PAULA DAIANA DE PAULO

IMPACT OF CLIMATE CHANGE FACTORS AND MANAGEMENT TOOLS ON THE FEEDING BEHAVIOR OF PHYTOSUCCIVOROUS INSECT PESTS

Thesis presented to the Universidade Federal de Viçosa, as part of the requeriments of the Entomology Graduate Program, to obtain title of *Doctor Scientiae*.

VIÇOSA
MINAS GERAIS - BRAZIL
2019

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa

T
P331i
2019

Paulo, Paula Daiana de, 1988-
Impact of climate change factors and management tools on the feeding behavior of phytosuccivorous insect pests / Paula Daiana de Paulo. – Viçosa, MG, 2019.
xi, 119 f. : il. (algumas color.) ; 29 cm.

Texto em inglês.
Orientador: Eugenio Eduardo de Oliveira.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Pragas agrícolas - Controle integrado. 2. Insetos - Controle - Métodos gráficos. 3. Mudanças climáticas. 4. Inseticidas. 5. *Bacillus thuringiensis* . I. Universidade Federal de Viçosa. Departamento de Entomologia. Programa de Pós-Graduação em Entomologia. II. Título.

CDD 22. ed. 632.9

PAULA DAIANA DE PAULO

IMPACT OF CLIMATE CHANGE FACTORS AND MANAGEMENT TOOLS ON THE FEEDING BEHAVIOR OF PHYTOSUCCIVOROUS INSECT PESTS

Thesis presented to the Universidade Federal de Viçosa, as part of the requeriments of the Entomology Graduate Program, to obtain title of *Doctor Scientiae*.

APPROVED: July 26st, 2019.

Paula Daiana de Paulo

Paula Daiana De Paulo
Author

Eugênio E. Oliveira

Eugênio Eduardo de Oliveira
Advisor

“Know all the theories, master all the techniques, but as you touch a human soul be just another human soul” *Carl Gustav Jung*

Acknowledgment

"GOD Thank you for giving me the strength and encouragement especially during all the challenging moments in completing this thesis. I am truly grateful for your exceptional love, faithfulness and grace during this entire journey"

To my parents, Júlio De Paulo and Vânia De Paulo, my sister, Paloma and niece Manuella, for all unconditional love, care, trust, joy transmitted and support.

To the Federal University of Viçosa and the Department of Entomology, for the opportunity to attend the doctorate studies and develop this work.

To Professor Dr. Eugênio Eduardo de Oliveira for having welcomed me in his laboratory, for the guidance, encouragement, opportunity, for all the teachings that contributed in a relevant way for my professional and personal development.

To Professor Dr. Eliseu Jose Guedes Pereira for the support and encouragement in this journey.

To Dr. Alberto Fereres, researcher of the Institute of Agricultural Sciences (ICA) CSIC, Madrid Spain, for the guidance, good reception and for offering me several learning opportunities during the period I was in his laboratory in Madrid.

To Dra. Elisa Garzo (ICA)-CSIC Madrid Spain, for the great support, patience and for all the teachings during the development of my work overseas.

To Dr. Khalid Haddi, for immense support, encouragement and each of his teachings during my training.

To Dr. Luis Viteri Jumbo for his teachings and support with LaTeX.

To those who trained me and collaborated with my academic career development, Dr. Germano Leão Demolin Leite, and Dr. Marcos Antonio Matiello Fadini.

To all colleagues and former laboratory members of the "Brain Phy", Edmar, Luís, Karla, Nathaly, Gaby, Wilson, Hígor, Pedro, Larine, Nadylla, Kamilla, Grazy, Javier, Micaela, Franciele, Ryan Jhoan, Viviana, Sara, Carol, Cecilia, Alvaro, Thiago, Lourenço, Emerson, Salinas, for their friendship, companionship and help during all this time. my gratitude will be forever to you all.

To all friends I made in Spain, Andrés, Irene, Tacina, Yuri, Marina Santos, Alan, Inés, Sandra, Pablo, Ane, Marina Díaz, Laura and Christian, thanks for all the help, fun moments and for such patience!

Contents

Abstract viii
Resumo x

General introduction **1**
References 6

1 Effects of elevated CO_2 on the biology and feeding behavior of *Bemisia tabaci* (Hemiptera: Aleyrodidae) B biotype on Bt Soybean Plants (*Glycine max* L. Merr.) **11**
Abstract 12
1.1 Introduction 14
Introduction 14
1.2 Material & Methods 18
Material & Methods 18
1.2.1 Plant material, whitefly population and CO_2 growth chamber conditions 18
Plant material, whitefly population and CO_2 growth chamber conditions 18
1.2.2 Whitefly life history 20
Whitefly life history 20
1.2.3 Probing and feeding behavior of *B. tabaci* using the Electrical Penetration Graphs (EPG) technique 20
Probing and feeding behavior of *B. tabaci* using the Electrical Penetration Graphs (EPG) technique 20
1.2.4 Analysis of Electrical Penetration Graphs (EPG) waveforms 21
Probing and feeding behavior of *B. tabaci* using the Electrical Penetration Graphs (EPG) technique 21
1.2.5 Plant biomass and foliar nutrient content 24
Plant biomass and foliar nutrient content 24
1.2.6 Statistical analyses 24

Statistical analyses 24
1.3 Results 25
Results 25
1.3.1 *Bemisia tabaci* life-history 25
Bemisia tabaci life-history 25
1.3.2 Probing and feeding behavior of *B. tabaci* on soybean plants 28
Probing and feeding behavior of *B. tabaci* on soybean plants 28
1.3.3 Plant above ground biomass and foliar nutrient content . . 33
Plant above ground biomass and foliar nutrient content 33
1.4 Discussion 37
Discussion 37
1.5 References 44
References 44

2 Detrimental effects of dual Bt genes maize on the preference, feeding and reproduction of the corn leaf aphid *Rhopalosiphum maidis* 60
Abstract 61
2.1 Introduction 62
Introduction 62
2.2 Material & Methods 64
Material & Methods 64
2.2.1 Plant material 64
Plant material 64
2.2.2 Aphid strain 64
Aphid strain 64
2.2.3 Feeding bioassays using the Electrical Penetration Graphs (EPG) technique 65
Feeding bioassays using the Electrical Penetration Graphs (EPG) technique 65
2.2.4 Analysis of Electrical Penetration Graphs (EPG) waveforms 66
Analysis of Electrical Penetration Graphs (EPG) waveforms 66
2.2.5 Reproductive output of *R.maidis* 69
Reproductive output of *R.maidis* 69
2.2.6 Choice assays 69
Choice assays 69
2.2.7 Statistical analyses 69

Statistical analyses 69
2.3 Results 70
Results 70
2.3.1 Probing and feeding behavior of *R. maidis* on Bt and non-Bt maize Plants 70
Probing and feeding behavior of *R. maidis* on Bt and non-Bt maize Plants 70
2.3.2 Reproductive out put of *R.maidis* 76
Insecticidal activityReproductive out put of *R.maidis* 76
2.3.3 Choice assays 78
Choice assays 78
2.4 Discussion 80
Discussion 80
2.5 References 85
References 85

3 Sublethal effects of imidacloprid associated with NaCl on the feeding behavior of the *Bemisia tabaci* on Bt soybean plants **93**
Abstract 94
3.1 Introduction 95
Introduction 95
3.2 Material & Methods 97
Material & Methods 97
3.2.1 Plant material and whitefly population 97
Plant material and whitefly population 97
3.2.2 Concentration-mortality bioassays 97
Concentration-mortality bioassays 97
3.2.3 Probing and feeding behavior of *B. tabaci* on plants treated with sublethal dose of imidacloprid using the Electrical Penetration Graphs (EPG) technique 99
Probing and feeding behavior of *B. tabaci* on plants treated with sublethal dose of imidacloprid using the Electrical Penetration Graphs (EPG) technique 99
3.2.4 Analysis of Electrical Penetration Graphs (EPG) waveforms 100
Analysis of Electrical Penetration Graphs (EPG) waveforms 100
3.2.5 Statistical analyses 101

Statistical analyses 101
3.3 Results 102
Results 102
3.3.1 Concentration-mortality results 102
Concentration-mortality results 102
3.3.2 Probing and feeding behavior of *B. tabaci* on soybean plants exposed to LC_{10} of imidacloprid associated or not to NaCl . 104
Probing and feeding behavior of *B. tabaci* on soybean plants exposed to LC_{10} of imidacloprid associated or not to NaCl . 104
3.4 Discussion 110
Discussion 110
3.5 References 113
References 113

Final considerations **118**

Abstract

DE PAULO, Paula Daiana, D.Sc., Universidade Federal de Viçosa, July, 2019. **Impact of climate change factors and management tools on the feeding behavior of phytosuccivorous insect pests**. Advisor: Eugênio Eduardo de Oliveira. Co-Advisors: Eliseu Jose Guedes Pereira and Mauricelia Ferreira Almeida Laranjeiras.

The population dynamics of phytosuccivorous pests may be impacted by factors associated to climate changes (e.g., elevated CO_2: eCO_2). eCO_2 induces alterations in plant metabolism, and indirectly, herbivorous insects performance, for instance, the feeding behavior of these insects may be affected by changes in phyisiology of plants, due to altered nutritional quality and traces of resistance mediated by these change. In addition, tools used for pest management in the current scenario may contribute to changing the status of non-target secondary pest populations (e.g., Phytosucciivorous). One of the most widely used management tools current is transgenic crops expressing *Bacillus thuringiensis* (Bt) insecticidal proteins for the control of Lepidoptera and Coleoptera pests. One of the shortcomings of Bt technology is whether the expression of these insecticidal proteins in plant tissues can further non-target pests (e.g., phytosuccivorous). Considering the potential risks of losses caused by sap-sucking insects on Bt crops, there is a need to improve management strategies, and the proper use of insecticides is still the most effective tool for the control of these pests. The application of neonicotinoid insecticides alone and in combination with sodium chloride (NaCl) is a common practice in Brazilian agricultural (e.g., soybean) production. The use of the NaCl (0.5% w/v) associated with neonicotinoid insecticides has effectively suppressed target herbivores, and guaranteed greater sustainability (e.g., delaying the development of pesticide resistance and reducing the quantities of products used). However, the potential effects of this practice on non-target organisms (including secondary pests) have not been the subject of further studies. Given the above considerations, the study of interactions of eCO_2, Bt plants, insecticidal molecules, and sap-sucking insects is crucial. The elucidation of such knowledge gaps will also facilitate the development of new management strategies in order to mitigate negative effects in current and future scenarios. Phytosucciivorous insects such as aphids and whiteflies have been reported causing considerable problems in maize and soybean crops, respectively. These insects cause direct and indirect damage to plants by sucking nutrients from the phloem sap and being vectors of viruses. Therefore, the present study takes an approach with investigations conducted to better understand the interaction of factors associated with climate change (eCO_2), genetically modified Bt plants (i.e., maize and soybean) and insecticidal molecules (i.e., imidacloprid associated with NaCl) in aphids and whiteflies. In the first chapter We examined the effects of eCO_2 on the morphology and biochemistry of a Bt soybean cultivar and the indirect effects on whiteflies life history and feeding behavior. Our results showed that there was a reduction in the nutritional quality of plants that indirectly affected biological parameters of *B. tabaci*. In addition, through the analysis of the whitefly feeding behavior, we verified changes in the traces of resistance of the plants. The second chapter deals with the interaction of Bt maize expressing two different toxins and the aphid *Rhopalosiphum maidis*. This work was carried out to evaluate the preference, performance and feeding behavior of *R.maidis* in maize plants with dual Bt genes (Cry1Ab and Cry1F) and in its non-Bt isoline. Our results showed that *R.maidis* presented a lower preference for the pyramided Bt maize, resulting in a lower number of aphids in these plants. During the feeding behavior test, the duration of the non-probe (insect without contact with the stylet in the plant) was significantly higher in Bt plants. Respectively, the frequency of activities related to phloem sap ingestion (E2 and E2s) was significantly lower in Bt maize expressing two different toxins. In the third chapter, used Bt soybean plants to evaluate the effects of a sublethal concentration of imidacloprid alone and in combination with NaCl to *B. tabaci*. The association of NaCl did not modify the toxicity of solutions containing imidacloprid, however it was possible to observe a more evident stress (i.e., alterations in the feeding behavior) in insects that fed on plants treated with CL_{10} in the absence of NaCl. Our results provide important information that can corroborate with the development of strategies for the integrated management of phytosuccivorous insects in current and future scenarios.

Resumo

DE PAULO, Paula Daiana, D.Sc., Universidade Federal de Viçosa, julho de 2019. **Impactos de fatores associados a alterações climáticas e ferramentas de manejo de pragas no comportamento alimentar de fitosuccívoros**. Orientador: Eugênio Eduardo de Oliveira. Coorientadores: Eliseu Jose Guedes Pereira e Mauricelia Ferreira Almeida Laranjeiras.

A dinâmica populacional de insetos fitosuccívoros pode ser afetada por fatores associados a mudanças climáticas (por exemplo, elevado CO_2: eCO_2). eCO_2 pode provocar alterações no metabolismo das plantas e, indiretamente, afetar o desempenho de insetos herbívoros, por exemplo, o comportamento alimentar desses insetos pode ser afetado por alterações na fisiologia das plantas, devido a alteração da qualidade nutricional e traços de resistência mediados por essas mudanças. Além disso, ferramentas usadas para o manejo de pragas no cenário atual podem contribuir para mudança do status de populações de pragas secundárias não-alvo (por exemplo, insetos fitosuccívoros). Uma das ferramentas de manejo mais utilizadas atualmente são as culturas transgênicas expressando as proteínas inseticidas de *Bacillus thuringiensis* (Bt) para o controle de pragas da ordem Lepidoptera e Coleoptera, principalmente. Uma das lacunas da tecnologia Bt é verificar se a expressão dessas proteínas inseticidas nos tecidos vegetais podem colaborar com aumento populacional de pragas secundárias não alvos (e.g., sugadores de seiva). Tendo em vista os riscos potenciais de perdas por insetos sugadores nas lavouras com cultivo Bt, há uma necessidade de melhorar o manejo, e o uso adequado de inseticidas ainda é o mais efetivo para o controle dessas pragas. A aplicação de inseticidas neonicotinoides isoladamente e em combinação com sal de cozinha (NaCl) é prática comum na produção agrícola (e.g., cultivo de soja brasileira). O uso do sal (0,5% p/v) associado a esse inseticida tem suprimido eficazmente herbívoros-alvo, garantido maior sustentabilidade (i.e., maior tempo de utilização). No entanto, os potenciais efeitos desta prática em organismos não alvos (incluindo pragas secundárias) não tem sido objeto de maiores estudos. Diante o exposto, o estudo das interações de eCO_2, plantas Bt, moléculas inseticidas e insetos fitosuccívoros é crucial. A elucidação dessas lacunas do conhecimento também facilitará o desenvolvimento de novas estratégias de manejo, a fim de mitigar os efeitos negativos nos cenários atuais e futuros. Insetos fitossuccívoros como pulgões e moscas brancas têm sido relatados causando problemas consideráveis em lavouras de milho e soja, respectivamente. Estes insetos causam danos diretos e indiretos nas plantas por sugarem nutrientes da seiva do floema e serem vetores de viroses. Portanto, o presente estudo faz uma abordagem com investigações conduzidas objetivando melhor compreender a interação de fatores associados a alterações climáticas (i.e, CO_2 elevado), plantas Bt (i.e., milho e soja) e inseticidas (i.e., imidaclopride associado ou não ao NaCl) em insetos sugadores (e.g., pulgões e moscas brancas). No primeiro capítulo nós examinamos os efeitos do eCO_2 na morfologia e bioquímica de uma cultivar de soja Bt e os efeitos indiretos na história de vida e no comportamento alimentar de *B. tabaci*. Nossos resultados mostraram uma redução na qualidade nutricional das plantas, afetando indiretamente parâmetros biológicos de *B. tabaci*, além disso, através da análise do comportamento alimentar da mosca branca, verificamos alterações nos traços de resistência das plantas. O segundo capítulo aborda a interação milho piramidado com duplo gene Bt e o pulgão *Rhopalosiphum maidis*. O objetivo foi avaliar a preferência, desempenho e o comportamento alimentar de *R. maidis* em plantas de milho com duplo gene Bt (Cry1Ab e Cry1F) e na sua isolinha não-Bt. Nossos resultados mostraram que *R. maidis* apresentou menor preferência pelo milho Bt piramidado, resultando em menor permanência de pulgões nessas plantas. Durante o ensaio de comportamento alimentar, a duração da não sonda (inseto sem o contato com o estilete na planta) foi significativamente maior nas plantas Bt. Respectivamente, as atividades relacionadas a ingestão de seiva do floema (E2 e E2s) foi significativamente menor no milho com duplo gene Bt. No terceiro capítulo, utilizamos plantas de soja Bt para avaliarmos os efeitos da aplicação de uma concentração subletal do imidaclopride, associada ou não ao (NaCl), a mosca branca *B. tabaci*. A associação do NaCl não modificou a toxicidade das soluções contendo imidaclopride, no entanto foi possível observar um estresse mais evidente em moscas alimentadas em plantas tratadas com a CL_{10} na ausência do NaCl. Nossos resultados fornecem importantes informações que podem corroborar com o desenvolvimento de estratégias para o manejo integrado de insetos sugadores em cenários atuais e futuros.

General introduction

Global atmospheric CO_2 concentrations has risen by approximately 280 up to 400 ppm since the industrial revolution (Bonan and Doney, 2018). The latest forecast indicates that atmospheric CO_2 concentration will rise to approximately 900 ppm by the year 2100 (IPCC, 2014). The effects of elevated CO_2 on plants include increases in photosynthetic rate, biomass and carbon: nitrogen (C: N) ratio (Ainsworth and Rogers, 2007). The insect–host plant interactions can be altered with elevated CO_2 (eCO_2) due to changes in the nutritional quality and physiology of the host plants. Several species of herbivorous insects (e.g., sap-sucking) have shown changes in biology and behavior when fed on less nutritious host plants under elevated CO_2 conditions (Oehme et al., 2013; Satishchandra et al., 2018; Oehme et al., 2013).

In addition, tools used for pest management in the current scenario may contribute to changing the status of non-target secondary pest populations (e.g., Phytosucciivorous). One of the most widely used management tools current worldwide is the cultivation of plants (soybeans, maize, and others) expressing Cry proteins of *Bacillus thuringiensis* (James, 2011), not only due to their high effectiveness in pest control but also due to the ease of cultivation. These genetically modified plants express Cry proteins from the genes of *B. thuringiensis* specific for Lepidoptera and Coleoptera (Head et al., 2001). Bt maize and soybeans often in combination with the genetic modification for herbicide tolerance have been widely cultivated worldwide (Bortolotto et al., 2014; James, 2011).

However, the widespread adoption of the first generation of transgenic crops expressing only one Cry protein has led to intense selection in insect populations and accelerated the evolution of resistance (James, 2011; Santos-Amaya et al., 2015; Tabashnik et al., 2013; Carrière et al., 2016; Tabashnik and Carrière, 2017). One of the strategies adopted to overcome these evolving resistance cases was the use of "pyramid" Bt crops (Tabashnik et al., 2013; Brevault et al., 2013; Carrière et al., 2015). Pyramiding genes cause transgenic plants to express two or more Bt proteins that are different in mode of action but effective against the same target pest (Ghimire et al., 2011).

Although having high action specificity against Lepidoptera and Coleoptera, expression of Bt proteins in crops like maize and soybean can also produce unintended effects on individuals and populations of non-target insects as well as on the entire insect communities. The potential impacts of Bt plants on non-target phytophagous insects can be direct (by Bt toxin), indirect (by reducing competition with target insects), or a combination of both (Lumbierres et al., 2004). In fact, insertion of new genes may cause changes in plant quality as food for target and non-target herbivorous insects and thus altering reproductive ability or survival on the plant (Cuc et al., 2008). Therefore, it is important to understand the indirect impact of Bt plants on non-target species of pests as sap-sucking insects, where it is possible to occur population outbreaks that have often been neglected.

Considering the potential risks of losses by sap-sucking insects in crops of major economic importance with the adoption of high-cost technologies (e.g., Bt maize and soybeans), there is a need to improve the management of hemiptera insects in these crops. Thus, adequate use of insecticides is still the most effective for the control of these pests (Ghimire et al., 2011; Guedes, 2016). However, inadequate use of insecticides leads to substantial losses, such as the involvement of non-target organisms, the selection of resistant populations, and the occurrence of potential outbreaks of secondary pests (Sosa-Gómez and Silva, 2010; Guedes and Cutler, 2014; Tuelher et al., 2017; Castellanos et al., 2019).

Imidacloprid, the first neonicotinoid introduced, exhibits excellent contact and systemic activity and, therefore, has been largely used for the sustained management of sap-sucking in agronomic production systems worldwide (Jeschke and Nauen, 2008). With the degradation of neonicotinoids in large culture under field conditions, it is likely that highly polyphagous secondary pests (e.g., *B. tabaci*) will be exposed to the sublethal concentrations of neonicotinoids for relatively long periods. In addition, the use of sodium chloride (NaCl, 0.5% w/v) mixed with solutions containing organophosphorus insecticides leads to a 50 % reduction in the dose of insecticide to be used without loss of control efficiency (Coro and Gazzoni, 1998). However, little is known about the physiological basis of this synergistic effect, which requires more research. Previous studies have reported that neonicotinoid insecticides negatively affect the feeding behavior of several Hemiptera, including the whitefly (Nauen, 1995; Elbert and Nauen, 2004; Wang et al., 2017). However, the sublethal effect of neonicotinoids with NaCl yet to be reported.

Sap-sucking insects such as aphids and whiteflies have been reported causing considerable problems in maize and soybean crops, respectively (Suekane et al., 2013). These insects cause direct and indirect damage to plants by sucking nutrients from the phloem sap and are vectors of viruses (Fernandes et al., 2009; Ragsdale et al., 2011; Inoue-Nagata et al., 2016). In order to study the feeding behavior of phytosuccivorous, the technique of EPG (Electrical Penetration Graphs) (Tjallingii, 1988) has been widely used. The technique consists of incorporating the insect and the plant into an electrical circuit coupled to a measuring system and a computer, resembling an electrocardiogram. At the moment the insect inserts the stylet into the plant tissue, the circuit is closed and a voltage variation occurs which produces waveforms and patterns that correspond to the location and stylet activity (ingestion or salivation) during feeding.

Thus, this work of thesis approaches the study of the feeding behavior, through the EPG technique, the preference and performance of the *R. maidis* aphid in Bt maize plants with two resistance factors. In addition, the sublethal effect of the neonicotinoid insecticide imidacloprid, in association with or not to NaCl, and the effect of elevated CO_2 on Bt soybean plants in *B tabaci* were also well addressed in this study. The results of this work contribute to a better understanding of the interaction of genetically modified plants, insecticides associated with NaCl and, effects of elevated CO_2 in the feeding behavior of phytosuccivorous. In addition, our results will also facilitate the development of new management strategies in order to mitigate the negative effects in current and future scenarios.

Therefore, the objectives of this work were: evaluate the feeding behavior and population performance of *Bemisia tabaci* in Bt soybean plants, expressing the Cry1Ac toxin exposed to elevated concentrations of CO_2. Evaluate the preference and feeding behavior of *Rhopalosiphum maidis* in Bt maize plants expressing two Cry proteins (Cry1AB and Cry IF);and finally, evaluate the susceptibility and feeding behavior of *Bemisia tabaci* in soybean plants exposed to neonicotinoid imidacloprid, in association or not with NaCl.

References

Ainsworth, E. A. and Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. *Plant, Cell & Environment*, 30(3):258–270.

Bonan, G. B. and Doney, S. C. (2018). Climate, ecosystems, and planetary futures: The challenge to predict life in earth system models. *Science*, 359(6375):eaam8328.

Bortolotto, O., Bueno, A., Braga, K., Barbosa, G., and Sanzovo, A. (2014). Características biológicas de *Heliothis virescens* alimentados com bt-soybean MON87701× MON 89788 e sua isolinha convencional. *Anais da Academia Brasileira de Ciências*, 86(2).

Brevault, T., Heuberger, S., Zhang, M., Ellers-Kirk, C., Ni, X., Masson, L., Li, X., Tabashnik, B. E., and Carriere, Y. (2013). Potential shortfall of pyramided transgenic cotton for insect resistance management. *Proceedings of the National Academy of Sciences*, 110(15):5806–5811.

Carrière, Y., Crickmore, N., and Tabashnik, B. E. (2015). Optimizing pyramided transgenic bt crops for sustainable pest management. *Nature Biotechnology*, 33(2):161–168.

Carrière, Y., Fabrick, J. A., and Tabashnik, B. E. (2016). Can pyramids and seed mixtures delay resistance to bt crops? *Trends in Biotechnology*, 34(4):291–302.

Castellanos, N. L., Haddi, K., Carvalho, G. A., de Paulo, P. D., Hirose, E., Guedes, R. N. C., Smagghe, G., and Oliveira, E. E. (2019). Imidacloprid resistance in the neotropical brown stink bug Euschistus heros: selection and fitness costs. *Journal of Pest Science*, 92(2):847–860.

Coro, I. C. and Gazzoni, D. L. (1998). Sodium chloride: an insecticide enhancer for controlling pentatomids on soybeans. *Área de Informação da Sede-Artigo em periódico indexado (ALICE)*.

Cuc, N. T. T., Sujii, E. R., Wilson, L. J., Underwood, E., Andow, D. A., Van Hao, M., Zhai, B., and Van Chien, H. (2008). Potential effects of transgenic cotton on non-target herbivores in veitnam. *Ch*, 6:138–175.

Elbert, A. and Nauen, A. (2004). New applications for neonicotinoid insecticides using imidacloprid as an example. In *Insect Pest Management*, pages 29–44. Springer.

Fernandes, F. R., Cruz, A., Faria, J., Zerbini, F., and Aragão, F. J. (2009). Three distinct begomoviruses associated with soybean in central Brazil. *Archives of virology*, 154(9):1567–1570.

Ghimire, M. N., Huang, F., Leonard, R., Head, G. P., and Yang, Y. (2011). Susceptibility of Cry1ab-susceptible and -resistant sugarcane borer to transgenic corn plants containing single or pyramided *Bacillus thuringiensis* genes. *Crop Protection*, 30(1):74–81.

Guedes, R. N. C. (2016). Insecticide resistance, control failure likelihood and the first law of geography. *Pest Management Science*, 73(3):479–484.

Guedes, R. N. C. and Cutler, G. C. (2014). Insecticide-induced hormesis and arthropod pest management. *Pest Management Science*, 70(5):690–697.

Head, G., Brown, C. R., Groth, M. E., and Duan, J. J. (2001). Cry1ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. *Entomologia experimentalis et applicata*, 99(1):37–45.

Inoue-Nagata, A. K., Lima, M. F., and Gilbertson, R. L. (2016). A review of geminivirus diseases in vegetables and other crops in brazil: current status and approaches for management. *Horticultura Brasileira*, 34(1):8–18.

IPCC (2014). *IPCC. Climate Change 2014: Synthesis Report (eds Pachauri, R. K. & Meyer, L. A.)*.

James, C. (2011). *Global status of commercialized biotech/GM crops, 2011*, volume 44. ISAAA Ithaca, NY.

Jeschke, P. and Nauen, R. (2008). Neonicotinoids—from zero to hero in insecticide chemistry. *Pest Management Science: formerly Pesticide Science*, 64(11):1084–1098.

Lumbierres, B., Albajes, R., and Pons, X. (2004). Transgenic bt maize and *Rhopalosiphum padi* (Homoptera:Aphididae) performance. *Ecological Entomology*, 29(3):309–317.

Nauen, R. (1995). Behaviour modifying effects of low systemic concentrations of imidacloprid on *Myzus persicae* with special reference to an antifeeding response. *Pesticide Science*, 44(2):145–153.

Oehme, V., Högy, P., Zebitz, C. P., and Fangmeier, A. (2013). Effects of elevated atmospheric CO_2concentrations on phloem sap composition of spring crops and aphid performance. *Journal of Plant Interactions*, 8(1):74–84.

Ragsdale, D. W., Landis, D. A., Brodeur, J., Heimpel, G. E., and Desneux, N. (2011). Ecology and management of the soybean aphid in north america. *Annual review of entomology*, 56:375–399.

Santos-Amaya, O. F., Rodrigues, J. V. C., Souza, T. C., Tavares, C. S., Campos, S. O., Guedes, R. N., and Pereira, E. J. (2015). Resistance to dual-gene bt maize in *Spodoptera frugiperda*: selection, inheritance and cross-resistance to other transgenic events. *Scientific Reports*, 5(1).

Satishchandra, N. K., Vaddi, S., Naik, S. O., Chakravarthy, A. K., and Atlihan, R. (2018). Effect of temperature and CO_2 on population growth of south american tomato moth, *Tuta absoluta* (meyrick) (Lepidoptera: Gelechiidae) on tomato. *Journal of Economic Entomology*, 111(4):1614–1624.

Sosa-Gómez, D. R. and Silva, J. J. d. (2010). Neotropical brown stink bug (*Euschistus heros*) resistance to methamidophos in paraná, Brazil. *Pesquisa Agropecuária Brasileira*, 45(7):767–769.

Suekane, R., Degrande, P., de Lima Junior, I., de Queiroz, M., and Rigoni, E. (2013). Danos da mosca-branca *Bemisia tabaci* (genn.) e distribuição vertical das ninfas em cultivares de soja em casa de vegetação. *Arquivos do Instituto Biológico*, 80(2):151–158.

Tabashnik, B. E., Brévault, T., and Carrière, Y. (2013). Insect resistance to bt crops: lessons from the first billion acres. *Nature Biotechnology*, 31(6):510–521.

Tabashnik, B. E. and Carrière, Y. (2017). Surge in insect resistance to transgenic crops and prospects for sustainability. *Nature Biotechnology*, 35(10):926–935.

Tjallingii, W. (1988). Electrical recording of stylet penetration activities. In *Aphids, their biology, natural enemies and control*, pages 95–108. Elsevier Science Publishers.

Tuelher, E. S., da Silva, É. H., Freitas, H. L., Namorato, F. A., Serrão, J. E., Guedes, R. N. C., and Oliveira, E. E. (2017). Chlorantraniliprole-mediated toxicity and changes in sexual fitness of the neotropical brown stink bug *Euschistus heros*. *Journal of pest science*, 90(1):397–405.

Wang, X.-W., Li, P., and Liu, S.-S. (2017). Whitefly interactions with plants. *Current Opinion in Insect Science*, 19:70–75.

Chapter 1

Effects of elevated CO_2 on the biology and feeding behavior of *Bemisia tabaci* (Hemiptera: Aleyrodidae) B biotype on Bt Soybean Plants (*Glycine max* L. Merr.)

Effects of elevated CO_2 on the biology and feeding behavior of *Bemisia tabaci* (Hemiptera: Aleyrodidae) B biotype on soybean plants (*Glycine max* L. Merr.). Paula D.De Paulo, Elisa Garzo, Eugenio. E. Oliveira, Alberto Fereres; **Manuscript to be submited to Journal of Pest Science**.

Abstract

The performance of herbivore insects is modulated by the quality of their host plants. Elevated CO_2 induce alterations in plant metabolism, which indirectly, may alter the performance and feeding behaviour of herbivorous insects due to changes in the nutritional quality of their host plants. Understanding how pests respond to future climate boosted by increasing CO_2 is essential to predict how they may affect food security. We investigated the effects of elevated CO_2 (eCO_2) on a polyphagous insect pest, the whitefly MEAM1 species or B biotype of *Bemisia tabaci*, that transmits more than 300 plant virus species, some of them causing devastating diseases worldwide. We examined the effects of eCO_2 on the morphology and biochemistry of a *Bt*-soybean cultivar and the indirect effects on the life history and the feeding behavior of *B. tabaci*. Our results showed that changes in the C:N concentration and in plant morphology under eCO_2, alters whitefly life history and feeding behavior. We observed a significant increase of the egg to adult developmental time and a decrease in the fertility and fecundity of *B. tabaci* in plants under eCO_2. In addition, *B. tabaci* that fed on soybeans grown under eCO_2 negatively affected several parameters related to phloem sap ingestion and host plant resistence (e.g., reduction of E2, E2s, longest E2 and greater contribution of E1 to phloem ingestion). We expect that soybeans grown under future climate conditions will negatively affect the performance and feeding behavior of *B. tabaci* whiteflies, suggesting a reduction in the number of offspring and population growth.

Key words Integrated pest management, Electrical penetration graphs, whitefly, Carbon dioxide, Climate Change, Fitness

1.1 Introduction

Global atmospheric CO_2 concentrations has risen by approximately 280 up to 400 ppm since the industrial revolution (Bonan and Doney, 2018). The latest forecast indicates that atmospheric CO_2 concentration will rise to approximately 900 ppm by the year 2100 (IPCC, 2014). The effects of elevated CO_2 on plants include increases in photosynthetic rate, biomass and carbon: nitrogen ratio (C:N) (Ainsworth and Rogers, 2007). The assimilation and allocation of C and N resources within plant tissues under elevated CO_2 inevitably alters the host-plant physiology (mainly primary and secondary metabolites). This occurs, especially in C3 plants, in which the photosynthesis is limited by atmospheric concentrations of CO_2 (Gamage et al., 2018). In this way, the higher level of CO_2 in the atmosphere can contribute to higher yields in some plants (Korres et al., 2016; Ziska et al., 2007; Bisbis et al., 2018).

The increased CO_2 concentrations may promote higher yields in some plants and consequently, the nutritional quality for phytophagous insects may be altered. Generally, increases in carbon allocation reduce the amount of nitrogen in plant tissues, reducing the amount of soluble proteins and amino acids (Sun et al., 2009; Broberg et al., 2017). In addition, such changes may cause alteration in the production of secondary metabolites and plant defenses (Bidart-Bouzat and Imeh-Nathaniel, 2008; Casteel et al., 2008; Guo et al., 2014; DeLucia et al., 2012a; Zavala et al., 2013; Guo et al., 2014; Rajashekar, 2018; Teawkul and Hwang, 2018). Some studies have shown changes in sugar levels, flavonoids, caffeic acid, total phenolics, antioxidant capacity and jasmonic acid (JA) pathway in plants submitted to high concentration of CO_2 (Moretti et al., 2010; Jin et al., 2014; Becker and Kläring, 2016; Giri et al., 2016; Li et al., 2019).

The insect–host plant interactions can be altered with elevated CO_2 (eCO_2)

due to changes in the nutritional quality and physiology of their host plants. Several species of herbivorous insects have shown increased larval developmental duration when fed on less nutritious host plants under eCO_2 conditions (Coviella and Trumble, 1999; Satishchandra et al., 2018; Oehme et al., 2013a). Many plant-chewing insects increase their feeding rates on plants grown under eCO_2 to compensate for lower N levels in their host plant food source (Lindroth et al., 2001; Yin et al., 2010). In addition, Zavala et al. (2009) reported increased soybean susceptibility to two chewing insects, the Japanese beetle, and the western corn rootworm, by suppressing the expression of the proteinase inhibitor gene and other downstream genes of the JA pathway under eCO_2.

For sap-sucking insects these alterations in metabolic pathways and in plant nutritional quality can impact their behavior, biology, and their ability to transmit plant pathogens, in which the effects may differ according to the specific host plant (Coviella and Trumble, 1999; Robinson et al., 2012; DeLucia et al., 2012b; Dáder et al., 2016; Jones, 2016; Trebicki et al., 2017; Zavala et al., 2016). For example, the population abundance of *Rhopalosiphum maidis*, the feeding efficiency and fitness of *Myzus persicae* (Guo et al., 2017) and *B. tabaci* (biotype Q) (Li et al., 2011; Penalver-Cruz et al., 2019), has been positively affected when they fed of plants grown under eCO_2. Some other sap-sucking were not affected by eCO_2, such as *Aulacorthum solani* (Hughes and Bazzaz, 2001) and *B. tabaci* (biotype B) (Wang et al., 2014). Conversely, eCO_2 negatively altered the fecundity of *Rhopalosiphum padi* (Trebicki et al., 2016), body mass of *Brevicoryne brassicae* (Klaiber et al., 2012) and the populational abundance of *Actthosiphon pisum* (Hughes and Bazzaz, 2001). Additionally, elevated CO_2 reduced the efficiency of phloem sap ingestion by *Aphis craccivora*(Yan et al., 2018) and, decreased the fitness and transmission rate of Cucumber mosaic virus by *Myzus persicae* in bell pepper plants (Dader et al., 2016). In the face of what it is known, the impact of eCO_2 on the growth, development, and transmission of plant viruses by their insect vectors seems to be host-plant specific.

Members of the complex of cryptic species known as *Bemisia tabaci* (Hemiptera: Aleyrodidae) cause considerable economic losses worldwide (Oliveira et al., 2001; Gilioli et al., 2014). Untill now, at least 39 cryptic species have been found in the *B. tabaci* complex based on mtCOI sequence divergence (Firdaus et al., 2013; Boykin and Barro, 2014; da F Barbosa et al., 2014; Guo et al., 2015; Hu et al., 2017). Some species of the *B. tabaci* complex are highly polyphagous and invasive, particularly in the Middle East–Asia Minor 1 biotype (MEAM1, known as B biotype) (Oliveira et al., 2001; EFSA, 2013; Boykin, 2014). This species cause devastating losses on important agricultural crops (e.g., soybean) (Oliveira et al., 2012; Barro et al., 2011; Pozebon et al., 2019) due to damage caused through direct feeding (phloem sap-sucking), toxin injection into the plant vascular system, and mainly, due to their transmission of various plant viruses (Navas-Castillo et al., 2011; Polston et al., 2014; Wang et al., 2017; Guo et al., 2019)

Soybean [*Glycine max* (L.) Merr., Fabaceae] is one of the crops with the greatest introduction of technology for insect control, with the development of genetically modified plants. The cultivars of transgenic soybeans expressing proteins from cry genes of *Bacillus thuringiensis* and are agronomically called Bt soybeans. They were developed for effective pest control, this is to reduce the population size of serious target pests, from Lepdoptera order, in soybean fields (Yu et al., 2011, 2013, 2014; ISAAA, 2017).Is has been observed in recent years an increase in the number of Bt non-target pest species (e.g.,*B. tabaci* biotype B) in areas cultivated with Bt soybeans, mainly in the reproductive stages of the plant. It is consequence, perhaps, of the decrease in the applications of insecticides in areas with this technology and where *B. tabaci* was considered only as an occasional pest, until recently. Although, it is now considered a key pest of soybean in some regions (Lima et al., 2002; Vieira et al., 2011; Oliveira et al., 2018).

Considering the economic relevance of soybean and the insect pest *B. tabaci* (MEAM1) worldwide, the study of insect-plant interactions under eCO_2 is crucial to evaluate its consequences on the environment and ecosystems, as well as to develop new management strategies. Ramos et al. (2019) reports that by 2050 and 2070 it will increased the plant susceptibility to *B. tabaci* and viruses in some regions, in order to mitigate negative effects in future scenarios. Researches that seek to understand the direct impacts of eCO_2 on changes in plant physiology and biochemistry and its indirect effects on herbivores, such as *B. tabaci*, can support the improvement of pest management strategies in a future climate change scenario. Therefore, the present study examines the feeding behavior using the Electrical Penetration Graphs (EPG) technique and life history of *B. tabaci* MEAM1 in soybean Bt that were exposed to elevated level of CO_2 (eCO_2=700 ppm). We tested the hypothesis that elevated CO_2 increases plant biomass, C: N ratio, and changes in the chemical compound concentration of these plants, thus altering the feeding behavior habits and the population performance of *B. tabaci*.

1.2 Material & Methods

1.2.1 Plant material, whitefly population and CO_2 growth chamber conditions

Soybean plants (*Glycine max*) cv TMG 2185IPRO, were used to analyse the effects of elevated CO_2 on plant growth parameters, plant biochemistry , the fitness and feeding behavior of *B. tabaci* Middle East-Asia Minor 1 species – (MEAM1). These plants express the Cry1Ab Bt toxin for resistance to several caterpillar species that attack soybean plants (Miklos et al., 2007) and bears tolerance to the herbicide glyphosate (Monsanto, 2013). The experiments were conducted at the Institute of Agricultural Sciences of the Spanish National Research Council (ICA-CSIC, Madrid, Spain) in two growth chambers with different CO_2 regimes: ambient CO_2 (aCO_2; 450 ppm) and elevated CO_2 (eCO_2; 700 ppm). Growing conditions were a temperature of 24:20°C (day: night); relative humidity:66%; photoperiod: 14:10 h (light: dark), with three Philips Green Power LED Production Modules Deep Red/Blue 150 on each shelf providing 200 μmol.m^2.s^{-1} of light intensity at canopy level. The soybean seeds were sown into pots (13 cm of diameter) with a 1:2 mixiture of vermiculite (Asfaltex S.A., Barcelona, Spain) and soil substrate (GoV4, Jiffy International, A.S. Norway). After sowing the pots were immediately transferred to each of the two growth chambers set at 400 and 700 ppm. Seven days after sowing, the seedlings started to be watered three times a week using 20-20-20 (N-P-K) Nutrichem fertilizer (Miller Chemical Fertilizer Corp., Pennsylvania, USA) at a dose of 1 g/L during the entire bioassays. Water in trays was maintained at a similar level between treatments and plants were rotated within the chambers to ensure there was no positional influence.

Colony of *B. tabaci* MEAM1 were kindly provided by Dr. J. López-Moya, Center for Research in Agricultural Genomics (CRAG-CSIC) Barcelona, Spain in 2012. The population was reared on eggplants (*Solanum melongena* L.) within metal-frame cages covered by insect-proof net in greenhouse conditions (temperature ranges of 24:20 ± 2 °C (day: night); a photoperiod of 16:8 h (light: dark); relative humidity of 70-80%) under high-pressure sodium lights (Osram Plantastar 400W E40). Whiteflies were synchronized prior to bioassay to guarantee age homogeneity at the time of the experiment (approximately one week old adults were used).

1.2.2 Whitefly life history

For each treatment, twenty soybean plants with three trifoliate leaves fully developed (vegetative stage V3) per treatment, previously grown during 25 days under aCO_2 (450 ppm) and eCO_2 (700 ppm), were infested with ten couples of adult whiteflies placed in a clip-cage (2 cm of diameter) on the abaxial side of the second fully expanded leaves. After a period of 24 h, both clip-cages and whitefly adults were removed and the evolution of 10 eggs per plant was monitored daily until adult emergence in two environmentally-controlled climatic chambers (24:20 °C (day:night); RH:66%; photoperiod 14:10h) each under the two CO_2 concentration regimes (aCO_2 and eCO_2). The emerged whitefly adults (i.e, less than 24 h after emergence) were sexed and 20 couples (replicates) were formed for each treatment. Each whitefly couple was placed inside individual clip-cages on the second expanded leaf of a new set of plants that were previously grown during 25 days under either aCO_2 (450 ppm) or eCO_2 (700 ppm). Duration of the egg stage, duration of each nymph instars (1^{st}, 2^{nd}, 3^{rd} and pupa) as well as the fecundity (total number of eggs laid by adult females) during 26 days and the fertility (percentage of hatched eggs) were evaluated for each treatment.

1.2.3 Probing and feeding behavior of *B. tabaci* using the Electrical Penetration Graphs (EPG) technique

Thirty soybean plants with three trifoliate leaves fully developed (vegetative stage V3), previously grown during 25 days under either ambient (450 ppm) and elevated (700 ppm) CO_2 levels were used to study the probing and feeding behavior of *B. tabaci* (MEAM1). The whitefly adult females were monitored using the Electrical Penetration Graphs (EPG) technique for eight hours of recording. A thin gold wire (2 cm length × 12.5 μm in diameter) glued to a thin copper wire

(2 cm length) was attached to the pronotum of each whitefly with a tiny drop of water based silver-conducting glue paint (Rodríguez-López et al., 2011).

Whiteflies were acclimatized for approximately one-hour before the EPG recording and were placed on the abaxial side of the second youngest leaf of each soybean plant. Recordings were performed using two Giga-4 DC amplifiers with 1 Giga-Ohm input resistance (EPG Systems, Wageningen, The Netherlands). EPG output was set to 50x gain and data was acquired at 100 Hz using DATAQ Di 700-710 A/D data acquisition USB device card (Data Instruments, Ohio, USA). Recordings were conducted in laboratory conditions at 25 ± 2°C inside a Faraday cage to avoid electrical noise and interference. A new plant and insect was used for each recording. Data acquisition and EPG analysis from 15 individual adult whitefly females per treatment were conducted using Stylet+software (EPG Systems, Wageningen, The Netherlands). EPG variables related to the probing and feeding behavior of the whiteflies were processed and analysed using the EPG-Excel data Worksheet v 5.0 (Sarria et al., 2009).

1.2.4 Analysis of Electrical Penetration Graphs (EPG) waveforms

Thirty-five EPG sequential and non-sequential variables (Table 1.1) from the EPG-Excel data workbook v. 5.0 were selected for each treatment. The whitefly feeding associated waveforms were identified. For identification, were previously correlated with behavioral events of *Trialeurodes vaporariorum* (West.) by Janssen et al. (1989) and in *B. tabaci* by Jiang et al. (1999). These waveforms were: waveform np, non-probing behavior (no stylet contact with the leaf tissue); waveform C, intercellular apoplastic stylet pathway where the insects show a cyclic activity of mechanical stylet penetration and secretion of saliva; waveform pd (potential drop), represents brief (4 to 12 s intracellular stylet punctures during the pathway phase (C). Furthemore, there were two waveforms related with the phloem activity: waveform E1, salivation into phloem sieve elements at the beginning of the phloem phase; and waveform E2, correlated with passive phloem sap uptake from the sieve elements. The term “probe” refers to any type of event during the period, in which the stylet of an individual insect is located in the plant tissue. And “non-probe” refers to the event with no waveform (indicating that the individual has its stylet outside plant tissues).

The EPG variables (mean ± standard error) were calculated as described in Backus et al. (2007): PPW, proportion of individuals that produced the waveform type; NWEI, Number of Waveform Events per Insect was calculated using the sum of the number of events of a particular waveform divided by the total number of insects under each treatment; WDI, Waveform Duration per Insect was calculated using the sum of durations of each event of a particular waveform made by each individual insect that produced that waveform divided by the total number of insects under each treatment. If there was no event of a particular waveform then scored by 0, WDEI, Waveform Duration per Event per Insect was the sum of the duration of the events for a particular waveform divided by the total number of events of that particular waveform under each insect.

Table 1.1: Sequential and non-sequential EPG variables used to compare the probing and feeding behavior of *Bemisia tabaci* female adults on soybean plants grown under aCO_2 (450 ppm) and eCO_2 (700 ppm) concentrations.

Non-sequential variables	***Non-sequential variables %***
Number of np	% C
Number of probe	% E1
Number of pd	% E2
Number of C	% E2s
Number of E1	% Contribution of E1 to phloem phase
Number of single E1	***Index***
Number of E2	Potential E2 index
Total duration of np	
Total probing time	***Sequential variables***
Total duration of C	Time to 1st probe from Start of EPG
Total duration of pd	Time from 1st probe to 1st E
Total duration of E	Time from the Beginning of that probe to 1st E
Total duration of E1	E1 followed by first sustained E2 (>10 min)
Total duration of E2	Time to from start of EPG 1st sustained E2 (10 minutes)
Total duration of sustained E2 (longer than 10 minutes)	Time from 1st probe to 1st sustained E2 (10 minutes)
Duration of the longest E2	Time from 1st probe to 1st E2
Duration of nonphloematic phase	Time from the beginning of that probe to 1st E2
Duration of first E2	Time from the Beginning of that probe to 1st sustained E2 (>10 min)

1.2.5 Plant biomass and foliar nutrient content

The experiment was conducted after the life history and EPG studies, and soybean plants were evaluated at two different ages: V3 vegetative stage (three trifoliate leaves fully developed) on the EPG study, and V6 vegetative stage (five trifoliate leaves developed) on the life history study, which required a longer duration for the execution of assay .

Ten soybean plants of each assay (life history and EPG studies) were processed, each plant was considered a replicate, totaling 10 replicates per treatment. Leaves and stems of each plant were separated, the stem length was measured and the leaves counted. After the evaluation of the morphological parameters, the samples of each test were divided into two parts. Five soybean plants of each assay was used to determine fresh weight, and subsequently, dried at 60 °C for dry weight determination. Another five soybean plants were frozen at -80 °C for further chemical analysis, totaling five replicates of each treatment to both assay . For the chemical analysis, the frozen -80°C samples were lyophilized to remove frozen water without the liquid phase, subsequently the leaf and stem samples were macerated for analysis. The total carbon (C) and nitrogen (N) concentration in the leaf tissue were determined by dry combustion using a Thermo Flash 2000 NC soil analyzer. The C: N ratio was calculated by dividing the C concentration by the N concentration for each leaf sample.

1.2.6 Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics, version 24.0 (IBM Corp., Armonk, NY). Raw data were checked for normality and homogeneity of variance using the Shapiro-Wilk W-Test before performing the parametric test. Data were transformed with ln (x+1) or $\arcsin\sqrt{x+1}$, if need to reduce heteroscedasticity. The data that followed a Gaussian distribution were analyzed by Student *t*-Test ($P > 0.05$). When data did not follow the normality assumptions, a non-parametric Mann-Whitney *U*-test ($P < 0.05$) was performed.

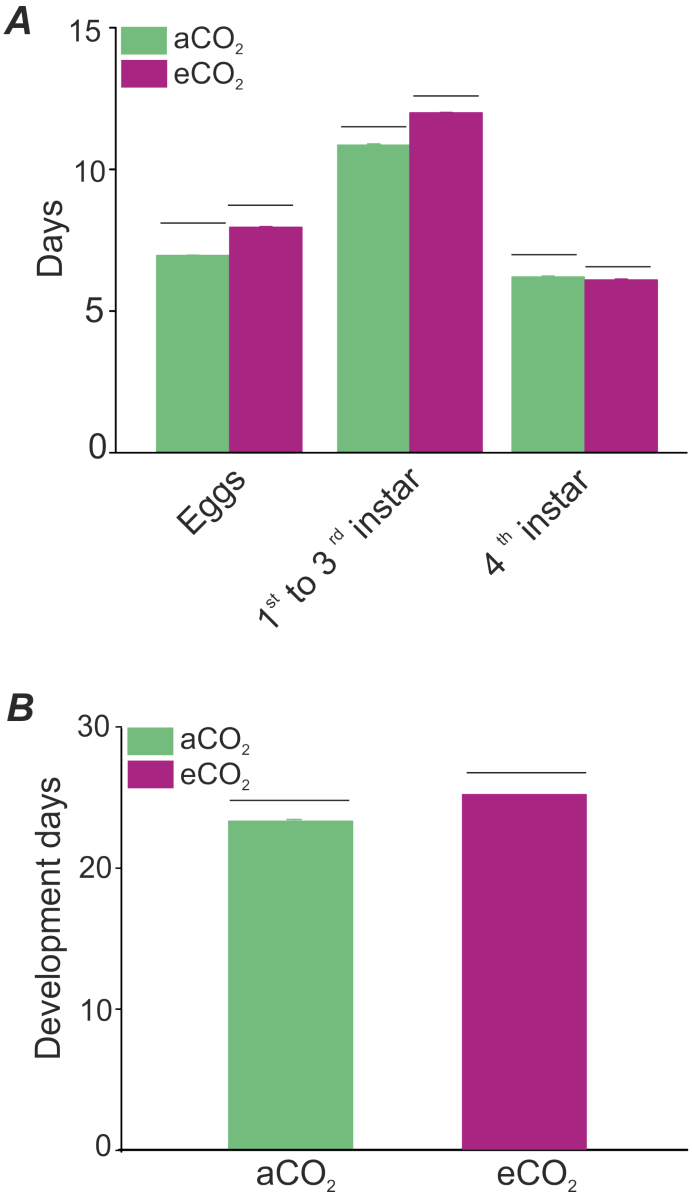
1.3 Results

1.3.1 *Bemisia tabaci* life-history

Elevated CO_2 levels on soybean plants affected all life-history parameters of *B. tabaci* (Figure 1.1). Developmental periods of immature stages of whitefly were negativaly affected by elevated CO_2. The mean duration of the embryonic period of laid eggs (aCO_2: 6.96 ± 0.01 days; eCO_2: 7.96 ± 0.01 days, U = 994.5, P = < 0.0001) and nymphal (1^{st} , 2^{nd} and 3^{rd} instars) developmental period (aCO_2: 10.86 ± 0.03 days; eCO_2: 12.00 ± 0.04 days, U = 4487.0, P= < 0.0001) were significantly longer on soybean exposed to elevated CO_2 than on the ones exposed to ambient CO_2. However, the development of the pupa stage was significantly longer in aCO_2 than in eCO_2 (aCO_2: 6.20 ± 0.03 days; eCO_2: 6.10 ± 0.03 days, U = 24143.5, P = 0.013) (Figure 1.1 A). In soybean plants exposed to eCO_2, the total development time (from egg to adult emergence) of *B. tabaci* was significantly higher when compared to ambient CO_2 (aCO_2: 24.04 ± 0.04 days; eCO_2: 26.08 ± 0.04 days, U = 971.00, P <0.001) (Figure 1.1 B).

The elevated CO_2 also affected negatively the fecundity and fertility of *B. tabaci* (MEAM1). The total number of eggs per female (fecundity) decreased in soybean plants under eCO_2 when compared to plants grown in aCO_2 (aCO_2: 226.56 ± 13.64; $eCO_{2:}$ 178.63 ± 10.54, t = 2.78, *df.* = 30, P = 0.009) (Figure 1.1 C). In addition, the percentage of eggs hatching (% fertility) of *B. tabaci* was also significantly reduced in soybean plants grown under eCO_2 compared to plants grown under aCO_2 (aCO_2: 94.71 ± 0.61; eCO_2: 90.38 ± 1.34, U = 55, P = 0.006)

(Figure 1.1 D).


A
15
10
5
0
Days
aCO₂
eCO₂
Eggs
1st to 3rd instar
4th instar
B
30
20
10
0
Development days
aCO₂
eCO₂
aCO₂
eCO₂

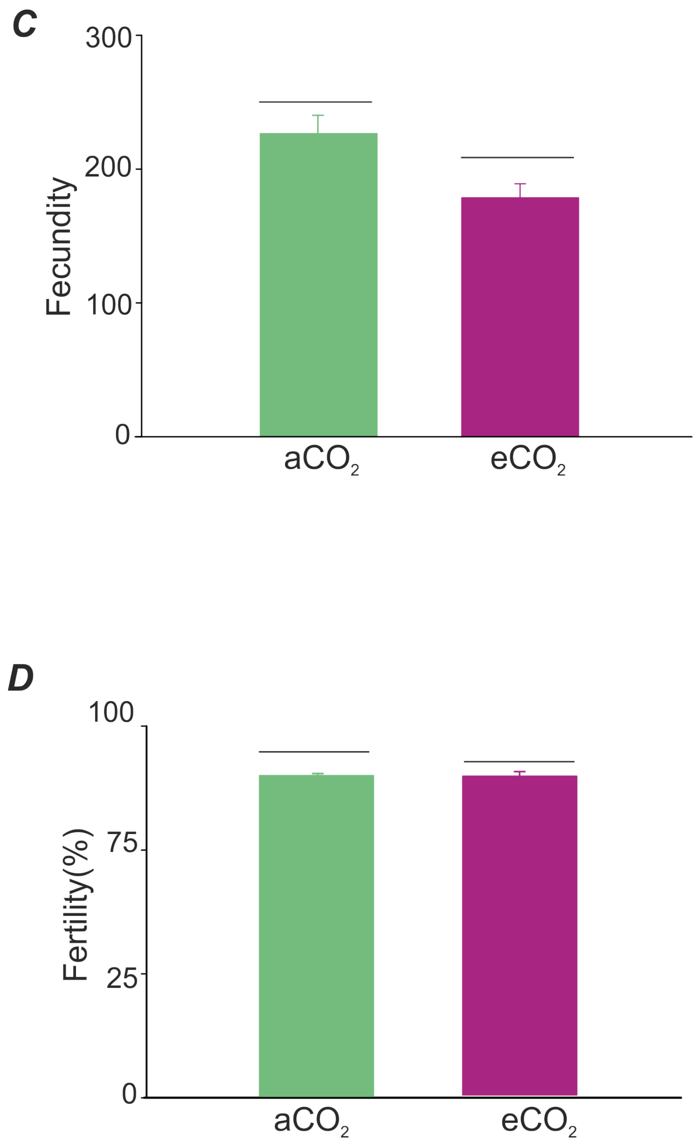


Figure 1.1: Results (mean ± SE) of the indirect effect of ambient CO_2 level (aCO_2=450 ppm) or elevated CO_2 level (eCO_2 = 700 ppm) CO_2 levels on different life parameters. (**A**) days duration of eggs to eclosion and developmental periods of inmature stages; (**B**) days duration of eggs to emergence adults; (**C**) Fecundity and (**D**) Fertility of *B. tabaci* on soybean plants.

1.3.2 Probing and feeding behavior of *B. tabaci* on soybean plants

Twenty five EPG variables were analyzed to study the probing and feeding behavior of *B. tabaci* on soybean plants grown under ambient and elevated CO_2 levels. Our results show that the feeding behavior of *B. tabaci* was altered on soybean plants grown under eCO_2. The temporal evolution is represented in (Figure 1.2). Its shows a specific feeding behavior of whitefly on soybean plants in 30-minutes intervals over a total recording time of 8 h. We observed that the percentage of whiteflies, that show phloem phase was dramatically reduced on plants exposed to eCO_2. Furthermore, significant differences were observed among treatments for some of the non-sequential and sequential EPG variables that are represented in (Figure 1.3).

The waveform duration (min/insect) (WDI) of sustained phloem sap ingestion (E2s) (aCO_2: 92.59± 27.21; eCO_2: 15.16 ± 10.10) and the duration of the longest E2 (aCO_2: 120.46 ± 32.64; eCO_2: 27.92 ± 16.23) decreased significantly in plants grown under eCO_2 (E2s: $U = 71$; $P = 0.016$; Longest E2: $U = 24.00$; $P = 0.029$) when compared with plants grown under aCO_2. Consequently the duration (min) of the non-phloematic phase was significantly higher when the whiteflies fed on soybean plants that were exposed to eCO_2 (aCO_2: 359.36 ± 31.62 ; eCO_2: 445.45 ± 19.29, $U = 35.00$; $P = 0.033$) compared to those that fed on plants grown under exposed to aCO_2 (Figure 1.3 A). In addition, the duration of the event (min/insect) (WDEI) of phloem ingestion (E2) (aCO_2: 71.10 ± 20.07 ; eCO_2:11.98 ± 5.33) and sustained phloem sap ingestion $>$ 10 minutes (E2s) (aCO_2: 145.68 ± 34.43; eCO_2: 64.44 ± 35.08) were significantly reduced on soybean exposed to eCO_2 (E2: $t = 2.63$; $d.f. = 19$; $P = 0.014$ and E2s: $t = 2.55$; $df = 11$; $P = 0.027$) (Figure 1.3 B). These results show that whiteflies reduce their ingestion of phloem sap on soybean plants that were grown under eCO_2.

The values of some sequential variables showed significant differences between the treatments. The whiteflies need more time to start phloem sap ingestion on soybean plants exposed to eCO_2. The duration from the first probe to the first phloem ingestion (E2) (aCO_2 : 230.51 ± 48.41 ; eCO_2: : 350.49 ± 32.94 min) and the time from the beginning of the recording to the first sustained phloem sap ingestion (Time from start EPG to 1^{st} E2 $>$ 10 min) (aCO_2: 316.58 ± 44.24 ; eCO_2: 357.41 ± 32.92) were significantly higher in plants exposed to eCO_2 (time from 1^{st} probe to 1^{st} E2: $U = 73.00$; $P = 0.040$ and time from start EPG to 1^{st} E2 $>$ 10 min: $U = 72.00$; $P = 0.035$ respectively) (Figure 1.3 C). In addition, the percentage of sustained phloem ingestion (E2s $>$ 10min) was significantly reduced ($U = 22.5$; $P = 0.017$) in whiteflies that fed on soybean plants under eCO_2 when compared to those whiteflies fed on soybean under aCO_2(aCO_2: 47.73 ± 10.06; eCO_2: 15.00 ± 6.31). Furthermore the contribution of salivation (E1) to phloem ingestion (E2) was significantly higher ($U = 26.00$; $P = 0.008$) in plants exposed to eCO_2 (aCO_2: 15.78 ± 8.86; eCO_2: 39.06 ± 10.42) (Figure 1.3 D), it should be emphasized that this last variable is an important indicator of plant resistance.

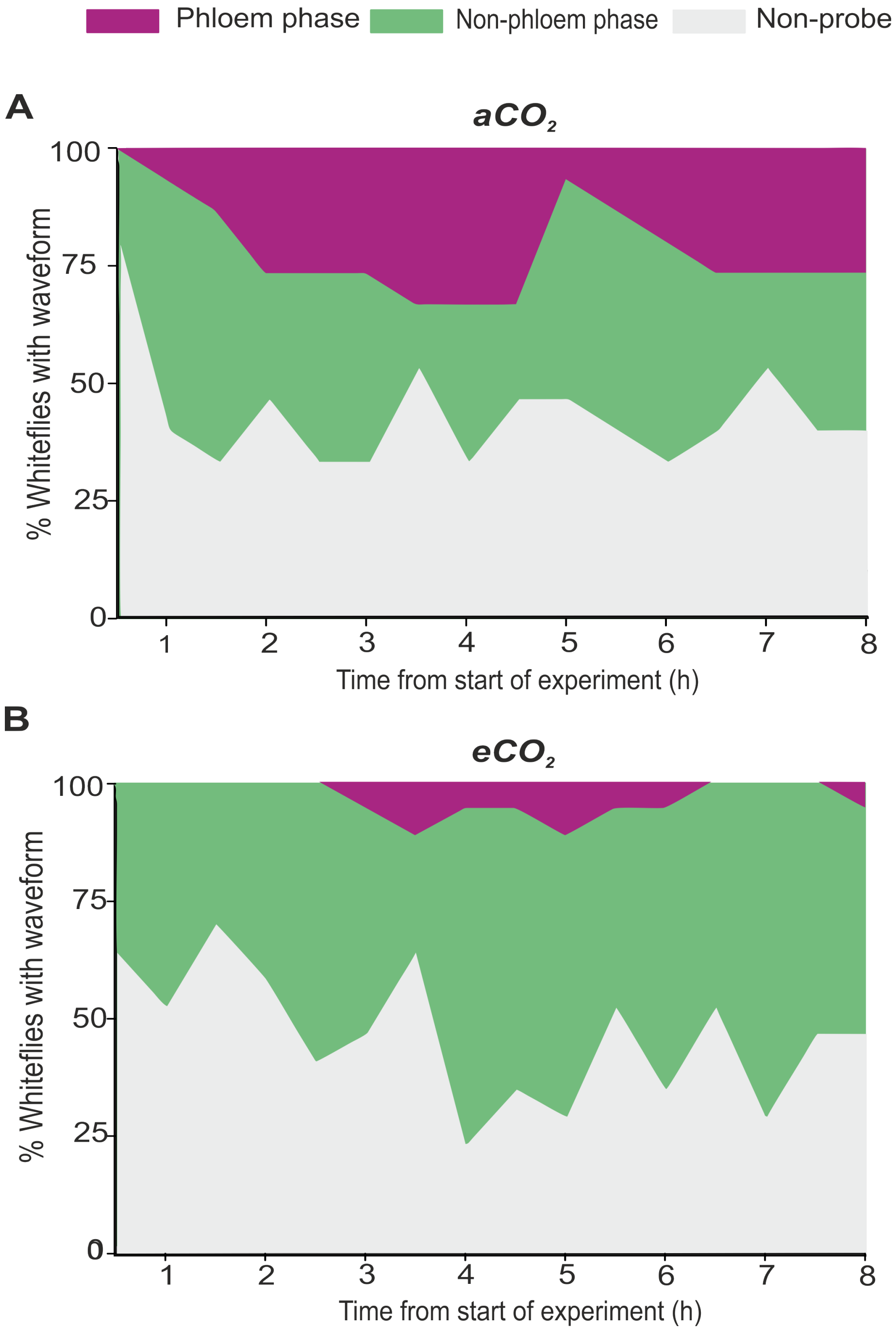
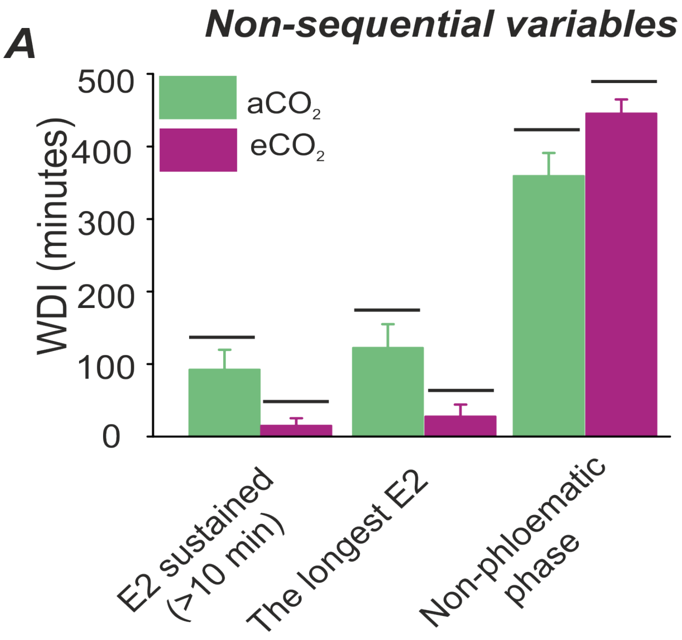
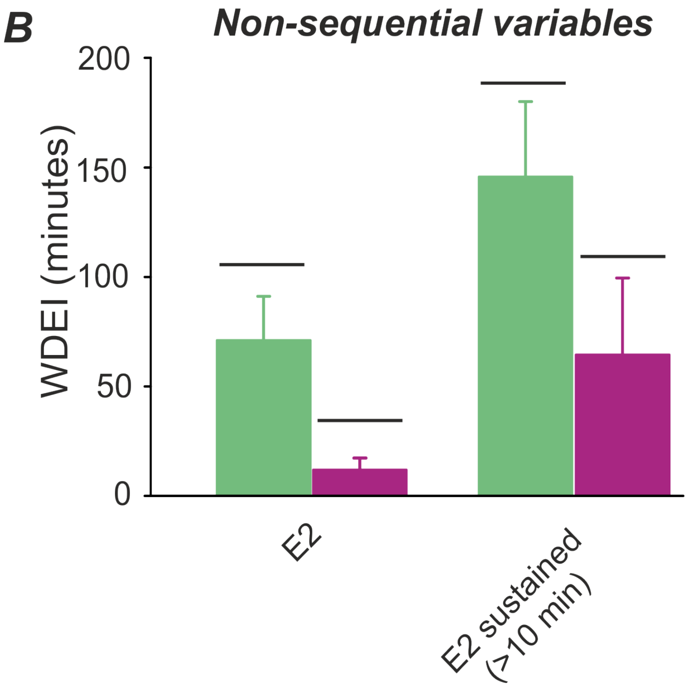


Figure 1.2: Percentage of witheflies (*Bemisia tabaci*) exhibiting specific electrical penetration graphs (EPG) waveforms in 30-min intervals over a total recording time of 8 h on soybean plants (*Glycine max*) under different levels of CO_2. Waveform np, nonprobing; waveforms of non-phloem phase (C, intercellular apoplastic stylet pathway; G, active xylem ingestion) and waveforms of phloem phase (E1, salivation and E2, passive phloem sap uptake from the sieve elements).


A
Non-sequential variables
aCO₂
eCO₂
WDI (minutes)
0
100
200
300
400
500
E2 sustained (>10 min)
The longest E2
Non-phloematic phase


B
Non-sequential variables
WDEI (minutes)
0
50
100
150
200
E2
E2 sustained (>10 min)

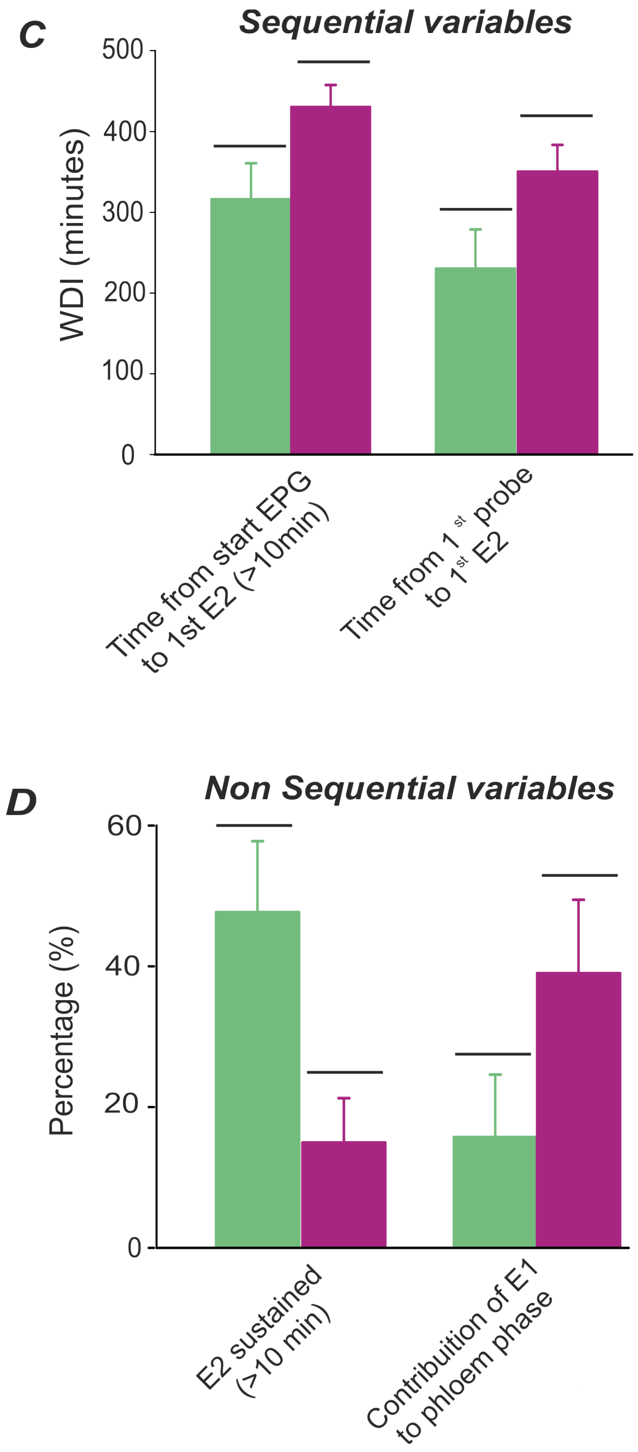


Figure 1.3: Mean± SE values of non-sequential EPG variables for the probing behavior of *B. tabaci* female adults on soybean plants grown under CO_2 concentrations (aCO_2 = 450 ppm and eCO_2 = 700 ppm). WDI: Waveform duration (min) per insect; WDEI: Waveform duration (min) per event per insect. E1, phloem salivation; E2, phloem ingestion; E2s, sustained E2 (>10 minutes)

1.3.3 Plant above ground biomass and foliar nutrient content

The results showed in (Figure 1.4) indicate that the morfology and foliar chemical composition of those soybean plants exposed to eCO_2 were significantly modified, in both phenological plant growth stages. For both plant growth stages (V3 and V6) the biosmass was significantly higher on plants grown under elevated CO_2 conditions. The most diferences was observed in the phenological stage V6. The number of leaves, the diameter and the fresh weight (FW) of stems were significantly higher on soybean plants exposed to eCO_2 in both phenological stages (Figure 1.4). The stem length (cm) of plants grown at eCO_2 was significantly higher only on those plants with phenological stage V3 (t = -4.03, df = 18, P = 0.001) (Figure 1.4).

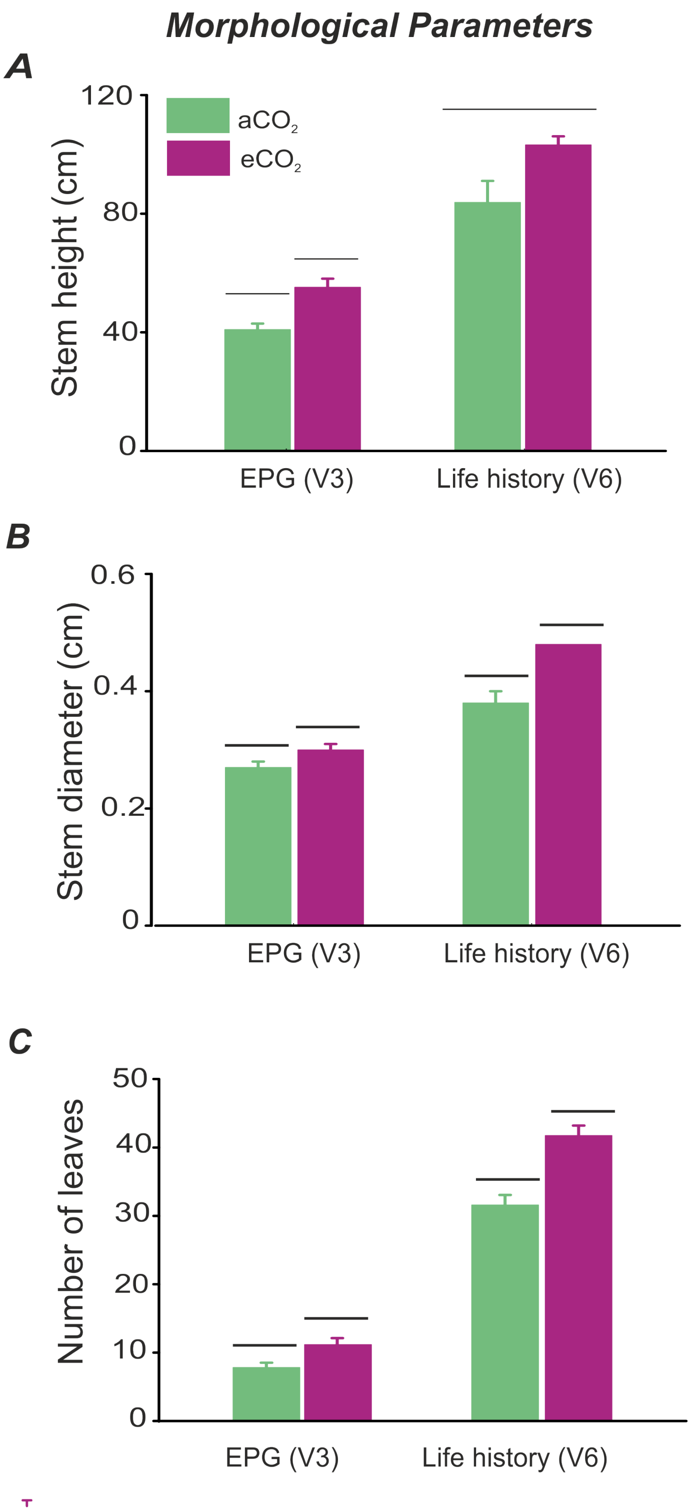


Figure 1.4: Mean ± SE values of plant growth parameters under ambiental CO_2 conditions (aCO_2: 450 ppm) and elevated CO_2 conditions (eCO_2: 700 ppm) in soybean plants with different phenology stage (V3 and V6): (A) Stem height (cm), (B) Stem diameter (cm) and (C) Number of leaves

Plant leaf content of carbon, nitrogen, and C:N ratio were evaluated in both phenological growth stage (V3 and V6), under both CO_2 levels (aCO_2 vs eCO_2) conditions (Fig 1.5). Only plants at V6 growth stage showed significant differences in the nitrogen content and C:N ratio. The carbon content was not significantly (t = -0.957; df = 8; P = 0.366) affected on those plants growing under both CO_2 levels (450 ppm and 700 ppm).The nitrogen (%) was significantly reduced (t = 3.15; df = 8; P = 0.014) on plants grown at eCO_2 conditions (700 ppm). And the C:N ratio was significantly higher (U = 2.00; P = 0.032) on those plants exposed to eCO_2 conditions than those plants grown under aCO_2 conditions (Figure 1.5).

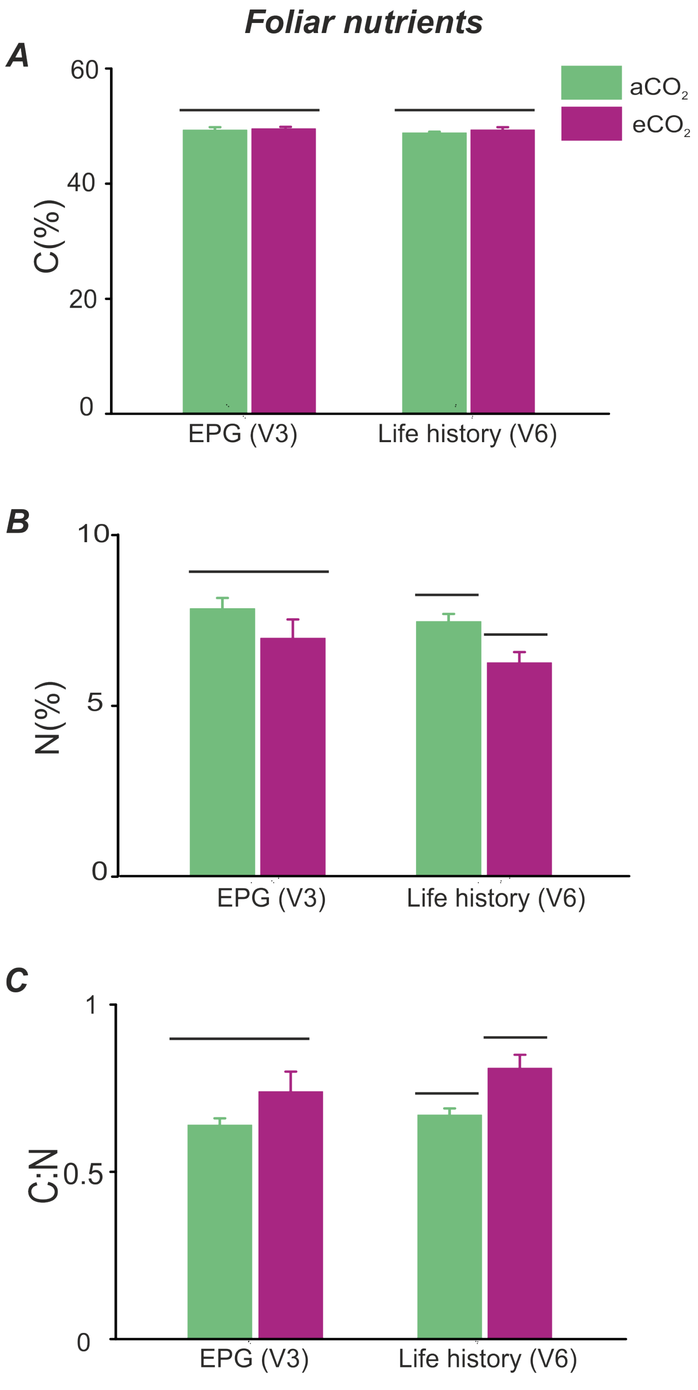


Figure 1.5: Mean ± SE values of plants nutrient content under ambiental CO_2 conditions (aCO_2: 450 ppm) and elevated CO_2 conditions (eCO_2: 700ppm) conditions in soybean plants with different phenology stage (V3 and V6):(A) % C on Leaves, (B) % N on Leaves, (C) C:N ratio on leaves

.

1.4 Discussion

This study shows that elevated CO_2 level modifies the morphology, and the biochemical composition of the plants, and indirectly these changes affected development time, fecundity, fertility and the feeding behavior of *B. tabaci* (MEAM1). The whiteflies wich developed on Bt soybean plants exposed to eCO_2 showed lower phloem ingestion; they required a longer time to complete the life cycle and the fecundity and fertility were affect negatively on these plants when compared with those whiteflies that developed on soybean plants exposed to aCO_2.

The indirect effects of elevated levels of CO_2 on sap-sucking insects are mainly mediated by their effects on host plant quality including changes in morphology, biochemistry, physiology and increase of the C:N ratio (Cornelissen et al., 2004; Finlay and Luck, 2011; Guo et al., 2013). Furthemore, it may be related to the reduction of amino acids in the phloem sap (Sun et al., 2009; Oehme et al., 2013b; Chen et al., 2019).

Generally, such changes are expected to vary with the plant species. Legumes often show an increase of nitrogen fixation via biological stimulation (Robinson et al., 2012) However, amino acid concentrations may vary according to legume species (Johnson et al., 2014). In some legume species, such as, *Trifolium repens*, *Lupinus albus*, *Pisum sativum* and *Glycine max*, the eCO_2 increase the size and number of nodules, and also nitrogen content (Zanetti et al., 1997; Ainsworth, 2003; Lee et al., 2003; Rogers et al., 2009; Li et al., 2017; Qiao et al., 2019).However, Kremer et al. (2018) reported that root nodulation on alfafa (*Medicago sativa*) was not affected under eCO_2, and they found amino acid in lower concentrations on plants without aphid infestation.

Our results showed that the N (%) was significantly lower in soybean plants exposed to eCO_2 at V6 growth stage, which may have caused negatively effects in the performance of *B. tabaci* (MEAM1) (e.g., increase in development time, fertility and fertility reduction) on these plants. These results may be associated with the planting of soybean in the autoclaved substrate and without Rhizobium, reducing the chances of biological fixation of N. Another factor that may have influenced the nitrogen reduction is the Bt soybean variety, some studies show that eCO_2 in Bt plants causes changes in the C: N ratio, significantly reducing the nitrogen, soluble sugars and starch contents. Jiang et al. (2017) showed the separate effect of CO_2 and N as limiting factors on the biomass accumulation in transgenic rice plants. The authors reported that the Bt protein content in rice plants under eCO_2 was higher than in ambient aCO_2, regardless of treatment with N. Chen et al. (2005) observed that at elevated CO_2 levels, transgenic Bt cotton had significantly lower nitrogen, soluble sugars, starch, and total non-structural carbohydrates than non-transgenic cotton. Furthermore, they found a significant decrease in Bt toxin on a cotton cultivar grown in elevated CO_2 compared to the one grown in ambient CO_2.

The transgenic Bt plants invest some nutrients in the production of specific toxic proteins [i.e. endotoxins of *Bacillus thuringiensis* (Bt)], which could alter the C: N balance of these plants, especially under altered abiotic conditions (Coviella et al., 2000; Coviella, 2002). Himanen et al. (2008) found that rape plants (*Brassica napus*) genetically modified with the Bt gene, grown under eCO_2, decreased the N content and increased the C:N ratio of Bt plants. These results may indicate that the expression of Bt protein in soybeans may have corroborated the change in the nutritional quality of soybean plants grown under eCO_2.These factors could contribute to an increasing in the C:N ratio (Figure 1.5 C), promoting a reduction of the nutritional quality of the phloem-sap, altering the concentration of amino acids (N-containing compounds) that are essential for the nutrition and performance of phloem sap-sucking insects (Douglas, 2006; Rashid et al., 2016).

In addition to changes in soybean leaf nutrient composition, eCO_2 in this study caused changes in the morphology of soybean plants, the number, and density of leaves and stems were significantly higher in soybean plants grown under eCO_2 (Fig 1.4). Similar results were found by Yifei et al. (2018), whom observed that eCO_2 had promoted increased biomass and productivity of soybean crops, and had negative effects on the development of *Spodoptera litura* (Fabricius). This increase in biomass may have resulted in physical and chemical barriers in these plants, making feeding by *B. tabaci* difficult. Guo et al. (2014) demonstrated that eCO_2 increased the density of non-glandular trichomes in young leaves and mature leaves, as well as that of glandular trichomes in mature leaves of *M. trunculata*. In their study, the time spent by the aphid for its first probe in the plant (i.e. to find the proper feeding site) increased with an eCO_2 concentration. In epidermis and mesophyll probing, any obstacles could impede localizing or reaching phloem vessels by the stylets (Will and van Bel, 2006; Walling and Thompson, 2012).

For example, Rodríguez-López et al. (2011) demonstrated a resistance based on glandular trichomes in tomato plants associated with pre-phloem factors (epidermis) that reduced the attractiveness and efficiency of the interaction of *B. tabaci*-phloem. Our results showed that *B. tabaci* took a similar period of time to introduce the stylet for the first probe on soybean grown under eCO_2 treatments. Therefore, we suggest that eCO_2 did not cause changes in the superficial epidermis of the leaf and that may have affected the probing behavior of the whitefly. On the other hand, the results obtained in the present study (Figure 1.1 C) show that whiteflies need a longer time to start phloem activities in plants exposed to elevated CO_2 level (i.e. Time from 1st probe to 1st E2 and Time to from start of EPG 1st E2 $>$10 minutes). Other factors such as changes in chemical on plants mesophyll may have affected the feed behavior of these insects.

In general, the stimulation of primary metabolism by elevated CO_2 enhances carbon flow towards the synthesis of secondary metabolites, most of these metabolites are related to plant defense against possible stressors (e.g., herbivorous insects) (Noctor and Mhamdi, 2017; Lu et al., 2018). The accumulation of allelochemicals has been previously associated with mesophyll defenses and antifeedant activities (Gabrys and Tjallingii, 2002; Kordan et al., 2012). About that, Hohenstein et al. (2019) reported soybean accumulated isoflavones phytoalexins in mesophyll cells as a mechanism of defense against aphids, deterring feeding before aphids reach the phloem. Zavala et al. (2013) indicated that plants cultivated under eCO_2 altereted the production of allelochemicals, thus, the longer time to start phloem activities by whiteflies in soybean cultivated under eCO_2 in this work may have due to the bigger to the accumulation of these compounds in the leaf mesophyll.

In addition, the results of the present study showed that the interaction phloem × *B.tabaci* was altered on soybean exposed to eCO_2. The amount of time spent in phloem activities was relatively lower, and had a greater contribution of salivation (E1) to phloem intake in plants exposed to under eCO_2((Figure 1.3 D). After locating and penetrating a single sieve element and before beginning phloem-sap ingestion (E2 waveform), aphids inject watery saliva (E1 waveform) to overcome potential plant resistance factors at the phloem level, such as by preventing sieve element sealing (Tjallingii, 2006). The duration and frequency of saliva secretion may indicate the suitability of the host plant and define non-host plants (Trebicki et al., 2012). Resistance factors in phloem have been suggested in different types of plant–aphid interactions (Diaz-Montano et al., 2007; Peng and Walker, 2018). For example, phloem protein coagulation and callose deposition (Tjallingii, 2006; Garzo et al., 2017), accumulation of lectins (Down et al., 1996; Gatehouse et al., 1997), protease inhibitors (Kehr, 2006), and secondary metabolites (Dinant et al., 2010). Previous works suggests that increased or extended salivation has been correlated with whiteflies feeding on the resistant plant (Jiang and Walker, 2007; Liu et al., 2013; McDaniel et al., 2016). Thus, in this study, the increase of salivation of *B. tabaci* in soybean plants cultivated under eCO_2 may have been due to an increase in resistance at the phloem level.

Our results show that parameters related to phloem ingestion (E2) were negatively affected on soybean plants exposed to elevated CO_2. The duration of E2, longer duration of E2 (phloem ingestion < 10 min) and the total duration of E2 sustained (E2s: phloem ingestion > 10 min) were significantly lower in plants exposed to elevated. Similar results were found by (Penalver-Cruz et al., 2019), in which the species of *B. tabaci* MED reached the phloem fewer times. They observed that the ingestion of phloem sap was delayed in plants that were previously exposed to high levels of CO_2 than in those that were grown under ambient CO_2. However, the authors reported that whiteflies that were indirectly affected by eggplants cultivated under eCO_2 levels showed longer episodes of sustained phloem ingestion and greater fertility. The authors suggested that *B. tabaci* MED was able to compensate the nutritional deficit of eggplant under eCO_2.

In contrast to that, Chen et al. (2019) found that corn leaf aphid *R. maidis* on eCO_2 barley seedlings, increased the time spending in passive phloem feeding, but it had lower in fecundity, intrinsic rate of natural increase and fresh body weight. The authors reported that the decrease in plant nutrient contents in eCO_2 barley seedlings may have increased the time spending in passive phloem feeding which was unfavorable for its reproductive and population abundance.

Similar results were also found by Trebicki et al. (2016). Their results indicated that *R. padi* reduced fecundity and increased feeding rates in wheat plants grown under eCO_2 in order to compensate the nutritional deficit of the plants exposed to eCO_2. Similarly, a study with *Acyrthosiphon pisum* feeding on *Medicago truncatula* indicated that eCO_2 reduced the stomatal opening of plants (Sun et al., 2015). This induced an increase in phloem and xylem sap ingestion by *A. pisum* as a result of the decrease in transpiration and increase water potential of *M. truncatula*.

Our results provide strong evidence that the nutritional quality of soybeans under eCO_2 is reduced, negatively affecting the feeding behavior of *B. tabaci* biotype B females, which drastically reduced phloem ingestion. These results indicate that *B. tabaci* biotype B did not demonstrate the ability to overcome the presumed stress that eCO_2 caused in soybean plants.

In addition to the changes caused by eCO_2 in the *B. tabaci* interaction and soybean plants, it is necessary to emphasize the importance of the eCO_2 interaction in virus transmission by insects that feed on sap. Infection with a pathogen can modify host appearance and physiology, making it more attractive to vectors (Fereres et al., 2016; Trebicki et al., 2016). Previous studies showed that eCO_2 promoted the ability of aphids (e.g. *M. persicae*) to transmit viruses (e.g. *Cucumber mosaic virus* or CMV) in bell pepper (Dader et al., 2016) and Potato virus Y (PVY) in tobacco (Bosquee et al., 2018). In view of the above, the interaction eCO_2, soybean and *B. tabaci* could differ in a scenario with the presence of the virus.

Based on the results presented in this research, the elevated CO_2 modifies the morphology, and the biochemical composition of the plants, and indirectly, these changes affected the feeding behavior and the biology (i.e., development time, fecundity and fertility) of the whitefly population. It was also demonstrated that *B. tabaci* MEAM1 was not able to overcome the decline of nutritional quality in soybean plants under eCO_2, where phloem ingestion was lower, and consequently delayed the life cycle, as well as reduced fecundity and fertility. Therefore, *B. tabaci* MEAM1 may not adapt to changes in abiotic factors compared to other herbivores as the climate continues to change. Our results may help in future studies involving climate change and virus transmission, as well as assist in new management strategies for populations of *B. tabaci* in future scenarios of climate change.

Acknowledgments The authors would like to acknowledge Institute of Agricultural Sciences of the Spanish National Research Council (CSIC), and the financial support from CAPES Foundation, and the National Council of Scientific and Technological Development (CNPq). The article is part of the first author's PhD thesis. The authors declare that there are no conflicts of interest.

1.5 References

Ainsworth, E. A. (2003). Variation in acclimation of photosynthesis in *Trifolium repens* after eight years of exposure to free air CO_2 enrichment (FACE). *Journal of Experimental Botany*, 54(393):2769–2774.

Ainsworth, E. A. and Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. *Plant, Cell & Environment*, 30(3):258–270.

Backus, E. A., Cline, A. R., Ellerseick, M. R., and Serrano, M. S. (2007). *Lygus hesperus* (Hemiptera: Miridae) feeding on cotton: New methods and parameters for analysis of nonsequential electrical penetration graph data. *Annals of the Entomological Society of America*, 100(2):296–310.

Barro, P. J. D., Liu, S.-S., Boykin, L. M., and Dinsdale, A. B. (2011). *Bemisia tabaci*: A statement of species status. *Annual Review of Entomology*, 56(1):1–19.

Becker, C. and Kläring, H.-P. (2016). CO_2 enrichment can produce high red leaf lettuce yield while increasing most flavonoid glycoside and some caffeic acid derivative concentrations. *Food Chemistry*, 199:736–745.

Bidart-Bouzat, M. G. and Imeh-Nathaniel, A. (2008). Global change effects on plant chemical defenses against insect herbivores. *Journal of Integrative Plant Biology*, 50(11):1339–1354.

Bisbis, M. B., Gruda, N., and Blanke, M. (2018). Potential impacts of climate change on vegetable production and product quality – a review. *Journal of Cleaner Production*, 170:1602–1620.

Bonan, G. B. and Doney, S. C. (2018). Climate, ecosystems, and planetary futures: The challenge to predict life in earth system models. *Science*, 359(6375):eaam8328.

Bosquee, E., Boullis, A., Bertaux, M., Francis, F., and Verheggen, F. J. (2018). Dispersion of textitMyzus persicae and transmission of potato virus y under elevated CO_2 atmosphere. *Entomologia Experimentalis et Applicata*, 166(5):380–385.

Boykin, L. M. (2014). *Bemisia tabaci* nomenclature: lessons learned. *Pest Management Science*, 70(10):1454–1459.

Boykin, L. M. and Barro, P. J. D. (2014). A practical guide to identifying members of the *Bemisia tabaci* species complex: and other morphologically identical species. *Frontiers in Ecology and Evolution*, 2.

Broberg, M., Högy, P., and Pleijel, H. (2017). CO_2 -induced changes in wheat grain composition: Meta-analysis and response functions. *Agronomy*, 7(2):32.

Casteel, C. L., O'neill, B. F., Zavala, J., Bilgin, D. D., Berenbaum, M. R., and DeLucia, E. H. (2008). Transcriptional profiling reveals elevated CO_2 and elevated O_3 alter resistance of soybean (*Glycine max*) to japanese beetles (*Popillia japonica*). *Plant, Cell & Environment*, 31(4):419–434.

Chen, F., Wu, G., Ge, F., Parajulee, M. N., and Shrestha, R. B. (2005). Effects of elevated CO_2 and transgenic bt cotton on plant chemistry, performance, and feeding of an insect herbivore, the cotton bollworm. *Entomologia Experimentalis et Applicata*, 115(2):341–350.

Chen, Y., Serteyn, L., Wang, Z., He, K., and Francis, F. (2019). Reduction of plant suitability for corn leaf aphid (Hemiptera: Aphididae) under elevated carbon dioxide condition. *Environmental Entomology*.

Cornelissen, T., Stiling, P., and Drake, B. (2004). Elevated CO_2 decreases leaf fluctuating asymmetry and herbivory by leaf miners on two oak species. *Global Change Biology*, 10(1):27–36.

Coviella, C. E. (2002). Plant allocation to defensive compounds: interactions between elevated CO_2 and nitrogen in transgenic cotton plants. *Journal of Experimental Botany*, 53(367):323–331.

Coviella, C. E., Morgan, D. J. W., and Trumble, J. T. (2000). Interactions of elevated CO_2 and nitrogen fertilization: Effects on production of *Bacillus thuringiensis* toxins in transgenic plants. *Environmental Entomology*, 29(4):781–787.

Coviella, C. E. and Trumble, J. T. (1999). Effects of elevated atmospheric carbon dioxide on insect-plant interactions. *Conservation Biology*, 13(4):700–712.

da F Barbosa, L., Marubayashi, J. M., Marchi, B. R. D., Yuki, V. A., Pavan, M. A., Moriones, E., Navas-Castillo, J., and Krause-Sakate, R. (2014). Indigenous american species of the *Bemisia tabaci* complex are still widespread in the americas. *Pest Management Science*, 70(10):1440–1445.

Dáder, B., Fereres, A., Moreno, A., and Trębicki, P. (2016). Elevated CO_2 impacts bell pepper growth with consequences to *Myzus persicae* life history, feeding behaviour and virus transmission ability. *Scientific Reports*, 6(1).

Dader, B., Fereres, A., Moreno, A., and Trebicki, P. (2016). Elevated CO_2 impacts bell pepper growth with consequences to *Myzus persicae* life history, feeding behaviour and virus transmission ability. *Scientific Reports*, 6(1).

DeLucia, E. H., Nabity, P. D., Zavala, J. A., and Berenbaum, M. R. (2012a). Climate change: Resetting plant-insect interactions. *Plant Physiology*, 160(4):1677–1685.

DeLucia, E. H., Nabity, P. D., Zavala, J. A., and Berenbaum, M. R. (2012b). Climate change: Resetting plant-insect interactions. *PLANT PHYSIOLOGY*, 160(4):1677–1685.

Diaz-Montano, J., Reese, J. C., Louis, J., Campbell, L. R., and Schapaugh, W. T. (2007). Feeding behavior by the soybean aphid (Hemiptera: Aphididae) on resistant and susceptible soybean genotypes. *Journal of Economic Entomology*, 100(3):984–989.

Dinant, S., Bonnemain, J.-L., Girousse, C., and Kehr, J. (2010). Phloem sap intricacy and interplay with aphid feeding. *Comptes Rendus Biologies*, 333(6-7):504–515.

Douglas, A. E. (2006). Phloem-sap feeding by animals: problems and solutions. *Journal of Experimental Botany*, 57(4):747–754.

Down, R. E., Gatehouse, A. M., Hamilton, W. D., and Gatehouse, J. A. (1996). Snowdrop lectin inhibits development and decreases fecundity of the glasshouse potato aphid (*Aulacorthum solani*) when administered in vitro and via transgenic plants both in laboratory and glasshouse trials. *Journal of Insect Physiology*, 42(11-12):1035–1045.

EFSA (2013). *Scientific Opinion on the risks to plant health posed by Bemisia tabaci species complex and viruses it transmits for the EU territory*. European Food Safety Authority (EFSA), Parma, Italy.

Fereres, A., Peñaflor, M., Favaro, C., Azevedo, K., Landi, C., Maluta, N., Bento, J., and Lopes, J. (2016). Tomato infection by whitefly-transmitted circulative and non-circulative viruses induce contrasting changes in plant volatiles and vector behaviour. *Viruses*, 8(8):225.

Finlay, K. and Luck, J. (2011). Response of the bird cherry-oat aphid (*Rhopalosiphum padi*) to climate change in relation to its pest status, vectoring potential and function in a crop–vector–virus pathosystem. *Agriculture, Ecosystems & Environment*, 144(1):405–421.

Firdaus, S., Vosman, B., Hidayati, N., Supena, E. D. J., Visser, R. G., and van Heusden, A. W. (2013). The *Bemisia tabaci* species complex: Additions from different parts of the world. *Insect Science*, 20(6):723–733.

Gabrys, B. and Tjallingii, W. (2002). The role of sinigrin in host plant recognition by aphids during initial plant penetration. *Entomologia Experimentalis et Applicata*, 104(1):89–93.

Gamage, D., Thompson, M., Sutherland, M., Hirotsu, N., Makino, A., and Seneweera, S. (2018). New insights into the cellular mechanisms of plant growth at elevated atmospheric carbon dioxide concentrations. *Plant, Cell & Environment*, 41(6):1233–1246.

Garzo, E., Fernández-Pascual, M., Morcillo, C., Fereres, A., Gómez-Guillamón, M. L., and Tjallingii, W. F. (2017). Ultrastructure of compatible and incompatible interactions in phloem sieve elements during the stylet penetration by cotton aphids in melon. *Insect Science*, 25(4):631–642.

Gatehouse, A. M., Davison, G. M., Newell, C. A., Merryweather, A., Hamilton, W. D., Burgess, E. P., Gilbert, R. J., and Gatehouse, J. A. (1997). Transgenic potato plants with enhanced resistance to the tomato moth, *Lacanobia oleracea*: growth room trials. *Molecular Breeding*, 3(1):49–63.

Gilioli, G., Pasquali, S., Parisi, S., and Winter, S. (2014). Modelling the potential distribution of Bemisia tabaci in europe in light of the climate change scenario. *Pest Management Science*, 70(10):1611–1623.

Giri, A., Armstrong, B., and Rajashekar, C. B. (2016). Elevated carbon dioxide level suppresses nutritional quality of lettuce and spinach. *American Journal of Plant Sciences*, 07(01):246–258.

Guo, H., Peng, X., Gu, L., Wu, J., Ge, F., and Sun, Y. (2017). Up-regulation of MPK4 increases the feeding efficiency of the green peach aphid under elevated CO_2 in *Nicotiana attenuata*. *Journal of Experimental Botany*, 68(21-22):5923–5935.

Guo, H., Sun, Y., Li, Y., Liu, X., Zhang, W., and Ge, F. (2013). Elevated CO_2 decreases the response of the ethylene signaling pathway in *Medicago truncatula* and increases the abundance of the pea aphid. *New Phytologist*, 201(1):279–291.

Guo, H., Sun, Y., Li, Y., Liu, X., Zhu-Salzman, P. W. K., and Ge, F. (2014). Elevated CO_2 alters the feeding behaviour of the pea aphid by modifying the physical and chemical resistance of *Medicago truncatula*. *Plant, Cell & Environment*, 37(9):2158–2168.

Guo, Q., Shu, Y.-N., Liu, C., Chi, Y., Liu, Y.-Q., and Wang, X.-W. (2019). Transovarial transmission of tomato yellow leaf curl virus by seven species of the *Bemisia tabaci* complex indigenous to china: Not all whiteflies are the same. *Virology*, 531:240–247.

Guo, T., Guo, Q., Cui, X.-Y., Liu, Y.-Q., Hu, J., and Liu, S.-S. (2015). Comparison of transmission of *Papaya leaf curl China virus* among four cryptic species of the whitefly *Bemisia tabaci* complex. *Scientific Reports*, 5(1).

Himanen, S. J., Nossinen, A., Dong, W.-X., Nerg, A.-M., Stewart, C. N., Poppy, G. M., and Holopainen, J. K. (2008). Interactions of elevated carbon dioxide and temperature with aphid feeding on transgenic oilseed rape: Are *Bacillus thuringiensis* (bt) plants more susceptible to nontarget herbivores in future climate? *Global Change Biology*, 14(6):1437–1454.

Hohenstein, J. D., Studham, M. E., Klein, A., Kovinich, N., Barry, K., Lee, Y.-J., and MacIntosh, G. C. (2019). Transcriptional and chemical changes in soybean leaves in response to long-term aphid colonization. *Frontiers in Plant Science*, 10.

Hu, J., Zhang, X., Jiang, Z., Zhang, F., Liu, Y., Li, Z., and Zhang, Z. (2017). New putative cryptic species detection and genetic network analysis of *Bemisia tabaci* (Hempitera: Aleyrodidae) in china based on mitochondrial COI sequences. *Mitochondrial DNA Part A*, 29(3):474–484.

Hughes, L. and Bazzaz, F. A. (2001). Effects of elevated CO_2 on five plant-aphid interactions. *Entomologia Experimentalis et Applicata*, 99(1):87–96.

IPCC (2014). *Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* IPCC, Geneva, Switzerland, pp. 151.

ISAAA (2017). *Global Status of Commercialized Biotech/GM Crops in 2017: Biotech Crop Adoption Surges as Economic Benefits Accumulate in 22 Years*. ISAAA Brief No. 53. ISAAA: Ithaca, NY.

Janssen, J., Tjallingii, W., and Lenteren, J. (1989). Electrical recording and ultrastructure of stylet penetration by the greenhouse whitefly. *Entomologia Experimentalis et Applicata*, 52(1):69–81.

Jiang, S., Lu, Y., Dai, Y., Qian, L., Muhammad, A. B., Li, T., Wan, G., Parajulee, M. N., and Chen, F. (2017). Impacts of elevated CO_2 on exogenous *Bacillus thuringiensis* toxins and transgene expression in transgenic rice under different levels of nitrogen. *Scientific Reports*, 7(1).

Jiang, Y. X., Lei, H., Collar, J. L., Martin, B., Muñiz, M., and Fereres, A. (1999). Probing and feeding behavior of two distinct biotypes of *Bemisia tabaci*

(Homoptera: Aleyrodidae) on tomato plants. *Journal of Economic Entomology*, 92(2):357–366.

Jiang, Y. X. and Walker, G. P. (2007). Identification of phloem sieve elements as the site of resistance to silverleaf whitefly in resistant alfalfa genotypes. *Entomologia Experimentalis et Applicata*, 125(3):307–320.

Jin, L., Zhang, H., Lu, Y., Yang, Y., Wu, K., Tabashnik, B. E., and Wu, Y. (2014). Large-scale test of the natural refuge strategy for delaying insect resistance to transgenic bt crops. *Nature Biotechnology*, 33(2):169–174.

Johnson, S., Ryalls, J., and Karley, A. (2014). Global climate change and crop resistance to aphids: contrasting responses of lucerne genotypes to elevated atmospheric carbon dioxide. *Annals of Applied Biology*, 165(1):62–72.

Jones, R. (2016). Future scenarios for plant virus pathogens as climate change progresses. In *Advances in Virus Research*, pages 87–147. Elsevier.

Kehr, J. (2006). Phloem sap proteins: their identities and potential roles in the interaction between plants and phloem-feeding insects. *Journal of Experimental Botany*, 57(4):767–774.

Klaiber, J., Najar-Rodriguez, A. J., Piskorski, R., and Dorn, S. (2012). Plant acclimation to elevated CO_2 affects important plant functional traits, and concomitantly reduces plant colonization rates by an herbivorous insect. *Planta*, 237(1):29–42.

Kordan, B., Dancewicz, K., Wroblewska, A., and Gabrys, B. (2012). Intraspecific variation in alkaloid profile of four lupine species with implications for the pea aphid probing behaviour. *Phytochemistry Letters*, 5(1):71–77.

Korres, N. E., Norsworthy, J. K., Tehranchian, P., Gitsopoulos, T. K., Loka, D. A., Oosterhuis, D. M., Gealy, D. R., Moss, S. R., Burgos, N. R., Miller, M. R., and

Palhano, M. (2016). Cultivars to face climate change effects on crops and weeds: a review. *Agronomy for Sustainable Development*, 36(1).

Kremer, J. M. M., Nooten, S. S., Cook, J. M., Ryalls, J. M. W., Barton, C. V. M., and Johnson, S. N. (2018). Elevated atmospheric carbon dioxide concentrations promote ant tending of aphids. *Journal of Animal Ecology*, 87(5):1475–1483.

Lee, T. D., Tjoelker, M. G., Reich, P. B., and Russelle, M. P. (2003). Contrasting growth response of an N2-fixing and non-fixing forb to elevated CO_2: dependence on soil N supply. *Plant and Soil*, 255(2):475–486.

Li, N., Li, Y., Zhang, S., Fan, Y., and Liu, T. (2017). Effect of elevated CO_2 concentration and temperature on antioxidant capabilities of multiple generations of *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae). *Journal of Insect Physiology*, 103:91–97.

Li, X., Zhang, L., Ahammed, G. J., Li, Y.-T., Wei, J.-P., Yan, P., Zhang, L.-P., Han, X., and Han, W.-Y. (2019). Salicylic acid acts upstream of nitric oxide in elevated carbon dioxide-induced flavonoid biosynthesis in tea plant (*Camellia sinensis* L.). *Environmental and Experimental Botany*, 161:367–374.

Li, Z.-Y., Liu, T.-J., Xiao, N.-W., Li, J.-S., and Chen, F.-J. (2011). Effects of elevated CO_2 on the interspecific competition between two sympatric species of *Aphis gossypii* and *Bemisia tabaci* fed on transgenic bt cotton. *Insect Science*, 18(4):426–434.

Lima, L., Campos, L., Moretzsohn, M., Návia, D., and de Oliveira, M. (2002). Genetic diversity of *Bemisia tabaci* (Genn.) populations in Brazil revealed by RAPD markers. *Genetics and Molecular Biology*, 25(2):217–223.

Lindroth, R. L., Kopper, B. J., Parsons, W. F., Bockheim, J. G., Karnosky, D. F., Hendrey, G. R., Pregitzer, K. S., Isebrands, J., and Sober, J. (2001).

Consequences of elevated carbon dioxide and ozone for foliar chemical composition and dynamics in trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). *Environmental Pollution*, 115(3):395–404.

Liu, B., Preisser, E. L., Chu, D., Pan, H., Xie, W., Wang, S., Wu, Q., Zhou, X., and Zhang, Y. (2013). Multiple forms of vector manipulation by a plant-infecting virus: *Bemisia tabaci* and *Tomato Yellow Leaf Curl Virus*. *Journal of Virology*, 87(9):4929–4937.

Lu, C., Qi, J., Hettenhausen, C., Lei, Y., Zhang, J., Zhang, M., Zhang, C., Song, J., Li, J., Cao, G., ul Malook, S., and Wu, J. (2018). Elevated CO_2 differentially affects tobacco and rice defense against lepidopteran larvae via the jasmonic acid signaling pathway. *Journal of Integrative Plant Biology*, 60(5):412–431.

McDaniel, T., Tosh, C. R., Gatehouse, A. M. R., George, D., Robson, M., and Brogan, B. (2016). Novel resistance mechanisms of a wild tomato against the glasshouse whitefly. *Agronomy for Sustainable Development*, 36(1).

Miklos, J. A., Alibhai, M. F., Bledig, S. A., Connor-Ward, D. C., Gao, A. G., Holmes, B. A., Kolacz, K. H., Kabuye, V. T., MacRae, T. C., Paradise, M. S., Toedebusch, A. S., and Harrison, L. A. (2007). Characterization of soybean exhibiting high expression of a synthetic transgene that confers a high degree of resistance to lepidopteran pests. *Crop Science*, 47(1):148.

Monsanto (2013). *Monsanto Company (2012) Science behind our products: breeding.* https://monsanto.com/products/Pages/breeding.aspx.

Moretti, C., Mattos, L., Calbo, A., and Sargent, S. (2010). Climate changes and potential impacts on postharvest quality of fruit and vegetable crops: A review. *Food Research International*, 43(7):1824–1832.

Navas-Castillo, J., Fiallo-Olivé, E., and Sánchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. *Annual Review of Phytopathology*, 49(1):219–248.

Noctor, G. and Mhamdi, A. (2017). Climate change, CO_2, and defense: The metabolic, redox, and signaling perspectives. *Trends in Plant Science*, 22(10):857–870.

Oehme, V., Högy, P., Zebitz, C. P., and Fangmeier, A. (2013a). Effects of elevated atmospheric CO_2 concentrations on phloem sap composition of spring crops and aphid performance. *Journal of Plant Interactions*, 8(1):74–84.

Oehme, V., Högy, P., Zebitz, C. P., and Fangmeier, A. (2013b). Effects of elevated atmospheric CO_2 concentrations on phloem sap composition of spring crops and aphid performance. *Journal of Plant Interactions*, 8(1):74–84.

Oliveira, C. E. D. S., Carneiro, D. E. F., Toscano, L. C., and dos Santos, R. M. F. (2018). Dinamica populacional de *Bemisia tabaci* biotipo b (Gennadius 1889) em cultivares de soja transgenica. *Journal of Neotropical Agriculture*, 5(2):1–5.

Oliveira, C. M., Auad, A. M., Mendes, S. M., and Frizzas, M. R. (2012). Economic impact of exotic insect pests in brazilian agriculture. *Journal of Applied Entomology*, 137(1-2):1–15.

Oliveira, M., Henneberry, T., and Anderson, P. (2001). History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop Protection*, 20(9):709–723.

Penalver-Cruz, A., Garzo, E., Prieto-Ruiz, I., Diaz-Carro, M., Winters, A., Moreno, A., and Fereres, A. (2019). Feeding behavior, life history, and virus transmission ability of *Bemisia tabaci* mediterranean species (Hemiptera: Aleyrodidae) under elevated CO_2. *Insect Science*.

Peng, H.-C. and Walker, G. P. (2018). Sieve element occlusion provides resistance against *Aphis gossypii* in TGR-1551 melons. *Insect Science*.

Polston, J. E., Barro, P. D., and Boykin, L. M. (2014). Transmission specificities of plant viruses with the newly identified species of the *Bemisia tabaci* species complex. *Pest Management Science*, 70(10):1547–1552.

Pozebon, H., Filho, A. C., Guedes, J. V., Ferreira, D. R., Marques, R. P., Bevilaqua, J. G., Patias, L. S., Colpo, T. L., and Arnemann, J. A. (2019). Distribution of *Bemisia tabaci* within soybean plants and on individual leaflets. *Entomologia Experimentalis et Applicata*, 167(5):396–405.

Qiao, Y., Miao, S., Li, Q., Jin, J., Luo, X., and Tang, C. (2019). Elevated CO_2 and temperature increase grain oil concentration but their impacts on grain yield differ between soybean and maize grown in a temperate region. *Science of The Total Environment*, 666:405–413.

Rajashekar, C. B. (2018). Elevated CO_2 levels affect phytochemicals and nutritional quality of food crops. *American Journal of Plant Sciences*, 09(02):150–162.

Ramos, R. S., Kumar, L., Shabani, F., and Picanço, M. C. (2019). Risk of spread of tomato yellow leaf curl virus (TYLCV) in tomato crops under various climate change scenarios. *Agricultural Systems*, 173:524–535.

Rashid, M. M., Jahan, M., and Islam, K. S. (2016). Effects of nitrogen, phosphorous and potassium on host-choice behavior of brown planthopper, *Nilaparvata lugens* (Stål) on rice cultivar. *Journal of Insect Behavior*, 30(1):1–15.

Robinson, E. A., Ryan, G. D., and Newman, J. A. (2012). A meta-analytical review of the effects of elevated CO_2 on plant-arthropod interactions highlights the importance of interacting environmental and biological variables. *New Phytologist*, 194(2):321–336.

Rodríguez-López, M. J., Garzo, E., Bonani, J. P., Fereres, A., Fernández-Muñoz, R., and Moriones, E. (2011). Whitefly resistance traits derived from the wild tomato *Solanum pimpinellifolium* affect the preference and feeding behavior of *Bemisia tabaci* and reduce the spread of *Tomato yellow leaf curl virus*. *Phytopathology*, 101(10):1191–1201.

Rogers, A., Ainsworth, E. A., and Leakey, A. D. (2009). Will elevated carbon dioxide concentration amplify the benefits of nitrogen fixation in legumes? *PLANT PHYSIOLOGY*, 151(3):1009–1016.

Sarria, E., Cid, M., Garzo, E., and Fereres, A. (2009). Excel workbook for automatic parameter calculation of EPG data. *Computers and Electronics in Agriculture*, 67(1-2):35–42.

Satishchandra, N. K., Vaddi, S., Naik, S. O., Chakravarthy, A. K., and Atlihan, R. (2018). Effect of temperature and CO_2 on population growth of south american tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomato. *Journal of Economic Entomology*, 111(4):1614–1624.

Sun, Y., Guo, H., Yuan, L., Wei, J., Zhang, W., and Ge, F. (2015). Plant stomatal closure improves aphid feeding under elevated CO_2. *Global Change Biology*, 21(7):2739–2748.

Sun, Y. C., Jing, B. B., and Ge, F. (2009). Response of amino acid changes in Aphis gossypii (Glover) to elevated CO_2 levels. *Journal of Applied Entomology*, 133(3):189–197.

Teawkul, P. and Hwang, S.-Y. (2018). Carbon dioxide- and temperature-mediated changes in plant defensive compounds alter food utilization of herbivores. *Journal of Applied Entomology*, 143(3):289–298.

Tjallingii, W. F. (2006). Salivary secretions by aphids interacting with proteins of phloem wound responses. *Journal of Experimental Botany*, 57(4):739–745.

Trebicki, P., Nancarrow, N., Perez, N. A. B., Rodoni, B., Aftab, M., Freeman, A., Yen, A., and Fitzgerald, G. J. (2017). Virus incidence in wheat increases under elevated CO_2 : A 4-year study of yellow dwarf viruses from a free air carbon dioxide facility. *Virus Research*, 241:137–144.

Trebicki, P., Tjallingii, W. F., Harding, R. M., Rodoni, B. C., and Powell, K. S. (2012). EPG monitoring of the probing behaviour of the common brown leafhopper *Orosius orientalis* on artificial diet and selected host plants. *Arthropod-Plant Interactions*, 6(3):405–415.

Trebicki, P., Vandegeer, R. K., Bosque-Perez, N. A., Powell, K. S., Dader, B., Freeman, A. J., Yen, A. L., Fitzgerald, G. J., and Luck, J. E. (2016). Virus infection mediates the effects of elevated CO_2 on plants and vectors. *Scientific Reports*, 6(1).

Vieira, S., Bueno, A., Boff, M., Bueno, R., and Hoffman-Campo, C. (2011). Resistance of soybean genotypes to *Bemisia tabaci* (Genn.) Biotype B (Hemiptera: Aleyrodidae). *Neotropical Entomology*, 40(1):117–122.

Walling, L. L. and Thompson, G. A. (2012). Behavioral and molecular-genetic basis of resistance against phloem-feeding insects. In *Phloem*, pages 328–351. Wiley-Blackwell.

Wang, G.-H., Wang, X.-X., Sun, Y.-C., and Ge, F. (2014). Impacts of elevated CO_2 on *Bemisia tabaci* infesting bt cotton and its parasitoid Encarsia formosa. *Entomologia Experimentalis et Applicata*, 152(3):228–237.

Wang, X.-W., Li, P., and Liu, S.-S. (2017). Whitefly interactions with plants. *Current Opinion in Insect Science*, 19:70–75.

Will, T. and van Bel, A. J. E. (2006). Physical and chemical interactions between aphids and plants. *Journal of Experimental Botany*, 57(4):729–737.

Yan, H., Guo, H., Yuan, E., Sun, Y., and Ge, F. (2018). Elevated CO_2 and O_3 alter the feeding efficiency of *Acyrthosiphon pisum* and *Aphis craccivora* via changes in foliar secondary metabolites. *Scientific Reports*, 8(1).

Yifei, Z., Yang, D., Guijun, W., Bin, L., Guangnan, X., and Fajun, C. (2018). Effects of elevated CO_2 on plant chemistry, growth, yield of resistant soybean, and feeding of a target lepidoptera pest, *Spodoptera litura* (Lepidoptera: Noctuidae). *Environmental Entomology*, 47(4):848–856.

Yin, J., Sun, Y., Wu, G., and Ge, F. (2010). Effects of elevated CO_2 associated with maize on multiple generations of the cotton bollworm, *Helicoverpa armigera*. *Entomologia Experimentalis et Applicata*, 136(1):12–20.

Yu, H., Li, Y., Li, X., Romeis, J., and Wu, K. (2013). Expression of cry1Ac in transgenic bt soybean lines and their efficiency in controlling lepidopteran pests. *Pest Management Science*, 69(12):1326–1333.

Yu, H., Li, Y., Li, X., and Wu, K. (2014). Arthropod abundance and diversity in transgenic bt soybean. *Environmental Entomology*, 43(4):1124–1134.

Yu, H.-L., Li, Y.-H., and Wu, K.-M. (2011). Risk assessment and ecological effects of transgenic *Bacillus thuringiensis* crops on non-target organisms. *Journal of Integrative Plant Biology*, 53(7):520–538.

Zanetti, S., Hartwig, U. A., van Kessel, C., Lüscher, A., Hebeisen, T., Frehner, M., Fischer, B. U., Hendrey, G. R., Blum, H., and Nösberger, J. (1997).

Does nitrogen nutrition restrict the CO_2 response of fertile grassland lacking legumes? *Oecologia*, 112(1):17–25.

Zavala, J., Gog, L., and Giacometti, R. (2016). Anthropogenic increase in carbon dioxide modifies plant-insect interactions. *Annals of Applied Biology*, 170(1):68–77.

Zavala, J. A., Casteel, C. L., Nabity, P. D., Berenbaum, M. R., and DeLucia, E. H. (2009). Role of cysteine proteinase inhibitors in preference of japanese beetles (*Popillia japonica*) for soybean (*Glycine max*) leaves of different ages and grown under elevated CO_2. *Oecologia*, 161(1):35–41.

Zavala, J. A., Nabity, P. D., and DeLucia, E. H. (2013). An emerging understanding of mechanisms governing insect herbivory under elevated CO_2. *Annual Review of Entomology*, 58(1):79–97.

Ziska, L. H., Epstein, P. R., and Rogers, C. A. (2007). Climate change, aerobiology, and public health in the northeast united states. *Mitigation and Adaptation Strategies for Global Change*, 13(5-6):607–613.

Chapter 2

Detrimental effects of dual Bt genes maize on the preference, feeding and reproduction of the corn leaf aphid *Rhopalosiphum maidis*

Detrimental effects of dual Bt genes maize on the preference, feeding and reproduction of the corn leaf aphid *Rhopalosiphum maidis*. Paula D. De Paulo, Khalid Haddi, Mauricelia Fernandes, Eliseu J. G. Pereira, Eugenio E. Oliveira; **Manuscript to be submitted to Journal of Pest Science**.

Abstract

Insect-resistant Bt plants play an important role in crop production due to highly specific activities mainly against Lepidoptera and Coleoptera insect pests. However, with large-scale areas devoted to transgenic cultivars, the impact of this technology on other invertebrate communities (e.g., non-target sap-sucking species) is yet to be fully clarified. Here, EPG technique was used to compare the effects of pyramided Bt (Cry1Ab and Cry1F) and non-Bt maize plants on the feeding behavior of the corn leaf aphid *Rhopalosiphum maidis* (Hemiptera: Aphididae). Further bioassays were conducted to compare the preference and the reproductive outputs (e.g., daily number of nymphs per adult, accumulated number of produced nymphs and percentage of nymphs reaching adulthood) of *R. maidis* on these plant types. Our results showed that *R. maidis* adults not only spent longer time in non-probing activities (i.e., moving and finding feeding site) when feeding on maize pyramided Bt plants, but also exhibited significantly lower frequency of sap ingestion activities once their stylets reached transgenic plants phloem. Furthremore, *R. maidis* presented lower preference and reduced reproductive outputs in pyramided Bt plants when compared to their performance on the non-Bt plants. Thus, by revealing detrimental responses on feeding behaviors, preference and reproductive outputs of *R. maidis*, our findings demonstrated reduced risk of these phytosuccivorous insects becoming an economically important pest in pyramided transgenic maize.

Key words: sap-sucking insects, electrical penetration graphs, genetically modified plants.

2.1 Introduction

Insect-resistant crops engineered to express insecticidal proteins derived from the soil bacteria *Bacillus thuringiensis* (Bt) have been in use for decades. The Bt endotoxins are encoded by various Cry genes and have specific activities against insect species belonging to different orders (Svobodová et al., 2017). The widespread adoption of the first generation of transgenic crops expressing only one Cry protein has led to intense selection in insect populations and accelerated the evolution of resistance (James, 2010; Tabashnik et al., 2013; Santos-Amaya et al., 2015; Carrière et al., 2016; Tabashnik and Carrière, 2017). One of the strategies adopted to overcome these evolving resistance cases was the use of "pyramid" Bt crops (Tabashnik et al., 2013; Brevault et al., 2013; Carrière et al., 2015; Jin et al., 2014; Carrière et al., 2016). Pyramiding genes cause transgenic plants to express two or more Bt proteins that are different in mode of action but effective against the same target pest (Ghimire et al., 2011; Monsanto, 2012).

Although having high action specificity against Lepidoptera and Coleoptera, expression of Bt proteins in crops like maize can also produce unintended effects on individuals and populations of non-target insects as well as on the entire insect communities. The potential impacts of Bt maize on non-target phytophagous insects can be direct (by Bt toxin), indirect (by reducing competition with target insects), or a combination of both (Lumbierres et al., 2004). In fact, insertion of new genes may cause changes in plant quality as food for target and non-target herbivorous insects and thus altering reproductive ability or survival on the plant (Cuc et al., 2008). Furthermore, changes in plant/insect interactions can result from the expression of a new protein leading to shifts in the preference and reproductive behaviors of females, with further impact at populations level of these insect species (Fontes et al., 2002). Moreover, gene pyramiding has been related with the possibility of synergistic interactions between different Cry proteins. Such interactions may trigger unexpected effects that are not observed when expression occurs of only one Cry protein (Hilbeck and Otto, 2015). In this way, populations of secondary pests that previously had little relative importance under one Cry protein scenario can change the status to become more important pests Lu et al. (2010).

Among the phytophagous insect pests encountered in both Bt and non Bt maize,aphids, like corn leaf aphid *Rhopalosiphum maidis*, constitute a group of active phloem-feeding and virus vector species that cause variable damages and yield losses from year to year (Caballero et al., 2001; Carena and Glogoza, 2004; Meihls et al., 2013). Studies analyzing the impact of transgenic plants expressing Cry toxins on aphids are contrasting and report effects ranging from detrimental to survival and fecundity to beneficial on populations (Raps et al., 2001; Ashouri et al., 2001; A, 2004; Mellet and Schoeman, 2007; Burgio et al., 2007; Faria et al., 2007; Shu et al., 2018, 2019). While previous investigations suggested that, aphids may present better performance when feeding on Bt plants than on non Bt plants (Faria et al., 2007), others reported toxicities of Bt toxins (e.g., Cry2, Cry3A and Cry4) against potato aphid, *Macrosiphum euphorbiae* (Walters and English, 1995; Porcar et al., 2009) and even high toxicity of Cry4 or Cry11 proteins to the pea aphid, *Acyrthosiphon pisum* (Li et al., 2011). The vast majority of these studies has been carried out on plants expressing only one Cry protein, focused on reporting the negative or positive effects and only speculated about the mechanisms underlaying the reported effects.

Thus, hypothetizing that potential effects of Bt proteins on sap sucking aphids would influence their reproduction through beahavioral alterations of host selection and feeding, the present work, firstly verified the reproductive performance and preference of the *R. maidis* aphid on Bt maize plants expressing two Cry proteins (Cry1AB and Cry IF) and in its non-Bt isoline and secondly, used the Electrical Penetration Graphs (EPG); one of the most rigorous methods to identify arthropod feeding behaviors; to compare the effects of pyramided Bt technology on the feeding activity of the aphids.

2.2 Material & Methods

2.2.1 Plant material

Maize plants (*Zea mays* L.) expressing the pyramided Cry1Ab and Cry 1F Bt toxins (Bt 30F53YH) as well as the plants of non-Bt isoline (cv 30F53) were used in this work.The maize seeds (one seed per pot) were sown into plastic pots (15 cm of diameter, 1.400 L of volume:) filled with Terral Solo® substrate soil and kept under greenhouse conditions of temperature (25:20 ± 2°C day:night), relative humidity (70-80%) and photoperiod (14:10 h light:dark). Fifteen days after sowing, the seedlings started to be watered three times a week using 20-20-20 (N-P-K) at a dose 1 g/L in the amount of 500 mL per pot during the entire bioassays. Irrigation was performed through a dripping system, maintained at a similar level of water between treatments and plants were rotated within the greenhouse to ensure there was no positional influence.

2.2.2 Aphid strain

The stock colonies of apterous aphids of *R. maidis* were initially generated from clones obtained from Embrapa Milho e Sorgo (Sete Lagoas-MG) and were kept in sorghum (*Sorghum bicolor*) and barley (*Hordeum vulgare*) plants in a greenhouse. For the experiments, colonies of *R. maidis* were kept in conventional non Bt maize plants in plastic pots, isolated with a transparent plastic cylinder

(60 cm of height and 15 cm of diameter) closed in the upper part with a nylon fabric under controlled conditions of temperature (25 ± 2 °C), relative humidity (70-80 %) and photoperiod (16:8 h light: dark). The age of *R. maidis* aphids used in all tests was previously synchronized. Twenty five paternogenic adults were inserted into clipcage cages for 48 hours in Sorghum plants before being removed and the produced nymphs were left until reaching the adulthood (approximately 8 days) and the used in subsequent tests.

2.2.3 Feeding bioassays using the Electrical Penetration Graphs (EPG) technique

The effect of Bt and Non-Bt cultivars on the probing and feeding behavior of *R. maidis* adults was examined by the Electrical Penetration Graphs (EPG) technique (Tjallingii, 1988, 1978). Maize plants of each cultivar (Bt and Non-Bt) at vegetative stage V6 (six leaves fully developed) previously grown during 25 days in a greenhouse were used. Apterous aphid adults reared on *Sorghum* sp. were carefully collected by using a brush and then starved for about 30 min. The adult aphids were tethered from their dorsum to 3 cm long, 20 micron diameter gold wire using conductive water-based silver glue. The other end of the gold wire was connected to the EPG amplifier with a copper wire attached to a copper nail. Sets of eight plants x aphid combinations were used in a completely randomized design for each recording. Continous 8 hour recordings were performed using one Giga-8 DC amplifiers with 1 Giga-Ohm input resistance (EPG Systems, Wageningen, The Netherlands). EPG output was set to 50x gain and data were acquired at 100 Hz using DATAQ Di 700-710 A/D data acquisition USB device card (Data Instruments, Ohio, USA). Recordings were conducted under laboratory conditions at 25 ± 2 °C inside a Faraday cage to avoid electrical noise and interference. Data acquisition and EPG analysis from 23 individual adults, *R.maidis* for the control treatment (Isoline non-Bt) and 25 for the Bt maize were conducted using Stylet+ software (EPG Systems, Wageningen, The Netherlands). EPG variables related to the probing and feeding behavior of the aphids were processed and analysed using the EPG-Excel data Worksheet v 5.0 (Sarria et al., 2009).

2.2.4 Analysis of Electrical Penetration Graphs (EPG) waveforms

Thirty EPG sequential and non-sequential variables (Table 2.1) from the EPG-Excel data workbook v. 5.0 were selected for each treatment. The *R. maidis* feeding-associated EPG waveforms previously described for aphids (Tjallingii, 1988), were identified. These waveforms were: non-probing waveform (np) where the insect is showing a behavior with no stylet contact with the leaf tissue, C waveform indicating intercellular apoplastic stylet pathway where the insects show a cyclic activity of mechanical stylet penetration and secretion of saliva, and potential drop waveform (pd) representing brief (4 to 12 s) intracellular stylet punctures during the pathway phase (C). In addition, there are two waveforms related with the phloem activity: waveform E1 with salivation into phloem sieve elements at the beginning of the phloem phase and waveform E2 correlated with passive phloem sap uptake from the sieve elements. The term “probe” refers to any type of event during the period in which the stylet of an individual insect is located in the plant tissue, and “non-probe” refers to the event with no waveform (indicating that the individual has its stylet outside plant tissues). Other nonsequential EPG variables (mean ± standard error) calculated as described in Backus et al. (2007) included the proportion of individuals that produced the waveform type (PPW), the number of waveform events per insect (NWEI) calculated using the sum of the number of events of a particular waveform divided by the total number of insects under each treatment, the waveform duration per insect (WDI) calculated using the sum of durations of each event of a particular waveform made by each individual insect that produced that waveform divided by the total number of insects under each treatment. If there is not evento of a particular waveform then scored by 0; and the waveform duration per event per insect (WDEI) which is the sum of the duration of the events for a particular waveform divided by the total number of events of that particular waveform under each insect.

Table 2.1: Sequential and non-sequential EPG variables used to compare the probing and feeding behavior of *R. maidis* adults on maize expressing dual cry protein (Cry 1F and Cry 1Ab) or isoline Non-Bt

Non-sequential variables	***Non-sequential variables %***
Number of np	% C
Number of probe	% E1
Number of pd	% E2
Number of C	% E2s
Number of E1	% Contribution of E1 to phloem phase
Number of single E1	***Index***
Number of E2	Potential E2 index
Total duration of np	
Total probing time	***Sequential variables***
Total duration of C	Time to 1st probe from Start of EPG
Total duration of pd	Time from start of EPG to 1st E
Total duration of E	Time from the Beginning of that probe to 1st E
Total duration of E1	E1 followed by first sustained E2 (>10 min)
Total duration of E2	Time to from start of EPG 1st E2
Total duration of sustained E2 (longer than 10 minutes)	Time to from start of EPG 1st sustained E2 (10 minutes)
Duration of the longest E2	Time from 1st probe to 1st E2
Duration of nonphloematic phase	Time from the beginning of that probe to 1st E2
Duration of first E2	

2.2.5 Reproductive output of *R.maidis*

Using clipcages, groups of aphid adults (n = 21) (i.e., one nymph in each plant) were individualized in Bt maize leaves and in their isoline (i.e., 21 plants of each variety). Twenty-four hours later, adult aphids were removed leaving only one nymph per clipcage. The nymphs were observed daily until reaching adulthood. After emergence, adult fecundity was observed daily by recording the daily number of nymphs for eight days. After evaluating the total number of nymphs generated during eight days in this experiment, the percentage of nymphs reaching adulthood age in each treatment (Bt and non-Bt maize) was recorded for period pre-reproductive. The experiments were performed in an air-conditioned room with temperature (25 ± 2 °C), relative humidity (70-80 %) and photoperiod 14:10 h (light:dark).

2.2.6 Choice assays

The free choice assays were performed using four leaf discs (2.0 cm of diameter) of Bt and non-Bt maize that were placed equidistantly from the center of Petri dishes (10 cm of diameter x 1.7 cm of height) on filter paper moistened with 1.0 ml of distilled water. Fifteen aphid adults were transferred to the center of the petri dishes containing the four leaf discs (two leaf discs of each maize variety Bt and non Bt). The number of aphids on each leaf disc was recorded at 2, 6, 12 and 24 hours post-release. Twenty-two Petri dishes (technical replicas) were tested in parallel.

2.2.7 Statistical analyses

The data of the free choice assay were submitted to repeated measures ANOVA using PROC ANOVA (SAS, 2013). The number of *R. maidis* in maize leaf discs within each time (h) was tested for normality by Shapiro-Wilk and accordingly compared by either *t*-test or the non-parametric Mann-Whitney test ($p \leqslant 0.05$) using SigmaPlot software version 12.5.

Statistical analyses of the reproductive output (i.e., average daily number of nymphs per adult, accumulated number of nymphs produced over the eight-days period and percentage of nymphs reaching adulthood) and feeding behavior of *R. maidis* were performed using IBM SPSS Statistics, version 24.0 (IBM Corp., Armonk, NY). The raw data were checked for normality and homogeneity of variance before performing the parametric tests. The statistical differences were evaluated with Student *t*-test. If the data did not follow a normal distribution according to the Shapiro-Wilk normality test, a non-parametric Mann-Whitney *U* test ($p \leqslant 0.05$) was performed. Complementary regression analyses were eventually carried out.

2.3 Results

2.3.1 Probing and feeding behavior of *R. maidis* on Bt and non-Bt maize Plants

The overall results of the EPG variables showed that the feeding behavior of *R.maidis* was altered on pyramided Bt maize compared to the non-Bt isoline (Figure 2.1, 2.2 and 2.3).

From the temporal evolution, that represents specific feeding behaviors of *R. maidis* in 30-minute intervals over a total recording time of 8 h, the percentage of *R.maidis* showing non-probe phase was expressively higher on pyramided Bt maize than on non-Bt isoline (Figure 2.1).

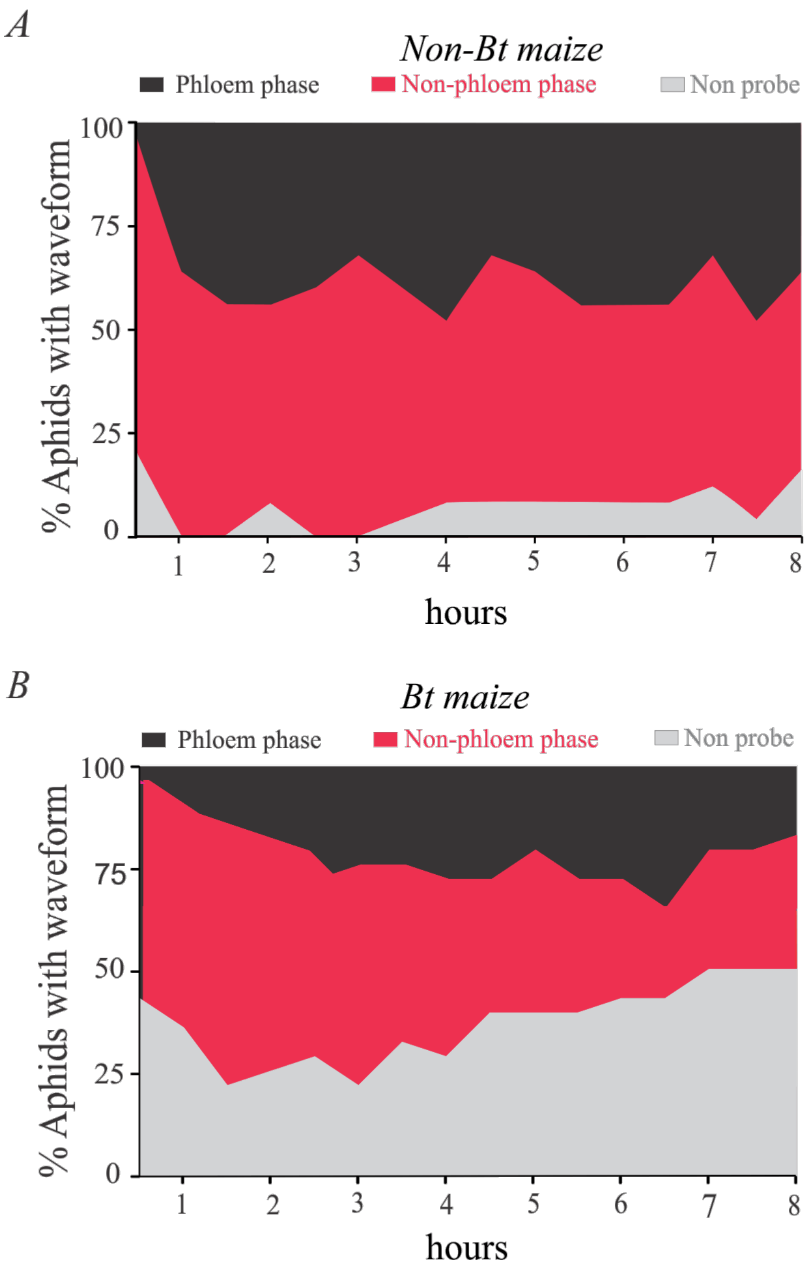


Figure 2.1: Percentage of Aphids (*Rhopalosiphum maidis*) exhibiting specific electrical penetration graphs (EPG) waveforms in 30-min intervals over a total recording time of 8 h on pyramid Bt maize expressing two Cry toxins (Cry 1F and Cry 1Ab) or non-Bt isoline (Cv maize). Waveform np, nonprobing; waveforms of non-phloem phase (C, intercellular apoplastic stylet pathway; G, active xylem ingestion) and waveforms of phloem phase (E1, salivation and E2, passive phloem sap uptake from the sieve elements).

Furthermore, meanwhile no differences were found in the sequencial variables evaluated to compare the effect of the cultivars on the probing and feeding behavior of *R.maidis*, significant differences were observed for some non-sequential EPG variables, related to phloem (Figure 2.3) and non-phloem activities (Figure 2.2) on the two cultivars.

In non-phloem variables, the number of waveformes per event per insect (NWEI) did not differ between the treatments for the non-probe, probe, "Pd" and C wavefroms (Figure 2.2 A). Significant differences were found for the duration in seconds per insect (WDI) of the non-probe, probe and "Pd" waveforms (Figure 2.2 B) and for waveform duration per event (WDE) of the non-probe and probe waveforms (Figure 2.2 C).

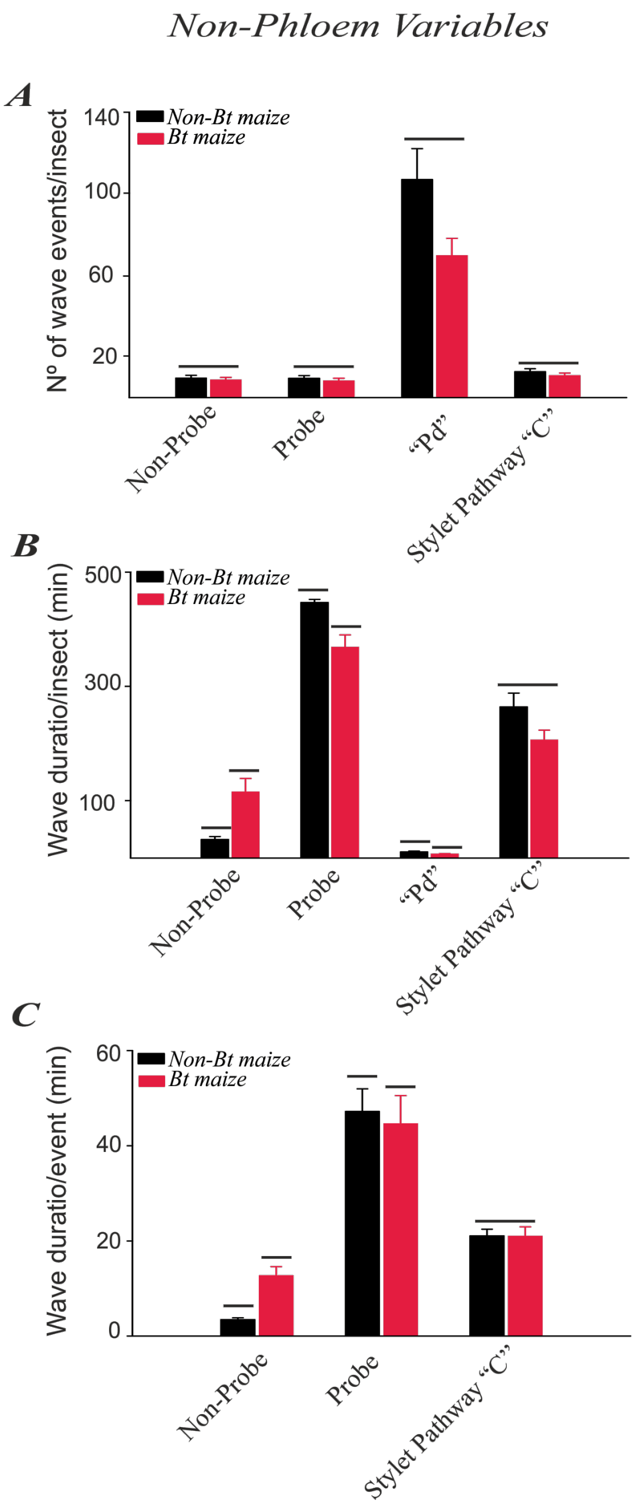


Figure 2.2: Mean (± SE) values of non-phloem (A,B,C) for the probing behavior of *Rhopalosiphum maidis* adults on pyramid Bt maize expressing two Cry toxins (Cry 1F and Cry 1Ab) and non-Bt isoline. Parameter bars under different horizontal lines are statistically different ($p \leqslant 0.05$).

In phloem variables, the NWEI (Non-Bt: 4.20 ± 0.58; Bt: 2.65 ± 0.43) and WDI (Non-Bt: 299.20 ± 60.63 s; Bt: 156.23 ± 41.88 s) of salivation (E1) were significantly reduced on pyramided Bt maize (NWEI: U = 186.00; P= 0.034; WDI: U = 173.00; P = 0.018). Concomitantly, the NWEI of phloem sap ingestion (E2) (2.56 ± 0.30; Non-Bt: 1.65 ± 0.23) and sustained phloem sap ingestion (E2s) (Non-Bt: 2.16 ± 0.23; Bt: 1.48 ± 0.22) were significantly lower on pyramided Bt maize (E2: U = 193.00, P = 0.044; E2s: U = 192.00, P = 0.041) when compared to non-Bt isogenic line. Generally, these results show that aphids reached the phloem sieve tubes of non-Bt maize more often (Figure 2.3).

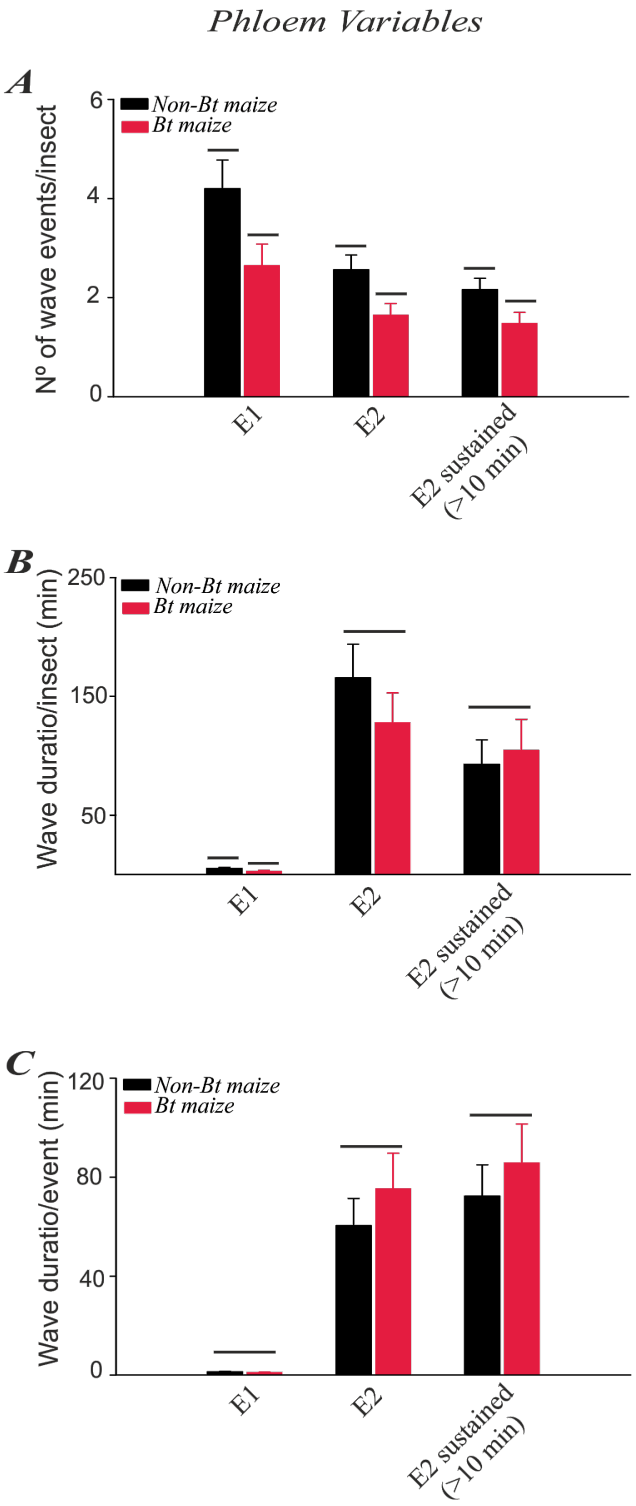


Figure 2.3: Mean (± SE) values of phloem variables (A,B,C) the probing behavior of *Rhopalosiphum maidis* adults on pyramid Bt maize expressing two Cry toxins (Cry 1F and Cry 1Ab) and non-Bt isoline. Parameter bars under different horizontal lines are statistically different ($p \leqslant 0.05$).

2.3.2 Reproductive out put of *R.maidis*

The fecundity of *R.maidis* adults over the eight days of evaluation deferred between the two cultivars (Figure 2.4). The accumulated number of nymphs produced by aphid adults on Bt maize was lower than on non-Bt maize (Figure 2.4 A). In addition, the average number of nymphs produced per aphid adults in Bt maize was significantly lower compared to non-Bt isoline (U = 4245.0, df = 344, $P < 0.001$) (Figure 2.4 B). Furthermore, a lower percentage of nymphs reached adulthood in pyramidal Bt maize plants (t = 10.62, df = 40, $P < 0.001$) (Figure 2.4 C).

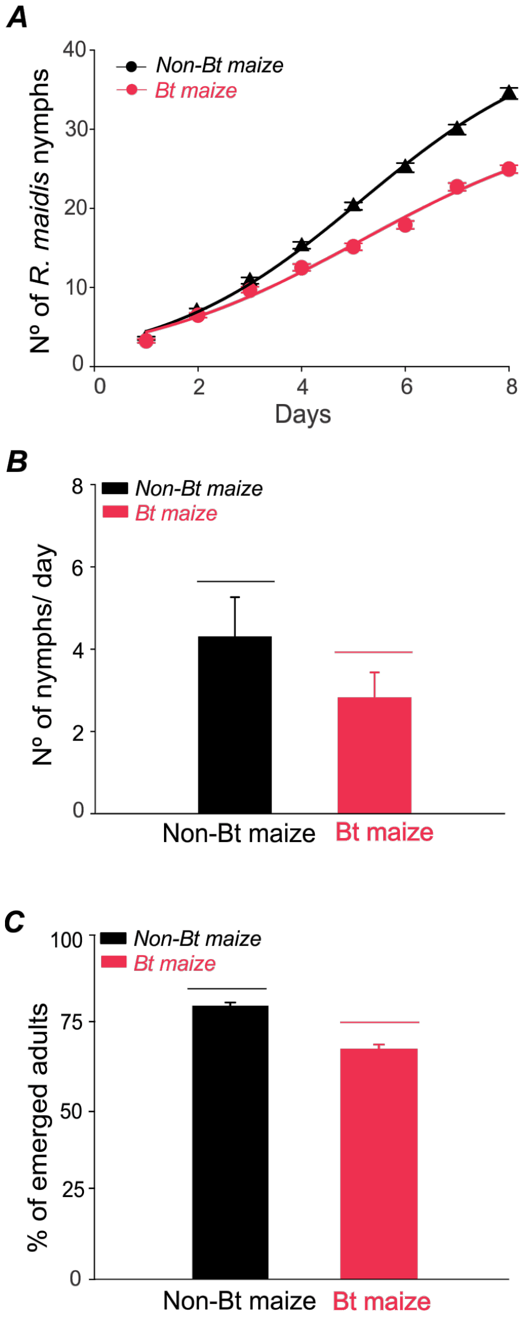


Figure 2.4: Cumulative number produced over eight day period (A) and average number of (± SE) nymphs per day (B) and percentage of emerged adults (C) of *Rhopalosiphum maidis* on pyramided Bt maize expressing two Cry toxins (Cry 1F and Cry 1Ab) or non-Bt isoline.Parameter bars under different horizontal lines are statistically different ($p \leqslant 0.05$).

2.3.3 Choice assays

Repeated measures ANOVA showed that the number of *R. maidis* adults choosing either leaf discs was significatively affected by the maize cultivar, the hour of evaluation and the interaction between the cultivars and hours of evaluation (Table 2.2) (Figure 2.5). Two hours after insect release, the number of *R. maidis* found in leaf disks of pyramided Bt maize expressing the toxins Cry 1F and Cry 1Ab was significantly smaller compared to the non-Bt isoline. Subsequently, the number of aphid on the Bt maize disks significantly decreased over time while in the non-Bt isoline the aphid number increased between two and six hours and plateaued after that (Figure 2.5).

Table 2.2: Repeated measures ANOVAs for the number of adults *Rhopalosiphum maidis* choosing leaf discs of either Bt maize expressing dual Cry protein (Cry 1F and Cry 1Ab) or isoline Non-Bt over a 24 hours period)

Variation source	*df*	*F*	*P*
Between subjects			
Maize Cultivar (MC)	1	46.50	<0.001
Evaluation hours (Eh)	3	165.68	0.029
MC vs Eh	1	0.22	<0.001
Error	126	-	-

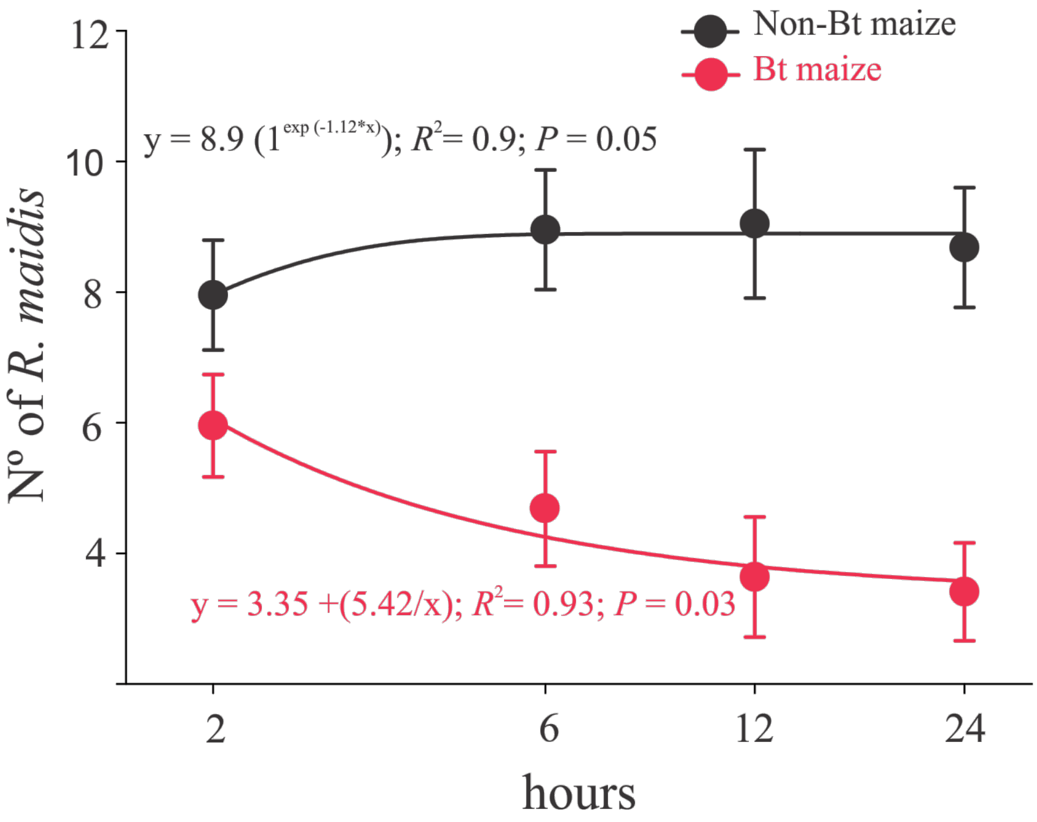


Figure 2.5: Mean of number (± SE) of *Rhopalosiphum maidis* adults choosing leaf discs of pyramid Bt maize expressing two Cry toxins (Cry 1F and Cry 1Ab) or Non-Bt isoline in different times.

2.4 Discussion

Using Electrical Penetration Graphs technique, the present study aimed to investigate the effects of pyramided Bt maize plants on some biological traits of the corn leaf aphid *R.maidis*. The results demonstrated the existence of negative interaction between *R. maidis* aphid and pyramided maize expressing dual-gene Bt (Cry 1Ab + Cry 1F). The aphids showed a decreased reproductive performance in addition to lower preference and reduced feeding activities on pyramided Bt maize plants compared to the non-Bt isoline.

It is widely accepted that the fitness of aphid clones is influenced by the ability to satisfactorily obtain the required nutrients. Furthermore, both their reproductive performance and fitness are determined by a combination of plant nutritional quality and their physiological capacity to obtain sufficient nutrients (Powell et al., 2006). In this context, since initiation of reproduction is a clear indication of host plant acceptance by aphids (Powell et al., 2006), the decreased reproductive output found here suggests low preference and reduced acceptance of *R.maidis* aphids for pyramided Bt maize plants.

Phytophagous insects like aphids generally present a number of different sensory and behavioural mechanisms to locate, detect and confirm the suitability of plant hosts in terms of nutritional quality and defense (Schoonhoven et al., 2005; Powell et al., 2006). In our free choice assay, the preference of aphids was altered in maize plants with a dual Bt genes. Throughout the 24-hour evaluation, the number of *R. maidis* in pyramided maize leaf discs decreased, while in the non-Bt isoline the number of insects remained constant. The differences in aphid Bt and non-Bt maize preferences may be linked to changes in host plant quality caused by pleiotropic effects of the genetic modification (Lumbierres et al., 2004; Arpaia et al., 2017). In fact, the insertion of Bt genes into the genome of agricultural crops may induce alterations at a structural level of different traits of genetically modified plants including changes in plant composition and structure (e.g. lignin, cuticle, hairiness, etc.) (Arpaia et al., 2017). For example, higher content of lignin was reported in Bt maize (Saxena and Stotzky, 2001; Poerschmann et al., 2005) making it different in some way from non-Bt maize since lignin is a major structural component conferring strength and rigidity to cells. Differences in leave parameters like color, topology, texture, epicuticular waxes and trichome exudates influence aphid behavior before stylet insertion (Bruce et al., 2005; Powell et al., 2006) and could explain the lower preference of *R. maidis* to the Bt plants.

However, although cues detected before stylet insertion are fundamental for the host selection process, the major factors influencing plant preference by aphids are actually detected after stylet insertion (Powell et al., 2006). Therefore, in order to address how such factors in pyramided maize plant influence aphid's preference and acceptance, detailed analysis of interaction between aphid stylets with Bt plant tissues is of great importance. Historically, the electrical penetration graph (EPG) technique has been a suitable tool for monitoring feeding activity and studying interactions between stylets of phytophagous hemipteran insects like aphids with plant tissues (Backus and Bennett, 1992; Backus et al., 2019).

Our EPG recordings of feeding behavior for *R.maidis* in pyramided Bt and non Bt maize plants showed characteristic waveforms associated with various feeding phases including non-probing, pathway and phloematic activities (Backus et al., 2005). Furthermore, differences in various parameters of these feeding phases demonstrated a detrimental effect of pyramided Bt technology on the corn leaf aphid probing and sap ingestion.

A high percentage of *R.maidis* showed extended non-probe phase on pyramided Bt maize compared to non-Bt isoline and when probing activity occurred the total probing time of *R.maidis* was significantly shorter on pyramided Bt maize. The extended time of non-probe activity suggests the existence of resistance factors in the epidermis and mesophyll of pyramided Bt leaves as any obstacles could impede localizing or reaching phloem vessels by the stylets (Will and van Bel, 2006; Walling and Thompson, 2012). Extended time from the start of EPG to the first probe are generally associated with mechanical stimuli on the leaf surface, resulting from the presence of trichomes and the thickness of the epidermis (Helden and Tjallingii, 1993; ten Broeke et al., 2013; Benatto et al., 2018). Thicker upper epidermis and large number of trichomes in the leaves of a cultivar of *Brassica napus* have been reported to considerably delay the initiating of the first probing by the cabbage aphid *Brevicoryne brassicaee* (Hao et al., 2019). Furthermore, in the mesophyll, the total duration of the apoplastic stylet pathway (C waveforms) spent by the aphids was similar among the two maize cultivars but the duration of intracellular stylet punctures (Pds waveforms) was lower on Bt plants indicating that some mesophyll-related hindrance occurred which may explain the lower feeding preference of aphids in transgenic plants. Moreover, after their stylets entered into the phloem, aphids spent similar time in ingestion although they made more E1, E2, and E2s and spent a substantial proportion of time in salivation when feeding on Non-Bt plants. Similar durations of phloem-feeding on the two cultivars suggest that the aphids did not encounter any feeding deterrents that may impede their settling (Dreyer et al., 1987; Goławska and Łukasik, 2009).

During the short cell punctures (Pds), the aphid maxillary stylets pierce the main storage sites (i.e., the protoplast and the vacuole) of secondary metabolites of plants (potential allelochemicals) to obtain additional cues for phloem finding (Van Emden and Harrington, 2017). Therefore, the high possibility that the aphids ingest the Bt toxin during the intracellular tests (Pds) in the host plant selection process can explain the hypothesized mesophyll-related hindrance Powell et al. (2006). However, phloem does not contain significant amounts of Bt proteins (Head et al., 2001; Dutton et al., 2002; Romeis and Meissle, 2011; Meissle and Romeis, 2017) and previous studies indicated that Cry toxins under the prevailing acidic pH conditions in the gut of hemipteran insects, show almost no toxicity despite being activated (Li et al., 2011; Shao et al., 2013).

On the other hand, transgene integration may cause unintended effects such as the formation of new metabolites, or alteration levels and/or biochemistry of existing ones (Herman and Price, 2013) that could hamper nutrients ingestion with potential extended effects on reproduction. In their metabolomics assay, Hao et al. (2017) found that four transgenic maize varieties had significantly altered metabolites when compared to their respective isolines. Furthermore, genetic modification can influence phloem sap composition through alterations in amino acid characteristics and C:N balance (Coviella, 2002; Faria et al., 2007) resulting in a changed aphid performance (Hunt et al., 2006) and corroborating the negative effect on the reproductive performance of the *R. maidis* on maize plants expressing dual Cry proteins (Cry 1Ab + Cry 1 F) found in our study. Our results provide evidence that Bt maize plants expressing dual genes (Cry 1Ab+Cry 1F) reduced fecundity of *R. maidis* by affecting the preference and the feeding behavior. Although these results indicate that *R. maidis* do not present a risk of becoming economically important pests on pyramided Bt maize crops, further studies related to changes in the biochemistry and physiology of these transgenic plants should be performed in order to better understand their interaction with non-target insect species.

Acknowledgments

The authors would like to acknowledge the financial support from CAPES Foundation, and the National Council of Scientific and Technological

Development (CNPq). The article is part of the first author's PhD thesis. The authors declare that there are no conflicts of interest.

2.5 References

A, A. (2004). *Seasonal occurrence and relative abundance of aphids on potato plants with classical and transgenic characters of resistance to Colorado potato beetle Leptinotarsa decemlineata (Say).*

Arpaia, S., Birch, A. N. E., Kiss, J., van Loon, J. J., Messéan, A., Nuti, M., Perry, J. N., Sweet, J. B., and Tebbe, C. C. (2017). Assessing environmental impacts of genetically modified plants on non-target organisms: The relevance of in planta studies. *Science of The Total Environment*, 583:123–132.

Ashouri, A., Michaud, D., and Cloutier, C. (2001). Unexpected effects of different potato resistance factors to the colorado potato beetle (coleoptera: Chrysomelidae) on the potato aphid (homoptera: Aphididae). *Environmental Entomology*, 30(3):524–532.

Backus, E. A. and Bennett, W. H. (1992). New ac electronic insect feeding monitor for fine-structure analysis of waveforms. *Annals of the Entomological Society of America*, 85(4):437–444.

Backus, E. A., Cervantes, F. A., Guedes, R. N. C., Li, A. Y., and Wayadande, A. C. (2019). AC–DC electropenetrography for in-depth studies of feeding and oviposition behaviors. *Annals of the Entomological Society of America*, 112(3):236–248.

Backus, E. A., Cline, A. R., Ellerseick, M. R., and Serrano, M. S. (2007). *Lygus hesperus* (Hemiptera: Miridae) feeding on cotton: new methods and parameters for analysis of nonsequential electrical penetration graph data. *Annals of the Entomological Society of America*, 100(2):296–310.

Backus, E. A., Serrano, M. S., and Ranger, C. M. (2005). Mechanisms of hopperburn : an overview of insect taxonomy, behavior, and physiology. *Annual Review of Entomology*, 50(1):125–151.

Benatto, A., Mogor, A. F., Penteado, S. C., Pereira, L. S., Salas, F. J. S., and Zawadneak, M. A. C. (2018). Influence of trichomes in strawberry cultivars on the feeding behavior of *Chaetosiphon fragaefolii* (cockerell) (hemiptera: Aphididae). *Neotropical Entomology*, 47(4):569–576.

Brevault, T., Heuberger, S., Zhang, M., Ellers-Kirk, C., Ni, X., Masson, L., Li, X., Tabashnik, B. E., and Carriere, Y. (2013). Potential shortfall of pyramided transgenic cotton for insect resistance management. *Proceedings of the National Academy of Sciences*, 110(15):5806–5811.

Bruce, T. J., Wadhams, L. J., and Woodcock, C. M. (2005). Insect host location: a volatile situation. *Trends in Plant Science*, 10(6):269–274.

Burgio, G., Lanzoni, A., Accinelli, G., Dinelli, G., Bonetti, A., Marotti, I., and Ramilli, F. (2007). Evaluation of bt-toxin uptake by the non-target herbivore, *Myzus persicae* (Hemiptera: Aphididae), feeding on transgenic oilseed rape. *Bulletin of Entomological Research*, 97(2):211–215.

Caballero, P. P., Ramirez, C. C., and Niemeyer, H. M. (2001). Specialisation pattern of the aphid *Rhopalosiphum maidis* is not modified by experience on a novel host. *Entomologia Experimentalis et Applicata*, 100(1):43–52.

Carena, M. J. and Glogoza, P. (2004). *Resistance of maize to the corn leaf aphid: a review*. Department of Plant Sciences, North Dakota State University, Fargo, ND 58105, USA.

Carrière, Y., Crickmore, N., and Tabashnik, B. E. (2015). Optimizing pyramided transgenic bt crops for sustainable pest management. *Nature Biotechnology*, 33(2):161–168.

Carrière, Y., Fabrick, J. A., and Tabashnik, B. E. (2016). Can pyramids and seed mixtures delay resistance to bt crops? *Trends in Biotechnology*, 34(4):291–302.

Coviella, C. E. (2002). Plant allocation to defensive compounds: interactions between elevated CO2 and nitrogen in transgenic cotton plants. *Journal of Experimental Botany*, 53(367):323–331.

Cuc, N. T. T., Sujii, E. R., Wilson, L. J., Underwood, E., Andow, D. A., Van Hao, M., Zhai, B., and Van Chien, H. (2008). Potential effects of transgenic cotton on non-target herbivores in Veitnam. *Ch*, 6:138–175.

Dreyer, D. L., Jones, K. C., Jurd, L., and Campbell, B. C. (1987). Feeding deterrency of some 4-hydroxycoumarins and related compounds: Relationship to host-plant resistance of alfalfa towards pea aphid (*Acyrthosiphon pisum*). *Journal of Chemical Ecology*, 13(4):925–930.

Dutton, A., Klein, H., Romeis, J., and Bigler, F. (2002). Uptake of bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. *Ecological Entomology*, 27(4):441–447.

Faria, C. A., Wäckers, F. L., Pritchard, J., Barrett, D. A., and Turlings, T. C. (2007). High susceptibility of bt maize to aphids enhances the performance of parasitoids of lepidopteran pests. *PLoS ONE*, 2(7):e600.

Fontes, E. M., Pires, C. S., and Panizzi, E. R. S. A. R. (2002). The environmental effects of genetically modified crops resistant to insects. *Neotropical Entomology*, 31(4):497–513.

Ghimire, M. N., Huang, F., Leonard, R., Head, G. P., and Yang, Y. (2011). Susceptibility of cry1ab-susceptible and -resistant sugarcane borer to transgenic corn plants containing single or pyramided *Bacillus thuringiensis* genes. *Crop Protection*, 30(1):74–81.

Goławska, S. and Łukasik, I. (2009). Acceptance of low-saponin lines of alfalfa with varied phenolic concentrations by pea aphid (Homoptera: Aphididae). *Biologia*, 64(2).

Hao, W., Li, F., Yan, W., Li, C., and Hao, D. (2017). Comparative metabolic profiling of four transgenic maize lines and two non-transgenic maize lines using high-performance liquid chromatography mass spectrometry. *Acta Physiologiae Plantarum*, 39(8).

Hao, Z.-P., Zhan, H.-X., Wang, Y.-L., and Hou, S.-M. (2019). How cabbage aphids *Brevicoryne brassicae* (L.) make a choice to feed on *Brassica napus* cultivars. *Insects*, 10(3):75.

Head, G., Brown, C. R., Groth, M. E., and Duan, J. J. (2001). Cry1ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. *Entomologia Experimentalis et Applicata*, 99(1):37–45.

Helden, M. and Tjallingii, W. F. (1993). Tissue localisation of lettuce resistance to the aphid *Nasonovia ribisnigri* using electrical penetration graphs. *Entomologia Experimentalis et Applicata*, 68(3):269–278.

Herman, R. A. and Price, W. D. (2013). Unintended compositional changes in genetically modified (gm) crops: 20 years of research. *Journal of agricultural and food chemistry*, 61(48):11695–11701.

Hilbeck, A. and Otto, M. (2015). Specificity and combinatorial effects of *Bacillus Thuringiensis* cry toxins in the context of GMO environmental risk assessment. *Frontiers in Environmental Science*, 3.

Hunt, E., Pritchard, J., Bennett, M., Zhu, X., Barrett, D., Allen, T., Bale, J., and Newbury, H. (2006). The *Arabidopsis thaliana/Myzus persicae* model system demonstrates that a single gene can influence the interaction between a plant and a sap-feeding insect. *Molecular Ecology*, 15(13):4203–4213.

James, C. (2010). A global overview of biotech (GM) crops: Adoption, impact and future prospects. *GM Crops*, 1(1):8–12.

Jin, L., Zhang, H., Lu, Y., Yang, Y., Wu, K., Tabashnik, B. E., and Wu, Y. (2014). Large-scale test of the natural refuge strategy for delaying insect resistance to transgenic bt crops. *Nature Biotechnology*, 33(2):169–174.

Li, H., Chougule, N. P., and Bonning, B. C. (2011). Interaction of the *Bacillus thuringiensis* delta endotoxins Cry1ac and Cry3aa with the gut of the pea aphid, Acyrthosiphon pisum (Harris). *Journal of Invertebrate Pathology*, 107(1):69–78.

Lu, Y., Wu, K., Jiang, Y., Xia, B., Li, P., Feng, H., Wyckhuys, K. A. G., and Guo, Y. (2010). Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of bt cotton in china. *Science*, 328(5982):1151–1154.

Lumbierres, B., Albajes, R., and Pons, X. (2004). Transgenic bt maize and *Rhopalosiphum padi* (Homoptera : Aphididae) performance. *Ecological Entomology*, 29(3):309–317.

Meihls, L. N., Handrick, V., Glauser, G., Barbier, H., Kaur, H., Haribal, M. M., Lipka, A. E., Gershenzon, J., Buckler, E. S., Erb, M., Kollner, T. G., and Jander, G. (2013). Natural variation in maize aphid resistance is associated with 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside methyltransferase activity. *The Plant Cell*, 25(6):2341–2355.

Meissle, M. and Romeis, J. (2017). Transfer of cry1ac and cry2ab proteins from genetically engineered bt cotton to herbivores and predators. *Insect Science*, 25(5):823–832.

Mellet, M. A. and Schoeman, A. S. (2007). *Effect of Bt-cotton on chrysopids, ladybird beetles and their prey: Aphids and whiteflies*.

Monsanto (2012). *Monsanto Company (2012) Science behind our products: breeding.*

Poerschmann, J., Gathmann, A., Augustin, J., Langer, U., and Górecki, T. (2005). Molecular composition of leaves and stems of genetically modified bt and near-isogenic non-bt maize—characterization of lignin patterns. *Journal of Environment Quality*, 34(5):1508.

Porcar, M., Grenier, A.-M., Federici, B., and Rahbe, Y. (2009). Effects of *Bacillus thuringiensis* - endotoxins on the pea aphid (*Acyrthosiphon pisum*). *Applied and Environmental Microbiology*, 75(14):4897–4900.

Powell, G., Tosh, C. R., and Hardie, J. (2006). Hot plant selection by aphids: Behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology*, 51(1):309–330.

Raps, A., Kehr, J., Gugerli, P., Moar, W. J., Bigler, F., and Hilbeck, A. (2001). Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and of the nontarget herbivore *Rhopalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. *Molecular Ecology*, 10(2):525–533.

Romeis, J. and Meissle, M. (2011). Non-target risk assessment of bt crops - cry protein uptake by aphids. *Journal of Applied Entomology*, 135(1-2):1–6.

Santos-Amaya, O. F., Rodrigues, J. V. C., Souza, T. C., Tavares, C. S., Campos, S. O., Guedes, R. N., and Pereira, E. J. (2015). Resistance to dual-gene bt maize in *Spodoptera frugiperda*: selection, inheritance and cross-resistance to other transgenic events. *Scientific Reports*, 5(1).

Sarria, E., Cid, M., Garzo, E., and Fereres, A. (2009). Excel workbook for automatic parameter calculation of EPG data. *Computers and Electronics in Agriculture*, 67(1-2):35–42.

Saxena, D. and Stotzky, G. (2001). Bt corn has a higher lignin content than non-bt corn. *American Journal of Botany*, 88(9):1704–1706.

Schoonhoven, L. M., Van Loon, B., van Loon, J. J., and Dicke, M. (2005). *Insect-plant biology*. Oxford University Press on Demand.

Shao, E., Liu, S., Lin, L., and Guan, X. (2013). Proteolytic processing of *Bacillus thuringiensis* toxin Cry1Ab in rice brown planthopper, *Nilaparvata lugens* (Stål). *Journal of Invertebrate Pathology*, 114(3):255–257.

Shu, Y., Du, Y., and Wang, J. (2019). Presence of cry1ab in the bt maize - aphid (*Rhopalosiphum maidis*) - ladybeetle (*Propylea japonica*) system has no adverse effects on insect biological parameters. *Entomologia Experimentalis et Applicata*.

Shu, Y., Romeis, J., and Meissle, M. (2018). No interactions of stacked bt maize with the non-target aphid *Rhopalosiphum padi* and the spider mite *Tetranychus urticae*. *Frontiers in Plant Science*, 9.

Svobodová, Z., Shu, Y., Habuštová, O. S., Romeis, J., and Meissle, M. (2017). Stacked bt maize and arthropod predators: exposure to insecticidal cry proteins and potential hazards. *Proceedings of the Royal Society B: Biological Sciences*, 284(1859):20170440.

Tabashnik, B. E., Brévault, T., and Carrière, Y. (2013). Insect resistance to bt crops: lessons from the first billion acres. *Nature Biotechnology*, 31(6):510–521.

Tabashnik, B. E. and Carrière, Y. (2017). Surge in insect resistance to transgenic crops and prospects for sustainability. *Nature Biotechnology*, 35(10):926–935.

ten Broeke, C. J., Dicke, M., and van Loon, J. J. (2013). Performance and feeding behaviour of two biotypes of the black currant-lettuce aphid, *Nasonovia ribisnigri*, on resistant and susceptible *Lactuca sativa* near-isogenic lines. *Bulletin of Entomological Research*, 103(5):511–521.

Tjallingii, W. (1988). Electrical recording of stylet penetration activities. In *Aphids, their biology, natural enemies and control*, pages 95–108. Elsevier Science Publishers.

Tjallingii, W. F. (1978). Eletronic recording of penetration behaviour by aphids. *Entomologia Experimentalis et Applicata*, 24(3):721–730.

Van Emden, H. F. and Harrington, R. (2017). *Aphids as crop pests*. Cabi.

Walling, L. L. and Thompson, G. A. (2012). 16 behavioral and molecular-genetic basis of resistance against phloem-feeding insects. *Phloem: molecular cell biology, systemic communication, biotic interactions*, page 328.

Walters, F. S. and English, L. H. (1995). Toxicity of *Bacillus thuringiensis*-endotoxins toward the potato aphid in an artificial diet bioassay. *Entomologia Experimentalis et Applicata*, 77(2):211–216.

Will, T. and van Bel, A. J. E. (2006). Physical and chemical interactions between aphids and plants. *Journal of Experimental Botany*, 57(4):729–737.

Chapter 3

Sublethal effects of imidacloprid associated with NaCl on the feeding behavior of the *Bemisia tabaci* on Bt soybean plants

Sublethal effects of imidacloprid associated with NaCl on the feeding behavior of the *Bemisia tabaci* on Bt soybean plants. Paula D. de Paulo, Khalid Haddi, Tiago M. de Oliveira, Eliseu J. G. Pereira, Eugenio E. Oliveira; **Manuscript to be submitted to Pest Management Science**.

Abstract

The application of neonicotinoid insecticides individually and in combination with Sodium Chloride (NaCl) is common practice in Brazilian agricultural (e.g., soybean) production. The use of the salt (0.5% w/v) associated with this insecticide has effectively suppressed target herbivores, and guaranteed greater sustainability (i.e., longer time of use) of insecticidal molecules. However, the potential effects of this practice on non-target organisms (including secondary pests), it has not been the subject of investigation. Thus, we evaluate the toxicity of neonicotinoid imidacloprid, in association with or not to NaCl, the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae). Subsequently, *B. tabaci* feeding behavior was evaluated through the EPG technique in soybean plants exposed to LC_{10} of imidacloprid with or without association with NaCl to investigate possible sublethal effects on the feeding behavior of these insects. The association of NaCl did not modify the toxicity of the solutions containing imidacloprid (LC_{50} = 0.031 (0.027-0.039). However, we observed some disturbances in the feeding behavior of *B. tabaci* caused by sublethal concentration (LC_{10}) (e.g., salivation "E1" and ingestion of phloem "E2") though, these disturbances were not observed when NaCl was added in the formulations of insecticides. In this way, we concluded that the LC_{10} without the addition of NaCl was able to cause more evident stress in the feeding behavior of *B. tabaci*. More studies are needed to evaluate possible effects of this practice, and thus, endorse strategies in integrated pest management.

Key words:Integrated pest management, Electrical penetration graphs, whitefly

3.1 Introduction

Soybean [*Glycine max* (L.) Merr., Fabaceae] is one of the crops with the greatest introduction of technology for insect control, with the development of genetically modified plants. The cultivars of transgenic soybeans expressing proteins from cry genes of *Bacillus thuringiensis* agronomically called Bt soybeans, were developed for pest control effective measure to reduce the population size of serious target pests in soybean fields of the Lepdopteran order (Yu et al., 2014). Although Bt technology on soybean is extremely effective in controlling pest targets of the order lepdoptera, the crop is still vulnerable to severe attacks of important pests of the order Hemipetera (e.g., "stink bug complex") (Corrêa-Ferreira, 2005; Tuelher et al., 2016).

Besides that, what has been observed in recent years also is, an increase of others insects of order Hemiptera (e.g., *Bemisia tabaci* biotype B) in areas cultivated with Bt soybeans, mainly in the reproductive stages of the plant. Until very recently, *B. tabaci* was considered only as an occasional pest; however, it is now considered a key pest of soybean in some regions (Lima et al., 2002; Oliveira et al., 2018). *B. tabaci* complex are highly polyphagous and invasive, particularly the Middle East–Asia Minor 1 biotype (MEAM1, known as B biotype) (Oliveira et al., 2001; Boykin, 2014). This species cause devastating losses on important agricultural crops (e.g., Soybean plants) (Barro et al., 2011; Oliveira et al., 2012) due to damage caused through direct feeding (phloem sap-sucking), toxin injection into the plant vascular system, but most importantly because of their transmission of various plant viruses (Navas-Castillo et al., 2011; Polston et al., 2014; Guo et al., 2019).

Considering the potential risks of losses caused by sap-sucking insects on Bt soybean crops, there is a need to improve the management of these pests and the proper use of insecticides is still the most effective tool for the control of these pests (Ghimire and Woodward, 2013; Guedes, 2016). Imidacloprid is a systemic insecticide that is readily absorbed in plant tissues, nectar, and pollen (Bonmatin et al., 2014; Codling et al., 2016), and is one of the most widespread neonicotinoid insecticides in the world; it affects the cholinergic pathways, disrupting the insect nervous system and acting as a neurotoxin (Wood and Goulson, 2017; Blacquière et al., 2012; Jeschke et al., 2011).

With the degradation of neonicotinoids in soybean plants, *B. tabaci* are likely to be exposed to sublethal concentration of neonicotinoids over relatively long periods. It is therefore expected that neonicotinoids, aside from a direct (lethal) effect on the whitefly, may also cause a range of sublethal effects on this pest. In addition, the use of sodium chloride (NaCl, 0.5% w/v) mixed with insecticide emulsion containing organophosphate insecticides leads to 50% reduction of the insecticide dose to be used without loss of control efficiency (Corso and Gazzoni, 1998; Sosa-Gómez et al., 2009). However, little is known about the physiological basis of this synergistic effect, which requires further investigation.

Previous studies have reported that neonicotinoid insecticides negatively affect the feeding behavior of several hemipterans, including whitefly (Nauen, 1995; Elbert and Nauen, 2004). However, the sublethal effect of neonicotinoids with NaCl yet to be reported. Thus, in this work, we evaluated the mortality response by concentration of imidacloprid with or without association with NaCl to *B.tabaci* whitefly, aiming to establish sublethal and median concentrations (LC_{10} and LC_{50}). Our study focuses on the effects of the sublethal concentration (LC_{10}) of the insecticide associated with NaCl on the feeding behavior of *B tabaci*. Consequently, this study provides some information for the effects of neonicotinoids in the presence of NaCl to better understand the effect of this practice and to endorse strategies in integrated pest management.

3.2 Material & Methods

3.2.1 Plant material and whitefly population

For all toxicity and feeding behavior assays, soybean plants (*Glycine max*) from the TMG 2185IPRO cultivar were used. These plants express the Cry1Ac toxin conferring resistance to several caterpillar species that attack soybean plants and also bear Roundup Ready (RR2) confers tolerance to the herbicide glyphosate (Monsanto, 2013). Colony of *B. tabaci* MEAM1 was reared on eggplants (*Solanum melongena* L.) and cabagge (*Brassica oleracea* var. capitata) within metal-frame cages covered by insect-proof net under greenhouse conditions (temperature ranges of 24 ± 2°C (day: night), relative humidity (70-80%) and photoperiod of 16:8 h (light: dark). Cohorts of whiteflies used in bioassays were synchronized for age (approximately one week old adults) to guarantee homogeneity at the time of the experiment.

3.2.2 Concentration-mortality bioassays

A commercial formulation of the neonicotinoid insecticide imidacloprid (water dispersible granules at 700 g active ingredient (i.e.)/L; Bayer CropScience, Sao Paulo, Brazil) was used in distilled water + Tween (0.1%). The effects of imidacloprid were assessed with and without salt addition (NaCl, 0.5% w/v). Two controls consisting of water and Tween (0.1% w/v %) or water and Tween (0.1% w/v %) and NaCl were used. At least five concentrations of the neonicotinoid insecticide imidacloprid diluted in distilled water and Tween a (0.1%) with or without salt addition were used. The concentrations ranged from 10 % to 80 % of the recommended field dose (i.e., 4.2 μg / cm^2).

The concentration assay was performed using two different methodologies using leaves on plants or cut leaflets in petri dishes. In the first one, adult insects were exposed to treatments on plant leaflets (vegetative stage V3). Briefly, the second fully expanded trifoliolate leaves were used and the highlighted leaflets were immersed in the insecticidal solutions and then drying was allowed for approximately two hours at room temperature (25°C ± 2°C). Soybean plants previously grown during 25 days under greenhouse conditions (temperature ranges of 24 ± 2 °C [day: night]; a photoperiod of 16:8 h [light: dark]) with three trifoliate leaves fully developed (growth stage V3) were utilized. The adults were placed on the abaxial side of leaves previously treated and isolated with clipcages.

The second method used cut leaflets kept in Petri dishes. The exposure was by immersion in insecticidal solutions as described above. The treated leaflets were inserted into the Petri dishes (10 cm of diameter × 1.7 cm of height) on a filter paper moistened with 1 ml of distilled water.

In both methods, five replicates, each with fifteen adults (approximately one week old) for each concentration. Insects were counted as dead when they were unable to move their body when stimulated with a fine bristle brush after a 24 h exposure to treatments.

3.2.3 Probing and feeding behavior of *B. tabaci* on plants treated with sublethal dose of imidacloprid using the Electrical Penetration Graphs (EPG) technique

The second fully expanded leaf of each plant was treated with LC_{10} (0.01071 mg/L; see result section) of imidacloprid associated or not to NaCl, and the respective controls H_20 + Tween) and (H_20 + Tween + NaCl). The insecticidal solutions and the respective controls were applied by the immersion method, and after two hours (drying time) the plants were used to study the probing and feeding behavior of *B. tabaci* (MEAM1). The whitefly adult females previously kept in soybean plants without insecticides were monitored using the Electrical Penetration Graphs (EPG) technique for eight hours of recording. A thin gold wire (2 cm length × 12.5 μm in diameter) glued to a thin copper wire (2 cm length) was attached to the pronotum of each whitefly with a tiny drop of water based silver-conducting glue paint (Rodríguez-López et al., 2011). Whiteflies were placed on the abaxial side of the second youngest leaf of each soybean plant with the respective treatments. Recordings were performed using two Giga-8 DC amplifiers with 1 Giga-Ohm input resistance (EPG Systems, Wageningen, The Netherlands). EPG output was set to 50 × gain and data were acquired at 100 Hz using DATAQ Di 700-710 A/D data acquisition USB device card (Data Instruments, Ohio, USA). Recordings were conducted under laboratory conditions at 25 ± 2 °C inside a Faraday cage to avoid electrical noise and interference. A new plant of each treatment and insect was used for each recording. Data acquisition and EPG analysis from 19 individual adult whitefly females per treatment (LC_{10} and control); and 17 individual adult whitefly females per treatment (LC_{10} + NaCl and control) were conducted using Stylet+ software (EPG Systems, Wageningen, The Netherlands). EPG variables related to the probing and feeding behavior of the whiteflies were processed and analysed using the EPG-Excel data Worksheet v 5.0 (Sarria et al., 2009).

3.2.4 Analysis of Electrical Penetration Graphs (EPG) waveforms

EPG sequential and non-sequential variables from the EPG-Excel data workbook v. 5.0 were selected for each treatment. The whitefly feeding-associated waveforms, which were previously correlated with behavioral events of *Trialeurodes vaporariorum* (West.) by Janssen et al. (1989) and in *B. tabaci* by Jiang et al. (1999), were identified. These waveforms were: waveform np, non-probing behavior (no stylet contact with the leaf tissue); waveform C, intercellular apoplastic stylet pathway where the insects show a cyclic activity of mechanical stylet penetration and secretion of saliva; waveform pd (potential drop), represents brief (4 to 12 s intracellular stylet punctures during the pathway phase (C). There are two waveforms related with the phloem activity: waveform E1, salivation into phloem sieve elements at the beginning of the phloem phase; waveform E2, correlated with passive phloem sap uptake from the sieve elements. The term “probe” refers to any type of event during the period in which the stylet of an individual insect is located in the plant tissue, and “no-probe” refers to the event with no waveform (indicating that the individual has its stylet outside plant tissues).

The EPG variables (mean ± standard error) were calculated as described in (Backus et al., 2007): PPW, proportion of individuals that produced the waveform type; NWEI, Number of Waveform Events per Insect was calculated using the sum of the number of events of a particular waveform divided by the total number of insects under each treatment; WDI, Waveform Duration per Insect was calculated using the sum of durations of each event of a particular waveform made by each individual insect that produced that waveform divided by the total number of insects under each treatment. If there is not event for a particular waveform then scored by 0; WDEI, Waveform Duration per Event per Insect is the sum of the duration of the events for a particular waveform divided by the total number of events of that particular waveform under each treatment.

3.2.5 Statistical analyses

Concentration-mortality data were subjected to probit analysis using SAS software, and 95% confidence intervals for resistance ratios were estimated following (Robertson et al., 2017) and considered significant when not including the value 1. Feeding behavior parameters were submitted to univariate analysis of variance (ANOVA), multiple pairwise comparisons, where necessary, were performed using the Tukey's test ($P < 0.05$). Kruskal-Wallis and multiple pairwise comparisons using Dunn's test were performed when the premises of normality and homogeneity were not satisfied. Statistical analyses were performed using SigmaPlot 12.5 (Systat Software, San Jose, CA, USA).

3.3 Results

3.3.1 Concentration-mortality results

The toxicity of imidacloprid was similar for both the bioassays performed on soybean plants (LC_{50} = 0.023 [0.021-0.028 mg/L] and Petri dishes (LC_{50} = 0.024 [0.019-0.025] mg/L) (Figure 3.1). The association of NaCl did not impact the toxicity of the solutions containing imidacloprid independently of the exposure method (Soybean plants: LC_{50} = 0.031 [0.027-0.039] mg / L) (Petri dishes: 0.025 [0.021-0.029] mg/L). The results showed that the two forms of exposure did not result in differences in the susceptibility of *B. tabaci* to imidacloprid and that the association of NaCl did not potentiate the insecticidal action of imidacloprid against whiteflies in 24 hours (Figure 3.1).

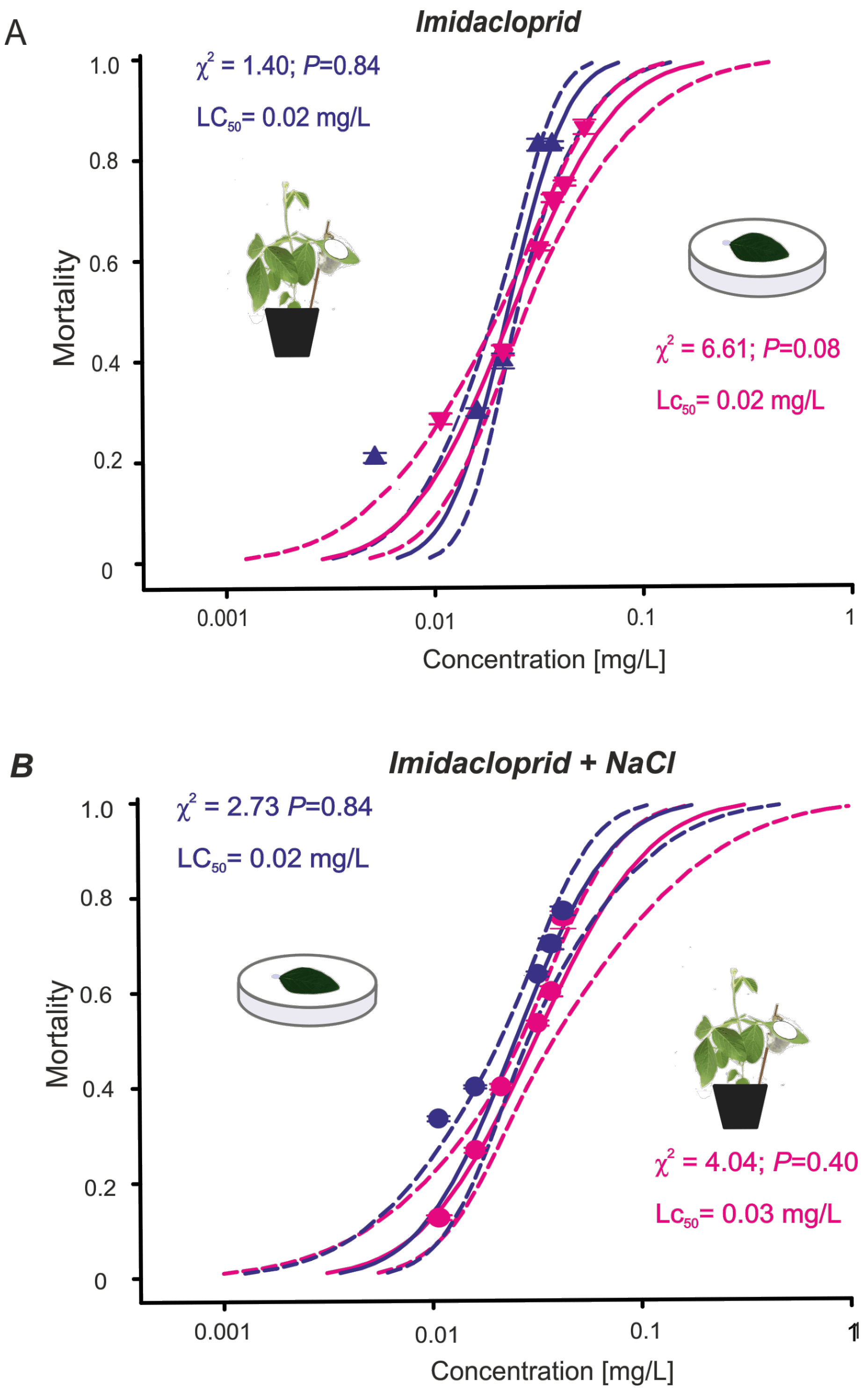


Figure 3.1: Toxicity of imidacloprid to witheflies *Bemisia tabaci*. Lethal concentration (LC) values were estimated based in concentration–mortality bioassays using probit analysis. A: Imidacloprid and B: Imidacloprid + NaCl. The symbols show the averaged mortality, and the vertical bars represent the standard error (SE).

3.3.2 Probing and feeding behavior of *B. tabaci* on soybean plants exposed to LC_{10} of imidacloprid associated or not to NaCl

EPG variables related to non-phloem and phloem phases were analyzed to study the probing and feeding behavior of *B. tabaci* on soybean plants. Our results show that some parameters of whitefly feeding behavior were altered on soybean plants exposed to LC_{10} of imidacloprid associated or no to NaCl. In non-phloem variables (Figure 3.2 A), the number of waveformes per event per insect (NWEI) did not differ between the treatments for the non-probe (F = 1.9, *df* = 3, *P* = 0.14) and pathway stylet "C" waveforms (F = 2.2, *df* = 3, *P* = 0.10). However, the number of "Pd" waveforms was differ (H_2O = 18.8 ± 4.4; H_2O + NaCl = 7.9 ± 1.8; LC_{10} = 5.2 ± 1.6; LC_{10} + NaCl = 7.6 ± 2.1) (H = 9.36, *df* = 3, *P* = 0.025). Whitefly maintained in plants treated with LC_{10} performed lower Pd's than whiteflies fed on plants treated with LC_{10} + NaCl and the respective controls.

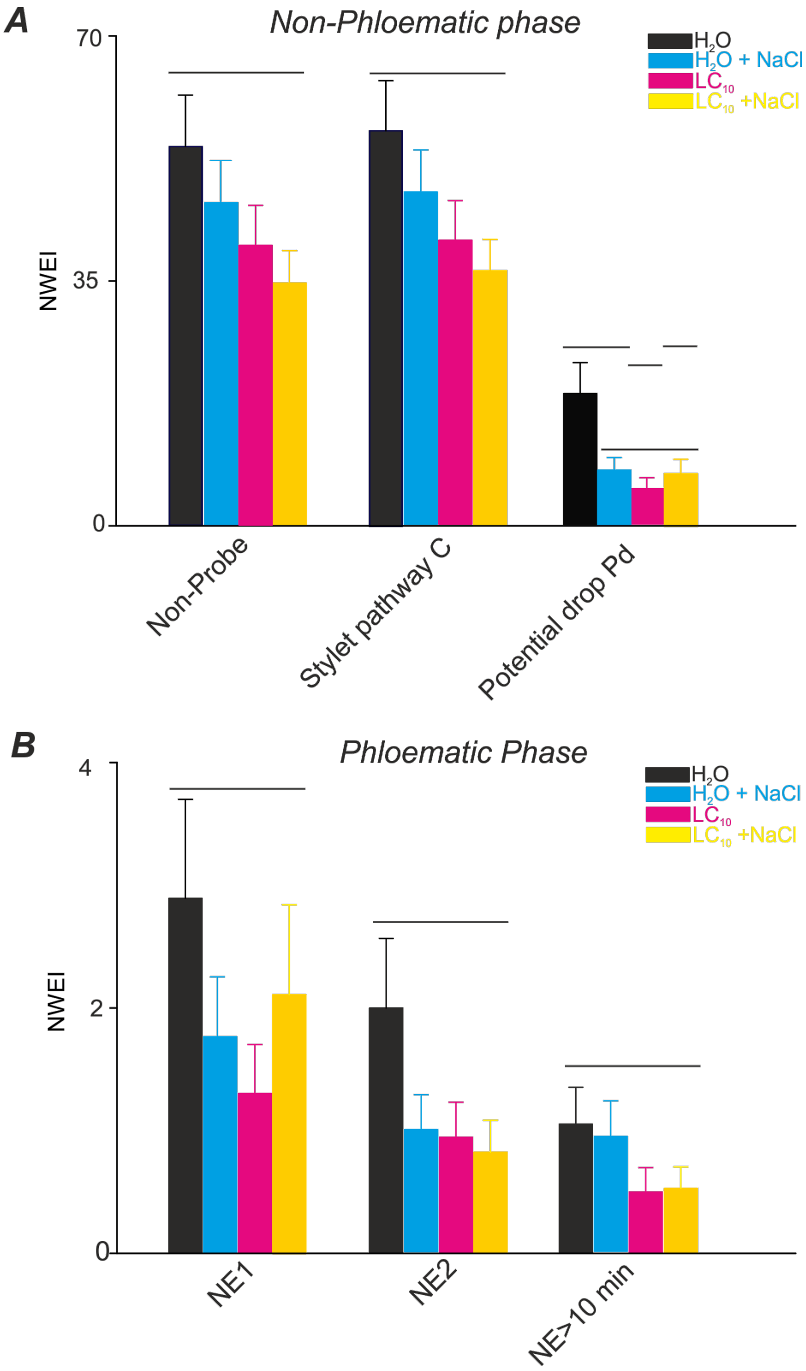


Figure 3.2: Mean ± SE values of EPG variables for the probing behavior (Probe and phloem Ingetion) of *Bemisia tabaci* female adults on soybean plants exposed LC_{10} of imidacloprid with or without NaCl. NWEI: Number of Waveform Events per Insect. A: (Non-probe, Stylet pathway and Potential drop "Pd") B: (E1, phloem salivation; E2, phloem ingestion; E2s, sustained E2 (>10 minutes). Parameter bars under different horizontal lines are statistically different ($p \leqslant 0.05$).

Similarly the duration in minutes per insect (WDI) did not differ between the treatments for the non-probe (F = 0.446, *df* = 3, *P* = 0.707), although, the duration of stylet pathway "C" waveforms (H_2O = 222.3 ± 22.04 ; H_2O + NaCl = 248.3 ±22.2; LC_{10} = 301.0 ± 23.0; LC_{10} + NaCl = 249.5± 18.3 min) (F = 2.98, *df* = 3,*P* = 0.037) and "Pd" waveforms was differ (H_2O = 1.76 ± 0.43; H_2O + NaCl = 0.92 ± 0.22; ; LC_{10} = 0.50 ± 0.16; LC_{10} + NaCl = 0.92 ± 0.3 min) (H = 9.86, *df* = 3, *P* = 0.031) (Figure 3.3 A and B). *B. tabaci* maintained in plants treated with LC_{10} performed Stylet pathway "C" for longer time and Pd's for less time, than whiteflies fed plants treated with LC_{10} + NaCl and the respective controls.

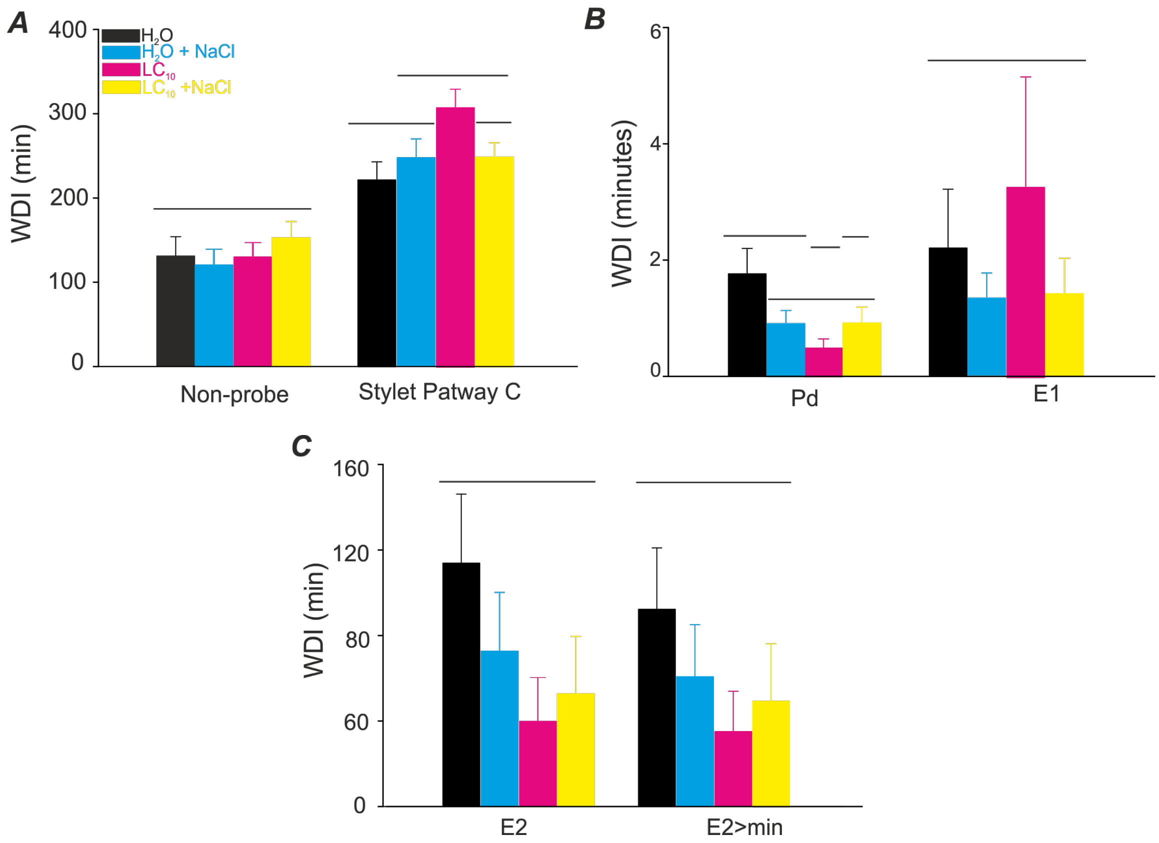


Figure 3.3: Mean±SE values of EPG variables for the probing behavior (Probe and phloem Ingetion) of *Bemisia tabaci* female adults on soybean plants exposed CL10 of imidacloprid with or without NaCl. WDI: Waveform Duration per Insect. A: (Non-probe and Stylet pathway) B: Potential drop "Pd" and E1, phloem salivation) C: (E2, phloem ingestion; E2s, sustained E2 (>10 minutes). Parameter bars under different horizontal lines are statistically different ($p \leqslant 0.05$).

Significant differences were found for the duration per event per insect (WDE) of the non-probe (H_2O = 3.9 ± 0.4; H_2O + NaCl = 2.6 ± 0.3; LC_{10} = 3.1 ± 0.3; LC_{10} + NaCl = 4.5 ± 0.5 min) (H = 24.7, *df* = 3, $P < 0.001$), Stylet pathway "C" (H_2O = 3.9 ± 0.25; H_2O + NaCl = 5.2 ± 0.5 ; LC_{10} = 7.1 ± 0.8; LC_{10} + NaCl = 6.8 ± 0.7 min) (H = 60.7, *df* = 3, $P < 0.001$) and "Pd" waveforms (H_2O = 0.09 ± 0.002; H_2O + NaCl = 0.12 ± 0.003; LC_{10} = 0.09 ± 0.003; LC_{10} + NaCl = 0.12 ± 0.003 min) (H = 45.8, *df* = 3, $P < 0.001$) (Figure 3.4 A and B).

In phloem variables, the salivation (E1), phloem sap ingestion (E2) and sustained phloem sap ingestion (E2s), the NWEI and WDI were not significantly diference (Figure 3.4 B, 3.3 A and C). Significant differences were found for the duration per event per insect (WDE) in: (E1) [H_2O = 0.47 ± 0.05; H_2O + NaCl = 0.75 ± 0.1; LC_{10} = 2.5 ± 1.1; LC_{10} + NaCl = 0.7 ± 0.07 (H = 21.4; *df* = 3; $P < 0.001$)] and (E2) [H_2O = 56.5 ± 15.9; H_2O + NaCl = 72. 7 ± 22.7; LC_{10} = 41.9 ± 19.3; LC_{10} + NaCl = 63.95 ± 31.6; (H = 7.9; *df* = 3; *P* = 0.05)].

Significant differences were not found for the sustained phloem sap (E2s) [H_2O = 105.5 ± 26.0; H_2O + NaCl = 76.6 ± 23.82; LC_{10} = 77.6 ± 33.5; LC_{10} + NaCl = 96.7 ± 46.4) (H = 0.95; *df* = 3; *P* = 0.82)] (Figure 3.4 A and C).

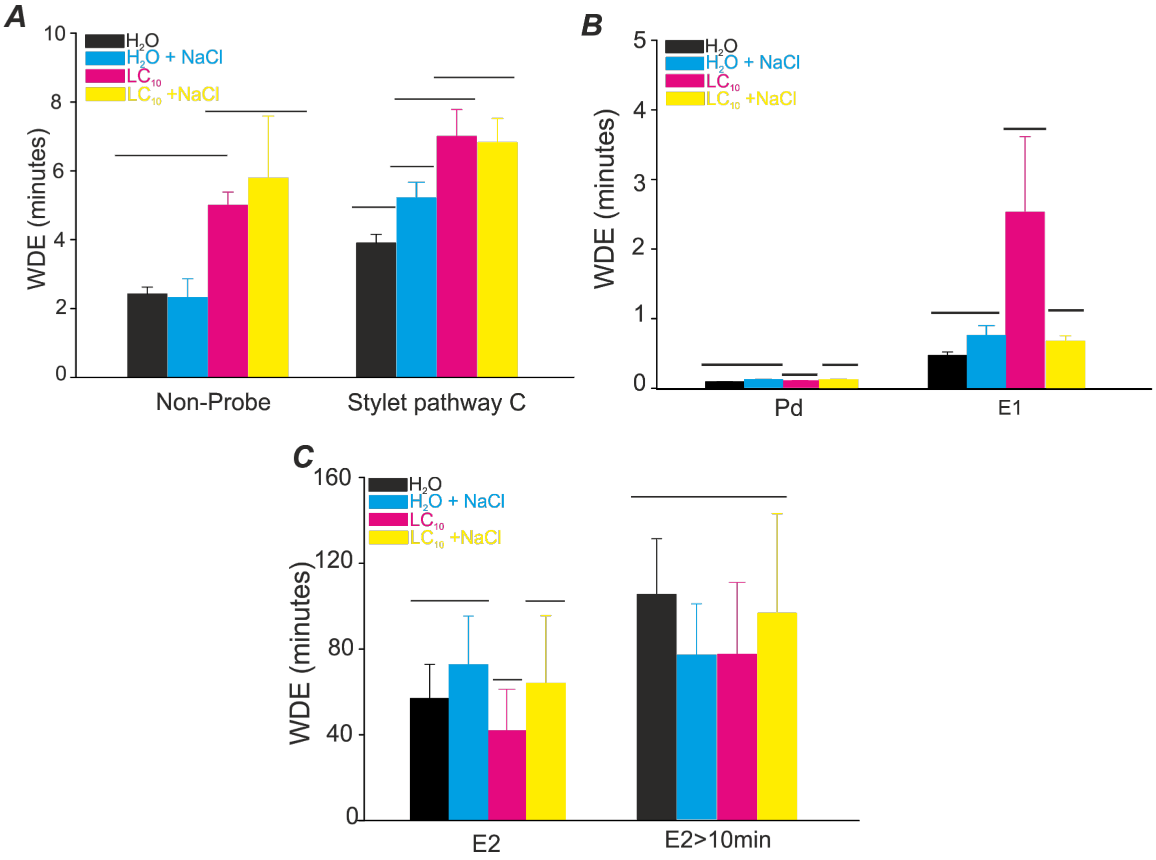


Figure 3.4: Mean$\pm$standard error values of EPG variables for the probing behavior (Probe and phloem Ingetion) of *Bemisia tabaci* female adults on soybean plants exposed CL_{10} of imidacloprid with or without NaCl. WDE: Waveform duration (min) per event. A: (Non-probe and Stylet pathway) B: Potential drop "Pd" and E1, phloem salivation) C: (E2, phloem ingestion; E2s, sustained E2 (>10 minutes) Parameter bars under different horizontal lines are statistically different ($p \leqslant 0.05$).

3.4 Discussion

The results of this study suggest that the addition of sodium chloride (NaCl) to the insecticidal solution did not promote an increase in mortality of *B.tabaci* adults in 24 h independent of methodology (Figure 3.1). However, it was possible to observe insecticide-mediated stress to sublethal concentration (LC_{10}) without the association of the NaCl in soybean plants. Some parameters of feeding behavior differed between the treatments (e.g, Non probe, Stylet pathway "C", potential drop “Pd”, and phloem ingestion "E2").

Our results show that the number and duration of intracellular stylet punctures (pds) were significantly lower in soybean plants treated with only LC_{10} of imidacloprid. Garzo et al. (2015) showed that the aphid *Myzus persicae* susceptible to imidacloprid reduces the time in intercellular apoplastic stylet pathway C, which includes intracellular stylet punctures (pds), on imidacloprid-treated. Aphids are able to ingest small amounts of cell contents during Subphase II-3 of intracellular stylet punctures (Pds) (Mart et al., 1997). *B tabaci* produces pds without clear distinct subphases, although two subphases could be distinguished for some potential drops (Jiang et al., 1999; Johnson and Walker, 1999) thus, the reduction of intracellular puncture may have elapsed from the ingestion of small amounts of insecticide acquired from the cellular content. *B tabaci* that fed of Soybean plants treated with the insecticide solution NaCl did not reduce intercellular apoplastic stylet pathway C, which includes intracellular stylet punctures (Pds), the NaCl may have reduced the perception of the presence of the insecticide by these insects.

B tabaci that fed on soybean plants exposed to LC_{10} of Imidacloprid presented a difference in relation to the phloem-related activities. Salivation duration was significantly higher in plants exposed to LC_{10} without NaCl. Sap-sucking insects inject watery saliva (E1 waveform) to overcome potential plant resistance factors at the phloem level, such as by preventing sieve element sealing (Tjallingii, 2006). The duration and frequency of saliva secretion may indicate the suitability of the host plant and define non-host plants (Peng and Walker, 2018). In our study, the sublethal concentration of imidacloprid caused evident stress in whiteflies marked by increased salivation in plants treated with LC_{10}, this stress was not evidenced in plants treated with the insecticidal solution + NaCl.

In addition, whitefly fed on soybean plants treated only with LC_{10} significantly reduced E2 duration per event (WDE). On the other hand, *B. tabaci* fed on plants treated with LC_{10} + NaCl did not show reduction in phloem ingestion when compared to respective controls. In this way, we can infer that when there is the addition of NaCl on insecticide solution with imidacloprid, whiteflies were able to feed on the phloem longer (Figure 3.4).

Some studies has shown that areas treated with low concentrations of NaCl are preferred by *Euchistus heros* (Corso, 1990; Marcomini, 2016), which may be a response of neurons expressing Gr5a receptors which responds to the stimulation of low concentrations of NaCl - and / or Gr66a – resulting in the behavior of NaCl aversion in insects (Marella et al., 2006; Yarmolinsky et al., 2009). This physiological response may induce greater contact with the insecticidal molecule, thus reflecting on a behavioral response modifying the feeding behavior and consequently, occurring greater ingestion of insecticide (Corso and Gazzoni, 1998).

Our study shows that LC_{10} of imidacloprid affected probe activities (duration of intracellular stylet punctures "Pd", duration of Stylet Pathway "C"), as well as affected the duration of activities related to phloem intake (salivation E1 and ingestion of phloem E2). The addition of NaCl to imidacloprid did not affect the parameters of phloem intake, so our study shows that LC_{10} without the addition of NaCl was able to cause more evident stress in the feeding behavior of *B. tabaci*.

Acknowledgments

The authors would like to acknowledge the financial support from CAPES Foundation, and the National Council of Scientific and Technological Development (CNPq). The article is part of the first author's PhD thesis. The authors declare that there are no conflicts of interest.

3.5 References

Backus, E. A., Cline, A. R., Ellerseick, M. R., and Serrano, M. S. (2007). *Lygus hesperus* (Hemiptera: Miridae) feeding on cotton: new methods and parameters for analysis of nonsequential electrical penetration graph data. *Annals of the Entomological Society of America*, 100(2):296–310.

Barro, P. J. D., Liu, S.-S., Boykin, L. M., and Dinsdale, A. B. (2011). *Bemisia tabaci*: A statement of species status. *Annual Review of Entomology*, 56(1):1–19.

Blacquière, T., Smagghe, G., van Gestel, C. A. M., and Mommaerts, V. (2012). Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. *Ecotoxicology*, 21(4):973–992.

Bonmatin, J.-M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D. P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E. A. D., Noome, D. A., Simon-Delso, N., and Tapparo, A. (2014). Environmental fate and exposure neonicotinoids and fipronil. *Environmental Science and Pollution Research*, 22(1):35–67.

Boykin, L. M. (2014). *Bemisia tabaci* nomenclature: lessons learned. *Pest management science*, 70(10):1454–1459.

Codling, G., Naggar, Y. A., Giesy, J. P., and Robertson, A. J. (2016). Concentrations of neonicotinoid insecticides in honey, pollen and honey bees (*Apis mellifera* l.) in central saskatchewan, canada. *Chemosphere*, 144:2321–2328.

Corrêa-Ferreira, B. S. (2005). Suscetibilidade da soja a percevejos na fase anterior ao desenvolvimento das vagens. *Pesquisa Agropecuária Brasileira*, 40(11):1067–1072.

Corso, I. (1990). Uso de sal de cozinha na redução da dose de inseticida para controle de percevejos da soja. *Embrapa Soja-Comunicado Técnico (INFOTECA-E)*.

Corso, I. C. and Gazzoni, D. L. (1998). Sodium chloride: an insecticide enhancer for controlling pentatomids on soybeans. *Área de Informação da Sede-Artigo em periódico indexado (ALICE)*.

Elbert, A. and Nauen, A. (2004). New applications for neonicotinoid insecticides using imidacloprid as an example. In *Insect Pest Management*, pages 29–44. Springer.

Garzo, E., Moreno, A., Hernando, S., Mariño, V., Torne, M., Santamaria, E., Díaz, I., and Fereres, A. (2015). Electrical penetration graph technique as a tool to monitor the early stages of aphid resistance to insecticides. *Pest Management Science*, 72(4):707–718.

Ghimire, N. and Woodward, R. T. (2013). Under-and over-use of pesticides: An international analysis. *Ecological economics*, 89:73–81.

Guedes, R. N. C. (2016). Insecticide resistance, control failure likelihood and the first law of geography. *Pest Management Science*, 73(3):479–484.

Guo, Q., Shu, Y.-N., Liu, C., Chi, Y., Liu, Y.-Q., and Wang, X.-W. (2019). Transovarial transmission of tomato yellow leaf curl virus by seven species of the *Bemisia tabaci* complex indigenous to china: Not all whiteflies are the same. *Virology*, 531:240–247.

Janssen, J., Tjallingii, W., and Van Lenteren, J. (1989). Electrical recording and ultrastructure of stylet penetration by the greenhouse whitefly. *Entomologia Experimentalis et Applicata*, 52(1):69–81.

Jeschke, P., Nauen, R., Schindler, M., and Elbert, A. (2011). Overview of the status and global strategy for neonicotinoids. *Journal of Agricultural and Food Chemistry*, 59(7):2897–2908.

Jiang, Y., Lei, H., Collar, J. L., Martin, B., Muñiz, M., and Fereres, A. (1999). Probing and feeding behavior of two distinct biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae) on tomato plants. *Journal of Economic Entomology*, 92(2):357–366.

Johnson, D. and Walker, G. (1999). Intracellular punctures by the adult whitefly *Bemisia argentifolii* on DC and AC electronic feeding monitors. *Entomologia Experimentalis et Applicata*, 92(3):257–270.

Lima, L., Campos, L., Moretzsohn, M., Návia, D., and De Oliveira, M. (2002). Genetic diversity of *Bemisia tabaci* (genn.) populations in Brazil revealed by rapd markers. *Genetics and Molecular Biology*, 25(2):217–223.

Marcomini, M. C. (2016). *Efeito do NaCl no comportamento alimentar de Euschistus heros (Hemiptera: Pentatomidae).* Encontro Anual de Iniciação Científica - Universidade Estadual de Londrina. 2016.

Marella, S., Fischler, W., Kong, P., Asgarian, S., Rueckert, E., and Scott, K. (2006). Imaging taste responses in the fly brain reveals a functional map of taste category and behavior. *Neuron*, 49(2):285–295.

Mart, B., Collar, J., Tjallingii, W., Fereres, A., et al. (1997). Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of nonpersistently transmitted plant viruses. *Journal of General Virology*, 78(10):2701–2705.

Nauen, R. (1995). Behaviour modifying effects of low systemic concentrations of imidacloprid on *Myzus persicae* with special reference to an antifeeding response. *Pesticide Science*, 44(2):145–153.

Navas-Castillo, J., Fiallo-Olivé, E., and Sánchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. *Annual Review of Phytopathology*, 49(1):219–248.

Oliveira, C. E. D. S., Carneiro, D. E. F., Toscano, L. C., and dos Santos, R. M. F. (2018). Dinâmica populaciona de *Bemisia tabaci* biótipo b (Gennadius, 1889) em cultivares de soja transgênica. *Journal of Neotropical Agriculture*, 5(2):1–5.

Oliveira, C. M., Auad, A. M., Mendes, S. M., and Frizzas, M. R. (2012). Economic impact of exotic insect pests in brazilian agriculture. *Journal of Applied Entomology*, 137(1-2):1–15.

Oliveira, M., Henneberry, T., and Anderson, P. (2001). History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop Protection*, 20(9):709–723.

Peng, H.-C. and Walker, G. P. (2018). Sieve element occlusion provides resistance against *Aphis gossypii* in TGR-1551 melons. *Insect Science*.

Polston, J. E., Barro, P. D., and Boykin, L. M. (2014). Transmission specificities of plant viruses with the newly identified species of *Bemisia tabaci* species complex. *Pest Management Science*, 70(10):1547–1552.

Robertson, J. L., Jones, M. M., Olguin, E., and Alberts, B. (2017). *Bioassays with arthropods*. CRC press.

Rodríguez-López, M. J., Garzo, E., Bonani, J. P., Fereres, A., Fernández-Muñoz, R., and Moriones, E. (2011). Whitefly resistance traits derived from the wild tomato *Solanum pimpinellifolium* affect the preference and feeding behavior of *Bemisia tabaci* and reduce the spread of *Tomato yellow leaf curl virus*. *Phytopathology*, 101(10):1191–1201.

Sarria, E., Cid, M., Garzo, E., and Fereres, A. (2009). Excel workbook for automatic parameter calculation of EPG data. *Computers and Electronics in Agriculture*, 67(1-2):35–42.

Sosa-Gómez, D. R., Silva, J. J. D., de Oliveira Negrao Lopes, I., Corso, I. C., Almeida, A. M. R., Moraes, G. C. P. D., and Baur, M. E. (2009). Insecticide susceptibility of ieuschistus heros/i (heteroptera: Pentatomidae) in brazil. *Journal of Economic Entomology*, 102(3):1209–1216.

Tjallingii, W. F. (2006). Salivary secretions by aphids interacting with proteins of phloem wound responses. *Journal of Experimental Botany*, 57(4):739–745.

Tuelher, E. S., da Silva, É. H., Freitas, H. L., Namorato, F. A., Serrão, J. E., Guedes, R. N. C., and Oliveira, E. E. (2016). Chlorantraniliprole-mediated toxicity and changes in sexual fitness of the neotropical brown stink bug *Euschistus heros*. *Journal of Pest Science*, 90(1):397–405.

Wood, T. J. and Goulson, D. (2017). The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. *Environmental Science and Pollution Research*, 24(21):17285–17325.

Yarmolinsky, D. A., Zuker, C. S., and Ryba, N. J. (2009). Common sense about taste: From mammals to insects. *Cell*, 139(2):234–244.

Yu, H., Li, Y., Li, X., and Wu, K. (2014). Arthropod abundance and diversity in transgenic bt soybean. *Environmental Entomology*, 43(4):1124–1134.

Final considerations

Here was evaluated the preference and feeding behavior of *Rhopalosiphum maidis* in Bt maize plants expressing two Cry proteins (Cry1AB and Cry IF). The susceptibility and feeding behavior of *Bemisia tabaci* in soybean plants exposed to neonicotinoid imidacloprid, in association or not with NaCl. Moreover, was evaluated the feeding behavior and population performance of *Bemisia tabaci* in Bt soybean plants, expressing the Cry1Ab toxin exposed to elevated concentrations of CO_2.

Bt maize plants expressing dual genes (Cry 1Ab+Cry 1F) reduce the preference, affecting the feeding behavior and fecundity of *R. maidis*. These results indicate that *R. maidis* do not present a risk of becoming economically important pests on pyramided Bt maize crops, further studies related to changes in the biochemistry and physiology of these transgenic plants should be performed in order to better understand their interaction with non-target insect species.

The association of NaCl did not modify the toxicity of the solutions containing imidacloprid to *Bemisia tabaci*. However, some disturbances in the feeding behavior of *B. tabaci* caused by sublethal concentration (LC_{10}) were observed.

Was also observed that changes in the C:N concentration and in plant morphology under eCO_2, alters whitefly life history and feeding behavior. We observed a significant increase of the egg to adult developmental time and a decrease in the fertility and fecundity of *B. tabaci* in plants under eCO_2. In addition, *B. tabaci* that fed on soybeans grown under eCO_2 negatively affected several parameters related to phloem sap ingestion and host plant resistence (e.g., reduction of E2, E2s, longest E2 and greater contribution of E1 to phloem ingestion). We expect that soybeans grown under future climate conditions will negatively affect the performance and feeding behavior of *B. tabaci* whiteflies, suggesting a reduction in the number of offspring and population growth.