

ELIZEU DE SÁ FARIAS

**NOVAS FERRAMENTAS PARA O MANEJO INTEGRADO DE PRAGAS EM
CULTIVOS DE BRÁSSICAS**

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

Orientador: Marcelo Coutinho Picanço

Coorientadores: Ricardo Siqueira da Silva
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**VIÇOSA - MINAS GERAIS
2021**

**Ficha catalográfica elaborada pela Biblioteca Central da Universidade
Federal de Viçosa - Campus Viçosa**

T

F224n Farias, Elizeu de Sá, 1990-
2021 Novas ferramentas para o manejo integrado de pragas em
cultivos de brássicas / Elizeu de Sá Farias. – Viçosa, MG, 2021.
85 f. : il. (algumas color.) ; 29 cm.

Orientador: Marcelo Coutinho Picanço.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. *Brassica oleracea* - Doenças e pragas - Controle integrado. 2. Inteligência artificial. I. Universidade Federal de Viçosa. Departamento de Entomologia. Programa de Pós-Graduação em Entomologia. II. Título.

CDD 22. ed. 635.3497

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APROVADA: 30 de abril de 2021.

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Elizeu de Sá Farias
Autor



Marcelo Coutinho Picanço
Orientador

AGRADECIMENTOS

Ao Senhor Deus, pelo dom da vida e por ser o meu pastor e nada me faltar.

À minha esposa Aline, por topar casar comigo e por estar do meu lado em todos os momentos.

Aos meus pais Nonô e Rosa, pelo amor incondicional e pelo exemplo de vida.

Aos meus irmãos (Sara, Elias, Célia, Eliene e Matheus), pela amizade e apoio.

As duas vírgulas seguidas servem para indicar o meu lugar no meio dessa turma.

À Universidade Federal de Viçosa, por ser minha segunda casa e que, durante os anos de curso técnico e graduação, foi literalmente a minha casa. São 15 anos de vínculo e sou extremamente grato à Universidade e às pessoas que a representam pela estrutura e condições fornecidas.

Ao meu orientador, professor Marcelo Picanço, pela amizade, conselhos e lições.

Aos integrantes atuais e egressos do laboratório MIP-UFV, pelo auxílio na coleta de dados e pelas conversas que me enriqueceram técnica e pessoalmente. É muita gente para agradecer... Mas a você do lab MIP, meu muito obrigado!

Ao Programa de Pós-Graduação em Entomologia da UFV, incluindo professores, técnicos e estudantes, por prover um ambiente saudável e enriquecedor.

Ao senhor Luís Carlos e sua esposa Rosália, por gentilmente permitirem o monitoramento de pragas de brássicas em sua propriedade.

Aos servidores e demais colaboradores do Vale da Agronomia, por cederem a área usada para estabelecimento dos experimentos de tabela de vida e pelas limpezas periódicas dos arredores da área.

Aos taxonomistas, pelo trabalho voluntário e indispensável para a elaboração desta tese. São eles: Dra. Angélica Pentead-Dias (UFSCar) e Dr. Marcelo Tavares (UFES), responsáveis pela identificação dos parasitóides; Dr. Jacques Delabie (CEPEC/CEPLAC) e Me. Julio Chaul (UFV), responsáveis pela identificação de formigas; e Dra. Sthefane D'ávila (UFJF), responsável pela confirmação da identificação das lesmas.

Aos membros da comissão examinadora, pela disponibilidade de fazerem parte da banca e pelas sugestões que contribuíram para a melhoria do texto da tese.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pela concessão da bolsa de estudos (processo: 140983/2017-1).

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

RESUMO

FARIAS, Elizeu de Sá, D.Sc., Universidade Federal de Viçosa, abril de 2021. **Novas ferramentas para o manejo integrado de pragas em cultivos de brássicas.** Orientador: Marcelo Coutinho Picanço. Coorientadores: Ricardo Siqueira da Silva e Paulo Antonio Santana Junior.

O manejo integrado de pragas (MIP) reúne processos que visam reduzir populações de pragas a uma densidade economicamente aceitável, aliando diferentes métodos de controle. A elaboração de sistemas de MIP requer estudos detalhados sobre a bioecologia da praga-alvo e de eficácia dos métodos de controle. Esta tese propõe novas ferramentas para manejo eficiente e sustentável de pragas de brássicas, um dos principais grupos de hortaliças cultivados no mundo. Organismos desfolhadores como a traça (*Plutella xylostella*, Lepidoptera: Plutellidae), a falsa-medideira (*Trichoplusia ni*, Lepidoptera: Noctuidae) e a lesma *Deroceras laeve* (Stylommatophora: Agriolimacidae) são pragas destrutivas de brássicas, pois causam redução de volume produzido e no valor de mercado dos produtos. O primeiro capítulo desta tese descreve a elaboração de tabelas de vida ecológicas para *P. xylostella* e a identificação dos principais inimigos naturais desse lepidóptero na região de estudo, Viçosa-MG. A predação foi o fator primário de mortalidade de *P. xylostella*, independente do estágio da praga e da estação do ano. Formigas (*Brachymyrmex bruchi*, *Camponotus* spp., *Solenopsis saevissima* e *Tetramorium simillimum*) e aranhas foram os principais predadores. As maiores taxas de mortalidade por parasitismo, sobretudo por *Oomyzus sokolowskii* (Hymenoptera: Eulophidae), ocorreram na primavera. O segundo capítulo determina o relacionamento das densidades de *P. xylostella* com a produtividade de brócolis e o impacto da herbivoria dessa praga em plantas de diferentes estágios fenológicos. A produtividade de brócolis foi linear e negativamente relacionada com níveis de infestação por *P. xylostella*. O estágio pré-formação de cabeça foi determinante para a produtividade de plantas de brócolis sob ataque de *P. xylostella*. O terceiro capítulo descreve o uso de redes neurais artificiais (RNAs) como modelos preditivos de dinâmicas sazonais de *T. ni*. A RNA com melhor desempenho (coeficiente de correlação de Pearson = 0,71) foi elaborada para prever densidades de ovos de *T. ni* com 15 dias de antecedência. Os preditores

com maior importância relativa foram identificados por meio do algoritmo de Olden e de análises de sensibilidade. No último capítulo, a calda bordalesa (CB) é estudada como método de controle da lesma *D. laeve* em plantas de repolho. A CB apresentou efeitos letais ($CL_{50} = 28,15\%$ e o $TL_{50} = 8,83$ horas) e alta eficácia de controle (mortalidade $> 90\%$) até o 7º dia após a pulverização das plantas. Adicionalmente, a CL_{25} da CB reduziu o consumo foliar de *D. laeve* em 3,31 vezes. Como conclusão, os resultados desta tese possibilitam o manejo mais eficiente e sustentável das principais pragas de brássicas.

Palavras-chave: *Brassica oleraceae*. Calda bordalesa. *Deroceras laeve*. *Plutella xylostella*. Rede neural artificial. Tabela de vida ecológica. *Trichoplusia ni*.

ABSTRACT

FARIAS, Elizeu de Sá, D.Sc., Universidade Federal de Viçosa, April, 2021. **New tools for integrated pest management in brassica crops.** Adviser: Marcelo Coutinho Picanço. Co-advisers: Ricardo Siqueira da Silva and Paulo Antonio Santana Junior.

Integrated Pest Management (IPM) involves a set of processes to reduce pest populations to economically acceptable levels, using a combination of different management methods. The development of IPM systems requires detailed studies on the bioecology of pests and the effectiveness of management methods. This thesis presents new tools for an effective and sustainable management of pests of brassicas, one of the main groups of vegetables worldwide. Defoliating organisms such as the diamondback moth (*Plutella xylostella*, Lepidoptera: Plutellidae), the cabbage looper (*Trichoplusia ni*, Lepidoptera: Noctuidae), and the marsh slug *Deroceras laeve* (Stylommatophora: Agriolimacidae) are destructive pests of brassica because they reduce both volume produced and crop marketability. The first chapter of this thesis describes the development of ecological life tables for *P. xylostella* and the identification of the main natural enemies of this lepidopteran in the study area, Viçosa-MG. Predation was the major mortality factor for *P. xylostella*, irrespective of pest stage and season. Ants (*Brachymyrmex bruchi*, *Camponotus* spp, *Solenopsis saevissima* and *Tetramorium simillimum*) and spiders were the primary predators. The highest mortality rates due to parasitism, especially by *Oomyzus sokolowskii* (Hymenoptera: Eulophidae), occurred in the spring. The second chapter determines the relationship between the *P. xylostella* densities and the productivity of broccoli, and the impact of this pest herbivory on plants of different phenological stages. Broccoli productivity was linear and negatively related to *P. xylostella* infestation levels. Pre-heading was the determining stage for the head weight loss of plants under *P. xylostella* attack. The third chapter describes the use of artificial neural networks (ANNs) as predictive models of *T. ni* seasonal dynamics. The best performing ANN (Pearson's correlation coefficient = 0.71) was designed to predict *T. ni* egg densities 15 days in advance. The predictors with the greatest relative importance were identified using Olden's algorithm and sensitivity analyses. In the final chapter, the Bordeaux mixture (BM)

was studied as a method of controlling *D. laeve* in cabbage plants. BM exhibited lethal effects ($LC_{50} = 28.15\%$ and $LT_{50} = 8.83$ hours) and high control efficacy (mortality $> 90\%$) up to the 7th day after plant spraying. In addition, the BM LC_{25} reduced the leaf consumption of *D. laeve* by 3.31-fold. In conclusion, the findings from this thesis contribute to more effective and sustainable management of the main brassica pests.

Keywords: *Brassica oleraceae*. Bordeaux mixture. *Deroceras laeve*. *Plutella xylostella*. Artificial neural network. Ecological life table. *Trichoplusia ni*.

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INTRODUÇÃO GERAL

O manejo integrado de pragas (MIP) constitui o conjunto de processos que visa reduzir populações de artrópodes pragas a uma densidade economicamente aceitável através do alinhamento de diferentes métodos de controle (Kogan, 1998). Embora o manejo de pragas date de séculos atrás, sendo iniciado com a prática da agricultura a cerca de 10000 anos, a sistematização dos conceitos atuais de MIP é relativamente recente (Pedigo et al., 1986; Stern et al., 1959). O paradigma central do MIP é a menor perturbação possível do agroecossistema, objetivo implementado sobretudo por meio de sistemas de tomada de decisão e uso de táticas de controle que sejam compatíveis com agentes naturais reguladores de pragas (Castle and Naranjo, 2009; Kogan, 1998). Como resultado, sistemas de tomada de decisão têm sido desenvolvidos para diversas pragas no mundo, ao mesmo tempo que inseticidas de largo espectro (e.g., carbamatos e organofosforados), largamente utilizados no passado, têm sido gradualmente substituídos por princípios ativos mais específicos e seletivos (Castle and Naranjo, 2009). A elaboração de sistemas de MIP requer estudos detalhados sobre a biologia da praga-alvo, dos fatores que afetam a ocorrência e distribuição da praga e métodos de controle eficazes e menos danosos à saúde humana e ao ambiente, especialmente sobre populações de inimigos naturais (Castle and Naranjo, 2009).

As brássicas compreendem um dos principais grupos de hortaliças no mundo. Devido ao seu elevado valor nutricional e benefícios à saúde humana (Lin and Chang, 2005; Reis et al., 2015), brássicas, sobretudo da espécie *Brassica oleracea* L. (e.g., repolho, couve-flor e brócolis) são largamente consumidas e cultivadas. As brássicas constituem um importante modelo para a implementação de MIP, pois reúnem características tais como cultivo durante todo o ano em regiões tropicais, o que permite investigar criteriosamente os fatores que afetam a abundância de pragas (Campos et al., 2006; Fidelis et al., 2019; Marchioro and Foerster, 2016). Além disso, esses cultivos sofrem perdas por pragas generalistas; assim, estudos gerados para esse grupo de culturas podem ser passo inicial para a implementação de processos de manejo em outras culturas.

Fitófagos desfolhadores são importantes causadores de perdas em brássicas, tanto em termos de volume produzido quanto em valor de produção.

A desfolha contínua por esses organismos reduzem a área fotossintética de plantas, enquanto a presença de imaturos em órgãos comercializados (e.g., floretes de brócolis e couve flor) incorrem em perda na classificação do produto (Hooks and Johnson, 2002). A traça-das-crucíferas, *Plutella xylostella* (L.) (Lep.: Plutellidae), é a praga mais destrutiva de brássicas no mundo (Furlong et al., 2013; Philips et al., 2014). Lagartas de *P. xylostella* são especialistas, alimentando-se exclusivamente de folhas de plantas cultivadas e espontâneas da família Brassicaceae (Philips et al., 2014). Por sua vez, a falsa-medideira (*Trichoplusia ni* Hübner, Lep.: Noctuidae) é um fitófago generalista. Esse organismo se alimenta de plantas de 36 famílias, incluindo soja, algodão, tomate e seu hospedeiro primário (*B. oleracea*) (Coapio et al., 2018; Martin et al., 1976). As lesmas (Gastropoda: Pulmonata), particularmente *Deroceras laeve* (Müller) (Stylommatophora: Agriolimacidae), são outro grupo de pragas generalistas (Nash et al., 2007; South, 1992). Lesmas são conspícuas em cultivos de hortaliças. Em grandes culturas como soja e milho, lesmas causam redução do estande de plantio ao se alimentarem de sementes e plântulas, sobretudo em sistemas de cultivo em plantio direto (Douglas and Tooker, 2012; Faberi et al., 2006).

Visando suprir as lacunas no conhecimento dos principais fatores reguladores de populações de *P. xylostella* na região de estudo (Viçosa-MG), o primeiro capítulo desta tese descreve a elaboração de tabelas de vida ecológicas para *P. xylostella* visando, com base nessas informações, prover subsídios para a preservação de inimigos naturais dessa praga em cultivos de brássicas. Tabelas de vida ecológicas auxiliam na determinação de inimigos naturais importantes na regulação de pragas. A partir do acompanhamento de uma coorte (população de indivíduos de mesma idade) ao longo dos estágios do ciclo de vida, os principais fatores de mortalidade (e.g., inimigos naturais) podem ser determinados (Morris and Miller, 1954; Southwood and Henderson, 2000). Com base nessas informações, medidas podem ser adotadas para preservar e/ou aumentar a atuação de organismos reguladores de pragas, estabelecendo o que é conhecido como controle biológico de conservação (Araújo et al., 2017; Rezende et al., 2014).

O segundo capítulo visa a determinação do relacionamento entre densidades de *P. xylostella* com a produtividade de brócolis, etapa primária no

desenvolvimento de índices de tomada de decisão (Pedigo et al., 1986). Adicionalmente, o impacto da herbivoria de *P. xylostella* em diferentes estágios fenológicos de brócolis foi estudado, com implicações para o foco do monitoramento dessa praga para os estágios fenológicos da cultura mais afetados (Ayalew, 2006; Vyavhare et al., 2015).

O terceiro capítulo deste trabalho busca aplicar ferramentas de inteligência artificial para a elaboração de modelos preditivos de dinâmicas sazonais de *T. ni* (falsa-medideira). Redes neurais artificiais (RNAs) foram usadas para esse fim devido à sua adequação para se ajustar a modelos complexos com múltiplos preditores (Watts and Worner, 2008). Variáveis climáticas, plantas hospedeiras e idade das plantas constituíram as variáveis de entrada. Diferentes topologias de ANNs foram testadas, buscando selecionar a rede com maior habilidade preditiva da densidade de *T. ni*. O estabelecimento de modelos preditivos é importante no MIP, podendo ser considerado como um componente de sistemas de tomada de decisão, uma vez que tais modelos auxiliam na concentração de esforços de amostragem e de controle (D'Auria et al., 2016; Fidelis et al., 2019).

O quarto e último capítulo aborda o uso de calda bordalesa, fungicida comumente utilizado em cultivos orgânicos (Nuñez et al., 2018), como método de controle da lesma *D. laeve*. Efeitos letais (curvas de concentração- e tempo-mortalidade, além do residual de controle) e anti-alimentares da calda bordalesa em *D. laeve* foram determinados, visando prover subsídios para o uso desse método no manejo de lesmas em brássicas.

Referências

- Araújo, T.A., Picanço, M.C., Ferreira, D.O., Campos, J.N., Arcanjo, L.P., Silva, G.A., 2017. Toxicity and residual effects of insecticides on *Ascia monuste* and predator *Solenopsis saevissima*. *Pest Manag. Sci.* 73, 2259–2266.
- Ayalew, G., 2006. Comparison of yield loss on cabbage from diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) using two insecticides. *Crop Prot.* 25, 915–919.
- Campos, W.G., Schoederer, J.H., Souza, O.F., 2006. Seasonality in neotropical populations of *Plutella xylostella* (Lepidoptera): Resource availability and migration. *Popul. Ecol.* 48, 151–158.
- Castle, S., Naranjo, S.E., 2009. Sampling plans, selective insecticides and sustainability: The case for IPM as 'informed pest management.' *Pest Manag. Sci.* 65, 1321–1328.

- Coapio, G.G., Cruz-López, L., Guerenstein, P., Malo, E.A., Rojas, J.C., 2018. Oviposition preference and larval performance and behavior of *Trichoplusia ni* (Lepidoptera: Noctuidae) on host and nonhost plants. *Arthropod. Plant. Interact.* 12, 267–276.
- D’Auria, E.M., Wohleb, C.H., Waters, T.D., Crowder, D.W., 2016. Seasonal population dynamics of three potato pests in Washington state. *Environ. Entomol.* 45, 781–789.
- Douglas, M.R., Tooker, J.F., 2012. Slug (Mollusca: Agriolimacidae, Arionidae) ecology and management in no-till field crops, with an emphasis on the mid-Atlantic region. *J. Integr. Pest Manag.* 3, C1–C9.
- Faberi, A.J., López, A.N., Manetti, P.L., Clemente, N.L., Castillo, H.Á., 2006. Growth and reproduction of the slug *Deroceras laeve* (Müller) (Pulmonata: Stylommatophora) under controlled conditions. *Spanish J. Agric. Res.* 4, 345–350.
- Fidelis, E.G., Farias, E.S., Lopes, M.C., Sousa, F.F., Zanuncio, J.C., Picanço, M.C., 2019. Contributions of climate, plant phenology and natural enemies to the seasonal variation of aphids on cabbage. *J. Appl. Entomol.* 143, 365–370.
- Furlong, M.J., Wright, D.J., Dossall, L.M., 2013. Diamondback moth ecology and management: Problems, progress, and prospects. *Annu. Rev. Entomol.* 58, 517–541.
- Hooks, C.R.R., Johnson, M.W., 2002. Lepidopteran pest populations and crop yields in row intercropped broccoli. *Agric. For. Entomol.* 4, 117–125.
- Kogan, M., 1998. Integrated pest management: Historical perspectives and contemporary developments. *Annu. Rev. Entomol.* 43, 243–270.
- Lin, C.-H., Chang, C.-Y., 2005. Textural change and antioxidant properties of broccoli under different cooking treatments. *Food Chem.* 90, 9–15.
- Marchioro, C.A., Foerster, L.A., 2016. Biotic factors are more important than abiotic factors in regulating the abundance of *Plutella xylostella* L., in Southern Brazil. *Rev. Bras. Entomol.* 60, 328–333.
- Martin, P.B., Lingren, P.D., Greene, G.L., 1976. Relative abundance and host preferences of cabbage looper, soybean looper, tobacco budworm, and corn earworm on crops grown in Northern Florida. *Environ. Entomol.* 5, 878–882.
- Morris, R.F., Miller, C.A., 1954. The development of life tables for the spruce budworm. *Can. J. Zool.* 32, 283–301.
- Nash, M.A., Thomson, L.J., Hoffmann, A.A., 2007. Slug control in Australian canola: Monitoring, molluscicidal baits and economic thresholds. *Pest Manag. Sci.* 63, 851–859.
- Núñez, A.M.P., Rodríguez, G.A.A., Monteiro, F.P., Faria, A.F., Silva, J.C.P., Monteiro, A.C.A., Carvalho, C. V., Gomes, L.A.A., Souza, R.M., Souza, J.T., Medeiros, F.H.V., 2018. Bio-based products control black rot (*Xanthomonas campestris* pv. *campestris*) and increase the nutraceutical and antioxidant components in kale. *Sci. Rep.* 8, 1–11.
- Pedigo, L.P., Hutchins, S.H., Higley, L.G., 1986. Economic injury levels in theory and practice. *Annu. Rev. Entomol.* 31, 341–368.
- Philips, C.R., Fu, Z., Kuhar, T.P., Shelton, A.M., Cordero, R.J., 2014. Natural

- history, ecology, and management of diamondback moth (Lepidoptera: Plutellidae), with emphasis on the United States. *J. Integr. Pest Manag.* 5, 1–11.
- Reis, L.C.R., Oliveira, V.R., Hagen, M.E.K., Jablonski, A., Flôres, S.H., Rios, A.O., 2015. Carotenoids, flavonoids, chlorophylls, phenolic compounds and antioxidant activity in fresh and cooked broccoli (*Brassica oleracea* var. *Avenger*) and cauliflower (*Brassica oleracea* var. *Alphina F1*). *LWT - Food Sci. Technol.* 63, 177–183.
- Rezende, M.Q., Venzon, M., Perez, A.L., Cardoso, I.M., Janssen, A., 2014. Extrafloral nectaries of associated trees can enhance natural pest control. *Agric. Ecosyst. Environ.* 188, 198–203.
- South, A., 1992. Control of slug pests, in: South, A. (Ed.), *Terrestrial slugs*. Springer Netherlands, Dordrecht, Netherlands, pp. 322–347.
- Southwood, T.R.E., Henderson, P.A., 2000. The construction, description, and analysis of age-specific life-tables, in: Southwood, T.R.E., Henderson, P.A. (Eds.), *Ecological Methods*. Blackwell Science, Oxford, UK, pp. 404–436.
- Stern, V.M., Smith, R.F., van den Bosch, R., Hagen, K.S., 1959. The integrated control concept. *Hilgardia* 29, 81–101.
- Vyavhare, S.S., Way, M.O., Medina, R.F., 2015. Determination of growth stage-specific response of soybean to redbanded stink bug (Hemiptera: Pentatomidae) and its relationship to the development of flat pods. *J. Econ. Entomol.* 108, 1770–1778.
- Watts, M.J., Worner, S.P., 2008. Using artificial neural networks to determine the relative contribution of abiotic factors influencing the establishment of insect pest species. *Ecol. Inform.* 3, 64–74.

CAPÍTULO 1

**Life tables for the diamondback moth
(*Plutella xylostella*) in southeast Brazil
indicate ants and spiders as leading
mortality factors**

Annals of Applied Biology, 2021, 178(3), pp. 498–507

<https://doi.org/10.1111/aab.12656>

Life tables for the diamondback moth (*Plutella xylostella*) in southeast Brazil indicate ants and spiders as leading mortality factors

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Abstract

The diamondback moth (DBM), *Plutella xylostella* (Lep.: Plutellidae), causes significant losses in *Brassica* crops. Leaf consumption by the larvae reduces plant yield and causes the contamination of heading brassicas. Chemical control usually provides unsatisfactory results due to the quickly-developed resistance of DBM populations to insecticides. Thus, natural control by biotic factors (predators, parasitoids, and entomopathogens) is crucial and should be managed upon knowledge of their role as DBM regulators. The leading mortality factors of DBM across the annual seasons in southeast Brazil were investigated by conducting 57 field life tables for two years. The highest and lowest total mortalities of DBM occurred in winter (99.7%) and autumn (94%). Predation was the main mortality factor in all seasons. Marginal parasitism rates were higher in spring and lower in autumn, and *Oomyzus sokolowskii* (Hym.: Eulophidae) accounted for almost 90% of total parasitism. The highest mortalities by rainfall were recorded in spring (44.5%), while physiological death and infection caused low mortalities ($\leq 6\%$) throughout the seasons. In addition, models were built to investigate the main predators acting on the DBM life stages; Araneae, *Brachymyrmex bruchi*, and *Tetramorium simillimum* (Hym.: Formicidae) were the predators associated with egg/L1 predation whereas *Solenopsis saevissima*, *T. simillimum*, and *Camponotus* spp. (Hym.: Formicidae) were the ones responsible

for L4/pupa predation. The L2/L3 stage was the least affected by predation, and the final model for this stage did not include any predator. In light of these findings, habitat manipulation and insecticide selectivity are discussed as measures to preserve the main predators and parasitoid.

Keywords: apparent mortality, conservation biological control, Formicidae, k -values, marginal attack rate

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lep.: Plutellidae), is a destructive pest of *Brassica* crops (Furlong et al., 2013; Philips et al., 2014). *Plutella xylostella* larvae cause damage through defoliation, which reduces plant yield (Philips et al., 2014). Besides, contamination of heading brassicas (e.g., cabbage, cauliflower, and broccoli) with DBM larvae can render the product unmarketable (Vail et al., 1989). Insecticide-resistant DBM populations are reported throughout the world (Bacci et al., 2018; Zago et al., 2014; Steinbach et al., 2017). The often perceived inefficacy of various insecticides in controlling the DBM has underlined the importance of naturally-occurring mortality factors on this pest management.

Climate is one of the main factors regulating insect populations (Peacock et al., 2006). The combined action of weather variables (e.g., temperature and rain) affects the pest both directly (e.g., causing mortality and physiological disorders, Pereira et al., 2007) and indirectly (e.g., affecting the duration of the pest life-cycle and the foraging activity of natural enemies) (Fidelis et al., 2019). Also, predators, parasitoids, and entomopathogens act on pests, reducing their populations. The importance of natural mortality factors varies considerably with the pest species. For instance, predators cause up to 77% and 26% mortality on *Planococcus citri* (Risso) (Hem.: Pseudococcidae) and *Neoleucinodes elegantalis* (Guenée) (Lep.: Crambidae), respectively (Silva et al., 2017; Rodrigues-Silva et al., 2017). Conversely, rainfall causes significant mortality (20%) on *Leucoptera coffeella* (Guérin-Méneville) (Lep.: Lyonetiidae) (Pereira et al., 2007), but not on *N. elegantalis* (Silva et al., 2017).

Ecological life tables are valuable tools for assessing the importance of the natural mortality factors of pests. By estimating the mortality by the acting factors throughout the pest life stages, one can determine the leading mortality causes

(Southwood & Henderson, 2000; Morris & Miller, 1954). Based on this knowledge, measures can be undertaken to preserve/enhance the leading mortality factors (Naranjo, 2001; Rosado et al., 2013). Life tables for the DBM were generated in some countries viz. Canada, Australia, and Japan (Harcourt, 1963; Dancau et al., 2020; Furlong et al., 2004). However, to the best of our knowledge, none has been developed in Brazil.

We recently described how DBM populations fluctuate over the year in southeast Brazil due to the variation of weather variables (Farias et al., 2020). However, an understanding of the leading mortality factors of DBM is still lacking, especially regarding the forces regulating its population. Therefore, we developed life tables to determine the main natural enemies and their importance over the seasons in southeast Brazil. These findings could be used to implement conservation measures to preserve these natural enemies.

Material and methods

Study site

This study was conducted from May 2017 to February 2019 in the experimental field (-20.767271, -42.868931) at the Universidade Federal de Viçosa, Viçosa, Minas Gerais state, Brazil. The region's climate is characterized as Cwb (dry winter and warm summer), according to the Köppen class (Peel et al., 2007). The area was surrounded by fragments of native vegetation (seasonal semi-deciduous forest) and small plots of corn, coffee, soybean, and vegetables (tomato, brassicas, and cucurbits).

Life tables

Horizontal life tables were carried out to assess the natural factors causing mortalities on DBM stages [egg/L1 (1st instar larva), L2/L3, and L4/pupa] over the seasons. The dates of beginning and end of the life tables, and the temperature and rainfall data (recorded by an automated station located 200 m from the study site, INMET, 2019) are presented in table 1. Eight replicates were held per season; however, some of them were defoliated by *Atta* sp. (Hym.: Formicidae) and the bird *Penelope jacquacu* (Spix's guan), resulting in a total of 57 replicates ($n = 14, 15, 13,$ and 15 for winter, spring, summer, and autumn, respectively).

Potted cabbage plants (cv. Astro Plus) with eight leaves were kept in the lab (25 ± 5 °C, RH $65 \pm 10\%$) for 12 hours in wooden cages (two plants per cage) containing ~ 80 DBM adults from a laboratory reared-population. The plants were then carefully transported to the study site, sunken (protruding the plastic rim two cm below the soil) into the ground, and thoroughly inspected for DBM eggs. Two to four leaves (from different plant portions viz. upper, median, and lower) were selected, and eggs from other leaves were removed using a fine brush (size 4). Eggs from the selected leaves were counted, and this reading constituted the initial number of eggs (l_0). Subsequent assessments were performed twice daily (at 8 am and 4 pm) by counting both the number of DBM (dead and alive) at each stage and predators on plants. Due to the small size of eggs and the leaf-mining habit of L1, plants were closely examined for egg/L1 mortality using illuminated hand-held magnifiers (30× magnification). The study plot contained 40 plants, including the infested ones, spaced 0.5×0.8 m apart. The plants were watered twice daily and did not receive any fertilizer or pesticide application.

Throughout the experiment, mortality was attributed to the acting factors (d_xFs) according to the following protocol: *i*) individuals disappearing after rainfall events were considered dead by rain washing; *ii*) individuals dead by physiological disorders, infection (brownish colored-colored and soften larvae, and pupae covered by white spores), and parasitism were directly quantified in the assessments (figure S1 – supporting information); *iii*) predation by certain groups (such as Araneae, figure S2 – supporting information) could be directly measured in the field, but for most cases (e.g., Formicidae, figure S3 – supporting information), the predator did not leave any DBM body remnants. Therefore, individuals disappearing in the absence of rainfall were taken as dead by predation. The assessments ended when all pupae either died or emerged as adults, leaving an easily-distinguishable empty cocoon (figure S4 – supporting information).

Given the initial number of eggs (l_0), individuals entering each DBM stage (l_x), and dead individuals at each stage x (d_x), apparent and real mortalities (q_x and r_x , respectively) were calculated using formulas 1 and 2 (Southwood & Henderson, 2000):

$$1) q_x = d_x \div l_x$$

$$2) r_x = d_x \div l_0$$

Also, marginal mortality (*MM*) rates were estimated. The use of *MM* allowed us to attribute to each mortality cause (d_xF) its real importance, by estimating the mortality due to that factor in the absence of other synchronous d_xFs (Royama, 1981). For instance, measuring parasitism directly in the field can lead to an underestimation of this factor's role (Royama, 1981), since parasitized individuals can disappear by the action of other contemporaneous d_xFs . Hence, *MM* for parasitism was measured in the lab by collecting, at each season, five leaves (containing at least eight individuals) per life stage and rearing them (25 ± 5 °C, RH $65 \pm 10\%$, and 12 h photoperiod) until adulthood (or parasitoid emergence). Also, *MM* for physiological death was measured separately (i.e., devoid of other d_xFs), by placing five plants (per season) individually into wooden cages (rain-protected by transparent plastic) and counting individuals dying at each stage. For rainfall, *MM* was taken as the apparent mortality (q_x) for this d_xF , since no other factor masks its action. Marginal mortality rates of predation and infection were derived from their own and relevant contemporaneous d_xFs apparent mortality (q_x) using formula 3 (Elkinton et al., 1992; Naranjo & Ellsworth, 2005):

$$3) \text{ } MM = qB \div (1 - qA)$$

where $qB = q_x$ of the factor (d_xF) of interest, and $qA =$ sum of q_x of contemporaneous d_xFs potentially obscuring qB . For predation and infection, qA was attributed as being rainfall and rainfall + predation (i.e., only rainfall obscuring predation, and rainfall + predation obscuring infection), respectively.

From the *MM*, we calculated *k*-values (killing power) for each d_xF using the formula $k = -\log(1 - MM)$ (Royama, 1981). *k*-values are a practical presentation of *MM*, since they can (contrarily to *MM*) be added across pest stages and d_xFs , making them useful for further analyses or graphical representations. Also, provided the survival rates (emerging adults $\div l_0$), sex ratio ($SR = 0.5$; Harcourt, 1957), and fecundity [based on the mean temperatures recorded in each season, according to Ngowi et al. (2017)], the net reproductive rate (R_0 , female offspring/female/generation) was calculated as:

$$4) R_0 = (\text{survival} \times SR \times \text{fecundity}) \div l_0$$

Natural enemies' identification

The relevant natural enemies (field-collected predators and parasitoids emerging in the lab) were kept in 90% ethanol. Parasitoids and Formicidae were identified by taxonomic experts. Other predators (Coleoptera, Hemiptera, Syrphidae, Neuroptera, and Vespidae) were identified by comparing the collected specimens with exemplars of the Regional Museum of Entomology at the UFV.

Statistical analyses

All analyses were performed using R (R Core Team, 2019). Firstly, we analyzed R_0 and k -values (pooled across all stages and d_xFs) as a function of season (models 1 and 2) using generalized linear mixed models (GLMMs with gamma distribution and log-link; Bates et al., 2015) which included year as random effect (1|year). Then, we checked for differences on k -values (pooled across all d_xFs) among DBM stages (egg/L1, L2/L3, and L4/pupa – model 3) through GLMM with gamma distribution (log-link) including both year and season as crossed random effects (1|year + 1|season). Gamma distribution was adopted in both cases (models for R_0 and k -values) due to the right-skewed distribution of the response variables. Lastly, MM by predation (therein marginal predation rates), for each stage, were regressed against predator densities (models 4 to 6) by linear mixed models (including year and season as crossed random effects), in order to identify the predators most accountable for that stage's predation. In this analysis, we only included predators whose frequency was $> 1\%$ in the assessments. Inasmuch as the models had several predictors, they were simplified using a stepwise procedure (in both forward and backward directions) to identify the set of predictors yielding the lowest AIC value (i.e., the most parsimonious models).

All models were singular (i.e., with non-significant random effects), the reason why the random terms were dropped. Significances (P -values) of the models were obtained by likelihood ratio tests (*lrtest* function, *lmtest* package; Zeileis & Hothorn, 2002) of the full models against null models (having only intercept). Significant differences among selected levels of nominal variables (DBM stage and season) were tested using pairwise differences of LS means (*lsmeans* package; Lenth, 2016). All models were checked for homoscedasticity

and normality by checking residual plots. A detailed description of the models used in this manuscript is given in the supplementary Table S1.

Results

Natural enemies

Five hymenopteran species were found parasitizing DBM viz. *Cotesia* sp. Cameron (Braconidae), *Conura pseudofulvovariiegata* (Becker) (Chalcididae), *Aprostocetus* sp. Westwood and *Oomyzus sokolowskii* (Kurdjimov) (Eulophidae), and *Hyposoter* sp. Forster (Ichneumonidae) (Table 2). Thirty-six percent of all collected DBM were parasitized, and *O. sokolowskii* accounted for almost 90% of total parasitism.

Ants (Formicidae) and spiders (Araneae) were the most abundant predators, and appeared in 54.41 and 20% of the assessments, respectively (Table 3). Among the Formicidae, *Tetramorium simillimum* (Smith) was the most abundant, followed by *Brachymyrmex bruchi* Forel, *Pheidole* spp., *Camponotus* spp., *Solenopsis saevissima* (Smith), *Cardiocondyla emeryi* Forel, and *Dorymyrmex brunneus* Forel. Syrphidae and Staphylinidae occurred in 4.73 and 1.51% of the readings, respectively, and the other predators were scarce (< 1% frequency).

Life tables

The total mortality rates of DBM were not less than 94% over the entire study period, being slightly higher in winter (99.7%) and spring (99.6%) (Table 4). Based on the sum of real mortality (r_x) across the DBM stages, predation was the main mortality factor (d_xF) in all seasons. The highest mortalities by predation occurred in summer (96.1%) and winter (93.2%), followed by autumn (87.9%) and spring (54.9%). Mortality rates by parasitism were low (sum of $r_x \leq 2.2\%$), irrespective of the season, since this factor was obscured by other contemporaneous d_xFs . Marginal rates (MM) of parasitism occurred in the L2/L3 and L4/pupa stages, and were higher in spring and lower in autumn. The highest mortalities by rainfall were recorded in spring (44.5%). Physiological death appeared across all stages and seasons causing low mortality rates (sum of $r_x \leq 6\%$), whereas infection occurred low rates ($\leq 0.4\%$) only in autumn and winter (L4/pupa).

Net reproductive rates and k-values

The net reproductive rate (R_0) and mean k -values (pooled across all d_xF_s and pest stages) of DBM varied among seasons (R_0 : $\chi^2(3) = 30.55$, $P < 0.001$; k -values: $\chi^2(3) = 43.64$, $P < 0.001$). R_0 was significantly higher in autumn (7.630 female offspring/female/generation) compared to the other seasons (winter = 0.230, spring = 0.383, and summer = 0.718) (Figure 1). Conversely, k -values increased from winter to spring and then decreased towards autumn (Figure 2a). k -values also differed among the DBM stages ($\chi^2(2) = 17.65$, $P < 0.001$), being lower in L2/L3 compared to the other stages (Figure 2b). Predation was the prevailing d_xF in all stages. For egg/L1, rainfall was the second factor in importance, whereas parasitism was second to predation for the L2/L3 and L4/pupa stages.

Marginal predation rates vs. predator densities

The final model for marginal predation rates of egg/L1 included Araneae, *B. bruchi*, and *T. simillimum* as predictors, all contributing positively to the dependent variable (Table 5). When *B. bruchi* densities increase from 0 to 2 individuals per assessment, marginal predation rates increase from 72.3 to 95.5% (Figure 3b). The curves for Araneae and *T. simillimum* are slightly steeper, showing that when densities increase from 0 to 1 individuals per assessment, predation rates are predicted to increase from 73.6 to 96.0% and 67.1 to 88.6%, respectively (Figure 3a and 3c). The model for L2/L3 only had Syrphidae, which was negatively related to marginal predation rates (Table 5). The model for L4/pupa had the ants *S. saevissima* and *Camponotus* spp. (both marginally significant) and *T. simillimum* affecting the predation rates positively, while *B. bruchi* was negatively related to the dependent variable. An increment of 19.6, 16.7, and 17.0% on L4/pupa marginal predation is expected when the densities of *S. saevissima*, *T. simillimum*, and *Camponotus* spp. increase from 0 to 1, 0 to 1, and 0 to 0.5 individuals per assessment, respectively (Figure 3d-f).

Overall, winter and summer had higher predator densities than spring and autumn (Figure 4). Spiders' densities varied little among the seasons. Conversely, ants' densities fluctuated considerably over the year, with *T. simillimum* and *B. bruchi* being more abundant in the summer, and winter having the highest densities of *S. saevissima* and *Camponotus* spp.

Discussion

Over the life tables, five hymenopteran species were found parasitizing DBM. Another study in Brazil reported a similar composition of DBM parasitoids in Central-West Brazil, with species from the genus *Apanteles*, *Cotesia*, and *Conura*, *Diadegma leontinae* Brethes (an Ichneumonidae not found during our assessments), and *O. sokolowskii* (Guilloux et al., 2003). However, contrary to them, which reported *D. leontinae* and *Apanteles* sp. as the dominant parasitoids, *O. sokolowskii* was the prevailing species in our study, contributing almost 90% to DBM parasitism. As for the DBM predators, some of them (Acari, Araneae, Miridae, and Formicidae) were seen preying upon DBM during the assessments (Figures S2 and S3 – supporting information). Others (Carabidae, Coccinellidae, Neuroptera, Syrphidae, Staphylinidae, and Vespidae) were reported as DBM predators in previous studies (Miranda et al., 2011; Hosseini et al., 2012). Araneae and Formicidae were the most abundant predators. We could not sort by family all the collected Araneae, but three individuals found preying on DBM larvae were representatives of Linyphiidae (two individuals) and Dictynidae. Besides, *Cheiracanthium inclusum* (Hentz) (Eutichuridae), a reported DBM predator (Silva-Torres et al., 2010), appeared in our assessments.

The total DBM mortality ($\sum r_x$) varied over the seasons, ranging from 94.0 to 99.7%. These values are comparable to those reported in life tables conducted in Canada ($\geq 99.96\%$) (Dancau et al., 2020) and Japan ($\geq 94.21\%$) (Wakisaka et al., 1991), and higher than those found in Australia (56 to 87% mortality) (Furlong et al., 2004). Irrespective of the season, predation was the prevailing d_xF in our study (especially in winter and summer). Among the DBM stages, L2/L3 was the one presenting the lowest MM rates. The k -value for the most important d_xF (predation) was lower for this stage. A reasonable explanation for this is the shorter duration of this stage compared to the others; L2/L3 comprises 21.11% of the total DBM developmental time, whereas egg/L1 and L4/pupa represent 32.74 and 46.15%, respectively (Steinbach et al., 2017). This indicates that artificial control measures (e.g., use of selective and bio-rational insecticides), when needed, should be focused on the L2/L3 stage, especially during autumn, to complement the action of naturally-occurring mortality factors.

During spring, rainfall (44.5% mortality) was one of the leading mortality factors (together with predation), particularly in earlier stages (egg/L1). Wakisaka

et al. (1991), investigating the effect of artificial precipitation (by means of water sprinkling) on DBM stages, reported up to 30% of eggs washed-off from the plants after water sprinkling; for eggs laid on the upper leaf side, this value was even higher (47%). Besides displacing eggs and larvae from the plant, rain droplets accumulate on the lipophilic surface of brassica leaves, forming larger drops that kill young larvae by drowning. Another indirect effect is the resulting preying of rain-dislodged larvae by soil-dwelling arthropods (Dancau et al., 2020). We also observed substantial pupa mortalities ($q_x = 11.6\%$) following heavy rains during summer. The higher importance of rain mortality in spring compared to summer (both comprising the rainy season for the study region), is due to the higher frequency of heavy rains (precipitation > 10 mm/hour, Jarraud, 2008) on the former.

Based on its low r_x rates, parasitism was strongly obscured by the other d_xFs . Overall, the highest and lowest k -values for parasitism occurred in spring and autumn, respectively. Also, k -values of parasitism (mainly represented by *O. sokolowskii*) were higher for L4/pupa than for L2/L3. Studies accessing the *O. sokolowskii* preference for different DBM stages report higher parasitism in L4 compared to L2 and L3 (Talekar & Hu, 1996; Sow et al., 2013), which support our findings.

When regressing marginal predation rates against densities of the most frequent predators, no predator was positively related to L2/L3 predation. For the other stages, spiders and *T. simillimum* were the predators associated with egg/L1 predation, and *S. saevissima*, *T. simillimum*, and *Camponotus* spp. were the ones responsible for L4/pupa predation. The importance of ants to DBM control has been shown by a comprehensive study by Agarwal et al. (2007). Using sentinel larvae and exclusion experiments, they found six species (including two species of *Camponotus*) foraging on DBM larvae, with *Pheidole* sp. and *Tetramorium* sp. being the primary species involved. Also, previous studies have found *T. simillimum* to predate on larvae of the curculionids *Hypothenemus hampei* (Ferrari) and *Diaprepes abbreviatus* (L.) (Armbrecht & Gallego, 2007; Whitcomb et al., 1982). As for the spiders, their role in the biological control of pests, including DBM, has been extensively acknowledged (Ma et al., 2005; Suenaga & Hamamura, 2015; Quan et al., 2011).

In light of these findings, conservation measures are important for maintaining natural enemies' populations in brassica crops. Crop cultivation (e.g., plowing and grass-cutting) contributes to reducing spider densities in agroecosystems, both by causing direct mortality and leading spiders to move out of the area (Thorbek & Bilde, 2004; Bogya & Markó, 1999). Therefore, in brassica crops from the study region, tilling should be avoided and ground cover (e.g., grassy or non-brassica weeds on the inter-rows) be maintained to preserve spider populations. Besides, the use of selective insecticides (IGRs, diamides, spinosyns, and *Bt*-based products) is a measure that could be deployed to preserve both predatory ants (Barros et al., 2018; Araújo et al., 2017) and spiders (Pekar, 2002; Markó et al., 2009). During spring (season with higher parasitism rates), chemical control should be avoided to the maximum extent, since *O. sokolowskii* is reportedly sensible to several insecticides (Bacci et al., 2018; Cordero et al., 2007; Haseeb et al., 2005), including IPM-compatible ones (e.g., spinosyns and IGRs). Habitat diversification is another means of enhancing pest biological control (Wilkinson & Landis, 2005). By increasing habitat complexity (through intercropping and polyculture, among other practices), alternative resources such as pollen, nectar, and shelter are provided, which increase the abundance and diversity of natural enemies (Rezende et al., 2014; Togni et al., 2019).

Estimates of net reproductive rates (R_0) were low from winter to summer (exhibiting an increasing pattern) and plainly higher in autumn. Contrariwise, the mean k -values (pooled across all $d_x F_s$ and pest stages) follow a reverse direction, decreasing towards autumn. R_0 is a parameter that measures the tendency of the population to either increase in size ($R_0 > 1$), decrease ($R_0 < 1$), or remain stable ($R_0 = 1$) (Southwood & Henderson, 2000). Based on the values found in our study, DBM populations would exhibit higher growth during autumn, decrease drastically in winter, and grow steadily in the spring and summer towards autumn. However, the climate plays a major role in regulating DBM dynamics. Lower values of temperature and relative humidity favor this pest, which results in higher DBM densities in winter, decreasing towards autumn (Farias et al., 2020). Our study shows how the natural mortality factors of DBM would act if this pest was equally abundant in all seasons. By artificially infesting plants with DBM in periods in which the pest densities were supposed to be low (summer and autumn), a

temporal asynchrony of DBM with their main natural enemies (i.e., high DBM densities and low predator densities) might have occurred (Van Nouhuys & Lei, 2004; Roy et al., 2005). This is evidenced by the lowest predator densities in spring and autumn, the seasons with lowest mortalities by predation.

In summary, this study describes how natural mortality of DBM fluctuates due to the acting factors whose intensities vary across seasons. Predation, the leading mortality factor throughout the year, is exerted mainly by ants and spiders. Parasitism, in turn, is particularly important in regulating DBM during spring. Conservation measures should be undertaken to preserve predators (especially spiders and ants) and parasitoids (mostly represented by *O. sokolowskii*).

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, and Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG. ESF gratefully acknowledges the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the scholarship grant (process number: 140983/2017-1). Special thanks to the taxonomic experts Angélica Pentead-Dias (UFSCAR, Brazil) and Marcelo Tavares (UFES, Brazil) for parasitoid identification; and Jacques Delabie (CEPEC/CEPLAC, Brazil) and Julio Chaul (UFV, Brazil) for Formicidae identification.

Conflict of interest

The authors have no conflicts of interest to declare.

References

- Agarwal V.M., Rastogi N., Raju S.V.S. (2007) Impact of predatory ants on two lepidopteran insect pests in Indian cauliflower agroecosystems. *Journal of Applied Entomology*, **131**, 493–500.
- Araújo T.A., Picanço M.C., Ferreira D.O., Campos J.N., Arcanjo L.P., Silva G.A. (2017) Toxicity and residual effects of insecticides on *Ascia monuste* and predator *Solenopsis saevissima*. *Pest Management Science*, **73**, 2259–2266.
- Armbrecht I., Gallego M.C. (2007) Testing ant predation on the coffee berry borer in shaded and sun coffee plantations in Colombia. *Entomologia Experimentalis et Applicata*, **124**, 261–267.

- Bacci L., Rosado J.F., Picanço M.C., Gonring A.H.R., Galdino T.V.S., Martins J.C. (2018) Failure control of *Plutella xylostella* (Lepidoptera: Plutellidae) and selectivity of their natural enemies to different insecticides. *Journal of Plant Protection Research*, **58**, 161–167.
- Barros E.M., Silva-Torres C.S.A., Torres J.B., Rolim G.G. (2018) Short-term toxicity of insecticides residues to key predators and parasitoids for pest management in cotton. *Phytoparasitica*, **46**, 391–404.
- Bates D., Mächler M., Bolker B., Walker S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67**, 1–48.
- Bogya S., Markó V. (1999) Effect of pest management systems on ground-dwelling spider assemblages in an apple orchard in Hungary. *Agriculture, Ecosystems and Environment*, **73**, 7–18.
- Cordero R.J., Bloomquist J.R., Kuhar T.P. (2007) Susceptibility of two diamondback moth parasitoids, *Diadegma insulare* (Cresson) (Hymenoptera; Ichneumonidae) and *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera; Eulophidae), to selected commercial insecticides. *Biological Control*, **42**, 48–54.
- Dancau T., Haye T., Cappuccino N., Mason P.G. (2020) Something old, something new: Revisiting the diamondback moth (Lepidoptera: Plutellidae) life table after 65 years. *Canadian Entomologist*, **152**, 70–88.
- Elkinton J.S., Buonaccorsi J.P., Bellows T.S., Van Driesche R.G. (1992) Marginal attack rate, *k*-values and density dependence in the analysis of contemporaneous mortality factors. *Researches on Population Ecology*, **34**, 29–44.
- Farias E.S., Santos A.A., Ribeiro A.V., Carmo D.G., Paes J.S., Picanço M.C. (2020) Climate and host plants mediating seasonal dynamics and within-plant distribution of the diamondback moth (*Plutella xylostella*). *Crop Protection*, **134**, 105172.
- Farias, E.S., Santos, R.C., Carmo, D.G., Soares, J.R., Costa, T.L., Santos, A.A., Picanço, M.C. (2021). Life tables for the diamondback moth (*Plutella xylostella*) in southeast Brazil indicate ants and spiders as leading mortality factors. *Annals of Applied Biology*, **178**, 498–507.
- Fidelis E.G., Farias E.S., Lopes M.C., Sousa F.F., Zanuncio J.C., Picanço M.C. (2019) Contributions of climate, plant phenology and natural enemies to the seasonal variation of aphids on cabbage. *Journal of Applied Entomology*, **143**, 365–370.
- Furlong M.J., Shi Z., Liu S.-S., Zalucki M.P. (2004) Evaluation of the impact of natural enemies on *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) populations on commercial *Brassica* farms. *Agricultural and Forest Entomology*, **6**, 311–322.
- Furlong M.J., Wright D.J., Dossdall L.M. (2013) Diamondback moth ecology and management: Problems, progress, and prospects. *Annual Review of Entomology*, **58**, 517–541.
- Guilloux T., Monnerat R., Castelo-Branco M., Kirk A., Bordat D. (2003) Population dynamics of *Plutella xylostella* (Lep., Yponomeutidae) and its parasitoids in the region of Brasilia. *Journal of Applied Entomology*, **127**, 288–292.
- Harcourt D.G. (1957) Biology of the diamondback moth, *Plutella maculipennis*

(Curt.) (Lepidoptera: Plutellidae), in eastern Ontario. II. Life-history, behaviour, and host relationships. *The Canadian Entomologist*, **89**, 554–564.

Harcourt D.G. (1963) Major mortality factors in the population dynamics of the diamondback moth, *Plutella maculipennis* (Curt.) (Lepidoptera: Plutellidae). *The Memoirs of the Entomological Society of Canada*, **95**, 55–66.

Haseeb M., Amano H., Liu T.X. (2005) Effects of selected insecticides on *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) and *Oomyzus sokolowskii* (Hymenoptera: Eulophidae), parasitoids of *Plutella xylostella* (Lepidoptera: Plutellidae). *Insect Science*, **12**, 163–170.

Hosseini R., Schmidt O., Keller M.A. (2012) Detection of predators within *Brassica* crops: A search for predators of diamondback moth (*Plutella xylostella*) and other important pests. *African Journal of Agricultural Research*, **7**, 3473–3484.

INMET (2019) BDMEP - Banco de Dados Meteorológicos para Ensino e Pesquisa. Available at: <http://www.inmet.gov.br/projetos/rede/pesquisa/> [Accessed May 11, 2019].

Jarraud M. (2008) *Guide to meteorological instruments and methods of observation (WMO-No. 8)*. Geneva, Switzerland: World Meteorological Organisation.

Korner-Nievergelt F., Roth T., von Felten S., Guelat J., Almasi B., Korner-Nievergelt P. (2015) *Bayesian data analysis in ecology using linear models with R, BUGS and Stan*. Elsevier.

Lenth R. V. (2016) Least-squares means: The R package lsmeans. *Journal of Statistical Software*, **69**, 1–33.

Ma J., Li D., Keller M., Schmidt O., Feng X. (2005) A DNA marker to identify predation of *Plutella xylostella* (Lep., Plutellidae) by *Nabis kinbergii* (Hem., Nabidae) and *Lycosa* sp. (Aranaea, Lycosidae). *Journal of Applied Entomology*, **129**, 330–335.

Markó V., Keresztes B., Fountain M.T., Cross J. V. (2009) Prey availability, pesticides and the abundance of orchard spider communities. *Biological Control*, **48**, 115–124.

Miranda F., Bylund H., Grönberg L., Larsson L., Björkman C. (2011) Population density and killing capacity by predators of eggs and larvae of the diamondback moth in Nicaragua. *Environmental Entomology*, **40**, 333–341.

Morris R.F., Miller C.A. (1954) The development of life tables for the spruce budworm. *Canadian Journal of Zoology*, **32**, 283–301.

Naranjo S.E. (2001) Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. *Crop Protection*, **20**, 835–852.

Naranjo S.E., Ellsworth P.C. (2005) Mortality dynamics and population regulation in *Bemisia tabaci*. *Entomologia Experimentalis et Applicata*, **116**, 93–108.

Ngowi B. V., Tonnang H.E.Z., Mwangi E.M., Johansson T., Ambale J., Ndegwa P.N., Subramanian S. (2017) Temperature-dependent phenology of *Plutella xylostella* (Lepidoptera: Plutellidae): Simulation and visualization of current and future distributions along the Eastern Afromontane. *PLOS ONE*, **12**, e0173590.

Van Nouhuys S., Lei G. (2004) Parasitoid-host metapopulation dynamics: The

- causes and consequences of phenological asynchrony. *Journal of Animal Ecology*, **73**, 526–535.
- Peacock L., Worner S., Sedcole R. (2006) Climate variables and their role in site discrimination of invasive insect species distributions. *Environmental Entomology*, **35**, 958–963.
- Peel M.C., Finlayson B.L., McMahon T.A. (2007) Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences*, **11**, 1633–1644.
- Pekar S. (2002) Susceptibility of the spider *Theridion impressum* to 17 pesticides. *Journal of Pest Science*, **75**, 51–55.
- Pereira E.J.G., Picanço M.C., Bacci L., Della Lucia T.M.C., Silva E.M., Fernandes F.L. (2007) Natural mortality factors of *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) on *Coffea arabica*. *Biocontrol Science and Technology*, **17**, 441–455.
- Philips C.R., Fu Z., Kuhar T.P., Shelton A.M., Cordero R.J. (2014) Natural history, ecology, and management of diamondback moth (Lepidoptera: Plutellidae), with emphasis on the United States. *Journal of Integrated Pest Management*, **5**, 1–11.
- Quan X., Wu L., Zhou Q., Yun Y., Peng Y., Chen J. (2011) Identification of predation by spiders on the diamondback moth *Plutella xylostella*. *Bulletin of Insectology*, **64**, 223–227.
- R Core Team. (2019) *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rezende M.Q., Venzon M., Perez A.L., Cardoso I.M., Janssen A. (2014) Extrafloral nectaries of associated trees can enhance natural pest control. *Agriculture, Ecosystems and Environment*, **188**, 198–203.
- Rodrigues-Silva N., Campos S.O., Farias E.S., Souza T.C., Martins J.C., Picanço M.C. (2017) Relative importance of natural enemies and abiotic factors as sources of regulation of mealybugs (Hemiptera: Pseudococcidae) in Brazilian coffee plantations. *Annals of Applied Biology*, **171**, 303–315.
- Rosado J.F., Bacci L., Martins J.C., Silva G.A., Gontijo L.M., Picanço M.C. (2013) Natural biological control of green scale (Hemiptera: Coccidae): A field life-table study. *Biocontrol Science and Technology*, **24**, 190–202.
- Roy M., Brodeur J., Cloutier C. (2005) Seasonal activity of the spider mite predators *Stethorus punctillum* (Coleoptera: Coccinellidae) and *Neoseiulus fallacis* (Acarina: Phytoseiidae) in raspberry, two predators of *Tetranychus mcdanieli* (Acarina: Tetranychidae). *Biological Control*, **34**, 47–57.
- Royama T. (1981) Evaluation of mortality factors in insect life table analysis. *Ecological Monographs*, **51**, 495–505.
- Silva-Torres C.S.A., Pontes I.V.A.F., Torres J.B., Barros R. (2010) New records of natural enemies of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in Pernambuco, Brazil. *Neotropical Entomology*, **39**, 835–838.
- Silva E.M., Silva R.S., Silva N.R., Milagres C.C., Bacci L., Picanço M.C. (2017) Assessment of the natural control of *Neoleucinodes elegantalis* in tomato cultivation using ecological life tables. *Biocontrol Science and Technology*, **27**,

525–538.

Southwood T.R.E., Henderson P.A. (2000) The construction, description, and analysis of age-specific life-tables, In *Ecological methods*, pp. 404–436. Eds T. R. E. Southwood and P. A. Henderson. Oxford, UK: Blackwell Science.

Sow G., Arvanitakis L., Niassy S., Diarra K., Bordat D. (2013) Life history traits of *Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae), a parasitoid of the diamondback moth. *African Entomology*, **21**, 231–238.

Steinbach D., Moritz G., Nauen R. (2017) Fitness costs and life table parameters of highly insecticide-resistant strains of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) at different temperatures. *Pest Management Science*, **73**, 1789–1797.

Suenaga H., Hamamura T. (2015) Effects of manipulated density of the wolf spider, *Pardosa astrigera* (Araneae: Lycosidae), on pest populations and cabbage yield: A field enclosure experiment. *Applied Entomology and Zoology*, **50**, 89–97.

Talekar N.S., Hu W.J. (1996) Characteristics of parasitism of *Plutella xylostella* (Lep., Plutellidae) by *Oomyzus sokolowskii* (Hym., Eulophidae). *Entomophaga*, **41**, 45–52.

Thorbeck P., Bilde T. (2004) Reduced numbers of generalist arthropod predators after crop management. *Journal of Applied Ecology*, **41**, 526–538.

Togni P.H.B., Venzon M., Souza L.M., Santos J.P.C.R., Sujii E.R. (2019) Biodiversity provides whitefly biological control based on farm management. *Journal of Pest Science*, **92**, 393–403.

Vail K.M., Kok L.T., Lentner M. (1989) Broccoli yield response to selected levels of cabbage looper (Lepidoptera: Noctuidae) larvae in Southwestern Virginia. *Journal of Economic Entomology*, **82**, 1437–1443.

Wakisaka S., Tsukuda R., Nakasuji F. (1991) Life tables of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) and effects of rainfall, temperature and host plants on survival and reproduction. *Japanese Journal of Applied Entomology and Zoology*, **35**, 115–122.

Whitcomb W.H., Gowan T.D., Buren W.F. (1982) Predators of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) larvae. *The Florida Entomologist*, **65**, 150–158.

Wilkinson T.K., Landis D.A. (2005) Habitat diversification in biological control: the role of plant resources, In *Plant-provided food for carnivorous insects: a protective mutualism and its applications*, pp. 305–325. Eds F. L. Wackers, P. C. J. van Rijn, and J. Bruin. Cambridge: Cambridge University Press.

Zago H.B., Siqueira H.A.A., Pereira E.J., Picanço M.C., Barros R. (2014) Resistance and behavioural response of *Plutella xylostella* (Lepidoptera: Plutellidae) populations to *Bacillus thuringiensis* formulations. *Pest Management Science*, **70**, 488–495.

Zeileis A., Hothorn T. (2002) Diagnostic checking in regression relationships. *R News*, **2**, 7–10

Table 1. Weather conditions and duration of the field experiments in Viçosa, MG, Brazil. In the table is presented the dates of beginning and end of the assessments in each season, mean daily temperature (°C) and temperature range (minimum and maximum), and mean daily precipitation (mm/day) and percentage of days with heavy rains (precipitation > 10 mm/hour).

| Year, season | Date | Temperature | | Rainfall | |
|-------------------|-----------------------|-------------|------------|---------------|-----------------|
| | | Mean (°C) | Range (°C) | Mean (mm/day) | Heavy rains (%) |
| 2017, autumn | 5/25/2017-6/21/2017 | 17.6 | 7.3-28.9 | 0.51 | 0 |
| 2017, winter | 9/2/2017-9/21/2017 | 18.7 | 7.5-30.2 | 0 | 0 |
| 2017, spring | 11/30/2017-12/19/2017 | 22 | 16-31.3 | 13.73 | 12 |
| 2017/2018, summer | 2/17/2018-3/2/2018 | 22.7 | 17.4-31.4 | 3.5 | 0 |
| 2018, autumn | 4/28/2018-6/5/2018 | 18.0 | 5.5-29.1 | 0.33 | 0 |
| 2018, winter | 8/14/2018-9/21/2018 | 18.3 | 8.7-30.6 | 1.33 | 0 |
| 2018, spring | 11/13/2018-11/26/2018 | 21.4 | 15.8-29.8 | 10.71 | 14 |
| 2018/2019, summer | 2/8/2019-2/25/2019 | 22.8 | 17.1-34.5 | 4.29 | 6 |

Table 2. Percentage parasitism of hymenopteran species associated with *Plutella xylostella* collected on cabbage. Parasitism is expressed as the proportion of each species on total cases (parasitized and non-parasitized *P. xylostella*, $n = 576$) and on parasitized *P. xylostella* (within brackets, $n = 110$). Viçosa, MG, Brazil. 2017-2019.

| Parasitoid | Stage | Parasitism (%) | |
|---------------|---|-----------------|---------------|
| Braconidae | <i>Cotesia</i> sp. Cameron | Larval | 0.53 (2.82) |
| Chalcididae | <i>Conura pseudofulvovariegata</i> (Becker) | Hyperparasitoid | 1.07 (5.63) |
| Eulophidae | | | 17.11 (90.14) |
| | <i>Aprostocetus</i> sp. Westwood | Hyperparasitoid | 0.53 (2.82) |
| | <i>Oomyzus sokolowskii</i> (Kurdjimov) | Larval-pupal | 16.58 (87.32) |
| Ichneumonidae | <i>Hyposoter</i> sp. Forster | Larval-pupal | 0.27 (1.41) |
| Total | | | 36.10 |

Table 3. Percentage abundance and frequency of *Plutella xylostella* predators on cabbage ($n = 808$ individuals on 465 assessments). The percentage values are the proportion of each species on the total number of predators (for abundance) and the number of predator appearances on the total number of assessments. Viçosa, MG, Brazil. 2017-2019.

| Predator | Abundance (%) | Frequency (%) |
|---|---------------|---------------|
| Acari: Trombidiformes | 0.50 | 0.86 |
| Araneae | 13.37 | 20.00 |
| Coleoptera: Carabidae <i>Elysius</i> sp. Walker | 0.25 | 0.43 |
| Coleoptera: Coccinellidae <i>Harmonia axyridis</i> (Pallas) | 0.37 | 0.65 |
| Coleoptera: Staphylinidae <i>Paederus</i> sp. | 0.87 | 1.51 |
| Diptera: Syrphidae <i>Allograpta</i> spp. | 3.22 | 4.73 |
| Hemiptera: Miridae <i>Tupiocoris</i> sp. China & Carvalho | 0.50 | 0.43 |
| Hymenoptera: Formicidae | 80.07 | 54.41 |
| <i>Camponotus</i> spp. | 7.55 | 12.04 |
| <i>Solenopsis saevissima</i> (Smith) | 4.21 | 5.81 |
| <i>Pheidole</i> spp. | 14.85 | 11.61 |
| <i>Dorymyrmex brunneus</i> Forel | 1.11 | 0.86 |
| <i>Brachymyrmex bruchi</i> Forel | 17.95 | 10.54 |
| <i>Tetramorium simillimum</i> (Smith) | 32.43 | 25.16 |
| <i>Cardiocondyla emeryi</i> Forel | 1.98 | 0.86 |
| Hymenoptera: Vespidae | 0.37 | 0.65 |
| <i>Polybia</i> sp. | 0.25 | 0.43 |
| <i>Protonectarina sylveirae</i> (de Saussure) | 0.12 | 0.22 |
| Neuroptera: Hemerobiidae | 0.50 | 0.86 |
| Total | | 76.77 |

Table 4. Life table parameters of *Plutella xylostella* on cabbage for the different seasons of the years 2017 and 2019 in Viçosa, MG, Brazil. $n = 14, 15, 13,$ and 15 life tables for winter, spring, summer, and autumn, respectively.

| Season/ x | Infection | | Parasitism | | Physiological death | | Predation | | Rainfall | | Σr_x |
|---------------|----------------|-------|----------------|-------|---------------------|-------|----------------|-------|----------------|-------|--------------|
| | $q_x (r_x)$ | MM | $q_x (r_x)$ | MM | $q_x (r_x)$ | MM | $q_x (r_x)$ | MM | $q_x (r_x)$ | MM | |
| <u>Winter</u> | <u>(0.001)</u> | | <u>(0.003)</u> | | <u>(0.06)</u> | | <u>(0.932)</u> | | <u>(0.002)</u> | | <u>0.997</u> |
| Egg/L1 | | | | | 0.045 (0.045) | 0.001 | 0.810 (0.810) | 0.811 | 0.002 (0.002) | 0.002 | |
| L2/L3 | | | 0 (0) | 0.489 | 0.092 (0.013) | 0 | 0.699 (0.101) | 0.699 | 0 (0) | 0 | |
| L4/Pupa | 0.015 (0.001) | 0.054 | 0.086 (0.003) | 0.320 | 0.088 (0.003) | 0.160 | 0.719 (0.022) | 0.719 | 0 (0) | 0. | |
| <u>Spring</u> | <u>(0)</u> | | <u>(0)</u> | | <u>(0.001)</u> | | <u>(0.549)</u> | | <u>(0.445)</u> | | <u>0.996</u> |
| Egg/L1 | | | | | 0 (0) | 0.057 | 0.305 (0.305) | 0.545 | 0.441 (0.441) | 0.441 | |
| L2/L3 | | | 0 (0) | 0.440 | 0 (0) | 0.072 | 0.717 (0.182) | 0.717 | 0 (0) | 0 | |
| L4/Pupa | 0 (0) | 0 | 0 (0) | 0.929 | 0.02 (0.001) | 0.339 | 0.861 (0.062) | 0.921 | 0.065 (0.005) | 0.065 | |
| <u>Summer</u> | <u>(0)</u> | | <u>(0.011)</u> | | <u>(0.003)</u> | | <u>(0.961)</u> | | <u>(0.016)</u> | | <u>0.991</u> |
| Egg/L1 | | | | | 0 (0) | 0.060 | 0.843 (0.843) | 0.843 | 0 (0) | 0 | |
| L2/L3 | | | 0 (0) | 0.200 | 0.016 (0.002) | 0 | 0.554 (0.087) | 0.59 | 0.061 (0.01) | 0.061 | |
| L4/Pupa | 0 (0) | 0 | 0.194 (0.011) | 0.697 | 0.007 (0) | 0.077 | 0.53 (0.031) | 0.6 | 0.116 (0.007) | 0.116 | |
| <u>Autumn</u> | <u>(0.004)</u> | | <u>(0.022)</u> | | <u>(0.036)</u> | | <u>(0.879)</u> | | <u>(0)</u> | | <u>0.940</u> |
| Egg/L1 | | | | | 0.011 (0.011) | 0 | 0.517 (0.517) | 0.517 | 0 (0) | 0 | |
| L2/L3 | | | 0 (0) | 0.020 | 0.03 (0.014) | 0.012 | 0.365 (0.172) | 0.365 | 0 (0) | 0 | |
| L4/Pupa | 0.013 (0.004) | 0.037 | 0.076 (0.022) | 0.090 | 0.04 (0.011) | 0.190 | 0.663 (0.189) | 0.663 | 0 (0) | 0 | |

$x = Plutella xylostella$ stage; $d_x F$ = mortality factor; q_x = apparent mortality; r_x = real mortality; MM = marginal mortality, Σr_x = summation of r_x in the respective season, which corresponds to the total mortality.

Table 5. Summary of linear models of marginal predation rates of *Plutella xylostella* life stages (egg/L1, L2/L3, and L4/pupa) against densities of predators (Araneae, Formicidae, Staphylinidae, and Syrphidae). Viçosa, MG, Brazil. 2017-2019.

| <i>P. xylostella</i> stage | Term ^a | Slope ± SE | t-value | P |
|----------------------------|-------------------------------|------------|---------|--------|
| Egg/L1 | Intercept | 0.62±0.05 | 11.92 | <0.001 |
| | Araneae | 0.22±0.15 | 1.49 | 0.143 |
| | <i>Brachymyrmex bruchi</i> | 0.12±0.07 | 1.72 | 0.093 |
| | <i>Tetramorium simillimum</i> | 0.22±0.08 | 2.71 | 0.010 |
| L2/L3 | Intercept | 0.66±0.04 | 18.09 | <0.001 |
| | Syrphidae | -0.47±0.17 | -2.77 | 0.008 |
| L4/Pupa | Intercept | 0.71±0.04 | 19.90 | <0.001 |
| | <i>Solenopsis saevissima</i> | 0.20±0.12 | 1.64 | 0.108 |
| | <i>Brachymyrmex bruchi</i> | -0.05±0.03 | -1.87 | 0.068 |
| | <i>Tetramorium simillimum</i> | 0.17±0.08 | 2.13 | 0.039 |
| | <i>Camponotus</i> spp. | 0.34±0.18 | 1.92 | 0.062 |

^a For all stages, full models included Araneae, *S. saevissima*, *Pheidole* spp., *B. bruchi*, *T. simillimum*, *Camponotus* spp., Staphylinidae, and Syrphidae. A stepwise procedure combining both forward and backward selection was used to select the most parsimonious combination of predictors.

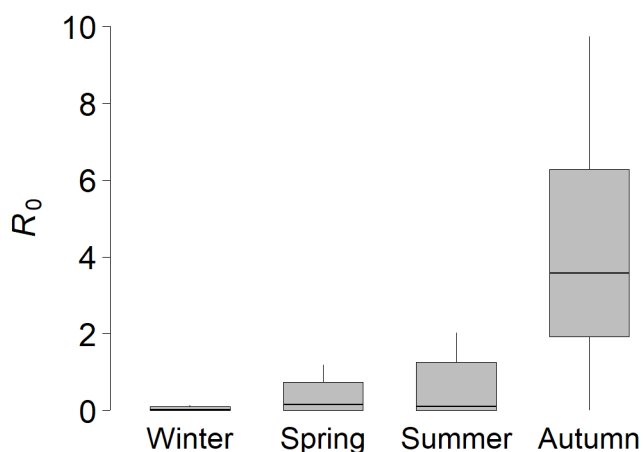


Figure 1. Box plots of R_0 (net reproductive rate of the population) for *Plutella xylostella* over the seasons (winter, spring, summer, and autumn) in Viçosa, MG, Brazil. Box plots show median values (horizontal line), 25–75% interquartile range (box bounds), and 10th and 90th percentiles (whiskers).

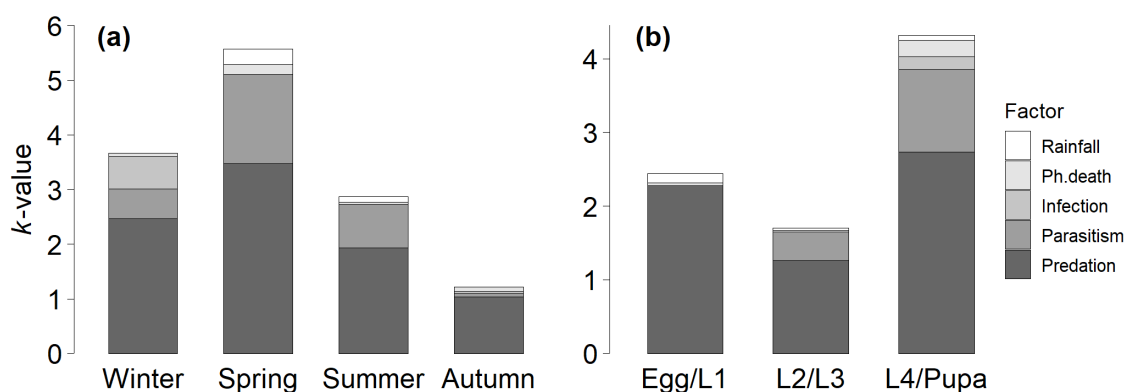


Figure 2. k -values (a) over the seasons (winter, spring, summer, and autumn) and (b) within each *Plutella xylostella* life stage (egg/L1, L2/L3, and L4/pupa) in Viçosa, MG, Brazil. Ph. death = physiological death. L1 = 1st instar larva, L2 = 2nd instar larva, L3 = 3rd instar larva, L4 = 4th instar larva.

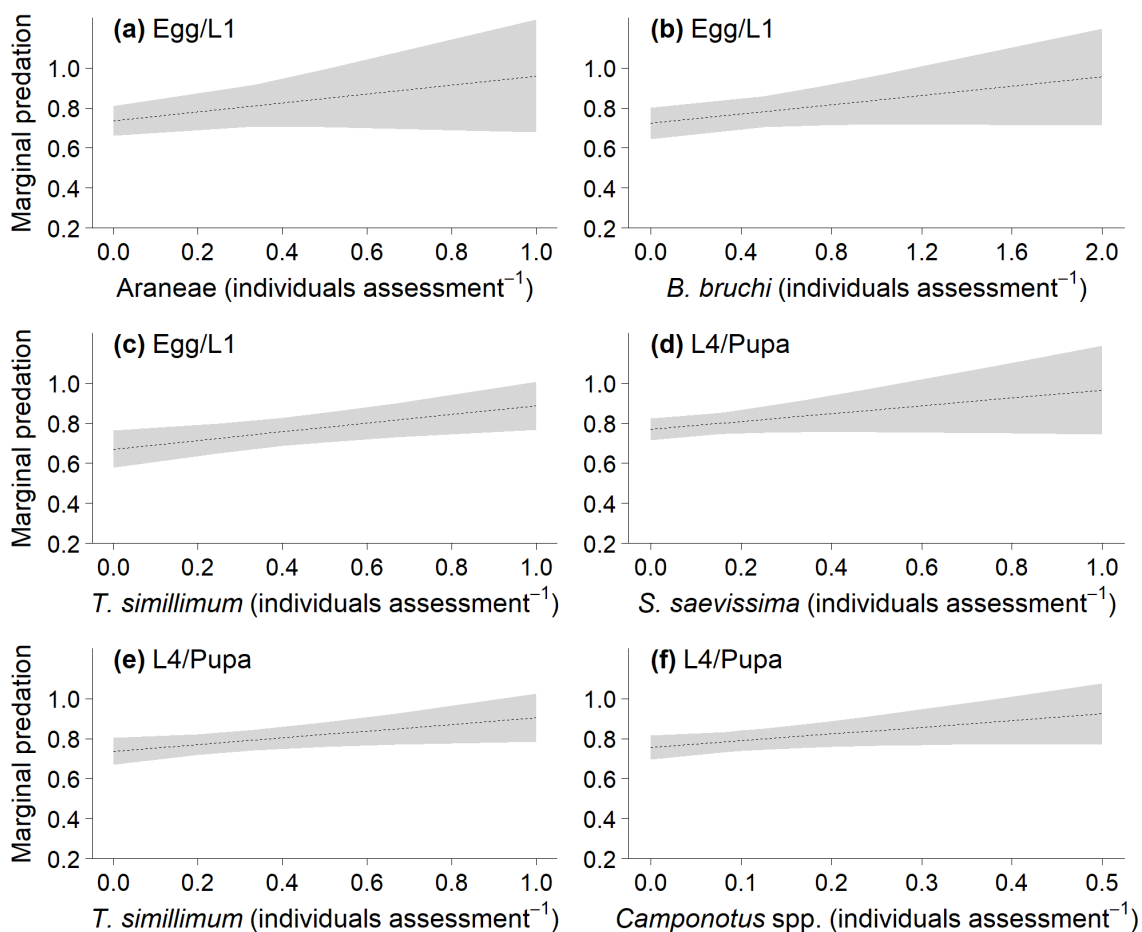


Figure 3. Plots showing the effect of predator (*Araneae*, *Brachymyrmex bruchi*, *Tetramorium simillimum*, *Solenopsis saevissima*, and *Camponotus* spp.) densities on marginal predation rates of *Plutella xylostella* life stages (egg/L1, L2/L3, and L4/pupa). Plots include model estimates (dashed black lines) and 95% confidence intervals (gray shadings). L1 = 1st instar larva, L2 = 2nd instar larva, L3 = 3rd instar larva, L4 = 4th instar larva.

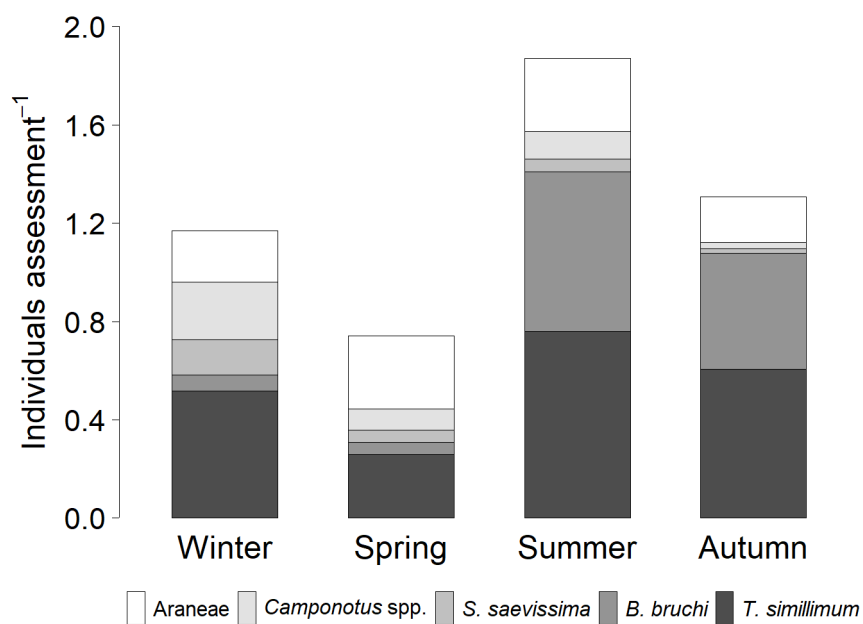


Figure 4. Densities of predators (*Araneae*, *Camponotus* spp., *Solenopsis saevissima*, *Brachymyrmex bruchi*, and *Tetramorium simillimum*) on cabbage across seasons (winter, spring, summer, and autumn) in Viçosa, MG, Brazil.

Supporting information

Table S1. Description of the models used in this manuscript, including the error distribution (and link function), the full models, and the optimal models.

| Model | Distribution | Full model | Optimal model |
|-------|-----------------------------|---|---|
| 1 | Gamma (log link) | $R0_i \sim \alpha + \text{season}_i + (1 \text{year}) + \epsilon_i$ | $R0_i \sim \alpha + \text{season}_i + \epsilon_i$ |
| 2 | Gamma (log link) | $k_i \sim \alpha + \text{season}_i + (1 \text{year}) + \epsilon_i$ | $k_i \sim \alpha + \text{season}_i + \epsilon_i$ |
| 3 | Gamma (log link) | $k_i \sim \alpha + \text{stage}_i + (1 \text{year}) + (1 \text{season}) + \epsilon_i$ | $k_i \sim \alpha + \text{stage}_i + \epsilon_i$ |
| 4 | Gaussian (identity link) | $MPre_i \sim \alpha + Ara_i + Sol_i + Phe_i + Bra_i + Tet_i + Cam_i + Sta_i + Syr_i + (1 \text{year}) + (1 \text{season}) + \epsilon_i$ | $MPre_i \sim \alpha + Ara_i + Bra_i + Tet_i + \epsilon_i$ |
| 5 | Gaussian (identity link) | $MPre_i \sim \alpha + Ara_i + Sol_i + Phe_i + Bra_i + Tet_i + Cam_i + Sta_i + Syr_i + (1 \text{year}) + (1 \text{season}) + \epsilon_i$ | $MPre_i \sim \alpha + Syr_i + \epsilon_i$ |
| 6 | Gaussian (identity link) | $MPre_i \sim \alpha + Ara_i + Sol_i + Phe_i + Bra_i + Tet_i + Cam_i + Sta_i + Syr_i + (1 \text{year}) + (1 \text{season}) + \epsilon_i$ | $MPre_i \sim \alpha + Sol_i + Bra_i + Tet_i + Cam_i + \epsilon_i$ |

MPre = marginal predation, Ara = Araneae, Sol = *Solenopsis saevissima*, Phe = *Pheidole* spp., Bra = *Brachymyrmex bruchi*, Tet = *Tetramorium simillimum*, Cam = *Camponotus* spp., Sta = Staphylinidae, Syr = Syrphidae.

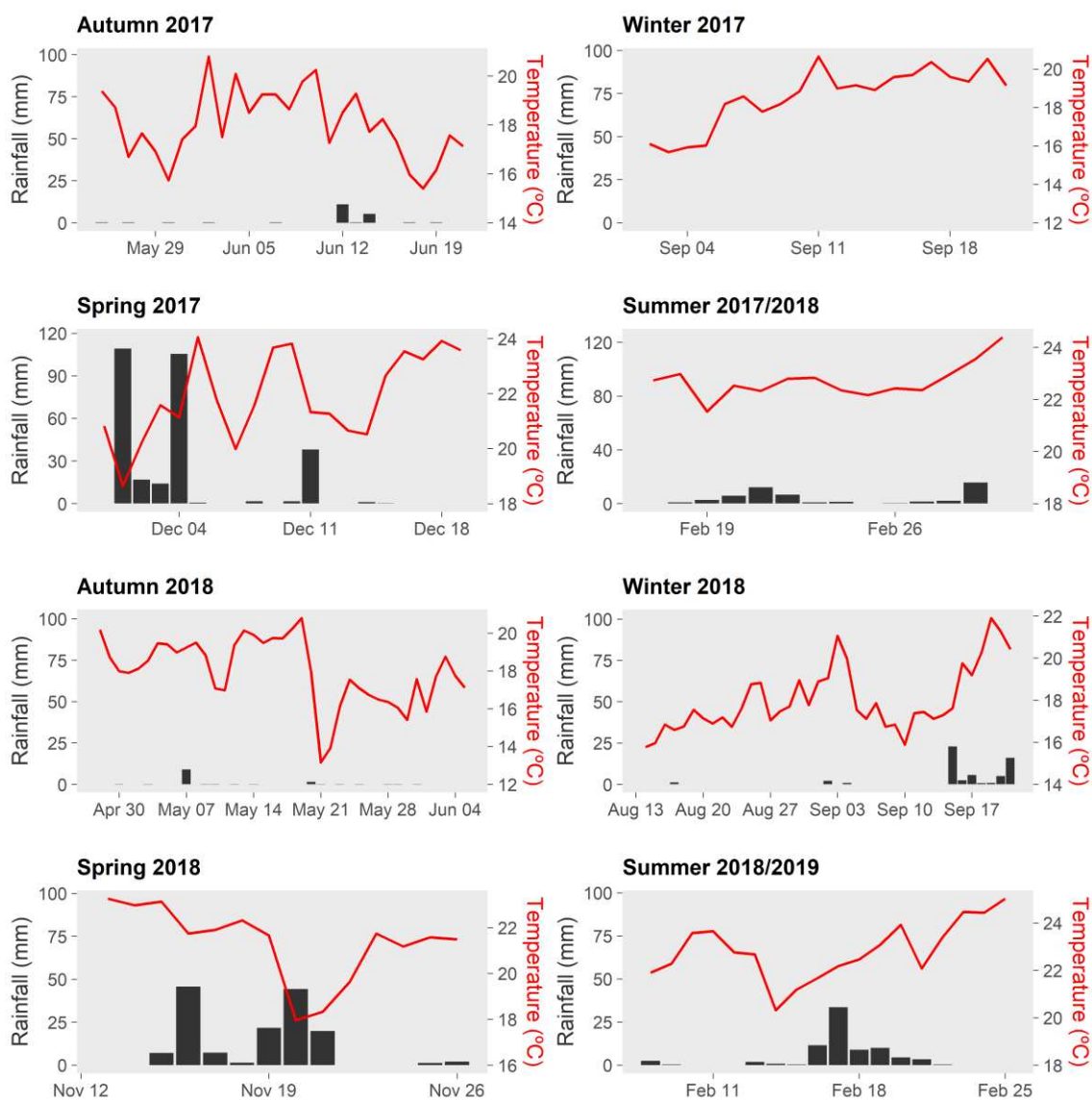


Figure S1. Daily values of weather variables (mean temperature and rainfall) throughout the field experiments in Viçosa, MG, Brazil.



Figure S2. a) L4 *Plutella xylostella* dead due to *Cotesia* sp. parasitism; b) *Cotesia* sp. pupa; c) *P. xylostella* pupa dead by *Oomyzus sokolowskii* parasitism; and d) *O. sokolowskii* emerging from a dead *P. xylostella* pupa.



Figure S3. Araneae preying on a) L2, b) L4, c) pupa of *Plutella xylostella*. d) Pupa attacked by Araneae and e-f) predatory mites preying on pupa and L4 *P. xylostella*, respectively.

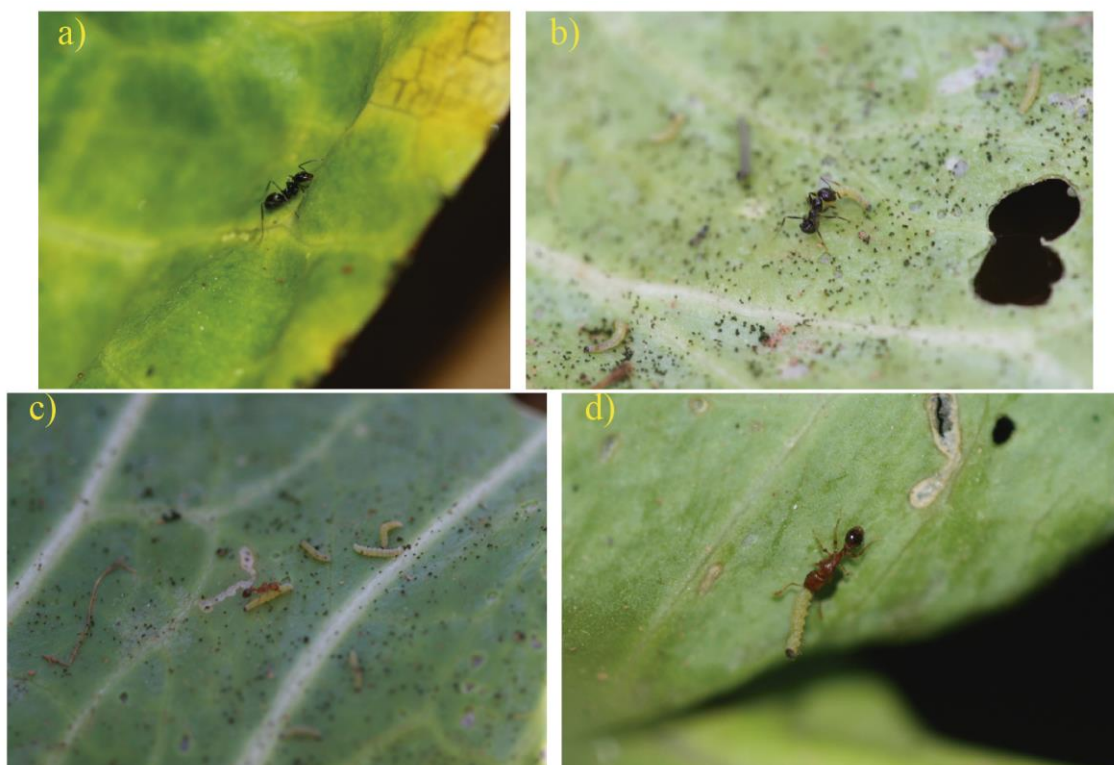


Figure S4. Formicidae foraging on *Plutella xylostella*. a-b) *Dorymyrmex brunneus* preying on *P. xylostella* eggs and L3, respectively; c-d) *Tetramorium simillimum* preying on *P. xylostella* L3.



Figure S5. Empty cocoons of *P. xylostella* from which adults emerged.

CAPÍTULO 2

Impact of diamondback moth density and infestation timing on broccoli yield

Neotropical Entomology, 2021, 50(2), pp. 298–302

<https://doi.org/10.1007/s13744-021-00849-3>

Impact of diamondback moth density and infestation timing on broccoli yield

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Abstract

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lep.: Plutellidae), is an important pest of broccoli (*Brassica oleracea* L. var. *italica* Plenck). Few studies have focused on the real DBM impact on broccoli yield. We performed greenhouse studies to assess the effect of DBM densities and infestation timing (at pre-heading, heading or during the entire cycle) on broccoli head weight. Polynomial trend analysis revealed a downward linear response of head weight to DBM densities, indicating that broccoli is susceptible to DBM attack. As for the infestation timing, infestations during the pre-heading stage significantly impacted head weight, whereas the same did not occur for infestations during the heading stage. DBM density did not affect plant height or total leaves but was upward-related to plant defoliation, and head weight correlated negatively with plant defoliation. These findings indicate that pre-heading is the determining stage for head weight loss of plants under DBM attack. In order to prevent quantitative losses, efforts for DBM management should be focused on this particular stage.

Keywords: *Brassica oleracea*, Defoliation, *Plutella xylostella*, Polynomial trend analysis

Introduction

Broccoli (*Brassica oleracea* L. var. *italica* Plenck) is a vegetable grown in various regions of the world (Dixon 2007, Johansen *et al* 2017). In Brazil, broccoli cultivation is more concentrated in the South and Southeast regions (IBGE 2019). This vegetable is highly appreciated because of its benefits to human health. A diet rich in broccoli has been reported to prevent diseases such as cancer, diabetes, and hypertension due to the high contents of antioxidants (carotenoids, flavonoids, and vitamins) (Lin and Chang 2005, Reis *et al* 2015).

Lepidopteran pests are important causes of yield losses in broccoli, both in volume produced or in crop marketability. The continuous leaf consumption by the larvae reduces broccoli yield, and the presence of larvae in the florets can result in market rejection (Hooks and Johnson 2002). The diamondback moth (DBM), *Plutella xylostella* (L.) (Lep.: Plutellidae), is a major pest of broccoli and other Brassicaceae crops worldwide (Furlong *et al* 2013, Philips *et al* 2014). Damage by the DBM occurs due to leaf-mining by first instar larvae and intense leaf tissue consumption by later instars (especially third and fourth instar) (Philips *et al* 2014).

Although DBM is extensively studied worldwide, few works have focused on its impact on broccoli yield. Studying the crop response to pest insect levels is a primary step to develop decision-making systems, including action thresholds (Pedigo *et al* 1986). Crop response to insect injury may differ with respect to the growth stage (Costa *et al* 2019). Once determined the crop stage most impacted by insect injury, management strategies can be focused on that particular stage to prevent economic losses (Ayalew 2006, Vyavhare *et al* 2015).

This study aimed to determine the response of broccoli yield to DBM attack. Besides, the impact of infestation timing (infestations initiating on pre-heading, heading or during the entire cycle) on broccoli head weight was assessed.

Materials and methods

General conditions

This study was conducted from April to July 2019 (autumn to winter) in a greenhouse located in Viçosa, MG, Brazil (20°45'28.3"S, 42°52'10.1"W). Temperatures in the greenhouse ranged from 9 to 41°C. Broccoli seedlings (cv.

Master) with three true leaves were transplanted into 8-liter plastic pots. Plants were fertilized with 7.5, 10.6, and 6.5 g of N, P₂O₅, and K₂O per plant, respectively (Ribeiro *et al* 1999). At pre-planting, 20, 100, and 20% of the recommended doses of N, P₂O₅, and K₂O, respectively, were applied. Top-dressing fertilization with N and K₂O was split into 14 applications at weekly intervals. Four applications of a foliar fertilizer containing boron (0.2%) and molybdenum (0.05%) were performed at 55, 65, 75, and 95 days after transplanting (DAT). Plants were spaced 0.5 × 0.8 m and hose-watered twice daily.

The experiment was performed in an RCBD with four replicates (plants) and a factorial design 3 × 7 (infestation timing × DBM density). Plants were infested at the pre-heading stage (from 7th to 63rd DAT), at the heading stage (from 63rd to 91st DAT), or during the entire broccoli cycle (from 7th to 91st DAT). Infestations were performed weekly with third instar DBM at seven densities (0, 1, 2, 4, 6, 8, and 12 larvae per plant) placed on the middle portion of the plants. Before each infestation, pupae from the previous infestation were counted and removed. To account for larva establishment failure, the mean number of pupae recovered for each infestation level (0.16±0.10, 0.63±0.06, 1.35±0.10, 2.16±0.17, 3.37±0.21, 4.1±0.27, 6.01±0.36, respectively), rather than the number of inoculated larvae, was used in the analyses.

Head weight as a function of DBM levels and infestation timing

At 106 DAT, broccoli heads of all treatments were harvested and weighed using an electronic balance (± 1 g). A two-way ANOVA (*stats* package from R, *lm* function, R Core Team 2019). was performed to assess the effect of infestation timing, DBM density, and their interaction on head weight. For each DBM infestation timing, orthogonal polynomial trend analysis (*stats* package, *poly* function) was performed to identify the most suitable order (linear, quadratic, cubic, or quartic) fitting head weight on DBM levels. Subsequently, head weight for each broccoli stage was regressed versus pest density, and the confidence interval (CI₉₅) of model slopes was used to compare the curves.

Plant phenology and defoliation as a function of DBM density

Assessments were performed at 14, 28, 42, 56, 64, 77, and 92 days after the first infestation. The total number of fully expanded leaves was counted, and

plant height (from ground level to the apical bud) was tape-measured. Defoliation (D%) by each infestation level was determined using a scale defined in previous experiments (Fig S1). To maintain uniformity, only the first author performed the defoliation assessments. At each evaluation date, all leaves of two plants randomly selected were photographed, and leaf length (LL) and width (LW) were measured using ImageJ (version 1.50i, National Institutes of Health, Bethesda, USA). Leaf area (LA) was then calculated based on the formula: $LA = 5.97 \times 0.058 \times LL \times LW$ (Olfati *et al* 2010). Subsequently, D% was converted into defoliation area (DA, expressed in cm²) using the equation: $DA = D\% \times LA \div 100$.

Total leaves, plant height, and defoliation area were plotted against time (days after first infestation, DAFI), and the area under the curve (AUC) of these variables was calculated using the function *AUC* from *DescTools* R package (Signorell 2019). Later, linear models (*stats* package from R, *lm* function) were tested to verify the effect of pest densities on the response variables' AUC.

Results

Both the infestation timing (entire cycle, pre-heading, and heading) and its interaction with the pest density affected the head weight (ANOVA, $F_{2, 75} = 2.10$ and $F_{2, 75} = 5.70$, $P < 0.05$, respectively). Polynomial trend analysis revealed a linear relationship of head weight against DBM infestation levels (Table 1). Linear models were significant for plants infested during the entire cycle ($r^2 = 0.42$, $P = 0.01$) and during the pre-heading stage ($r^2 = 0.53$, $P < 0.01$) (Fig 1a and 1b). The same did not occur for infestations in the heading stage ($r^2 = 0.25$, $P = 0.15$) (Fig 1c). The slope of the curve of plants infested during the pre-heading phase (-28.20, $CI_{95} = -40.34 - -16.06$) did not differ from that of plants infested throughout the cycle (-26.43, $CI_{95} = -41.65 - -11.22$).

Irrespective of the DBM infestation level, total leaves increased linearly up to 60 days after first infestation and then stabilized, while plant height grew steadily throughout the assessments (Fig 2a). The infestation levels did not affect the AUC of total leaves ($r^2 = 0.08$, $P = 0.82$) or plant height ($r^2 = 0.10$, $P = 0.70$) (Fig 2b). Conversely, AUC of defoliation varied with the number of larvae per plant ($r^2 = 0.87$, $P < 0.001$). A significant and negative correlation between AUC of defoliation and head weight was found ($r = -0.62$, $n = 24$, $P = 0.001$).

Discussion

The crop yield response to insect levels might assume linear (the crop is susceptible to the pest) or non-linear (the crop is either tolerant or hyper susceptible to pest injury) fashions (Buntin 2001). In our case, crop response was best represented by a linear model, indicating that broccoli does not exhibit any degree of tolerance nor is highly susceptible to DBM. Although more common to direct pests (i.e., pests damaging harvestable products, e.g. Fernandes *et al*, 2011), the linearity of the crop yield/pest levels relationship is not rare for indirect pests (Nabirye *et al* 2003, Tuan *et al* 2017), and has been reported for DBM in cauliflower (Subramanian *et al* 2010).

When analyzing the outputs from plants infested during the entire cycle, the weight loss per one larva was 26 grams (12.27%), as indicated by the linear curve of head weight against DBM levels. Based on this model, head weight is predicted to decrease in 24.6, 49.1 and 73.6% for mean DBM densities of 2, 4, and 6 larvae per plant. In Brazil, DBM populations are higher during winter and spring (Marchioro and Foerster 2016, Farias *et al* 2020), but this pest is found all year round. Due to the high value of broccoli in the Brazilian market, especially during summer, weight reduction can lead to substantial economic losses.

Differences in head weight from different infestation levels can be explained mainly by plant defoliation, as indicated by the high negative correlation between the two variables. At the end of the assessments, leaf consumption by 6.01, 3.37, and 0.63 larvae corresponded to the area of 1.6, 0.6, and 0.2 leaves, respectively. Therefore, the reduced yield of plants under DBM attack is likely related to the reduction in photosynthesizing leaf area due to the foliar tissue loss (Peterson 2001). To a lesser extent, nutrient allocation for plant regrowth (Caldwell *et al* 1981, Belsky *et al* 1993) and production of secondary compounds to fight DBM herbivory (Simms and Rausher 1987, Vos *et al* 2013) might also have contributed to the lower yield of plants under DBM attack.

Infestation timing impacted broccoli response to DBM. Head weight of plants infested during the heading stage was not affected by DBM. Conversely, DBM herbivory during the pre-heading phase was determining for inflorescence size. A field study with cabbage reports a similar pattern; DBM control during pre-heading provided a higher yield than control only during the heading phase (Ayalew 2006). The differences in plant susceptibility among broccoli stages

might be related to sink-source relations, which differ from a phase to another. During pre-heading, plants use nutrients for vegetative growth (leaf and stem formation). During heading, vegetative growth is interrupted, as depicted in figure 2, with the total leaves stabilizing at the end of the crop cycle. At this point, nutrients from leaves and stems (metabolic sources) and photoassimilates are allocated to the inflorescence (metabolic sink) (Li *et al* 2011). Therefore, DBM defoliation during pre-heading reduced plant photosynthesis; this caused a lower accumulation of nutrients in the leaves and stems, leading to a smaller inflorescence size.

Our study focused on the quantitative impacts of DBM on broccoli yield. However, another factor determining broccoli marketability is the head contamination with DBM larvae (Hooks and Johnson 2002). This qualitative loss was not quantified in our study, since larvae were not found in broccoli heads at harvest. Thus, although DBM infestation during heading does not impact broccoli yield quantitatively, it could potentially cause qualitative losses, depending on market requirements regarding inflorescence contamination.

In summary, our study shows that broccoli plants are susceptible to DBM attack, as indicated by the downward linear relationship of crop yield with DBM levels. Also, DBM attack during pre-heading is determining for broccoli head weight. These findings indicate that DBM should be monitored throughout the cycle, in order to prevent quantitative losses during pre-heading and inflorescence contamination with DBM larvae during heading.

Authors' Contributions

ESF and MCP conceived the research. ESF, LCSS, JBM, and PASJ conducted experiments. ESF conducted statistical analyses. ESF wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgments

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process number: 140983/2017-1), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG.

References

- Ayalew G (2006) Comparison of yield loss on cabbage from diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) using two insecticides. *Crop Prot* 25:915–919
- Belsky AJ, Carson WP, Jensen CL, Fox GA (1993) Overcompensation by plants: Herbivore optimization or red herring? *Evol Ecol* 7:109–121
- Buntin GD (2001) Techniques for evaluating yield loss from insects. In: Peterson RKD, Higley LG (eds) *Biotic stress and yield loss*. CRC Press, Boca Raton, pp 23–41
- Caldwell MM, Richards JH, Johnson DA, Nowak RS, Dzurec RS (1981) Coping with herbivory: Photosynthetic capacity and resource allocation in two semiarid *Agropyron* bunchgrasses. *Oecologia* 50:14–24
- Costa TL, Sarmiento RA, Araújo TA, Pereira PS, Silva RS, Lopes MC, Picanço MC (2019) Economic injury levels and sequential sampling plans for *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotype B on open-field melon crops. *Crop Prot* 125:104887
- Dixon GR (2007) *Vegetable brassicas and related crucifers*. CABI, Wallingford, UK
- Farias ES, Santos AA, Ribeiro AV, Carmo DG, Paes JS, Picanço MC (2020) Climate and host plants mediating seasonal dynamics and within-plant distribution of the diamondback moth (*Plutella xylostella*). *Crop Prot* 134:105172
- Farias ES, Sant'ana LCS, Melo JB, Santana PA, Picanço MC (2021) Impact of diamondback moth density and infestation timing on broccoli yield. *Neotrop Entomol* 50:298–302
- Fernandes FL, Picanço MC, Campos SO, Bastos CS, Chediak M, Guedes RN, Silva RS (2011) Economic injury level for the coffee berry borer (Coleoptera: Curculionidae: Scolytinae) using attractive traps in Brazilian coffee fields. *J Econ Entomol* 104:1909–1917
- Furlong MJ, Wright DJ, Dossall LM (2013) Diamondback moth ecology and management: Problems, progress, and prospects. *Annu Rev Entomol* 58:517–541
- Hooks CRR, Johnson MW (2002) Lepidopteran pest populations and crop yields in row intercropped broccoli. *Agric For Entomol* 4:117–125
- IBGE (2019) Censo Agropecuário 2017. <https://sidra.ibge.gov.br/pesquisa/censo-agropecuario/censo-agropecuario-2017>. Accessed 10 Oct 2019
- Johansen TJ, Mølmann JA, Bengtsson GB, Schreiner M, Velasco P, Hykkerud AL, Cartea E, Lea P, Skaret J, Seljåsen R (2017) Temperature and light conditions at different latitudes affect sensory quality of broccoli florets (*Brassica oleracea* L. var. *italica*). *J Sci Food Agric* 97:3500–3508
- Li H, Jiang H, Li T (2011) Broccoli plant nitrogen, phosphorus, and water relations at field scale and in various growth media. *Int J Veg Sci* 17:190–210
- Lin C-H, Chang C-Y (2005) Textural change and antioxidant properties of broccoli under different cooking treatments. *Food Chem* 90:9–15

- Marchioro CA, Foerster LA (2016) Biotic factors are more important than abiotic factors in regulating the abundance of *Plutella xylostella* L., in Southern Brazil. *Rev Bras Entomol* 60:328–333
- Nabirye J, Nampala P, Kyamanywa S, Ogenga-Latigo MW, Wilson H, Adipala E (2003) Determination of damage-yield loss relationships and economic injury levels of flower thrips on cowpea in eastern Uganda. *Crop Prot* 22:911–915
- Olfati JA, Peyvast G, Shabani H, Nosrati-Rad Z (2010) An estimation of individual leaf area in cabbage and broccoli using non-destructive methods. *J Agr Sci Tech* 12:627–632
- Pedigo LP, Hutchins SH, Higley LG (1986) Economic injury levels in theory and practice. *Annu Rev Entomol* 31:341–368
- Peterson RKD (2001) Photosynthesis, yield loss, and injury guilds. In: Peterson RKD, Higley LG (eds) *Biotic stress and yield loss*. CRC Press, Boca Raton, FL, pp 83–97
- Philips CR, Fu Z, Kuhar TP, Shelton AM, Cordero RJ (2014) Natural history, ecology, and management of diamondback moth (Lepidoptera: Plutellidae), with emphasis on the United States. *J Integr Pest Manag* 5:1–11
- R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
- Reis LCR, Oliveira VR, Hagen MEK, Jablonski A, Flôres SH, Rios AO (2015) Carotenoids, flavonoids, chlorophylls, phenolic compounds and antioxidant activity in fresh and cooked broccoli (*Brassica oleracea* var. *Avenger*) and cauliflower (*Brassica oleracea* var. *Alphina F1*). *LWT - Food Sci Technol* 63:177–183
- Ribeiro AC, Guimarães PTG, Alvarez VH (1999) Recomendação para o uso de corretivos e fertilizantes em Minas Gerais: 5a. aproximação. Comissão de fertilidade do solo do estado de Minas Gerais, Viçosa, Brazil
- Signorell A (2019) DescTools: Tools for descriptive statistics. <https://cran.r-project.org/package=DescTools>
- Simms EL, Rausher MD (1987) Costs and benefits of plant resistance to herbivory. *Am Nat* 130:570–581
- Subramanian S, Rabindra RJ, Sathiah N (2010) Economic threshold for the management of *Plutella xylostella* with granulovirus in cauliflower ecosystem. *Phytoparasitica* 38:5–17
- Tuan SJ, Lee CC, Tang LC, Saska P (2017) Economic injury level and demography-based control timing projection of *Spodoptera litura* (Lepidoptera: Noctuidae) at different growth stages of *Arachis hypogaea*. *J Econ Entomol* 110:755–762
- Vos IA, Pieterse CMJ, van Wees SCM (2013) Costs and benefits of hormone-regulated plant defences. *Plant Pathol* 62:43–55
- Vyavhare SS, Way MO, Medina RF (2015) Determination of growth stage-specific response of soybean to redbanded stink bug (Hemiptera: Pentatomidae) and its relationship to the development of flat pods. *J Econ Entomol* 108:1770–1778

Table 1. Polynomial trend analysis for each infestation timing (entire cycle, pre-heading, and heading) of broccoli head weight as a function of *Plutella xylostella* infestation levels.

| Infestation timing | Order | <i>t</i> | <i>P</i> |
|--------------------|-------|----------|----------|
| Entire cycle | 1th | -3.495 | 0.002 |
| | 2nd | 1.253 | 0.224 |
| | 3rd | 0.407 | 0.688 |
| | 4th | -0.093 | 0.927 |
| Pre-heading | 1th | -4.687 | <0.001 |
| | 2nd | -0.908 | 0.375 |
| | 3rd | -0.805 | 0.430 |
| | 4th | 0.627 | 0.538 |
| Heading | 1th | 0.218 | 0.829 |
| | 2nd | 1.160 | 0.260 |
| | 3rd | -0.238 | 0.814 |
| | 4th | 0.098 | 0.923 |

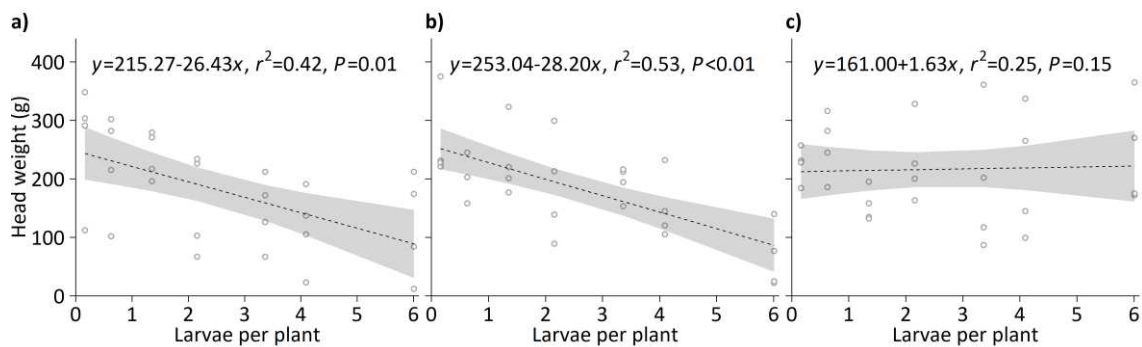


Fig 1 *Plutella xylostella* densities affecting head weight of broccoli plants infested during a) the entire cycle, b) pre-heading stage, and c) heading stage. Plots include observed values (white circles), model estimates (dashed black lines), and 95% confidence intervals (gray shadings).

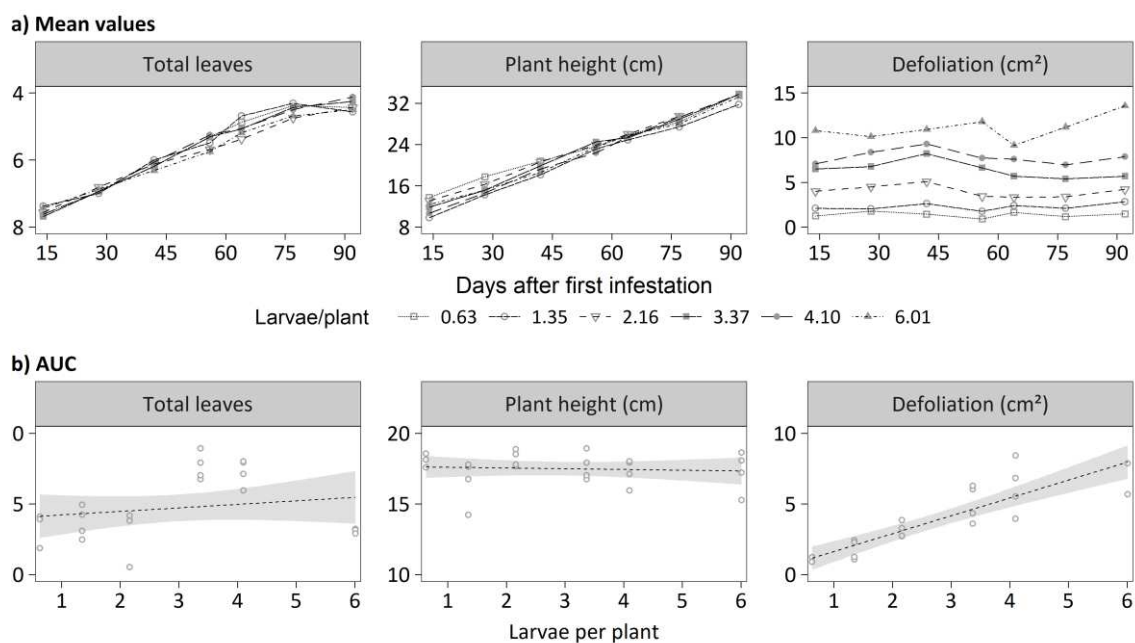


Fig 2 a) Mean values and b) area under the curve (AUC) of total leaves, plant height (cm) and defoliation (cm²) of broccoli plants under varying *Plutella xylostella* infestation levels (0.63, 1.35, 2.16, 3.37, 4.10 and 6.01 larvae per plant). Measures were taken at 14, 28, 42, 56, 64, 77, and 92 days after the first infestation. Plots of AUC include observed values (white circles), model estimates (dashed black lines), and 95% confidence intervals (gray shadings). Equations for AUC models: Total leaves ($y=13.95+0.25x$, $r^2 = 0.08$, $P = 0.82$); Plant height ($y=17.87-0.05x$, $r^2 = 0.10$, $P = 0.70$); Defoliation ($y=0.87+1.45x$, $r^2 = 0.87$, $P < 0.001$).

Supporting information

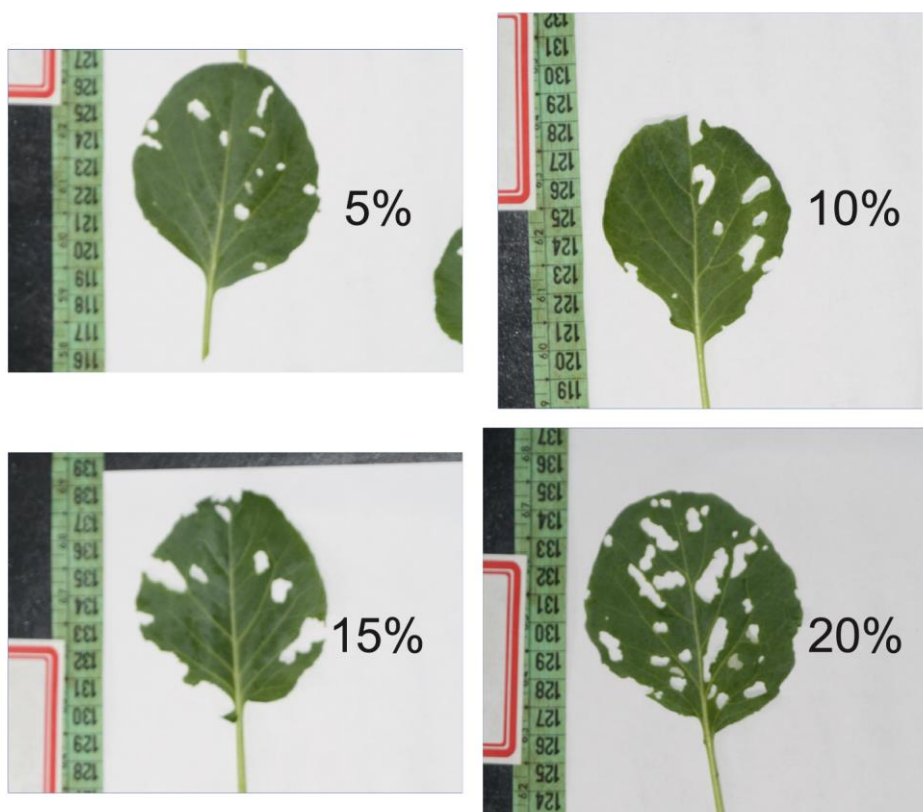


Fig S1 Defoliation scale for broccoli exposed to *Plutella xylostella* larvae. The consumed area (%) was quantified using ImageJ (version 1.50i, National Institutes of Health, Bethesda, USA).

CAPÍTULO 3

**Forecasting the seasonal dynamics of
Trichoplusia ni (Lep.: Noctuidae) on three
Brassica crops through neural networks**

Forecasting the seasonal dynamics of *Trichoplusia ni* (Lep.: Noctuidae) on three *Brassica* crops through neural networks

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Abstract

The cabbage looper, *Trichoplusia ni* Hübner (Lep.: Noctuidae) is a destructive pest of *Brassica* crops. Their larvae feed on leaves, and such defoliation reduces plant yield. The understanding and modelling of pest seasonal dynamics is central to management programs, because it allows one to set up sampling and control efforts. This study aimed to train, with field collected data, artificial neural networks (ANN) for *T. ni* forecasting on *Brassica* crops. ANNs were used due to their suitability to fit complex models with multiple predictors. Three weather variables (air temperature, rainfall, and relative humidity lagged at different intervals from the day of pest assessment) and three host plants (broccoli, cabbage and cauliflower) along with another plant-related variable (days after transplanting) were used as input variables to build ANNs with different topologies. Two outputs (*T. ni* eggs or larvae) were tested to verify which one would yield more precise models. ANNs forecasting *T. ni* eggs performed better, based on Pearson's correlation (r_V) of observed with fitted values. The winning ANN ($r_V = 0.706$) had weather data lagged by 15 days, 2 neurons in the hidden layer, hyperbolic tangent as the activation function, and resilient backpropagation as the learning algorithm. Broccoli and cauliflower were the hosts with major

contributions for *T. ni* occurrence. Rainfall was the primary environmental predictor and affected negatively *T. ni*. Therefore, the winning ANN may be used to forecast *T. ni* egg densities, 15 days in advance, allowing for timely management of this pest.

Keywords: Cabbage looper; integrated pest management; sensitivity analysis; supervised learning

Introduction

The cabbage looper, *Trichoplusia ni* Hübner (Lep.: Noctuidae), is a generalist cosmopolitan pest of field and greenhouse crops (Martin et al., 1976). *Trichoplusia ni* larvae feed on 150 cultivated and weed species from 36 families, including cotton, tomato and *Brassica* species, their primary hosts (Coapio et al., 2018; Martin et al., 1976). Eggs are found singly in both leaf surfaces, and they hatch about two to five days post-oviposition (Toba et al., 1973). The larval stage has five instars with 3 to 4 cm in length when mature, and feeds on leaves, causing extensive economic damages. The economic threshold for *T. ni* is estimated at 0.1 larvae per plant in cabbage (Greene, 1972).

Pest insect populations fluctuate throughout the year as do their rates of migration, reproduction, and mortality (Campos et al., 2006). Knowing the most favorable periods for pest occurrence allows one to plan ahead the sampling and control efforts (D'Auria et al., 2016; Fidelis et al., 2019). Timely application of curative measures (e.g., insecticide spraying) fosters, in many cases, the lower use of insecticides, contributing to environmental and human safety (D'Auria et al., 2016), and reduces the risk of selecting insecticide-resistant pest strains (Silva et al., 2011).

Climate variables and host plants play a significant role amid factors that affect seasonal variations of pest populations. Climate variables have primary importance in pest regulation (Peacock et al., 2006), since they act directly (by affecting survival, reproduction and behavior, e. g., Farias et al., 2020; Pereira et al., 2007) and indirectly on insects (by regulating the availability and nutritional quality of host plants and natural enemy abundance, e. g., Fidelis et al., 2019; Pereira et al., 2007). The abundance of pest insects is also dependent upon the presence of host plants in favorable nutritional conditions (Marchioro & Foerster, 2014; Teixeira et al., 2013). Thus, models determining the contribution of weather

variables and host plants to the *T. ni* dynamics are important for improved IPM programs.

Artificial neural networks (ANN) are computing systems that simulate the functioning of biological neurons (Russell & Norvig, 2010). These networks have several applications within the field of insect dynamic modelling. They can yield predictive models which account for nonlinear relationships between input and output variables, and fit complex models with multiple predictors (Watts & Worner, 2008). A typical feedforward ANN consists of an input layer (with input data), one or more hidden layers, and an output layer (Tarca et al., 2007). Each neuron in one layer has connections to the neurons in the next layer. The neurons receive a set of inputs with weights, calculate a weighted sum of these signals, and then use an activation function to generate an output (Tarca et al., 2007). During training, the weights are iteratively updated (by using algorithms such as back-propagation) to improve the ANN performance (i.e., to reduce predictive error).

Although ANN uses have increased in diverse research fields, this is not widespread in IPM to predict population density (Skawsang et al., 2019; Tonnang et al., 2017). To gain this insight, we tested whether ANN could be a reliable tool to predict the cabbage looper's seasonal dynamics based on weather data and three *Brassica* hosts (broccoli, cabbage, and cauliflower).

Material and methods

Data collection

We assessed nine plots of each host (broccoli cv. Master, cabbage cv. Astro Plus, and cauliflower cv. Verona CMS) from May 2017 to March 2019. The fields were located in Coimbra (-20.840998, -42.773570), Minas Gerais state, Brazil, and measured ca. 200 m². The region's climate is tropical and falls into the Köppen class Cwb (Peel et al., 2007), with alternating rainy summers and dry winters. Plants were spaced 0.4 (within-row) × 0.7 m (inter-row) and grown without pesticide application. Agronomical practices were performed following local recommendations (Ribeiro et al., 1999).

The plots were surveyed approximately fortnightly, providing a total of 121 data sets. At each date, twenty plants (on average) were thoroughly inspected by counting *T. ni* (eggs and larvae) on each leaf in each host. Daily weather data

(rainfall, relative humidity, air temperature, solar irradiance, dew point, and atmospheric pressure) were retrieved from the Instituto Nacional de Meteorología (INMET, 2019).

Data pre-processing

All procedures were performed in R v. 3.5.3 and RStudio v. 1.2.5001 (R Core Team, 2019). First, we performed Pearson's correlations between input features to reduce data dimensionality (i.e., the number of predictors). Dew point ($r = 0.73$) and atmospheric pressure ($r = -0.68$) were highly correlated with air temperature, and solar irradiance with relative humidity ($r = -0.77$) (supplementary material, figure S1). Thus, dew point, atmospheric pressure, and solar irradiance were removed from the predictors. As a result, the input variables included the host plant (a nominal input later converted into three dummy variables), days after transplanting (DAT), air temperature, rainfall, and relative humidity. We also examined if the different pest variables (eggs and larvae) would impact model performance. Thus, counts of eggs and larvae were tested separately as response variables.

The effect of varying time lags of weather predictors on ANN performance was also studied. Five time intervals of weather variables, spaced 15, 30, 45, 60, and 90 days apart from the field assessments, were used. Before ANN modelling, data were filtered by time lag (providing six datasets), and, for each data set, data were partitioned into training (70%) and validation (30%) (function *sample.split*, package *caTools*) (Tuszynski, 2019). Input variables were centered (by subtracting the mean) and normalized (by converting them into z-scores) (function *scale*, package *stats*). This procedure is required in ANN models to avoid overweighting wider measurements in the learning process. Also, the response variables (eggs and larvae of *T. ni*) were scaled from zero to one (function *rescale*, package *scales*) (Wickham, 2018).

Data analysis

The ANNs were developed using the function *mip* from *RSNNS* package (Bergmeir & Benitez, 2012). Four activation functions (exponential, hyperbolic tangent, identity, and logistic) and three learning algorithms (resilient backpropagation, scaled conjugate gradient, and standard backpropagation)

were tested. The maximum number of iterations was fixed at 100, and the ANN size (number of neurons in the hidden layer) ranged from 1 to 11. The initial ANN weight was randomly generated (*initFunc = "Randomize_Weights"*), and the remaining arguments were default.

In total, 1320 ANNs were built (4 activation functions \times 3 learning algorithms \times 11 ANN sizes \times 2 response variables \times 5 time lags). The models were scored by comparing the observed values of the response variables against those fitted by the ANNs. Pearson's correlation (r_v) and root mean square error ($RMSE_v$) were estimated for the validation set. Our core goal was to select a single best ANN configuration yielding the most accurate model (i.e., the one with the lowest $RMSE_v$ and highest r_v). Once selected the winning ANN, the relative importance of the predictors was estimated using the Olden's algorithm (function *olden*, package *NeuralNetTools*). Olden's method calculates the relative importance of the predictors based on the product of the input-hidden and hidden-output connection weights and the sum of these products across all hidden neurons (Costa et al., 2021; Olden et al., 2004). The winning ANN was run 250 times, each with a random set of starting weights, and Olden's estimates were recorded and combined to estimate the 95% confidence interval (CI_{95}) (Beck, 2018). A predictor was significant when the CI_{95} bounds did not include zero (Beck, 2018). Finally, sensitivity analyses were performed using partial dependence plots (function *partial*, package *pdp*). Partial dependence plots show the relationship of the response variable with a particular predictor while taking into account the average effect of other factors (Greenwell, 2017).

Results

The ANNs forecasting *T. ni* eggs had better results, based on their r_v and $RMSE_v$ values. The ANN configuration containing weather data lagged 15 days, 2 neurons in the hidden layer, hyperbolic tangent activation function, and learning algorithm resilient backpropagation had the best performance ($r_v = 0.71$ and $RMSE_v = 0.15$) (Table 1). The topology of the winning network is depicted in Figure 1.

The relative importance (calculated from the neuron connection weights) of each predictor to the response variable is displayed in Figure 2. Predictors were significant to the winning ANN's performance when the CI_{95} of their

importance did not include zero. Broccoli (H_Bro) and cauliflower (H_Cau) were the hosts most favorable for *T. ni*. Rainfall was the primary environmental predictor affecting *T. ni* dynamics and, together with DAT, was negatively related to the response variable. Temperature (C_Temp) and relative humidity (C_RH) had marginal importance on *T. ni* abundance.

Figure 3 illustrates the average behavior of *T. ni* counts in response to predictors' levels. The pest had a peak relationship with DAT (Fig. 3a) and responded negatively to rainfall (Fig. 3b).

Feedback testing was performed to assess the predictive performance of the ANN in the entire dataset. Fitted values closely matched ($r = 0.69$) the observed data (Fig. 4).

Discussion

Our study reports a neural network to be used as a predictive tool for *T. ni* management. A set of drivers, including host plant traits (*Brassica oleraceae* variety and plant age) and weather variables lagged by 15 days, were applied to predict *T. ni* densities. Recent studies indicate that such combination allows for better model predictions than those containing only weather data because it reflects the interaction among abiotic and biotic factors, including plant-related traits, on pest dynamics (Skawsang et al., 2019; Tonnang et al., 2017). The predictors were important for the ANN performance, based on Olden's analysis, with *T. ni* displaying, in general, non-linear response to them. Based on these findings, the winning ANN may be a powerful tool to predict cabbage looper seasonal dynamics, thus improving pest management programs on *Brassica* crops.

The best ANN configuration had two neurons in the hidden layers, which is following the parsimony principle. By selecting the least number of hidden neurons providing proper predictive performance, overfitting (which could potentially incur a loss of the ANN's generalization power) is minimized (Carmo et al., 2020; Dahmani et al., 2014; Ibrahim et al., 2005). Therefore, the winning ANN is expected to perform well on data they have not been trained on.

Rainfall was the primary environmental predictor affecting *T. ni* dynamics and presented a linear downward relationship with *T. ni* densities. This is likely related to the mortality and dislodging effects of rainfall on eggs and young larvae.

Trichoplusia ni eggs are laid on both leaf sides, which makes them highly prone to be displaced by rain splash. Similarly, young larvae might be washed-off from plants or drowned in rain droplets accumulated on plant leaves as noted to *Plutella xylostella* L. (Farias et al., 2020) and *Ascia monuste orseis* Godart (Santos et al., 2020), which contributes to population reduction during the wet season. Another indirect effect of rainfall is the increased infection of larvae by entomopathogens such as viruses, triggered by wet periods (Hofmaster, 1961). We found temperature and relative humidity having a lesser role in *T. ni* abundance compared to rainfall.

Among the host plants, broccoli and cauliflower were the major contributors to *T. ni* abundance. A previous study found an interaction between the host plant and plant age on *T. ni* preference (Boling & Pitre, 1971). 15-wk old broccoli plants, cabbage, and cauliflower were equally abundant on *T. ni* eggs and larvae. Conversely, older plants (21-wk) of broccoli and cauliflower were preferred over cabbage (Boling & Pitre, 1971). When performing sensitivity analyses, *T. ni* displayed a hump-shaped response to plant age, indicating that the population built following plant phenology and, at mid-cycle, decreased and plateaued until harvest. This might be related to leaf quality, which declines when the plant starts to form inflorescence. At this point, plants direct their resources to head expansion, and the leaves become poorer in nutrients, compromising phytophagous' performance (Li et al., 2011). Besides, natural enemy populations also increase, following pest buildup, and might reduce pest populations at plant mid-cycle onwards (Fidelis et al., 2019; Haddad et al., 2016).

When pooling the whole data set, our ANN predicted fairly well ($r = 0.60$) *T. ni* egg density, especially on cauliflower ($r = 0.70$). The network was designed to predict egg density, a stage representative of pest abundance which, at the same time, does not cause plant injury (Silva et al., 2019). Therefore, managers could potentially forecast egg densities, 15 days in advance, to concentrate sampling protocols and control measures (Barzman et al., 2015; Maelzer & Zalucki, 2000) in order to avoid economic losses from larvae feeding on leaves. The establishment of a decision-making system, comprised of sampling plans for scouting eggs and action thresholds (Silva et al., 2019; Zehnder et al., 1995), is required to implement this management program.

In summary, we provide an ANN to predict *T. ni* population density on *Brassica* crops to be incorporated in their IPM systems. The network presents a biological meaning as a result of the inclusion of factors that influence *T. ni* population size. Thus, we confirm the use of ANN as a powerful tool to predict pest population dynamics in tropical regions.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, and Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG. ESF gratefully acknowledges the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the scholarship grant (process number: 140983/2017-1).

References

- Barzman, M., Bàrberi, P., Birch, A. N. E., Boonekamp, P., Dachbrodt-Saaydeh, S., Graf, B., Hommel, B., Jensen, J. E., Kiss, J., Kudsk, P., Lamichhane, J. R., Messéan, A., Moonen, A.-C., Ratnadass, A., Ricci, P., Sarah, J.-L., & Sattin, M. (2015). Eight principles of integrated pest management. *Agronomy for Sustainable Development*, *35*(4), 1199–1215.
- Beck, M. W. (2018). NeuralNetTools: Visualization and analysis tools for neural networks. *Journal of Statistical Software*, *85*(11), 1–20.
- Bergmeir, C., & Benitez, J. M. (2012). Neural networks in R using the Stuttgart neural network simulator: RSNNS. *Journal of Statistical Software*, *46*(7), 1–26.
- Boling, J. C., & Pitre, H. N. (1971). Hostal plant preference for oviposition by *Trichoplusia ni*, and efficiency of *Apanteles marginiventris* as a populational regulator of *Trichoplusia ni* in field-cage tests. *Journal of Economic Entomology*, *64*(2), 411–412.
- Campos, W. G., Schoereder, J. H., & DeSouza, O. F. (2006). Seasonality in neotropical populations of *Plutella xylostella* (Lepidoptera): Resource availability and migration. *Population Ecology*, *48*(2), 151–158.
- Carmo, D. G., Farias, E. S., Costa, T. L., Queiroz, E. A., Nascimento, M., & Picanço, M. C. (2020). Instar determination of *Blaptostethus pallescens* (Hemiptera: Anthocoridae) using artificial neural networks. *Annals of the Entomological Society of America*, *113*(1), 50–54.
- Coapio, G. G., Cruz-López, L., Guerenstein, P., Malo, E. A., & Rojas, J. C. (2018). Oviposition preference and larval performance and behavior of *Trichoplusia ni* (Lepidoptera: Noctuidae) on host and nonhost plants. *Arthropod-Plant Interactions*, *12*(2), 267–276.
- Costa, N. L., Lima, M. D., & Barbosa, R. (2021). Evaluation of feature selection methods based on artificial neural network weights. *Expert Systems with Applications*, *168*, 114312.

- Dahmani, K., Dizene, R., Notton, G., Paoli, C., Voyant, C., & Nivet, M. L. (2014). Estimation of 5-min time-step data of tilted solar global irradiation using ANN (Artificial Neural Network) model. *Energy*, *70*, 374–381.
- D'Auria, E. M., Wohleb, C. H., Waters, T. D., & Crowder, D. W. (2016). Seasonal population dynamics of three potato pests in Washington state. *Environmental Entomology*, *45*(4), 781–789.
- Farias, E. S., Santos, R. C., Carmo, D. G., Soares, J. R. S., Costa, T. L., Santos, A. A., & Picanço, M. C. (2020). Life tables for the diamondback moth (*Plutella xylostella*) in southeast Brazil indicate ants and spiders as leading mortality factors. *Annals of Applied Biology*, 1–10. <https://doi.org/10.1111/aab.12656>
- Fidelis, E. G., Farias, E. S., Lopes, M. C., Sousa, F. F., Zanuncio, J. C., & Picanço, M. C. (2019). Contributions of climate, plant phenology and natural enemies to the seasonal variation of aphids on cabbage. *Journal of Applied Entomology*, *143*(4), 365–370.
- Greene, G. L. (1972). Economic damage threshold and spray interval for cabbage looper control on cabbage. *Journal of Economic Entomology*, *65*(1), 205–208.
- Greenwell, B. M. (2017). pdp: An R package for constructing partial dependence plots. *The R Journal*, *9*(1), 421–436.
- Haddad, C. R., Brabec, M., Pekár, S., & Fourie, R. (2016). Seasonal population dynamics of a specialized termite-eating spider (Araneae: Ammoxenidae) and its prey (Isoptera: Hodotermitidae). *Pedobiologia*, *59*(3), 105–110.
- Hofmaster, R. N. (1961). Seasonal abundance of the cabbage looper as related to light trap collections, precipitation, temperature and the incidence of a nuclear polyhedrosis virus. *Journal of Economic Entomology*, *54*(4), 796–798.
- Ibrahim, F., Taib, M. N., Abas, W. A. B. W., Guan, C. C., & Sulaiman, S. (2005). A novel dengue fever (DF) and dengue haemorrhagic fever (DHF) analysis using artificial neural network (ANN). *Computer Methods and Programs in Biomedicine*, *79*(3), 273–281.
- INMET. (2019). *BDMEP - Banco de Dados Meteorológicos para Ensino e Pesquisa*. <http://www.inmet.gov.br/projetos/rede/pesquisa/>
- Li, H., Jiang, H., & Li, T. (2011). Broccoli plant nitrogen, phosphorus, and water relations at field scale and in various growth media. *International Journal of Vegetable Science*, *17*(2), 190–210.
- Maelzer, D. A., & Zalucki, M. P. (2000). Long range forecasts of the numbers of *Helicoverpa punctigera* and *H. armigera* (Lepidoptera: Noctuidae) in Australia using the Southern Oscillation Index and the Sea Surface Temperature. *Bulletin of Entomological Research*, *90*(2), 133–146.
- Marchioro, M., & Foerster, L. A. (2014). Preference–performance linkage in the diamondback moth, *Plutella xylostella*, and implications for its management. *Journal of Insect Science*, *14*(85).
- Martin, P. B., Lingren, P. D., & Greene, G. L. (1976). Relative abundance and host preferences of cabbage looper, soybean looper, tobacco budworm, and corn earworm on crops grown in Northern Florida. *Environmental Entomology*, *5*(5), 878–882.

- Olden, J. D., Joy, M. K., & Death, R. G. (2004). An accurate comparison of methods for quantifying variable importance in artificial neural networks using simulated data. *Ecological Modelling*, *178*(3), 389–397.
- Peacock, L., Worner, S., & Sedcole, R. (2006). Climate variables and their role in site discrimination of invasive insect species distributions. *Environmental Entomology*, *35*(4), 958–963.
- Pereira, E. J. G., Picanço, M. C., Bacci, L., Lucia, T. M. C. D., Silva, É. M., & Fernandes, F. L. (2007). Natural mortality factors of *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) on *Coffea arabica*. *Biocontrol Science and Technology*, *17*(5), 441–455.
- R Core Team (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.
- Russell, S. J., & Norvig, P. (2010). *Artificial intelligence: A modern approach* (3rd ed.). New Jersey: Prentice Hall.
- Santos, A. A., Ribeiro, A. V., Groom, S. V. C., Farias, E. S., Carmo, D. G., Santos, R. C., & Picanço, M. C. (2020). Season and weather affect the mortality of immature stages of *Ascia monuste orseis* (Lepidoptera: Pieridae) caused by natural factors. *Austral Entomology*, *59*(4), 810–818.
- Silva, É. M., Crespo, A. L. B., Farias, E. S., Bacci, L., Queiroz, R. B., & Picanço, M. C. (2019). Conventional sampling plan for scouting *Neoleucinodes elegantalis* (Lepidoptera: Crambidae) eggs on tomato fruits. *Journal of Economic Entomology*, *112*(5), 2433–2440.
- Silva, G. A., Picanço, M. C., Bacci, L., Crespo, A. L. B., Rosado, J. F., & Guedes, R. N. C. (2011). Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, *Tuta absoluta*. *Pest Management Science*, *67*(8), 913–920.
- Skawsang, Nagai, Tripathi, & Soni. (2019). Predicting rice pest population occurrence with satellite-derived crop phenology, ground meteorological observation, and machine learning: A case study for the Central Plain of Thailand. *Applied Sciences*, *9*(22), 4846.
- Tarca, A. L., Carey, V. J., Chen, X., Romero, R., & Drăghici, S. (2007). Machine learning and its applications to biology. *PLOS Computational Biology*, *3*(6), e116.
- Teixeira, N. C., Santos, N. A., Maurício, R. M., Guedes, R. N. C., Oliveira, M. G. A., & Campos, W. G. (2013). Cabbage seasonal leaf quality mediating the diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) performance. *Neotropical Entomology*, *42*(6), 545–551.
- Toba, H. H., Kishaba, A. N., Pangaldan, R., & Vail, P. V. (1973). Temperature and the development of the cabbage looper. *Annals of the Entomological Society of America*, *66*(5), 965–974.
- Tonnang, H. E. Z., Hervé, B. D. B., Biber-Freudenberger, L., Salifu, D., Subramanian, S., Ngowi, V. B., Guimapi, R. Y. A., Anani, B., Kakmeni, F. M. M., Affognon, H., Niassy, S., Landmann, T., Ndjomatchoua, F. T., Pedro, S. A., Johansson, T., Tanga, C. M., Nana, P., Fiaboe, K. M., Mohamed, S. F., ... Borgemeister, C. (2017). Advances in crop insect modelling methods—Towards a whole system approach. *Ecological Modelling*, *354*, 88–103.

Tuszynski, J. (2019). *CaTools: Tools: Moving window statistics, GIF, Base64, ROC AUC, etc.* <https://cran.r-project.org/package=caTools>

Watts, M. J., & Worner, S. P. (2008). Using artificial neural networks to determine the relative contribution of abiotic factors influencing the establishment of insect pest species. *Ecological Informatics*, 3(1), 64–74.

Wickham, H. (2018). *scales: Scale Functions for Visualization.* <https://cran.r-project.org/package=scales>

Zehnder, G. W., Sikora, E. J., & Goodman, W. R. (1995). Treatment decisions based on egg scouting for tomato fruitworm, *Helicoverpa zea* (Boddie), reduce insecticide use in tomato. *Crop Protection*, 14(8), 683–687.

Table 1. Topology (number of neurons, activation function, and learning algorithm) and performance (r_v = Pearson's correlation of fitted and observed data, and $RMSE_v$ = root mean square error in the validation set) of artificial neural networks designed to predict *Trichoplusia ni* counts (eggs and larvae) in *Brassica* crops with weather variables lagged at different intervals from the day of pest scouting (15 to 90 days). The winning ANN is highlighted in bold.

| <i>y</i> | Lag | Neurons | Activation function | Learning function | r_v | $RMSE_v$ |
|-------------|-----------|----------|---------------------------|---------------------|--------------|--------------|
| Eggs | 15 | 2 | Hyperbolic tangent | Rprop | 0.706 | 0.151 |
| Eggs | 30 | 10 | Exponential | Std_Backpropagation | 0.516 | 0.228 |
| Eggs | 45 | 8 | Hyperbolic tangent | Rprop | 0.610 | 0.168 |
| Eggs | 60 | 2 | Logistic | Rprop | 0.546 | 0.180 |
| Eggs | 90 | 10 | Exponential | Rprop | 0.566 | 0.190 |
| Larvae | 15 | 2 | Logistic | SCG | 0.417 | 0.236 |
| Larvae | 30 | 4 | Logistic | SCG | 0.504 | 0.223 |
| Larvae | 45 | 10 | Hyperbolic tangent | Std_Backpropagation | 0.387 | 0.243 |
| Larvae | 60 | 9 | Hyperbolic tangent | Std_Backpropagation | 0.501 | 0.225 |
| Larvae | 90 | 2 | Hyperbolic tangent | Rprop | 0.370 | 0.270 |

Rprop = resilient backpropagation; SCG = scaled conjugate gradient; Std_Backpropagation = standard backpropagation.

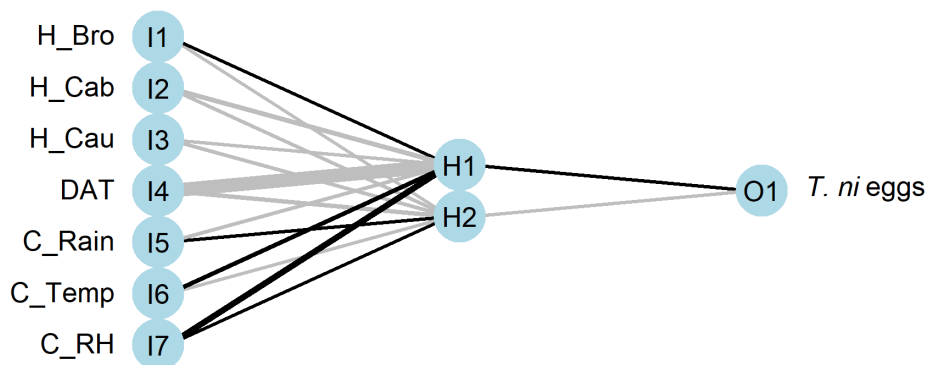


Figure 1. Schematic diagram of the winning artificial neural network (ANN). The ANN consists of an input layer with seven neurons, one hidden layer with two neurons, and a single-neuron output layer. H_Bro = Broccoli, H_Cab = Cabbage, H_Cau = Cauliflower, DAT = days after transplant, C_Rain = rainfall (mm/day), C_Temp = Mean daily temperature ($^{\circ}\text{C}$), C_RH = Mean daily relative humidity (%). Black lines represent positive weights between layers (grey lines otherwise), and line thickness corresponds to each weight's relative magnitude.

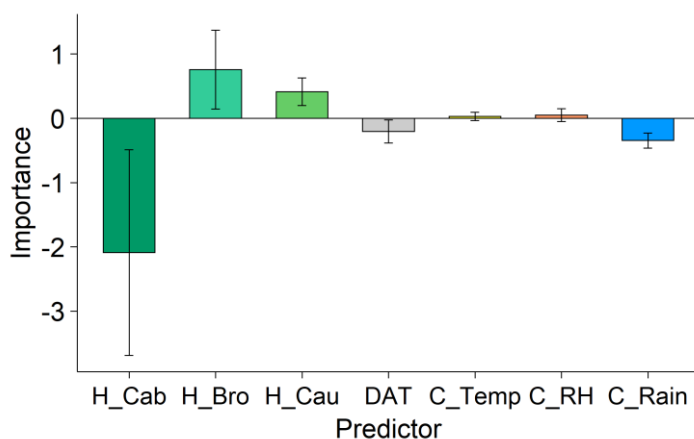


Figure 2. Relative importance (mean and 95% confidence interval) of predictors to the response variable as estimated from the winning artificial neural network (ANN) weights through the Olden method. 250 ANNs with different starting weights were created and the predictors' importance was calculated and stored to allow mean and confidence intervals' calculation.

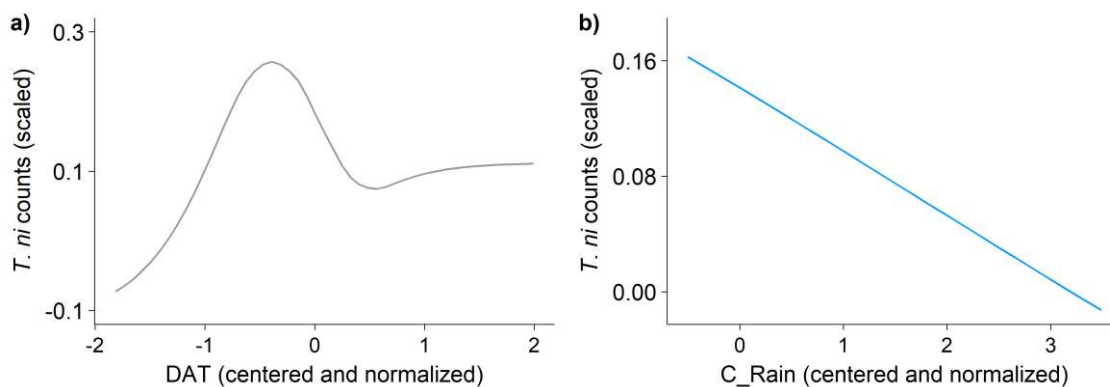


Figure 3. Partial dependence plots of the winning network showing the mean variation of the output (*Trichoplusia ni* egg counts) against levels of input variables (DAT = days after transplant, C_Rain = rainfall), while the other input variables are fixed at their mean values.

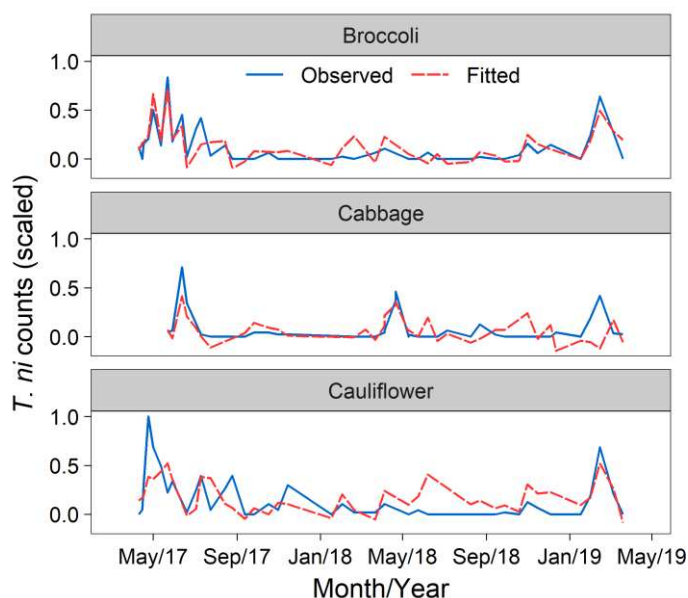


Figure 4. Predicted and observed counts of *Trichoplusia ni* eggs in three *Brassica* hosts (broccoli, cabbage, and cauliflower) in Viçosa, MG, Brazil.

Supporting information

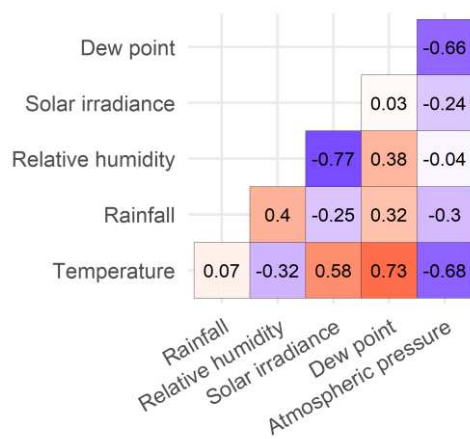


Figure S1. Pearson's correlation coefficients of weather variables among each other.

CAPÍTULO 4

Lethal and antifeedant effects of Bordeaux mixture on the marsh slug (*Deroceras laeve*)

Journal of Environmental Science and Health - Part B,
2021, 56(2), pp. 117–121

<https://doi.org/10.1080/03601234.2020.1852851>

Lethal and antifeedant effects of Bordeaux mixture on the marsh slug (*Deroceras laeve*)

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Abstract

The marsh slug, *Deroceras laeve* (Müller), is a generalist pest of agricultural crops. Slugs are mainly managed with synthetic pesticides that can also have high toxicity towards vertebrates and non-target arthropods. Besides, they are not labeled for use in organic crop systems. Bordeaux mixture (BM) is an alternative product often used in organic crops for controlling plant diseases. The molluscicidal activity of BM has been reported; however, to our knowledge, no study has determined its efficacy to control *D. laeve*. This study aimed to determine the lethal (concentration- and time-mortality curves, and foliar persistence) and antifeedant (reduction in leaf consumption) effects of BM-treated cabbage leaves on *D. laeve*. The LC₅₀ of BM was 28.15%, and the LT₅₀ was 8.83 hours. The BM LC₂₅ reduced *D. laeve* leaf consumption by 3.31-fold. Furthermore, high control effectiveness (mortality > 90%) was attained until the 7th day after spraying. These findings reveal that BM reduces damage by killing *D. laeve* in a fast faction and by reducing foliar consumption. Therefore, BM can be an alternative to *D. laeve* management in both conventional and organic systems.

Keywords: copper-based fungicides, Gastropoda, organic production, probit

Introduction

Herbivorous slugs (Gastropoda: Pulmonata) are destructive pests of cultivated crops in temperate and tropical regions.^[1,2] The marsh slug, *Deroceras laeve* (Müller) (Stylommatophora: Agriolimacidae) is native from North America and it is currently found worldwide, occurring predominantly in wet habitats.^[3] *Deroceras laeve* feeds on seeds and seedlings of crops such as soybean, corn, and vegetables. When in high population densities, feeding injuries may lead to severe plant defoliation and reduction in plant density.^[4] In addition, plant contamination by slug mucus and feces can result in market rejection. Therefore, control methods for *D. laeve* are required to reduce crop losses.

Slug control relies on the use of bait pellets or liquid formulations containing iron phosphate, metaldehyde, or methiocarb that act as stomach or contact toxicants.^[5-7] These active ingredients have a residual effect for controlling slugs under field conditions.^[1,2,8] However, they may be toxic to vertebrates and other non-target organisms, such as slug predators (e.g., carabids and staphylinids) and detritivorous arthropods.^[8-10] Besides, synthetic pesticides are not labeled for use in organic crop fields.

Bordeaux mixture (BM) is an alternative product widely used to control plant diseases.^[11] A recent study has found that, besides providing control of black rot (*Xanthomonas campestris* pv. *Campestris*), BM improves the nutritional value (i.e., foliar contents of phosphorus and antioxidant proteins) of brassica crops.^[12] The molluscicidal activity of BM dates several decades back.^[5] The effect of other copper-based fungicides (e.g., copper hydroxide) has been well reported,^[13-15] but few studies have addressed the potential of BM to manage gastropod pests. Recent reports indicate that BM enhances the efficacy of commercial products against *Deroceras reticulatum* (Müller).^[16] However, no information is available to guide farmers to implement BM on *D. laeve* control.

Therefore, this study aimed to determine the lethal (concentration- and time-mortality curves, and foliar persistence) and antifeedant effects of BM on *D. laeve*.

Material and methods

Slugs

Individuals of *D. laeve* were collected from November to December 2019 in organic broccoli fields located in Coimbra (Minas Gerais state, Brazil). The species identification was confirmed by Professor Sthefane D'ávila, and exemplars were deposited (accession number CMMPO10857) in the Museum of Malacology "Prof. Maury Pinto de Oliveira" at the Universidade Federal de Juiz de Fora, Brazil. After being collected, the slugs were brought to the laboratory and fed fresh broccoli leaves. On the same day of collections, uniform-sized individuals (ca. 1.7-cm long) were selected for the experiments. 125, 250, 90, and 250 slugs were used in the concentration-mortality, time-mortality, leaf consumption, and residual effect bioassays, respectively, totaling 715 individuals.

Bordeaux mixture

The field rate of BM was prepared by mixing 5 g of hydrated lime in 250 mL of water (2% m/v) and slowly adding 250 mL of a copper sulfate solution (2% m/v in water) with agitation.^[12,17] In order to enhance leaf wettability, 2.5 mL (0.5% v/v) of a surfactant (polyoxyethylene alkyl phenol ether 20% m/v) was added to the mixture.

Bioassays

All bioassays were performed immediately after BM preparation. For the control group, leaves were treated with water plus surfactant. In all experiments, mortality in control was below 4%. The research design is shown in Fig. 1S (supporting material).

Concentration- and time-mortality curves

The experimental unit comprised a treated leaf disk of cabbage placed in a Petri dish (9 cm diameter) to which five slugs were transferred. A plastic film and plate lid were used to cover the dishes. Half of a wet filter paper disk was placed at the bottom of the Petri dish to maintain humidity. Cabbage leaves were treated by the leaf-dipping method and air-dried. Slugs were exposed to four BM

concentrations (10, 25, 50, and 100% of the field rate), and their mortality was assessed after 72 hours. Five replications were used for each concentration.

For the time-mortality bioassay, individuals of *D. leave* were exposed to the field rate of BM and their mortality was assessed at five exposure intervals (2, 6, 24, 48, and 72 h). Five independent replications were used for each length of exposure.

Leaf consumption

A bioassay was conducted by exposing slugs to treated (LC₂₅ of BM) and untreated (control) cabbage leaves, then recording the leaf consumption (cm²). After 72 h, the leaf disks were photographed using an EOS Digital Rebel XS camera (Canon Inc., Taiwan, China). Leaf area consumed was calculated using the software ImageJ (version 1.50i;

National Institutes of Health, Bethesda, MD, USA). Fifteen replications were performed for each treatment using the procedure described in the first bioassay with a slight modification (three slugs were used per experimental unit).

Residual effect

The experiment was carried out in a greenhouse located at UFV (-20.757861, -42.869472). Temperature and relative humidity in the greenhouse ranged from 17.4 to 48.0 °C and 32 to 90%, respectively. Cabbage seedlings (cv. Astro Plus) were transplanted into 5-liter plastic pots, spaced 0.5 × 0.8 m, and watered twice a day. Fertilization with N, P₂O₅, and K₂O followed standard recommendations.^[18] Plants destined for spraying with BM (field rate) and control were separated (three meters apart) to avoid spraying drift. Five plants (20 days-old) were sprayed per treatment until run-off using a backpack sprayer. The treated leaves were tagged using white strings to avoid the collection of unsprayed (newly emerged) leaves. On the spraying day (i.e., day 0) and at the 3rd, 7th, 14th, and 21st days after, five leaves (one per plant) for each treatment were collected. The leaves were cut up into disks and placed in Petri dishes (9 cm diameter), to which five *D. leave* were transferred following the procedure described in the first bioassay. Throughout the experiment, BM-sprayed plants did not exhibit any symptoms of phytotoxicity.

Statistical analyses

Lethal concentrations (LC₂₅ and LC₅₀) and time (LT₅₀) for *D. laeve* exposed to BM were estimated by regression analysis of probit mortality versus log₁₀ of concentration (or length of exposure) (PROC PROBIT, SAS 9.2, SAS Institute Inc, Cary, NC, USA). Because data were not normally distributed, leaf consumption in BM-treated and untreated leaves were compared using Mann-Whitney *U*-test (*wilcox.test* function, *stats* package from R). The non-linear curve of BM efficacy over time and its confidence intervals (CI₉₅) was obtained using *nls* (*stats* package) and *predictNLS* functions (*propagate* package from R).^[19]

Results

Lethal effects

Pearson's Chi-square test statistic ($P > 0.05$) indicated that the data fitted the regression models for concentration-mortality and lethal-time curves. The LC₅₀ of BM was 28.15% (CI₉₅ = 21.97 – 35.55, Fig. 1a), and the LT₅₀ was 8.83 hours (CI₉₅ = 4.24 – 15.75, Fig. 1b).

Leaf consumption

Individuals exposed to the LC₂₅ (17.15% of the field rate) of BM presented a 3.31-fold reduction in leaf consumption compared to control (Mann-Whitney *U*-test, $U = 192.5$, $P < 0.001$, effect size $r = 0.61$, CI₉₅ of the difference: 1.50 – 4.99, Fig. 2).

Residual effect

Bordeaux mixture showed high control effectiveness of *D. laeve* (mortality > 90%) until the 7th day. After this period, the curve presented a slight decrease, with 50% efficacy on the 14th day, until reaching 20% efficacy at 20 days post-spraying (Fig. 3).

Discussion

The molluscicidal activity of BM has been reported in the past.^[5,16] However, no study has determined the effects of BM on *D. laeve*. Here, we estimated acute lethal effects (LC₅₀ and LT₅₀) of BM for *D. laeve*. We found that

BM has a satisfactory residual effect (90% of mortality) up to 7 days after spraying. Also, we showed that a sublethal concentration of BM reduces *D. laeve* foliar consumption.

The LC₅₀ for *D. laeve* was found to be 28.15% of the field rate of BM. Based on the concentration-mortality curve, the 75% concentration of BM causes 91% mortality. Brazilian legislation recommends the baseline of $90 \pm 10\%$ mortality to select effective concentrations of a candidate pesticide.^[20] Thus, the 75% BM concentration can be applied in cabbage fields, aiming to reduce spraying costs.

The LT₅₀ was 8.83 hours. Mortality caused by copper-based products is higher by the dermal pathway than by ingestion.^[21] Studies with other gastropods indicate that copper sulfate (a component of BM) penetrates through the foot and causes a disruption in epithelia, leading to internal damage and irritant effects.^[15,22] The time-mortality curve estimated in our study indicates a rapid effect of BM on *D. laeve*. They did not consume much of the leaves before dying because, sooner after contact with BM, the slugs exhibited an avoidance response toward the treated leaves and produced an excess of mucus. These findings reveal that BM reduces damage both by quickly killing the slugs and by reducing their consumption.

Other studies have reported the deterrent and repellent effects of copper-based fungicides.^[7,23] A previous study has reported that copper hydroxide formulations reduce *D. laeve* leaf consumption by up to 58% on lettuce.^[14] Here, we exposed the slugs to a sublethal concentration (LC₂₅ or 17.15% of the field rate) to confirm the potential of BM on reducing leaf consumption by *D. laeve*. We found that BM reduced consumption by 66% compared to control. These findings indicate that even when exposed to a sublethal concentration, such a few days after pesticide spraying,^[24] the residual BM will benefit crops by substantially reducing *D. laeve* injuries.

Bordeaux mixture showed a high control efficacy (> 90% of mortality) up to 7 days post-spraying. In another study, a copper hydroxide-based fungicide presented a persistence of 6 to 10 days as a feeding deterrent against the slug *Leidyula floridana* (Leid) and snail *Lissachatina fulica* (Ferussac).^[23] After one week, the reduced BM effectiveness results from its degradation by environmental factors and copper absorption by plants.^[10,25] Wash-off by rainfall

and degradation by sunlight and high temperatures are the major factors reducing the foliar persistence of pesticides in the field.^[26,27] Our experiment was conducted in a greenhouse where high relative humidity and temperature conditions occurred. These factors probably reduced the efficiency of BM on *D. laeve* over time. Our results indicate that, when necessary, BM spraying should be performed weekly to promote satisfactory control. However, BM application should be judicious and need-based to avoid BM accumulation on the soil. When the sprayed BM reaches the soil (either by spray droplets or rain removal), Cupper can be adsorbed by clay minerals and organic matter,^[28] and in high levels may exert toxic effects on plants and non-target organisms.^[29,30] Therefore, BM should not be used as a stand-alone measure against *D. laeve* to avoid soil accumulation and related adverse effects.

Conclusion

Altogether, our study shows the lethal and sublethal effects of BM on *D. laeve*. Our findings indicate that this product presents fast action, reduces leaf consumption, and persists (at a satisfactory level) up to 7 days in plants. These results are promising for BM use in both conventional and organic crop production systems.

Acknowledgments

The authors thank Prof. Sthefane D'ávila for species identification and the colleagues from the MIP-UFV lab for their assistance in collecting slugs. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

References

- [1] Nash, M.A.; Thomson, L.J.; Hoffmann, A.A. Slug control in Australian canola: Monitoring, molluscicidal baits and economic thresholds. *Pest Manag. Sci.* **2007**, *63*(9), 851–859.
- [2] South, A. Control of slug pests. In *Terrestrial Slugs*; South, A., Ed. Springer: Dordrecht, Netherlands, 1992; 322–347.
- [3] Douglas, M.R.; Tooker, J.F. Slug (Mollusca: Agriolimacidae, Arionidae)

ecology and management in no-till field crops, with an emphasis on the mid-Atlantic region. *J. Integr. Pest Manag.* **2012**, *3*(1), C1–C9.

[4] Faberi, A.J.; López, A.N.; Manetti, P.L.; Clemente, N.L.; Castillo, H.Á. Growth and reproduction of the slug *Deroceras laeve* (Müller) (Pulmonata: Stylommatophora) under controlled conditions. *Spanish J. Agric. Res.* **2006**, *4*, 345–350.

[5] Henderson, I.; Triebkorn, R. Chemical control of terrestrial gastropods. In *Molluscs as Crop Pests*; Barke, G.M., Ed. CAB International: Wallingford, UK, 2002; 1–31.

[6] Iglesias-Piñeiro, J.; Castillejo, J.; Ester, A. Laboratory evaluation of potential molluscicides for the control of eggs of the pest slug *Deroceras reticulatum* (Müller) (Pulmonata: Limacidae). *Int. J. Pest Manag.* **2002**, *48*(1), 19–23.

[7] Hollingsworth, R.G.; Armstrong, J.W. Effectiveness of products containing metaldehyde, copper or extracts of yucca or neem for control of *Zonitoides arboreus* (Say), a snail pest of orchid roots in Hawaii. *Int. J. Pest Manag.* **2003**, *49*(2), 115–122.

[8] Gavin, W.E.; Mueller-Warrant, G.W.; Griffith, S.M.; Banowetz, G.M. Removal of molluscicidal bait pellets by earthworms and its impact on control of the gray field slug (*Derocerus reticulatum* Mueller) in western Oregon grass seed fields. *Crop Prot.* **2012**, *42*, 94–101.

[9] Gall, M. Le; Tooker, J.F. Developing ecologically based pest management programs for terrestrial molluscs in field and forage crops. *J. Pest Sci.* (2004). **2017**, *90*(3), 825–838.

[10] Rae, R.G.; Robertson, J.F.; Wilson, M.J. Optimization of biological (*Phasmarhabditis hermaphrodita*) and chemical (iron phosphate and metaldehyde) slug control. *Crop Prot.* **2009**, *28*(9), 765–773.

[11] Pérez-Rodríguez, P.; Paradelo, M.; Rodríguez-Salgado, I.; Fernández-Calviño, D.; López-Periago, J.E. Modeling the influence of raindrop size on the wash-off losses of copper-based fungicides sprayed on potato (*Solanum tuberosum* L.) leaves. *J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes* **2013**, *48*(9), 737–746.

[12] Nuñez, A.M.P.; Rodríguez, G.A.A.; Monteiro, F.P.; Faria, A.F.; Silva, J.C.P.; Monteiro, A.C.A.; Carvalho, C. V.; Gomes, L.A.A.; Souza, R.M.; Souza, J.T.; Medeiros, F.H.V. Bio-based products control black rot (*Xanthomonas campestris* pv. *campestris*) and increase the nutraceutical and antioxidant components in kale. *Sci. Rep.* **2018**, *8*(1), 1–11.

[13] Moran, S.; Gotlib, Y.; Yaakov, B. Management of land snails in cut green ornamentals by copper hydroxide formulations. *Crop Prot.* **2004**, *23*(7), 647–650.

[14] Thompson, J.M.; Sibley, J.L.; Foshee, W.G.; Keever, G.J.; Appel, A.G. Effect of copper hydroxide on slug feeding. *J. Environ. Hortic.* **2005**, *23*(4), 167–170.

[15] Capinera, J.L. Evaluation of copper hydroxide as a repellent and feeding deterrent for cuban brown snail (Mollusca: Gastropoda: Pleurodontidae). *Florida Entomol.* **2018**, *101*(3), 369–372.

[16] Gavin, W.E.; Banowetz, G.M.; Griffith, S.M.; Mueller-Warrant, G.W.; Whittaker, G.W. Effects of baits and bait alternatives on slug mortality, egg production, and seedling survival. *Seed Production Research at Oregon State*

University, Ext/CrS 12, 12–17. 2007.

[17] Bettiol, W.; Ghini, R.; Galvão, J.A.H.; Siloto, R.C. Organic and conventional tomato cropping systems. *Sci. Agric.* **2004**, *61*(3), 253–259.

[18] Ribeiro, A.C.; Guimarães, P.T.G.; Alvarez, V.H. *Recomendação para o uso de corretivos e fertilizantes em Minas Gerais: 5a. aproximação*. Comissão de fertilidade do solo do estado de Minas Gerais: Viçosa, Brazil, 1999.

[19] Spiess, A.-N. propagate: Propagation of uncertainty. <https://cran.r-project.org/package=propagate>.

[20] MAPA. Manual de procedimentos para registro de agrotóxicos. <http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/insumos-agricolas/agrotoxicos/arquivos/manual-de-procedimentos-para-registro-de-agrotoxicos.pdf> (accessed Aug. 2019).

[21] Sullivan, J.T.; Cheng, T.C. Comparative mortality studies on *Biomphalaria glabrata* (Mollusca: Pulmonata) exposed to copper internally and externally. *J. Invertebr. Pathol.* **1976**, *28*(2), 255–257.

[22] Ryder, T.A.; Bowen, I.D. The slug foot as a site of uptake of copper molluscicide. *J. Invertebr. Pathol.* **1977**, *30*(3), 381–386.

[23] Capinera, J.L.; Dickens, K. Some effects of copper-based fungicides on plant-feeding terrestrial molluscs: A role for repellents in mollusc management. *Crop Prot.* **2016**, *83*, 76–82.

[24] Morales, S.I.; Martínez, A.M.; Figueroa, J.I.; Campos-García, J.; Gómez-Tagle, A.; Lobit, P.; Smagghe, G.; Pineda, S. Foliar persistence and residual activity of four insecticides of different mode of action on the predator *Engytatus varians* (Hemiptera: Miridae). *Chemosphere* **2019**, *235*, 76–83.

[25] Martins, V.; Teixeira, A.; Bassil, E.; Blumwald, E.; Gerós, H. Metabolic changes of *Vitis vinifera* berries and leaves exposed to Bordeaux mixture. *Plant Physiol. Biochem.* **2014**, *82*, 270–278.

[26] Brévault, T.; Oumarou, Y.; Achaleke, J.; Vaissayre, M.; Nibouche, S. Initial activity and persistence of insecticides for the control of bollworms (Lepidoptera: Noctuidae) in cotton crops. *Crop Prot.* **2009**, *28*(5), 401–406.

[27] Gautam, B.K.; Little, B.A.; Taylor, M.D.; Jacobs, J.L.; Lovett, W.E.; Holland, R.M.; Sial, A.A. Effect of simulated rainfall on the effectiveness of insecticides against spotted wing drosophila in blueberries. *Crop Prot.* **2016**, *81*, 122–128.

[28] Pérez-Rodríguez, P.; Soto-Gómez, D.; La Calle, I. De; López-Periago, J.E.; Paradelo, M. Rainfall-induced removal of copper-based spray residues from vines. *Ecotoxicol. Environ. Saf.* **2016**, *132*, 304–310.

[29] Rosa, V.M.D.; Scoriza, R.N.; Correia, M.E.F.; Baretta, D. Ecotoxicological potential of bordeaux mixture and Neem oil on non-target soil organisms. *Rev. Bras. Ciências Agrárias* **2017**, *12*(4), 470–474.

[30] Komárek, M.; Čadková, E.; Chrástný, V.; Bordas, F.; Bollinger, J.C. Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. *Environ. Int.* **2010**, *36*(1), 138–151.

[31] Farias, E.S.; Santos, A.A.; Carmo, D.G.; Melo, J.B.; Picanço, M.C. Lethal and antifeedant effects of Bordeaux mixture on the marsh slug (*Deroceras laeve*). *J. Environ. Sci. Heal. B.* **2021**, *56*(2), 117–121.

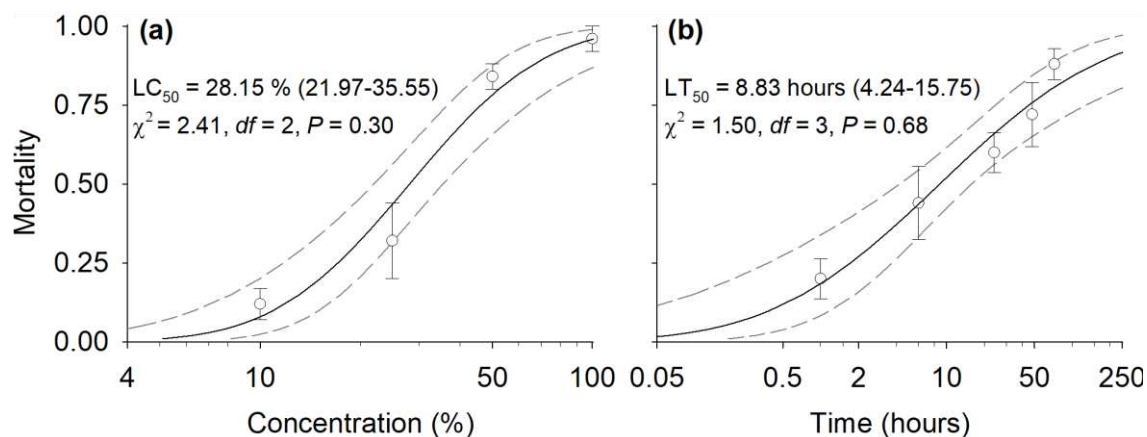


Figure 1. (a) Concentration-mortality curve of Bordeaux mixture (BM) to *Deroceras laeve* after 72 hours' exposure and (b) time-mortality curve of BM to *D. laeve*. Dashed lines represent the lower and upper confidence intervals (CI₉₅).

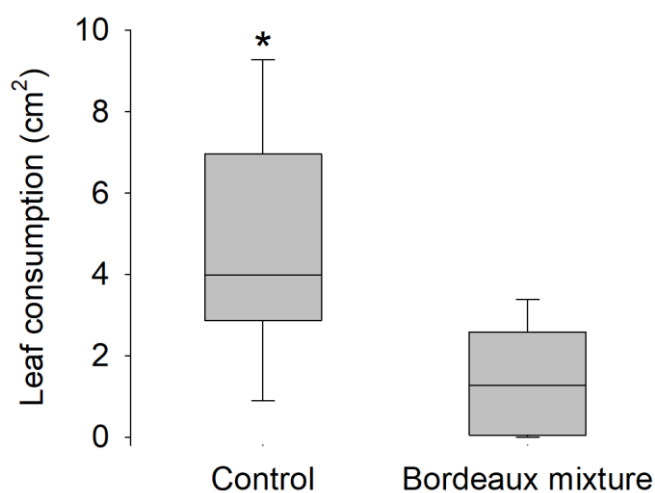


Figure 2. Leaf consumption by *Deroceras laeve* exposed to the LC₂₅ of Bordeaux mixture. Box plots show median values (horizontal line), 25–75% interquartile range (box bounds), and 10th and 90th percentiles (whiskers). The asterisk indicates a significant statistical difference to the control group by the Mann-Whitney *U*-test at the 5% level.

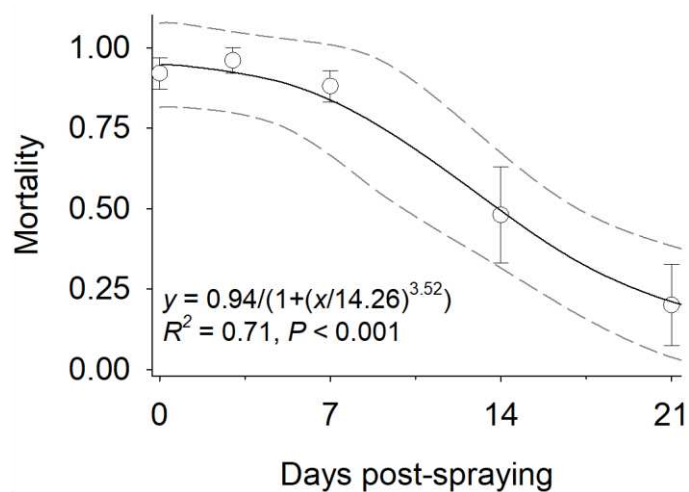


Figure 3. Mortality of *Deroceras laeve* as a function of time (in days) post-spraying of Bordeaux mixture. Dashed lines represent the lower and upper confidence intervals (CI₉₅).

Supporting material

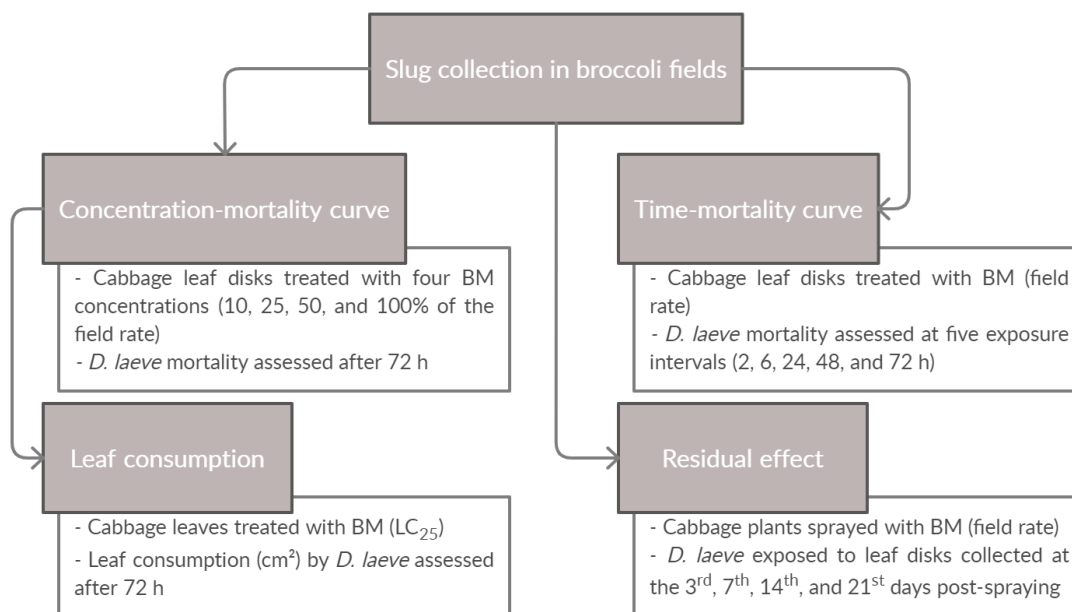


Figure 1S. Schematic illustration of the research design.

CONCLUSÕES GERAIS

Plutella xylostella apresenta elevadas taxas de mortalidade natural ($\geq 94\%$) no campo, sobretudo no inverno. A predação é o fator primário de mortalidade de *P. xylostella*, independente do estágio da praga e da estação. Formigas (*Brachymyrmex bruchi*, *Camponotus* spp., *Solenopsis saevissima* e *Tetramorium simillimum*) e aranhas são os principais predadores. As maiores taxas de mortalidade por parasitismo ocorrem na primavera e *Oomyzus sokolowskii* prevalece em importância entre os parasitoides.

A produtividade de brócolis é linear e negativamente relacionada com níveis de *P. xylostella*. O estágio pré-formação de cabeça é determinante para a produtividade de plantas de brócolis sob ataque dessa praga.

Redes neurais artificiais (RNAs) foram desenvolvidas para a predição, com 15 dias de antecedência, de populações de *T. ni* em cultivos de brássicas. A predição de densidades de ovos provê modelos com maior habilidade preditiva. Brócolis e couve-flor são os hospedeiros que mais contribuem para a ocorrência de *T. ni*. A ocorrência de chuvas é o principal preditor ambiental e afeta *T. ni* negativamente.

A calda bordalesa (CB) apresenta alta atividade moluscicida sobre *D. laeve*. A CB causa rápida mortalidade de *D. laeve* e reduz o consumo foliar desse gastrópode. Além disso, alta eficácia de controle (mortalidade $> 90\%$) é alcançada até o 7º dia após a pulverização das plantas.