**UNIVERSIDADE ESTADUAL PAULISTA - UNESP**

**CÂMPUS DE JABOTICABAL**

# PEANUT PLANT RESPONSE TO LEPIDOPTEROUS PESTS DEFOLIATION AND DETERMINATION OF THE ECONOMIC INJURY LEVEL

**José Ricardo Lima Pinto**

Engenheiro Agrônomo

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**José Ricardo Lima Pinto**

**Orientador: Prof. Dr. Odair Aparecido Fernandes**

**Coorientador: Prof. Dr. Scott Powell**

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção de título de Doutor em Agronomia (Entomologia Agrícola)

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**Câmpus de Jaboticabal**

## CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: PEANUT PLANT RESPONSE TO LEPIDOPTEROUS PESTS DEFOLIATION AND DETERMINATION OF THE ECONOMIC INJURY LEVEL

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Jaboticabal, 07 de abril de 2022

## DADOS CURRICULARES DO AUTOR

**JOSÉ RICARDO LIMA PINTO –** Nascido em João Pessoa – PB, no dia 17 de maio de 1993. Em 2011 ingressou no curso de Eng. Agronômica da Universidade Federal da Paraíba, e recebeu o título de Engenheiro Agrônomo em julho de 2016. Foi estagiário do laboratório de controle biológico da Associação de Plantadores de Cana-de-açúcar da Paraíba (ASPLAN). Posteriormente foi estagiário do laboratório de entomologia da Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA-Algodão), realizando pesquisas com manejo de pragas da cultura do amendoim. Durante o período da graduação trabalhou no laboratório de Zoologia de Invertebrados, sendo bolsista PIBIC pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Neste período trabalhou em pesquisas que avaliaram a análise faunística e flutuação populacional de moscas-das-frutas (Diptera: Tephritidae) e parasitoides em pomares frutícolas no brejo paraibano. Ingressou no curso de Pós-Graduação, em nível de Mestrado, em agosto de 2016, pela Faculdade de Ciências Agrárias e Veterinárias – FCAV/Unesp, Jaboticabal, na área de Entomologia Agrícola, sob a orientação do Prof. Dr. Odair Aparecido Fernandes, sendo bolsista pela CAPES. Defendeu a dissertação em Julho de 2018 com o título “Aspectos bioecológicos e manejo da lagarta-do-pescoço-vermelho na cultura do amendoim com uso de atrativo alimentar para adultos”. Em agosto de 2018 iniciou o Doutorado pelo mesmo programa e orientador. Durante este período, desenvolveu pesquisas que objetivaram caracterizar a respostas de plantas de amendoim a desfolha ocasionadas por lepidópteros. Além disso, por meio de parcerias internacionais e financiamento CAPES-PrInt, desenvolveu parte dos seus estudos doutorais nos Estados Unidos no período de Março/2020 à Fevereiro/2021 sob supervisão do Prof. Dr. Scott Powell pela Montana State University, onde aprimorou habilidades para integração de análise geoespaciais e inteligência artificial para o manejo integrado de pragas.

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**SUMÁRIO**

**RESUMO GERAL** ..... **1**

**GENERAL ABSTRACT** ..... **3**

**CHAPTER I- GENERAL INTRODUCTION** ..... **5**

**REFERENCES** ..... **10**

**CHAPTER II - SEASONAL DYNAMICS OF LEPIDOPTEROUS AND ITS PARASITOIDS IN PEANUT** ..... **13**

INTRODUCTION ..... 15

MATERIAL AND METHODS ..... 17

RESULTS ..... 22

DISCUSSION ..... 29

REFERENCES ..... 34

SUPPLEMENTARY MATERIAL ..... 39

**CHAPTER III - FOLIAGE CONSUMPTION AND BIOLOGICAL PARAMETERS OF LEPIDOPTEROUS IN PEANUT** ..... **42**

INTRODUCTION ..... 44

MATERIAL AND METHODS ..... 46

RESULTS ..... 49

DISCUSSION ..... 57

REFERENCES ..... 62

**CHAPTER IV - DETECTION OF DEFOLIATION INJURY IN PEANUT WITH HYPERSPECTRAL PROXIMAL REMOTE SENSING** ..... **69**

INTRODUCTION ..... 71

MATERIAL AND METHODS ..... 74

RESULTS ..... 81

DISCUSSION ..... 89

REFERENCES ..... 94

**CHAPTER V - DETERMINATION OF THE ECONOMIC INJURY LEVEL FOR LEPIDOPTEROUS PESTS IN PEANUT ..... 101**

INTRODUCTION ..... 103

MATERIAL AND METHODS ..... 105

RESULTS ..... 112

DISCUSSION ..... 125

REFERENCES ..... 129

SUPPLEMENTARY MATERIAL ..... 134

**CHAPTER VI – FINAL CONSIDERATIONS ..... 135**

## RESUMO GERAL

A agricultura mundial tem sofrido diversas transformações, baseadas principalmente na integração de novas tecnologias, com objetivo de garantir a segurança alimentar e a partir de sistemas de produção sustentáveis. Todavia, algumas culturas, como por exemplo do amendoim no Brasil, ainda carecem de estudos básicos que propiciem a correta adoção de práticas adequadas de manejo de pragas. Logo, devido à falta de estudos com relação a resposta de plantas de amendoim a desfolha provocada por lepidópteros pragas e ausência de ferramentas que auxiliem os produtores a determinar o momento ideal para tomada de decisão no controle desses insetos, o presente trabalho teve como objetivo estabelecer bases para a tomada de decisão de controle dos principais lepidópteros pragas que ocorrem na cultura do amendoim no Brasil. Dessa forma, no capítulo II caracterizamos, ao longo de duas safras de cultivo do amendoim, a abundância das espécies associadas ao amendoim e sua flutuação populacional. Observamos que *Stegasta bosqueella* (Chambers, 1875) (Lepidoptera: Gelechiidae) e o gênero *Spodoptera* são mais abundantes, com maior ocorrência durante a fase de floração (41 ± 70 Dias Após o Plantio (DAP)) e formação da vagem (71 ± 100 DAP). Uma vez que identificamos as espécies mais abundantes, no Capítulo III, avaliamos os aspectos biológicos e o comportamento alimentar das espécies, S. *bosqueella* e *Spodoptera cosmioides* (Walker, 1858) (Lepidoptera, Noctuidae) sob dois dos principais genótipos de amendoim atualmente adotados no Brasil, o IAC 503 e o IAC OL3. Concluímos que o potencial de consumo de folhagem para *S. cosmioides* foi 31,5 e 27,8 vezes maior, do que o observado em *S. bosqueella*, nos genótipos IAC 503 e IAC OL3, respectivamente. Além disso, o desenvolvimento de *S. bosqueella* foi superior sob IAC 503, enquanto, para *S. cosmioides*, IAC OL3 proporcionou melhores recursos

para o desenvolvimento larval. Após quantificar o potencial de consumo foliar das espécies mais abundantes em amendoim no Brasil, caracterizamos no capítulo IV, por meio de parâmetros de troca gasosa e sensoriamento remoto hiperespectral, como as plantas de amendoim respondem a herbivoria por *S. bosqueella* e *S. cosmioides*. Por fim, no capítulo V nós determinamos o nível de dano econômico (NDE) para *S. bosqueella* e *S. cosmioides* em amendoim, em diferentes estágios de desenvolvimento da cultura. Dessa forma, verificamos que o nível de dano econômico para *S. bosqueella* é de 14,65, 77,96 e 36,30 larvas por planta nos estágios vegetativo inicial, floração/frutificação e Formação e fixação de vagens, respectivamente. Já para *S. cosmioides*, o nível de dano econômico é de 0,74, 2,48 e 1,16 larvas por planta nos estágios vegetativo inicial, floração/frutificação e Formação e fixação de vagens, respectivamente. Os resultados gerados permitirão que extensionistas, produtores e consultores tomem decisões de manejo mais assertivas ao lidar com a desfolha do amendoim.

## GENERAL ABSTRACT

World agriculture has been undergoing several changes, driven mainly by the integration of new technologies, with the objective of guaranteeing food security and sustainable production systems. However, for some crops, such as peanuts, there is a lack of knowledge to provide the correct adoption of innovative pest management practices. Therefore, due to the lack of studies regarding peanut plants response to defoliation caused by lepidopterous pests and the absence of tools that help producers to determine the ideal moment for control decision-making of these insects, the present work aimed to establish the basis for control decision-making to the main lepidopterous pests that occur in Brazilian peanut crops. Thus, in Chapter II we characterized, over two peanut crop seasons, the abundance of species associated with peanuts and their population fluctuations. We observed that *Stegasta bosqueella* (Chambers, 1875) (Lepidoptera: Gelechiidae) and the genus *Spodoptera* are more abundant, with greater occurrence during the bloom/pegging stage (41 ± 70 Days After Planting (DAP)) and pod/setting stage (71 ± 100 DAP). Once we identified the most abundant species, in Chapter III, we evaluated the biological aspects and feeding behavior of the species, *S. bosqueella* and *Spodoptera cosmioides* (Walker, 1858) (Lepidoptera, Noctuidae) under two of the main peanut genotypes currently adopted in Brazil, the IAC 503 and the IAC OL3. We concluded that the foliage consumption of *S. cosmioides* was 31.5 and 27.8 times greater than that observed for *S. bosqueella*, in the IAC 503 and IAC OL3 genotypes, respectively. Furthermore, the development of *S. bosqueella* was superior under IAC 503, while, for *S. cosmioides*, IAC OL3 provided better resources for larval development. After quantifying the foliar consumption potential of the most abundant species in Brazilian peanuts, we characterized in Chapter IV, through gas exchange parameters and hyperspectral

remote sensing, how peanut plants respond to herbivory by *S. bosqueella* and *S. cosmioides*. Finally, in chapter V we determined the economic injury level (EIL) for *S. bosqueella* and *S. cosmioides* in peanuts at different stages of crop development. Thus, we verified that the economic injury level for *S. bosqueella* is 14.65, 77.96 and 36.30 larvae per plant in the early vegetative, bloom/pegging and pod/setting stages, respectively. For *S. cosmioides* the economic injury level is 0.74, 2.48 and 1.16 larvae per plant in the early vegetative, bloom/pegging and pod/setting stages, respectively. The generated results will allow extension personnel, producers, and consultants to make more assertive management decisions when dealing with peanut defoliation.

## CHAPTER I- GENERAL INTRODUCTION

In Brazil, since the so-called "Green Revolution" and the development of the agriculture in the “Cerrado” region, featured by great advances in the areas of agricultural mechanization, genetic improvement and the use of fertilizers and chemical pesticides, we have not seen so many changes in the production system. This new revolution in the agriculture production chain has been called Digital Agriculture, since it is supported by the integration between the agricultural processes, with advanced technologies. This approach focuses on the increase in productivity and profits through the capacity to translate a high amount of information acquired by sensors in the field into data driven knowledge by the aid of advanced processing algorithms. Furthermore, the demand for ‘‘sustainable intensification’’, which means, producing more food in the same area, at the same time, using low amounts of inputs (e.g., chemical insecticides and fertilizers) has increased in recent decades (Behmann et al. 2015).

Given this scenario, the adoption of new pest management practices becomes essential to achieve the actual agricultural practices demands. Insect pests are one of the main causes of losses in agriculture (Oerke 2006). Therefore, to avoid profitability loss due to unnecessary pesticides applications, the control decision making process must be developed based on the pest population size, inside the Integrated Pest Management (IPM) approach. Consequently, sampling is essential for IPM (Kogan and Jepson 2007, Pedigo and Rice 2014). However, traditional survey techniques are generally time consuming, costly and labor intensive. Recent studies have been demonstrating the potential to include new technologies, such as remote sensing, to optimize pest sampling efforts (Duarte et al. 2020, Sishodia et al. 2020). This approach, combined with the pest management knowledge, can provide accurate

control decision making in expanded coverage, provide time savings, agility and, above all, velocity in the detection of plants injured by insects.

Remote sensing has been a valuable source for IPM application in different approaches, such as: unmanned aerial vehicles (UAV) (Hunt and Rondon 2017), ground-based spectroradiometers (Prabhakar et al. 2011), hyperspectral imaging systems (Nguyen and Nansen 2020), thermal infrared scanners (Nguyen and Nansen 2020) and satellite imaging systems (Radeloff et al. 1999). However, using remote sensing in IPM programs is only feasible when the knowledge of how the target insect alters the physiological condition of plants is available, since plants can respond differently according to the stressor (Nansen and Elliott 2016). This knowledge drives the type of sensor to use in order to discern healthy from stressed plants (Abd El-Ghany et al. 2020).

In view of this, many challenges are involved in classifying pests, because in an agricultural crop system, not only the targeted species occur, but entomologists have to deal with multiple pests as well as non-targeted species, which can be numerous (Barbedo 2020). For this reason, before the application of any technological innovation into the decision-making process, the development of basic studies including abundance of pest species and behavior on the plant host are key processes (Higley and Pedigo 1996, Karban 2020). In addition, studies that evaluate insect population dynamics, the reproductive capacity, the rate of mortality and survival, the time of growth, and the life expectancy of the pest population are crucial in optimizing the decision making process (Henderson and Southwood 2016, Carey and Roach 2020)

Peanut (*Arachis hypogaea* L.) is considered one of the five most important legumes in the world, being an important source of vegetable oil, proteins and vitamins

(Fletcher and Shi 2016). In light of this, São Paulo, the most important Brazilian peanut producer state, comprises almost 90% of Brazilian peanut production (CONAB 2021). The development of new cultivars and yield loss reduction during mechanized harvesting, and improvement of the postharvest process, were the main practices that contributed to Brazilian peanut yield increase. However, the scarcity of new tools for pest management, combined with the lack of knowledge about the insect community and biology, increases the scenario of chemical pesticides spraying in a "scheduled" manner (usually every 15 days), which is responsible for approximately 20–30% of the peanut crop production costs (Garcia-casellas 2004, Michelotto et al. 2015). The abusive use of chemical pesticides, due to incorrect decision making, promotes higher production costs, imposes phytosanitary barriers for the export of products and increases the resistance of insects to chemical products (Dean et al. 2020). In this context, understanding the lowest population density capable of causing economic damage to plants, the Economic Injury Levels (EILs) and the population density at which control measures should be started to prevent reaching the EILs, the economic threshold (ET), together with studies that seek to verify the mechanisms by which insect herbivory physically and physiologically alters plants are very important, however, poorly understood for the Brazilian peanut crop.

In addition, other obstacles have affected the adoption of IPM in Brazilian peanuts, such as: (1) many growers rent land from sugar mills to grow peanuts and, in most cases, these peanut-growing areas are located far from each other demanding growers to move agricultural machines around to manage the crop; (2) the need for frequent fungicide application, since the varieties currently adopted are highly susceptible to fungal diseases; (3) low availability of phytosanitary products and increase in insect resistant populations. Therefore, the adoption of precision pest

management, a pest management strategy that employs scouting and precision targeting of pest control tactics (Weisz 1996) integrated with insect biology information, in a homogeneous manner and, in real time, allows for quick and effective management through information technology acquired in the field with sensors, using simple or more sophisticated equipment. This approach presents itself as the great vanguard for the management of agricultural pests in the coming decades.

Thus, our work aimed to provide the basis for the development of peanut IPM. Peanut can be infested by more than 360 species of insects (Smith and Barfield 1982), in which, *Stegasta bosqueella* (Chambers, 1875) (Lepidoptera: Gelechiidae) and *Spodoptera* genus are considered the most abundant lepidopterous defoliator species in peanut worldwide (Boiça Junior et al. 2011, Tojo et al. 2008, Tuan et al. 2017, Nigude et al. 2018, Pinto et al. 2020). However, it is still unclear what are the most abundant lepidopterous species that are current occurring in Brazilian peanut. Therefore, in the first part, during two crop growing seasons, we analyzed the lepidopterous pest population fluctuations in peanuts. In the second part, we examined the biological aspects and feeding behavior of the most abundant defoliators on different peanut genotypes, with special emphasis on its biotic potential and fertility under laboratory conditions. In the third part, we measured physiological and spectral responses of peanut plants infested with the most abundant peanut defoliators as well as developed basic information to help contribute to incorporating remote sensing as an instrument to leverage the adoption of IPM programs in peanut. Finally, we determined EIL's for *S. bosqueella* and *Spodoptera cosmioides* (Walker, 1858) (Lepidoptera, Noctuidae), the observed most abundant lepidopterous defoliators in peanut, at different crop development stages based on the products registered for the peanut crop with MAPA (Ministry of Agriculture, Planning and Supply). The information

generated by our study will contribute to enhance current peanut IPM programs and enable the adoption of new technologies for pest management, which based on experiences with other crops, can reduce production costs and reduce the impacts generated by successive applications of chemicals.

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## CHAPTER II - SEASONAL DYNAMICS OF LEPIDOPTEROUS AND ITS PARASITOIDS IN PEANUT

**RESUMO -** Estudos sobre a dinâmica populacional de pragas em amendoim são escassos, e há falta de conhecimento sobre as espécies de lepidópteros e inimigos naturais associados a cultura do amendoim no Brasil. Portanto, o objetivo do nosso estudo foi determinar a flutuação populacional de lepidópteros pragas (adultos e larvas) e seus parasitóides para caracterizar a abundância desses indivíduos em lavouras comerciais de amendoim localizadas em São Paulo, o maior estado produtor brasileiro de amendoim. Os ensaios de campo foram conduzidos em duas áreas comerciais diferentes ao longo de duas safras de amendoim 2017/2018 (área de 6 ha) e 2019/2020 (área de 8 ha). Em ambas as áreas, não foram aplicados produtos visando o controle de lepidópteros. A amostragem de adultos começou uma semana antes da semeadura e foi realizada com armadilhas contendo um atrativo comercial para mariposas. Para a amostragem de larvas, o levantamento de campo teve início 15 dias após o plantio (DAP) das plantas de amendoim e consistiu na avaliação dos folíolos ainda fechados. Tanto a avaliação de adultos como também de lagartas foi realizada duas vezes por semana. Verificamos que *Stegasta bosqueella* foi a espécie mais abundante, seguida por insetos pertencentes ao gênero *Spodoptera*. Modelos de regressão linear múltipla para *S. bosqueella* e *Spodoptera* spp. indicaram um incremento em adultos capturados nas armadilhas quando se observou um aumento na precipitação e umidade relativa do ar. Em relação às lagartas, a alta temperatura e umidade do ar foram um fator positivo, enquanto a chuva resultou num impacto negativo na abundância larval. A análise de componentes principais (ACP) mostrou que a abundância de adultos apresenta maiores ocorrências durante os estádios de floração/frutificação (41-70 DAP) e formação de vagens (71-100 DAP) do amendoim. Por fim, verificou-se baixa incidência de parasitóides relacionados aos lepidópteros pragas. Nosso estudo fornece informações que podem ser usadas como base para alavancar o controle e tomada de decisão de lepidópteros, bem como para melhorar as estratégias de controle que podem ser implementadas para programas de MIP no amendoim, como controle biológico e abordagens de controle baseado em adultos.

**Palavras-chave:** MIP amendoim, pragas do amendoim, flutuação populacional, amostragem de insetos

## CHAPTER II - SEASONAL DYNAMICS OF LEPIDOPTEROUS AND ITS PARASITOIDS IN PEANUT

**ABSTRACT -** Studies on pest population dynamics in peanuts are scarce, and there is a lack of knowledge about the lepidopterous species and their natural enemies associated with peanuts in Brazil. Therefore, the objective of our study was to determine population fluctuation of lepidopterous pests (adults and larvae) and their parasitoids to characterize the abundance of these species in commercial peanut crops located in São Paulo, the largest Brazilian peanut producer state. Field trials were conducted in two different commercial areas during two peanut growing seasons 2017/2018 (6-ha area) and 2019/2020 (8-ha area). In both areas, no larval control products were applied. Adult sampling started one week before sowing and was performed using traps containing a commercial moth attractant. For larval sampling, the field survey started 15 days after plant planting (DAP) of the peanut plants and consisted of still-closed leaflet evaluation. Both adult and larval assessment were performed twice a week. We verified that *Stegasta bosqueella* was the most abundant species, followed by insects belonging to the *Spodoptera* genus. Multiple linear regression models for *S. bosqueella* and *Spodoptera* spp. indicated an increase in trapped adults when an increase in rainfall and relative humidity was observed. Regarding larvae, high temperature and air humidity were a positive factor while rainfall had a negative impact on larval abundance. Principal component analysis (PCA) showed that adult abundance presents higher occurrences during the bloom/pegging stage (41-70 DAP) and pod-setting (71-100 DAP) peanut growth stages. Finally, low incidence of parasitoids related to the lepidopterous pests were verified. Our study provides information that can be used as a basis for leveraging lepidopterous decision making control as well as to improve control strategies that could be implemented for peanut IPM programs, such as biological control and adult-based control approaches.

**Key words:** Peanut IPM, groundnut pests, population fluctuation, insect sampling

## INTRODUCTION

Peanut (*Arachis hypogaea* L.) can be infested by more than 360 species of insects (Smith and Barfield 1982) that can reduce the crop yield due to direct feeding effects and increase the production costs related to insecticide application needs. Depending on where peanuts are produced, these insects can be considered either pests of primary or secondary importance. *Stegasta bosqueella* (Chambers, 1875) (Lepidoptera: Gelechiidae) is considered one of the most abundant lepidopterous defoliator species in North and South America (Boiça Junior et al. 2011, Pinto et al. 2020). However, in Asia the genus *Spodoptera* has a higher importance (Tojo et al. 2008, Tuan et al. 2017, Nigude et al. 2018). However, population dynamics of the target insect pest can vary over time and outbreaks of pests considered as secondary can occur, and compromise production, as previously reported (Wall and Berberet 1975, Kharboutli and Mack 1993, Teixeira et al. 2001).

In view of this, bioecological information about the species that are present in the crops, and their abundance over the growing season, are essential for the establishment of an Integrated Pest Management (MIP) program. Furthermore, studies of an insect's pest population dynamics and their natural enemies are crucial to informing the ideal timing for decision making (Pedigo and Rice 2014). Also, knowing the seasonal population dynamics of a target pest can enable the planning of the most effective management strategies, since tactics such as biological control and behavioral manipulation are highly dependent on the correct time of application (Heimpel and Mills 2017, Gregg et al. 2018). Unfortunately, studies on pest population dynamics in peanuts are scarce, and there is a lack of knowledge about the lepidopterous species and their natural enemies associated with peanuts in Brazil.

In addition, it is well established that the seasonality of biotic and abiotic factors (i.e., rainfall and temperature) can negatively or positively influence insect pest population dynamics (Peterson et al. 2009, Varella et al. 2015, Santos et al. 2017). Also, in peanuts, we have some evidence that the plant phenology (period of leaf emission and the development of reproductive stages) has a direct relationship with defoliator occurrence (Almeida 2015). However, particularly in Brazil, most of the previous research was developed in the last decades using plants of the Spanish and Valencia peanut vegetative group which have an erect growth habit (Castro et al. 1972, Almeida 2015). Nevertheless, these groups are not widely used anymore, and the Runner varieties that belong to the Virginia group, with a decumbent growth habit, are currently adopted by peanut farmers in Brazil and worldwide, since it has a greater yield potential (Gulluoglu et al. 2017, Pirotta et al. 2017). Consequently, because organisms can adapt in space and time (Krebs 2014) and could be highly affected by insect-plant interactions (Nabity et al. 2009, Karban 2020) studies that aim to understand pest population dynamics in peanuts on Runner cultivars become crucial. Also, even though biological control in Brazil has a long history of success for important crops such as soybean, sugarcane and maize (Parra and Coelho 2019), this control strategy has not been adopted by Brazilian peanut farmers. This information must be explained by the lack of knowledge about the natural enemies that are associated with the peanut lepidopterous pest in Brazil.

Therefore, due to the limited information on the overall diversity and population dynamics of defoliators and its natural enemies in peanut, the objective of our study was to determine the population fluctuation of lepidopterous pests (adults and larvae) and its parasitoids to characterize the abundance of these species in commercial peanut crops located in São Paulo State, Brazil, during two peanut crop seasons,. This

region was chosen because it comprises more than 90% of the Brazilian peanut production (CONAB 2021).

## MATERIAL AND METHODS

### Field Location

Field trials were conducted in commercial peanut areas located in the Northeastern region of São Paulo State, Brazil, during two peanut growing seasons (2017/2018 and 2019/2020). In both seasons, we selected fields with the cultivar IAC 503 that belongs to the decumbent vegetative group with “High Oleic” characteristic (70 to 80% oleic acid in the oil) (IAC 2018), cultivated in areas with crop rotation with sugarcane. The arrangement of 0.90 cm between rows and 18 seeds sown per meter (approximately 130,000 peanut plants per ha) was standard in both seasons. The seeds received chemical treatment, with a mixture containing insecticides fipronil (Fipronil Nortox 800 WG®, Nortox, Arapongas, Brazil) and fungicides pyraclostrobin (Opera®, BASF, Schwarzheide, Germany) and Carboxanilideand dimethyldithiocarbamate (Vitavax Thiram 200 SC, UPL-Brazil, Campinas, Brazil), as usually adopted by farmers in the region of Jaboticabal, São Paulo, Brazil. During the crop growing season, no products aiming to control the larvae were applied. However, for *Enneothrips enigmaticus* (Moulton, 1941) (Thysanoptera: Thripidae) control (high abundant insect in Brazilian peanut) applications were carried out using the insecticide thiamethoxan (Actara 250 WG®, Syngenta Crop Protection Agrochemicals, Greensboro, USA) and imidacloprid (Imidagold 700 WG®, UPL-Brazil, Campinas, Brazil), whenever necessary. A total of 7 applications were performed in each season.

During the first season (2017/2018) the experiment was carried out between the months of November/2017 and March/2018 (total of 27 samples) in a 6-ha

experimental area located in the municipality of Taquaritinga, São Paulo, Brazil (21.43016893S -48.60981799W). The study area was divided into six plots of 1 ha each. According to the Köppen classification, the region's climate is characterized as humid tropical with a rainy season in summer and drought in winter. The mean annual temperature in the region is 22.6 ºC, with an average annual rainfall of 1735 mm. In the second season (2019/2020), the experiment was conducted between the months of November/2019 and February/2020 (total of 25 samples) in an experimental area of 8 ha situated in the municipality of Dobrada, São Paulo, Brazil (21.49048298S, -48.38833998W). Similarly, as in the first season, the study area was divided into plots of 1 ha each (total of 8 plots). According to the Köppen climate classification, the region is classified as dry winter mesothermal with average temperatures of the hottest and coldest months, respectively, above 22 ºC and below 18 ºC and average annual rainfall between 1,300 and 1,500 mm.

**Adult Sampling**

For adult sampling, in the 2017/2018 season, Ajar® traps (delta type with an adhesive floor) and Isca Bola Funil® (composed of a plastic bowl with funnel and a collecting bag) (Isca Technologies, Riverside, United States) were used. In the 2019/2020 crop season, only the Ajar® trap was adopted for adult sampling, since it was efficient to represent the adults abundance in peanut and it is ease of handling in comparison to the Isca Bola Funil® (Pinto 2018). In both seasons, the traps were associated with the Noctovi®/Acttra® Noctuideo commercial insect attractant (Isca Technologies, Riverside, United States) and were positioned at the center of each experimental 1-ha plot. Adult population assessment started one week before sowing and trap surveys were carried out twice a week. The attractant, as well as the Ajar®

trap sticky floor and Isca Bola Funil® bag, containing the insects, were removed and replaced at each sampling and taken to the laboratory for identification and counting. Adult samples were grouped by morphological characteristics and sent for identification at a species level by Dr. Vitor Becker.

**Larval Sampling**

For larval population evaluation, the field survey started 15 days after planting (DAP) of the peanut plants. In the crop season 2017/2018 sampling was extended until 96 DAP (totaling 23 samples), whereas for the second season (2019/2020) sampling occurred until 86 DAP (totaling 22 samples). The field survey was performed according to the methodology recommended by Suassuna et al. (2014) and consisted of ten sampling points per plot. Each point was composed of three consecutive plants in a row from which one leaf (four closed leaflets) was randomly taken per plant totaling 120 leaflets per plot. Larvae were counted and transferred to laboratory facilities, where they were kept individually on double sheets of wet filter paper in plastic Petri dishes (2.5 × 11 cm in diameter), in order to allow the emergence of parasitoids associated with each larva. Peanut leaflets were added inside of each Petri dish as a food substrate and replaced daily to maintain appropriate food resources of the larvae. Emerged parasitoids were preserved in 70% alcohol for later identification. The identification was carried out with the aid of the identification keys of Sharkey et al. (2009) and Fakhruddin and Inayatullah (2015) for individuals belonging to the family Braconidae, and Khalaim (2011) for Ichneumonidae.

Also, the defoliation of the plants at the sampling point was estimated using visual injury scores attributed based on the scale proposed by Janini (2011). This scale, ranging from 1 to 5, established that rate 1 indicates absence of injury; rate 2,

leaflets with symptoms of larval consumption of up to 25%; rate 3, from 26 to 50%; rate 4, from 51 to 75%; and rate 5, from 76 to 100% of the leaflet consumed by the insect pest.

**Meteorological parameters**

The Meteorological data were acquired from the NASA Power platform (https://power.larc.nasa.gov/) because the nearest meteorological station was located beyond 10 km. The variables evaluated were daily maximum and minimum temperatures and temperature range (Maximum temperature – Minimum temperature) (ºC), rainfall (mm) and relative humidity (%).

**Data Analysis**

To avoid inaccurate assumptions, we have considered in our analysis only species that were simultaneously collected in both traps and fields. To evaluate adult population fluctuations, we used the average number of adults captured by trap in each survey. For larvae, our data were based on the average number of individuals captured in each plant (30 plants per 1-ha plot), on every sampling date.

Faunistic analysis (frequency and constancy) of immature species was performed based on the methodology proposed by Silveira Neto et al. (1976), following the equation: $Pi = ni/N$, in which, frequency (Pi) indicates the proportion of individuals of a species (ni) in relation to the total number of individuals in the sample (N). Constancy determines the percentage of samples in which a given species was present, being calculated according to the formula: C = p . 100/N, where: p = number of samples with the species and N = total number of samples evaluated. We

considered a species as constant (w) if the species was present in more than 50% of the samples; accessory (y) if a given species was present in 25-50% of the samples and accidental (z) if the species was present in less than 25% of samples.

Principal component analysis (PCA) was applied to verify the relationship of the peanut development stage with meteorological parameters and *S. bosqueella* and *Spodoptera* spp. adult and larva abundance, using data from the two-peanut seasons. Peanut growth stage was characterized following three growth stages: 1) the early vegetative state (2 weeks after planting to pre-flowering), 2) the bloom/pegging stage (41-70 days after planting (DAP)) and 3) the pod-setting stage (71-100 DAP). The growth stages were defined following Boote (1982) and Tuan et al. (2017). For this analysis, only *S. bosqueella* and *Spodoptera* spp. were chosen due to the high abundance observed on both seasons. PCA analysis was used because it allows us to summarize and extract patterns as well as to reduce the data dimensionality, contained in the n variables (here, n = 8) to two orthogonal variables called principal components. Principal components are linear combinations of the original variables that can be graphically visualized, with minimal loss of information (Husson et al. 2017). The PCA function in the FactoMineR (for the analysis) and factoextra (for graph display) packages in the statistical software R v4.0.3 (The R Foundation for Statistical Computing, 2019) were used for performing this analysis. Also, multiple stepwise linear regression analysis was performed to verify the most significant climate variables correlated to the adult population as well as to quantify these relationships. These analyzes were performed using PROC REG (SAS Institute Inc. 2015).

## RESULTS

### Faunistic analysis

The defoliating lepidopterous captured both in the peanut fields and traps were: *S. bosqueella*, *Spodoptera* spp., *Chrysodeixis includens* (Walker, 1858) (Lepidoptera, Noctuidae), *Anticarsia gemmatalis* Hübner 1818 (Lepidoptera, Erebidae) and *Helicoverpa armigera* (Hübner 1808) (Lepidoptera: Noctuidae). This was observed after a population survey during the two peanut growing seasons. *Stegasta bosqueella* was the most abundant species, followed by the armyworms belonging to the genus *Spodoptera*, represented by *Spodoptera cosmioides* (Walker 1858), *Spodoptera albula* (Walker 1857) and *Spodoptera frugiperda* (J.E. Smith 1797) (Lepidoptera: Noctuidae).

Among the defoliating insects, *S. bosqueella* and *Spodoptera* spp. were the most frequent species during both seasons (Table 1). During the first season, a total of 22 samples of larvae were taken and *S. bosqueella* and *Spodoptera* spp. were present in 95.65% and 73.91%, respectively. However, in the second season, these species were present on 100 % of the 22 samples. *S. bosqueella* and *Spodoptera* spp. can be characterized as constant species in Brazilian peanuts. *Chrysodeixis includens* was less frequent both in the first and second seasons and can be considered as an accidental species. A larger number of *A. gemmatalis* was observed only in the second season and it can be classified as an accessory species. Finally, even though we collected adults in both seasons, *H. armigera* larvae were present only on 7 of 22 samples (31.82 %) in the second season and, therefore, can be considered as an accessory species.

**Table 1.** Faunistic analysis of lepidopterous species collected in peanuts, during two peanut crop growing seasons in the northeastern region of São Paulo State, Brazil.

| **2017/2018 Growing Season** | | | | |
|---|---|---|---|---|
| **Species** | **N** | **Samples with the species** | **Frequency (%)** | **Constancy** |
| *S. bosqueella* | 529 | 22 | 80.40 | 95.65 W |
| *Spodoptera* spp. | 106 | 17 | 16.11 | 73.91 W |
| *A. gemmatalis* | 4 | 3 | 0.61 | 13.04 Z |
| *C. includens* | 19 | 4 | 2.89 | 17.39 Z |
| **2019/2020 Growing Season** | | | | |
| *S. bosqueella* | 776 | 22 | 50.79 | 100.00 W |
| *Spodoptera* spp. | 643 | 22 | 42.08 | 100.00 W |
| *A. gemmatalis* | 92 | 10 | 6.02 | 45.45 Y |
| *C. includens* | 2 | 2 | 0.13 | 9.09 Z |
| *H. armigera* | 15 | 7 | 0.98 | 31.82 Y |

N = Total of individuals collected; W = constant, Y = accessory, Z = accidental

## Seasonal population fluctuation

### Adults

The mean number of *C. includens, Spodoptera* spp., *A. gemmatalis*, and *H. armigera* adults were below 20 individuals/trap/date throughout the sampling dates in both seasons (Figure 1). On the other hand, *S. bosqueella* populations were collected during practically the entire experimental period, even in the first sampling before the emergence of plants. Three *S. bosqueella* adult population peaks were observed in both seasons, being between 40-45 DAP; second 60-70 DAP and third above 90 DAP.

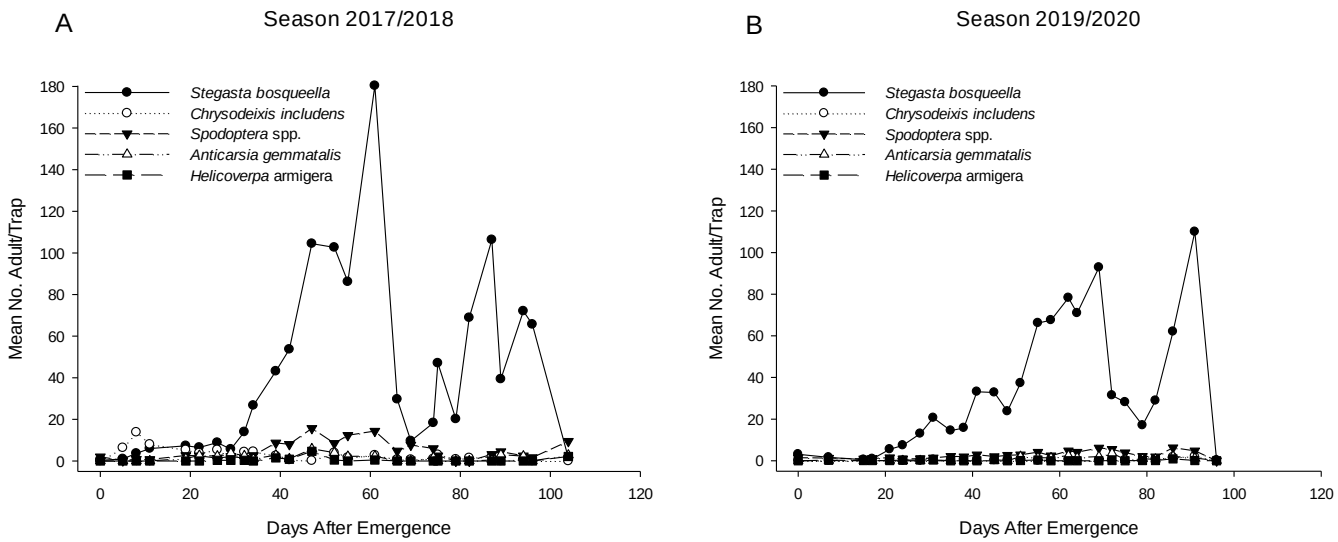


**Figure 1.** Average number of adults collected in peanut commercial fields during two growing seasons 2017/2018 (A) and 2019/2020 (B).

## Larvae

Similarly to the adults, a low number of *C. includens*, *A. gemmatalis* and *H. armigera* larvae was collected during the sampling dates in both seasons (Figure 2). However, the second season showed a greater number of *Spodoptera* spp. after 50 DAP. *Stegasta bosqueella* and *Spodoptera* spp. larvae were captured during almost the whole experimental period. Sampling of larvae was conducted from 15 to 96 DAP and from 15 to 86 DAP in the first and second seasons, respectively. Population fluctuation of larval species varied between the seasons, with the greatest occurrence of larvae observed in the periods of 30-40, 50-60 and 70-80 DAP in both seasons. However, the average number of larva/plant remained below 0.6 throughout the studies conducted during the two peanut seasons.

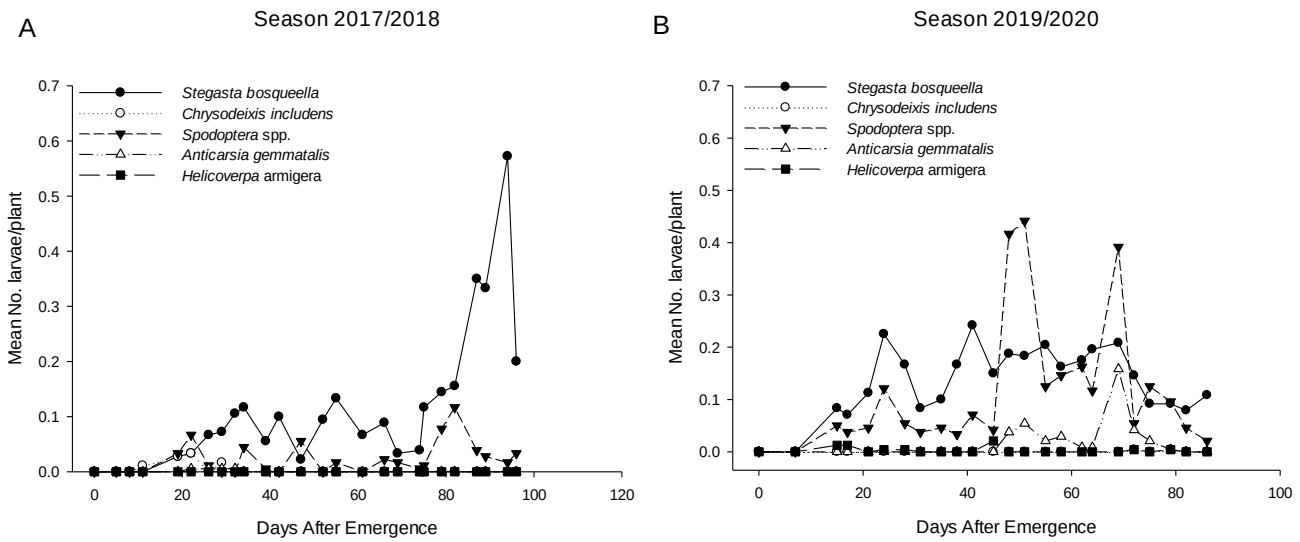


**Figure 2.** Average number of larvae collected in peanut commercial fields during two growing seasons 2017/2018 (A) and 2019/2020 (B).

The level of injury caused by larvae of lepidopterous pests in peanut plants, regardless of the season, remained between 1 and 50% of perforations in the newly opened leaflets (rates 1 and 2) (Figure 3). Also, the highest scores were observed between 40-60 DAP during which the highest abundance of larvae was observed.

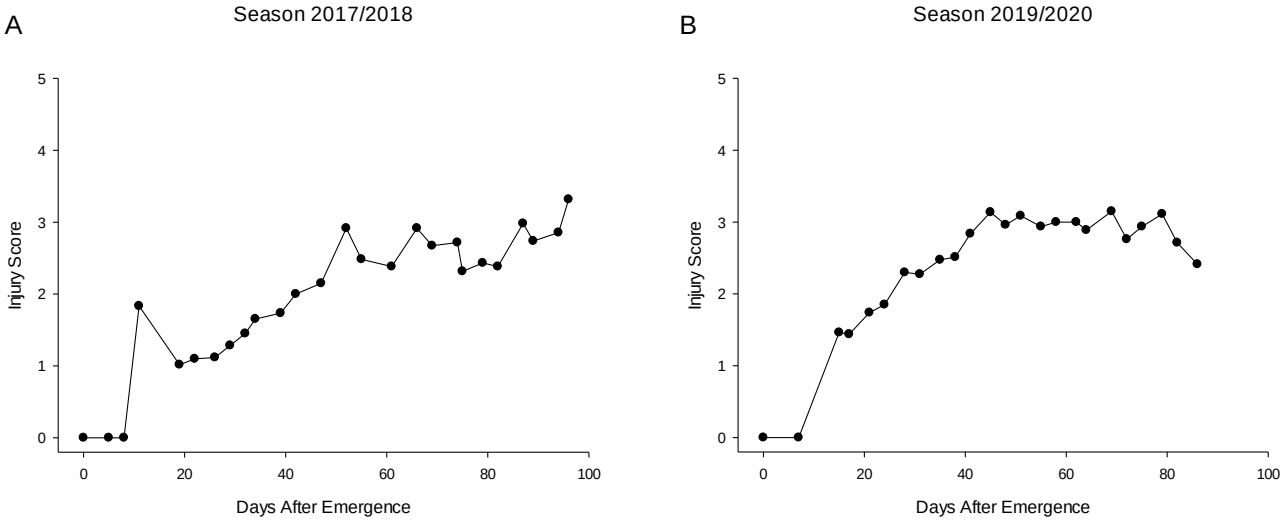


**Figure 3.** Mean injury score verified in peanut commercial fields during two growing seasons 2017/2018 (A) and 2019/2020 (B).

**Climate, peanut development stage and insect pest abundance**

Descriptive statistics and diagnostic plots of the model residuals suggested that the assumptions of normality, linearity, and homoscedasticity were reasonably met for both species (Supplementary 1, 2 and 3). The models for *S. bosqueella* indicate an increase in trapped adults when an increase in rainfall and relative humidity was observed (P<0.05, supplementary 2). Also, rainfall ($r^2$=0.4767; c(p) = 7.70 and P=0.0069) and temperature range ($r^2$= 0.3985; c(p) = 14.3214 and P= <.0001) were included by the multiple stepwise regression analysis (Stepwise selection) as the weightiest variable for *S. bosqueella* adults. Similarly, for *Spodoptera* spp., the selected multiple linear regression model suggests that adult population intensifications occur with an increase in rainfall (P<0.05, supplementary 2). In addition, multiple stepwise regression analysis also highlighted rainfall ($r^2$=0.50; c(p) = 5.66 and P<.0001) and temperature range ($r^2$=0.5380; c(p) = 2.7532 and P= 0.0307) as the most significant climate variables for *Spodoptera* spp. adult population development.

We also observed that climate variables seem to have a significant impact on larvae of both *S. bosqueella* and *Spodoptera* spp. species, in which high temperature and humidity are positive factors whereas rainfall has a negative impact (P<0.05, supplementary 3) on the population. Furthermore, minimum temperature was found to be the most important climate variable for *S. bosqueella* ($r^2$=0.11; c(p) = 29 and P=0.0347) and *Spodoptera* spp. ($r^2$=0.21; c(p) = 60.94 and P=0.0024) to explain population dynamics.

Principal components analysis also exhibited a high association between adult abundance and climate variables for *S. bosqueella* and *Spodoptera* spp., showing that the greatest adult abundance was driven by an increase in rainfall and relative

humidity and a decrease in temperature range. Furthermore, PCA analysis indicates that adult abundance presents higher abundance during the bloom/pegging and pod-setting peanut growth stages (Figure 4). This analysis accurately captured most of the field data variation, with 73.70 and 72.68% of the variance explained for *S. bosqueella* (Figure 4 A and B) and *Spodoptera* spp. (Figure 4 C and D), respectively.

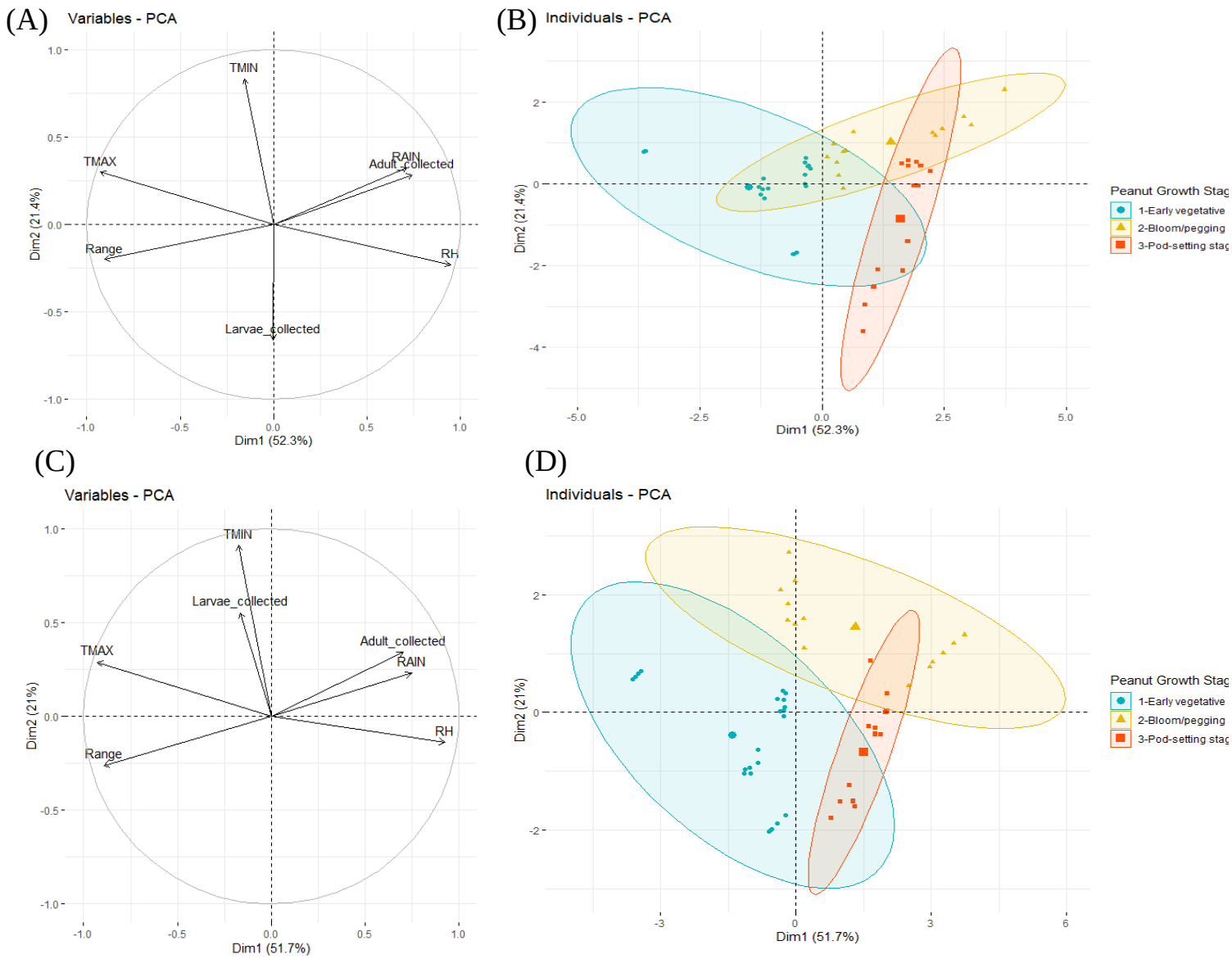


**Figure 4.** Principal component analysis representation of *Stegasta bosqueella* (A, B) and *Spodoptera* spp. (C, D) adult and larvae abundances with peanut growth stage and climate variables association over the two growing seasons 2017/2018 (A) and 2019/2020 (B).

**Biological control agents**

During the first season (2017/2018), the emergence of larval parasitoids was obtained only from *S. bosqueella* (nine individuals) and *Spodoptera* spp. (six individuals) larvae (Table 2). From the 635 larvae collected, we observed the presence of the species *Chelonus* sp. *Apanteles* sp. *Bassus* sp. (Braconidae) and *Temelucha* sp. (Ichneumonidae). The percentage of parasitism of *S. bosqueella* and *Spodoptera* spp. was 1.70 and 5.70, respectively (Table 2).

Similarly to the first season, larval parasitoids were obtained only from *S. bosqueella* (five individuals) and *Spodoptera* spp. (two individuals) larvae (Table 2) in the second season (2019/2020). However, *Chelonus* sp. was the only species verified from a total of 1,419 larvae captured. The percentage of parasitism of *S. bosqueella* and *Spodoptera* spp. was 0.64 and 0.31, respectively.

In addition to the larval parasitoids, other natural enemies were observed during field sampling and were adults of Syrphidae and Dolichopodidae (Diptera) family, green lacewings (Neuroptera: Chrysopidae), ladybugs (Coleoptera: Coccinellidae) on both crop season. Pirate bugs, *Orius* sp. (Hemiptera: Anthocoridae), were also observed, but only during the first season.

**Table 2.** Parasitoids of lepidopterous pests observed during two peanut growing seasons in the northeastern region of São Paulo State, Brazil.

| Growing season | Host | Family | Taxon | Total | Parasitism (%) |
|---|---|---|---|---|---|
| **2017/2018** | *Stegasta bosqueella* | Braconidae | *Apanteles* sp. | 1 ♀ | 1.70 |
| | | Braconidae | *Chelonus* sp. | 8 (5 ♀ and 3 ♂) | |
| | *Spodoptera* spp. | Braconidae | *Bassus* sp. | 1 ♀ | 5.70 |
| | | Braconidae | *Chelonus* sp. | 4 ♀ | |
| | | Ichneumonidae | *Temelucha* sp. | 1 ♀ | |
| **2019/2020** | *Stegasta bosqueella* | Braconidae | *Chelonus* sp. | 5 (3 ♀ and 2 ♂) | 0.64 |
| | *Spodoptera* spp. | Braconidae | *Chelonus* sp. | 2 (2♀) | 0.31 |

## DISCUSSION

An accurate quantification and categorization of the insect species associated with crops is considered one of the most important activities in ecological research and integrated pest management (Pedigo and Rice 2014). This information gives support for understanding the pest community and behavior, as well as to drive the selection of the optimum control decision making. Therefore, our results gathered over 2 years from two geographical locations evaluated the occurrence of lepidopterous species in peanuts, expressed by twice a week insect abundance assessment, along with climate and plant phenology associations. We observed that *S. bosqueella* and *Spodoptera* spp. are the most abundant lepidopterous in peanut fields, with a broad occurrence during the bloom/pegging (41-70 DAP) and pod-setting (71-100 DAP) peanut growth stages. Surprisingly, we also verified that rainfall has a positive influence on *S. bosqueella* and *Spodoptera* spp. adult populations in peanuts. Finally, low incidence of parasitoids related to the lepidopterous pests in commercial peanut fields were verified.

Although the presence of other important noctuid species such as *C. includens*, *A. gemmatalis* and *H. armigera* can still be observed, only *S. bosqueella* and *Spodoptera* spp. were considered as constant lepidopterous species over the two study locations and seasons. The fact that a species is categorized as constant is related to its high ability to use the host resources during the year, and this behavior is usually associated with abundant pest populations (Schowalter 2016). Historically, *S. bosqueella* has been reported as a key peanut pest in the Americas (Boiça Junior et al. 2011, Pinto et al. 2020). However, studies relating *Spodoptera* species in peanuts have exclusively focused on the assessment of feeding capacity under laboratory conditions (Boiça Junior et al. 2011, 2013). Therefore, this work confirms

the occurrence of *Spodoptera* species in peanuts as defoliators and indicates that they should be considered in Peanut-IPM programs in the state of São Paulo (which accounts for 90% of the Brazilian peanut production). The armyworms are no longer negligible species in peanut production. In addition, since armyworms species can also use other leguminous plants such as soybean (*Glycine max*) for development, consider the landscape will be crucial to predict infestation and design pest management programs in peanut.

Considering crop phenology, our results showed that *S. bosqueella* and *Spodoptera* spp. had a seasonal distribution being more prevalent between bloom/pegging (41-70 DAP) and pod-setting (71-100 DAP) peanut growth stages. In these growing stages, peanut plants grouped in the decumbent growth cultivars, has a significant increase in the Leaf Area Index (LAI) (Kiniry et al. 2005) and this must have favored the increase in the injury of defoliators due to the increase in plant biomass and, consequently, greater availability of food. The increase in importance of peanut pests related to biomass intensification was previously reported by Castro et al. (1972) in erect growth peanuts. Such an increase in armyworm population was also verified for *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) during the peanut reproductive stage in India (Tiwari et al. 2008). In addition, previous studies showed that both S. *bosqueella* and *Spodoptera* spp. species present preference and better development under closed leaflets in peanut plants (unpublished results). Consequently, as soon as the plant reduces the production of new leaflets, larval infestations decrease. Therefore, a higher abundance of adults in the pod-filling growth stage (final peanut development stage) could be related to adult dispersion to other areas to find new food resources.

In both seasons, we observed that adults were present in peanut sites at an early stage (before the emergence of the peanut plants). The early detection of adults could be mainly related to the presence of spontaneous peanut plants in the areas. This issue is related to the fact that peanuts are mainly used as crop rotation with sugarcane in São Paulo state (CONAB 2021). So, recently planted sugarcane areas whose land was previously used to grow peanuts usually contain these spontaneous peanut plants which, in turn, host and increase the presence of pest insects as well as diseases (Rivero 2018). However, even though the presence of adults were observed early, larvae infestation only starts to increase after 20 DAP. This should be related to a seed treatment with insecticides that is a common procedure applied by peanut farmers in Brazil to reduce initial problems with insect pests.

In our study, a strong pattern showed an increase in the adult population of both *S. bosqueella* and *Spodoptera* spp. species starting after 40 DAP. This period corresponded also to the time of more frequent precipitation during the rainy season, higher relative humidity and lower reduction of temperature range in both seasons. To our knowledge, this study is the first to evaluate the adult population dynamics in peanuts during an entire season and brings important findings to leverage lepidopterous pest management. As an example, this information provides an opportunity to assess pest population abundance using adult traps instead of the traditional larval sampling, which is laborious and costly. Also, it will be useful as the basis for the adoption of control strategies focusing on reducing the adult population (e.g., attract-and-kill) that can be implemented within a broader Peanut-IPM program.

Regarding larvae, we observed that *S. bosqueella* and *Spodoptera* spp. presented a positive linear relationship with high temperature and humidity and negative association with rainfall. Based on experience in other crops, there is some

evidence that the rainy season has a negative impact on the population of other lepidopterous insects, such as the tomato leafminer, *Tuta absoluta* (Meyrick, 1917) (Lepidotera, Gelechiidae), in tomatoes (Medeiros 2007) and the fall armyworm, *S. frugiperda*, in maize (Varella et al. 2015). However, further studies should address an ecological approach using demographic analytical methods to assess and quantify the mortality factors and their effect on larvae during peanut plant development.

For both *S. bosqueella* and *Spodoptera* spp., the percentage of parasitized larvae collected in peanuts was below 6% in the two peanut seasons. Traditionally, Brazilian farmers adopt the application of a mixture of insecticides and fungicides every other week to prevent pest problems. In general, the insecticides used are non-selective and incompatible with IPM programs. However, most insecticide applications are carried out along with fungicide sprays, which are currently necessary due to the incidence of foliar diseases whose peanut runner cultivars are susceptible (Michelotto et al. 2017). This mixture is usually adopted to optimize the machinery and logistics. The introduction of new disease-resistant cultivars such as the IAC-Top Verde (IAC 2019) could provide a great opportunity to reduce chemical control and consequently to leverage biological control in Brazilian peanuts. Thus, although we have observed a low number of natural enemies associated with lepidopterous in our study, the identification of these species can be used as guidance to enhance the natural enemies community conservation and augmentation in peanut, as well as to encourage the adoption of new studies and surveys that can contribute to IPM adoption by peanut farmers. We perceive a promising future for the adoption of biological control methods for lepidopterous control in peanuts, both through the launch of new cultivars that provide synergy with the IPM strategies as well as the

increase in work that has been recently developed (Pinto and Fernandes 2020, Pinto et al. 2020).

In general, IPM approaches by Brazilian peanut farmers have been poorly adopted. This is mostly related to the fact that sampling methods currently applied are quite laborious and costly. Thus, our study provides information that can be used as a basis for better lepidopterous control management strategies. Also, our findings can be used for control methods that use both larval and the adult stages as targets. In view of this, the possibility of population abundance assessment, using traps for adults, as well as its relationship with climatic variables, can encourage the adoption of both control and sampling methods, focused on the adult stage. These methodologies, based on experiences with other crops (Charmillot et al. 2000, Gregg et al. 2018) can be extremely advantageous, in addition to being more selective and compatible with other control methods, within the scope of IPM.

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## SUPPLEMENTARY MATERIAL

**Supplementary 1.** Descriptive statistics of lepidopterous, injury and climate parameters during two peanut growing seasons (n = 52 samples).

| | | | **Adult** | | |
|---|---|---|---|---|---|
| Variable | *S. bosqueella* | *C. includens* | *Spodoptera* spp. | *A. gemmatalis* | *H. armigera* |
| Mean | 38.30 | 1.57 | 3.92 | 1.20 | 0.29 |
| SD | 38.31 | 2.58 | 3.49 | 1.38 | 0.73 |
| Variance | 1467.72 | 6.67 | 12.17 | 1.91 | 0.54 |
| Maximum | 180.33 | 13.83 | 15.67 | 5.83 | 4.67 |
| Minimum | 0.33 | 0.00 | 0.17 | 0.00 | 0.00 |
| Kurtosis | 2.32 | 9.51 | 2.94 | 1.01 | 25.71 |
| Skewness | 1.41 | 2.73 | 1.74 | 1.11 | 4.72 |

| | | | **Larva** | | | |
|---|---|---|---|---|---|---|
| Variable | *S. bosqueella* | *C. includens* | *Spodoptera* spp. | *A. gemmatalis* | *H. armigera* | Injury |
| Mean | 0.14 | 0.00 | 0.07 | 0.01 | 0.00 | 2.39 |
| SD | 0.10 | 0.01 | 0.10 | 0.03 | 0.00 | 0.64 |
| Variance | 0.01 | 0.00 | 0.01 | 0.00 | 0.00 | 0.41 |
| Maximum | 0.57 | 0.03 | 0.44 | 0.16 | 0.02 | 3.32 |
| Minimum | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.02 |
| Kurtosis | 7.36 | 12.96 | 7.06 | 25.98 | 13.27 | -0.67 |
| Skewness | 2.17 | 3.62 | 2.67 | 4.75 | 3.55 | -0.68 |

| | | **Climate parameters** | | |
|---|---|---|---|---|
| Variable | Max. Temp. (°C) | Min. Temp. (°C) | RH (%) | Precipitation (mm) |
| Mean | 29.11 | 19.99 | 79.41 | 34.39 |
| SD | 1.53 | 1.27 | 6.46 | 38.17 |
| Variance | 2.33 | 1.62 | 41.74 | 1457.13 |
| Maximum | 32.57 | 22.26 | 88.14 | 234.12 |
| Minimum | 26.44 | 16.37 | 62.86 | 0.00 |
| Kurtosis | -0.41 | 0.53 | -0.15 | 14.15 |
| Skewness | 0.30 | -0.91 | -0.76 | 3.20 |

SD: standard deviation; CV, Max. Temp. : Maximum Temperature; Min. Temp.: Minimum Temperature, RH: Relative Humidity.

**Supplementary 2.** Coefficient estimates and SEs for selected climate parameters on *Stegasta bosqueella* and *Spodoptera* spp. adult in peanut. Bold values are representing climate parameters selected by the multiple stepwise regression analysis.

| **Ref. = *Stegasta bosqueella*** | **Estimate** | **Std. error** | **t Value** | **Pr > \|t\|** |
|---|---|---|---|---|
| Intercept | -1329.96053 | 566.66536 | -2.35 | 0.0228* |
| Maximum Temperature | 19.68256 | 13.28835 | 1.48 | 0.1447 |
| Minimum Temperature | 8.55927 | 5.70555 | 1.50 | 0.1397 |
| Relative Humidity | 7.47711 | 2.99441 | 2.50 | 0.0158* |
| **Rainfall** | **0.79781** | **0.25125** | **3.18** | **0.0025*** |
| **Ref. = *Spodoptera* spp.** | **Estimate** | **Std. error** | **F-value** | **Pr > F** |
| Intercept | 22.95310 | 50.94190 | 0.45 | 0.6542 |
| Maximum Temperature | -1.22081 | 1.19459 | -1.02 | 0.3116 |
| Minimum Temperature | 1.20033 | 0.51292 | 2.34 | 0.0232* |
| Relative Humidity | -0.15006 | 0.26919 | -0.56 | 0.5797 |
| **Rainfall** | **0.11540** | **0.02259** | **5.11** | **<0001*** |

* Statistically significant at α= 0.05.

**Supplementary 3.** Coefficient estimates and SEs for selected climate parameters on *Stegasta bosqueella* and *Spodoptera* spp. larva in peanut. Bold values are representing climate parameters selected by the multiple stepwise regression analysis.

| **Ref. = *Stegasta bosqueella*** | **Estimate** | **Std. error** | **t Value** | **Pr > \|t\|** |
|---|---|---|---|---|
| Intercept | -4.28429 | 1.56288 | -2.74 | 0.0094* |
| Maximum Temperature | 0.02342 | 0.03912 | 0.60 | 0.5530 |
| **Minimum Temperature** | **-0.04841** | **0.02215** | **4.78** | **0.0347*** |
| Temperature Range[1] | - | - | - | - |
| Relative Humidity | 0.04139 | 0.00939 | 4.41 | <0001* |
| Rainfall | 0.00518 | 0.00115 | -4.50 | <0001* |
| **Ref. = *Spodoptera* spp.** | **Estimate** | **Std. error** | **t-value** | **Pr > F** |
| Intercept | -9.98154 | 1.19371 | -8.36 | <0001* |
| Maximum Temperature | 0.14819 | 0.02988 | 4.96 | <0001* |
| **Minimum Temperature** | **0.06677** | **0.02055** | **10.55** | **0.0024*** |
| Temperature Range[1] | - | - | - | - |
| Relative Humidity | 0.05483 | 0.00717 | 7.64 | <0001* |
| Rainfall | -0.00276 | 0.00087867 | -3.14 | 0.0033* |

* Statistically significant at α = 0.05.

**Supplementary 4.** Climate parameters: maximum and minimum daily temperature, relative air humidity (%) and accumulated precipitation (mm), during the period of the experiment for peanut season 1 and season 2 collected from the NASA's Power platform.

| Season 1 – 2017/2018 | | | | | | Season 2 – 2019/2020 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Date** | **DAP[1]** | **TMAX[2] (°C)** | **TMIN[3] (°C)** | **RH[4] (%)** | **Rainfall (mm)** | **Date** | **DAP[1]** | **TMAX[2] (°C)** | **TMIN[3] (°C)** | **RH[4] (%)** | **Rainfall (mm)** |
| 17/11/2017 | 0 | 30.93 | 16.37 | 68.17 | 3.51 | 12/11/2019 | 0 | 32.10 | 21.29 | 66.92 | 21.11 |
| 22/11/2017 | 5 | 27.56 | 19.62 | 84.78 | 44.55 | 19/11/2019 | 7 | 32.04 | 19.73 | 64.24 | 27.40 |
| 25/11/2017 | 8 | 28.93 | 17.87 | 78.40 | 14.97 | 27/11/2019 | 15 | 32.57 | 19.98 | 62.86 | 22.63 |
| 28/11/2017 | 11 | 29.20 | 20.60 | 80.97 | 87.72 | 29/11/2019 | 17 | 31.03 | 21.37 | 75.30 | 26.18 |
| 06/12/2017 | 19 | 29.74 | 18.56 | 76.64 | 17.43 | 03/12/2019 | 21 | 29.91 | 20.45 | 77.06 | 26.95 |
| 09/12/2017 | 22 | 29.00 | 21.03 | 82.37 | 23.76 | 06/12/2019 | 24 | 29.66 | 19.97 | 75.27 | 12.77 |
| 13/12/2017 | 26 | 30.70 | 19.03 | 73.25 | 2.37 | 10/12/2019 | 28 | 31.47 | 20.52 | 70.62 | 14.58 |
| 16/12/2017 | 29 | 30.50 | 18.77 | 72.80 | 8.46 | 13/12/2019 | 31 | 26.61 | 20.82 | 85.64 | 33.44 |
| 19/12/2017 | 32 | 30.80 | 20.03 | 72.47 | 6.09 | 17/12/2019 | 35 | 28.87 | 20.93 | 80.56 | 20.50 |
| 21/12/2017 | 34 | 29.00 | 20.85 | 79.30 | 35.13 | 20/12/2019 | 38 | 28.58 | 20.52 | 79.52 | 23.29 |
| 26/12/2017 | 39 | 28.44 | 20.14 | 80.18 | 18.99 | 23/12/2019 | 41 | 27.40 | 20.35 | 83.98 | 12.28 |
| 29/12/2017 | 42 | 29.77 | 21.00 | 79.03 | 8.13 | 27/12/2019 | 45 | 30.51 | 19.98 | 73.85 | 22.94 |
| 04/01/2018 | 47 | 27.57 | 21.28 | 87.27 | 234.12 | 30/12/2019 | 48 | 31.95 | 20.83 | 69.81 | 7.04 |
| 09/01/2018 | 52 | 26.74 | 19.04 | 84.38 | 48.78 | 03/01/2020 | 51 | 29.69 | 21.46 | 77.72 | 35.82 |
| 12/01/2018 | 55 | 27.30 | 19.60 | 84.30 | 36.12 | 07/01/2020 | 55 | 29.30 | 19.49 | 81.08 | 48.14 |
| 18/01/2018 | 61 | 27.90 | 19.57 | 81.80 | 104.40 | 10/01/2020 | 58 | 28.08 | 21.77 | 86.84 | 85.61 |
| 23/01/2018 | 66 | 28.34 | 21.10 | 80.74 | 12.81 | 13/01/2020 | 61 | 29.58 | 22.26 | 83.79 | 39.41 |
| 26/01/2018 | 69 | 29.90 | 20.73 | 78.23 | 24.45 | 16/01/2020 | 64 | 30.53 | 20.94 | 80.10 | 18.87 |
| 31/01/2018 | 74 | 28.24 | 20.54 | 85.34 | 70.59 | 21/01/2020 | 69 | 29.84 | 20.24 | 80.85 | 27.21 |
| 02/02/2018 | 75 | 28.55 | 17.25 | 78.15 | 0.00 | 24/01/2020 | 72 | 28.86 | 20.11 | 84.39 | 99.39 |
| 06/02/2018 | 79 | 27.88 | 17.93 | 81.58 | 20.79 | 27/01/2020 | 75 | 29.32 | 17.47 | 72.11 | 8.41 |
| 09/02/2018 | 82 | 29.37 | 17.27 | 69.60 | 0.00 | 31/01/2020 | 79 | 28.61 | 21.21 | 84.07 | 38.45 |
| 14/02/2018 | 87 | 28.90 | 20.18 | 84.16 | 84.18 | 04/02/2020 | 82 | 28.49 | 21.33 | 86.77 | 44.00 |
| 16/02/2018 | 89 | 27.30 | 18.40 | 87.10 | 24.78 | 08/02/2020 | 86 | 26.51 | 20.10 | 86.70 | 32.59 |
| 21/02/2018 | 94 | 26.44 | 19.68 | 88.14 | 20.34 | 13/02/2020 | 91 | 27.65 | 19.47 | 86.19 | 70.62 |
| 23/02/2018 | 96 | 28.65 | 21.05 | 86.25 | 0.00 | | | | | | |
| 01/03/2018 | 104 | 27.07 | 19.18 | 87.45 | 15.93 | | | | | | |

**1 -DAP –** Days after planting**; 2- TMAX –** Maximum temperature**; 3- TMIN –** Minimum temperature **/ 4 RH –** Relative humidity

## CHAPTER III - FOLIAGE CONSUMPTION AND BIOLOGICAL PARAMETERS OF LEPIDOPTEROUS IN PEANUT

**RESUMO -** A lagarta do pescoço vermelho, *Stegasta bosqueella* e a lagarta militar do gênero *Spodoptera* são importantes desfolhadoras do amendoim. Infelizmente, devido à falta de conhecimento sobre a biologia e potencial de consumo desses insetos, os níveis limiares não estão disponíveis para produtores de amendoim que ainda dependem de aplicações calendarizadas de inseticidas químicos para controle. Assim, o objetivo deste trabalho foi avaliar aspectos biológicos e comportamento alimentar de *S. bosqueella* e *S. cosmioides* em dois dos principais genótipos de amendoim atualmente adotados no Brasil, o IAC 503 e o IAC OL3. Um total de 50 e 60 larvas foram avaliadas em cada genótipo de amendoim para *S. bosqueella* e *S. cosmioides*, respectivamente. Para cada espécie de lepidóptero, os parâmetros biológicos foram registrados e usados para construir tabelas de vida de fertilidade. Observou-se que a fertilidade e o desenvolvimento biológico de *S. bosqueella* e *S. cosmioides* variaram de acordo com o genótipo. Alta mortalidade larval (mais de 50%) foi verificada para *S. cosmioides* no 1º ínstar em ambos os genótipos de amendoim. No entanto, não foi observada diferença entre a sobrevivência larval de *S. bosqueella* e *S. cosmioides*. Além disso, o potencial de consumo de folhagem para *S. cosmioides* foi 31,5 e 27,8 vezes maior que o observado em *S. bosqueella*, nos genótipos IAC 503 e IAC OL3, respectivamente. Portanto, este trabalho fornece novas informações sobre o potencial biótico e parâmetros de reprodução de *S. bosqueella* e *S. cosmioides* em amendoim, bem como o potencial de consumo foliar para ambas as espécies de larvas permitindo o desenvolvimento de limiares econômicos.

**Palavras-chave:** potencial biótico, herbivoria, desempenho larval, MIP de amendoim, tabela de vida de fertilidade

## CHAPTER III - FOLIAGE CONSUMPTION AND BIOLOGICAL PARAMETERS OF LEPIDOPTEROUS IN PEANUT

**ABSTRACT -** The rednecked peanutworm, *Stegasta bosqueella*, and armyworms of the genus *Spodoptera* are important defoliators of peanuts. Unfortunately, due to lack of knowledge related to the biology and consumption potential of these insects, threshold levels are not available for peanut growers who are still dependent on scheduled applications of chemical insecticides for control. Thus, the objective of this study was to evaluate biological aspects and feeding behavior of *S. bosqueella* and *S. cosmioides* on two of the main peanut genotypes currently adopted in Brazil, the IAC 503 and IAC OL3. A total of 50 and 60 larvae were evaluated in each peanut genotype for *S. bosqueella* and *S. cosmioides*, respectively. For each lepidopterous species, biological parameters were recorded and used to build fertility life tables. It was observed that the fertility and biological development of *S. bosqueella* and *S. cosmioides* were varied according to the genotype. High larval mortality (more than 50%) was verified for *S. cosmioides* in the 1$^{st}$ instar in both peanut genotypes. However, no difference was observed between *S. bosqueella* and *S. cosmioides* larval survival. Also, the foliage consumption potential for *S. cosmioides* was 31.5 and 27.8-fold greater, than the observed in *S. bosqueella*, in IAC 503 and IAC OL3 genotypes, respectively. Therefore, this work provides novel information about the biotic potential and reproductivity parameters for *S. bosqueella* and *S. cosmioides* in peanuts, as well as the foliage consumption potential for both larvae species allowing the development of economic thresholds.

**Key words:** biotic potential, herbivory, larval performance, Peanut IPM, fertility life table

## INTRODUCTION

Peanut (*Arachis hypogaea* L.) is considered one the five most cultivated oilseed crops worldwide, and it is an important source of protein and oil (Fletcher and Shi 2016). The main peanut exporting countries China, United States, India, Argentina, and Brazil have shown increased productivity in recent years (USDA 2021). However, productivity, although essential in reducing production costs, is not enough to assure the competitiveness of agricultural products in the international and national markets, as there is a growing demand for foods that present low risks to consumers.

This crop can host several insect pests and diseases, which negatively influence the sustainability of the crop (Pirotta et al. 2017). Among the main lepidopterous pests, the rednecked peanutworm, *Stegasta bosqueella* (Chambers, 1875) (Lepidoptera: Gelechiidae), occurs in high abundance in numerous countries of the world (Mulder and Berberet 2001; Boiça Junior et al. 2011; Almeida 2015; Nogueira et al. 2016; Pinto et al. 2020a). The larva feeds on the youngest leaflets of peanut plants, which show symmetrical damages after being open. As a result of such an injury, peanut plants slow their vegetative development (Almeida 2015; Nogueira et al. 2016). In addition to the occurrence of *S. bosqueella*, high populations of individuals belonging to the genus *Spodoptera* (Lepidoptera: Noctuidae) in peanuts have been observed in several locations and outbreaks can occur (Kharboutli Marwan and Mack 1993; Tiwari et al. 2008; Nigude et al. 2018; Pinto 2018).

The use of scheduled chemical pesticides application is the main method adopted for the control of defoliators in peanuts (Garcia-Casellas 2004; Michelotto et al. 2017; Abbott et al. 2019). Thus, negative consequences such as phytosanitary barriers to peanut exports; insect resistance to chemical molecules and increase in

production costs can occur. Therefore, the knowledge of insect biology is essential to develop efficient management strategies within the framework of Integrated Pest Management (IPM). This information allows a better understanding of the target pest population dynamics and the ideal time for decision making. Consequently, it is possible to keep insect damage to acceptable levels and avoid increases in production costs due to unnecessary applications for pest control (Higley and Pedigo 1996; Pedigo and Rice 2014). However, to the best of our knowledge, no studies aimed to evaluate the reproductive capacity, the rate of mortality and survival, the time of growth, and the life expectancy of *S. bosqueella* and *S. cosmioides* in peanuts. These parameters are essential for understanding insect population dynamics, and they can be investigated through fertility life tables. Fertility life table is an important tool for analyzing the influence of the above ecological parameters on the growth and population dynamics of insects (Coppel and Mertins 1977; Maia et al. 2000; Ahmad et al. 2020) because it permits the comprehension of how the biological characteristics of a population are affected under a specific host when submitted to an appropriate environmental condition (Razmjou et al. 2014; Henderson and Southwood 2016; Barilli et al. 2019).

Furthermore, the type of host plant can play a significant role in the establishment of a phytophagous insect and can alter its population growth strategies (Forister et al. 2012; Karban 2020). Therefore, the comprehension of such an interaction is required for the enhancement of pest control. Quantitative analysis to measure the consumption potential and utilization of host plants by insect herbivores is a common approach used to evaluate plant insect interactions (Scriber and Slansky 1981; Greenberg et al. 2001; Peñalver-Cruz et al. 2020). Comprehending the

consumption potential of an individual is also crucial information for the development of a peanut IPM program, since the sensitivity to yield loss, and compensation for losses may vary according to genotypes (Prasad et al. 2007; Romero et al. 2020). We already know that *S. bosqueella* and *S. cosmioides* feed on a wide range of peanut genotypes (Boiça Júnior et al. 2013; Di Bello et al. 2015). However, the consumption potential of *S. bosqueella* and *S. cosmioides* in peanuts remains unclear. Therefore, the objective of this study was to evaluate the biological aspects and feeding behavior of *S. bosqueella* and *S. cosmioides* on different peanut genotypes, with special emphasis on its biotic potential and fertility life table parameters under laboratory conditions.

## MATERIAL AND METHODS

### Plant material and insects

The study was conducted with two of the main peanut genotypes currently adopted in Brazil, the IAC 503 and IAC OL3, belonging to the “high oleic” runner vegetative group (IAC 2018). Sowing was performed in 5-liter pots containing a mixture of soil, sand, and cattle manure (ratio of 2: 1: 1). The peanut plants were kept in an air-conditioned greenhouse facility (25±10 ºC, RH 70 ± 10%, and natural light). Planting was carried out weekly to ensure continuous availability of peanut plants for testing and insect rearing.

*Stegasta bosqueella* and *S. cosmioides* colonies were established according to the methodology adopted by Pinto and Fernandes (2020). *Stegasta bosqueella* adults (approximately 200 adults) were collected in a commercial peanut field located in the Jaboticabal municipality, São Paulo, Brazil. On the other hand, the initial *S. cosmioides*

colony (approximately 250 specimens) was provided by the Insect Biology Laboratory, University of São Paulo, Piracicaba, São Paulo state, Brazil. Both insect colonies were kept under controlled conditions (25 ± 2 ºC, 70 ± 10% RH, and 12L:12D). Insect of the F1 generation were developed using the peanut genotype IAC Runner 886 (IAC 2018) to avoid pre-imaginal conditioning. F2 generation was used for the experiment.

**Foliage consumption and biological aspects**

The experiment was carried out under completely randomized design with two treatments (peanut genotypes IAC 503 and IAC OL3). A total of 50 and 60 larvae were used in each peanut genotype for *S. bosqueella* and *S. cosmioides,* respectively. Freshly hatched larvae (<24 h) were individualized in Petri dishes (2.5×11 cm in diameter) and kept under controlled conditions (25 ± 2 ºC, 70 ± 10% RH, and 12L:12D). Individual closed peanut leaflets with minimal developmental stage R2 (40-45 days) were collected daily from IAC 503 and IAC OL3 peanut genotypes and used to feed *S. bosqueella* and *S. cosmioides* larvae. The leaflets used presented no injuries and were washed in a 1% sodium hypochlorite aqueous solution for three minutes and dried before offering them to the larvae. To standardize the age of the leaflets, only fully closed leaflets were collected, and they were used within 30 minutes after removal from plants. Once dried, the leaflets were individually inserted on double sheets of wet filter paper in plastic Petri dishes (2.5 × 11 cm in diameter) and replaced daily to avoid excessive dehydration. Upon replacement, the larval instar was determined by observing the presence of the cephalic capsule. Thus, for each lepidopterous species, the following biological parameters were recorded: larval period, larval viability, number of instars, sex ratio (number of females / total number of individuals), pupal period,

pupal viability, egg-adult period. The sex of both insects was verified by the evaluation of terminal portion of pupae.

The leaf area consumed by *S. bosqueella* and *S. cosmioides* was determined by digital photographs taken at every leaflet replacement. The image of the remaining leaf tissue was measured using the ImageJ program (Abràmoff et al. 2007). The daily consumption of *S. bosqueella* and *S. cosmioides* larvae was then accumulated to determine the consumption of each larval stage.

**Fertility life table**

Upon adult emergence, adult couples of *S. bosqueella* or *S. cosmioides* were inserted into PVC (polyvinyl chloride, 10 cm diameter × 20 cm height) cylindrical mating cages supported by a plastic cover (15 cm diameter × 2 cm height) and closed at the top with voile were prepared by genotype. Inside each cage, there was also a 15-day-old peanut seedling with leaflets, approximately 10 cm tall. This peanut stem served as an oviposition substrate for fertility assessments. Each cage contained only the plant corresponding to the treatment (genotype) was considered as our experimental unit. All the plants were replaced daily throughout the adult's lifespan. Adults were fed with 10% honey solution soaked in a piece of sponge also kept inside the cage.

Daily observations from the mating cages and previous assays allowed us to determine female fecundity (eggs/female), adult longevity and total life cycle (larval hatching to adult death). The experiment was carried out on a completely randomized design, in which each cage was considered a replication.

With the biological data from *S. bosqueella* and *S. cosmioides* obtained in the different genotypes, fertility life tables were constructed according to Birch (1948),

Silveira Neto (1976), Southwood (1978) and Price (1984). In addition, we calculated the time required for the population to double (TD), according to Krebs (1994).

## Statistical Analysis

The biological parameters including larval duration and viability, number of instars, pupal duration and viability, egg-adult duration and viability, female fecundity (eggs/female), adult longevity, total life cycle period (larval hatching to death of adults) and foliage consumption of *S. bosqueella* and *S. cosmioides* were subjected to analysis to evaluate the assumption of normality of the residuals and the homogeneity of the variances. Means were compared using the F test (p = 0.05). All analyses were performed using mixed models (PROC MIXED, SAS Institute, 2015).

Fertility life table parameters were estimated according to the Jackknife method to estimate parameter confidence intervals and comparisons between treatments were performed according to Maia et al. (2000), using SAS software (SAS Institute, 2015). The proportion of the number of surviving larvae in each treatment was compared using the Wilcoxon by the Kaplan-Meyer test with the aid of PROC LIFETEST (SAS Institute, 2015).

# RESULTS

## Biological aspects

### *Stegasta bosqueella*

It was verified that *S. bosqueella* larvae presented a faster development on IAC 503 genotype in comparison to IAC OL3 ($F_{1,43}$=4.17, P=0.0003) (Table 1). Similarly, *S.*

*bosqueella* had a shorter pupal period when fed on IAC 503 ($F_{1,37}$=7.55, P=0.0092). The sex ratio was 0.54 and 0.52 when larvae developed on IAC 503 and IAC OL3, respectively, and the time required to reach adulthood was shorter on IAC 503 compared to IAC OL3 ($F_{1,37}$=91.95, P <.0001) (Table 1). Regarding the other biological parameters, no significant differences were verified for larval ($F_{1,99}$=0.43, P=0.5127) and pupal viabilities ($F_{1,43}$=0.49, P=0.4863) and nor for egg-adult viability in both genotypes ($F_{1,98}$=0.04, P=0.8396) (Table 1)

**Table 1**. Biological parameters of the larval and pupal stages of *Stegasta bosqueella* and *Spodoptera cosmioides* on IAC 503 and IAC OL3 genotypes.

| Parameters | ***Stegasta bosqueella*** | | ***Spodoptera cosmioides*** | |
|---|---|---|---|---|
| | **IAC 503** | **IAC OL3** | **IAC 503** | **IAC OL3** |
| Larval period (days) | 13.96±0.39 b | 15.05±0.36 a | 19.00± 0.87 a | 17.11±0.45 a |
| Larval viability (%) | 48.56±7.07 a | 42.00±7.05 a | 26.66± 5.75 a | 43.33±6.45 a |
| Pupal period (days) | 7.40±0.30 b | 8.59±0.26 a | 15.84± 0.27 a | 15.47±0.24 a |
| Pupal viability (%) | 83.33±7.77 a | 90.48±6.56 a | 78.57±11.38 a | 65.38±9.51 a |
| Egg-Adult viability (%) | 40.00±6.99 a | 38.00±6.93 a | 21.66± 5.36 a | 28.33±5.86 a |
| Egg-Adult period (days) | 26.05±0.37 b | 30.63±0.30 a | 46.30± 0.35 a | 46.00±0.53 a |

Means (±EPM) followed by the same letter within rows for each species were not statistically different by the F test (p > 0.05).

*Stegasta bosqueella* larvae developed over 5 instars in both genotypes evaluated (Figure 1). The mean development time during the 1$^{st}$ ($F_{1,65}$=6.26, P=0.0148) and 3$^{rd}$ instars ($F_{1,48}$= 4.44, P=0.0403) was significantly greater on the IAC 503 genotype (3.26±0.10 and 3.04±0.12 days). In contrast, the mean development time for 5$^{th}$ instar was longer in the IAC OL3 genotype (3.95±0.32 days) ($F_{1,36}$=12.60, P=0.0011). There was no significant difference between the development time in both cultivars for the other instars.

Longevity of *S. bosqueella* adults was not affected by genotypes ($F_{1,8}$=1.93, P= 0.1747) (Table 2). However, the total life cycle (larval hatching to adult death) was higher on IAC OL3 in comparison to IAC 503 ($F_{1,30}$ =24.13, P<0.0001).

**Table 2**. Female fecundity (egg / female), adult longevity (days) and total cycle period (hatching larva to adult death) of *Stegasta bosqueella* and *Spodoptera cosmioides* on IAC 503 and IAC OL3 genotype.

| Characteristics | *Stegasta bosqueella* | | *Spodoptera cosmioides* | |
|---|---|---|---|---|
| | **IAC 503** | **IAC OL3** | **IAC 503** | **IAC OL3** |
| Fertility (eggs / female) | 218.4±7.0 a | 223.2±4.0 a | 1722.0±171.3 b | 2550.0±224.5 a |
| Adults Longevity (days) | 25.4±0.3 a | 24.7±0.4 a | 6.2±0.3 b | 8.1±0.4 a |
| Total Cycle Period (days) | 51.2±0.5 b | 55.2±0.6 a | 52.5±0.4 b | 54.1±0.5 a |

Means (±EPM) followed by the same letter within rows for each species were not statistically different by the F test (p > 0.05).

The evaluated genotypes did not affect the fecundity of *S. bosqueella* females ($F_{1,8}$ = 0.33, P = 0.5838) (Table 2). The largest number of eggs laid by *S. bosqueella* females was observed between days 2 and 7 in both cultivars and daily number of eggs varied from 7.4 to a maximum of 21 eggs/day in the IAC 503 genotype and from 1.6 to 27.2 eggs/day in the IAC OL3 (Figure 2). At the end of the 7th day, females of *S. bosqueella* accumulated a total of 98.80 and 106.40 eggs, on average, laid in both cultivars. In addition, for both genotypes, it was found that after the 7th day of oviposition the mean number of eggs per female decreased daily over time (Figure 2).

### *Spodoptera cosmioides*

*Spodoptera cosmioides* developed up to 7 instars under the different genotypes evaluated (Figure 1). The mean time of the 1$^{st}$ instar was longer in the IAC OL3 genotype ($F_{1,52}$=23.80, P<0.0001). The opposite was observed for the 3$^{rd}$ instar, when

*S. cosmioides* larvae presented higher developmental time in the IAC 503 in comparison to IAC OL3 ($F_{1,41}$=8.41, P=0.0060). However, for the other developmental stages, *S. cosmioides* larvae developed similarly in both genotypes (Figure 1).

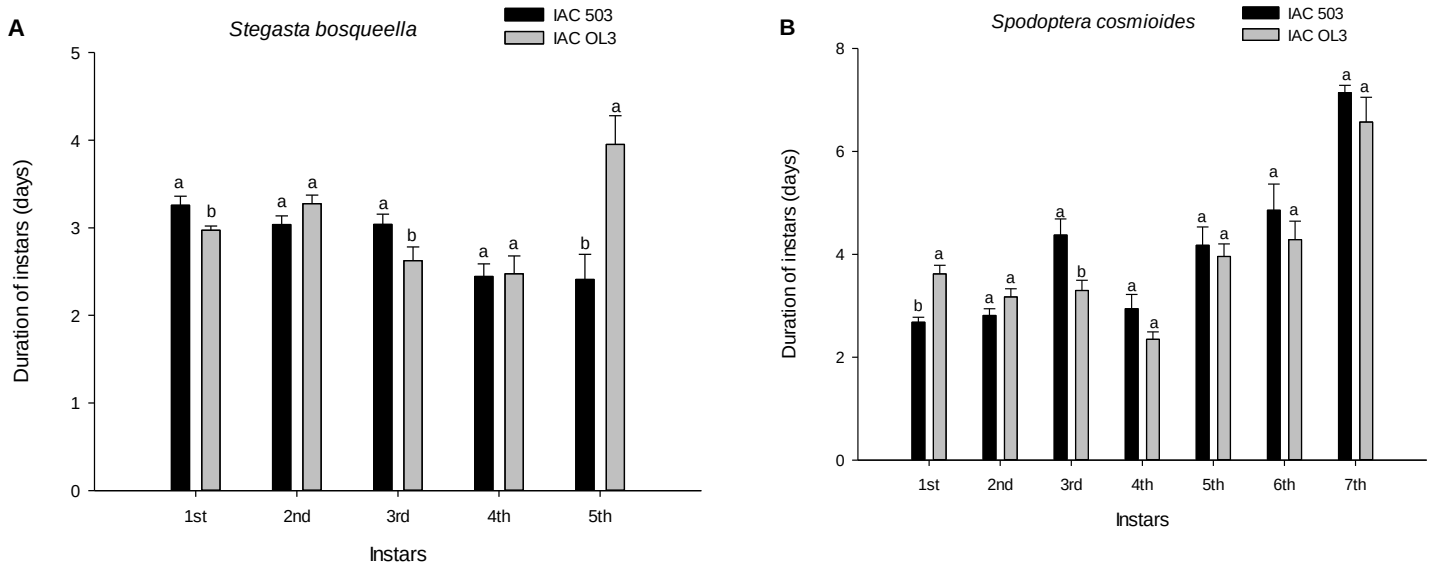


**Figure 1**. Mean time (days) (+EPM) of *Stegasta bosqueella* (A) and *Spodoptera cosmioides* (B) larval instars fed on IAC 503 and IAC OL3 genotype. Columns followed by the same letter are not different by Student F test (p > 0.05).

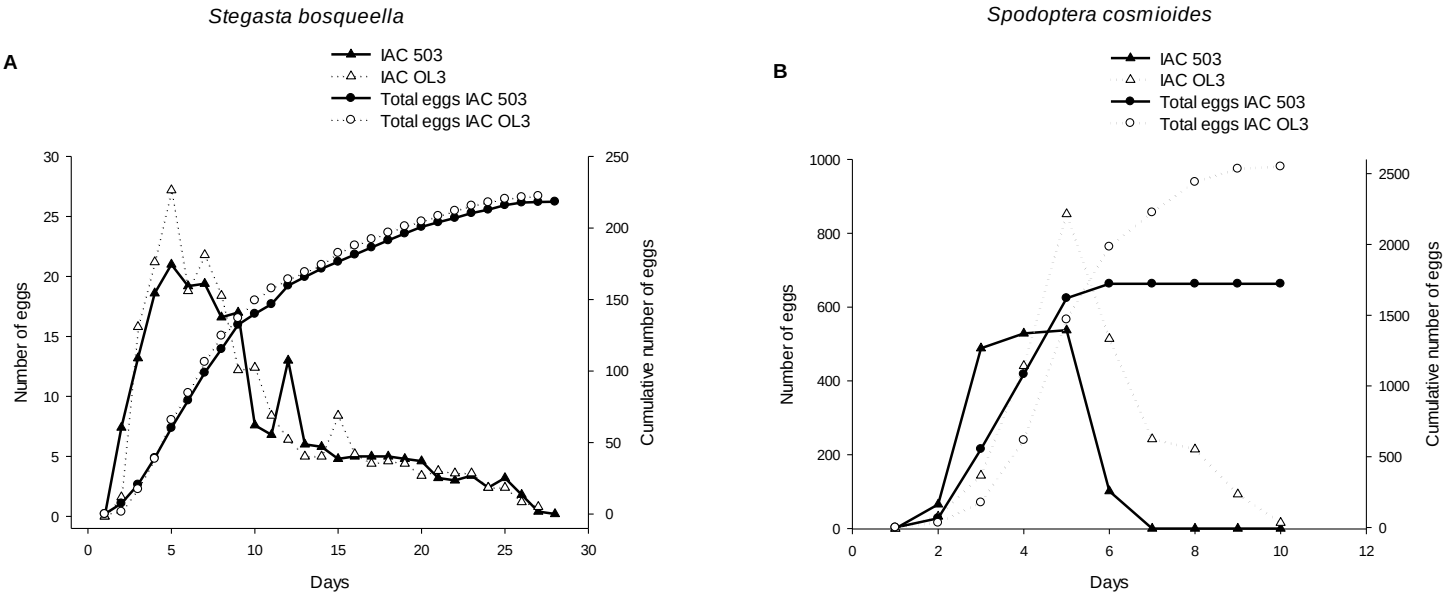


**Figure 2**. Mean number of eggs per day and total eggs per female of *Stegasta bosqueella* and *Spodoptera cosmioides* on IAC 503 and IAC OL3 genotypes.

For *S. cosmioides*, there was no significant difference between larval period ($F_{1,41}$ =3.71, P=0.0612), larval viability ($F_{1,118}$=3.72, P=0.0563), pupal period ($F_{1,28}$ = 1.05, P = 0.3148) and pupal viability ($F_{1,38}$=0.79, P=0.3796) under the different peanut genotypes evaluated (Table 1). We observed a sex ratio of 0.50 and 0.54 for IAC 503 and IAC OL3 respectively. Moreover, besides a low egg-adult viability of *S. cosmioides* 21.66±5.36 and 28.33±5.86 for IAC 503 and IAC OL3) was verified in both genotypes ($F_{1,118}$=0.70, P=0.4033), individuals who completed their development required approximately 46 days, on average, for the onset of adult emergence in both genotypes ($F_{1,28}$ =0.24, P=0.6303).

Longevity of *S. cosmioides* adults was shorter in IAC 503 ($F_{1,28}$=10.71, P=0.0028) (Table 2). Furthermore, the total life cycle (larval hatching to adult death) was greater in IAC OL3 in comparison to IAC 503 ($F_{1,28}$=4.77, P=0.0376) (Table 2). Significantly higher fecundity was found under the IAC OL3 genotype compared to IAC 503 ($F_{1,8}$=8.60, P=0.0189) (Table 2). Also, different from *S. bosqueella*, *S. cosmioides* females laid few eggs during the first days of life. *Spodoptera cosmioides* females presented a cumulative total of 1722±171.30 (IAC 503) and 2550±224.52 eggs laid (IAC OL3) after the 6th day of oviposition per female, on average. This total corresponds to 100 and 77.80% of the eggs laid by *S. cosmioides* females in the IAC 503 and IAC OL3 genotypes, respectively.

**Survival Analysis**

There was no significant difference between survival curves of *S. bosqueella* larvae developed under the different peanut genotypes according to the Wilcoxon test

(df = 1, $X^2$ = 2.1216, P = 0.1452) (Figure 3). In addition, there was a higher mortality in both genotypes during the first stages of development (1st and 2nd instars).

Similar results were observed for *S. cosmioides* larvae, in which both survival curves did not differ significantly by the Wilcoxon test (df = 1, $X^2$ = 1.0915, P = 0.2962) (Figure 3). Similarly to *S. bosqueella,* the highest mortality was concentrated in the early larval stages.

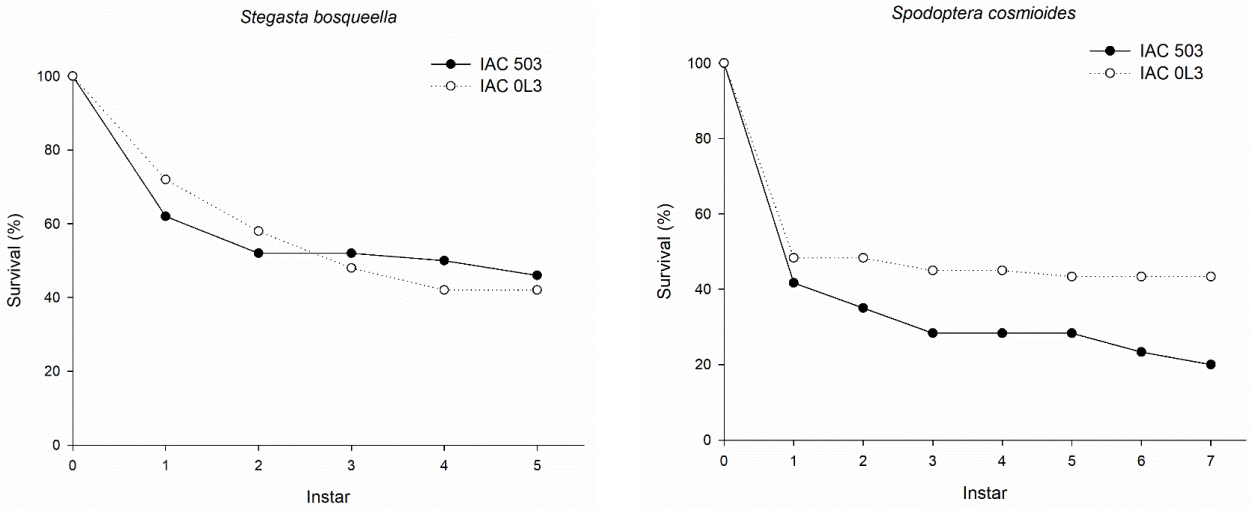


**Figure 3**. Survival curves of *Stegasta bosqueella* (A) and *Spodoptera cosmioides* larval instars fed on IAC 503 and IAC OL3 genotypes.

**Fertility life table analysis**

For *S. bosqueella*, the net reproduction rate (Ro) indicated that *S. bosqueella* when developed on the IAC 503 genotype may increase 57.78 times in each generation. This value was significantly 8.99 % higher than observed in the IAC OL3 genotype (Table 3). Furthermore, both the intrinsic population growth rate (rm) as well as the finite rate of increase (λ) and the mean time for population to double in number (TD) were also influenced by genotypes (Table 3). Higher values in IAC 503 were

observed when compared to IAC OL3. On the other hand, the average time between generations (T) was similar between genotypes (Table 3).

We verified *S. cosmioides* larvae (Table 3) developed on both peanut genotypes. Except for the average time between generations (T) which was not different, it was found that genotypes significantly affected reproduction and population development of *S. cosmioides*. The net reproduction rate (Ro) was 2.65 times higher in the IAC OL3 genotype compared to IAC 503. Similarly, the intrinsic population growth rate (rm) and the finite rate of increase (λ) were also greater in the genotype IAC OL3 (0.15 and 1.16 respectively). However, the mean time for population to double in number (TD) was longer when the IAC 503 genotype is the host plant.

**Table 3.** Parameters of the fertility life table of *Stegasta bosqueella* and *Spodoptera cosmioides* on IAC 503 and IAC OL3 genotypes[1].

| Parameters | *Stegasta bosqueella* | | *Spodoptera cosmioides* | |
|---|---|---|---|---|
| | **IAC 503** | **IAC OL3** | **IAC 503** | **IAC OL3** |
| $R_0$ | 57.78a | 48.79b | 223.86b[1] | 592.11a |
| | (52.62– 62.95) | (46.02–51.56) | (162.03– 285.7) | (447.18–737.04) |
| $r_m$ | 0.16a | 0.14b | 0.13b | 0.15a |
| | (0.15– 0.16) | (0.13– 0.15) | (0.12– 0.14) | (0.14– 0.16) |
| λ | 1.17a | 1.15b | 1.14b | 1.16a |
| | (1.16– 1.18) | (1.14– 1.16) | (1.13– 1.15) | (1.15– 1.17) |
| T | 25.19a | 26.89a | 40.94a | 42.19a |
| | (23.71– 26.66) | (24.52– 29.26) | (40.10– 41.79) | (40.28– 44.10) |
| TD | 4.30a | 4.79b | 5.23a | 4.57b |
| | (4.06– 4.53) | (4.38– 5.20) | (4.89– 5.58) | (4.25– 4.89) |

[1] Means (confidence interval at 95%) followed by the same letter within rows are not different by the Student t-test (P > 0.05).
$R_0 = \sum (lx.mx)$, female offspring per female per generation, when x = specific age, lx = proportion of mated females alive at age x, x = number of eggs produced per female at age x multiplied by the sex ratio (sex ratio: 0.54 and 0.53 for IAC 503 and IAC OL3 genotypes; $T = \sum (x.\ lx.mx) / \sum (lx.mx)$; $rm = \ln R_0/T$; $\lambda = erm$; $TD = \ln(2)/rm$. N = 50 individuals in each treatment.

## Foliage consumption

Except for the values observed during the 5th instar, the average leaf consumption by S*. bosqueella* larvae was significantly greater in the IAC 503 genotype (Figure 4). However, when observing the accumulated value for IAC 503 (4.56 ± 0.27 $cm^2$) and IAC OL3 (5.30 ± 0.39 $cm^2$), the consumption between genotypes is not different ($F_{1, 48} = 2.36$, P = 0.1313).

On the other hand, for *S. cosmioides*, it was found that except for the 1st, 3rd and 4th developmental stadia, no significant difference was observed in the average leaf consumption ($cm^2$) in both genotypes (Figure 4). Furthermore, as also observed for *S. bosqueella*, the accumulated consumption under the IAC 503 (143.53 ± 10.50 $cm^2$) and IAC OL3 (147.53 ± 9.54 $cm^2$) was not different ($F_{1,41} = 0.08$, P = 0.7996).

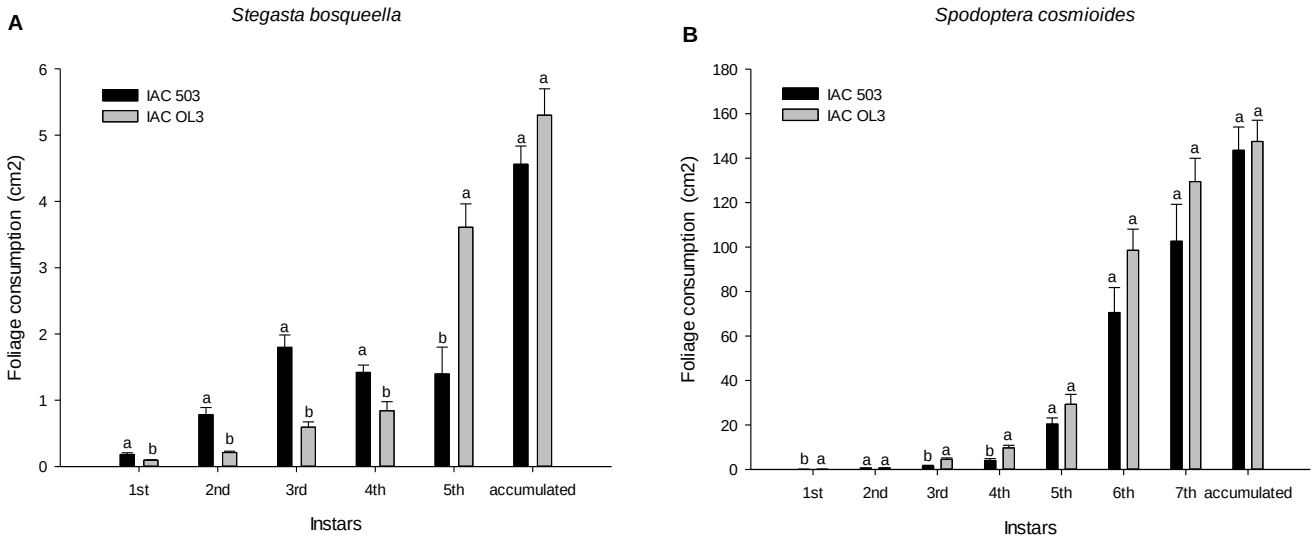


**Figure 4.** Mean leaf consumption (+EPM) of *Stegasta bosqueella* (A) and *Spodoptera cosmioides* (B) during larval period on peanuts genotypes.

## DISCUSSION

It was observed that the fertility and biological development of *S. bosqueella* and *S. cosmioides* are affected according to the peanut genotype. High larval mortality (more than 50%) was verified during *S. cosmioides* 1st instar in both peanut genotypes. However, in both genotypes, no significant difference was observed between *S. bosqueella* and *S. cosmioides* larval survival. The foliage consumption potential ($cm^2$) for *S. cosmioides* is 31.5 and 27.8-fold greater, than that observed in *S. bosqueella*, when submitted to IAC 503 and IAC OL3 genotypes, respectively. Therefore, this work provides novel information about the biotic potential and reproductivity parameters for *S. bosqueella* and *S. cosmioides* in peanuts, as well as the foliage consumption potential for both species. Furthermore, the quantification of foliage consumption and fertility as well as the biological development are crucial for peanut entomologists and growers, as bases for improving the decision-making process in IPM. In addition, this knowledge will contribute to the development of economic thresholds for defoliators in peanuts.

The highest larval mortality of *S. bosqueella* and *S. cosmioides* was concentrated in the early larval stages. According to Zalucki et al. (2002), mortality of lepidopterous foliage feeders is high during the first instar; however, early-stage mortality can be related to a combination of factors (e.g., weather, plant's integument, allelochemicals). In this context, the authors suggested that the degree of host "specialization" can be crucial for early-stage survivorship. Therefore, the fact that *S. bosqueella* is a specialist insect pest in peanuts can explain its higher egg-adult viability (1.84 and 1.34), when compared to *S. cosmioides*. In addition, the duration of first instars (1st and 2nd) was greater for *S. bosqueella* under IAC 503, while the opposite was verified for *S. cosmioides* (longer duration in IAC OL3). A

long time duration in the early-stage among the host plants is essential information for the pest management perspective, since most entomopathogens (e.g. *Bacillus thuringiensis* and virus) are more efficient when directed to first larval instars (Fiuza et al. 2017; Lacey 2017) and they can be applied for *S. bosqueella* and *S. cosmioides* management (Silva et al. 2020; Pinto et al. 2020b).

Moreover, host plants are important components of insect pest outbreaks (Barbosa et al. 2012) and the biological development of a particular insect can be used as a parameter to measure the suitability of the host plant to herbivores (Saeed et al. 2010a). For *S. bosqueella*, we observed that the larvae developed faster on IAC 503 and pupated compared to the IAC OL3 genotype. A higher development rate for *S. bosqueella* in the IAC 503 genotype had already been reported (Di Bello et al. 2015). In contrast, for *S. cosmioides*, IAC OL3 genotype is more suitable for its development as we verified greater fertility parameters. This variation observed between peanut genotypes can occur because the host quality affects key aspects of insect development, such as food acquisition, fecundity, longevity, and survival (Awmack and Leather 2002; Dogar et al. 2018). Moreover, the host plant components (e.g., the levels of nitrogen, carbon and defensive compounds) can negatively affect the feeding and performance of defoliators (Awmack and Leather 2002; Mbande et al. 2019). Unfortunately, in the present work, no chemical analyses of the cultivars were carried out to allow conclusions on these aspects, and further studies should be performed to investigate differences in chemical components between peanut genotypes.

Similarly, factors related to insect development, such as survival and fecundity parameters, on specific host are very important, since the developmental time can accelerate the population growth and might reduce generation time, which directly

affects the insect population dynamics (Schowalter 2016; Golizadeh et al. 2017; Truzi et al. 2017). Using the fertility life table, which is a criterion for evaluating factors related to insect development, we verified that when submitted to IAC 503, *S. bosqueella* has the probability of having more generations per season and greater intrinsic rate of increase ($r_m$). In contrast, *S. cosmioides* presented a higher intrinsic rate of increase ($r_m$), and almost 3-fold in number of times in which the population increases with each generation ($R_0$) on IAC OL3 in comparison to IAC 503. The life table parameters, especially net reproduction rate ($R_0$) and intrinsic rate of increase ($r_m$), have been used to evaluate the performance of pest populations in a specific host, because they reproduce the general effect of developing and survival rates, as well as the fecundity (population fitness) in different host plants. In this context, higher values of $R_0$ and $r_m$ indicate the susceptibility of phytophagous to a host plant to their attacks, whereas lower values show that the host plant can be resistant (Sedaratian et al. 2011; Golizadeh et al. 2017; Scheunemann et al. 2019). Therefore, according to our findings, IAC 503 is a better host for *S. bosqueella*, while IAC OL3 is more suitable for *S. cosmioides*. Therefore, this knowledge can help in the selection of peanut genotypes (IAC 503 or IAC OL3), according to the historical infestation of a specific larval species. Or else, growers can anticipate major species outbreaks based on the genotype chosen.

To develop the economic injury levels (EILs) as well as to contribute for accurate pest sampling programs, the information of feeding consumption is essential (Higley and Pedigo 1996; Kogan 1998; Pedigo and Rice 2014). For *S. bosqueella*, we observed that to complete the total development on both peanut genotypes, the rednecked peanutworm requires to consume 4.56 to 6 $cm^2$ of peanut leaflets. In contrast, the foliage consumption potential ($cm^2$) for *S. cosmioides* was

31.5 and 27.8-fold greater than the observed in *S. bosqueella*, for IAC 503 and IAC OL3 genotypes, respectively. The average amount of leaf consumed by *S. cosmioides* on IAC 503 and IAC OL3 (143.53±10.50 $cm^2$ and 147.53±9.54 $cm^2$, respectively) is also greater compared to other *Spodoptera* species in peanuts, such as *Spodoptera frugiperda* (J.E. Smith 1797) which was 94.56 $cm^2$ per larva (Garner and Lynch 1981) . Our results verified that the defoliation capacity by *S. bosqueella*, in comparison to another *Spodoptera* species in peanuts, is much lower. However, besides the reductions caused by defoliation, *S. bosqueella* has the potential to damage peanut plant buds (Wall and Berberet 1979; Pinto et al. 2020a) and it has to be considered for the pest management strategy, as well as in economic thresholds development.

Likewise, there is a large difference in the biological development parameters between both species. *Spodoptera cosmioides* needs almost twice the time (1.77 and 1.5) to reach adulthood in both peanut genotypes in comparison to *S. bosqueella*. However, the potential number of eggs laid by *S. cosmioides* is 7.8 and 11.4 times greater (1722 and 2550) than the values observed for *S. bosqueella* (218.40 and 223.20) in both peanut genotypes. In addition, for *S. bosqueella* or *S. cosmioides*, newly emerged adults (up to 7 days) have the potential to deposit the largest egg load.

This information about the fecundity can be used as the basis for adult control techniques (e.g., attract and kill and mating disruption) application. The goal of these strategies depends directly on a high reduction in the number of eggs laid into the crop (Gregg et al. 2018; Benelli et al. 2019). Therefore, according to our results, the impact of these control techniques can be increased if directs the application to the newly emerged adults.

Our results highlight the value of understanding *S. bosqueella* and *S. cosmioides* life cycles in order to estimate the biotic potential which is necessary to improve pest management control strategies. It is well known that understanding pest population development on different hosts have practical implications for the management of insect pests (Saeed et al. 2010b). Our study indicates that *S. bosqueella* development was higher under IAC 503; however, for *S. cosmioides*, IAC OL3 provided better resources for larval development. Therefore, our results are an important step for a better comprehension of *S. bosqueella* and *S. cosmioides* population dynamics, as well as the basis for EILs thresholds development to aid the adoption of peanut sampling programs within an IPM approach.

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# CHAPTER IV - DETECTION OF DEFOLIATION INJURY IN PEANUT WITH HYPERSPECTRAL PROXIMAL REMOTE SENSING

**RESUMO -** O sensoriamento remoto pode ser aplicado para otimizar a eficiência na detecção de pragas, como ferramenta de amostragem de insetos. Essa eficiência pode resultar em recomendações mais precisas para a tomada de decisão no manejo de pragas. A detecção de pragas com sensoriamento remoto geralmente é viável porque o estresse biótico das plantas causado pela herbivoria desencadeia uma resposta fisiológica defensiva nas plantas, que geralmente resulta em alterações na reflectância das folhas. Portanto, o objetivo principal deste estudo foi usar o sensoriamento remoto proximal hiperespectral e parâmetros de troca gasosa para caracterizar as respostas das folhas de amendoim à herbivoria por *Stegasta bosqueella* (Lepidoptera: Gelechiidae) e *Spodoptera cosmioides* (Lepidoptera: Noctuidae), duas principais pragas do amendoim na América do Sul. (*Arachis hypogaea*). O experimento foi conduzido em delineamento de blocos casualizados com esquema fatorial 2x3 (duas espécies de lepidópteros e 3 categorias de lesão). Os tratamentos foram: 1) infestação natural por 3º. instar de *S. bosqueella*, 2) infestação natural por 3º. instar de *S. cosmioides* e 3) simulação de lesão com tesoura para mimetizar lesão das lagartas. Verificamos que a reflectância da folha de amendoim é diferente entre a herbivoria das duas espécies de larvas, mas semelhante entre a desfolha real e simulada. Da mesma forma, observamos diferenças na taxa fotossintética, condutância estomática, transpiração e eficiência fotossintética do uso da água apenas entre as espécies, mas não entre a desfolha larval real e simulada. Nossos resultados fornecem informações essenciais para o desenvolvimento da amostragem e dos limiares econômicos de *S. bosqueella* e *S. cosmioides* em amendoim.

**Palavras-chave:** Sensoriamento remoto, lepidópteros desfolhadores, herbivoria, estresse biótico, MIP amendoim, manejo de pragas de precisão.

# CHAPTER IV - DETECTION OF DEFOLIATION INJURY IN PEANUT WITH HYPERSPECTRAL PROXIMAL REMOTE SENSING

**ABSTRACT -** Remote sensing can be applied to optimize efficiency in pest detection, as an insect sampling tool. This efficiency can result in more precise recommendations for decision making in pest management. Pest detection with remote sensing is often feasible because plant biotic stress caused by herbivory triggers a defensive physiological response in plants, which generally results in changes to leaf reflectance. Therefore, the key objective of this study was to use hyperspectral proximal remote sensing and gas exchange parameters to characterize peanut leaf responses to herbivory by *Stegasta bosqueella* (Lepidoptera: Gelechiidae) and *Spodoptera cosmioides* (Lepidoptera: Noctuidae), two major pests in South American peanut (*Arachis hypogaea*) production. The experiment was conducted in a randomized complete block design with a 2x3 factorial scheme (two lepidopterous species and 3 categories of injury). The injury treatments were: 1) natural infestation by 3rd instars of *S. bosqueella*, 2) natural infestation by 3rd instars of *S. cosmioides*, and 3) simulation of injury with scissors to mimic larval injury. We verified that peanut leaf reflectance is different between herbivory by the two larval species, but similar among real and simulated defoliation. Similarly, we observed differences in photosynthetic rate, stomatal conductance, transpiration, and photosynthetic water use efficiency only between species but not between real and simulated larval defoliation. Our results provide information that is essential for the development of sampling and economic thresholds of *S. bosqueella* and *S. cosmioides* on peanuts.

**Keywords:** Remote sensing; lepidopteran defoliator; herbivory; biotic stress; peanut IPM; precision pest management.

## INTRODUCTION

Producing more food without increasing the area of production is one of the great challenges for agriculture in the coming decades. By 2050, demand for food may increase by 70% to support the world population (FAO 2009). To meet this demand, productivity will need to be increased, guided mainly by technological innovations in production factors such as more efficient use of inputs (FAO 2009). The search for techniques to increase production is urgent, in particular those involved with minimizing damage associated with biotic (e.g. insect pests, pathogens, weeds) and abiotic factors (e.g. relative humidity, temperature, precipitation) (EMBRAPA 2014, Deutsch et al. 2018).

Precision agriculture strategies (e.g. the integration of different sensors, information, and management systems) can promote the efficient use of inputs and assist in decision making. Consequently, precision strategies may reduce environmental and economic impacts on production systems (Gebbers and Adamchuk 2010, Liu et al. 2017). Advanced remote sensing tools may enhance the adoption of Integrated Pest Management (IPM) programs (Behmann et al. 2015, Nansen and Elliott 2016, Liu et al. 2017). The progress in sensor technology, such as multi-spectral and hyperspectral, can provide high resolution data and offers new opportunities of remote sensing application in different crop systems. So, because remote sensing techniques are of extreme importance to attain sustainability in agriculture, this strategy has been inspected around the world in automating or supplementing IPM programs (Behmann et al. 2015, Nansen and Elliott 2016). In IPM, decision making about pests is often based on a population survey of pests, which then makes it possible to verify whether there is a need for management, and also to prioritize the types of pests to be managed (Pedigo et al. 1986, Pedigo and

Rice 2014). Remote sensing, as an insect sampling tool, can also optimize efficiency in pest detection, with precise recommendations for decision making in pest management (Hatfield and Pinter 1993, Hatfield et al. 2008, Hunt and Rondon 2017, Liu et al. 2018, Abd El-Ghany et al. 2020).

Pest detection with remote sensing is feasible because plant biotic stress caused by herbivory, for example, triggers a defensive physiological response in plants, which generally results in changes to leaf reflectance (Prabhakar et al. 2012, Behmann et al. 2015). Additionally, biochemical changes during plant growth, such as concentration of chlorophyll and water, affect the amount of electromagnetic radiation that is reflected, absorbed, or transmitted by plants (Carter and Miller 1994, Lillesand et al. 2004). Therefore, because insect defoliation it is a common form of herbivory, and it can alter the plant gas exchange parameters by removing photosynthetic tissue, understanding how herbivory influences key photosynthetic processes such as water-vapor transfer, respiration, and water-use efficiency is critical because these processes determine plant growth, development, and fitness (Peterson 2001, Schwachtje and Baldwin 2008). Furthermore, herbivory can represent short-and long-term consequences for plants and the magnitude of these consequences will vary depending on the type, timing, and intensity of the injury as well as plant tissues injured (Peterson 2001). So, investigating how plants respond to multiple species injury is essential.

The physiological mechanisms underlying peanut (*Arachis hypogaea*) responses to arthropod injury are poorly understood (War et al. 2012, Pinto et al. 2020). Furthermore, pest sampling in peanut crops is still carried out manually, making it extremely labor-intensive and costly (Almeida 2015, Abbott et al. 2019,

Pinto et al. 2020), leading to low adoption rates by producers which affects the sustainability of the peanut industry.

Therefore, in light of the worldwide importance of peanut (e.g. improving soil properties through atmospheric nitrogen fixation, and a functional food with high nutritional components) (Akram et al. 2018), the key objective of this study was to characterize peanut leaf responses to herbivory by *Stegasta bosqueella* (Chambers, 1875) (Lepidoptera: Gelechiidae) and *Spodoptera cosmioides* (Walker, 1858) (Lepidoptera, Noctuidae), two of the major pests in South American peanut production (Campos et al. 2011, Boiça Júnior et al. 2013, Pinto and Fernandes 2020). These larvae can occur in high abundance and caution is needed because they can cause intense defoliation with direct effect on plant development. In addition to defoliation, *S. bosqueella* can also feed directly on peanut buds affecting their growth and, therefore, peanut production. To accomplish this objective, we analyzed hyperspectral proximal remote sensing data for injured peanut leaves and collected information about leaf gas exchange response to real and simulated larval-induced herbivory. Since high spectral resolution requires advanced methods of data analysis (Behmann et al 2015) our study focused on the application of advanced machine learning algorithms such as Random Forest Classification and The Classification Tree analysis to comply with these demands. Understanding how herbivory by different organisms affects plant physiology is essential for grouping species in sampling programs that cause similar injuries (e.g., injury guilds), and to establish recommendations for the management of each species (Peterson 2001). Because this is the first study to characterize the effect of *S. bosqueella* and *S. cosmioides* on peanut plant reflectance and gas exchange, and it is well established that plant responses to insect injury are highly influenced by the intensity of injury,

the type of injury, and environmental factors factors (Peterson et al. 1992a, Macedo et al. 2005, 2007), our study was conducted in a greenhouse, where abiotic conditions can be readily monitored and controlled, allowing for more accurate characterization of the effects of herbivory.

## MATERIAL AND METHODS

### Experimental design

The experiment was conducted in a randomized complete block design with a 2x3 factorial scheme (two lepidopterous species and 3 categories of injury [insect-related injury, simulated injury and control]). The injury treatments were: 1) Natural infestation by 3rd instars of *S. bosqueella*, 2) Simulation of injury with scissors to mimic *S. bosqueella* larval injury, 3) Natural infestation by 3rd instars of *S. cosmioides*, 4) Simulation of injury with scissors to mimic *S. cosmioides* injury and 5) Non-Injured (control). Third instars were used because they cause representative and sufficient injuries that allow for measurement and evaluation (unpublished data). Each treatment consisted of ten 5-liter pots (replicates) containing a peanut plant 30 days after emergence (DAE).

### Test plants and insects

Studies were carried out with peanut cultivar IAC 503, which is a major peanut cultivar currently used in Brazil. IAC 503 belongs to the runner vegetative group with a “high oleic” characteristic (Godoy et al. 2018). For the experiments, peanut plants were grown in individual 5-liter pots containing a mixture of soil, sand, and cattle manure in a 1:2:1 proportion. Initially, 5 seeds were placed in each pot, and seedlings

were thinned after plant emergence, so that each pot contained only a single plant. Peanut plants were kept in an air-conditioned greenhouse facility (3.50 x 6.35 m in diameter, 25 ± 10 °C, RH 70 ± 10%, and natural light) at São Paulo State University, São Paulo, Brazil. Plants were irrigated daily using the same amount of water per plant.

In Brazil, both *S. bosqueella* and *S. cosmioides* prefer to feed on young peanut leaves, therefore, for insect colony establishment, planting was carried out weekly to ensure continuous availability of peanut leaflets. The *S. bosqueella* population (approximately 150 adults) was collected in a commercial peanut field located in Jaboticabal, São Paulo, Brazil, brought to the laboratory and kept in acrylic cages (30 × 30 × 30 cm) under controlled temperature conditions (12H:12D photoperiod, 25 ± 2 °C, and 60 ± 10% RH), according to Boiça Jr. et al. (2011). Therefore, the cultivar IAC 503 leaflets were used as feeding and oviposition substrates. The initial *S. cosmioides* colony (approximately 200 specimens) was provided by the Insect Biology Laboratory of the Superior School of Agriculture “Luiz de Queiroz”, São Paulo University, Piracicaba, state of São Paulo, Brazil and it was kept in the same controlled temperature conditions as *S. bosqueella*. Also. similar to *S. bosqueella*, IAC 503 leaflets were used as feeding and oviposition substrates. For our study, the F2 generation was used.

**Injury treatments**

Natural defoliation was performed 30 DAE based on the methodology of Delaney et al. (Delaney et al. 2008). Leaf cages composed of transparent plastic cylinders (6 cm in height x 4 cm in diameter) were used to restrict larvae to a single freshly opened leaflet and each cage was sealed with cardboard (5 x 5 cm in diameter)

(Figure 1, E). The same leaf position was adopted for all treatments. All larvae were removed after 24 h, while primary-leaf photosynthetic measurements were recorded 24 and 48 h after herbivores were introduced. The simulated herbivory (Figure 1, B and D) was carried out by removing part of the peanut leaflet corresponding to the injury observed in the natural defoliation caused by the larvae. This simulated larval defoliation was performed after removal of leaf cages with the aid of scissors in the same position as the injury caused by both *S. bosqueella* and *S. cosmioides* (Figure 1, A and C). In addition, control plants were used for all treatments. All treatments received leaf cages to control for any cage-effects on the experiments.

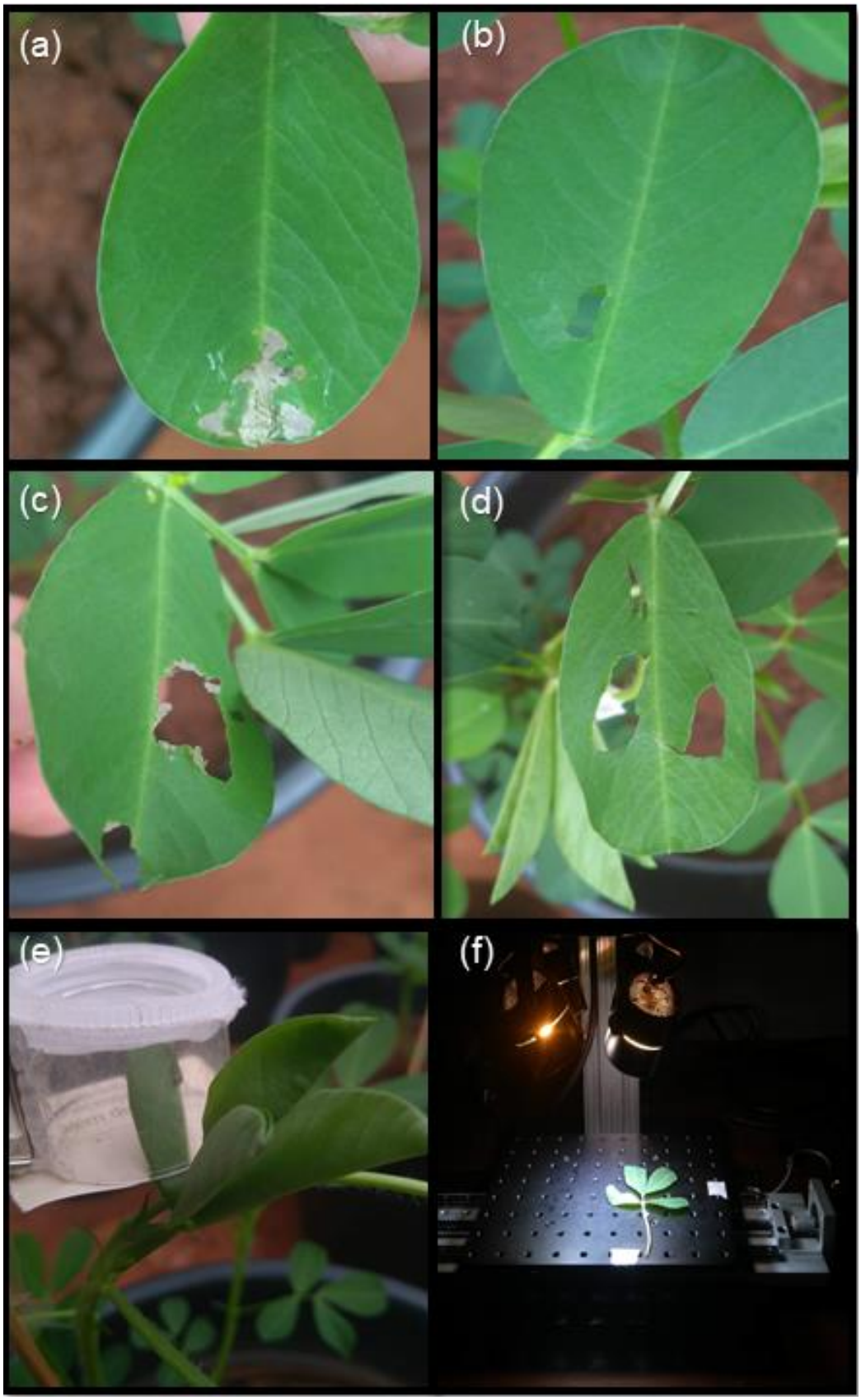


**Figure 1**. Types of defoliation caused by insects or simulated on peanuts. (**a**) *Stegasta bosqueella* larval herbivory, (**b**) *Stegasta bosqueella* simulated defoliation, (**c**) *Spodoptera cosmioides* larval herbivory, (**d**) *Spodoptera cosmioides* simulated defoliation (**e**) Larvae restricted to a single freshly opened leaflet and (**f**) hyperspectral image collection set up.

**Gas exchange measurements**

Gas exchange parameters were collected 24 h and 48 h after herbivore infestation. The following parameters were measured: intercellular $CO_2$ concentration (Ci) (µmol $CO_2$ $mol^{-1}$), assimilation/respiration (A) (µmol $CO_2$ $m^{-2}$ $s^{-1}$), stomatal conductance (GS) (mmol $H_2O$ $m^{-2}$ $s^{-1}$), transpiration (E) (mmol $H_2O$ $m^{-2}$ $s^{-1}$), leaf to air vapor pressure deficit (VPD) (kPa), photosynthetic water use efficiency (WUE) (mmol $CO_2$ $mol^{-1}$ $H_2O$) and leaf temperature (TLEAF) (ºC) through the CIRAS-3 Portable Photosynthesis System (PP Systems®, Amesbury, Massachusetts, USA). The $CO_2$ reference concentration and light intensity inside the leaf chambers were kept constant at 1500 µmol $m^{-2}$ $s^{-1}$ and 400 µmol $CO_2$ $mol^{-1}$, respectively.

**Hyperspectral remote sensing**

Hyperspectral remote sensing data were acquired from peanut plants (n = 50 leaflets) 72 h after treatment infestation. Plants were transferred to a climate-controlled room without artificial lighting (to ensure only camera lighting), where the hyperspectral imaging was performed using a benchtop system. Ambient climate conditions were 22-25 °C and 50-60% relative humidity. Acquisition of hyperspectral remote sensing data was performed from 8:00-11:00 am using a hyperspectral camera (PIKA L, Resonon Inc., Bozeman, Montana, USA) which acquires reflectance data in the 400-1000 nm spectral range. This camera has a 3.7 nm spectral resolution (Full Width Half Maximum - FWHM) and 281 spectral channels. The calibration surface used as a white reference was made of polyethylene plastic (Type 822, Spectronon Pro, Resonon, Bozeman, Montana, USA). The software

Spectronon Pro™ was used for camera control, data acquisition, and data processing.

We carefully dissected the portion of the plant where the injury occurred (Figure 1, F), and hyperspectral imaging was completed within 1-2 min. For this, with the aid of Spectronon Pro™ software, we manually collected the average reflectance by drawing a rectangle (length 1 cm x width 0.8) and mining the data only in the area of interest, thus ensuring greater homogeneity of data and the extraction of spectral responses. Thus, only the specific herbivory-injured leaflet information was evaluated per plant and used for data analysis.

**Data analysis**

For hyperspectral remote sensing data, univariate feature selection based on ANOVA F-value scores was used to create a variable importance plot to highlight the spectral regions useful in distinguishing between injured and non-injured (control) leaves. For this analysis, we used the “sklearn.feature_selection_Select KBest” library in Scikit-learn: Machine Learning in Pythonv3.6. (Pedregosa et al. 2011)

To test for treatment effects on peanut leaf reflectance, we used the near-infrared (NIR) spectral range (777.42-1000 nm) because it exhibited separation in sample reference spectra (Figure 2) and is sensitive to variation in plant stressors (Figure 3). For this, we performed comparative contrast analysis between groups using an F test ($p < 0.05$) (PROC MIXED) (SAS Institute 2015).

To evaluate the accuracy of prediction in discerning *S. bosqueella*, *S. cosmioides,* and uninfested leaves, a Random Forest Classification was performed using the Scikit-learn: Machine Learning in Pythonv3.6. (Pedregosa et al. 2011).

Random Forest is one of the more successful ensemble methods for pattern detection in a dataset as it conducts a random selection of all possible explanatory variables (here, hyperspectral bands) at each split (Breiman 2001). Due to the lack of experimental replicates in our study, the desire to reduce noise, and to respect the principles of parsimony and the Hughes Phenomenon (Hawkins 2004, Nansen and Elliott 2016), we used principal component analysis (PCA) along with Random Forest classification as a dimension reduction tool for all spectral bands (n=150) collected from the hyperspectral data. The main purpose in using PCA was to summarize all spectral dimensions (features) into three principal components, without losing information. The Random Forest Classification parameters were selected by a standard 5-StratifiedKFold cross-validation with GridSearchCV from Sklearn.model_selection library using Pythonv3.6. To evaluate the model robustness, we estimated the overall accuracy and the Kappa statistic from our Random Forest Classification model, using 70% of the data for training and 30% for validation (Adelabu et al. 2015).

To evaluate the effect of different larval species and herbivory-injury methods in peanut leaf gas exchange, we applied the multiple comparison Tukey test (p-value $< 0.05$) (PROC MIXED) (SAS Institute 2015). Similar to the hyperspectral analysis, we applied comparative contrast analysis between groups with an F test ($p < 0.05$), using the gas exchange parameters as explanatory variables to represent the variation between classes (injury categories). Aiming to understand peanut leaf gas exchange response to larval-induced herbivory, we developed a classification tree model with only injured and non-injured data as the response variable. We used the Sklearn.tree library to create the classification tree and a standard 5- StratifiedKFold cross-validation with GridSearchCV to define the maximum depth of the tree, the

minimum number of samples required to split an internal node and the function to measure the quality of a split. The classification tree analysis, a non-parametric statistical modeling technique, is widely used to identify a set of characteristics (here, gas exchange variables) that best distinguish individuals based on a categorical outcome variable (here, larval-induced-herbivory) (James et al. 2013). Therefore, we adopted the photosynthetic parameters assimilation/respiration (A- photosynthetic rate), stomatal conductance (GS), transpiration (E) and photosynthetic water use efficiency (WUE) as explanatory (predictor) variables.

## RESULTS

### Hyperspectral images

We verified that the average reference spectral signature for the different treatment groups illustrated the spectral regions that were crucial in their distinction and classification with low standard deviation (Figure 2, Appendix A). The reflectance profiles for each of the treatment groups appeared to be similar in the visible wavelengths, although spectral deviations occurred above 700 nm in the near-infrared (NIR) region. The spectral profile for control leaves exhibited the lowest values between 777.42-1000 nm, in contrast to an increase in reflectance in the stressed treatment classes. We also observed that the stressed classes based on the actual injury corresponding to each larval species exhibited different spectral responses in the NIR region. However, the simulated injury appeared to be similar to the injury made by the real larvae. Furthermore, the variable importance plot based on ANOVA F-values scores confirmed that the 777.42-1000 nm portion of the spectrum was crucial for spectral distinction among treatment groups (Figure 3).

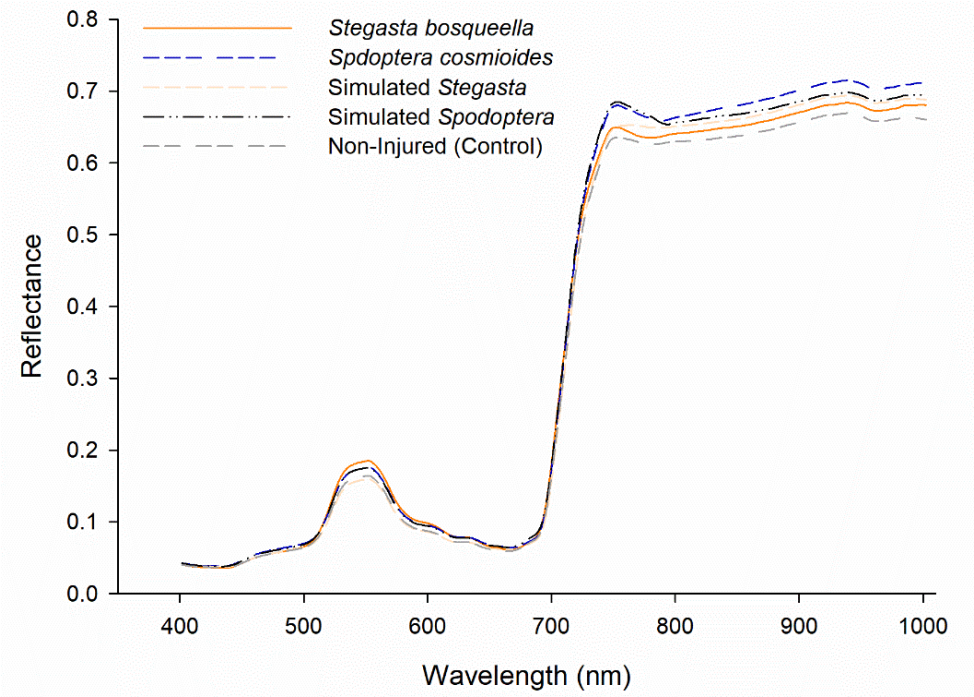


**Figure 2.** Average reference spectral reflectance for each of the five treatment groups.

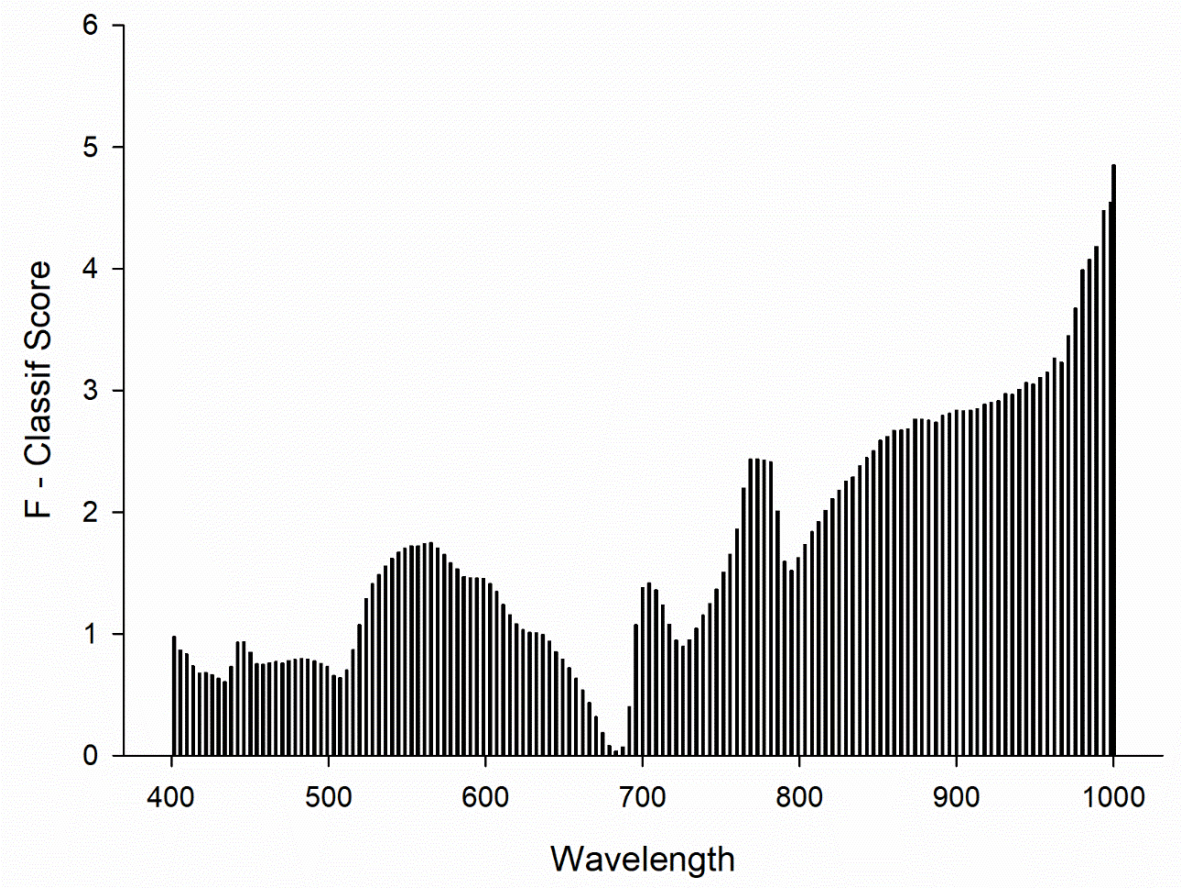


**Figure 3.** Variable importance plot based on ANOVA F-values scores.

However, because one of our main goals was to determine if leaf reflectance was different among larval species and injury categories, we performed contrast analysis with the 700-1000 nm spectral reflectance data. The contrast analysis showed that there was a difference between species' reflectance in the NIR region (777.42-1000 nm) ($F_{5, 3354}$ = 167.46, P= < 0.0001). However, there was no difference in peanut leaf reflectance between simulated or real injury ($F_{5, 3354}$ = 0.55, P= 0.4566). Conversely, peanut leaf reflectance changed when we compared injured vs non-injured leaves ($F_{5, 3354}$ = 356.40, P= < 0.0001) (Figure 2).

Our second goal was to build a Random Forest model to discern *S. bosqueella*, *S. cosmioides*, and uninfested leaves. Therefore, we performed PCA to condense the multidimensional spectral data into a three-dimensional environment (Figure 4). Principal components 1, 2, and 3 explained most of the variation in the data (95.52%), with PC1 responsible for 74% of the explained variance (Figure 4). For the Random Forest Classification model, using 70% of the dataset as training data and 30% as validation data, larval injury was classified with an overall accuracy of 92% and a Kappa statistic of 0.83 (Table 1). These results indicate that based on the 9 samples used for validation, our model correctly classified 8 of them.

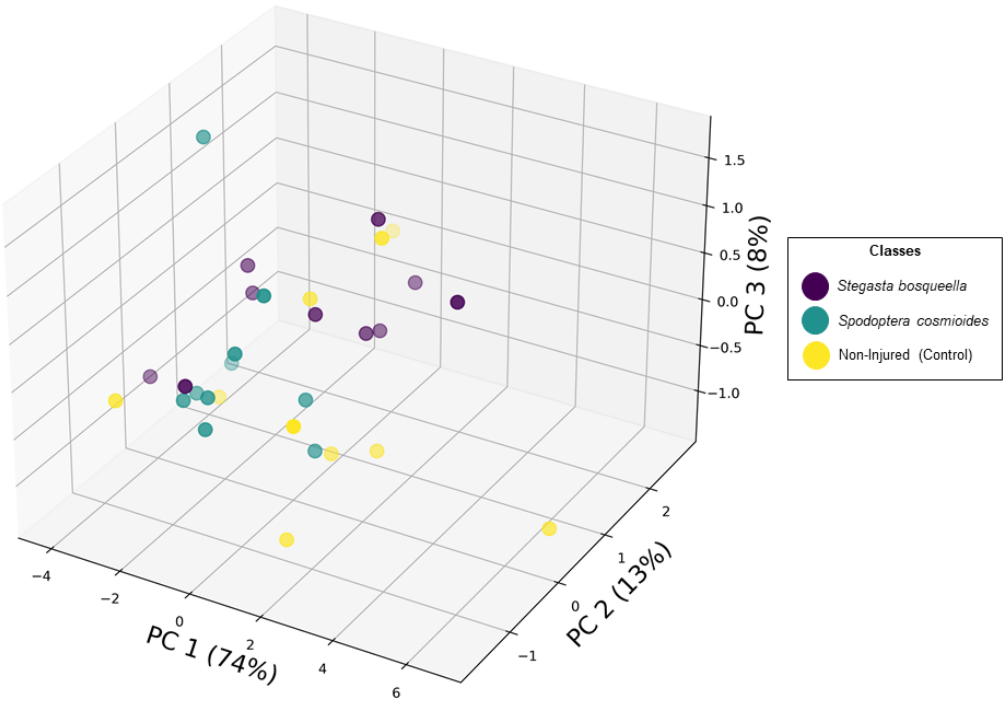


**Figure 4.** Principal component analysis representation of spectral reflectance data deriving from larvae stressed and non-injured peanut leaves.

**Table 1.** Confusion matrix for Random Forest Classification model.

| Treatments | Predicted Class | | | Classification overall | User Accuracy | Overall Accuracy |
|---|---|---|---|---|---|---|
| | *Stegasta bosqueella* | *Spodoptera cosmioides* | Non-Injured (Control) | | | |
| *Stegasta bosqueella* | 3 | 0 | 0 | 3 | 100% | 92% |
| *Spodoptera cosmioides* | 0 | 2 | 1 | 3 | 75% | |
| Non-Injured | 0 | 0 | 3 | 3 | 100% | |
| Truth overall | 3 | 2 | 4 | 9 | | |
| Kappa Statistic | 0.83 | | | | | |

## Gas exchange responses to herbivory measurements

Neither injury treatment significantly affected peanut leaf electrical conductivity in the primary-leaf photosynthetic measurements recorded 24 h after infestation (Table 2). Also, 24 h after the infestation, the contrast analysis showed no difference in injury caused by *S. bosqueella* and *S. cosmioides* and between real herbivory and simulated leaf injury gas exchange responses parameters (Table 2).

Treatment effects on peanut leaf gas exchange were only observed 48 h after the infestation (Table 3). Herbivory by *S. bosqueella* caused a low impact on peanut gas exchange and the photosynthetic rates were similar to the control. In contrast, injury by *S. cosmioides* reduced the photosynthetic rate by 3.97 μmol $CO_2$ $m^{-2}$ $s^{-1}$ compared to non-injured leaves. Contrast results showed differences in photosynthetic rate, stomatal conductance, transpiration, and photosynthetic water use efficiency only between species (Table 3). Similar to the hyperspectral image analysis, we verified that real and simulated injuries presented similar effects on peanut leaf gas exchange.

**Table 2.** The mean (± S.E.) effect of treatments on gas exchange parameter performance and treatment contrast for 24 hours after injury. Bolds value parameters indicate significant differences.

| | 24 hours after infestation | | | | | | |
|---|---|---|---|---|---|---|---|
| **Treatments** | **[1]A** ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) | **[2]Ci** ($\mu mol\ CO_2\ mol^{-1}$) | **[3]E** ($mmol\ H_2O\ m^{-2}\ s^{-1}$) | **[4]GS** ($mmol\ H_2O\ m^{-2}\ s^{-1}$) | **[5]Tleaf** ($^oC$) | **[6]VPD** (kPa) | **[7]WUE** ($mmol\ CO_2\ mol^{-1}\ H_2O$) |
| *Stegasta bosqueella* | 21.06 ± 2.67 a | 843.50 ± 15.06 a | 4.63 ± 0.33 a | 494.80 ± 76.17 a | 25.85 ± 0.22 a | 1.20 ± 0.09 a | 4.43 ± 0.40 a |
| *Spodoptera cosmioides* | 19.25 ± 1.53 a | 846.40 ± 10.12 a | 4.53 ± 0.27 a | 427.20 ± 54.21 a | 26.02 ± 0.18 a | 1.27 ± 0.07 a | 4.22 ± 0.17 a |
| Simulated *Stegasta bosqueella* | 19.46 ± 2.11 a | 860.90 ± 8.45 a | 5.03 ± 0.43 a | 611.30 ± 92.76 a | 25.88 ± 0.19 a | 1.14 ± 0.11 a | 3.89 ± 0.25 a |
| Simulated *Spodoptera cosmioides* | 18.67 ± 1.76 a | 852.70 ± 18.24 a | 4.57 ± 0.26 a | 459.80 ± 57.47 a | 25.79 ± 0.18 a | 1.20 ± 0.07 a | 4.11 ± 0.37 a |
| Non-Injured (Control) | 22.45 ± 2.25 a | 850.60 ± 14.04 a | 5.10 ± 0.31 a | 618.10 ± 88.52 a | 25.82 ± 0.30 a | 1.12 ± 0.11 a | 4.35 ± 0.27 a |
| | **A** ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) | **Ci** ($\mu mol\ CO_2\ mol^{-1}$) | **E** ($mmol\ H_2O\ m^{-2}\ s^{-1}$) | **GS** ($mmol\ H_2O\ m^{-2}\ s^{-1}$) | **Tleaf** ($^oC$) | **VPD** (kPa) | **WUE** ($mmol\ CO_2\ mol^{-1}\ H_2O$) |
| **Contrast** | **p value** | **p value** | **p value** | **p value** | **p value** | **p value** | **p value** |
| *Stegasta bosqueella* x *Spodoptera cosmioides* | 0.5405 | 0.8424 | 0.8291 | 0.5059 | 0.5886 | 0.6380 | 0.6418 |
| **Contrast** | **A** | **Ci** | **E** | **GS** | **Tleaf** | **VPD** | **WUE** |
| | **p value** | **p value** | **p value** | **p value** | **p value** | **p value** | **p value** |
| Real Injury x Simulated Injury | 0.6019 | 0.2547 | 0.5091 | 0.3016 | 0.6526 | 0.4804 | 0.3043 |

**[1]A** - assimilation/respiration; **[2]Ci** - intercellular CO2 concentration; **[3]E** - transpiration; **[4]GS** - stomatal conductance; **[5]Tleaf**- leaf temperature; **[6]VPD**- leaf to air vapor pressure deficit; **[7]WUE**- photosynthetic water use efficiency.

**Table 3.** The mean (± S.E.) effect of treatments on gas exchange parameters performance and treatments contrast for 48 hours after injury. Bolds values parameters indicate significant differences.

| | **48 hours after infestation** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Treatments** | **[1]A** ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) | **[2]Ci** ($\mu mol\ CO_2\ mol^{-1}$) | **[3]E** ($mmol\ H_2O\ m^{-2}\ s^{-1}$) | **[4]GS** ($mmol\ H_2O\ m^{-2}\ s^{-1}$) | **[5]Tleaf** ($^oC$) | **[6]VPD** (kPa) | **[7]WUE** ($mmol\ CO_2\ mol^{-1}\ H_2O$) |
| *Stegasta bosqueella* | 24.07 ± 1.35 a | 546.60 ± 16.52 a | 4.53 ± 0.17 a | 405.30 ± 33.78 a | 25.74 ± 0.09 a | 1.27 ± 0.05 a | 5.30 ± 0.22 a |
| *Spodoptera cosmioides* | 16.57 ± 1.05 b | 563.70 ± 22.71 a | 3.70 ± 0.28 a | 279.80 ± 34.92 a | 25.98 ± 0.11 a | 1.48 ± 0.07 a | 4.53 ± 0.12 a |
| Simulated *Stegasta bosqueella* | 20.68 ± 1.34 ab | 545.90 ± 18.16 a | 3.99 ± 0.30 a | 322.50 ± 52.98 a | 26.23 ± 0.20 a | 1.49 ± 0.10 a | 5.30 ± 0.27 a |
| Simulated *Spodoptera cosmioides* | 19.37 ± 2.22 ab | 567.20 ± 16.12 a | 3.84 ± 0.34 a | 308.80 ± 52.07 a | 26.01 ± 0.13 a | 1.48 ± 0.09 a | 4.97 ± 0.31 a |
| Non-Injured (Control) | 20.54 ± 1.86 ab | 542.20 ± 15.12 a | 4.22 ± 0.39 a | 383.90± 66.64 a | 26.04 ± 0.09 a | 1.39 ± 0.10 a | 4.95 ± 0.29 a |
| | **A** ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) | **Ci** ($\mu mol\ CO_2\ mol^{-1}$) | **E** ($mmol\ H_2O\ m^{-2}\ s^{-1}$) | **GS** ($mmol\ H_2O\ m^{-2}\ s^{-1}$) | **Tleaf** ($^oC$) | **VPD** (kPa) | **WUE** ($mmol\ CO_2\ mol^{-1}\ H_2O$) |
| **Contrast** | **p value** | **p value** | **p value** | **p value** | **p value** | **p value** | **p value** |
| *Stegasta bosqueella* x *Spodoptera cosmioides* | **0.0024** | 0.2956 | **0.0363** | **0.0495** | 0.1835 | 0.0504 | **0.0277** |
| **Contrast** | **A** | **Ci** | **E** | **GS** | **Tleaf** | **VPD** | **WUE** |
| | **p value** | **p value** | **p value** | **p value** | **p value** | **p value** | **p value** |
| Real Injury x Simulated Injury | 0.8570 | 0.9029 | 0.4487 | 0.5416 | 0.0449 | 0.1455 | 0.3582 |

**[1]A** - assimilation/respiration; **[2]Ci** - intercellular CO2 concentration; **[3]E** - transpiration; **[4]GS** - stomatal conductance; **[5]Tleaf**- leaf temperature; **[6]VPD**- leaf to air vapor pressure deficit; **[7]WUE**- photosynthetic water use efficiency.

The classification tree showed that injury by *S. bosqueella* presented high stomatal conductance and similar photosynthetic rate and water use efficiency when compared to non-injured leaflets (Figure 5). Alternatively, when infested with *S. cosmioides*, peanut leaflets showed decreased photosynthetic rate and water-use efficiencies and high stomatal conductance compared to *S. bosqueella* and the control.

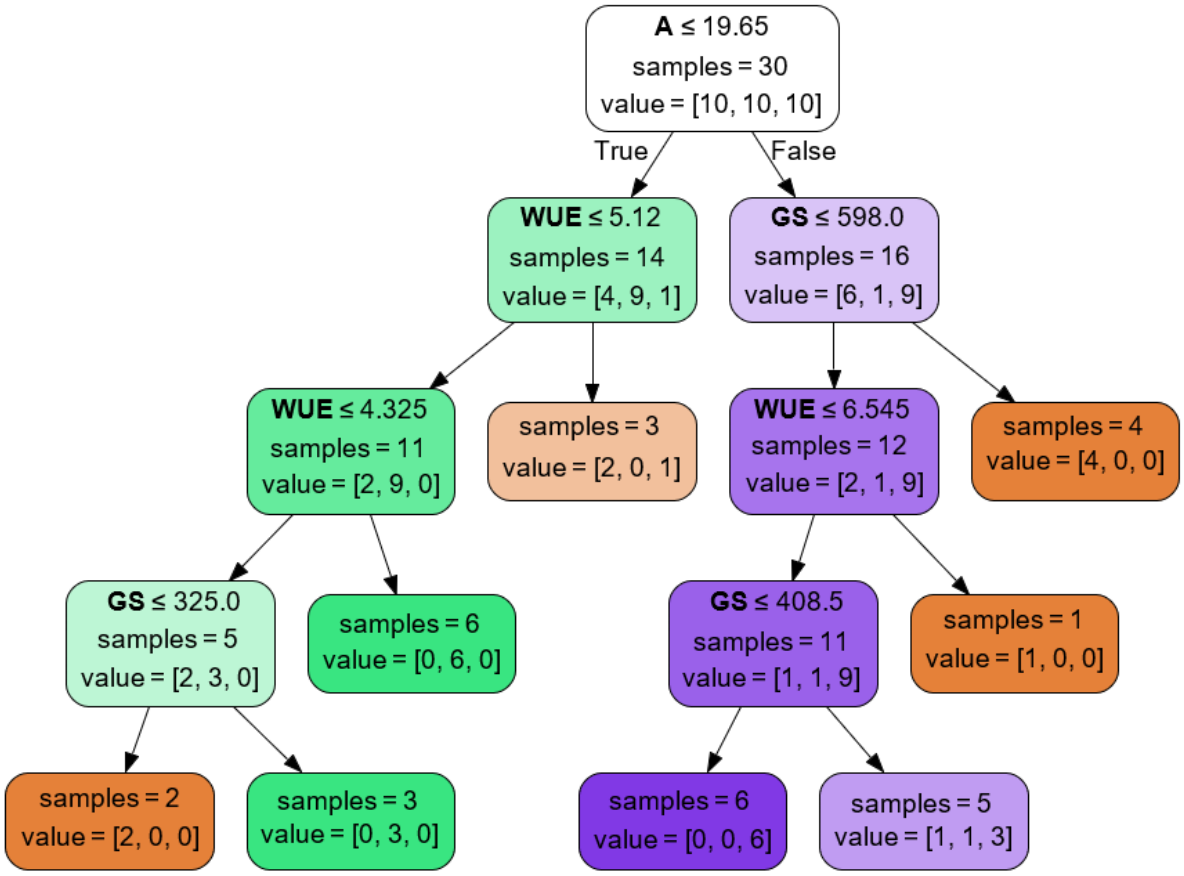


**Figure 5.** Classification tree for *Stegasta bosqueella* (orange), *Spodoptera cosmioides* (green) and non-injured (purple) leaf injury based on gas exchange parameters. **A** - assimilation/respiration (μmol $CO_2$ $m^{-2}$ $s^{-1}$); **GS** - stomatal conductance (mmol $H_2O$ $m^{-2}$ $s^{-1}$); **WUE**- photosynthetic water use efficiency (mmol $CO_2$ $mol^{-1}$ $H_2O$).

## DISCUSSION

The main purpose of this study was to characterize relationships between peanut leaf defoliation and leaf reflectance and gas exchange parameters, focusing on two major South American peanut defoliators, *S. bosqueella* and *S. cosmioides*. We demonstrated that peanut leaf reflectance is different between herbivory by the two larval species, but similar among real and simulated defoliation. Similar results were obtained based on peanut leaf gas exchange, in which differences were noted 48 h after injury. Based on these results, the potential of using changes in leaf reflectance simultaneously with gas exchange parameters as indicators of biotic stress in peanut is of particular interest to the study of plant-insect interactions and for incorporating remote sensing into peanut IPM programs.

We identified certain spectral regions that were especially sensitive to variation in plant stressors. Stress can be defined as “a departure from optimal physiological conditions” (Higley et al. 1993, Peterson and Higley 2001) and in our study, we observed that stressed leaves presented high reflectance in both the visible and NIR portions of the electromagnetic spectrum compared to non-stressed leaves. Generally, plant stress results in an increase in reflectance in the visible portion of the spectrum (400-700 nm) due to a decrease in chlorophyll a and b absorption; the opposite generally occurs in the NIR portion of the spectrum (750-2500 nm), with a decrease in reflectance driven by changes in the internal leaf structure (Carter and Knapp 2001, Prabhakar et al. 2012). The increase in NIR reflectance we observed in stressed peanut leaves could be related to variations in water content due to the infestation, which leads to a degeneration of the internal leaf structure at the cellular level (Zhang et al. 2012, Abdullah et al. 2018). Also, NIR reflectance is affected primarily by leaf

structure and is strongly associated with photosynthetic performance (Slaton et al. 2001).

In this context, low photosynthetic rates (A) and changes in water use efficiency (WUE) observed in leaves injured by *S. cosmioides* may explain the high reflectance in the NIR portion of the spectrum and could be a useful indicator of photosynthetic potential. These results suggest that the leaf internal structure and leaf pigments were important to the classification of the peanut leaflets infested by the most important defoliator larvae that occur in South American peanut crops.

Similarly, ANOVA F-values analysis suggested that the NIR spectral data can be used to detect stress between *S. bosqueella*, *S. cosmioides,* and non-infested leaves. The importance of the NIR portion of the spectrum in characterizing biotic stress caused by defoliators was reported by Huang et al. (2012) in rice leaves injured by *Cnaphalocrocis medinalis* (Lepidoptera: Crambidae) and Sudbrink et al. (2003) in cotton (*Gossypium hirsutum*) injured by *Spodoptera exigua* and *Trichoplusia ni* (Lepidoptera: Noctuidae). Our finding is an important one because a wide variety of vegetation indices combine reflectance data in the NIR region to detect biotic stress, including, for example, the Normalized Difference Vegetation Index (NDVI) and the Green Normalized Difference Vegetation Index (GNDVI) [48].

Random forest classification models of leaf reflectance provided a high accuracy of detection for *S. bosqueella* and *S. cosmioides* infestations. Also, the Kappa statistic values were greater than 0.80, representing good agreement between the reference and predicted data (Landis and Koch 1977). Random Forest Classification models have been successfully applied to a wide range of hyperspectral data analyses (Rodriguez-Galiano et al. 2012, Bellante et al. 2014, Du et al. 2015, Belgiu and Drăgu 2016, Fletcher and Reddy 2016, Vitrack-Tamam et al. 2020). It is important to

emphasize that in our study we controlled for the effects of environmental factors and reflectance measurements were obtained from plant leaves and not plant canopies. With this in mind, in-field remote sensing presents additional challenges, including the leaf angle to the sensor and leaf location in the canopy with respect to shadowing, as well as other environmental variables that could affect radiometric resolution and consequently classification accuracy (Campbell and Wynne. 2011, Fletcher and Reddy 2016, Hunt and Rondon 2017, Yang et al. 2017). Also, due to recent advances in spatial classification algorithms, additional studies must evaluate spatial image classification for pest detection in peanuts.

Similar to what has been observed in other studies (Davidson and Milthorpe 1966, Poston et al. 1976, Hall and Ferree 1976, Welter 1991, Higley 1992, Peterson et al. 1992b, 1996, 2004, Burkness et al. 1999, Macedo et al. 2007), we did not observe significant effects on either the assimilation/respiration (photosynthetic rate) or in stomatal conductance, intercellular $CO_2$ concentration, and transpiration at 24 h after defoliation by leaf-mass consumers. Differences in gas exchange of peanut leaves were only observed 48 h after infestation in the *S. cosmioides* injury treatment.

Therefore, in contrast to *S. cosmioides*, our results suggest that the principal effect of *S. bosqueella* larval-induced herbivory in peanut leaflets seems to be a leaf-mass reduction, not reduction of photosynthetic capacity of remaining leaf tissue. However, the injury by *S. bosqueella* is visually different. Rather than gross leaf-mass consumption like *S. cosmioides*, *S. bosqueella* leaves a layer of interveinal tissue, referred to as “windowpane injury” (Fig. 1, a). In addition, *S. bosqueella* feeds on meristematic tissues (buds), which can reduce plant development and alter plant architecture (Wall and Berberet 1979, Pinto et al. 2020).

According to Higley et al. (1993) and Peterson (2001), insect herbivores can be grouped into categories known as injury guilds based on plant response, such as population or stand reduction, leaf-mass reduction, leaf photosynthetic-rate reduction, leaf senescence alteration, light reduction, assimilate removal, water-balance disruption, seed or fruit destruction, architecture modification, and phenological disruption. From an agronomic and pest management perspective, injury guilds can be used to develop multiple-species economic injury levels (EILs), as well contribute to accurate pest sampling programs (Hutchins et al. 1988, Higley and Pedigo 1996, Peterson 2001, Pedigo and Rice 2014). However, results from this study suggest that injury from *S. bosqueella* and *S. cosmioides* most likely cannot be placed in the same injury guild for peanut cultivars that belongs to the runner vegetative group.

Because peanut leaves respond similarly to both real and simulated injury, both spectrally and physiologically, it is possible to use simulated injury to evaluate leaf-mass consumption *from S. cosmioides*. This is important because simulating insect defoliation offers distinct advantages in evaluating plant primary physiology, growth, and yield (Baldwin 1990). Importantly, however, our data here are restricted to only a particular developmental stage (i.e., flowering). In addition, because our experiment was conducted in controlled conditions, other studies will need to assess if environmental factors could have influence on peanut plant spectral and biotic responses in field conditions.

We determined that remote sensing can be used to differentiate injured and non-injured leaves. That is an important step to use remote sensing in peanut sampling programs. However, for decision making, more information is needed (e.g. inferences about the presence of insects which are the target of control measures). Consequently,

new studies should address this to improve remote sensing-based sampling programs in peanuts.

Our results reported here support the hypothesis that remote sensing in the NIR (777.42-1000 nm) portion of the spectrum can be used for classification of *S. bosqueella* and *S. cosmioides* injury. Also, we verified that *S. bosqueella* and *S. cosmioides* should be considered separately in sampling programs, and studies with simulated injury can be used to evaluate defoliator-induced stress in peanuts. This information will contribute to incorporating remote sensing as an instrument to leverage the adoption of IPM programs for peanuts.

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## CHAPTER V - DETERMINATION OF THE ECONOMIC INJURY LEVEL FOR LEPIDOPTEROUS PESTS IN PEANUT

**RESUMO -** Um passo fundamental no desenvolvimento de ferramentas de Manejo Integrado de Pragas (MIP) é determinar experimentalmente uma relação quantitativa entre a densidade de pragas e o rendimento das culturas, porque nem todos os insetos herbívoros são pragas e demandam controle. Portanto, para estimular uma abordagem de MIP pelos produtores de amendoim, nosso estudo quantificou a relação da desfolha do amendoim e a perda de produtividade, durante duas safras (2019/2020 e 2020/2021) ao longo de três diferentes estágios de crescimento do amendoim. Análises quantitativas e qualitativas foram realizadas e o Nível de Dano Econômico (NDE) e o Limiar Econômico (LE) para *Stegasta bosqueella* (Chambers, 1875) (Lepidoptera: Gelechiidae) e *Spodoptera cosmiodes* (Walker, 1858) (Lepidoptera Noctuidae) foram estimados para o amendoim. Observamos que houve influência significativa da desfolha no número de vagens com três, dois e um grão e vagens deformadas. Observou-se uma relação linear negativa entre a porcentagem de desfolha e a perda de produtividade nas duas safras avaliadas. Além disso, os estágios de desenvolvimento vegetativo (15 DAP) e formação de vagens (71-100 DAP) foram os estádios de crescimento do amendoim mais sensíveis à desfolha, indicando a maior suscetibilidade à desfolha durante esses períodos. O nível de dano econômico para *S. bosqueella* é de 14,65, 77,96 e 36,30 larvas por planta, enquanto para *S. cosmioides* é de 0,74, 2,48 e 1,16 larvas por planta nos estágios de desenvolvimento vegetativo, floração/frutificação e formação de vagens, respectivamente. Os resultados gerados permitirão que os profissionais do setor do amendoim tomem decisões mais assertivas de manejo de insetos ao lidarem com a desfolha do amendoim.

**Palavras-chave:** MIP amendoim, lepidópteros desfolhadores, limiar econômico

# CHAPTER V - DETERMINATION OF THE ECONOMIC INJURY LEVEL FOR LEPIDOPTEROUS PESTS IN PEANUT

**ABSTRACT -** A key step in developing Integrated Pest Management (IPM) tools is to determine experimentally a quantitative relationship between pest density and crop yield, because not all herbivorous insects are pests and require control. Therefore, to encourage an IPM approach by peanut farmers our study quantified the relationship of peanut defoliation and yield loss, during two growing seasons (2019/2020 and 2020/2021) over three different peanut growth stages. Quantitative and qualitative analysis were performed and economic injury level (EIL) and economic threshold (ET) for both *Stegasta bosqueella* (Chambers, 1875) (Lepidoptera: Gelechiidae) and *Spodoptera cosmioides* (Walker, 1858) (Lepidoptera Noctuidae) were estimated for peanuts. We observed that there was a significant influence of defoliation on the number of pods with three, two and one grain, and deformed pods. A negative linear relationship was observed between the percentage of defoliation and yield loss during both crop seasons. Also, the vegetative (15 DAP) and pod/setting stages (71-100 DAP) were the most sensitive peanut growth stages to defoliation indicating the highest susceptibility to defoliation during these periods. The economic injury level for *S. bosqueella* is 14.65, 77.96 and 36.30 larvae per plant whereas for *S. cosmioides* it is 0.74, 2.48 and 1.16 larvae per plant in the early vegetative, bloom/pegging and pod/setting stages, respectively. The generated results will allow peanut stakeholders to make more and better assertive management decisions when dealing with peanut defoliation.

**Key words:** Peanut IPM, lepidopterous defoliator, economic threshold

## INTRODUCTION

Integrated Pest Management (IPM) can be defined as a set of tactics and strategies that aim to keep the pest population below levels at which they can cause production losses (Pedigo 1995, Kogan 1998). The IPM concept is based on the premise that plants accept the presence of a tolerable pest density, without economically significant yield reductions, that preserves environmental quality and can leverage users' profits (Higley and Pedigo 1996, Pedigo and Rice 2014). However, a key step in developing IPM tools is to determine experimentally a quantitative relationship between pest density and crop yield, because not all herbivorous insects will be pests and require control (Peterson and Higley 2001).

In this context, for a better understanding of pest status in a crop as well as the ideal moment for pest control, the concept of economic injury level (EIL) was developed to determine the lowest pest population density capable of causing economic damage to the crop (Stern et al. 1959). To avoid reaching the EIL, it is crucial to understand the appropriate time to start pest control, which is defined as the economic threshold (ET). Economic threshold is the population density at which control measures must be initiated to prevent the insect population from reaching the EIL (Higley and Pedigo 1996). However, even though IPM strategies have been in development since the 1960s and are proven to be valuable for a wide range in varieties of crops and locations (Kogan 1998, Peshin and Dhawan 2009), surprisingly, for some important crops, such as peanut in Brazil, there is no information in the literature about the EIL for any defoliating species.

Brazil is one of the main peanut exporting countries along with China, United States, India and Argentina (USDA 2021). It is estimated that peanut cultivated area

has increased 43%, and the yield is 13% higher for season 2020/2021, in comparison to season 2011/2012, particularly in São Paulo State, that concentrates almost 90% of Brazilian peanut production (CONAB 2013, CONAB 2021). However, the increase in productivity could be even higher if a pest and disease management program were implemented, since it is estimated that 20–30% of the peanut crop production costs are associated with insect pests and diseases management in both North and Latin America (Garcia-casellas 2004, Michelotto et al. 2015). The absence of EILs for important peanut pests in Brazil represents one important limiting factor for a peanut IPM program development.

Furthermore, pest surveys carried out in the last peanut crop seasons (2017/2018, 2018/2019 and 2019/2020) have shown an increase in problems with lepidopterous pests in Brazilian peanut cultivated areas (unpublished data).The rednecked peanutworm, *Stegasta bosqueella* (Chambers, 1875) (Lepidoptera: Gelechiidae), and the armyworms of the genus *Spodoptera* (Lepidoptera: Noctuidae) are the most abundant lepidopterous species. These insects can cause intense defoliation and reduce plant development as well as pod quality. Bi-monthly chemical insecticides sprays are usually the unique control methods adopted by growers, and generally no other tools have been applied to control these defoliators in Brazilian peanuts.

Therefore, to avoid several of these unnecessary and wasteful applications, it is essential to establish appropriate economic thresholds for each specific insect pest. In this context, particularly for *S. bosqueella*, the larvae can feed directly on the tips of peanut vines reducing their growth and, hence, peanut yield (Pinto et al. 2020b). Furthermore, defoliation and yield loss caused by disease pathogens have received more attention than from insects in peanuts (Backman and Crawford 1984, Bourgeois

et al. 1991, Anco et al. 2020) and to our knowledge, no studies aimed to quantify the impact of feeding in the axillary bud region along with the defoliation are available. For peanuts, previous studies demonstrated that the estimation of yield reduction from defoliation depends on the extent of damage and the age of the plant (Williams et al. 1976, Wall and Berberet 1979, Tuan et al. 2017). Therefore, evaluating the impact of damage in the axillary bud region along with the defoliation in different stages of peanut growth becomes crucial to avoid underestimation of the EIL levels.

By having EILs developed, decision making can be taken based on pest abundance or injury and the control tactics be timely and accurately established. In view of this, we quantified the relationship of peanut defoliation and yield loss during two growing seasons (2019/2020 and 2020/2021) over different peanut growth stages. Based on this relationship we estimated EIL and ET for both *S. bosqueella* and *Spodoptera cosmiodes* (Walker, 1858) (Lepidoptera Noctuidae) in peanuts. These thresholds should be used by peanut stakeholders (extension personnel, growers, and crop consultants) to make more assertive management decisions when dealing with peanut defoliators.

## MATERIAL AND METHODS

### Study location and field characterization

The studies were conducted in an area of approximately 0.8 ha (21°14'23.1”S and 48°17'26.3”W), located next to the Department of Agricultural Sciences, School of Agricultural and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil. All experiments were carried out during two growing seasons (2019/2020 and 2020/2021).

Agronomic practices were adopted following common strategies recommended to peanut farmers in São Paulo, Brazil (Costa et al. 2019). For land preparation, plowing followed by a leveling harrowing was performed. In season 2019/2020, the arrangement of 0.90 cm between rows and sowing of 18 seeds per meter, was standard. For season 2020/2021, the row spacing of 0.90 cm and 18 seeds planted per meter were also adopted, but plants were thinned when they were 2 weeks old to ensure a final stand of one plant per meter.

For both seasons, the field trails were not irrigated and both fertilizer 2-20-20 (N:P:K) and herbicide treatments [Chloroacetanilide (1.2 l/ha, Dual Gold, Syngenta®) + Trifluralin (1.2 l/ha, NORTOX S/A) + Glyphosate (Roundup, Bayer®)] were adopted before planting time. Peanut cultivar IAC 503 that belongs to the runner vegetative group (decumbent growth habit) with “Hight Oleic” characteristic (70 to 80% oleic acid in the oil) (IAC 2018) was adopted during both crop seasons. Seeds received chemical treatment, with a mixture containing insecticides Fipronil (NORTOX 800 WG) and fungicides Pyraclostrobin (Opera®, BASF) and Carboxanilida and Dimetilditiocarbamato (Vitavax Thiram 200 SC, UPL). Furthermore, phytosanitary treatment was carried out throughout the experiment with applications of insecticides, herbicides and fungicides following the scheduled protocol adopted by producers in the region (Costa et al. 2019). Planting dates for each season as well as climate records are reported in supplementary 1.

**Experiment design**

For season 2019/2020, the experimental design consisted of a 5x3 factorial (5 levels of defoliation x 3 peanut growth stages) in a randomized complete block design

(RCBD), with five replications. For this purpose, defoliation was carried out on 3 adjacent plants and the experimental plot size was 5 x 5 meters.

During the season 2020/2021, a 5x2x3 factorial (5 levels of defoliation; 2 injury category [Simulated injury and Simulated injury + *S. bosqueella* injury on vine tips] x 3 peanut growth stages) in a randomized complete block design (RCBD) was adopted and the experimental plot size was 4.95 x 3.15 meters. For *Stegasta bosqueella* injury on vines, 25% of the buds were removed, based on preliminary tests conducted in a greenhouse condition (unpublished data). Therefore, in such treatment, plants were artificially defoliated and had 25% of the buds removed. Buds located in the tips were selected and manually removed.

For both seasons, five levels of defoliation 0, 25, 50, 75 and 100% were adopted through manual removal of leaflets, according to the established level, simulating the lepidopterous injury (Figure 1). The simulated injury was adopted because peanut leaves respond similarly to both real and simulated injury as described by Pinto et al. (2020a).


**Figure 1.** Defoliation levels adopted to simulate the injury of lepidopterous in peanuts. The X represents how the defoliation levels were applied to peanut plants.

**Artificial defoliation simulating larvae damage**

The defoliation was carried out only once during three different development stages following Boote (1982) Tuan et al. (2017) which were: 1) the early vegetative stage (2 weeks after planting to pre-flowering), 2) the bloom/pegging stage (41-70 days after planting (DAP)) and 3) the pod/setting stage (71-100 DAP). For the 2019/2020 season, defoliation occurred simulating a competitive environment (peanut plants present in the all experimental plot) (Figure 2, A). For the 2020/2021 season, plants were thinned and only those established to receive the treatments were kept (Figure 2, B). The purpose of this was to standardize the peanut plant development and architecture.


**Figure 2.** Defoliation of a peanut plant, but with all plants in a row in season 2019/2020 (A) and with only treated plant in season 2020/2021 (B).

After verifying the maturation of the pods (close to 70%), through sentinel plants following Williams and Drexler (1981), peanut plants were harvested and transferred to the laboratory, where the pods were separated for quantitative and qualitative analysis. Pods were then kept in a lab oven at 60°C for 3 days, until constant weight

was obtained. Moisture content was calculated as indicated by BRASIL (2009), using the formula:

$$\%\, moisture = \frac{100\ (P - p)}{P - t}$$

Where:
P=initial weight (weight of the container and its lid plus the weight of the wet seed)
p=final weight (container weight and lid plus seed dry weight);
t= weight of the container and its lid.

**Data analysis**

In this study, both qualitative and quantitative analysis were performed. For qualitative analysis the weight in grams/plant was calculated using the corrected moisture values (for season 2019/2020, the average of three defoliated plants was calculated). Then, the parameters: number of pods with three, two and one grain, as well as deformed pods were quantified. To analyze the effect of the fixed effect factors in season 2019/2020 (defoliation levels and peanut stage) and season 2020/2021 (defoliation levels, injury category, and peanut stage), the Multivariate Analysis of Variance (MANOVA) in factorial scheme under RCBD design was adopted. This analysis was performed using the package MultivariateAnalysis in the statistical software R v4.0.3 (The R Foundation for Statistical Computing, 2019). MANOVA was used because the dependent variables were more than one (i.e. number of pods with three, two and one grains and deformed pods). Before computing MANOVA, we ensured that no outliers values were present in the dataset. We considered p value < 0.05 as statistically significant.

In evaluating the relationship between defoliation and production, a regression analysis was performed using Proc REG (SAS Institute, 2015). The probability of fixed effect factors was tested using the F-statistic (P=0.05). The average percentage yield

loss in relation to the control treatment (when there was no defoliation) was estimated using the mean values of the treatments. The degrees of freedom were adjusted and the decomposition using the orthogonal polynomials method was used to select the model that best represented the relationship between the independent variables (here defoliation) and yield. From the observed values generated, the adjusted regression curve was obtained and the data from the regression, and more specifically the slopes, were used in the calculation of EILs.

The EIL for the four growth stages was calculated according to the methodologies proposed by Stone and Pedigo (1972) and Higley and Pedigo (1993), as follows:

**Gain Threshold (GT)**

Gain Threshold can be defined as the amount of damage that could justify the insect pest control. It is calculated by dividing the management costs by the market crop price. For this calculation we considered the product Racio®, composed of acephate, in Soluble Powder (SP) formulation, which are properly registered for use in peanuts according to the Brazilian Ministry of Agriculture - MAPA. We have quantified the cost of application per hectare including tractor and operational labor. For the market value, the price indicated by CEAGESP (Company of Depository and General Warehouses of São Paulo) in dollars (8.83US$) was adopted (2020 [https://www.noticiasagricolas.com.br/cotacoes/amendoim/amendoim-ceasas/2020-4-30] and 2021[https://www.noticiasagricolas.com.br/cotacoes/amendoim/amendoim-ceasas/2021-04-30]. The calculation was performed using the following formula:

GT= Cost of control/ha / Market value/kg/ha [Eq. 1]

**Percentage yield loss equivalent to the Gain Threshold**

To calculate the percentage, on the total production potential, the average peanut production in the state of São Paulo in the seasons 2019/2020 and 2020/2021 was considered (IEA, http://www.iea.sp.gov. br/ftpiea/AIA/AIA-11-2021.pdf). The calculation was performed using the following formula:

% yield loss necessary = 100 * [GT/projected yield] [Eq. 2]

**Determination of the percent defoliation necessary to cause that percent yield loss from the regression equations of yield loss on defoliation.**

To calculate the percentage of area that needs to be removed to cause the percentage loss in production, the loss data in peanut grams for different defoliation levels (0; 25, 50, 75, 100%) were used. This allowed us to obtain the coefficients of the linear equation according to the following formula:

$Y = a+bx$ [Eq. 3]

Where Y is the percentage of loss in production and X is the percentage of defoliation.

**Absolute amount of leaf tissue equivalent to the percentage of defoliation**

To calculate the absolute amount of leaf tissue equivalent to the percentage of defoliation calculated in the previous item, the peanut leaf area value was obtained from plants in each crop season and at each peanut development stage. This estimate was obtained by collecting and measuring the leaf area of two plants weekly throughout

their development. The leaf area was measured using LICOR LI-3100 (LICOR Nebraska, USA). Thus, the calculation was performed as follows:

Absolute Defoliation = Percent defoliation * Total foliage [Eq. 4]

**Number of insects needed to consume the corresponding leaf area (EIL)**

For this calculation, the average values of individual consumption of both *S. bosqueella* and *S. cosmioides* obtained in Chapter 2 were used. The calculation was performed using the following formula:

EIL per plant = (Absolute Defoliation / Consumption per insect) [Eq. 5]

## RESULTS

### Qualitative analysis

We observed that there was a significant influence of defoliation level on the combined dependent variables: number of pods with three, two and one grain, and deformed pods (Pillai's Trace = 0.5245, $F_{4,56}$=2.11, P = 0.0087, Table 1). This suggests that 50% of multivariate variance of the overall quality pods of peanut plants was influenced by defoliation level. Therefore, it was found that the removal of 50, 75 and 100% of the peanut leaves reflected a reduction of pods with 2 grains and an increase in the number of deformed pods (Figure 3). This effect was observed regardless of the time at which the defoliation was performed, once no significant influence of developmental stage was observed ($F_{2,56}$=1.39, P=0.2052). This was also confirmed

by the lack of significant interaction ($F_{8,56}$=1.13, P=0.1200, Table 1) between both evaluated fixed factors (defoliation levels and peanut development stage).

In contrast, during the season 2020/2021, both defoliation levels ($F_{4,142}$ = 3.41, P<0.0001) and peanut development stage ($F_{2,142}$ = 5.05, P < 0.0001) presented a high significant influence in the combined dependent variables (Table 1). However, no significant interaction was observed, which shows that both factors influence pod quality independently ($F_{8,142}$ = 1.04, P = 0.4109). In view of this, we verified that similarly to the season 2019/2020, defoliation of at least 50% negatively affects the quality of pods. Moreover, the effect is even greater when *S. bosqueella* injuries the vine tips in the early vegetative stage (15 DAP) and pod/setting stage (71-100 DAP) (Figure 4). This effect was evidenced by the significant interaction between Peanut stage**S. bosqueella* damage ($F_{2,142}$ = 3.52, P = 0.0006, Table 1). Furthermore, one can clearly notice that the number of deformed pods increases when defoliation occurs at the pod/setting stage (71-100 DAP) (Figure 4).

**Table 1.** Multivariate effect of defoliation levels, peanut stage and *S. bosqueella* damage on overall pods with three, two and one grains and deformed pods.

| **Season 2019/2020** | | | |
|---|---|---|---|
| **Effect** | **Pillai's Trace Value** | **df** | **P** |
| Defoliation levels | 0.5245 | 4 | 0.0087 |
| Peanut stage | 0.1877 | 2 | 0.2052 |
| Defoliation levels*Peanut stage | 0.6393 | 8 | 0.1200 |
| **Season 2020/2021** | | | |
| **Effect** | **Pillai's Trace Value** | **df** | **P** |
| Defoliation levels | 0.3512 | 4 | <0.0001 |
| Peanut stage | 0.2525 | 2 | <0.0001 |
| *S. bosqueella* damage | 0.0372 | 1 | 0.2569 |
| Defoliation levels*Peanut stage | 0.2211 | 8 | 0.4109 |
| Defoliation levels**S. bosqueella* damage | 0.0782 | 4 | 0.7870 |
| Peanut stage**S. bosqueella* damage | 0.1831 | 2 | 0.0006 |

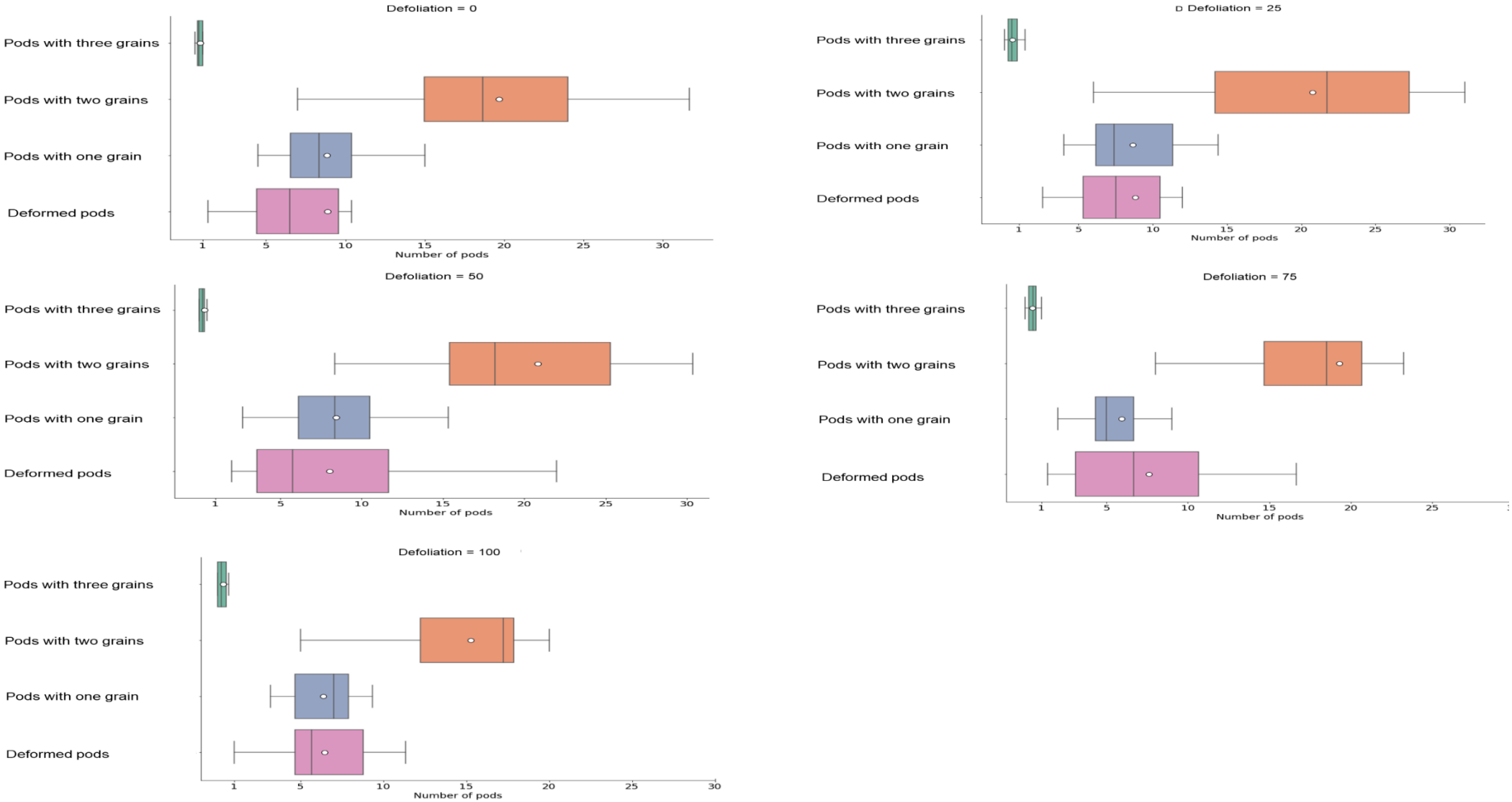


**Figure 3.** Qualitative analysis of peanut production (number of pods with three, two and one grain, and deformed pods) in different defoliation levels during season 2019/2020.

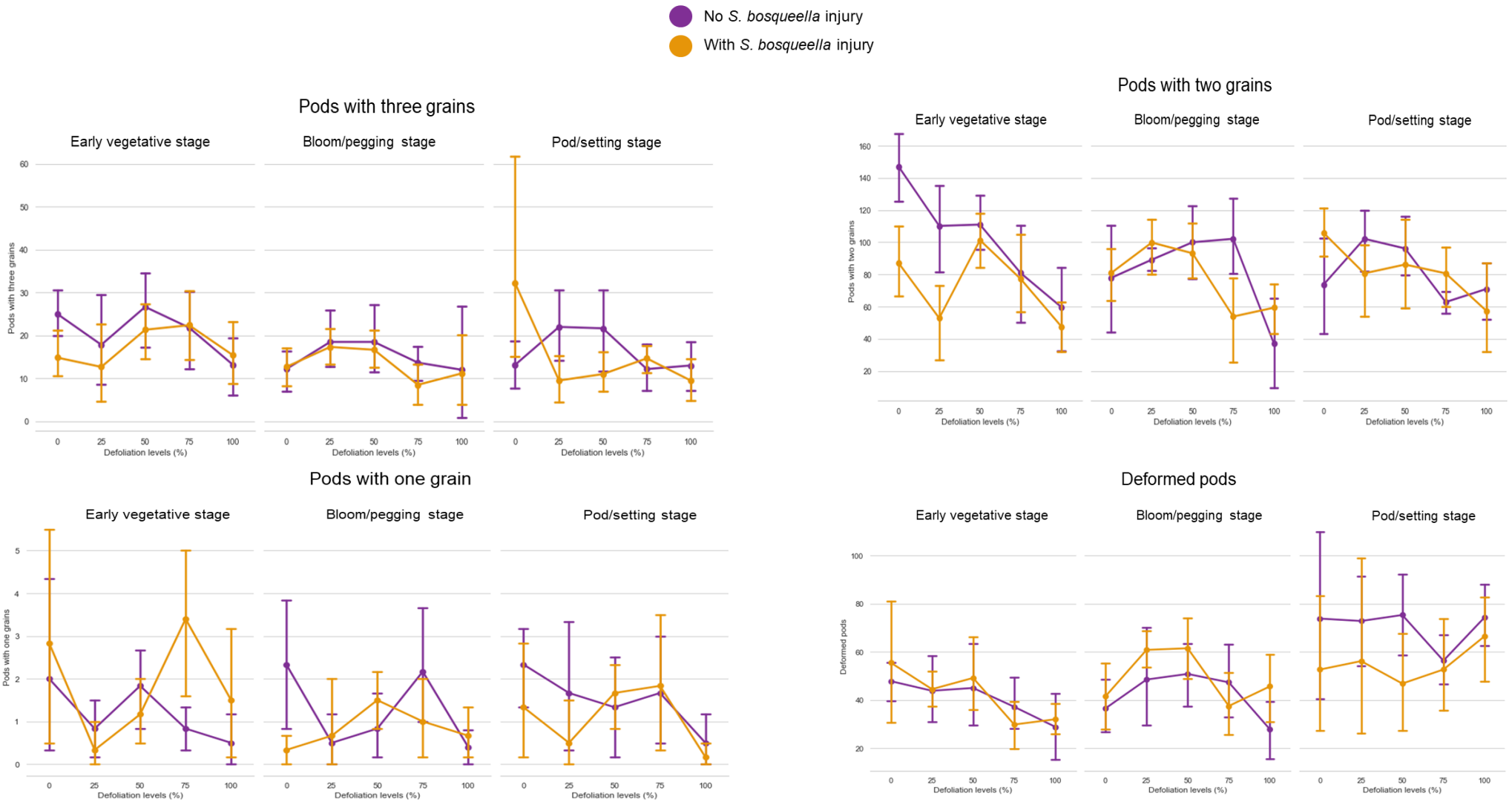


**Figure 4.** Qualitative analysis for number of pods with three, two and one grain, as well as deformed pods (+MSE) inside each defoliation levels, injury category, and peanut stage for season 2020/2021.

**Quantitative analysis**

A negative linear relationship was observed between the percentage of defoliation and yield loss during the both crop seasons (Figure 5). To assess this relationship, different levels of defoliation were simulated by removing peanut leaflets from plants in two distinct conditions (season 2019/2020 in a competitive environment and season 2020/2021 with isolated plant). We noticed that except for bloom/pegging stage (41±70 DAP) in season 2020/2021, when the defoliation was performed in a non-competitive environment, the relationship between defoliation and yield loss was linear and statistically significant ($P<0.05$), suggesting that the defoliation impacts peanut production. Therefore, we observed that at bloom/pegging stage (41-70 DAP) peanuts present a higher tolerance to defoliation.

Similarly to observations in the qualitative analysis, the regression equations indicated that the vegetative (15 DAP) and pod/setting stages (71-100 DAP) were the most sensitive peanut growth stages to defoliation, suggesting the highest grain yield loss. For season 2019/2020 in a competitive environment, in general, the regression equation indicated a yield decrease of 0.16 g/plant and 0.35 g/plant for every 1% increase in canopy defoliation, respectively for vegetative (15 DAP) and pod/setting stages (71-100 DAP). Once we used a planting density to finish with 130,000 peanut plants per hectare, this leads to a yield loss of 20.8 and 45.5 kg/ha (e.g. (0.16/1000)*130,000=20.8). In contrast, during the same peanut growth stages, when the defoliation was applied to isolated plants in season 2020/2021, the yield decreases of 0.93 and 0.92 g/plant for every 1% increase in canopy defoliation.

The yield obtained in the control treatment without defoliation was 50.80 ± 3.41 g/plant for season 2019/2020 and 159.35 ± 65.05 g/plant and 184.32 ± 75.25 g/plant

for season 2020/2021 with and without *S. bosqueella* injury to vine tips. For *S. bosqueella* damage, 25% of the buds were removed, based on preliminary tests conducted in a greenhouse condition. Therefore, we observed that, when *S. bosqueella* was present, the yield loss increased by 24, 11 and 11%, for the early vegetative; bloom/pegging and the pod/setting peanut growth stages, respectively (Figure 5). It is important to highlight that the yield difference between the crop growing seasons could be related to the different methodology adopted. Therefore, in a non-competitive environment (season 2020/2021) peanut plant was exposed to a higher yield potential.

With the percentage yield loss data obtained from different peanut growth stages, the adjusted regressions were generated, and are described in Figure 6. Linear and quadratic regression models were used for modeling the percentage yield loss. A high coefficient of determination indicates good model assumptions. Furthermore, defoliation sensitivity changes according to the peanut plant stage, where defoliation at the beginning of the vegetative and pod/setting stage appears to be more critical for peanut plants, resulting in greater yield loss.

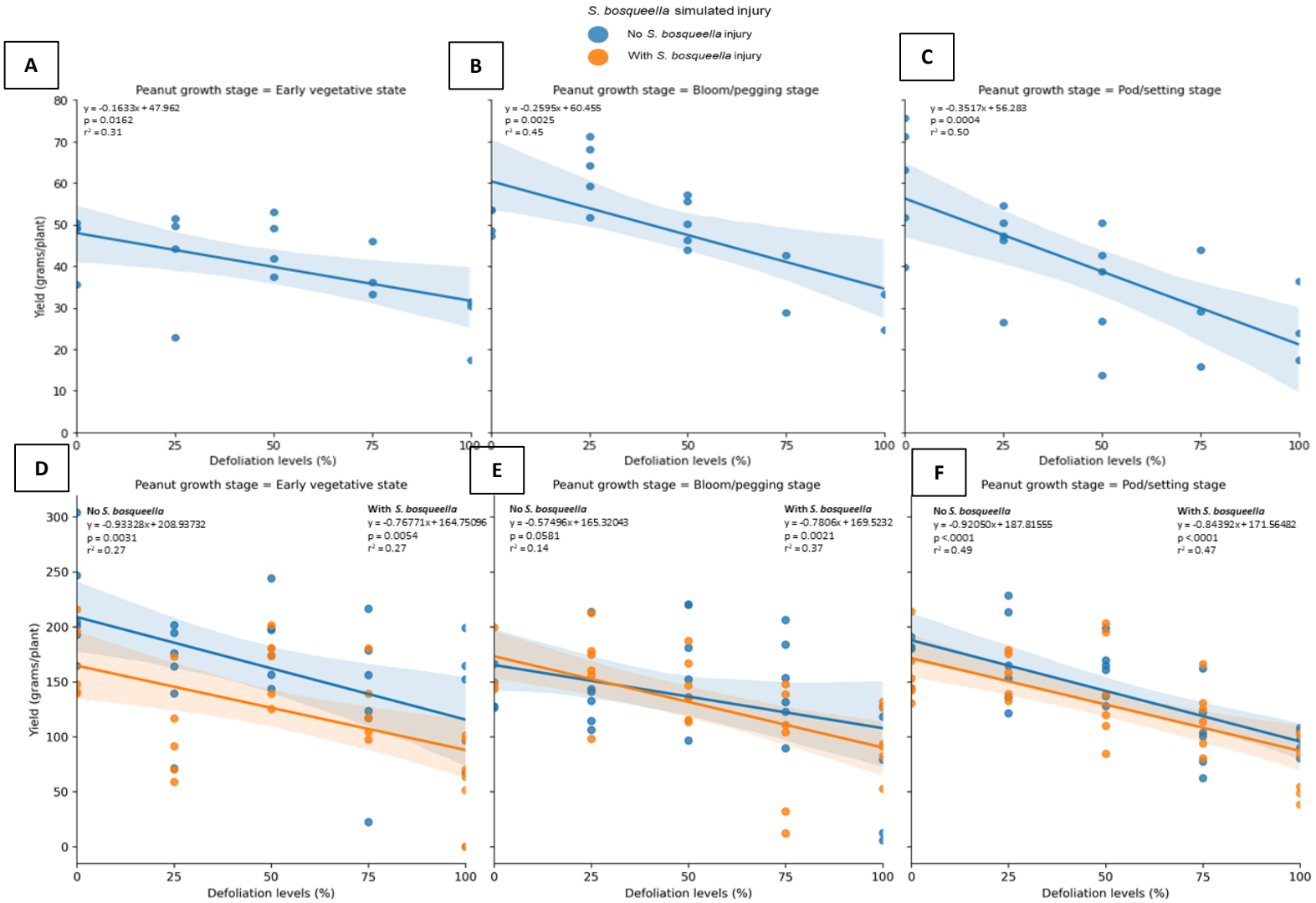


**Figure 5.** Relationships between different levels of simulated injury in peanut early vegetative state (A, D), bloom/pegging stage (B,E) and pod/setting stage (C,F) in relation to yield loss (grams/plant) and its regression models and confidence intervals for seasons 2019/2020 (A, B and C) and 2020/2021 (D, E and F).

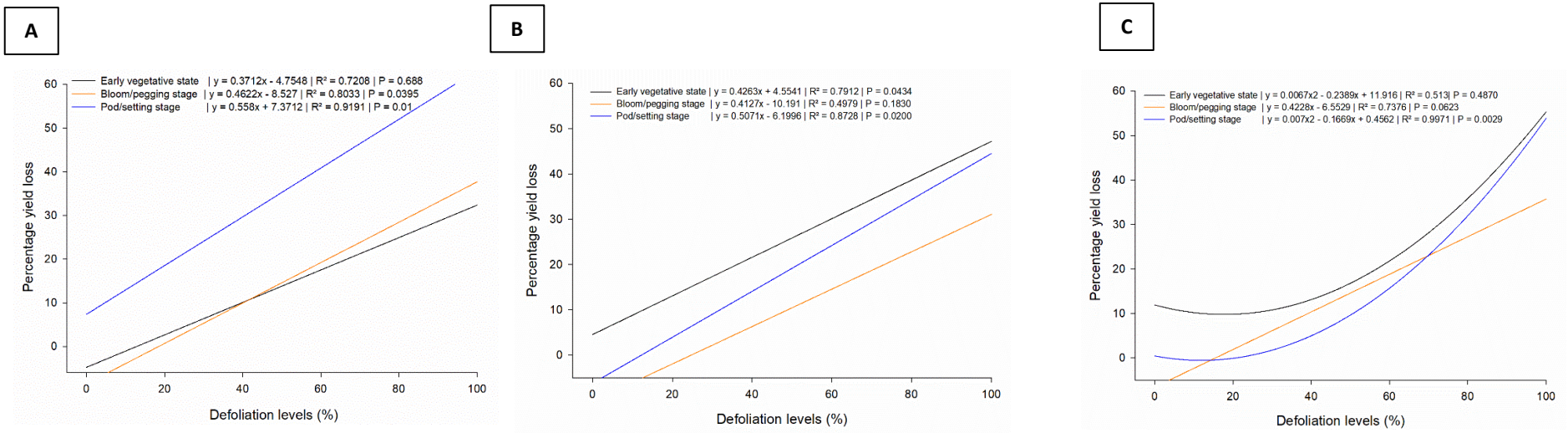


**Figure 6.** Regression of yield loss on defoliation of peanut growth stages for season 2019/2020 (A) and 2020/2021 without (B) and with (C) *S. bosqueella* damage (B).

## Economic injury levels for peanut defoliators

### Gain Threshold (GT)

The Gain Threshold for using Acephate (Racio®) in Soluble Powder (SP) formulation was $6.79 kg/ha. The Gain Threshold represents the relationship between the cost of treatment and the market value of peanuts. We considered R$ 48.00/ha, as the value for the control cost per hectare of the product Racio® at a dosage of 0.8 c.p./ha (The tractor-implement set and cost of labor, was also incorporated in this value). For the market value of peanuts, the value paid by CEAGESP (Company of Depository and General Warehouses of São Paulo) in April 2020 and 2021 was used for peanuts in 25/kg bags of shells (R$200.00 for 2020 and R$250.00 for 2021). All these values were converted into dollars at the exchange rate of R$5.43 for each US$1.00 (https://valorpro.globo.com).

### Percentage yield loss equivalent to the Gain Threshold

The percentage of total production potential equivalent to the Gain Threshold for the product Racio® was 0.17. For this calculation, the average peanut production in the state of São Paulo during 2019/2020 and 2020/2021 crop seasons was considered. Then, the percentage defoliation necessary to cause that percent yield loss from the regression equations of yield loss on defoliation was determined.

**Determination of the percent defoliation necessary to cause that percent yield loss from the regression equations of yield loss on defoliation.**

According to the adjustments for the regressions between yield loss obtained in Figure 7, the percentage of area needed to be removed to cause the percentage yield loss equivalent to the Gain Threshold was 12.84, 18.47 and 0.02% in season 2019/2020 and 4.63,10.26 and 6.29% (without the addition of 25% reduction of shoots) and 36.35, 6.48 and 24.81% (with the addition of 25% reduction of shoots) for the 2020/2021 season, in the early vegetative, bloom/pegging and pod/setting stages, respectively (Table 2).

**Absolute amount of leaf tissue equivalent to the percentage of defoliation**

The absolute defoliation calculated from the leaf area that needs to be removed to cause yield loss was 65.46, 358.94, and 1.33 $cm^2$ for season 2019/2020 and 68.04, 352.01, and 329.73 $cm^2$ (without the addition of 25% reduction of shoots) and 534.66, 222.36, and 1301.81 $cm^2$ (with the addition of 25% reduction of shoots) for the 2020/2021 season, in the early vegetative, bloom/pegging and pod/setting stages, respectively (Table 2).

**Number of insects needed to consume the corresponding leaf area (EIL)**

The economic injury level per plant considering the average leaf consumption of *S. bosqueella* under the cultivar IAC 503 (4.56 cm2) was 14.36, 78.71 and 0.29 insects per plant for season 2019/2020 and 14.93, 77.20, 72.31 and 117.25, 48.76 and 285.48 for season 2020/2021 without and with the addition of 25% reduction of shoots (Table 2) in the early vegetative, bloom/pegging and pod/setting stages, respectively.

For *S. cosmioides*, the economic injury level per plant considering the average leaf consumption of 143.53 cm2, was 0.46, 2.50 and 0.01 insects per plant for season 2019/2020 and 0.47, 2.45 and 2.30 and 3.73, 1.55 and 9.07 for season 2020/2021 without and with the addition of 25% reduction of shoots (Table 2) in the early vegetative, bloom/pegging and pod/setting stages, respectively.

**Proposed economic injury level**

For the proposed injury level, we averaged the results over the two peanut growing seasons 2019/2020 and 2020/2021. Therefore, the economic injury level for *S. bosqueella* is 14.65, 77.96 and 36.30 larvae per plant in the early vegetative, bloom/pegging and pod/setting stages, respectively. For *S. cosmioides* the economic injury level is 0.74, 2.48 and 1.16 larvae per plant in the early vegetative, bloom/pegging and pod/setting stages, respectively.

**Table 2.** Total leaf area and economic injury levels for *S. bosqueella* and *S. cosmioides* in peanut.

| Season | Leaf area (total cm²) | | | Percent defoliation necessary to cause yield loss (%) | | | Absolute amount of leaf tissue equivalent to the percentage of defoliation ($cm^2$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Early vegetative | Bloom/pegging | Pod/setting | Early vegetative | Bloom/pegging | Pod/setting | Early vegetative | Bloom/pegging | Pod/setting |
| **2019/2020** | 509.63 | 1943.51 | 5842.08 | 12.84 | 18.47 | 0.02 | 133.75 | 1267.36 | 12.02 |
| **2020/2021[1]** | 1470.90 | 3430.69 | 5246.24 | 4.63 | 10.26 | 6.29 | 68.04 | 352.01 | 329.73 |
| **2020/2021[2]** | 1470.90 | 3430.69 | 5246.24 | 36.35 | 6.48 | 24.81 | 534.66 | 222.36 | 1301.81 |

| Season | *Stegasta bosqueella* – EIL per plant | | | *Spodoptera cosmioides* – EIL per plant | | |
|---|---|---|---|---|---|---|
| | Early vegetative | Bloom/pegging | Pod/setting | Early vegetative | Bloom/pegging | Pod/setting |
| **2019/2020** | 14.36 | 78.71 | 0.29 | 0.46 | 2.50 | 0.01 |
| **2020/2021[1]** | 14.92 | 77.20 | 72.31 | 0.47 | 2.45 | 2.30 |
| **2020/2021[2]** | 117.25 | 48.76 | 285.48 | 3.73 | 1.55 | 9.07 |

1 – Without *S. bosqueella* damage only one plant was used per row analysis / 2 – With *S. bosqueella* damage only one plant was used per row analysis.

## DISCUSSION

There are no current action thresholds reported in the literature to control *S. bosqueella* and *S. cosmiodes* in peanuts. Moreover, the adoption of insecticides on a schedule basis, commonly used by Brazilian farmers, may not always be the best choice, or even necessary or effective to control these pests, especially if their population is low at the time of application (Poston et al. 1983, Pedigo 1995, Peterson and Hunt 2003, Damos and Savopoulou-Soultani 2010). Therefore, decision tools based on population size are indispensable for IPM strategies and could lead to a successful control of *S. bosqueella* and *S. cosmiodes* in peanuts, by reducing unnecessary pesticide applications and increasing farmer's profits.

We verified that when the defoliation is equal or above half of the leaves (50, 75 and 100%) the peanut pod quality decreases. Also, the reduction is higher when *S. bosqueella* damage (25% of plant buds removal) is present during the early vegetative stage (15 DAP) pod/setting stage (71-100 DAP). This result emphasizes the importance of having a decent canopy architecture during the growth of peanut plants. Similar results were observed by Enyi (1975), in which the author suggested that reduction of leaf area in earlier stages, lead to a reduction in the peanut plant architecture, because in the early stages, peanut plants use the assimilates produced by the leaves mainly to develop the main and side stems and to produce new leaves. Furthermore, we observed that the pod/setting stage is critical for grain yield and quality and the increase in the number of deformed pods after the intense defoliation, could be related to the fact that newly formed pods had their growth arrested due to restricted availability of internal supplies (Hodgson and Blackman 1956, Enyi 1975).

Similarly to pod quality, we observed that the growth stage of the plants drives the intensity that the grain yield is affected. Our results indicated that Pod/setting stage (71-100 DAP) is the most sensitive time for peanut defoliation, where the highest percent yield loss was observed. This result was also observed by other studies (Campbell 1978, Turner 1982, Endan et al. 2006). In addition, we observed that the yield decreases about 0.35 g/plant (45.5 kg/ha) and 0.92 g/plant for every 1% increase in canopy defoliation, respectively for season 2019/2020 (high competitive environment) and 2020/2021(isolated plants) when the defoliation occurs in Pod/setting stage (71-100 DAP). Also, we observed that defoliation at bloom/pegging stage (41-70 DAP) has a low effect in pod yield (not statistically significant ($P<0.05$)). Abbott et al. 2019, after performing six levels of defoliation (0, 20, 40, 60, 80 and 100%) by two defoliation timing (40 and 90 DAE) in the Georgia-06G runner-type peanut cultivar suggested that defoliation at 40 DAE did not affect pod yield, but when damage occurred 80 DAE, pod yield was reduced 18.6 kg/ha for every 1% increase in defoliation. Many other studies tried to correlate peanut defoliation with yield loss, particularly to understand pathogens damage. Previous studies have reported a yield loss rate of 57 kg/ha per unit defoliation increase (Backman and Crawford 1984), whereas Nutter and Littrell (1996) estimated yield losses in different locations/years varying from 33 to 99 kg/ha and Anco et al. 2020 an equivalent of 11 kg/ha per unit defoliation. Therefore, the degree of yield loss related to peanut defoliation can be different between cultivars, years and locations.

It is important to highlight that Brazilian farmers generally adopt a sowing density of 18 seeds/meters to compensate for the low viability of peanut seeds and it is well established that the environment in which the peanut plants are exposed, such as plant density, has a direct association to the plant yield (Morla et al. 2018). According to Onat

et al. (2017), pod number and pod weight per plant increases as soon as the plant density decreases. The referred authors, using a 5-25 cm plant density range, concluded that 5 cm between the peanut plants resulted in an average yield of 26.7 g/plant, while 25 cm showed a yield of 96.2 g/plant. Therefore, plant density will vary throughout the area and must be considered for pest control decisions, since the 100 cm between the peanut plants in season 2019/2020 adopted in our study, increased the potential for plant development and, consequently, plant yield.

Regarding the determination of EIL, the estimated parameters are dependent on the local costs for insecticide application and local crop values (Pedigo et al. 1986, Bueno et al. 2013). Our results showed that *S. bosqueella* can be an important pest in early vegetative state, because it can increase the yield loss by 25% due to feeding in the axillary bud region and will be a defoliator problem only in very high population (above 20 larvae per row). This result will address an important finding to understanding the impact of this key pest in Brazilian peanuts. For *S. cosmiodes* we noticed that, due to its high consumption capacity, the presence of at least 1 larva at pod/setting stage (71-100 DAP) is sufficient to cause economic loss. According to Deitz et al. (1992) appropriate thresholds for some Noctuidae species such as *Spodoptera frugiperda* (J. E. Smith 1797) and *Helicoverpa zea* (Boddie 1850) (Lepidoptera: Noctuidae) are 13 larvae per row meter in South Carolina, while the recommended ET for caterpillars in Alabama is four or more caterpillars per foot of row (Alabama Cooperative Extension System 2020). However, these are general recommendations and not stage specific as suggested in our study. Also, the lower tolerance observed in our study in comparison to the literature, could be related to the cost of control and quantity and value of the insecticides recommended, as well as to the higher potential consumption of *S. cosmiodies* in peanut (143.53 $cm^2$ per larva), since previous studies

documented that *S. frugiperda* feeding capacity in peanut is 94.56 $cm^2$ per larva (Garner and Lynch 1981).

In summary, our study demonstrated that protecting the plant canopy during the vegetative stage is essential to provide good plant architecture with a decent number of stems and pegs. Also, defoliation can inflict considerable loss to peanuts when plants are infested during pod/setting stage (71-100 DAP). Using the EILs generated in this study the decision making in peanuts requires a farmer to periodically scout peanut fields to assess *S. bosqueella* and *S. cosmioides* population size. However, once the sampling method currently recommended for peanuts is labor intensive and onerous, an improvement is essential to leverage the adoption for a Peanut IPM program. The ongoing improvement of digital agriculture will be essential to optimize insect pest sampling and provide real-time information and, therefore, leverage the decision making.

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## SUPPLEMENTARY MATERIAL

**Supplementary 1.** Monthly meteorological data for the peanut season 2019-2020 and 2020-2021 in Jaboticabal, São Paulo, Brazil.

| **Season 2019/2020 – planting date – 15/11/2019** | | | | | | |
|---|---|---|---|---|---|---|
| Month | Tmax | Tmin | Tavr | RH | Precipitation | DWR |
| November | 31.7 | 20.1 | 25.0 | 70.4 | 103.7 | 12 |
| December | 30.6 | 20.5 | 24.8 | 76.7 | 167.7 | 17 |
| January | 31.6 | 20.5 | 24.8 | 79.9 | 350.4 | 20 |
| February | 29.8 | 20.3 | 24.0 | 82.9 | 181.1 | 18 |
| March | 31.4 | 18.5 | 23.9 | 70.6 | 101.9 | 6 |
| April | 30.1 | 16.1 | 22.5 | 67.2 | 32.6 | 1 |
| **Season 2020/2021 – planting date – 11/12/2020** | | | | | | |
| Month | Tmax | Tmin | Tavr | RH | Precipitation | DWR |
| December | 31.4 | 20.2 | 24.5 | 76.7 | 312.4 | 22 |
| January | 31.8 | 20.7 | 25.2 | 73.6 | 138.8 | 15 |
| February | 31.4 | 19.7 | 24.6 | 73.4 | 91.7 | 12 |
| March | 31.5 | 19.8 | 24.8 | 72.2 | 65.2 | 8 |
| April | 30.1 | 16.2 | 22.5 | 65.2 | 32.3 | 4 |

Tmax: maximum temperature; Tmin: minimum temperature; Tavr: average temperature; RH: relative humidity; ND: number of rainy days.

## CHAPTER VI – FINAL CONSIDERATIONS

Brazil exported, on average, US$ 4.76 million in peanut products (grain and processed) between 1997 and 2001. Twenty years later, in 2020, this export value reached US$ 443 million, an amount almost ten times higher. Similarly, Brazil exported peanuts to about 56 markets in 2010, but, in 2020, this number practically doubled, reaching 100 different locations, in which, Russia, Algeria and the European Union are the main destinations for peanuts exported by Brazil (COMEX STAT http://comexstat.mdic.gov.br/pt/home).

Therefore, it is observed that Brazilian peanuts have been receiving more space in the international market due to the quality of the grain production. To accomplish this, there was a high investment in the peanut production chain, mainly in the sense of genetic improvement of cultivars and seed quality, management and cultivation techniques, farming mechanization, professionalization and specialization of production and quality control campaigns (e.g. product quality assurance certification seals). These practices resulted in higher quality products and food safety.

Although there was a recognized improvement in these sectors in recent years, it was realized that the current model used by peanut farmers does not maintain the balance between agricultural production and sustainability, as they leave aside the knowledge of cultural and phytosanitary management strategies. The main method of phytosanitary control in the peanut crop occurs through scheduled application, without considering the population size of the pest. As a consequence, in addition to the increase in the production costs and the possibility of chemical residues in the products, studies indicate that some insecticides are no longer effective in controlling peanut pests. Along with this, during in-person conversations with farmers during field

days, they raised questions about the difficulty of controlling certain pests, in which they had sprayed various products without getting the expected efficacy. Thus, this behavior may be a first indication of the presence of a resistant population. Consequently, it is clear that the development of more efficient management practices in Brazilian peanuts is essential. However, for the success of any pest control strategy, the knowledge on how a plant responds to injury of the target insect pest is essential. In this context, our findings will address many unclear questions that could leverage the adoption of the Integrated Pest Management (IPM) in peanut production systems.

One of the first measures of IPM is to recognize the main pests affecting the crop. In addition, as mentioned in previous chapters, the pest control decision in an IPM approach should be based on pest population size. Therefore, knowing the behavior of the pest throughout the cropping system is essential for successful control. Thus, in chapter II, we verified that *S. bosqueella* was the most abundant species, followed by insects belonging to the *Spodoptera* genus. Also, we showed that adult abundance presents higher occurrences during the bloom/pegging stage (41-70 DAP) and pod-setting (71-100 DAP) peanut growth stages.

Furthermore, within the IPM scope, the knowledge of insect biology and consumption potential is essential to develop efficient management strategies, since this information allows a better understanding of the target pest population dynamics and the ideal time for decision making. In addition, the comprehension of how the biological characteristics of a population are affected under a specific host is essential, since the plant host can alter the insect population growth strategies. So, in chapter III we observed that the IAC 503 peanut genotype is a better host for *S. bosqueella*, while IAC OL3 is more suitable for *S. cosmioides*. Also, the foliage consumption potential for

*S. cosmioides* was 31.5 and 27.8-fold greater than that observed in *S. bosqueella*, in IAC 503 and IAC OL3 genotypes, respectively.

Finally, in Chapters IV and V we characterized how peanut plants respond to defoliation by the major lepidopterous defoliators and estimated the lowest pest population density capable of causing economic damage to the crop, i.e., the economic injury level (EIL). Furthermore, once recent studies have demonstrated that the new technologies can optimize efficient pest detection, with precise recommendations for decision making in pest management, we identified certain spectral regions that were especially sensitive to measure peanut plant response to herbivory. Our results support the hypothesis that remote sensing in the NIR (777.42-1000 nm) portion of the electromagnetic spectrum can be used for classification of *S. bosqueella* and *S. cosmioides* injury. Also, we verified that *S. bosqueella* and *S. cosmioides* should be considered separately in sampling programs, and studies with simulated injury can be used to evaluate defoliator-induced stress in peanuts. The economic injury level for *S. bosqueella* is 14.65, 77.96 and 36.30 larvae per plant whereas for *S. cosmioides* is 0.74, 2.48 and 1.16 larvae per plant in the early vegetative, bloom/pegging and pod/setting stages, respectively.

The generated results will allow peanut stakeholders to make more and better assertive management decisions when dealing with peanut defoliation. However, it is important to highlight that additional studies at the field level must be carried out so that the findings of this and other studies are validated, as well as demonstrating to the producer that pest management based on technical recommendations in the peanut crop is possible. In addition, it is essential that there should be an integration between researchers, extension workers, cooperatives, entrepreneurs, and producers to overcome the challenges related to the adoption of peanut IPM.