0.04 and the TSS was 0.95 ± 0.03. Our forecast indicates greater potential damage from *E. heros* in the center-west of Brazil and in the west of Bahia, while *D. melacanthus* concentrates greater potential damage in the center-south of the country. This thesis then shows that despite the greater impact of *D. melacanthus* on pod reduction, *E. heros* was more competitive than *D. melacanthus* in the presence of both species, which justifies its abundance; however, *D. melacanthus* may prove to be a major problem in the absence of *E. heros*. Furthermore, this study also showed the impact of insecticides on both species and indicates that some molecules may not be more effective at commonly used doses and that their detoxification may be increased at low temperatures. Finally, we indicate, through ecological niche models, safe areas and areas with greater risks of soy and corn production against the attack of these insects. All studies carried out here suggest tools to be incorporated into a bed bug MIP program, helping with decision-making and contributing to the management of these pests in production fields.

Keywords: *Euschistus heros*. *Diceraeus* (*Dichelops*) *melacanthus*. Enzymatic detoxification. Interspecific competition. Ecological niche modeling.

RESUMO

RODRIGUES, Hígor de Souza, D.Sc., Universidade Federal de Viçosa, agosto de 2021. **Ecological dynamics of stink bugs that attack soybean and corn fields in the Neotropical region.** Orientador: Eugenio Eduardo de Oliveira. Coorientadores: Edson Hirose e Gislaine Aparecida Carvalho.

A soja e o milho são as duas das mais representativas culturas do agronegócio brasileiro. No entanto, durante o ciclo de desenvolvimento da cultura fatores como o ataque de insetos-praga, com destaque para o percevejo-marrom da soja, *Euschistus heros*, e o percevejo barriga-verde, *Diceraeus melacanthus*, podem reduzir a produtividade e requerem cuidados. Com isso, ferramentas para compor um programa de manejo integrado de pragas (MIP) se tornam necessárias para minimizar as perdas provocadas por esses artrópodes. Neste sentido, essa tese foi construída com os objetivos de avaliar a habilidade competitiva de *E. heros* e *D. melacanthus*, averiguar a toxicidade de diferentes grupos inseticidas e a capacidade de detoxificação desses insetos sob diferentes temperaturas e gerar produtos (i.e., mapas de adequabilidade) que indiquem locais com mais segurança e com maior risco de cultivo frente ao ataque desses dois pentatomídeos com base no clima atual, gerando assim ferramentas preventivas de controle para um programa de MIP de percevejos. *Euschistus heros* foi mais competitivo que *D. melacanthus* visto que a presença de seu heteroespecífico não impactou o número de insetos vivos. Todavia, quando não houve presença do heteroespecífico, *D. melacanthus* provocou menor produção de vagens e aumentou sua taxa de crescimento. *Diceraeus melacanthus* também se mostrou a espécie mais tolerante a imidaclopride comparada a *E. heros* [RR_{50}=15.32 (4.9-48.1)], e foi a espécie que mais teve detoxificação enzimática quando submetido a mudanças de temperatura (i.e., 20 °C) com a mortalidade na CL_{50} reduzida em 47.96%. Outro resultado produzido neste trabalho foram modelos de previsão (i.e., modelos de nicho ecológico) baseados em clima. Os indicadores dos modelos foram satisfatórios para ambas as espécies: para *E. heros*, o AUC (Area Under Curve) foi 0.99 ± 0.01 e o TSS (True Skill Statistic) foi 0.95 ± 0.03; para *D. melacanthus*, o AUC foi 0.94 ± 0.04 e o TSS foi 0.95 ± 0.03. Nossa previsão indica maiores danos potenciais de *E. heros* no centro-oeste do Brasil e no Oeste da Bahia, já *D. melacanthus* concentra maiores danos potenciais no centro-sul do país. Esta tese então mostra que apesar do maior impacto de *D. melacanthus* na redução de vagens, *E. heros* foi mais competitivo que *D. melacanthus* na presença das duas espécies, o que justifica sua abundância; porém, *D. melacanthus* pode vir a ser um grande problema na ausência de *E. heros*. Além disso, este estudo também mostrou o impacto de inseticidas nas duas espécies e indica que algumas moléculas podem não ser mais eficazes em doses comumente usadas e ainda que sua detoxificação pode ser aumentada em baixas temperaturas. Por fim, indicamos através de modelos de nicho ecológico, áreas seguras e àreas com maiores riscos de produção de soja e milho frente ao ataque desses insetos. Todos os estudos realizados aqui sugerem ferramentas a serem incorporadas em um programa de MIP de percevejos, ajudando nas tomadas de decisão e contribuindo para o manejo dessas pragas nos campos de produção.

Palavras-chave: *Euschistus heros*. *Diceraeus* (*Dichelops*) *melacanthus*. Detoxificação enzimática. Competição interespecífica. Modelagem de nicho ecológico.

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SUMMARY

GENERAL INTRODUCTION

The cultivation of soybean (*Glycine max* L.) and corn (*Zea may*s L.), two important Brazilian commodities, represents a huge slice of the country's agribusiness (IBGE, 2019, CONAB, 2021). In addition to being a huge share of the national PIB (of portuguese, Gross Interno Product), the entire production chain of these crops generates direct and indirect jobs. In addition, they are important for the national supply for both human and animal feed, as these are the main inputs. Furthermore, grains produced here are also of international importance, as they meet this need in several other countries such as China, as Brazil is a major exporter of these grains (ANEC, 2019, Oliveira and Schneider, 2016).

However, the development of these crops may be strongly affected by attack by pest insects, among them potentially harmful to the development and productivity of the crop, such as the Neotropical brown stink bug *Euschistus heros* (Fabricius, 1794) and the Neotropical green-belly stink bug *Diceraeus melacanthus* (Dallas, 1851) (Heteroptera: Pentatomidae) (Corso and Gazzoni 1998, Bueno et al. 2012, Sosa-Gomez et al. 2014; Sosa-Gómez et al., 2020). Thus, it is necessary that actions to clarify why these species are harmful to soybeans and corns to avoid risks such as loss of production and possible losses to the country's economy.

To optimize the management of these and other pests that attack production fields, many producers have increasingly adopted Integrated Pest Management (IPM), which is based on different pillars of action and uses different control strategies to optimize the efficiency of the tools and reduce the potential environmental impact these tools can have (Wearing 1998, Ehler 2006, Bueno et al., 2017). The IPM adopts preventive and curative control tools; however, it must be accepted that preventive tools are highly appreciated and can be better managed and used in the context of pest management (Wearing 1998, Baker et al., 2002).

Along with this information, the objectives of this thesis were: (I) to evaluate the competitive ability of *E. heros* and *D. melacanthus* and their potential interactions, (II) to investigate the effect of different insecticide molecules and the detoxification capacity of the two species and whether different temperatures influenced this process and (III) generate information on dispersion and possible places of occurrence to predict management actions based on climate and ecological niche modeling. From that, this thesis intends to generate preventive tools for IPM (i.e., species competitive ability, detoxification of different insecticides and ecological niche modeling) in order to contribute to the management of these two pest species.

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CHAPTER 1. COMPETITION BETWEEN NEOTROPICAL STINK BUGS *EUSCHISTUS HEROS* AND *DICERAUS MELACANTHUS* IN SOYBEAN FIELDS

COMPETITION BETWEEN NEOTROPICAL STINK BUGS *EUSCHISTUS HEROS* AND *DICERAUS MELACANTHUS* IN SOYBEAN FIELDS

Abstract

The changes in Brazilian's soybean, *Glycine max* (L.), cultivation in the last decades impacted the distribution and ecological dynamic of Neotropical brown stink bug, *Euschistus heros* (F.), Southern green stink bug *Nezara viridula* (L.) and allowed that Neotropical green-belly stink bug, *Diceraeus melacanthus* (West.) – important corn's pest, explore this crop. With this scenario, we evaluated the competitiveness of the two main stink bug species of soybean currently, *E. heros* and *D. melacanthus*. Mixed infestation was realized in field cages fixing a species and varying other and these insects were kept in competition until 45 days after infestation (DAI); number of live insects and yield parameters of soybean were evaluated. *E. heros* had more competitive ability that *D. melacanthus* because reduced the number of live insects of your heterospecific while was not impacted by your presence; however, the increment of competition of *D. melacanthus* with conspecific positively affected the growth rate, which did not happen with *E. heros*. Furthermore, lower pod production was observed with *D. melacanthus* without presence of heterospecific (i.e. *E. heros*). The competitiveness of *E. heros* is superior that your heterospecific and justifies the higher abundance in soybean fields; however, *D. melacanthus* is more harmful in the absence of its competitor (i.e. *E. heros*).

Keywords: Stink bug, Pentatomidae, phytophagous competition, interspecific competition, intraspecific competition, population growth.

1.1 Introduction

In insects' communities there are several ecological relations. One of the main ones is competition, intra- or interspecific, which can directly interfere in abundance of a species, in geographical distribution and diversity of species (Denno et al., 1995, Kaplan et al., 2001, Bird et al., 2019). This evidence has been reported in several studies but focuses on leaf-chewing insects and insects that feed on grains (vanVeen et al., 2006; Oliveira et al., 2007) and investigations of intra- and interspecific competition in field of seed-sucking insects are poor explored Zeillinger et al., 2011, Tuelher et al., 2016) mainly in Pentatomids (e.g., soybean stink bug complex).

Stink bug complex in soybean is formed mainly by native insects in the Neotropics as Neotropical brown stink bug *Euschistus heros* (Fabricius) (Hemiptera: Pentatomidae) and Neotropical green-belly stink bug *Diceraeus melacanthus* (Dallas) and stink bugs originating in other regions as Southern green stink bug *Nezara viridula* (Linnaeus) and redbanded stink bug *Piezodorus guildinii* (Westwood) (Conte et al., 2014; Conte et al., 2015; Conte et al., 2016, 2017, 2018; Panizzi et al., 2012; Panizzi and Lucini, 2016; Panizzi et al., 2000; Sosa-Gómez et al., 2020). These ones develop and compete for resources in different wild plants species; however, high population and, consequently, strong competition happens in the soybean due abundant resource by size of the Brazilian planted area which generates a niche overlap (Bird et al., 2019; Panizzi and Lucini, 2016). This competition not only impacts on loss of productivity but also on strategies for IPM (Integrated Pest Management) (Panizzi, 2013), as well as consuming a large amount of insecticides that are the main control agents this pest insects and still impacts the (world?) economy, whereas that Brazilian soybeans figure as an important cereal in the international market with exportation volume of 84 million of ton in the last year, supplying several Asian countries such as China (ANEC,2018; Oliveira and Schneider, 2016).

In order to reach this level of productivity as one of the largest exporters in the world, soybean cultivation in Brazil has underwent some changes, such as the advance of production areas to the Middle West of Brazil in a different biome than was usually cultivated (i.e., Brazilian cerrado), new cultivars and forms of planting (i.e., no-tillage cultivation system) and temporal shortening of the cultivation cycle and intensification in planting in the soybean-corn production (Bertrand and Théry, 2006; Dall'Agnol, 2016; Galford et al., 2008; Galvão et al., 2015). These changes directly impacted the dynamics of stink bug in the crop, for example: *N. viridula* was the main stink bug in the soybean fields in the 70's and 80's and from the 90's to today this place is occupied by *E. heros* because of these factors and their competing ability (Conte et al., 2014; Conte et al., 2015; Conte et al., 2016, 2017, 2018; Panizzi and Slansky Jr, 1985; Panizzi and Lucini, 2016; Tuelher et al., 2016). Moreover, this temporal shortening between soybean and corn cultivation allowed *D. melacanthus*, an important corn pest, explore more soybean cultivation and to be an important component of the stink bug complex competing directly with *E. heros* (Chocorosqui and Panizzi, 2008; Conte et al., 2014; Conte et al., 2015; Conte et al., 2016, 2017, 2018; Crosariol Netto et al., 2015; Gomez, 1998; Panizzi et al., 2015).

Thus, the present study aims to investigate the role of competition in the stink bug complex in soybean emphasizing the two most occurring species in the production fields currently: *E.*

heros and *D. melacanthus*. In addition, we investigated whether *D. melacanthus* has a competitive ability to potentially overcome *E. heros* in soybean crops in the next agricultural crops.

1.2 Materials and Methods

1.2.1 Insects

Colonies of *Euschistus heros* and *Diceraeus melacanthus* used in experiments were maintained at Embrapa Rice and Bean (Santo Antônio de Goiás, GO, Brazil). The colony of *E. heros* starting from insects maintained at Embrapa Genetic Resources and Biotechnology (Brasília, DF, Brazil). The colony of *D. melacanthus* starting from insects maintained at Embrapa Soybean (Londrina, PR, Brazil). All the developmental stages of both stink bugs were under controlled conditions (27±2 °C, 60±20% relative humidity and with a photoperiod of 14 h) and following methods previously described (Borges et al., 2006; Silva et al., 2008; Silva et al., 2011). Routinely, introduction of field-insects was doing in the laboratory colonies to increase the genetic variability of insects used in the experiments.

1.2.2 Cultivation condition and experimental plots

The soybean variety BRS 8170 IPRO ('Intacta') (Embrapa Soybean, Londrina, Brazil), maturity group 8.1, was sowed under conventional area at the experimental farm of Embrapa Rice and Bean (16° 29´ 30.8´´ S, 49° 17´ 41.7´´ W) on 15 December 2017. Common agronomic practices to soybean production in Brazilian 'cerrado' were used. In the seeding was utilized in the cultivation line fipronil (4 g ha-1, Basf, São Paulo, Brazil), thiophanate methyl (45 mL ha-1, IHARA, São Paulo, Brazil) and the seeds were inoculated with Bradyrhizobium elkanii (6 mL kg−1 seeds, Nitragin®, Novozymes, São Paulo, Brazil) and sowed at a rate of 15 seeds m-1 with a 0.50 m row spacing.

When soybeans reached the R3 stage, field cages (1.5 x 1.0 x 1.0 m, height x width x length) were placed under two rows of soybean plants and, from there, were maintained only 4 plants per cage.

To control weeds, was used diclosulam (42 g i.a ha-1, Dow AgroSciences, São Paulo, Brazil) and glyphosate (1 L ha-1, Monsanto, São Paulo, Brazil). A week before stink bug infestation, were sprayed inside cages lambda-cialotrina (Kaiso 250 CS, Nufarm Ind. Quim. e Farm.) to prevent damage caused by other insects.

1.2.3 Competition experiments

The bioassay was realized in a single season once the multiples densities of both insect species to contain the species composition and the effect expected in different years. Fifty-five days after sowing, adults sexually mature (12-14 days after emergence) of *E. heros* and *D. melacanthus* were confined inside cages previously inspected (to ensure that there were no insects developing there) in the sex ratio of 1: 1 (male: female). Four soybean field (cages) were used as replicate each containing each treatment. The control treatment was conducted with cages without insects. Two days after insect infestation, dead insects were replaced to maintain the initial proportion.

The competition experiments were designed for *E. heros* and its heterospecific competitor *D. melacanthus* using an additive series (Snaydon, 1991). Mixed infestations were established in each cage (experimental unit) containing four soybean plants. The initial number of insects of one specie was fixed at 10 adults, while the other specie had increment in the number of adults varying from 0 to 10, with proportions of 0, 0.17, 0.29, 0.38 and 0.50. The insects were kept confined in competition and the assessments were realized with 15, 30 and 45 days after infestation (DAI), where the number of insects (adults and nymphs) of each species were evaluated. The instantaneous rate of population increase (ri) for each stink bug specie in each experimental unit was performed at 30 and 45 DAI using the formula $ri = \frac{ln(\frac{Nf}{Ni})}{\Delta T}$, where Nf and Ni are the final and initial number of live insects, respectively, and ΔT is the duration of the experiment in days (Stark and Banks, 2003; Walthall and Stark, 1997).

At the end of the soybean development cycle, all four plants that formed each experimental unit were harvested and the yield and grain quality parameters as number of empty pods, number of damaged grains, number of grains per plant and total grain yield were evaluated.

1.2.4 Statistical analysis

Repeated-measures analysis of variance were performed to assessment the number of live insects in each cage, once the same experimental units were used at each evaluation date (Green, 1993; Paine, 1996), avoiding problems of pseudorepetition in time (Paine 1996; Hurlbert 1984;

Stewart-Oaten et al. 1986). For this analysis was used the PROC MANOVA procedure with the PROFILE statement, as suggested by (von Ende, 1993). Post hoc Tukey's HSD tests (0.05) were used to compare means whenever necessary.

The analyses of covariance (PROC GLM procedure) was utilized to analysis the instantaneous rate of increase in each specie at 45 DAI, where the presence specie was utilized as independent variable and the proportional increase in density as a covariate. When necessary, complementary regression analyses were performed (PROC REG procedure).

PROC ANOVA procedure and post hoc Tukey's HSD tests (0.05) were performed to comparisons of soybean yields and quality parameters among the treatment means.

The assumptions of normality and homogeneity of variance were checked, and no data transformation was necessary (UNIVARIATE procedure). All statistical procedures above were performed using SAS/STAT software for Windows (SAS Institute Inc, 2008).

1.3 Results

1.3.1 Population growth over time

Repeated-measures ANOVA for the number of live insects after 45 days of competition showed significant results (Table **??**). The number of live *D. melacanthus* indicated significant effects in the presence (P = 0.0015) and in interaction of the presence and increasing of initial population of co-occurring specie (P = 0.0246) (Table **??**). Furthermore, significant effects were also found for evaluation of days of competition (time) (P = 0.0023) (Table **??**). As shown in Fig. 1.1A, the number of *D. melacanthus* was unaffected by increment of *E. heros* in the 15 first days of competition, but after the 30th and 45th days of competition the number of lives *D. melacanthus* was significant reduced according with increasing of the heterospecific. When it is only observed the increment of the initial proportion of *D. melacanthus* (Fig. 1.1B), exist an increment in the lives *D. melacanthus* in 15 first days of competition, which does not happen in the remaining period of competition.

These results from the repeated-measures ANOVA, however, showed that to the number of lives *E. heros* neither to species that had their initial proportion changed (P = 0.50) nor to the initial proportion of each species (P = 0.76) nor to their interaction (P = 0.39) no significant effects were observed (Table **??**). To number of lives *E. heros* still, no significant effect of time and your interactions was observed (Table **??**).

Tabela 1.1: Repeated-measures ANOVA for live stink bugs after 45 days of competition experiments between *Euschistus heros* and *Diceraeus melacanthus* in the 2017/2018 growing season.

Sources of variation	df	*Euschistus heros*			*Diceraeus melacanthus*		
			F	*P*		*F*	*P*
Between samples							
Species (S)	1		0.46	0.50		11.85	0.0015*
Initial proportion (P)	5		0.52	0.76		1.11	0.37
S x P	5		1.07	0.39		2.95	0.0246*
Error	36		-	-		-	-
Sources of variation	$\text{df}_{num}/\text{df}_{den}$	Wilks′lambda	*F*	*P*	Wilks′lambda	*F*	*P*
Within samples							
Time (T)	2/35	0.97	0.48	0.62	0.71	7.28	0.0023*
T x S	2/35	0.96	0.81	0.45	0.91	1.64	0.21
T x P	10/70	0.71	1.32	0.24	0.77	0.97	0.48
T x S x P	10/70	0.84	0.64	0.77	0.82	0.73	0.69

* Significant at $P < 0.05$.

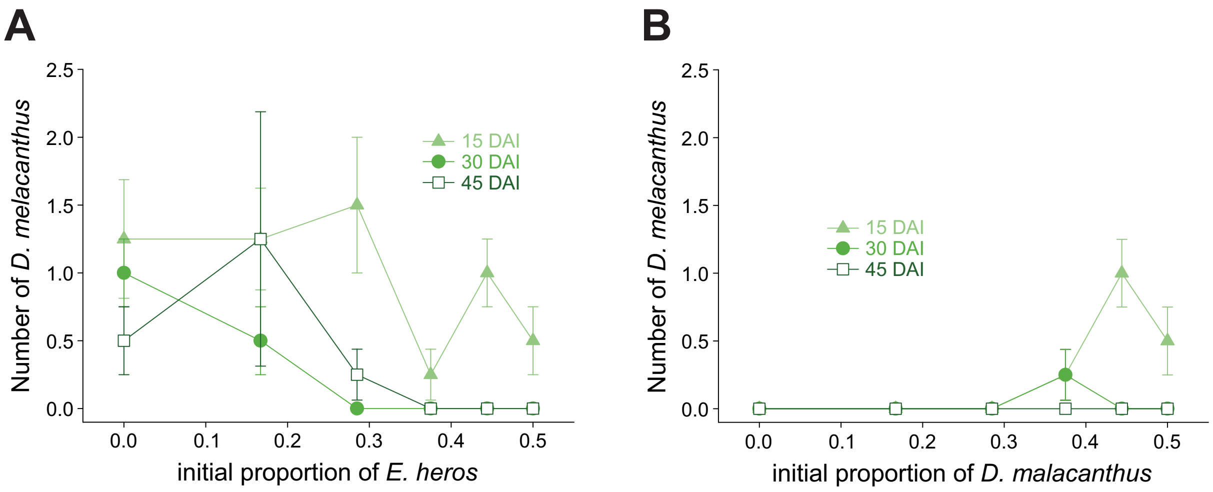

Figura 1.1: Total number of *Diceraeus melacanthus* obtained after 45 days of competition between *Euschistus heros* and *D. melacanthus*. (A) The number of *D. melacanthus* was not influenced by the increase of *E. heros* in the first evaluation (i.e., 15 DAI), but was significantly reduced in the other evaluations (i.e. 30 and 45 DAI) by increasing the initial proportion of co-occurring. (B) Increase in the initial proportion of *D. melacanthus* caused a significant increase in the number of lives *D. melacanthus* only at the beginning of the competition (i.e. 15 DAI). Linked symbols represent the mean of four replicates in 15 (light green), 30 (green) and 45 (dark green) DAI and the vertical bars represent the standard error.

1.3.2 *Competition and the instantaneous rate population increase (ri)*

Only the model of the analysis of covariance for the population growth rate of *D. melacanthus* after 30 and 45 days of competition was significant, while no significant difference was observed for *E. heros* (Table 1.2). Despite the growth rate of *D. melacanthus* has been significant for interaction between the presence of the species that exhibited an increased proportion and the effect of the increasing proportion, only the increasing the competition level by conspecifics positively affected the growth rate of *D. melacanthus* in both scenarios (i.e. 30 and 45 days of competition) (Table 1.2, Fig. 1.2).

Tabela 1.2: Analyses of covariance for the total number of live insects after 30 and 45 days of competition between *Euschistus heros* and *Diceraeus melacanthus* in the 2017/2018 growing season.

		30 days of competition			
Sources of variation		*Euschistus heros*		*Diceraeus melacanthus*	
	df	*F*	*P*	*F*	*P*
Model	3	0.96	0.43	12.97	<0.001*
Error	20	-	-	-	-
Species with increasing density	1	0.16	0.69	7.57	0.0123*
Initial proportion	1	1.36	0.26	15.36	<0.001*
Interaction	1	1.33	0.26	15.36	<0.001*
		45 days of competition			
		Euschistus heros		*Diceraeus melacanthus*	
	df	*F*	*P*	*F*	*P*
Model	3	0.76	0.53	4.14	0.019*
Error	20	-	-	-	-
Species with increasing density	1	0.30	0.59	2.06	0.17
Initial proportion	1	1.78	0.20	5.08	0.035*
Interaction	1	0.17	0.68	5.08	0.035*

* Significant at *P* <0.05.

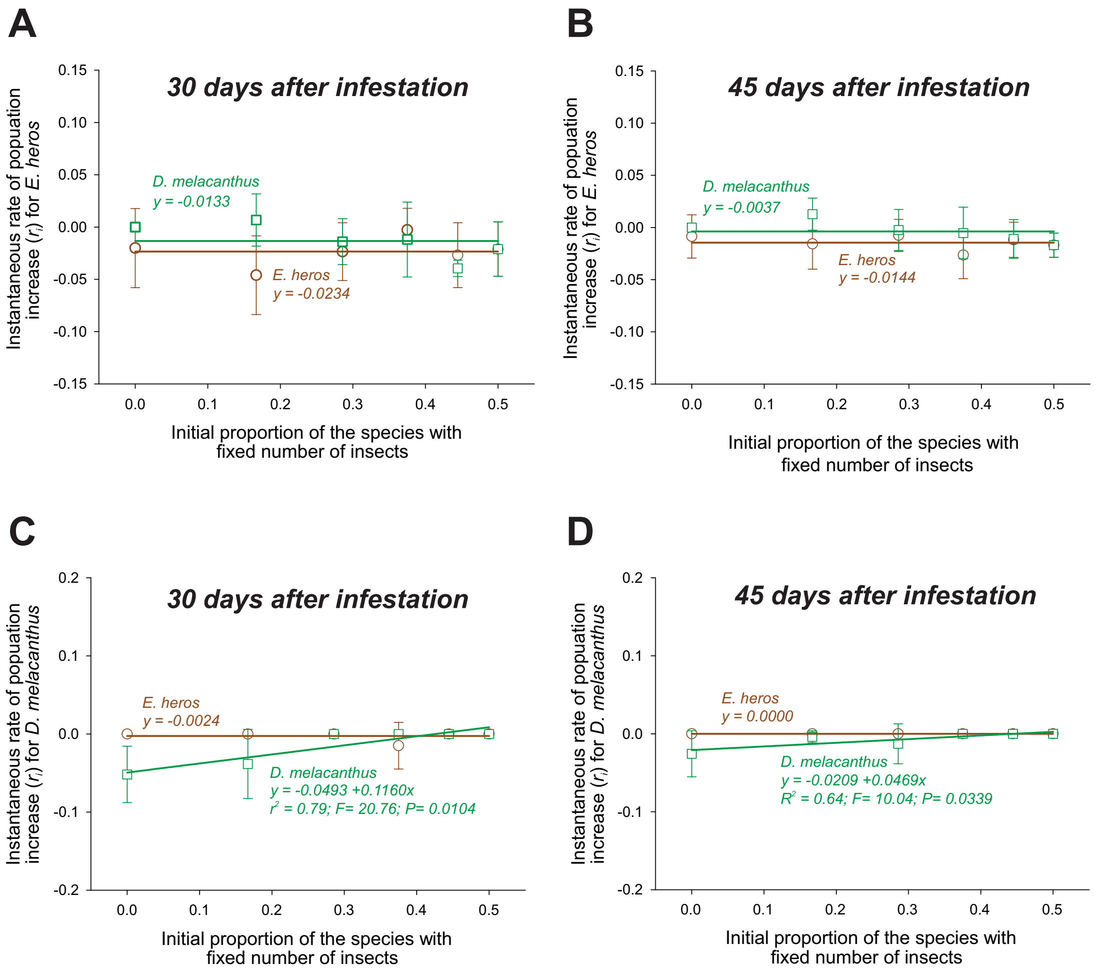

Figura 1.2: Instantaneous rate of population increase (ri) of *Euschistus heros* (A and B) and *Diceraeus melacanthus* (C and D) obtained after 30 and 45 days of competition between *E. heros* and *D. melacanthus*. The curves refer to the species with a fixed number of insects and the species with a variable number of insects, as indicated on each curve. The symbols represent the mean of four replicates and the vertical bars represent the standard error.

1.3.3 Yield parameters

The presence of stink bugs significantly enhances the production of empty pods (ANOVA, F11,33= 2,56, P = 0.018, Fig. 1.3A). Without the presence of conspecific, *D. melacanthus* caused higher production of empty pods (t test, t = 2.82, df = 6, *P* = 0.0152) but when had the presence of *E. heros*, this difference not was observed (ANOVA, F8,26= 1,09, *P*= 0.40) (Fig. 1.3A). Another observation when *D. melacanthus* is the specie with fixed number of insects, is that there is a tendency that higher proportions of *D. melacanthus* (e.g., 71:29, *D. melacanthus: E. heros*) may produce smaller amounts of empty pods (Fig. 1.3A).

The production of grains was reduced in the presence of *D. melacanthus* without conspecific comparing to control (i.e. treatment without stink bugs) (t test, t = 3.55, df = 6, *P* = 0.0006) (Figure 1.3B). The increasing of initial proportion of conspecific *E. heros* caused the growth of production of grains in experimental units (Fig. 1.3B). The presence of stink bugs did not affect the 100 grains weight (F = 1.86, *P* = 0.093). However, both species caused around 20% of commercial defects in the grains comparing with no stink bugs infestation (ANOVA, F11,34 = 3.76, *P* = 0.001) (Fig. 1.3C).

1.4 Discussion

We evaluated in this study the direct competition between Neotropical stink bugs *Euschistus heros* and *Diceraeus melacanthus* in the soybean crop. The development of *E. heros* is not affected by its heterospecific; however, *D. melacanthus* is disadvantaged by the presence of *E. heros* despite having a high capacity to reduce grain yield in the crop.

The phytophagous studied here are natural of Neotropics and coexist in soybean fields since 70's (Corrêa-Ferreira, 1986; Panizzi, 1977; Panizzi and Slansky Jr, 1985; Panizzi and Smith, 1976). In past studies, *E. heros* was related how soybean pest but had little importance in this time figuring as secondary pest. However, since 90's this species changed the scenarios of importance and figure as major stink bug pest of soybean (Panizzi and Lucini, 2016). Though considered rare and irrelevant, *D. melacanthus* was present in soybean fields; however, since 2015 this status was changed and in current crops it is the main competitor of *E. heros* in soybean (Conte et al., 2014; Conte et al., 2015; Conte et al., 2016, 2017) (Supplementary Figure 1).

Some suspicions are attributed to the increase of the population of *E. heros* in Neotropical soybeans. The mainly are the heavy use of herbicides in the cropping system excluding host

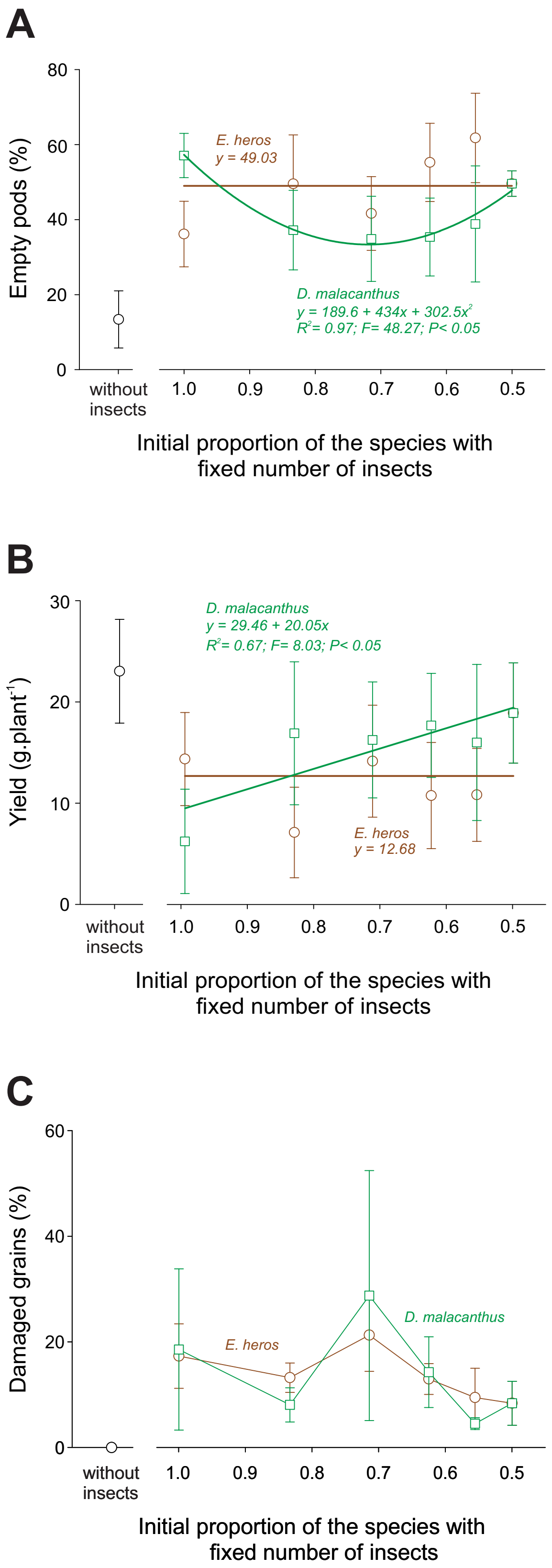

Figura 1.3: Percentage of production of empty pods (A), production (B) and damage grains caused (C) in soybean plants obtained after 45 days of competition between *Euschistus heros* and *Diceraeus melacanthus* and in the absence these stink bugs. The yield parameters of non-infested plants are indicated as black symbols. The curves and the linked symbols refer to the species with a fixed number of insects and the species with a variable number of insects, i.e. *E. heros* (brown) and *D. melacanthus* (green). The symbols represent the yield of infested plants (i.e. mean of four replicates) and the vertical bars represent the standard error.

plants to *N. viridula* since *E. heros* besides being less polyphagous, can survive in the absence of food plants and the no-tillage cultivation that provides shelter as well as fallen seeds for food to insects that live part of their life on the soil litter as *E. heros* (Panizzi et al., 2015; Panizzi and Hirose, 1995; Panizzi and Lucini, 2016). Thus, we expected that *E. heros* to be a better competitor than its heterospecific and our findings showed that our expectations were correct, and its competitive ability was not exceeded.

Other factors can be attributed to this success. The elevation of global temperature due to global climate change may have affect the distribution this species for creating more zones of climate adaptability for *E. heros*, including the conditions found in the Brazilian cerrado where it became the main site of soybean production, favoring its biology by allow more generations per year. In addition, few studies comparing the susceptibility to insecticides in different species this complex were performed and *E. heros* showed more tolerant to these chemical molecules, which may be a great contributor to the prevalence of this species in the soybean fields (Santos et al., 2016; Snodgrass et al., 2005; Sosa-Gómez et al., 2009; Sosa-Gómez and Silva, 2010; Tuelher et al., 2018; Willrich et al., 2003).

It is important to note that *D. melacanthus* has also benefited for these conditions - since it also lives part of his life in the soil litter - and mainly for intensity of the multiple cropping systems (i.e., temporal shortening between soybean and corn cultivation), allowing the greater exploration of double crop (soybean-corn) system. Our assumptions were that as happened the suppression of *N. viridula* and *P. guildinii* by, between other factors, better competitiveness of *E. heros* (Panizzi and Lucini, 2016; Tuelher et al., 2016), *D. melacanthus* could come to overshadow the competitive abilities of *E. heros* and become the main stink bug in the stink bug complex of soybean. On hand in our results not is possible affirm still that *D. melacanthus* can overcome *E. heros* in soybean fields but is necessary attention because the proportion of *D. melacanthus* grows with each crop season in Parana state (Supplementary Figure 1).

Although not be still a better competitor, *D. melacanthus* have a great capacity of development in soybean, getting to develop and reproduce (Chocorosqui and Panizzi, 2008). Furthermore, in the last 20 years the production process was totally modified compared what is currently and these changes will continue both the cultivation methods and the pest control methods, scenario that allowed a change in the past (i.e., *N. viridula* by *E. heros*) (Panizzi and Lucini, 2016) and may allow a change in the future since the control tools will be directed to *E. heros* reducing its development and allowing the emergence of another species as the main stink bug of the stink bug complex of soybean.

Thus, our study indicates that *D. melacanthus* is more harmful than *E. heros* in soybean without the presence of any competitor; however, when there is the presence of the heterospecific, the competitive ability of *E. heros* is superior and inhibits the actions of *D. melacanthus*. It is important to note, however, that *D. melacanthus* shows itself as a pest insect alarming for corn crops mainly in the soybean-corn succession. However, more studies are required (i.e., insecticide tolerance and resistance, impact of global climate change in stink bug's distribution) to understand this phenomenon what is happening with stink bug complex in Brazilian soybean fields in the last decade and to elucidate the combination of factors that allow this greater competitive capacity of *E. heros* and whether it can be overcome by some other species of the stink bug complex of soybean (i.e., *D. melacanthus*) or other species of the soybean's pest complex to generate results that will help in the IPM of this commodity so representative in the Brazilian economy.

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1.6 Supplementary Material

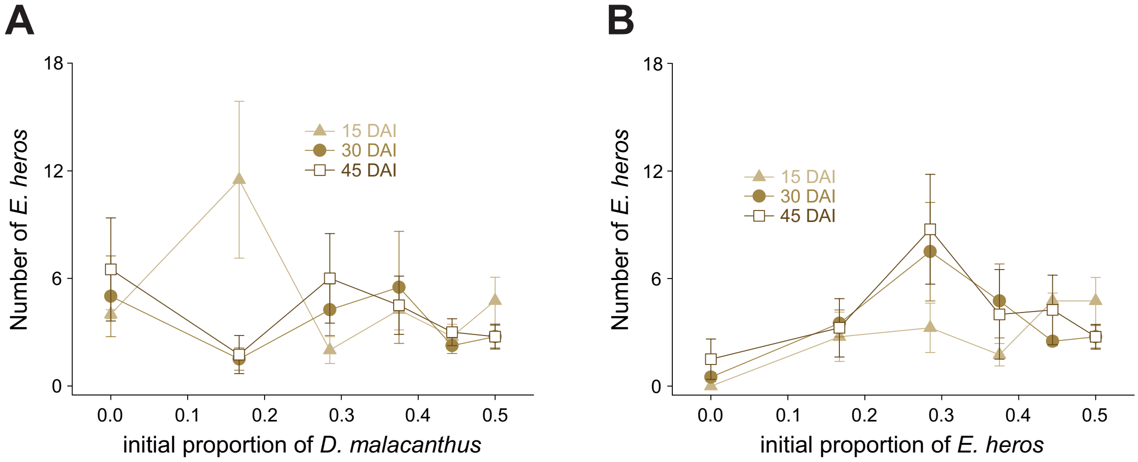

Figura 1.4: Total number of *Euschistus heros* obtained after 45 days of competition between *Diceraeus melacanthus* and *E. heros*. (A) The number of *E. heros* was influenced by the increase of *D. melacanthus* in the first evaluation (i.e., 15 DAI), but was not significantly reduced in the other evaluations (i.e. 30 and 45 DAI) by increasing the initial proportion of co-occurring. (B) Increase in the initial proportion of *E. heros* not caused a significant increase in the number of lives *E. heros*. Linked symbols represent the mean of four replicates in 15 (light brown), 30 (brown) and 45 (dark brown) DAI and the vertical bars represent the standard error.

CHAPTER 2. RESPONSES OF DETOXIFICATION ENZYMES OF *EUSCHISTUS HEROS* AND *DICERAUS MELACANTHUS* UNDER DIFFERENT TEMPERATURE REGIMES

CHAPTER 2. RESPONSES OF DETOXIFICATION ENZYMES OF *EUSCHISTUS HEROS* AND *DICERAUS MELACANTHUS* UNDER DIFFERENT TEMPERATURE REGIMES

Abstract

To control pests such as *Euschistus heros* and *Diceraeus melacanthus* in soybean production fields, producers use many insecticides as neonicotinoids, pyrethroids and organophosphorus, but in some cases the effectiveness of control is not satisfactory because these pests showed insecticide resistance and/or tolerance, especially with enhanced detoxification by cytochrome P450 (CYP) enzymes. In addition, changes in temperature can modulate these enzymatic actions and influence in mortality. Here, was firstly conducted toxicological studies for *D. melacanthus* (i.e., LC curves), not reported in previous work and, with the obtained results to imidacloprid [RR_{50} = 15.32 (4.88-48.13)] in contrast with *E. heros*, measured the enzymatic activity of CYP, glutathione-S-transferase (GST) and pNPA-esterase and the impact of different temperatures in the imidacloprid detoxification process. Our data report toxicological studies with three different active ingredients for *D. melacanthus* and a possible resistance and/or tolerance of this species to imidacloprid in relation to *E. heros*. Furthermore, showed that both *E. heros* and *D. melacanthus*, CYP is involved in enzymatic detoxification and in low temperatures (i.e., 20 °C) this enzymatic complex is efficient to the point of reducing the mortality comparing the LC_{50} in 47.96% and 25.26%, respectively. These findings add to other reports and help in the decision making regarding the use of insecticidal molecules, mainly neonicotinoids, contributing to the sustainability of these curative methods of control.

Keywords: Neonicotinoid, cytochrome P450, thermal tolerance, stink bug, Pentatomidae.

2.1 Introduction

Soybean is an important crop in Brazil and its cultivation was modified in the last decades. This modification allowed that stink bugs how Neotropical brown stink bug, *Euschistus heros*, and green-belly stink bug, *Diceraeus melacanthus*, explore with more efficacy this cultivation; *E. heros* is the most important stink bug in soybean and *D. melacanthus*, important corn pest, currently is the second most important stink bug in soybean because of approaching soybean cultivation with corn cultivation, situation that favored the attack of this pest (Bertrand and Théry, 2006;

Conte et al., 2014; Conte et al., 2015; Conte et al., 2016, 2017, 2018; Galford et al., 2008; Galvão et al., 2015; Sosa-Gómez et al., 2020).

Insecticides (e.g., neonicotinoids, pyrethroids, organophosphorus) are widely used to prevent losses caused by this pest insects on most farms (Sosa-Gómez and Silva, 2010; Tuelher et al., 2018). However, while the toxicity of these molecules to E. heros is known (Haddi et al., 2015; Pitta et al., 2018; Santos et al., 2016a; Santos et al., 2016b; Tuelher et al., 2017; Rodrigues et al., 2021), little is known about the toxicologic effect of these compounds on *D. melacanthus* (Chocorosqui and Panizzi, 2008). Besides that, it's not known if this may be a factor that allows the growth of *D. melacanthus* to the detriment of the growth of other species as *Nezara viridula* and *Piezodorus guildinii* – members of soybean stink bug complex (Conte et al., 2014; Conte et al., 2015; Conte et al., 2016, 2017, 2018), since that *D. melacanthus* may be more tolerant than the others for a determined insecticide and no toxicological study has been performed for this species so far to investigate.

The use these insecticides molecules, in most cases, allow efficient control of *E. heros*, but a risk of control failure and morfological alterations was reported in main production areas like Brazilian cerrado indicating possible insecticide-resistant populations (Guedes, 2017; Tuelher et al., 2018; Castellanos et al., 2019; Castellanos et al., 2021; Tibola et al., 2021). Insecticide resistance or tolerance is widely studied and mechanisms as altered target-site insensitivity, behavioral and enhanced detoxification resistance are described how insecticidal action suppressants (Bass et al., 2015; Nauen and Denholm, 2005; Puinean et al., 2010; Perez Campos et al., 2021). However, metabolic resistance as enhanced activity of detoxifying enzymes such as cytochrome P450 (CYP) and glutathione-S-transferase (GST) are the main pathways for resistance to imidacloprid, molecule more investigated in this work (Bass et al., 2011; Puinean et al., 2010; Yang et al., 2013; Yang et al., 2016; Zhang et al., 2016). In addition, it's known that temperature can influence this process of detoxification (Foster et al., 1997; Guo et al., 2018; Tiwari et al., 2015; Whiten and Peterson, 2015; Zhang et al., 2015) increasing the enzymatic activity of certain enzymes and providing reduction in mortality, but little is known about this influence in metabolic process of insecticide detoxification in stink bugs.

Thus, this study was conducted with the aim of providing toxicological studies on *D. melacanthus* and comparing them with *E. heros*. As *D. melacanthus* showed to be more tolerant to imidacloprid [RR = 15.32 (4.88-48.13)], we investigated the metabolic contribution of the main detoxification enzymes and how the temperature impacts this process and in mortality comparing both species.

2.2 Materials and Methods

2.2.1 Insects

All insects used in this experiment originated from a colony of *Euschistus heros* and *Diceraeus melacanthus* maintained at Embrapa Rice and Bean (Santo Antônio de Goiás, GO, Brazil). The colony of *E. heros* starting from insects maintained at Semiochemical Laboratory of the EMBRAPA Natural Resources and Biotechnology (Brasília, DF, Brazil). The colony of *D. melacanthus* starting from insects maintained at Embrapa Soybean (Londrina, PR, Brazil). The colony was reared to prevent diapause. All the developmental stages of both stink bugs were under controlled conditions (27±2 °C, 60±20% relative humidity and with a photoperiod of 14 h) and following methods previously described (Borges et al., 2006; Silva et al., 2008; Silva et al., 2011). Routinely, introduction of field-insects from soybean farms was doing in the laboratory colonies to increase the genetic variability of insects used in the experiments.

2.2.2 Insecticides

The insecticides used were imidacloprid (Evidence 700 WG, Bayer CropScience); thiamethoxam (Actara 250 WG, Syngenta Crop Protection); lambda-cyhalothrin (Kaiso 250 CS, Nufarm Ind. Chem. and Farm.); beta-cyfluthrin (Bulldock 125 SC, Bayer CropScience) and acephate (Cefanol, Sipcam Agro Brazil).

2.2.3 Concentration-mortality bioassay

Bioassays with all insecticide groups followed methods that were adapted from toxicological studies in *E. heros* carried with glass bottles (Santos et al., 2016b; Snodgrass et al., 2005; Tuelher et al., 2017; Willrich et al., 2003). Insecticides were used to coat the inner walls of 250 ml clear glass vials. Water (distilled and deionized) will be used as a vehicle for the commercial formulation of insecticide. A control treatment (distilled and deionized water) and different concentrations of insecticides were used to estimate the concentration-mortality curve. Ten replicates, each with 10 newly emerged adults (< 24h), were used at each concentration. The period of exposition was 48 h, and the insects were counted as dead when unable to walk the length of their body. Mortality data were corrected for natural mortality (i.e., control treatment).

2.2.4 *Enzymatic activity determination bioassay*

Enzyme extraction and different temperatures used

In all enzymatic bioassay were used the fat body homogenized of five newly emerged adults ($\leq$ 24 h old) of both *E. heros* and *D. melacanthus*. Furthermore, the same procedures of enzymatic activity determination were repeated in four different temperatures (i.e., 20, 25, 30 and 35 °C) with use of incubators.

Total protein content

The Bradford method (Bradford, 1976) was used to determine the total protein contents and the standard curve for protein content was performed from serial dilutions of bovine albumin.

Cytochrome P450 (CYP) activity

CYP activity was determined by indirect measure using heme peroxidation with 3,3´,5,5´-tetramethylbenzidine (TMBZ) as the substrate (Brogdon et al., 1997). In the reaction were added to TMBZ an aliquot of enzyme solution, potassium phosphate buffer (1 M) and hydrogen peroxide (3%). Phosphate buffer was used as control and the absorbance was read at 650 nm. The results obtained from the readings were compared with a standard curve of absorbance with known values of cytochrome C.

Glutathione-S-transferase (GST) activity

GST activity was measured according to Habig et al. (Habig et al., 1974), using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate. In the reaction, together with CDNB, were mixed enzyme solution, phosphate buffer (0.1 M) and GSH solution (150 mM). The control consisted of phosphate buffer, CDNB solution and GSH solution. The absorbance was read at 340 nm and the changes in absorbance every 30 s (until 90 s) determined GST activity.

pNPA-esterase activity

pNPA-esterase activity was measured according to (Hemingway, 1998), using pNPA/Na in acetonitrile (100 mM) as the substrate. In the reaction, together with substrate, were mixed enzyme solution and phosphate buffer (50 mM). The control consisted of phosphate buffer and the absorbance was read at 405 nm and the changes in absorbance every 15 s (until 120 s) determined pNPA-esterase activity.

2.2.5 *Bioassay of mortality in different temperatures*

The same exposition method described above was utilized to evaluate the mortality in different temperatures. Here, was used LC50 (by lower confidence interval) of more promisor molecule (i.e., imidacloprid) and distilled and deionized water as control. The insects were confined 48 h in incubators (Eletrolab, model 202/4, SP, Brazil) at following temperatures: 20, 25, 30 and 35 °C, and the mortality was recorded. The mortality was corrected with the control by Henderson-Tilton's formula (Henderson and Tilton, 1955).

2.2.6 *Statistical analysis*

The data for estimate the curves of concentration-mortality were submitted to probit analysis using the PROC PROBIT procedure (SAS Institute, 2008). The ratio resistance (RR) was estimated by 95% confidence intervals (CI) as described by Robertson et al. (2007) considering significant if were greater than the value 1. The data of biochemical bioassays (i.e., detoxifying enzyme activity) were subjected to univariate analysis of variance (ANOVA), or a Kruskal–Wallis test by ranks when the assumptions of normality and homoscedasticity were not satisfied. The mortality data in different temperatures were corrected with natural mortality and compared by ANOVA. Regression analyses were performed to detect trends using the curve-fitting procedure of SigmaPlot 12.5. The regression model was chosen based on parsimony, lower standard errors, and steep increases in R^2 with model complexity.

2.3 Results

2.3.1 Concentration-mortality

The concentration-mortality results, estimated based on concentration-mortality bioassays, were satisfactory by probit model ($P > 0.05$) for both species and all insecticides. The lethal concentration (LC; in g a.i./cm2) of imidacloprid was showed in Fig 2.1A. The LC_{50} for *E. heros* was 0.61 (0.26-1.28) and for *D. melacanthus* was 9.28 (6.60-12.85); the RR_{50} was 15.32 (4.88-48.13) (Fig 2.1A). The LC of another neonicotinoid, thiamethoxam, was showed in Fig 2.1B. The LC_{50} this molecule for *E. heros* was 0.13 (0.10-0.16) and for *D. melacanthus* was 0.13 (0.10-0.18) (Fig 2.1B).

The lethal concentration of two pyrethroid molecules were showed in Fig 2.1C and Fig 2.1D. The LC_{50} for *E. heros* was 0.05 (0.03-0.06) and *D. melacanthus* was 0.11 (0.06-0.16) to lambda-cyhalothrin (Fig 2.1C) and for another molecule this group, betacifluthrin, the LC_{50} for *E. heros* was 0.38 (0.24-0.51) and *D. melacanthus* was 0.18 (0.14-0.24) (Fig 2.1D). In both cases, any species was more tolerant than the other despite *E. heros* showed a trend of higher tolerance than *D. melacanthus* to betacifluthrin.

Acephate was the unique molecule organophosphorus used in our concentration-mortality bioassay and showed LC_{50} for *E. heros* of 0.022 (0.014-0.034) and *D. melacanthus* of 0.018 (0.015-0.021).

2.3.2 Enzymatic activity

The specific activity of main detoxification enzymes was measured with basis in the biochemical bioassay (Fig 2.2). The estimated curves showed a similar pattern for the two species for both cytochrome P450 and pNPA-esterase (Fig 2.2, Table 2.1). However, for glutathione-S-transferase there was a change in the pattern of activities between *E. heros*, greater activity at lower temperatures, and *D. melacanthus*, greater activity at higher temperatures (Fig 2.2, Table 2.1).

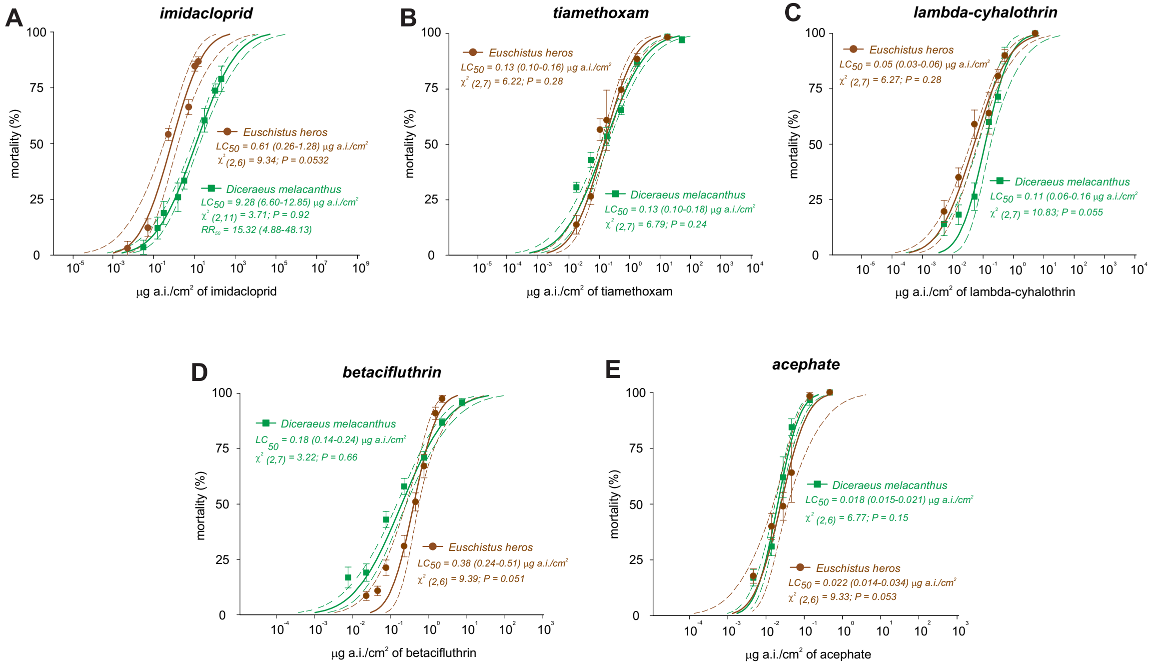

Figura 2.1: Toxicity of imidacloprid (A), tiamethoxam (B), lambda-cyhalotrin (C), betacifluthrin (D) and acephate (E) to stink bugs *Euschistus heros* and *Diceraeus melacanthus*. Lethal concentration (LC) values were estimated based on concentration-mortality bioassays using probit analyses. Dotted lines denote 95% confidence intervals.

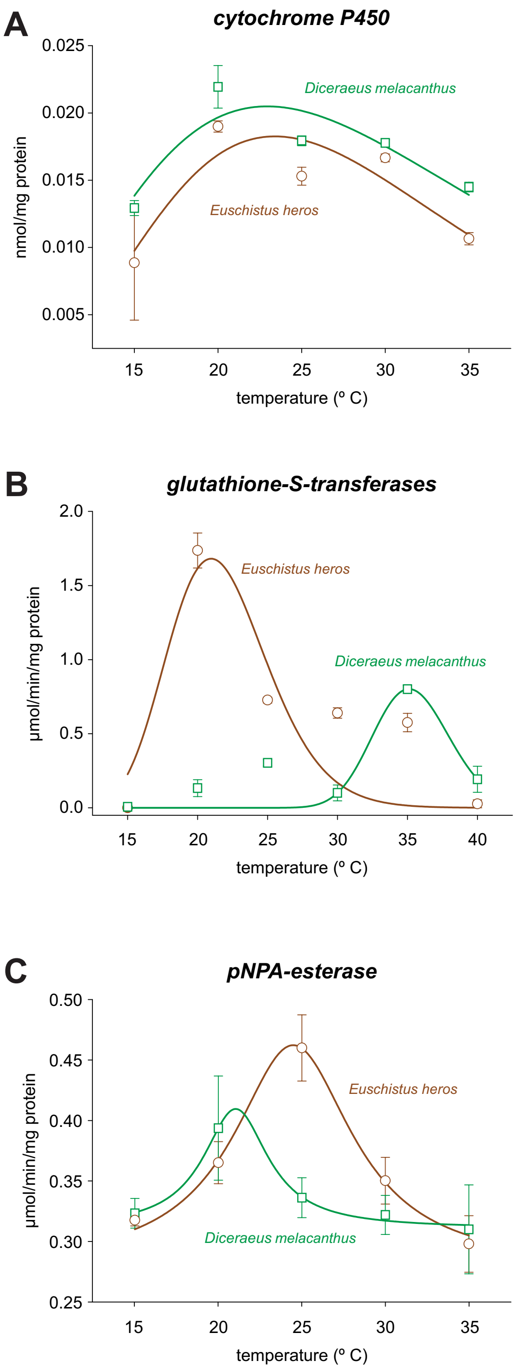

Figura 2.2: Activity of cytochrome P450 (A), glutathione-S-transferases (B) and pNPA-esterase (C) of *Euschistus heros* and *Diceraeus melacanthus* under different temperatures. The lines represent the non-linear regression fit of the enzyme activity results. Symbols represent the mean of three independent replicates (± SE). The parameters of the equation are shown in Table 2.1.

The activity of cytochrome P450 was higher both for *E. heros* ($F_{4,10}$ = 7.11; P = 0.013) and *D. melacanthus* ($F_{4,10}$ = 19.84; P < 0.0001) at 20 °C and lower at 15 and 35 °C (Fig 2.2A). The activity of glutathione-S-transferases was higher for *E. heros* at 20 °C and lower at 15 and 40 °C ($F_{5,12}$ = 119.26; P < 0.0001; Fig2.2B); for *D. melacanthus* was higher at 35 °C and in the other temperatures did not differ ($F_{5,12}$ = 32.34; P < 0.0001; Fig 2.2B). The better activity of pNPA-esterase was different for each specie (Fig 2.2C); for *E. heros* was 25 °C ($F_{4,10}$ = 5.43; P = 0.014) and for *D. melacanthus* was 20 °C ($F_{4,10}$ = 4.12; P = 0.036).

2.3.3 *Influence of temperature on mortality*

The temperature influences in mortality of *E. heros* and *D. melacanthus* to imidacloprid (Fig 2.3). Adults of *E. heros* exposed to LC_{50} of imidacloprid [0.61 (0.26-1.28)] had mortality reduced at 20 °C and increase at 35 °C (Fig 2.3, Table 2.2). The exposition in the same temperatures of *D. melacanthus* for its LC_{50} [9.28 (6.60-12.85)] exhibited the same results (i.e., reduced at 20 °C and increase at 35 °C) but an inverse behavior of the quadratic curve with mortality differing between the species in extreme temperatures tested (Fig 2.3, Table 2.2).

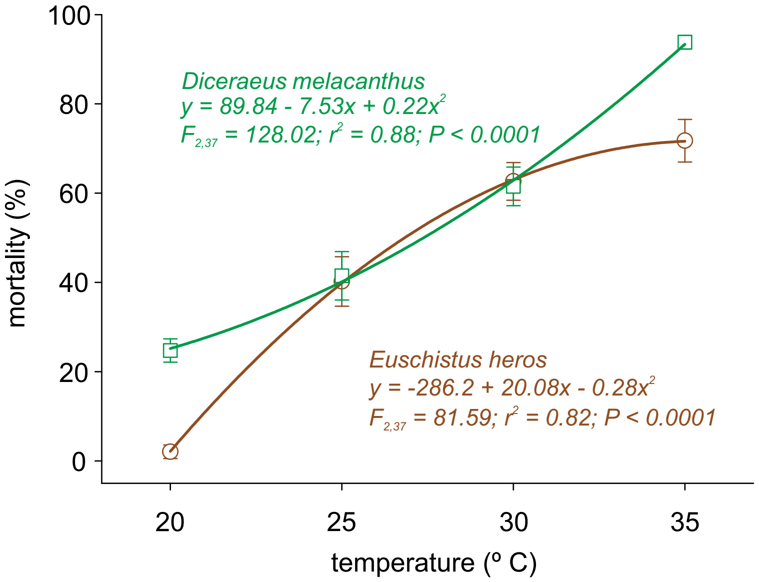

Figura 2.3: Toxicity of LC_{50} of imidacloprid to stink bugs *Euschistus heros* [LC_{50} = 0.61 (0.26-1.28)] and *Diceraeus melacanthus* [LC_{50} = 9.28 (6.60-12.85)] to four different temperatures (20, 25, 30 and 35 °C). The symbols represent the mean mortality of ten replicates and the vertical bars represent the mean standard error (± SE). Lines represent quadratic regressions.

Tabela 2.1: Summary of non-linear regression analyses of detoxification enzymes parameters (shown in Fig 2.2).

Variable	**Model**	**Species**	**Estimated parameters* (± SE)**				**df**	**F**	**P**	**R^2**
			$\boldsymbol{a}$	$\boldsymbol{b}$	$\boldsymbol{x_0}$	$\boldsymbol{y_0}$				
cytochrome P450	$y = (x \leq 0; 0; ae^{(\frac{-0.5(\frac{ln(\frac{x}{x0})}{b})^2}{x})}$	*Euschistus heros*	0.46 (0.25-0.67)	0.40 (0.04-0.75)	27.40 (17.04-37.75)	-	2	3.64	<0.05	0.78
		Diceraeus melacanthus	0.53 (0.04-0.70)	0.48 (0.06-0.90)	28.88 (15.51-42.26)	-	2	3.32	<0.05	0.77
glutathione-S-transferases	$y = (x \leq 0; 0; ae^{(\frac{-0.5(\frac{ln(\frac{x}{x0})}{b})^2}{x})}$	*Euschistus heros*	35.75 (0.84-70.66)a	0.17 (-0.04-0.37)a	21.56 (17.19-25.93)a	-	2	3.05	<0.05	0.67
		Diceraeus melacanthus	28.22 (6.34-50.10)a	0.08 (0.00-0.15)a	35.32 (30.86-39.78)b	-	2	4.03	<0.05	0.73
pNPA-esterase	$y = y_0 + \frac{a}{1+(\frac{x-x_0}{b})^2}$	*Euschistus heros*	0.18 (-0.05-0.42)	4.35 (-5.94-14.65)	24.48 (19.34-29.62)	0.28 (0.07-0.49)	3	45.94	<0.05	0.99
		Diceraeus melacanthus	0.10 (-0.32-0.52)	2.37 (-12.18-16.92)	21.04 (11.87-30.21)	0.31 (0.23-0.39)	3	40.59	<0.05	0.99

*Parameter values followed by different letters in the columns were significantly different (based on non-overlapping of confidence limits).

Tabela 2.2: Summary of quadratic regression analyses of mortality in different temperatures (showed in Fig 2.3).

Species	Model	Estimated parameters* (± SE)			df	F	P	R^2
		y_0	a	b				
Euschistus heros	$y = y_0 + ax + bx^2$	-286,2 (-348.6 – -223.8)a	20.1 (15.4 – 24.8)a	-0.28 (-0.36 – -0.20)a	37	81.59	<0.001	0.82
Diceraeus melacanthus		89.8 (41.2 – 138.4)b	-7.5 (-11.1 – -3.9)b	0.2 (0.13 – 0.27)b	37	128.02	<0.001	0.88

*Parameter values followed by different letters in the columns were significantly different (based on non-overlapping of confidence limits).

2.4 Discussion

The results showed the LC curves to five main insecticide molecules used in soybean crops for *Euschistus heros* and *Diceraeus melacanthus* and that *D. melacanthus* is more tolerant than *E. heros* to imidacloprid [RR = 15.32 (4.88-48.13)] – it's noteworthy that here we show for the first time toxicological studies (i.e., LC curves) with *D. melacanthus*. Moreover, our results indicate that low temperatures (e.g., 20 °C) reduced the mortality of both species potentially by increased in enzymatic activity of cytochrome P450 (CYP) and glutathione-S-transferases (GST).

Despite the large number of studies showing effect of several insecticides on *E. heros* (Haddi et al., 2015; Pitta et al., 2018; Santos et al., 2016a; Santos et al., 2016b; Tuelher et al., 2017), very few have shown these results for *D. melacanthus*, the second most important stink bug of soybean currently (Chocorosqui and Panizzi, 2008; Conte et al., 2018), and our result contributes to the rational use and rotation of effective insecticidal molecules for showing the effectiveness these compounds.

The other important result this work is the influence of temperature in activity of main enzymatic detoxification and, consequently, in mortality of stink bugs *E. heros* and *D. melacanthus* (Fig 2.2 and Fig 2.3). Enhanced detoxification activity was reported as the major mechanism involved in the imidacloprid resistance (Bass et al., 2015; Nauen and Denholm, 2005; Yang et al., 2013). Our data showed that *E. heros* seems to use the enzymes CYP and GST for detoxification of imidacloprid since this species shows higher enzymatic activity and lower mortality at 20 °C and lower enzymatic activities and higher mortalities at 30 and 35 °C (Fig 2.2 and Fig 2.3). Our results also indicate that CYP is involved in the process of imidacloprid detoxification in *D. melacanthus* since that, also at 20 °C, there was greater enzymatic expression and lower mortality; GST and total esterase appear not to be involved in this process in this species since their greatest activities were at 35 °C, temperature with the highest observed mortality (Fig 2.2 and Fig 2.3).

In phytophagous insects, specially generalist, CYP are a superfamily of enzymes that regulate numerous physiological processes including metabolism of plant secondary metabolites encountered in the diet, xenobiotic metabolism and insecticide resistance (Castellanos et al., 2019; Feyereisen, 2006; Scott and Wen, 2001; Yu et al., 2015; Zuo and Chen, 2014). For the neonicotinoid imidacloprid, in example, the resistance was associated more than 20 P450 genes from the families CYP4, CYP6 and CYP9, and its metabolism was confirmed in hemipteran insects as *Nilaparvata lugens* (CYP6AY1) and *Bemisia tabaci* (CYP6CM1vQ), and another order as *Drosophila melanogaster* (CYP6G1) (Bass et al., 2011; Bass et al., 2015; Clements et al., 2017; Clements et al., 2016; Daborn et al., 2001; Ding et al., 2013; Højland et al., 2014; Karunker et al., 2009; Zhang et al., 2016; Zhu and Luttrell, 2015).

The activity of esterase was high to both species and differ only at 25 °C in *D. melacanthus* comparing with *E. heros* (Fig 2.2). This detoxification enzyme is attributed how cause of resistance to imidacloprid in some insects (Romero and Anderson, 2016; Safi et al., 2017) but its major contribution in detoxification process is to confer resistance to carbamates, organophosphorus and pyrethroids in many insect species due its carboxylase activity (Achaleke et al., 2009; Bass et al., 2014; Lee, 1997; Li et al., 2007; Lilly et al., 2016; Liu et al., 2006; Montella et al., 2012). GST also is responsible for developing resistance in some insects directly (phase I) or by the metabolism of secondary products generated from other detoxification enzymes, such as P450s (phase II) (Pavlidi et al., 2018; Romero and Anderson, 2016). For *E. heros* and hemipterans like *Lygus lineolaris* this enzyme has no role in imidacloprid detoxification even in selected resistant populations, but our results show that this enzyme contributes to the action of CYP and in none of these previous studies was a temperature gradient correlating mortality as our findings show (Castellanos et al., 2019; Zhu and Luttrell, 2014).

Changes in temperature was described causing a reduction in mortality in previous studies (Foster et al., 1997; Guo et al., 2018; Tiwari et al., 2015; Whiten and Peterson, 2015; Zhang et al., 2015). Both *E. heros* and *D. melacanthus* were more tolerant to imidacloprid at 20 °C suggesting a possible lower efficacy of this mode of action during winter temperatures, how related to other insects (Foster et al., 1997; Foster et al., 1996; Tiwari et al., 2015). It is known that temperature affects catalytic reactions of the enzymes and the binding of a substrate to the enzyme (Hochachka and Somero, 1984; Hoffmann, 1984) and here in this study we found that the highest activity and possibly better binding with the substrate happens at 20 °C (Fig 2.2), collaborating with the greater susceptibility of both species to imidacloprid (Fig 2.3). In addition, other mechanisms such as altered membrane permeability, reduced penetration and/or transport to the target site modulated by temperature (Bielza, 2008; Braeckman et al., 1998; Gilby, 1980; Narahashi, 1976; Szeicz et al., 1973) can be contributing to this lower toxicity.

Thus, this work report by first time a toxicological study of three insecticidal groups in *D. melacanthus* and provides evidence that changes in temperature can increase the detoxification process in *D. melacanthus* and *E. heros*, showing that cytochrome P450 and glutathione-S-transferase are involved. However, more investigations are needed to explore the toxicological impacts of insecticides in *D. melacanthus* (e.g., sublethal effects), as well as to elucidate which resistance and/or tolerance mechanism are involved and what the temperature cause in this process.

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CHAPTER 3. CLIMATE SUITABILITY OF NEOTROPICAL STINK BUGS ALERTS CORN AND SOYBEAN-PRODUCING AREAS IN BRAZIL

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Abstract

Brazil is an important soybean and corn producer and stink bugs are important reduction factor in production. Two species this complex, *Diceraeus melacanthus* and *Euschistus heros*, currently are more worrisome due to their aggressiveness and comprehensiveness. These stink bugs are widely distributed in soybean and corn production fields in Brazil and it is expected that the climate, as well as host plants, may interfere with their distribution and to reduce productivity and increase production costs by use of insecticides. Thus, adoption of tools like suitability maps they can assist decision making, public polices and contribute to increasing the productivity and sustainability of Brazilian grain production. Thereby, the objective of this work is to indicate areas of soybean and corn production safe and with high risk of attack by these insects through Ecological Niche Modeling (ENM). The produced models have a great predictability. The average AUC value to *D. melacanthus* and *E. heros* were 0.94 ± 0.04 and 0.99 ± 0.01, respectively. The average TSS value were 0.82 ± 0.14 to *D. melacanthus* and 0.95 ± 0.03 to *E. heros*. These models indicate that production fields in the south and middle west of Brazil were highly suitable to *D. melacanthus*. Our forecast also indicates that *E. heros* is highly dispersed throughout all Brazilian soybean and corn fields and has a high risk of attack in these fields, which can substantially reduce productivity. Still shown that *E. heros* can be a limiting factor of production also in cultivation areas that may appear in the country, in the north and northeast regions. Our findings indicate, by species distribution models, the regions most impacted by each pest at the municipality level, generating a tool to compose integrated pest management programs.

Keywords: Ecological niche modelling, insect distribution, ENMTML package, *Euschistus heros*, *Dichelops (Diceraeus) melacanthus*, soybean stink bug, green belly stink bug.

3.1 Introduction

The production of food for the almost 7 billion people on Earth pressures an increase in agriculture production. The International Food Policy Research Institute has drawn attention to the world situation, with 800 million people chronically malnourished (IFPRI, 2020). Grains and cereals are the most important source of calories for most of the world's population. Nearly 60% of calories in developing countries are derived directly from these foods, with values above 80% in the poorest countries (WHO, 2003). The soybean crop from Brazil figures as one important commodities in the international market with an export volume of 84 million tons in the last year (ANEC, 2019; Oliveira and Schneider, 2016). Nevertheless, crop pests like stink bug cause significant production loss both directly (e.g., consuming leaf and pods) and indirectly (e.g., by increased use of agrochemicals) (Corso and Gazzoni, 1998; Ghimire and Woodward, 2013; Guedes et al., 2016; Panizzi et al., 2012; Sosa-Gómez et al., 2014). For instance, the costs by a hectare of soybean and corn pests are near to R$ 400,00 and R$ 250,00 in Brazil, respectively, a factor that increases proportionally with the revenue of each crop (Artuzo et al., 2017). These numbers highlight the importance of pest control in the scenario of food production.

Stink bug attacks are reported in all regions in Brazil where soybean and corn are cropped, but the distribution of this species is mainly determined by ecophysiological traits according to environmental conditions (Panizzi, 2013; Soares et al., 2018; Tuelher et al., 2018). Our current theoretical understanding of species distribution supports a conceptual model – known as the BAM diagram (Soberón, 2007; Soberon and Peterson, 2005) – based on the effect of abiotic conditions, biotic interactions, and spatial accessibility. For a pest species, this may be translated into the importance of environmental conditions, host plant traits, potential natural enemies, and dispersal constraints to shape the present and future distribution of the species (Steinhaus, 1960; Tremmel and Müller, 2012; Pélissié et al., 2018; Stout, 2014; Agosta, 2006; Mazzi and Dorn, 2012; Bebber et al., 2014). A particular aspect of this model is that the accessible area for a pest species unites the dispersion capacity of the pest and intense commercial exchanges provided by human distribution in the modern world (Soberón, 2007; Soberon and Peterson, 2005). Thus, pest species may be viewed as potentially invasive species (Worner and Gevrey, 2006) with very low constraints imposed by spatial accessibility. This may represent that the prediction of its distribution – and especially its changes related to global climate changes or unintended transportation – may be simpler and related to fewer parameters.

Recent advances based on species distribution theory create a huge set of modelling procedures collectively identified by Ecological Niche Models' broad umbrella (ENM). ENM are efficient procedures to approach the geographic distribution of species (Soberón, 2007; Soberón and Nakamura, 2009; Soberón, 2010). Shortly, ENM process consists of obtaining geographic points of occurrence of the species under study and relevant georeferenced abiotic data (e.g., temperature and precipitation) and submitting them to algorithms capable of finding areas environmentally similar to those where the species currently occurs (Andrade et al., 2020; Peterson et al., 2011; Venette et al., 2010). It was proved to be an essential tool for studies on biological conservation (Diniz et al., 2020, Velazco et al., 2019), disease spread (Kim et al., 2019, Huang et al., 2019, Ivorra et al., 2020), ecological restoration, only to show few examples. Nevertheless, it has not been used so intensively to model pest species and help understand their possible effects. In the context of pest management and control science, ENM may provide an efficient tool to determine which areas have higher suitability for a particular pest within current know occurrence areas, to identify possible areas that a pest could efficiently colonize outsize its current distribution, or evaluate possible distributional shifts related to climate change scenarios. All those applications may help identify potential threats to agriculture production and the development of efficient action plans. Nevertheless, a search at the ISI web of science platform found only 60 papers that deal with ENM for pest species in agriculture environments (search at 05/08/2020). Thus, there is a large space for using those techniques to be explored.

Soybean cultivation in Brazil has undergone a geographical expansion towards middle-west, from the Cerrado biome's consolidated areas but moving to new areas at the Amazonia. There is also the increase use of new cultivars, new forms of planting (i.e., no-tillage cultivation system), temporal shortening of the cultivation cycle and intensification practices. As the host plant is a major influence on the distribution of a species, the distribution of stink bugs has certainly been modified according to those changes in agricultural practices (Bertrand and Théry, 2006; Dall'Agnol, 2016; Galford et al., 2008; Galvão et al., 2015). Stink bugs have reached enormous importance and control measures are needed to reduce impacts on loss of productivity and generate tools for IPM (Integrated Pest Management) strategies since traditional management involves consuming a large number of insecticides – primary control method for these pest insects (Panizzi, 2013) – going against the goals of the United Nations (UN) for 2030 (UN, 2019).

Efforts to suppress the spread of stink bugs in Cerrado are needed, and the first step for this effort is to gather information about the new potential areas where the pest can disperse considering its climatic suitability. This information is especially important since it allows governmental pest management agencies to implement programs to shape public incentive in the agricultural sector related to soy and even assist in the decision-making of bank financing and agricultural insurance. Analytical tools and products that help decision-making to guide public policies, such as ENM modeling, are essential to direct incentives and integrate this entire production chain. Mapping areas where the suitability of mainly stink bug pests will be high, in this scenario, can bring an inexorable contribution to direct government efforts, especially at this time when soybean crops expand to areas not yet cultivated. Thus, this study aims to evaluate the possible present effects of the two most important stink bug pests, *Euschistus heros* and *Diceraeus melacanthus*, in new recent established frontier areas for soybean plantations in Brazil. These species were choose because they are the major insect pest of soybean in Brazilian's fields.

3.2 Materials and Methods

3.2.1 The modeled species

We modeled *E. heros* (neotropical brown stink bug) and *D. melacanthus* (green-belly stink bug), which are both native Pentatomidae species of the Neotropics. *E. heros* was the secondary pest in soybean in the past when the attention was in *N. viridula*; however, it is currently the main pest in soybean fields due to the expansion and management modifications in crop fields (Panizzi, 2013; Panizzi and Lucini, 2016; Tuelher et al., 2016). *D. melacanthus* is a pest known in the corn crops but, with the increase adoption of the soybean-corn cultivation system, it is also reported as a serious problem in soybean fields (Chocorosqui and Panizzi, 2008; Conte et al., 2018; Crosariol Netto et al., 2015; Panizzi et al., 2015). Thus, these species account for a representative loss of soybean production and represent a great risk to cultivation.

3.2.2 Occurrence records and data cleaning

The dataset used in this work was a compiled of 222 occurrence records for *E. heros* and 35 occurrence records for *D. melacanthus* in Neotropic region from data sources that included biological database [GBIF – Global Biological Information Facility (www.gbif.org)] and the search for scientific information on Web of Science, Science Direct, Google Scholar and PubMed (Table S1, Table S2, Fig 3.1). The keywords used during the search were "*Euschistus heros*", "Neotropical brown stink bug", "occurrence *Euschistus heros*", "*Dichelops melacanthus*", "green belly stink bug", "occurrence *Dichelops melacanthus*", and its translation to Portuguese. In the Supplementary Material, there is a list of all occurrences used and its source.

Bias in the occurrence records are detected and are common, but to correct this bias in the geographical space is necessary and there are some approaches to this (Fourcade et al., 2014; Varela et al., 2014). For this, we used the ENM procedures that had a function that filtered the occurrences of species by randomly sampling one occurrence in each cell of the grid.

3.2.3 *Environmental data*

The current climatic conditions data for our analysis were the 19 bioclimatic variables in WorldClim 2.0 with 5 arc-min (c. 10 km) (Table 3.1) (Fick and Hijmans, 2017). Climatic conditions are

Figura 3.1: Points of occurrence of *Euschistus heros* and *Diceraeus melacanthus* collected and used to perform the ENM and to produce the suitability maps.

expected to be highly important affecting insect species and may determine their distribution and abundance. Nevertheless, this climate information is assumed to have a high level of collinearity among the variables, which are expected to affect the modeling procedures (Graham, 2003, Dorman et al 2013). Following De Marco & Nobrega (2018), we use a principal component analysis of the original variables to derive new uncorrelated variables to the model. Thus, we use factor scores of the first six components representing 96.1% or the climatic information of the original matrix (Supplementary material 1).

3.2.4 *Species distribution modeling techniques*

With the advancement of distribution modeling techniques, several methods with different objectives have been proposed to construct ENM obtaining different performances (Zhu and Peterson, 2017). Thus, models that build results based on different algorithms are recommended to have a result that makes the best of each mathematical model (Araújo and New, 2007). We used seven different modeling techniques to generate our species distribution models: Maxent Simple, Maximum Likelihood, Support Vector Machine, Random Forest, Generalizes Additive Model, Generalized Linear Model, Gaussian Process.

The extent to fit ENM has important effects on model results. In special, the accessible area defined as a cell within the extend reachable by species propagules may be important to avoid significant overprediction errors. Otherwise, we assume that these pest species may reach most of suitable areas both by its intrinsic dispersal capabilities as by human-facilitated dispersal caused by soybean plantations. Under this premise, we use the entire neotropical region as accessible are for this.

As the focus of this work is on predicting areas at risk of attack by stink bugs in soybean crops in Brazil, we defined the Brazilian territorial limit assuming that these areas were accessible to species since to adjust the ENM forecasts, the area used it must cover the regions accessible to the species for a period of time as shown in component M of the BAM diagram (Soberon and Peterson, 2005).

Tabela 3.1: Bioclimatic variables derived from the monthly temperature and rainfall values from WordClim (http://worldclim.org/bioclim).

Bioclimatic variables	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**	**Axis 5**	**Axis 6**
Annual Mean Temperature	0.2691	0.2557	-0.0965	0.0645	0.0728	-0.0172
Mean Diurnal Range (Mean of monthly (max temp - min temp))	-0.2040	0.2065	-0.0671	-0.4946	-0.0865	-0.4810
Isothermality (BIO2/BIO7) (*100)	0.2421	-0.0034	0.3326	-0.0539	0.0635	-0.5174
Temperature Seasonality (standard deviation *100)	-0.2454	0.0327	-0.3949	-0.0218	-0.1650	0.1107
Max Temperature of Warmest Month	0.1360	0.3698	-0.3448	-0.0267	-0.0996	-0.0430
Min Temperature of Coldest Month	0.3007	0.1203	0.0642	0.1816	0.0787	0.0061
Temperature Annual Range (BIO5-BIO6)	-0.2544	0.1191	-0.3162	-0.2298	-0.1613	-0.0372
Mean Temperature of Wettest Quarter	0.2057	0.3057	-0.1845	-0.0646	0.1945	-0.0429
Mean Temperature of Driest Quarter	0.2689	0.1781	-0.0175	0.1637	-0.1018	-0.0535
Mean Temperature of Warmest Quarter	0.1887	0.3176	-0.3277	0.0791	-0.0244	0.0348
Mean Temperature of Coldest Quarter	0.2923	0.1830	0.0644	0.0641	0.1039	-0.0543
Annual Precipitation	0.2754	-0.1998	-0.0787	-0.2092	-0.1376	0.1047
Precipitation of Wettest Month	0.2790	-0.0765	0.0625	-0.3085	-0.2443	0.2901
Precipitation of Driest Month	0.1515	-0.3554	-0.2861	-0.0005	0.1062	-0.3546
Precipitation Seasonality (Coefficient of Variation)	-0.0392	0.2935	0.3837	-0.3858	-0.1191	-0.0967
Precipitation of Wettest Quarter	0.2801	-0.0858	0.0587	-0.3059	-0.2399	0.2755
Precipitation of Driest Quarter	0.1622	-0.3531	-0.2846	-0.0052	0.0792	-0.3191
Precipitation of Warmest Quarter	0.1738	-0.1846	-0.1516	-0.4834	0.5281	0.2034
Precipitation of Coldest Quarter	0.2113	-0.2041	-0.0645	0.0704	-0.6383	-0.1641
Accumulated percentage explained by each axis	51.8549	72.1720	84.1314	89.6846	93.6490	96.1324

3.2.5 Model evaluation and ensemble forecast

Two metrics will be used to evaluate the models. The AUC (Area Under Curve) will be used as a metric independent of the cut-off threshold, normally used for presence and absence data, it is recommended for the evaluation of environmental niche models (Fielding and Bell, 1997). Its values vary between 0 and 1, when used with presence-only data, the maximum values should theoretically be minus 1. TSS (True Skill Statistic) will also be used and works as an assessment measure dependent on the cut-off threshold. The TSS represents the average success rate of the forecast with values ranging between -1 and 1 (Liu et al., 2011). As our approach is based only on presence data, TSS, Jaccard and AUC will be calculated using background data such as pseudo-absence, which is a common procedure in the literature.

Data processing, construction of the ENMs and analyses were conducted in the R environment 3.6.2 with the ENMTML package's aid, a package in the R software for integrated construction of ecological niche models (Andrade et al., 2020) . The maps of climatic suitability were generated by the ENMTML package of R software and later processed and analyzed spatially in the software QGIS 3.10 (QGIS Development Team, 2009).

3.2.6 Analytical procedures

Through production data and planted area for each municipality made available on the IBGE data platform (https://www.ibge.gov.br/), we generated a current map of soybean and corn productivity for Brazil to be used in some correlations of this study.

We obtained climatic suitability for the two pest species studied in all Brazilian municipalities. For this, we carried out the ENM with a 10x10 km grid and, subsequently, we performed the interpolation of the suitability data with the municipality area to find out the average suitability of the stink bugs in each Brazilian municipality.

Finally, we cross these two datasets (e.g., distribution of each species with current soybean productivity) where we infer safe areas for soybean cultivation and areas with high suitability of stink bugs and possible risk for planting this cultivation.

The analytical procedures were performed in R software 3.6.2 using the packages "raster", "SDMTools", "rgdal", "classInt", "dismo", "XML", "maps" and "sp", and processed with QGIS 3.10.

3.3 Results

The average productivity of soybean in Brazil was 3.25 ton/ha, and the highest productivity achieved was 5.40 ton/ha, and the average productivity of corn was 3.01 ton/ha, according to IBGE data (IBGE, 2019). The states that concentrated the largest grain production are located in the middle-west and south of Brazil (IBGE, 2019).

The results of the models produced by the average of the best-evaluated algorithms have a high degree of predictability (AUC > 0.85; Table 3.2). The models to *D. melacanthus* achieved a mean AUC value of 0.94 ± 0.04 (mean ± standard deviation) and the mean TSS value of 0.82 ± 0.14 while the models to *E. heros* achieved a mean AUC value of 0.99 ± 0.01 and mean TSS value of 0.95 ± 0.03 (Table 3.2). In the current scenario, *D. melacanthus* has greater suitability in the south-central region of Brazil, being limited to a few other Brazilian soybean-producing municipalities (Fig 3.2C). However, *E. heros* has high climatic suitability not only in central region of Brazil, but also in several other soybean producing areas. In addition, its suitability is high in potential fields of expansion of soybean cultivation (Fig 3.2A). Based on these results, it is possible to affirm that soybean-producing areas located in the center-south of Brazil are areas with a higher risk of attack by these stink bugs species and, thus, more subject to productivity losses.

The Figure 3.3 and 3.4 shows this risk perspective more clearly by grouping different climate suitability categories and corn and soybean production (e.g., a municipality with high soybean productivity and high climate suitability). In this perspective, the suitability of *D. melacanthus* is greater than for producing areas in the central region of Brazil towards the south, with these areas being identified as risk areas for this species. However the central region towards the north show good productive capacity and reflect lower climatic suitability for this species, being treated as safe conditions. When it comes to *E. heros*, the situation is more worrying. According to our models, most of the soybean production fields have high climatic suitability for this species, and these producing fields are classified as risk areas. Few production areas indicate safe locations, with low climatic suitability.

Figure 3.3A and 3.3C shows the potential impact of *D. melacanthus* on corn and soybean crops, respectively. It is important to note that there is a great geographical overlap of the impact on the two crops, however, the potential impact on the corn crop may be much greater for this species since we can lessen that there is occurrence in the most productive areas with

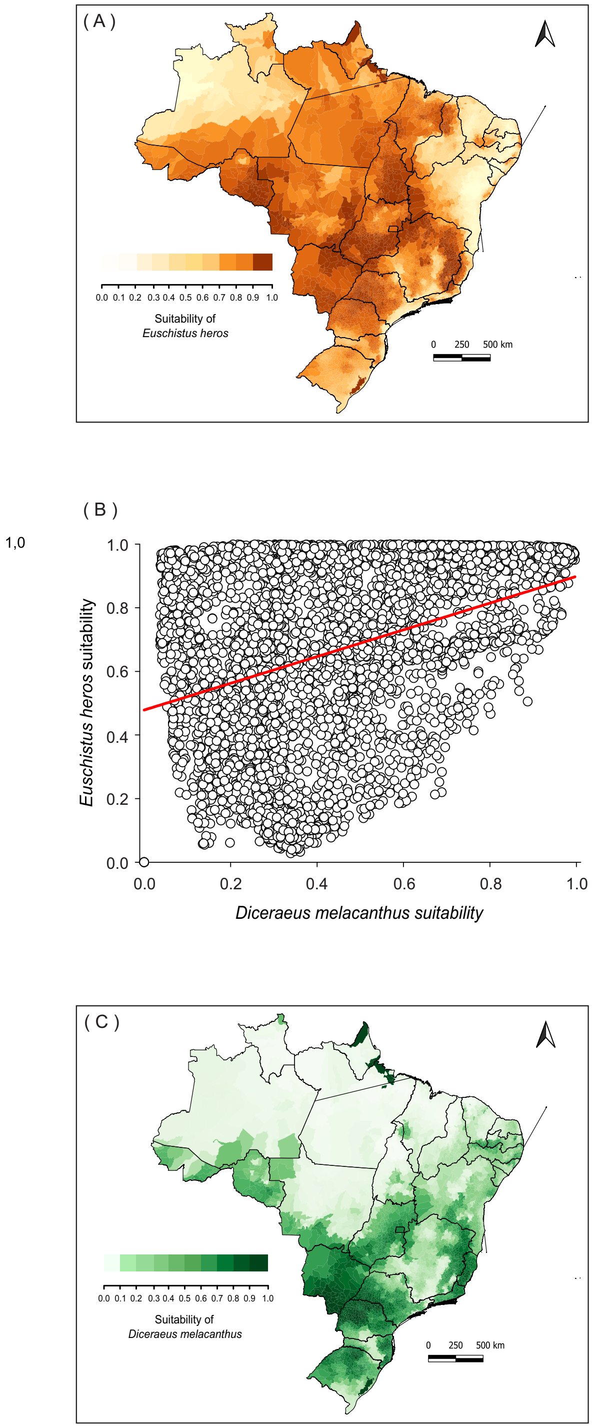


Figura 3.2: Average climatic suitability of *Euschistus heros* (A) and *Diceraeus melacanthus* (C) for each Brazilian municipality under current climatic scenario and the correlation between the climatic suitability of both species (B).

Tabela 3.2: Evaluation of models with used algorithms for *Euschistus heros* (148 unique occurrences) and *Diceraeus melacanthus* (27 unique occurrences).

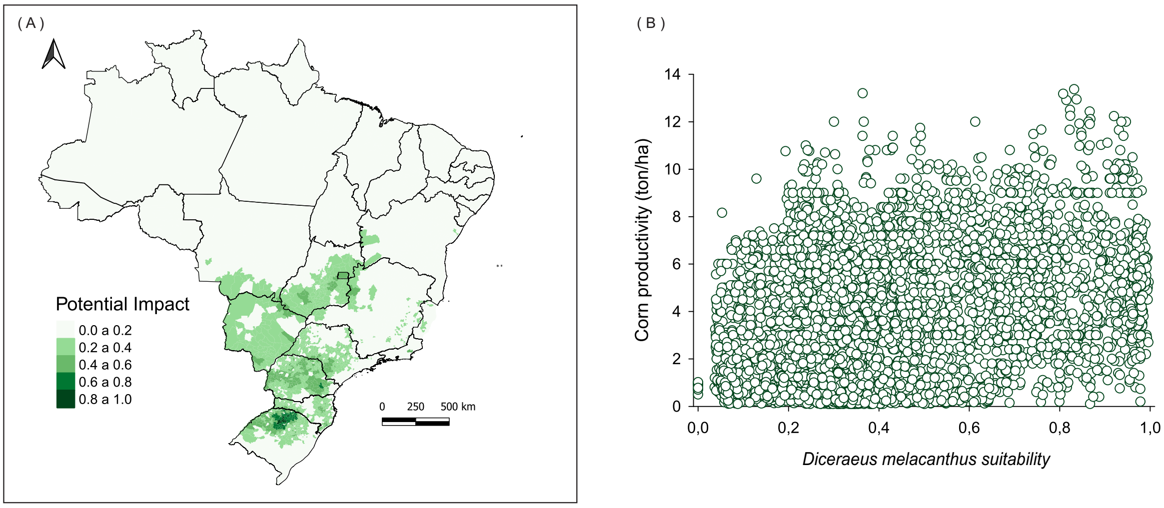
Species	**Algorithm**[1]	**AUC ± SD**[2]	**TSS ± SD**[3]	**Jaccard ± SD**[4]	**Sorensen ± SD**[5]	**Fpb ± SD**[6]
Dichelops melacanthus	MXS	0.93±0.05	0.82±0.14	0.82±0.14	0.71±0.00	0.92±0.00
	MLK	0.89±0.08	0.20±0.19	0.20±0.19	0.07±0.00	0.33±0.00
	SVM	0.90±0.06	0.66±0.13	0.66±0.13	0.57±0.00	0.75±0.00
	RDF	0.87±0.11	0.74±0.24	0.74±0.24	0.57±0.00	0.92±0.00
	GAM	0.92±0.09	0.77±0.08	0.77±0.08	0.71±0.00	0.83±0.00
	GLM	0.73±0.21	0.48±0.27	0.48±0.27	0.29±0.00	0.67±0.00
	GAU	0.89±0.13	0.74±0.24	0.74±0.24	0.57±0.00	0.92±0.00
	SUP	0.94±0.04	0.82±0.14	0.82±0.14	0.71±0.00	0.92±0.00
Euschistus heros	MXS	0.97±0.04	0.88±0.13	0.88±0.13	0.97±0.00	0.78±0.00
	MLK	0.93±0.01	0.39±0.56	0.39±0.56	0.78±0.00	0.00±0.00
	SVM	0.99±0.00	0.95±0.01	0.95±0.01	0.95±0.00	0.94±0.00
	RDF	0.99±0.0	0.95±0.04	0.95±0.04	0.98±0.00	0.92±0.00
	GAM	0.91±0.13	0.82±0.23	0.82±0.23	0.98±0.00	0.66±0.00
	GLM	0.87±0.16	0.76±0.27	0.76±0.27	0.95±0.00	0.57±0.00
	GAU	0.98±0.02	0.88±0.06	0.88±0.06	0.92±0.00	0.83±0.00
	SUP	0.99±0.01	0.95±0.03	0.95±0.03	0.97±0.00	0.92±0.00

[1] Algorithms used in processing of models. MXS – Maxent Simple, MLK – Maximum Likelihood, SVM – Support Vector Machine, RDF – Random Forest, GAM – Generalizes Additive Model, GLM – Generalized Linear Model and GAU – Gaussian Process. SUP is the ensemble of average of the best models. [2] Area under curve ± standard deviation. [3] True Skill Statistic ± standard deviation. [4] Jaccard index ± standard deviation. [5] Sorensen index ± standard deviation. [6] Fpb statistic ± standard deviation.

great frequency. There is no correlation that very productive areas are highly suitable (Fig 3.3B and 3.3D), but the maps of potential impact show that there are a large number of municipalities that have high grain production and that have a high suitability of this pest, which can, ultimately, reducing the productivity of the production fields.

In Figure 3.4B we can see a strong correlation in the potential impact of *E. heros* in the soybean culture since the highest concentration of municipalities with high productivity have a high suitability for this species.

Potential impact of Diceraeus melacanthus in corn production



Potential impact of Diceraeus melacanthus in soybean production

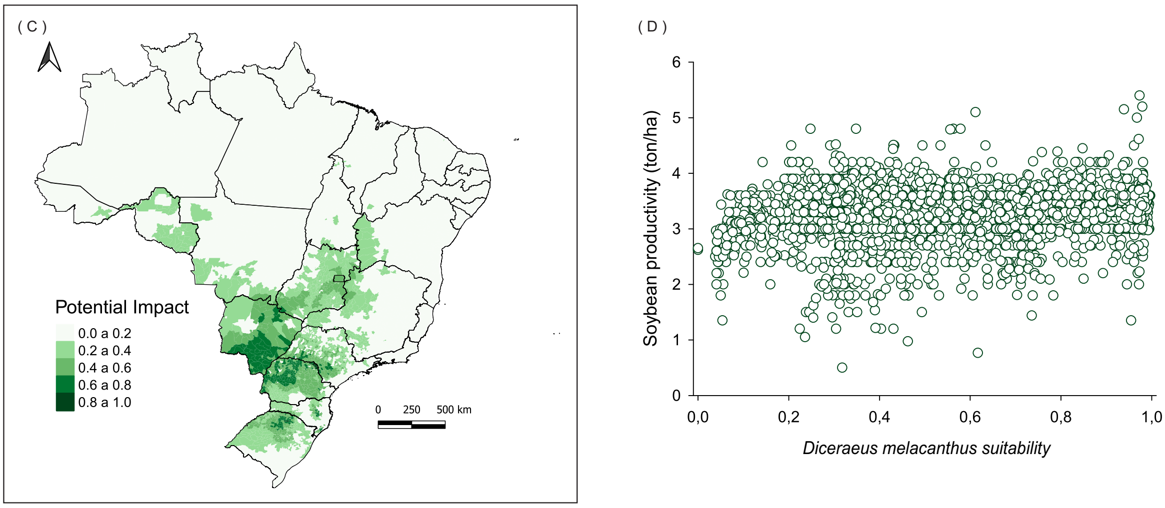
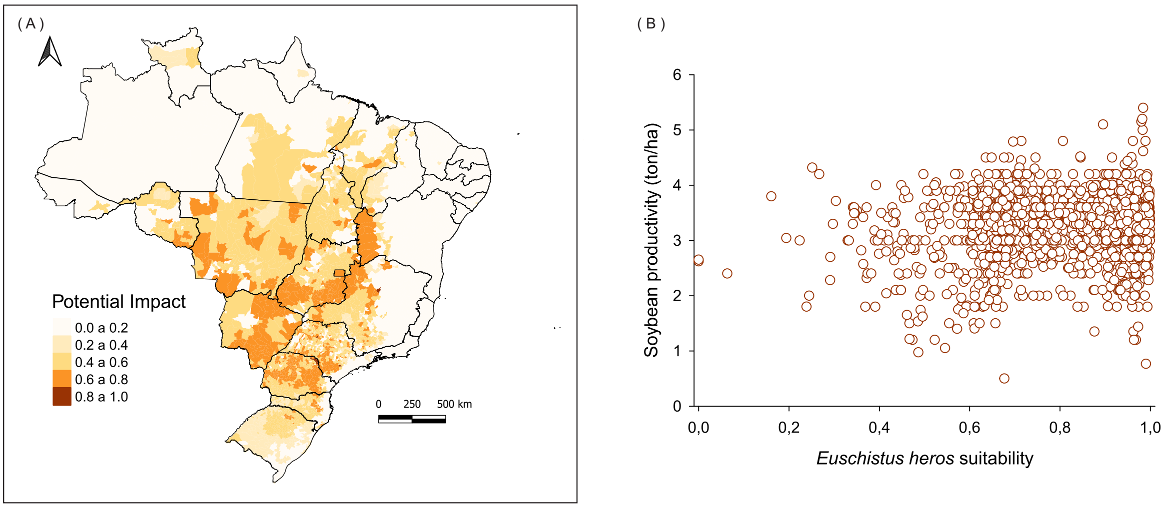


Figura 3.3: Interaction between suitability of *Diceraeus melacanthus* with soybean and corn productivity, shown areas at risk of attack of each species (high climatic suitability and high soybean or corn productivity) and safe areas to soybean production (low climatic suitability and high soybean or corn productivity).

Potential impact of Euschistus heros in soybean production



Potential impact of Euschistus heros in corn production

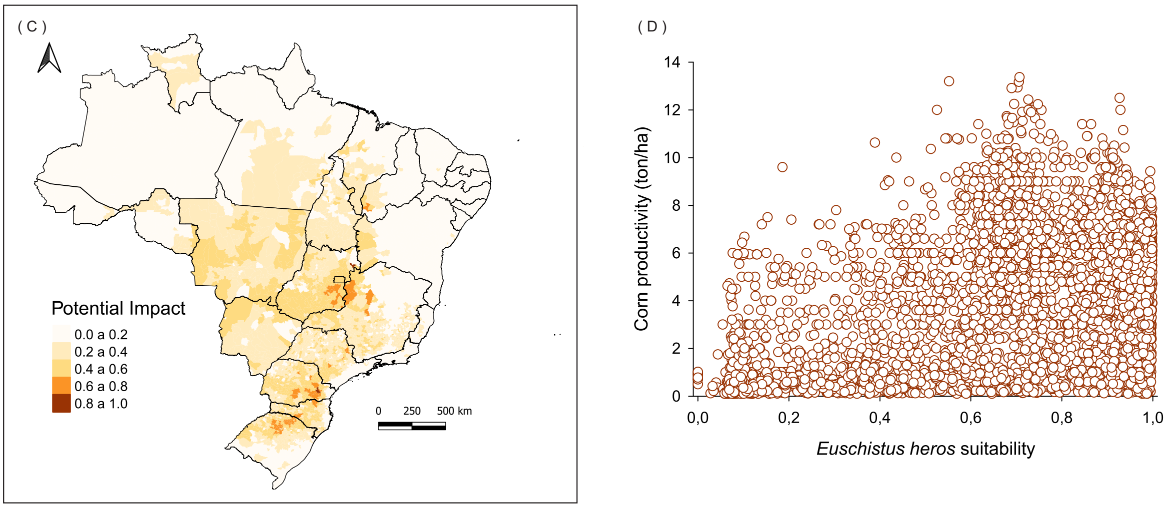


Figura 3.4: Interaction between suitability of *Euschistus heros* with soybean and corn productivity, shown areas at risk of attack of each species (high climatic suitability and high soybean or corn productivity) and safe areas to soybean production (low climatic suitability and high soybean or corn productivity).

3.4 Discussion

In general, there is no strong association between sites with high suitability for *E. heros* and *D. melacanthus*. Both species had large climatically suitable areas, suggesting that they could even expand from its current distribution. Integrating pest climate suitability and current procution we were able to crease a risky map for impact in production that we hope be useful to provide best planning for management practices. These results creates concern mainly for the grain production chain in two harvests (places that grow soybeans in the first harvest and corn in the second harvest), since suitability values for both species greater than 0.8 - values considered as high suitability - cover a large number of municipalities. We also show that suitability maps have high overlapping areas occur with current crops, and where consequently more grains are produced, such as MT, GO, PR and MS.

Modelling pest species is still an ongoing research agenda. There are some very important contribution in applying the species distribution modelling approach to those species (Aragon et al., 2010; Dupin et al., 2011; Wang et al., 2021), but there is still many interestinc conceptual and practical challenges to be faced. Theoretically, we expect that pest species will behave closest to invasive species and may represent pose similar challenges to modelling (Andrade et al., 2020). We expect that both invasive and pest species are selected to have broad environmental niches and conform as r-strategists in thei demographic and niche properties. Our result support this general expectation with broad environmental niches for those species. A second important parallel is the high expected dispersal capacity that is expected for both pest and invasive species (Nylin, 2001). This also means that we expect that pest species easily reach those accessible areas even if they are distant from current occupied areas in time. Thus, pest expansion to novel suitable areas, including those created due to climate change, should be considered only a matter of time, both due to its own ecological capabilities as due to commercial and antropic-driven dispersal.

Our contribution is mostly related to the relvance of SDM approach for practical aspects of pest control. An interesting observation from this perspective refers to areas in agricultural expansion in Brazil, which are related to areas of high suitability for these pests under study. In an area known for its agricultural expansion each year popularly called MATOPIBA (formed by the states of Maranhão, Tocantins, Piauí and Bahia) in which the crops are successively soybean and corn, there is high climatic suitability for *E. heros* and, in a few points, for *D. melacanthus*. In addition, expanding areas in other regions such as the states of Rondônia and Acre, and others that have practiced agriculture for decades, such as Minas Gerais, but where soybean is not very present in crop succession, are areas that generate some concern for have high suitability for the development of the two species in this study. In the state of Minas Gerais, there is a concern for direct damage, such as the spread and population increase of the pest in the most preferred crop such as soybean and corn, and indirect damage, by migration to other crops, since the pest is highly polyphagous and the region it has different crops at different times of the year. Furthermore, the region is a large producer of corn for animal feed throughout the year and, as it is highly suitable for *D. melacanthus*, it could also cause problems for this production chain.

The planting of soybean and corn in succession began around the 1990s with the release of soybean varieties with increasingly shorter cycles to allow this strategy (Duarte et al., 2007). From this moment on, the second corn crop has taken on great importance and is the most planted corn crop since 2006/2007, mainly in the Brazilian Midwest (CONAB, 2020). From a productive perspective, this gain in one crop in the year allowed for an increase in grain production and greater land use, which is a very positive factor for Brazilian agriculture in general. However, when thinking about pest management, this factor was essential for the population increase of several species because there is an abundance of food, shelter and green bridge in this cultivation system. Added to this, other factors such as biotechnologies developed for corn and soybean crops that had caterpillars as their main target allowed the outbreak of stink bugs, which were secondary pests in the past and are now the main pests in both crops. Many of these areas of successive cultivation report the attack of stink bugs annually. This is largely due to abiotic factors, such as abundant food and green bridge, not being development-limiting factors. Our suitability maps also suggest that there may be concerns for these areas of successive cultivation also due to biotic factors, since we rely on them to estimate our projections, which raises a great concern about the continued attack of these studied species if food sources are maintained.

All these factors imply economic losses for the grain production chain. Our correlations and projections indicating the potential impact of pests on each crop bring these perspectives of economic losses as we relate productivity and suitability. In our maps, we see that *D. melacanthus* has a greater impact potential in south-central Brazil in the two analyzed crops and *E. heros* has a potential impact in a large part of the cultivated areas throughout the Brazilian territory, with a greater impact on the soybean crop. These indications bring to government agencies, technicians and all those involved in grain production the main places to intensify integrated actions for the management of these species, with the intention of increasingly reducing productivity losses. In this way, we can understand that species distribution modeling tools, such as the ones we use, can help several areas of study, such as understanding the patterns of loss in agriculture by pest insects and providing bases for actions to mitigate these losses. In addition to giving us guidance in the current climate, these tools can guide us in the face of climate change so that we can anticipate actions aimed at managing pests.

Our findings indicate that *E. heros* has high climatic suitability in all grain producing sites and

D. melacanthus has high climatic suitability concentrated in south-central Brazil. In addition, we show through species distribution models the regions most impacted by each pest at the municipality level (i.e., high climate suitability and high productivity), generating yet another tool to compose integrated pest management programs to be used mainly by government institutes.

3.5 Acknowledgments

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3.7 Supplementary Material

Tabela 3.3: Geographical coordinates used from the occurrence points of *Euschistus heros* and your source.

Year of occurrence	Longitude (W)	Latitude (S)	References
1993	-47.5011	-15.5994	(Medeiros et al., 1998)
1993	-51.1793	-23.1884	(Panizzi and Niva, 1994)
1998	-47.9167	-15.7833	(Costa et al., 1998)
1999	-54.7814	-22.0761	(Degrande et al., 2000)
1999	-67.721	-10.1499	(Thomazini and Thomazini, 2001)
1999	-67.7383	-10.1525	(Thomazini, 2001)
2001	-62.1067	-32.7197	(Molinari, 2013)
2001	-54.5844	-19.3925	(Godoy et al., 2005)
2001	-46.0333	-7.51667	(Panizzi, 2002)
2002	-50.4525	-21.2036	(Belorte et al., 2003)
2003	-54.1667	-27.3333	(Schmidt and Barcellos, 2007)
2003	-52.7778	-27.0972	(Baldo, 2013)
2004	-53.5564	-30.5369	(Schmidt and Barcellos, 2007)
2004	-54.1667	-27.3333	(Medeiros and Megier, 2009)
2005	-54.2436	-27.7536	(Medeiros and Megier, 2009)
2005	-52.4029	-28.2238	(Scarparo et al., 2015)
2005	-54.1667	-27.3333	(Schmidt and Barcellos, 2007)
2005	-50.2197	-22.7878	(Sosa-Gómez et al., 2009)
2005	-50.2044	-23.6333	(Sosa-Gómez et al., 2009)
2005	-50.3881	-22.7467	(Sosa-Gómez et al., 2009)
2005	-50.7917	-22.7433	(Sosa-Gómez et al., 2009)
2005	-50.7894	-22.8161	(Sosa-Gómez et al., 2009)
2005	-51.1592	-23.3097	(Sosa-Gómez et al., 2009)
2005	-50.7969	-22.8169	(Sosa-Gómez et al., 2009)
2005	-51.1475	-23.3294	(Sosa-Gómez et al., 2009)
2005	-51.1475	-23.3294	(Sosa-Gómez et al., 2009)
2005	-50.2050	-22.6325	(Sosa-Gómez et al., 2009)
2005	-50.7872	-22.8189	(Sosa-Gómez et al., 2009)
2005	-50.2247	-22.7886	(Sosa-Gómez et al., 2009)
2005	-50.9975	-23.4572	(Sosa-Gómez et al., 2009)
2005	-51.1592	-23.3097	(Sosa-Gómez et al., 2009)
2005	-50.2047	-22.6322	(Sosa-Gómez et al., 2009)
2005	-51.1692	-23.3050	(Sosa-Gómez et al., 2009)
2005	-51.2419	-22.9961	(Sosa-Gómez et al., 2009)
2006	-60.7314	2.7575	(Marsaro Jónior et al., 2010)
2006	-53.9922	-28.5186	(Medeiros and Megier, 2009)
2006	-52.6361	-27.0844	(Chiaradia et al., 2011)
2006	-50.7894	-22.8161	(Sosa-Gómez et al., 2009)
2006	-51.1592	-23.3097	(Sosa-Gómez et al., 2009)
2006	-51.0408	-23.0589	(Sosa-Gómez et al., 2009)
2006	-50.3881	-22.7467	(Sosa-Gómez et al., 2009)
2006	-50.2197	-22.7878	(Sosa-Gómez et al., 2009)
2006	-51.0397	-23.0531	(Sosa-Gómez et al., 2009)
2006	-51.2858	-23.2792	(Sosa-Gómez et al., 2009)
2006	-51.1553	-23.2822	(Sosa-Gómez et al., 2009)
2006	-50.3931	-22.7456	(Sosa-Gómez et al., 2009)
2006	-51.6739	-24.2442	(Sosa-Gómez et al., 2009)
2006	-51.1553	-23.2822	(Sosa-Gómez et al., 2009)
2006	-50.7872	-22.8189	(Sosa-Gómez et al., 2009)
2006	-51.1692	-23.305	(Sosa-Gómez et al., 2009)
2006	-51.1692	-23.305	(Sosa-Gómez et al., 2009)
2006	-50.3883	-22.7469	(Sosa-Gómez et al., 2009)

Tabela 3.4: Geographical coordinates used from the occurrence points of *Diceraeus melacanthus* and your source.

Year of occurrence	**Longitude (W)**	**Latitude (S)**	**References**
1998	-51,1825	-23,1917	(Chocorosqui and Panizzi, 2008)
1999	-51,1825	-23,1917	(Chocorosqui and Panizzi, 2003)
2001	-51,1825	-23,1917	(Manfredi-Coimbra et al., 2005)
2002	-50,4525	-21,2036	(Belorte et al., 2003)
2004	-53,6022	-28,6397	(Brondani et al., 2008)
2005	-54,7964	-22,1281	(Carvalho, 2007)
2005	-54,5986	-22,2972	(Carvalho, 2007)
2005	-55,3761	-22,4442	(Carvalho, 2007)
2007	-54,8169	-22,2731	(Duarte et al., 2010)
2007	-51,1694	-23,3044	(Silva et al., 2013)
2008	-51,1456	-23,3000	(Silva et al., 2013)
2009	-52,3778	-24,0439	(Copatti and Oliveira, 2011)
2009	-52,3453	-23,9153	(Copatti and Oliveira, 2011)
2010	-51,1000	-25,0500	(Bridi et al., 2016)
2010	-51,1456	-23,3000	(Panizzi et al., 2015)
2011	-35,7833	-9,5500	(Firmino et al., 2017)
2011	-51,1692	-23,3042	(Cantone et al., 2012)
2011	-50,0406	-23,2444	(Sosa-Gómez et al., 2011)
2011	-50,9789	-23,1825	(Brustolin et al., 2011)
2011	-52,4067	-28,2628	(Smaniotto and Panizzi, 2015)
2012	-51,1799	-23,1900	(Kuss et al., 2012)
2014	-53,6217	-28,5697	(Engel et al., 2018)
2015	-52,4036	-28,2311	(Marsaro Júnior et al., 2017)
2015	-55,0412	-22,1796	(Silva et al., 2018a)
2015	-52,5747	-18,7996	(Silva et al., 2018a)
2015	-54,9407	-20,9516	(Silva et al., 2018a)
2015	-53,1079	-18,5372	(Silva et al., 2018a)
2016	-51,1753	-23,2022	(Schoavengerst and Corrêa-Ferreira, 2017)
2016	-48,4136	-15,2069	(Aquino et al., 2019)
2016	-47,4904	-15,6789	(Aquino et al., 2019)
2016	-47,5538	-16,6363	(Aquino et al., 2019)
2016	-54,0572	-24,5553	(Modolon et al., 2016)
2018	-47,9167	-15,7833	(Moraes, 2019)
2019	-52,4077	-28,2193	(Bianchi et al., 2019)
2019	-52,4000	-28,2500	(Panizzi and Lucini, 2019)

GENERAL CONCLUSION

General conclusion

The competitive ability of *Diceraeus melacanthus* in soybean surpasses *Euschistus heros* when there is no competitive presence. However, when heterospecifics exist in the same environment, *E. heros* not only has more ability to exploit resources in the soybean crop, but also inhibits the actions of *D. melacanthus*.

In this work we also show toxicological curves for *E. heros* and *D. melacanthus* for three insecticide groups, indicating that changes in temperature can increase insecticidal detoxification, especially when this process involves cytochrome P450 and glutathione-S-transferase, a tool that can help management in regions with different climates.

Finally, we show at the municipality level the potential impact of each species on the production of corn and soybeans, indicating places with greater safety and places that cause greater concerns in terms of attacking these pentatomids, generating a tool that can be used even by government institutes for adopting strategies.

This study has a practical implication for the management of *D melacanthus* and *E. heros*. We believe that these tools can be incorporated into the decision-making of an Integrated Pest Management program in order to optimize the management of these species and increase the productivity of these grains, which are so important to the Brazilian economy.