Autochthonous biological resources: the potential of the mirid *Dicyphus cerastii* for biological control in protected crops

Gonçalo Nuno Abraços Henriques Gomes Duarte

SCIENTIFIC ADVISORS:
PhD Elisabete Tavares Lacerda de Figueiredo
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Abstract

Dicyphine mirids (Hemiptera: Miridae: Bryocorinae: Dicyphini) are important biological control agents (BCAs) in protected crops. Despite this, only two species are commercially available in Europe, *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. The former establishes slowly, and the latter can cause damage to tomato crops. *Dicyphus cerastii* occurs along the Mediterranean basin and spontaneously colonizes protected tomato crops in Portugal, where it often co-occurs with *N. tenuis*. *Dicyphus cerastii* has been previously observed feeding on several tomato pests. However, many aspects of its biology and ecology remain poorly studied. This thesis intended to better understand the role of *D. cerastii* as BCA, and whether it can be an alternative or a complement to *N. tenuis*, by gathering information on its biological traits, predatory interactions, and phytophagous behaviour.

Our results indicate that *D. cerastii* may be a valuable predator in tomato crops since it showed high voracity on important pests even when compared to other dicyphines. Moreover, we observed that, despite being able to damage fruit and cause flower abortion, the impact of *D. cerastii* on the tomato plant is less severe than *N. tenuis*. Though mirids only engaged in intraguild predation in absence of extraguild prey, *D. cerastii* preyed more often on *N. tenuis* than otherwise. We also found that *D. cerastii* had a longer developmental period, higher mortality, and lower reproductive rate than *N. tenuis*, particularly above 20 °C, which could be the main reason behind the apparent seasonal competitive displacement by *N. tenuis*.

Overall, this thesis contributed to increase the knowledge on the dicyphine complex of protected crops by understanding the services and disservices provided by *D. cerastii*. Given its better performance at lower temperatures and high voracity, *D. cerastii* may complement *N. tenuis* in spring conditions in Portugal, which further evidences the importance of conservation of autochthonous biological resources.

Keywords: conservation, Dicyphini, predator, tomato, zoophytophagy

Resumo

Os mirídeos da tribo Dicyphini (Hemiptera: Miridae: Bryocorinae: Dicyphini) são importantes agentes de proteção biológica (APB) em culturas protegidas. Contudo, apenas duas espécies são comercializadas na Europa, *Macrolophus pygmaeus* e *Nesidiocoris tenuis*. A primeira espécie tende a demorar a estabelecer-se, e a segunda pode originar estragos na cultura do tomateiro.

Dicyphus cerastii ocorre na bacia mediterrânica e coloniza espontaneamente culturas protegidas em Portugal, coocorrendo frequentemente com *N. tenuis*. *Dicyphus cerastii* foi previamente observado a alimentar-se de várias pragas de tomateiro. Todavia, diversos aspetos da sua biologia e ecologia encontram-se pouco estudados. Esta tese procurou compreender melhor o papel de *D. cerastii* como APB, e se poderá ser uma alternativa/complemento a *N. tenuis*, através da recolha de informação sobre biologia, interações de predação e comportamento fitófago.

Os resultados deste trabalho indicam que *D. cerastii* poderá ser um predador importante em tomateiro, dado que exibiu voracidade elevada sobre pragas importantes desta cultura, mesmo comparando com outros Dicyphini. Apesar de poder originar estragos em frutos e aborto floral, o impacto de *D. cerastii* sobre a planta de tomateiro foi menos severo que o de *N. tenuis*. Apesar de os mirídeos estudados apenas terem exibido predação intraguilda na ausência de presa extraguilda, *D. cerastii* predou sobre *N. tenuis* mais vezes do que o contrário. Também se observou que, comparando com *N. tenuis*, *D. cerastii* apresentou período de desenvolvimento mais longo, maior mortalidade e menor taxa de reprodução, particularmente acima de 20 °C. Esta poderá ser a principal razão para a aparente substituição sazonal por *N. tenuis*.

Esta tese contribuiu para aumentar o conhecimento do complexo dos Dicyphini de culturas protegidas através da compreensão dos serviços e desserviços prestados por *D. cerastii*. Dado o seu melhor desempenho a temperaturas mais baixas, *D. cerastii* poderá complementar *N. tenuis* em condições de primavera em Portugal, o que evidencia a importância da conservação de recursos biológicos autóctones.

Palavras-chave: conservação, Dicyphini, predador, tomateiro, zoofitofagia

Resumo alargado

Os mirídeos, em particular os pertencentes à tribo Dicyphini, são importantes agentes de proteção biológica em diversas culturas agrícolas. A sua relevância é particularmente notória no caso do tomateiro, cujos tricomas glandulares reduzem a eficácia ou impedem o estabelecimento de muitos outros inimigos naturais.

Os Dicyphini possuem adaptações comportamentais e morfológicas que lhes permitem explorar os recursos alimentares associados a plantas com tricomas glandulares. Por esta razão, são o grupo de predadores mais frequentemente associado à cultura do tomateiro. Apesar desta capacidade, na Europa, apenas existem duas espécies produzidas em massa e comercialmente disponíveis; *Macrolophus pygmaeus* (Rambur) e *Nesidiocoris tenuis* (Reuter). As duas espécies proporcionam importantes serviços de regulação de populações de pragas, mas também têm desvantagens associadas e específicas. No caso de *M. pygmaeus*, bastante utilizado em culturas de tomateiro protegido no centro/norte da Europa, tende a demorar demasiado tempo a formar populações suficientemente elevadas em culturas mediterrânicas. Já *N. tenuis*, por vezes, exibe um comportamento fitófago pronunciado que origina perdas económicas na cultura do tomateiro, justificando intervenções com inseticidas para regulação das suas populações.

Além das espécies já mencionadas, em Portugal, no complexo de Dicyphini da cultura de tomateiro protegido ocorre frequentemente *Dicyphus cerastii* Wagner. Esta espécie está presente na região mediterrânica e, em Portugal, coloniza frequentemente estufas de tomateiro em que se alimenta de várias pragas desta cultura. Apesar disto, os estudos incidentes sobre *D. cerastii*, em particular sobre a sua biologia e ecologia, têm sido insuficientes. Esta informação é ainda mais relevante uma vez que a abundância relativa das espécies no complexo de Dicyphini em tomateiro protegido em Portugal parece estar a sofrer alterações beneficiando *N. tenuis* em detrimento de *D. cerastii*. Torna-se assim necessário compreender o contributo de *D. cerastii* para a proteção biológica do tomateiro bem como os factores que poderão explicar a alteração da sua abundância.

Esta tese tem como objetivo compreender melhor o papel de *D. cerastii* como agente de proteção biológica e se este mirídeo poderá constituir uma alternativa ou um complemento a *N. tenuis* na cultura do tomateiro. Para tal, estudaram-se diferentes aspetos importantes na seleção de agentes de proteção biológica, em particular parâmetros biológicos, interações de predação e o comportamento fitófago de *D. cerastii*.

Este trabalho está estruturado em cinco seções. A primeira seção, a introdução, descreve o estado da arte da proteção biológica e o impacto agronómico dos Dicyphini, bem como alguns critérios a considerar na avaliação destes agentes de proteção biológica. Os capítulos das seções II, II e IV são apresentadas como artigos científicos, cada um procurando responder a diferentes questões. A segunda

seção aborda a biologia de *D. cerastii* e, em particular, o impacto de diferentes hospedeiros vegetais no desenvolvimento, sobrevivência e longevidade. Os parâmetros demográficos de *D. cerastii* em tomateiro, a várias temperaturas, são também aqui investigados. A terceira seção explora tipos diferentes de interações de predação de *D. cerastii*. Primeiro, no terceiro capítulo, a capacidade de predação de *D. cerastii* sobre diferentes espécies de presas é avaliada, bem como a sua resposta à variação na densidade de presas. Em segundo lugar, no quarto capítulo, é estudada a interação entre *D. cerastii* e outros Dicyphini, considerando *D. cerastii* como presa ou predador. A quarta seção é dedicada à exploração do comportamento fitófago de *D. cerastii*, comparado ao de *N. tenuis*.

Como esperado, observou-se que para *D. cerastii*, o período de desenvolvimento, a sobrevivência e longevidade decresceram com o aumento da temperatura. Apesar de algumas diferenças significativas em algumas características, os três hospedeiros vegetais testados (fisális, tabaco e tomateiro) foram relativamente semelhantes, particularmente na presença de presa. Observou-se também que *D. cerastii* não conseguiu completar o seu desenvolvimento na ausência de presas em nenhum dos hospedeiros testados, mas sobreviveu mais tempo em tomateiro.

No geral, observou-se que *D. cerastii* teve um pior desempenho comparativamente ao que é reportado para *N. tenuis*, particularmente em temperaturas mais elevadas. Enquanto as ninfas de *D. cerastii* apresentaram um desenvolvimento mais rápido do que as de *N. tenuis* a 15 °C, esta tendência alterou-se a partir de 20 °C. A 30 °C; *D. cerastii* exibiu mortalidade elevada durante o desenvolvimento e não se reproduziu, ao contrário de *N. tenuis* que consegue reproduzir-se a 35 °C. *Dicyphus cerastii* exibiu menores limites de temperatura de desenvolvimento. No entanto, apresentou menor taxa de crescimento intrínseco e maior tempo de geração comparativamente ao que está descrito para *N. tenuis*.

Em relação à sua capacidade de predação, *D. cerastii* revelou ter potencial como agente de proteção biológica na cultura do tomateiro, considerando as elevadas taxas de predação verificadas para todas as presas testadas. Este predador alimentou-se prontamente das quatro presas testadas, consumindo diariamente uma média de 88,8 ninfas de *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), 134,4 ovos de *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), 37,3 ninfas de *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) ou 172,3 ovos de *Phthorimaea absoluta* Meyrick (Lepidoptera: Gelechiidae). O predador exibiu resposta funcional do tipo II para todas as presas e factores como o tamanho da presa e a sua mobilidade afetaram a sua capacidade de predação.

A elevada capacidade de predação de *D. cerastii* foi também confirmada ao observar interações de predação intraguilda com os mirídeos *M. pygmaeus* e, particularmente, *N. tenuis.* Apesar de tanto predação intraguilda como o canibalismo apenas terem ocorrido na ausência de presa alternativa, *D. cerastii* predou mais vezes *N. tenuis* do que o contrário. As interações de predação entre *D. cerastii* e *M.*

pygmaeus foram mútuas e simétricas e as interações de canibalismo foram semelhantes nas três espécies de predadores.

Um factor muito relevante na seleção de inimigos naturais zoofitófagos é a importância do seu comportamento fitófago. No trabalho final desta tese, foi possível confirmar que o impacto da alimentação de *D. cerastii* na planta de tomateiro é menos severo do que o de *N. tenuis*. No entanto, *D. cerastii* produziu estragos nos frutos e, no caso de aborto floral, verificou-se a mesma intensidade que com *N. tenuis*, o que sugere a necessidade de realização de mais estudos e algum cuidado com a promoção deste predador em estratégias de proteção biológica em tomateiro.

No geral, os resultados desta tese sugerem que a performance de *D. cerastii* a temperaturas mais elevadas, em particular o maior período de desenvolvimento e mortalidade, poderão constituir um fator de desvantagem competitiva em relação a *N. tenuis*, que além de ser mais termófilo é também introduzido com frequência nas culturas. Por sua vez, o resultado de interações diretas poderá não ter importância para explicar a desvantagem competitiva observada na região Oeste nos últimos anos dado que apenas ocorreu na ausência de presas e, ainda assim, favoreceu *D. cerastii* em relação a *N. tenuis*.

Atendendo ao seu desempenho térmico, a capacidade de predação e menor impacto sobre a planta de tomateiro, os resultados obtidos nesta tese indicam que em Portugal, *D. cerastii* poderá ser, pelo menos, complementar a *N. tenuis* no início da primavera. Nesta época, *N. tenuis* tem frequentemente dificuldade em instalar-se em culturas protegidas de tomateiro devido às temperaturas mais baixas que vulgarmente ocorrem.

Esta tese contribuiu para ampliar o conhecimento sobre *D. cerastii*, permitindo inferir sobre o seu potencial papel equanto agente de proteção biológica na cultura de tomateiro protegido, e sobre que ações se poderão tomar para promover os serviços prestados por este recurso biológico autóctone.

Palavras-chave: conservação, Dicyphini, predador, tomateiro, zoofitofagia

Dicyphus cerastii female, author's original

Section I – Introduction

Chapter 1 – Introduction

Chapter 1 – Introduction

1.1. Biological control

Biological control can be defined as the use of living organisms to supress or reduce the population density or negative impact of other organisms, making them less abundant than they would otherwise be (Eilenberg et al. 2001). Biological control comprises three main modalities: conservation, classical, and augmentative biological control.

Conservation biological control includes the modification of the environment and/or practices to protect and enhance natural enemies populations (Eilenberg et al. 2001). This modality considers the reduction and careful selection of pesticides, but also enhancement of habitat conditions. Such improvements may include the provision of alternative prey or hosts, complementary food sources such as nectar/polen, and refuge (van Driesche and Bellows 1996).

Classical biological control consists in the introduction of an exotic biological control agent (aiming at permanent establishment and longterm control of pest organisms (Eilenberg et al. 2001). Classical biological control agents often co-evolved with the species they are introduced to control. One of such examples is the introduction of the vedalia beetle *Novius* (=*Rodolia*) *cardinalis* (Mulsant) (Coleoptera: Coccinelidae) to California from Australia in 1888 and to Europe, through Portugal, in 1897 (Amaro, 2003) to control *Icerya purchasi* Maskell (Hemiptera: Monophlebidae). A successful introduction that was then replicated around the world wherever *I. purchasi* caused damage to crops (Caltagirone and Doutt 1989).

Augmentative biological control is considered as the introduction of mass-reared organisms into crops. It is considered inoculative when organisms are released in order to establish and provide control over a non-permanent, extended period (Eilenberg et al. 2001; van Lenteren and Bueno 2003). Differently, augmentative biological control is considered inundative when large numbers of organisms are released to provide sufficient and fast control of pest organisms (Eilenberg et al. 2001; van Lenteren and Bueno 2003). Augmentative biological control has been used at least since 300 AD when Chinese farmers near Canton, in China, would aquire nests of the ant *Oecophylla smaragdina* Fabricius (Hymenoptera: Formicidae) to regulate the populations of leaf feeding insects in citrus trees (McCook 1882; Clausen 1956). In the last 100 years, augmentative biological control has been increasingly implemented on different crops, following technological developments in mass production, shipment, and release methods. As a result, the global agricultural surface that relies on augmentative biological control is estimated to be around 30 million ha, and around 350 species of natural enemies have already been used (van Lenteren et al. 2018).

The interest in biological control keeps increasing around the world due to different factors such as pesticide resistance management, environmental impact, and human health concerns. As a result, there is an increase in market demand for produce with lower pesticide residue, and more regulatory policies that limit or withdraw biocidal active substances from the market. The latter is particularly relevant in Europe since the European Commission (2020) proposed the Farm to Fork Strategy which aims, among other measures, at a reduction of 50% of the use and risk of pesticides by 2030, further increasing market interest for biological control agents.

Besides regulatory pressures, technical success examples from augmentative biological control also drive farmers' interest. A paradigmatic example is the introduction of *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) in protected sweet pepper crops in Almería, Spain, in the early 2000s. Releases of *A. swirskii* greatly replaced the use of pesticides for control of thrips and whiteflies on this crop (Calvo et al. 2015). Currently, many other species are widely used on many crops and often replace pesticide use such as *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) against spider mites (Acari: Tetranychidae) or *Orius* spp. (Hemiptera: Anthocoridae) against thrips (Thysanoptera). The success obtained from augmentative biological control in some pest-crop systems combined with pressure from the market and regulatory pressures sets growers' expectations for the future and demands new research on biological control agents.

Since the 2000s, growers of protected crops in Europe have been increasingly interested in native natural enemies and, particularly, on generalist predators that can target different pests on various crops (van Lenteren et al. 2020). Among generalists, hemipteran predators have been in the forefront of research interest for predators already used or candidates for augmentative biological control, particularly those in the Miridae family (van Lenteren et al. 2020).

1.2. Mirids (Heteroptera: Miridae)

Mirids (Hemiptera: Miridae) are tipically small, delicate insects. Generally, they can be distinguished from other heteropterans by the absence of ocelli, and four segmented antennae and labium. On the anterior wings, the corium presents a distinctive triangular cuneus, and one or two closed cells are also present (Cassis and Schuh 2012).

Mirids are very diverse and represent the largest family of heteropterans with more than 11000 species, having developed different feeding preferences and behaviors such as phytophagy, mycetophagy, zoophagy, and zoophytophagy (Cassis and Schuh 2012). This diversity among feeding strategies is relevant in an agronomical context, with many species regarded as important economic pests in both food and fiber crops (Wheeler 2000a, 2001), while others are valued as key biological control agents regulating several pests on various crops (Wheeler 2000b, 2001).

Being such a diversely adapted group, mirid species tend to have a high degree of plant-host specifity, and most species are associated to a single host species (Cassis and Schuh 2012).

Within the mirid subfamily Bryocorinae, the dicyphine tribe (Miridae: Bryocorinae: Dicyphini) includes species that are particularly adapted to plants that bear glandular trichomes due to morphological (long legs and curved and elongated pretarsal claws) and behavioural (minimizing contact with trichome exudates) adaptations (Wheeler 2001; Voigt 2005) (**Figure 1**).

Figure 1. *Dicyphus cerastii* walking among tomato trichomes (author's original).

Dicyphine species are typically zoophytophagous (Wheeler 2001; Cassis and Schuh 2012), being able to exploit both animal and plant food sources. Animal resources consist of living prey, but also on dead prey, often an abundant resource on glandular trichomes bearing plants (Carvalho and Mexia 2000; Wheeler and Krimmel 2015) (**Figure 2**). Dicyphine species also feed on plant resources that may consist of polen (Perdikis and Lykouressis 2000), nectar (Portillo et al. 2012), and plant sap (Bennett et al. 2009).

Because of their plant related unique traits, dicyphine species have been extensively and increasingly studied (**Figure 3**) and used as biological control agents in important crops (van Lenteren 2012; van Lenteren et al. 2018). Their flexible feeding regime allows them to survive and remain in crops during periods of prey scarcity (Gabarra et al. 2004; Castañé et al. 2011), and dicyphine adaptations to hairy plants make them particularly relevant on important crops like tomato, which glandular trichomes often entrap biological control agents commonly used successfully in other crops (ex. other generalist predators, hymenopteran parasitoids) (Kennedy 2003).

Figure 2. *Dicyphus cerastii* feeding on a dead hoverfly (author's original).

In Europe, the dicyphine genera *Dicyphus* Fieber, *Macrolophus* Fieber, *Nesidiocoris* Kirkaldy includes the species that are most used as biological control agents in protected crops such as tomato, eggplant, and ornamentals. In central and northern Europe *Macrolophus pygmaeus* (Rambur) is the most commercially produced and introduced dicyphine species, whereas in southern Europe and in Mediterranean countries growers rely mostly on *Nesidiocoris tenuis* (Reuter). There are currently no *Dicyphus* species under mass production in Europe, and species of this genus are mostly regarded through conservation biological control practices (Castañé et al. 2004; Ingegno et al. 2017b).

In Portugal, the dicyphine complex in protected crops, especially on tomato, consists of *Dicyphus cerastii* Wagner, *Macrolophus pygmaeus, Macrolophus costalis* and *Nesidiocoris tenuis* (Carvalho and Mexia 2000; Luz 2001; Figueiredo et al. 2012b, 2016) (**Figure 4**). From north to south Portugal, *D. cerastii* often spontaneously colonizes garden tomato and enters tomato greenhouses when pesticides are not used frequently. This species was reclassified as *D. umbertae* Sanchez & Cassis in 2006 (Sanchez et al. 2006), but again reclassified as *D. cerastii* Wagner following a major revision of *Dicyphus* genus in 2018 (Sanchez and Cassis 2018).

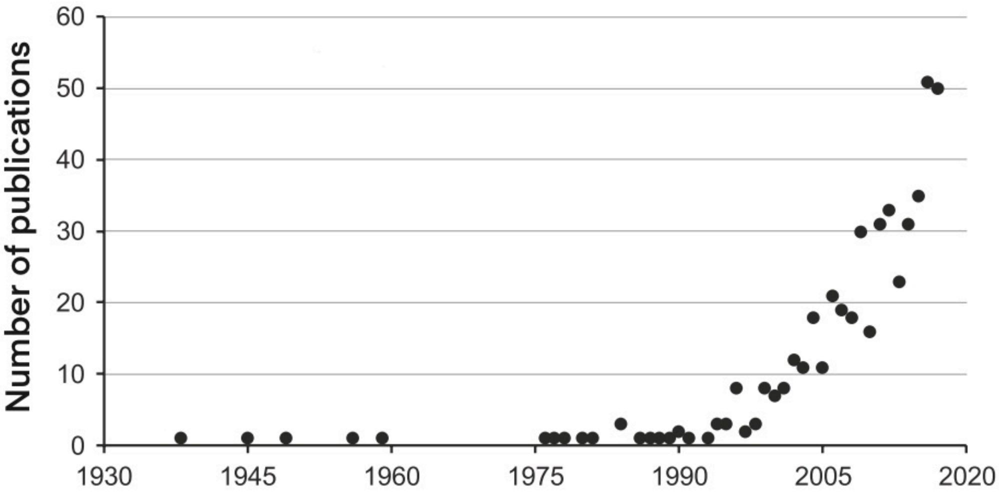

Figure 3. Number of publications indexed in the Web of Science that include the search terms "*Macrolophus*", "*Dicyphus*" or "*Nesidiocoris*" from van Lenteren et al. (2020).

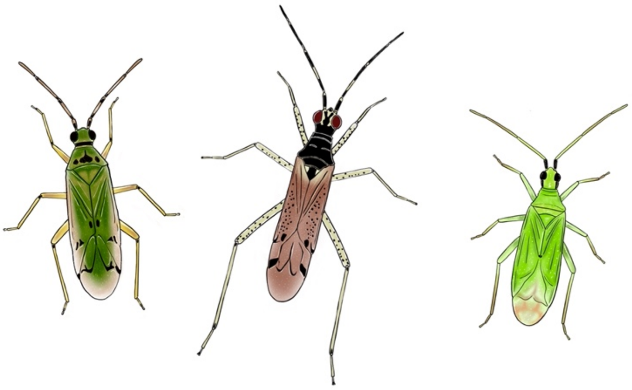

Figure 4. Three species found in the Portuguese dicyphine complex of tomato crops: *Nesidiocoris tenuis* (left); *Dicyphus cerastii* (center) and *Macrolophus pygmaeus* (right) (author's original).

Studies from the 1990s report *D. cerastii* as the most abundant dicyphine species in protected tomato crops in the Oeste Portuguese region, while *N. tenuis* was also present but less abundantly and more so during the warmer months of the year (Silva 1996; Carvalho 1999; Carvalho and Mexia 2000; Luz 2001, Figueiredo et al. 2012a). During the 2000s Portuguese and Spanish growers tried to implement augmentative biological control using the only comercially available mirid at the time (*M. pygmaeus*) on tomato greenhouses. However, contrarily to other European regions, this species has always struggled to establish sufficient populations in Iberian greenhouses despite being naturally present in the region. Since being commercialy available, *N. tenuis* has been widely adopted by Iberian growers as the main dicyphine for augmentative biological control in tomato crops. This species establishes better than *M. pygmaeus* and is even released in seedling nurseries to shorten the establishment period in the crop.

In recent years there was an increasing trend in augmentative biological control adoption with *N. tenuis* in the protected tomato sector in Portugal, mainly due to increasing pesticide restrictions and market requirements for produce with less residue. This may be a part of the explanation for the generalized presence of *N. tenuis* observed in the main production areas, particularly in the Portuguese Oeste region, where it may be displacing *D. cerastii* (Figueiredo et al. 2012a, Figueiredo et al.2014).

1.2.1. Dicyphine services

The main service provided by dicyphines is direct regulation of pest populations through predation. These generalist predators are known to feed and control populations of several important pests on different crops. However, their adaptations to glandular trichomes make these predators particularly relevant on tomato crops, where they regulate the populations of whiteflies (Hemiptera: Aleyrodidae), especially *Trialeurodes vaporariorum* (Westwood) (McGregor et al. 1999; Perdikis and Lykouressis 2002; López et al. 2012; Hassanpour et al. 2016; Ingegno et al. 2017a), and *Bemisia tabaci* (Gennadius) (Bonato et al. 2006; Calvo et al. 2009, 2016; Bouagga et al. 2018a; López et al. 2019).

Dicyphines are also widely reported as important regulators of *Phthorimaea* (=*Tuta*) *absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Different African, European and Neotropical dicyphine species have been described to effectively prey on the eggs and young larvae of this important tomato pest (Urbaneja et al. 2009; Ferracini et al. 2012; van Lenteren et al. 2016; Ingegno et al. 2017a, 2019; López et al. 2019; Garba et al. 2020).

Other tomato pests regulated by dicyphine predators include thrips (Thysanoptera) (Castañé et al. 1996; Blaeser et al. 2004; Shipp and Wang 2006; Messelink and Janssen 2014; Ingegno et al. 2017a; Bouagga et al. 2018a; Yano et al. 2020), spider mites (Acari: Tetranychidae)(McGregor et al. 1999; Enkegaard et al. 2001; Gavkare et al. 2017; López et al. 2019), leafminers (Diptera: Agromyzidae) (Parrella et al. 1982; Carvalho and Mexia 2000), aphids (Hemiptera: Aphididae) (Lykouressis et al. 2000; Perdikis and Lykouressis 2002; Messelink and Janssen 2014; López et al. 2019) (**Figure 5**), and psylids (Hemiptera: Triozidae) such as *Bactericera cockerelli* (Šulc) (Calvo et al. 2018; Veronesi et al. 2021).

Dicyphine predators may also contribute to biological control by acting as vectors of insect pathogens. This has been recently demonstrated with *N. tenuis* and *Engytatus varians* (Distant) that are capable of dispersing lepidopteran viruses through their feces, after feeding on infected prey or treated plants (Martínez et al. 2022; Gutiérrez-Cárdenas et al. 2023).

Aside from their direct effect on pest populations, dicyphine mirids also provide biological control through indirect effects. Recent research has demonstrated that dicyphines are able to induce plant defenses through their phytophagous behaviour. This happens mainly through the activation of abscisic

(ABA) and jasmonic (JA) acid pathways (Pappas et al. 2015; Pérez-Hedo et al. 2015a, b; Bouagga et al. 2018b). This induction of defenses by mirids can result in physiological changes in plants that reduce their nutritional value and consequent herbivore performance (Pappas et al. 2015).

Mirid phytophagy also induces the emission of herbivore-induced plant volatiles (HIPVs) (Bouagga et al. 2018b; Pérez-Hedo et al. 2018), compounds that affect the activity of herbivores and natural enemies. Bouagga et al. (2018b) showed that feeding activity of *N. tenuis* and *M. pygmaeus* on sweet pepper plants induced the production of HIPVs that attracted the parasitoid *E. formosa*, whereas phytophagous insects like *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and *B. tabaci* preferred plants on which no mirids had been feeding. Similarly, *Tetranychus urticae* Koch (Acari: Tetranychidae) and *F. occidentalis* preferred clean sweet pepper plants over plants previously exposed to *M. pygmaeus* (Zhang et al. 2019).

The blend of mirid induced HIPVs and their role in tritrophic interactions is also species-dependent, even among close (dicyphine) species like *M. pygmaeus* and *N. tenuis* (Pérez-Hedo et al. 2015a, 2018).

Figure 5. *Nesidiocoris tenuis* adult feeding on *Macrosiphum euphorbiae* on tomato. Author's original.

1.2.2. Dicyphine disservices

Despite being positive from a biological control perspective, omnivory can have a detrimental side if predators engage in predatory interactions with other biological control agents. This type of interaction, intraguild predation (IGP), tends to be particularly common among generalist predators (Polis et al. 1989; Lucas 2005).

The impact of intraguild predation maybe particularly evident with dicyphine predators when they face vulnerable intraguild prey such as sessile or slow moving stages, as demonstrated by the high capacity of *M. pygmaeus* to feed on eggs of hoverflies (Diptera: Syrphidae) (Fréchette et al. 2007), and those of *Adalia bipunctata* (L.) (Coleoptera: Coccinelidae), and larvae of *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecydomyiidae) (Devee et al. 2018). Moreno-Ripoll et al. (2012b) found molecular evidence of intraguild predation on whitefly parasitoids *Eretmocerus mundus* (Mercet) and *Encarsia pergandiella* Howard (Hymenoptera: Aphelinidae) by the mirid predators *M. pygmaeus* and *N. tenuis.* Taylor et al. (2005) also found that *D. hesperus* does not discriminate between healthy pupae of *T. vaporariorum* or those parasitized by *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae).

Intraguild predation among dicyphines has also been demonstrated for *M. pygmaeus-N. tenuis* (Moreno-Ripoll et al. 2012a; Perdikis et al. 2014), *D. tamaninii - M. pygmaeus* (Lucas and Alomar 2000), and *D. maroccanus* (*Syn. D. bolivari*) - *N. tenuis* (Salas Gervassio et al. 2017). Besides intraguild predation, cannibalistic interactions are also documented on several dicyphine species such as *M. pygmaeus*, *N. tenuis*, *D. tamaninii*, *D. errans*, and *D. hesperus* (Castañé et al. 2002; Laycock et al. 2006; Moreno-Ripoll et al. 2012a; Salas Gervassio et al. 2017; Arvaniti et al. 2019). However, both IGP and cannibalism between dicyphine species are generally reduced by the presence of an extraguild prey.

Indirect interactions between dicyphines may also be negative if one species displaces others through competition, creating a more simplified system. *Nesidiocoris tenuis*, for instance, is reported as having a better capacity to outcompete other species such as *D. maroccanus* and *M. pygmaeus*, given its shorter developmental time and higher reproductive capacity (Michaelides et al. 2017; Salas Gervassio et al. 2017). Competitive displacement between dicyphines may be even more problematic if the dominant species displays particularly negative traits like higher phytophagy.

Being zoophytophagous, dicyphines feed on both animal and plant resources. Phytophagy is an advantageous feeding strategy when there is low abundance of prey (Castañé et al. 2011; Gillespie et al. 2012), but also to obtain water (Sinia et al. 2004), and complementary nutrients (Gillespie and Mcgregor 2000). Despite its adaptational advantages, plant feeding can also represent a disservice in an agronomical context.

Plant feeding on sub-optimal plant hosts may result in poorer performance such as reduced predatory activity which, in turn, may lead to insufficient regulation of pest populations (Gillespie and Mcgregor

2000; Han et al. 2015). Conversely, direct damage inflicted by dicyphine mirids through their phytophagy can also be problematic as it may have economic importance. Typical dicyphine damage includes necrotic rings in stems and leaves, young shoot wilting, flower and fruit abortion, and fruit blemishing and puncturing (Sanchez 2009; Castañé et al. 2011; Moerkens et al. 2016, 2020; Puentes et al. 2018). Different factors affect the type and intensity of plant damage such as host plant species (Castañé et al. 2011), abundance of prey (Sanchez 2009), mirid species (Castañé et al. 2011; Roda et al. 2020; Pérez-Hedo et al. 2021), and population origin (Dumont et al. 2017; Chinchilla-Ramírez et al. 2020).

Perhaps the most controversial dicyphine species is *N. tenuis* because of its particularly aggressive phytophagous behaviour. This species produces characteristic necrotic rings in stems and leaves, flower abortion and fruit blemishing on tomato (Arnó et al. 2006, 2010; Castañé et al. 2011; Chinchilla-Ramírez et al. 2021). This dicyphine is regarded as a pest in Northwest Europe (Moerkens et al. 2020), despite being widely used and valued as a biological control agent in southern European protected crops. This regional difference in status may result from distinct practices in each zone, like cropping season, cycle length and technological intensification such as use of heating and artificial lighting (usually in Northwest Europe) (Moerkens et al. 2020).

Despite its contribution to biological control, the economic importance of *N. tenuis* damage often, and paradoxically, justifies the use of pesticides to regulate its populations, a practice that disrupts integrated pest management strategies. Because of this, recent research aims at reducing the negative impact of *N. tenuis* through different approaches, such as nutritional compensation (Urbaneja-Bernat et al. 2019), provision of alternative hosts (Biondi et al. 2016; Kouassi et al. 2021; Castillo et al. 2022), selection of less phytophagous strains (Chinchilla-Ramírez et al. 2020), and development of semiochemical lures (Hall et al. 2021).

Unlike *N. tenuis*, the phytophagy of *D. cerastii* has been poorly studied previously. This species has been observed to produce chlorotic punctures on tomato leaves (Figueiredo et al. 2016) but the damage potential of *D. cerastii* in different plant organs has not been studied yet.

1.3. Evaluation of mirids as biological control agents

In the past 20 years, generalist heteropteran predators, including dicyphine mirids, have become increasingly relevant in augmentative biological control in important crops (van Lenteren 2012; van Lenteren et al. 2018). Nowadays, dicyphines are still the subject of great research interest for new biological control agents (van Lenteren et al. 2020), and the evaluation of candidate species for augmentative biological control depends primarily on several factors related to biology and economy (van Lenteren et al. 2019).

First, candidate biological control agents should be evaluated for their ability to control pest populations. It is important that biological control agents display seasonal synchronization with target pests, as is generally the case with dicyphine species found in protected crops (Castañé et al. 2004; Figueiredo et al. 2012b; Bueno et al. 2018; Garba et al. 2020), since these insects are often integrated in tritrophic systems that are overall dependent on the plant host.

In the case of generalist predator biological control agents, the ability to effectively exploit different prey pests is also advantageous. Dicyphine predators are usually versatile predators of different arthropods found on their host plants, however their predatory efficacy depends on different factors including their species (Blaeser et al. 2004; Pérez-Hedo and Urbaneja 2015; Ingegno et al. 2019). Therefore, new dicyphine candidate species should be studied for their predation capacity on main important pests of target crops, but also and very importantly, on how effectively they respond to changes in prey density.

Low prey density may negatively affect predators and their capacity to maintain populations. As such, the ability to develop and survive in the crop on alternative food must also be considered when evaluating biological control agent candidates. Being zoophytophagous, dicyphines have the advantage of obtaining nutrition from different sources including their plant host. However, this trade-off must also be carefully examined when assessing new candidate species, given the risk of plant damage, as most species feed on plants, which can potentially originate damage (Sanchez 2009; Castañé et al. 2011; Moerkens et al. 2016, 2020; Puentes et al. 2018). Aditionally, other possible detrimental effects of dicyphines such as negative impact on other natural enemies should be considered given their generalist feeding regime.

To be considered effective biological control agents, candidate species should establish sufficient populations on the target crop. As such, candidate species should be well adapted to the plant host and perform well in the target crop environmental conditions. Therefore, understanding the influence of key-factors like temperature and host plant on development and reproduction is determinant in the evaluation of a candidate biological control agent.

The cost-effectiveness of mass rearing is also determinant for a candidate biological control agent species intended for augmentation. It is primarily affected by intrinsic biological factors like reproduction capacity, temperature, photoperiod, and diet requirements. Other abiotic requirements such as technology, materials, and rearing methods also condition the economic aspects of mass rearing. Finally, but very importantly, the market potential must also be thouroughly assessed (van Lenteren et al. 2019).

Biological control agents that are not cost-effective to mass produce, or for which there is only a limited market potential, may still be interesting to consider for conservation biological control strategies. For this, pesticide selectivity, and habitat management practices such as the creation of ecological infrastructures with alternative host plant species should be further investigated.

1.4. Thesis objectives and structure

The overarching objective of this thesis is to better understand if *D. cerastii* could be considered either an alternative or a complement to *N. tenuis* in protected tomato, given that this species' phytophagy and consequent use of pesticides demand for research on other dicyphine biological control agents.

Dicyphus cerastii has received little attention compared to other dicyphine species that colonize greenhouse crops both commercialized and not. Despite being a common dicyphine species in Portuguese tomato greenhouses, information on *D. cerastii* is still limited. Previous studies focused mostly on its predatory capacity on leafminers and whiteflies and its presence and abundance in tomato greenhouses (Silva 1996; Carvalho 1999; Carvalho and Mexia 2000; Luz 2001; Figueiredo et al. 2012b, 2016). Moreover, a detailed exploration of its biology, and potential role as as a biological control agent in protected crops was still lacking. Therefore, this thesis aims at further exploring the biology of *D. cerastii*, its predatory capacity, interaction with other natural enemies, and potential damage to tomato plants. This information will be the essential foundation for further research on the potential of *D. cerastii* as a biological control agent, either through mass production or conservation approaches.

This thesis is divided in five sections. The first section, introduction, describes the state of the art in biological control and the agronomical impact of mirids, particularly dicyphines, as well as considerations on which traits should be observed in the evaluation of these biological control agents.

Sections II, III and IV are presented as scientific articles, each aiming at answering different research questions. The second section explores the biology of *D. cerastii*, particularly the impact of different host plants and temperatures on nymphal development, survival, and longevity. The life history parameters of *D. cerastii* on tomato at different temperatures are also evaluated. The article that consists of Chapter 2 was the last to be submitted since more time was required to finalize all experiments, and its discussion already includes information from the following chapters that were published previously. However, in this thesis, it is presented before as it makes sense to start with the information on the biology of *D. cerastii.*

The third section explores different types of predatory interactions of *D. cerastii*. First, the third chapter assesses the predatory capacity of this predator over different prey species, and its response to changes in prey density. Secondly, in the fourth chapter, the interaction between *D. cerastii* and other dicyphines is considered, both as intraguild prey, and predator. The fourth section is dedicated to the exploration of the phytophagous behaviour of *D. cerastii* compared to that of *N. tenuis*. Finally, the fifth section offers a general discussion (Chapter 6) and conclusions (Chapter 7) obtained from the main findings from each of the previous sections.

The main objectives of this thesis were:

- Knowing how host plant and temperature influence development, survival, longevity, and assessing the reproductive capacity and demographic parameters of *D. cerastii* (Chapter 2).
- Studying the functional response of *D. cerastii* on different prey, and how prey traits affect predation (Chapter 3).
- Assessing if direct interactions such as intraguild predation with commercially introduced dicyphines may negatively impact *D. cerastii* (Chapter 4).
- Describing and comparing the type of plant damage and factors affecting phytophagy by *D. cerastii* compared to *N. tenuis* (Chapter 5).

We expect this thesis to contribute to further understanding the potential of *D. cerastii* as a biological control agent, enabling better decision making regarding the type of biological control strategy and best suited mirid species to promote/introduce in target crops such as tomato.

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Dicyphus cerastii egg, author's original

Section II – Biological traits of *Dicyphus cerastii* Wagner

Chapter 2. *Dicyphus cerastii* Wagner (Hemiptera: Miridae) as candidate biological control agent: data on development, survival, and reproduction

Abraços-Duarte G, Madeira F, Souto P, Silva EB, Figueiredo E. *Dicyphus cerastii* (Wagner) (Hemiptera: Miridae) as candidade biological control agent: data on development, survival, and reproduction. PlosOne(submitted)

Chapter 2. *Dicyphus cerastii* Wagner (Hemiptera: Miridae) as candidate biological control agent: data on development, survival, and reproduction

Gonçalo Abraços-Duarte, Filipe Madeira, Paula Souto, Elsa Borges da Silva, Elisabete Figueiredo

2.1. Abstract

Dicyphus cerastii Wagner (Hemiptera: Miridae) is an important predator of pests on horticultural crops. We investigated the influence of host (tomato, tobacco, and Cape gooseberry) and temperature (15.0, 20.0, and 25.0±1 °C) on nymphal development, survival, and adult longevity, both with and without prey. Without prey, nymphs didn't complete development in any of the hosts but survived longer on tomato. With prey, nymphal development, survival, and longevity decreased with increasing temperature on all hosts. The development and longevity of *D. cerastii* were further examined on tomato, with prey, at a wider range of temperatures (15.0, 20.0, 25.0, 27,5, 30.0, 32.5, and 35.0±1 °C). The reproductive capacity was measured at 20.0, 25.0, 30.0±1 °C, on tomato on the same diet. Egg development ranged from 30.6 days at 15.0 °C to 9.7 days at 32.5 °C. Nymph development decreased from 40.0 days at 15.0 °C to 16.4 days at 30.0 °C. Nymphal mortality was lowest at 20 °C and highest at 30 °C, and no nymphs completed development above 30.0 °C. The estimated optimal temperature for development from egg to adult was 29.2 °C, while the minimum temperature threshold for immature development was around 7.0 °C. The thermal constant for development were 230.36 degree-days for eggs, and 393.98 degree-days for nymphs. Adult longevity ranged from 158.6 days at 15.0 °C to 13.8 days at 30.0 °C. The net reproductive rate (R_0) and generation time (T) were higher at 20 °C whereas the intrinsic rate of increase (r_m) was highest at 25 °C. Reproduction at 30 °C was neglectable. We suggest that *D. cerastii* may be better suited for cooler conditions, as it may be outcompeted by other more thermophilous mirids like *Nesidiocoris tenuis* (Reuter) that have a better performance at higher temperatures.

Keywords: Biological control, dicyphini, mirid predators, development, fertility

2.2. Introduction

Dicyphine mirids (Miridae: Dicyphini) are important natural enemies on horticultural crops. This tribe comprises many zoophytophagous predators that are widely recognized for their role in biological control of protected crops throughout the world (Arnó et al. 2010; Gabarra et al. 2010; Calvo et al. 2018; van Lenteren et al. 2018).

In Europe, important dicyphine predators usually belong to the genera *Dicyphus* Fieber, *Macrolophus* Fieber and *Nesidiocoris* Kirkaldy. Some species like *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) are currently mass reared and commercialized for augmentation strategies (van Lenteren 2012). Differently, European *Dicyphus* species are mostly regarded in conservation biological control (CBC) (Alomar et al. 2002; Ingegno et al. 2017).

In Portugal and Spain, growers resort mostly to commercial releases of *N. tenuis* to control whiteflies (Hemiptera: Aleyrodidae) and *Phthorimaea absoluta* Meyrick (Lepidoptera: Gelechiidae) on protected tomato crops. Despite its good performance during summer, this predator often struggles to establish sufficient populations in early spring tomato crops, and it is considered to be more thermophilous than other European dicyphines (Sanchez et al. 2009; Ingegno et al. 2021). Another downside is that despite being a valuable predator, *N. tenuis* can also feed on, and damage tomato plants (Arnó et al. 2010; Castañé et al. 2011). Because of this behaviour, tomato growers often resort to pesticides to manage *N. tenuis* populations (Figueiredo et al. 2016), disturbing biological control services provided by other natural enemies. Consequently, there has been an increasing interest in evaluating other dicyphine species that may be an alternative to *N. tenuis* (Abbas et al. 2014; Madeira et al. 2019b; Ingegno et al. 2021).

Dicyphus cerastii Wagner is vastly distributed across the Mediterranean region (Kerzhner and Josifov 1999; Sanchez and Cassis 2018). In Portugal, this species is commonly found in protected tomato crops under low pesticide usage (Carvalho and Mexia 2000; Figueiredo et al. 2012, 2016). This generalist predator feeds on several horticultural pests (Carvalho and Mexia 2000; Francisco 2019; Abraços-Duarte et al. 2021; Rodrigues, 2022) and exhibits particularly high predation rates over economically important pests such as *P. absoluta* and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), even when compared with other dicyphines (Abraços-Duarte et al., 2021). Being zoophytophagous, *D. cerastii* can also originate plant damage resulting from its phytophagous behaviour, but still less severe compared to *N. tenuis* (Souto et al. 2022).

Dicyphus cerastii used to be the predominant mirid species in Portuguese tomato greenhouses, but *N. tenuis* has recently become more abundant than *D. cerastii* (Carvalho and Mexia, 2000; Figueiredo et al., 2016). A seasonal abundance shift has also been observed, with *D. cerastii* populations decreasing during summer whereas those of *N. tenuis* increase (Figueiredo et al., 2012).

Given its wide distribution, predatory capacity, and lower phytophagy, *D. cerastii* can be considered a candidate biological control agent (BCA) for protected crops like tomato, in both conservative and augmentative biological control strategies (Abraços-Duarte et al., 2021). This species could, presumably, be an alternative or a complement to *N. tenuis* in Portuguese greenhouses. However, there is only limited information about its life history parameters, and the influence of external factors like host plant and/or temperature on its biology remains largely unknown.

Autochthonous species, that are not currently mass produced, provide services through CBC strategies, which often considers habitat enrichment by providing alternative host plants (Balzan 2017; Perrin et al. 2019). In dicyphines, host plant species can influence different traits such as predation (López et al. 2012), survival (Urbaneja et al. 2005), and reproduction (Biondi et al. 2016; Nguyen-Dang et al. 2016). Therefore, information on how alternative hosts affect the performance of BCAs, is fundamental to select plants that provide the best conditions for population build up and establishment on crops. Dicyphines are specialists of glandular trichome bearing plants (Wheeler and Krimmel 2015), and *D. cerastii* is commonly found on tobacco (*Nicotiana tabacum* L.) and Cape gooseberry (*Physalis peruviana* L.) in gardens (our pers. obs.). To know how these host species influence *D. cerastii*, we compared the post-embryonic period, survival and longevity on tomato, tobacco, and Cape gooseberry at 15.0, 20.0 and 25.0±1.0 °C.

As for most insects, temperature plays a determinant role in the biological traits and, consequently, on the geographical distribution of dicyphines. Even co-occurring species display different immature development and thermal activity thresholds, which make them differently adapted for distinct climates or growing seasons (Ingegno et al., 2021). Temperature also influences the predatory capacity of these mirids (Hughes et al. 2010; Ziaei Madbouni et al. 2017), and the damage induced by their phytophagy (Sanchez 2008; Siscaro et al. 2019). Therefore, when evaluating new candidate BCA species, like *D. cerastii*, it is determinant to understand how temperature influences its life history parameters. In this context, we studied the development (embryonic and post-embryonic), survival and longevity of *D. cerastii* at a wide range of constant temperatures (15.0, 20.0, 25.0, 27.5, 30.0, 32.5, and 35.0±1.0 °C), on tomato, to allow modeling the temperature-dependent development rate. Finally, reproduction parameters were also observed on tomato at 20.0, 25.0 and 30±1.0 °C, to obtain the demographic parameters of *D. cerastii*.

2.3. Materials and Methods

2.3.1. Insects and host plants

Dicyphus cerastii was originally collected from different geographical sites in Portugal: Fataca (Odemira, Beja district) (collected from Cape gooseberry and *Pelargonium* spp. in gardens); Ferreira do Zêzere (Santarém district) (collected on garden Cape gooseberry and tomato), Lisbon and Sintra (Lisbon district) (collected on Cape gooseberry and tomato), and Torres Vedras (Lisbon district) (always collected on commercial tomato greenhouses either on tomato or tobacco), and Póvoa de Varzim (Porto district) (from commercial tomato greenhouses on tomato/tobacco). From these populations, a mixed population colony was started, which was frequently refreshed with wild individuals, to prevent lack of genetic diversity.

Rearing units were kept in 40x40x60 cm mesh cages (Entosphinx, Pardubice, Czech Republic) with tobacco plants (ca. 20-30 cm high). These units were fed weekly with a mix of *Artemia* sp. (Anostraca: Artemiidae) cysts and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (Entofood ®, Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands) provided *ad libitum*. Bee pollen (Serramel, Euromel Apicultores, Penamacor, Portugal) was also sprinkled on tobacco leaves at the time of feeding.

To obtain 1st instar nymphs for experiments, *D. cerastii* adults were kept in a cage for 2 weeks for oviposition. After that newly emerging nymphs were collected daily for use in biological assays and to refresh the rearing units.

All rearing units were kept under laboratory conditions in the insectary at Instituto Superior de Agronomia, University of Lisbon (ISA) at 25±2 °C, 65±5% h.r. and a 14 h photoperiod.

All plants were grown at ISA's facilities and were not sprayed with pesticides. Plants were fertilized once a week and watered as needed. To prevent contamination from fungi or arthropods, host plant leaves were washed with abundant water, then bathed in a 5% solution of sodium hypochlorite for 10 min, and rinsed in water again, before being used in bioassays.

2.3.2. Nymphal development, survival, and adult longevity on different host plants

Nymphal development was compared between the host plants tomato, Cape gooseberry and tobacco at three temperatures (15.0, 20.0, 25±1.0 °C). For these experiments, plastic 100 ml Deli cups, with a meshed lid were used. For all three host plants, leaf discs (Ø 30 mm) were placed in the Deli cups, abaxial side up, on top of a moistened cotton disc (Ø 60 mm). Factitious prey (Entofood®) food was provided on a 2 cm^2 sticky paper, placed in the cup.

First instar nymphs (<24 h old) were collected from rearing cages and placed individually in a Deli cup. These cups were kept in climatic chambers (Fitoclima S600; Aralab, Rio de Mouro, Portugal) at each of the three selected temperatures. These bioassays were performed in both presence and absence of factitious prey (Entofood®), in which case no sticky paper was added to the cup.

Nymphs were checked every 24 h and the respective instar and mortality were recorded. Every two days, the insects were moved into a new cup, with a new cotton disc, fresh leaf disc, and sticky paper with food. This procedure was maintained until the death of adult insects to determine longevity. At least 40 *D. cerastii* first instars were used for each temperature, host, and prey availability experiment.

2.3.3. Life history parameters on tomato

Nymphal survival without prey was higher on tomato, and given the economic importance of this crop, life history parameters were further studied on this host in a wider range of temperatures, to allow modeling temperature-dependent development rate.

2.3.3.1 Embryonic development on tomato

To determine the embryonic development period, 3-4 weeks old tomato plants *cv* San Pedro (Vilmorin Iberica S.A., Alicante, Spain) were placed inside plastic cups (300 ml). A hole was created in the bottom of the cup, and the plant stem was pushed through it. This cup was then placed in a smaller (200 ml) cup which had water accessible only to the roots. In each cup, factitious prey (Entofood®) was provided on a 2 cm^2 sticky paper. Plastic cups were placed for 24 h in a mesh cage (35x35x35 cm) (Entosphinx, Pardubice, Czech Republic) containing ca. 50 adult couples of *D. cerastii*. After this period, adult mirids were removed, and the cups were placed in climatic chambers at different temperatures (15.0, 20.0, 25.0, 27.5, 30.0, 32.5, 35.0±1.0 °C) and were daily inspected to record the number of nymphs emerging.

2.3.3.2 Post-embryonic development and adult longevity on tomato

Development was observed on tomato *cv* San Pedro at the constant temperatures of 15.0, 20.0, 25.0, 27.5, 30.0, 32.5, and 35.0±1.0 °C. These bioassays followed the same procedure as previously described for different host plants, except that, in this experiment, the Deli cups at higher temperatures (27.5, 30.0, 32.5, and 35.0 °C) were changed every other day as the cotton disc would dry too quickly. At least 40 *D. cerastii* first instars were used for each temperature tested.

2.3.3.3 Reproduction on tomato

Reproductive parameters were observed on tomato at three different temperatures (20.0, 25.0 and 30±1.0 °C). Adult couples used in these experiments were obtained by placing first instar nymphs (< 24 h old) in identical conditions to those previously described for rearing units, in climatic chambers at each of the tested temperatures (20.0, 25.0 and 30.0 ±1.0 °C), 60±10 % r.h., and 14 h photoperiod. The cages were observed daily and emerging adult couples (< 24 h old) were collected and placed in plastic cups (identical to those used in embryonic development bioassays) with a tomato plant cv San Pedro and a

2 cm2 sticky paper with factitious prey (Entofood ®). The couples were daily moved into new cups (with new plant and fresh prey) until nymphs started to emerge, to record the pre-oviposition period. After this, couples were moved into new cups every three days until female death. Males that died during experiments were replaced with others from the respective temperature. At least 20 couples were tested for each temperature.

The eggs of dicyphines are laid inside plant tissue and difficult to count. In preliminary experiments, we observed that the number of emerged nymphs was often higher than that of counted eggs. Therefore, in this work, we used the number of emerged nymphs rather than eggs to infer the fertility of *D. cerastii*. Because of this, it must also be noted that the pre-oviposition period we recorded corresponds to the period before the emergence of the first nymphs and not of the first eggs laid.

2.3.4. Data analysis

For the comparison of different host plants, the duration of nymphal development and adult longevity were calculated. These response variables were analyzed using Generalized Linear Models (GLM) based on a Gaussian distribution with an identity link function. For each of the response variables, the explanatory variables in the model were 'host' (tomato, Cape gooseberry, tobacco), 'temperature' (15.0 °C, 20.0 °C and 25.0 °C) and 'sex' (male and female), and their interactions. The models were simplified with a stepwise model selection based on the Akaike information criterion (AIC) using the 'stepAIC' function of the *MASS* package in the R software (Venables and Ripley 2002). When significant differences were detected, post-hoc comparisons of mean values were performed with the Tukey HSD method (*agricolae* R package).

All survival curves of *D. cerastii* at each temperature and host were estimated using the Kaplan-Meier method. Log-rank tests were used to compare survival curves. For this, the R packages *survival* (Therneau et al. 2022) and *survminer* (Kassambara et al. 2021) were used.

For tomato, the duration of egg, each nymphal instar, total nymph development time and adult longevity were calculated for all the temperatures that allowed completion of immature development. These response variables were also analyzed using GLM based on a Gaussian distribution with an identity link function. For egg development the explanatory variable in the model was 'temperature' (15.0, 20.0, 25.0, 27.5, 30.0, 32.5 °C). For nymphal instar, post-embryonic development time and adult longevity the explanatory variables in the model were 'temperature' (15.0, 20.0, 25.0, 27.5, 30.0 °C) and 'sex' (male, female), and their interactions. As previously described, models were simplified with a stepwise model selection based on the AIC. The Tukey HSD method was used to perform post-hoc mean values comparisons when significant differences were detected.

To describe the temperature-dependent developmental rate relationship, three mathematical models were used, a linear model (Campbell et al. 1974) and two non-linear ones (Lactin-2, Brière-1) (Lactin et al. 1995; Brière et al. 1999). Lower, optimal and upper temperatures were obtained for all immature stages. The two non-linear models were chosen since they are commonly used for modeling insect developmental rates (Liu et al. 2015; Lumbierres et al. 2021) having been used for Heteroptera (Nielsen et al. 2008; Martínez-García et al. 2017). Only the non-linear models allowed calculating the optimum temperature (t_{opt}) and the maximum (t_{max}) developmental threshold, whereas for all the models the lower developmental threshold (t_{min}) was estimated as the value intercepting the temperature axis. The goodness of fit was evaluated by the coefficient of determination for both linear and non-linear models (R^2, higher value indicated better fitting), the residual sum of squares (RSS, lower value indicated better fitting), the AIC (lower value indicated better fitting), and by biological criteria. Data was fitted using the *devRate* R package (Rebaudo and Rabhi 2018). Initial parameter estimation for the Lactin-2 model was made following the suggestions of Logan (1988). All statistical analyses referring to model adjustment and comparisons were performed using R version 3.5.2 (R Core Team 2020). The thermal constant (K) of each immature stage of *D. cerastii* was also calculated; this constant was estimated using the linear model as the reciprocal of the slope b (K=1/b). To adjust the linear and Brière-1 models, last data values, 32.5 °C and 35.0 °C, were omitted. This was necessary for the correct calculation of the parameters K and t_{min} in the case of the linear model (Clercq and Degheele 1992) and to estimate the lower developmental threshold (t_{min}), the optimum temperatures (t_{opt}), and the maximum lethal temperature (t_{max}) for the Brière-1 model. The equations of the linear and each of the two non-linear models are detailed in **Table S.1**.

Finally, for tomato, a life table was built with data from immature development and reproduction bioassays at 20 °C and 25 °C, but not from 30 °C since fertility was very low. Sex ratio, adult daily survival, the pre-oviposition time, and the number of offspring produced by the females were recorded. The net reproductive rate (R_0; female offspring per female), generation time (T; days), the intrinsic rate of increase (r_m; females per female per day), doubling time (DT; days), and the finite rate of increase (λ; females per female per day) were calculated according to Birch (1948). The standard error associated with R_0, T, r_m, DT and λ was estimated by bootstrapping (100.000 replications). To compare differences between temperatures, paired bootstrap tests were used ($\alpha = 0.05$).

2.4. Results

2.4.1. Nymphal development, survival, and longevity on different host plants

In absence of factitious prey, *D. cerastii* nymphs were unable to complete development on all host plants. Nymphs survived longer on tomato compared to Cape gooseberry and tobacco (**Table 1**, **Figure 1**). On tomato, some nymphs could reach the 5th instar, whereas on Cape gooseberry none could complete the 2nd instar, and on tobacco one nymph reached the 3rd instar but didn't complete it. Temperature also influenced nymph survival on each host differently: on tomato survival decreased only at 25 °C; on Cape gooseberry, survival decreased at 20 °C and was similar at 25 °C; on tobacco, survival decreased as temperature increased (**Table 1**, **Figure 1**, **Table S.2**).

In the presence of factitious prey, survival decreased with increasing temperature, on all hosts. The longest survival was observed on Cape gooseberry at 15 °C and the shortest on tomato at 25 °C (**Table 1**, **Figure 2**, **Table S.3**).

Table 1 – Survival in days (mean ± SE) of *Dicyphus cerastii* at three different temperatures (15.0, 20.0, 25.0±1.0 °C) on three different hosts (tomato, Cape gooseberry, tobacco), both without and with factitious prey.

	Without factitious prey			With factitious prey		
Host	15 °C	20 °C	25 °C	15 °C	20 °C	25 °C
Tomato	17.9±1.7aA	13.3±2.1aA	6.8±1.0bA	198.6±11.8aAB	96.1±8.0bAB	55.7±3.8cA
Cape gooseberry	5.1±0.4aB	3.7±0.3bB	4.0±0.3bB	218.6±10.2aA	103.2±6.3bA	66.5±4.4cAB
Tobacco	5.3±0.6aB	3.8±0.3bB	3.1±0.2cB	194.9±23.1aB	103.0±6.2aB	70.4±4.7bB

Means followed by different lowercase letters within rows, or uppercase within columns, correspond to groups among which survival curves are significantly different for Log-Rank comparison test (p<0.05) (within treatments: without or with factitious prey).

In the presence of prey, nymph mortality was generally under 20% (except for tobacco at 15 °C, and tomato and Cape gooseberry at 25 °C) (**Table 2**). Host, temperature, and their interaction influenced the developmental period of *D. cerastii* nymphs, whereas sex did not. On all hosts, post-embryonic development time decreased with increasing temperature. At 15 °C development was fastest on tomato, and no differences were observed between Cape gooseberry and tobacco; at 20 °C there were no differences between hosts; and at 25 °C development time was shortest on tobacco (**Table 2**, **Table S.4**).

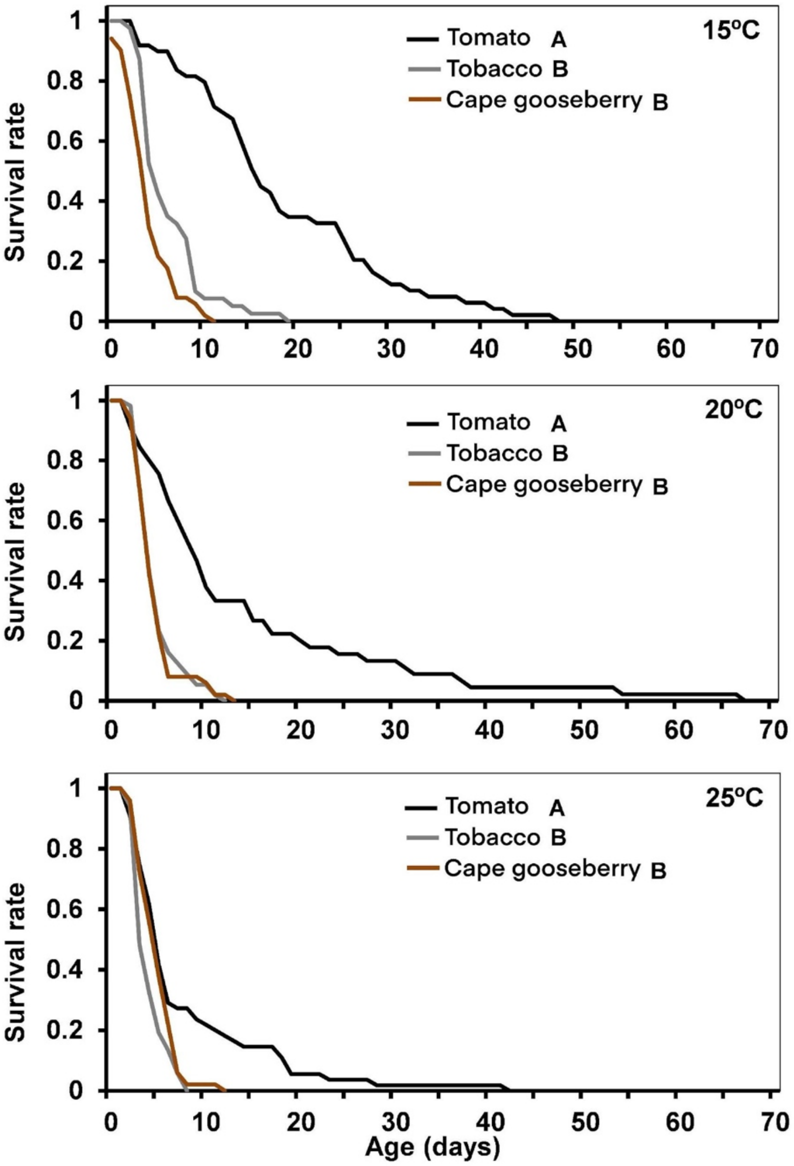

Figure 1 – Survival (days) of *Dicyphus cerastii* nymphs on three plant hosts (tomato, tobacco, Cape gooseberry) at three different temperatures (15.0, 20.0, 25.0±1.0 °C) without factitious prey. For each temperature, different letters in front of host species correspond to significantly different survival curves for Log-Rank comparison test (p<0.05).

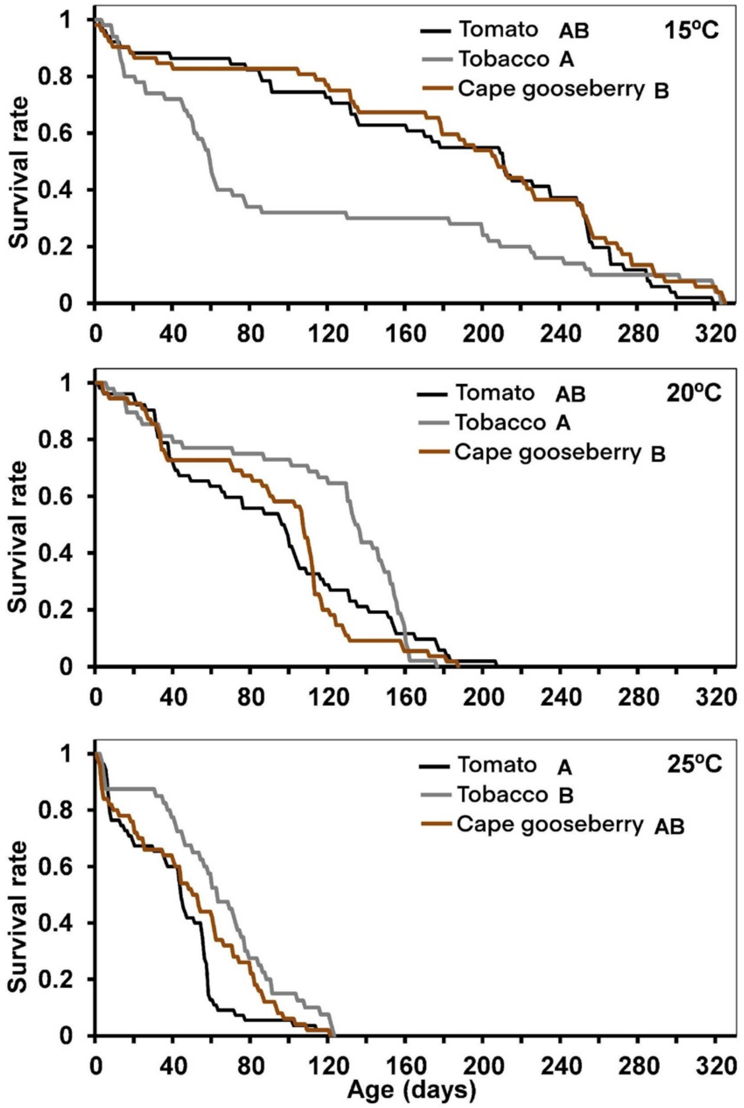

Figure 2 – Survival (days) of *Dicyphus cerastii* on three plant hosts (tomato, tobacco, Cape gooseberry) at three different temperatures (15, 20, 25 °C) with factitious prey. For each temperature, different letters in front of host species correspond to significantly different survival curves for Log-Rank comparison test ($p<0.05$).

Adult longevity was not significantly influenced by the host alone (**Table S.4**). However, temperature, sex, and the interactions of the host with temperature, and sex with temperature were significant (**Table 2, Table S.4**). Both male and female longevity decreased with increasing temperature. At 15.0 °C and 25.0 °C male longevity was higher than that of females, whereas at 20.0 °C there were no significant differences (**Table S.5**).

2.4.2. Life history parameters on tomato

2.4.2.1 Embryonic and post-embryonic development, longevity, and survival on tomato

Eggs hatched at all temperatures except at 35.0 °C. Embryonic development was influenced by temperature and was longest at 15.0 °C and shortest at 30.0 °C, however there were no significant differences between 27.5 °C, 30.0 °C and 32.5 °C (**Table 3**, **Table S.6**).

As observed above, for host comparison (Cape gooseberry, tobacco), sex had no influence on post-embryonic development time (**Table S.6**), which decreased with increasing temperature, but there were no significant differences between 27.5 °C and 30.0 °C (**Table 3**). *Dicyphus cerastii* could not complete development at 32.5 °C and 35.0 °C. In fact, at 32.5 °C some individuals were able to reach the 3rd instar whereas at 35.0 °C only three individuals were able to complete the 1st instar.

Nymph mortality varied among temperatures. At 15.0 °C and 20.0 °C more nymphs were able to complete development compared to the other temperatures. Among the temperatures that allowed development, the lowest survival was recorded at 30.0 °C (**Table 3**, **Table S.7**). Overall survival curves (from hatching to adult death) differed among all temperatures, except between 27.5 °C and 30 °C (**Figure 3**, **Table S.8**).

Temperature also influenced adult longevity, whereas sex did not. Longevity was highest at 15.0 °C, followed by 20.0 °C, and there were no significant differences between 25.0 °C, 27.5 °C and 30.0 °C (**Table 3**, **Table S.6**)

All the three models used provided a good fit to the relationship between temperature and the development rates of egg, each nymphal instar, post-embryonic or total immature (**Table S.9**). Despite this, the Brière-1 model provided negative minimum thresholds (t_{min}) for the first and fifth nymphal instar, and for total post-embryonic development. Fitting the Linear model presented lower minimum development thresholds (t_{min}) compared to the Lactin-2 model for both post-embryonic (4.74 and 6.00 °C, respectively) and total immature (6.26 and 7.50 °C, respectively). The Lactin-2 model estimated the optimal temperature for total development (t_{opt}) to be at 29.20 °C. The thermal constant K values were 230.36 degree-days (DD) for eggs, 393.98 DD for nymphs, and 584.76 DD for total egg to adult development (**Table S.9**).

Table 2– Duration in days (mean ± SE) of post-embryonic development, adult longevity, and percentage of nymph mortality of *Dicyphus cerastii* at three different temperatures (15, 20, 25 °C) on three different hosts (tomato, Cape gooseberry, tobacco) with factitious of prey.

Host	15 °C			20 °C			25 °C		
	Development (days)	Nymph mortality (%)	Longevity (days)	Development (days)	Nymph mortality (%)	Longevity (days)	Development (days)	Nymph mortality (%)	Longevity (days)
Tomato	40.0±0.5aA	12.0	158.6±12.0aA	25.1±0.3bA	7.7	70.9±8.2bA	20.0±0.3cA	32.7	35.7±4.0cA
Cape gooseberry	44.7±0.6aB	17.3	173.7±10.4aA	25.7±0.5bA	14.5	77.2±6.4bA	19.6±0.6cAB	26.0	46.7±4.5cAB
Tobacco	45.2±0.7aB	54.2	149.2±23.8aA	25.8±0.4bA	16.7	106.3±6.4bB	18.6±0.3cB	10.3	51.7±4.8cB

Means followed by the same lowercase letter within rows, or uppercase within columns, correspond to groups (within each response variable) among which means are not significantly different for Tukey HSD test (p<0.05)

Table 3 – Duration in days (mean ± SE) of the egg, nymphal instars, total nymphal development, adult longevity, percentage of nymphal mortality, and sex ratio (proportion of females) of *Dicyphus cerastii* at seven different temperatures (15.0, 20.0, 25.0, 27.5, 30.0, 32.5, 35.0 °C) on tomato with factitious prey.

TºC	Egg	Instar					Nymph mortality (%)	Total	Sex ratio	Longevity
		1st	2nd	3rd	4th	5th				
15.0 °C	30.6±0.2a	7.5±0.2a	6.4±0.1a	6.2±0.2a	7.4±0.2a	12.6±0.2a	12.0ab	40.0±0.5a	0.32	158.6±12.0a
20.0 °C	16.5±0.1b	4.5±0.1b	3.7±0.1b	3.7±0.1b	4.7±0.1b	8.0±0.1b	7.7a	25.1±0.3b	0.60	70.9±8.2b
25.0 °C	11.2±0.1c	4.1±0.1b	2.8±0.1c	3.0±0.1c	3.6±0.1c	6.5±0.1c	32.7ce	20.0±0.3c	0.57	35.7±4.0c
27.5 °C	9.8±0.1d	3.0±0.1c	2.3±0.1c	2.5±0.1c	3.2±0.1c	5.0±0.1d	25.0bc	16.1±0.2d	0.51	12.4±1.1c
30.0 °C	9.6±0.1d	3.1±0.1c	2.7±0.1c	2.7±0.1c	3.3±0.1c	4.6±0.2d	51.9de	16.4±0.3d	0.42	13.8±1.6c
32.5 °C	9.7±0.1d	n.d.	n.d.	n.d.	n.d.	n.d.	100.0*	n.d.		
35.0 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	100.0*	n.d.		

Means followed by the same letter within columns correspond to groups among which values are not significantly different for Tukey HSD multiple comparison test (p<0.05). Nymph mortality percentages followed by the same letter within columns, correspond to groups among which survival curves are not significantly different for Log-Rank comparison test (p<0.05).* - not considered in the analysis; n.d. – no development

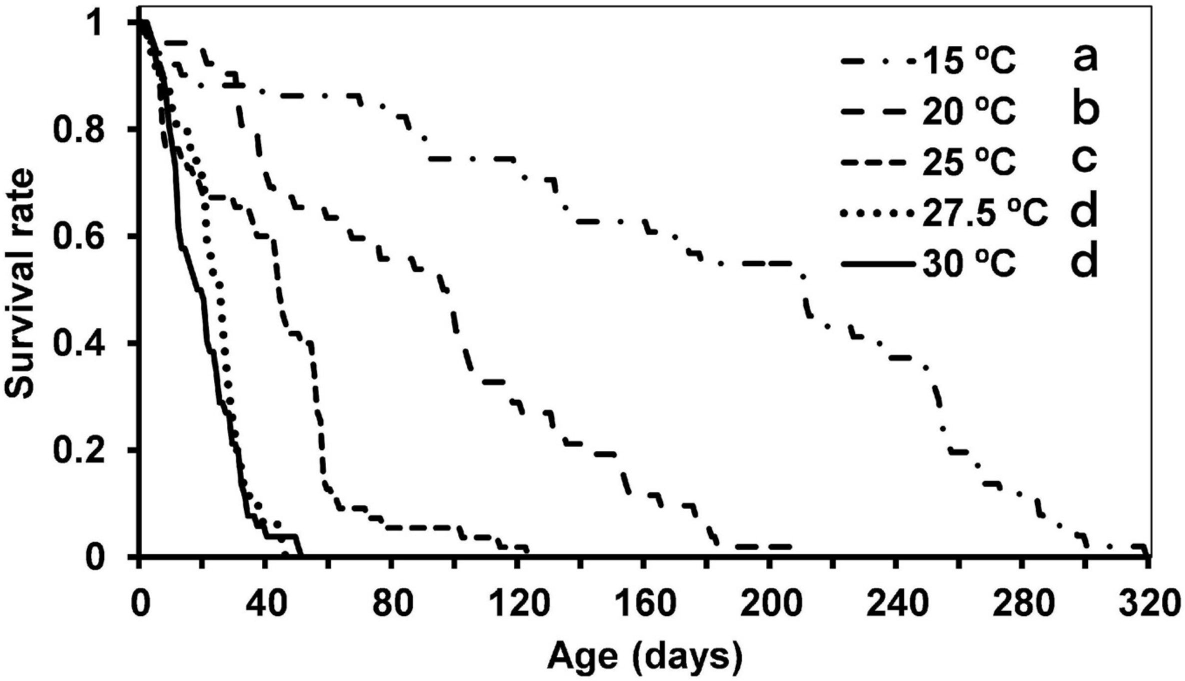

Figure 3 – Survival (days) of *Dicyphus cerastii* on tomato at five different temperatures (15.0, 20.0, 25.0, 27.5 and 30.0 °C) with factitious prey. Different letters in front of each temperature correspond to significantly different survival curves for Log-Rank comparison test (p<0.05).

2.4.2.2 Reproduction and demographic parameters

At 20 °C, individual *D. cerastii* females produced 159.6 ± 23.7 (n=18) nymphs during their 87.8 ± 6.5 days lifespan, while at 25 °C they generated 116.5 ± 15.0 (n=20) nymphs in their 41.6 ± 3.8 days lifespan, but there were no significant differences between the number of nymphs produced (U=141.5, p=0.260). These values resulted in a fertility rate of 2.1 ± 0.3 and 3.7 ± 0.4 nymphs per female per day at 20 °C and 25 ºC, respectively, which were significantly different (U=74.0, *p*=0.002). Couples kept at 30 °C produced very few nymphs (only 3 nymphs were obtained from 21 couples).

Temperature influenced the demographic parameters. The pre-oviposition (emergence of the first nymphs) as well as the oviposition period were longer at 20 °C compared to 25 °C (**Figure 4**, **Table 4**). The net reproductive rate (R_0), mean generation time (T), and doubling time (DT) of *D. cerastii* were higher at 20 °C whereas the intrinsic rate of increase (r_m), and the finite rate of increase (λ) were higher at 25 °C (**Table 4**).

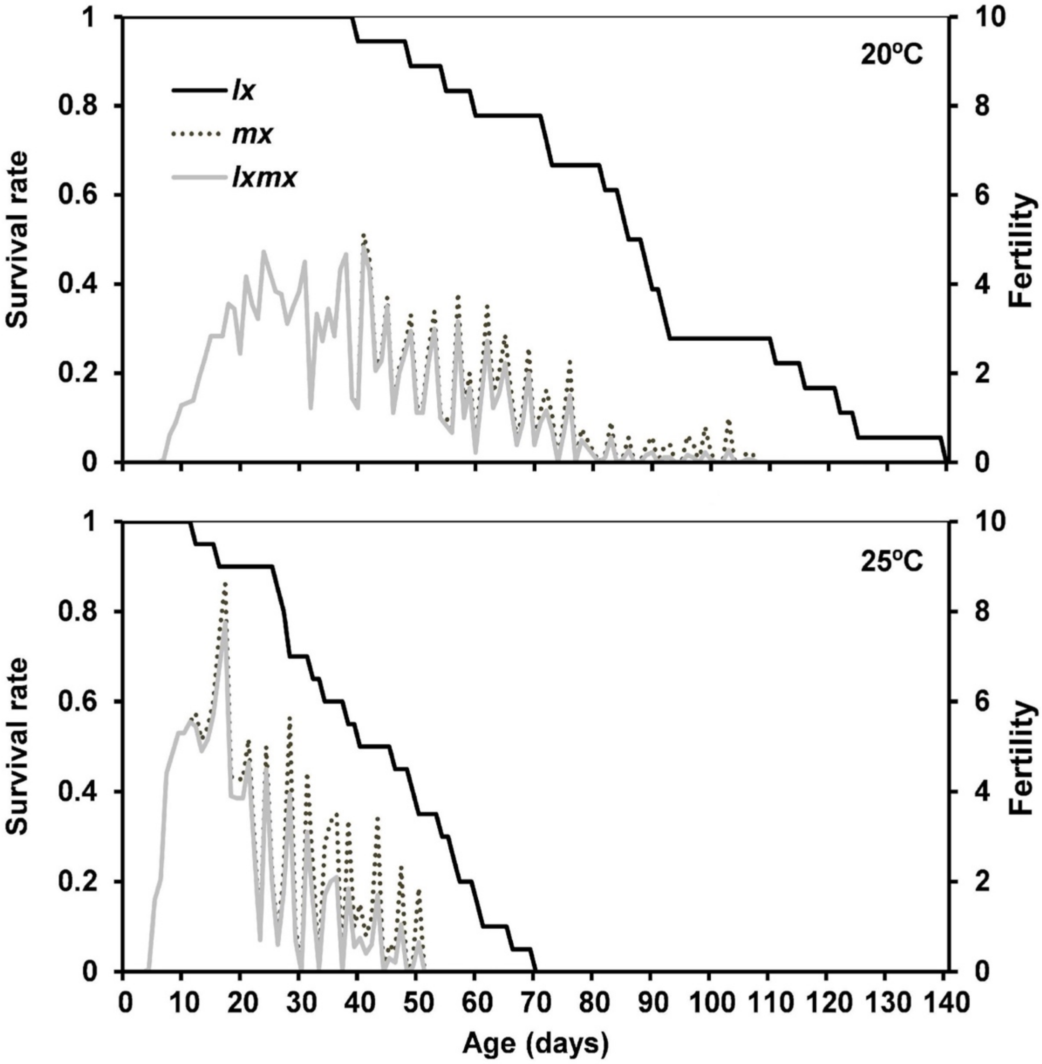

Figure 4 – Age-specific survival rate (*lx*), age-specific fertility (*mx*) and age-specific net maternity (*lxmx*) of *Dicyphus cerastii* females at 20 °C and 25 °C on tomato with factitious prey.

Table 4. Mean (±SE) pre-oviposition time (days), net reproductive rate (R_0, female offspring per female), mean generation time (T, days), intrinsic rate of increase (r_m, day^{-1}) doubling time (DT, days) and finite rate of increase (λ, day^{-1}) of *D. cerastii* on tomato with factitious prey at 20 °C and 25 °C. SE was estimated by bootstrapping (100.000 replications). p = P-value calculated using paired bootstrap tests.

	Temperature		
Demographic parameters	**20** °C	**25** °C	p
Pre-oviposition	17.3±2.0	8.6±1.5	0.0026
R_0	88.96±0.41	44.24±0.18	0.0039
T	62.85±0.05	39.03±0.08	0.0402
r_m	0.067±0.000	0.091±0.000	0.0008
DT	10.91±0.23	8.20±0.018	0.0108
λ	1.07±0.0001	1.10±0.00	0.0007

2.5. Discussion

The performance of zoophytophagous dicyphines is largely dependent on the availability of prey. Several studies report that when animal prey is absent, nymphs are either unable to complete development or display lower survival rates (Perdikis and Lykouressis 2000; Sanchez et al. 2004; Urbaneja et al. 2005; Abbas et al. 2014; Beitia et al. 2016; Biondi et al. 2016; Arvaniti et al. 2018). In the present study, *D. cerastii* was also unable to complete development in the absence of prey on all the hosts tested.

Without prey, *D. cerastii* nymphs survived longer on tomato at all the tested temperatures. A similar trend was reported for *N. tenuis* that survived longer on tomato compared to eggplant and pepper, in absence of prey (Urbaneja et al., 2005). Contrary to these results, the predator *Dicyphus hesperus* Knight (Heteroptera: Miridae) survived longer on tobacco than on tomato in the absence of prey (Sanchez et al., 2004). Plant host species can influence survival, development, and performance in dicyphines (Nakano et al., 2021; Sanchez et al., 2004; Urbaneja et al., 2005; Voigt, 2019), therefore, *D. cerastii* nymphs may have obtained better nutrition from tomato, over tobacco and Cape gooseberry.

Besides species, other plant attributes influence the performance of dicyphine mirids, such as cultivar (Siscaro et al., 2019) and organs. For example, *Dicyphus tamaninii* Wagner (Heteroptera: Miridae), in the absence of prey, can complete development while feeding on tomato fruits but not on leaves (Lucas and Alomar 2001). *Nesidiocoris tenuis* also shows better developmental success when nymphs are reared on whole tomato plants compared to excised leaves (Perdikis and Arvaniti 2016). Pollen and nectar also have nutritional value to dicyphines (Perdikis and Lykouressis 2000; Vandekerkhove and De Clercq 2010; Portillo et al. 2012; Arvaniti et al. 2018) and *D. cerastii* may also benefit from floral resources as it is observed feeding on flowers of tobacco and tomato, and recently, also on those of *Calendula officinalis* L., *Fagopyrum esculentum* Moench, and *Phacelia tanacetifolia* Benth. (our pers. obs.). Therefore, though our results suggest that *D. cerastii* may tend to the zoophagous side of the zoophytophagous spectrum, the effect of more complete plant resources on its performance and development in the absence of prey should be investigated in future research.

Development time was shorter with increasing temperature, as is often noted in other studies (Ingegno et al., 2021; Martínez-García et al., 2017; Perdikis and Lykouressis, 2000). At 15 °C, development was fastest on tomato, in which case *D. cerastii* nymphs took approximately 40 days to complete development, which is shorter than the 55.9 days that *N. tenuis* nymphs need (Sanchez et al., 2009), but similar to other dicyphines like *M. pygmaeus*, *Dicyphus eckerleini* Wagner and *Dicyphus errans* Wolff that take 42-43 days (Ingegno et al., 2021). At 20 °C there were no differences among hosts, and *D. cerastii* took 25.1 days to complete development on tomato. At this temperature it is already surpassed by *N.*

tenuis and *M. pygmaeus* that require only 21.2 and 22 days, respectively (Ingegno et al., 2021; Sanchez et al., 2009). However, at 20 °C, *D. cerastii* remains similar to *D. eckerleini* and *D. errans* (25-26 days) but faster than *Dicyphus bolivari* Lindberg (28 days) and *Dicyphus flavoviridis* Tamanini (37 days) (Ingegno et al., 2021). At 25 °C development was faster on tobacco (18.6 days) than on tomato (20.0 days) but neither were significantly different from Cape gooseberry (19.6 days). The difference between *D. cerastii* and *N. tenuis* further increases at 25 °C since the latter needs only about 13 days (Ingegno et al., 2021; Sanchez et al., 2009). At this temperature, and depending on the host, *D. cerastii* can be slower or similar to *M. pygmaeus* (ca. 17 days) (Perdikis and Lykouressis 2002b; Ingegno et al. 2021), D. eckerleini (16 days), *D. errans* (16.3-17 days) (Madeira et al. 2019a; Ingegno et al. 2021), and *D. bolivari* (ca.19 days) (Ingegno et al., 2021; Madeira et al., 2019a). The nymphs of *D. cerastii* took 16.4 days to complete development at 30 °C and had a high mortality rate (51.9%). At this temperature, *N. tenuis* needs only about 9 days (Ingegno et al., 2021; Sanchez et al., 2009), *M. pygmaeus* and *D. errans* are also faster requiring just 13 days (Ingegno et al., 2021), However *D. cerastii* was faster than *D. bolivari* that needs 18 days (Ingegno et al., 2021). At 35 °C *D. cerastii* was unable to complete development, similarly to several other European dicyphine species that cannot tolerate this temperature, with the exception *D. bolivari* (Barcelona strain) and *N. tenuis* (Perdikis and Lykouressis 2002a; Sanchez et al. 2009; Gavkare and Sharma 2017; Ingegno et al. 2021).

Adult longevity was also reduced with increasing temperature. At 15 °C, longevity was similar among hosts. At 20 °C it was highest on tobacco, and at 25 °C it was also highest on tobacco but not significantly different from Cape gooseberry. Differences in longevity between host plants have also been reported in other mirid species (Sanchez et al. 2004; Pandey et al. 2020). Males of *D. cerastii* exhibited higher longevity than females at all temperatures. However, at 20 °C this difference was not significant. Other dicyphine males like those of *M. pygmaeus* and *M. costalis* also live longer than females (Perdikis and Lykouressis 2002b; Margaritopoulos et al. 2003). By contrast, there were no differences in longevity between sexes, when tomato was further studied at a wider range of temperatures.

As expected, embryonic development was also faster as temperature increased. Similarly to *M. pygmaeus* (Perdikis and Lykouressis 2002a), *D. cerastii* eggs didn't hatch at 35 °C, whereas eggs of *N. tenuis* can still hatch at this temperature (Gavkare and Sharma, 2017; Sanchez et al., 2009). At 32.5 °C, *D. cerastii* eggs hatched but no nymph completed development. This suggests that *D. cerastii* eggs may be more suited to survive extreme conditions derived from high temperatures than young nymphs, as dicyphine eggs are protected within plant tissues.

The minimum developmental thermal threshold estimated by the Lactin-2 model for *D. cerastii* eggs was higher (8.4 °C) than that of the linear model (6.1 °C), the latter being similar to *M. pygmaeus* (6.9 °C), but lower than *N. tenuis* (9.8-12.1 °C), *M. caliginosus* (8.7 °C) and *D. hesperus* (7.3 °C) (Hart et al. 2002;

Perdikis and Lykouressis 2002a; Gillespie et al. 2004; Sanchez et al. 2009; Martínez-García et al. 2016). Similarly, the Lactin-2 model also presented a higher threshold for nymphal development (6.0 °C) than the linear model (4.7 °C) for *D. cerastii*. In both cases these values were lower than what is reported for other species like *M. pygmaeus* (9.2 °C), *N. tenuis* (11.7 °C), *M. caliginosus* (7.2 °C) and *D. hesperus* (7.8-8.01 °C) (Gillespie et al., 2004; Hart et al., 2002; Martínez-García et al., 2016; Perdikis and Lykouressis, 2002b; Sanchez et al., 2009). For total development (egg to adult) the Lactin-2 model estimated 7.5 °C whereas the Linear model estimated 6.26 °C. This was also lower than what is reported for other dicyphine species such as *M. pygmaeus* (8.8 °C), *M. caliginosus* (7.7 °C), and *N. tenuis* (10.9 °C) (Hart et al. 2002; Perdikis and Lykouressis 2002a; Mirhosseini et al. 2018)

The thermal constant K for *D. cerastii* eggs (230.36 DD) was higher than that described for *M. pygmaeus* (182 DD), *M. caliginosus* (184.8 DD), and *N. tenuis* (148.6 DD) (Hart et al., 2002; Perdikis and Lykouressis, 2002a; Sanchez et al., 2009), but identical to that of *D. hesperus* (ca. 230 DD) (Gillespie et al., 2004). Nymphs of *D. cerastii* (393.98 DD) also have higher thermal constants than *M. pygmaeus* (253 DD), *M. caliginosus* (270.3 DD), *D. hesperus* (274.4-301.9 DD), and *N. tenuis* (182.3 DD) (Gillespie et al., 2004; Hart et al., 2002; Perdikis and Lykouressis, 2002a; Sanchez et al., 2009). The same trend was found for egg to adult development, since *D. cerastii* (584.76 DD) showed higher needs than *M. pygmaeus* (431 DD), *M. caliginosus* (495 DD), and *N. tenuis* (318.4 DD) (Hart et al., 2002; Mirhosseini et al., 2018; Perdikis and Lykouressis, 2002a).

Females of *D. cerastii* produced 116.5 nymphs in their lifetime at 25 °C, which was more than other dicyphines like *D. maroccanus* (Syn. *D. bolivari*) (50.8), *M. pygmaeus* (49.2-55), *N. tenuis* (83.7), *Campyloneuropsis infumatus* (Carvalho) (81.3), but similar to *Engytatus varians* (Distant) (106.9), and *M. basicornis* (124.1) (Abbas et al. 2014; Urbaneja-Bernat et al. 2015; Silva et al. 2016; Sylla et al. 2016). *Dicyphus cerastii* daily fertility at 20 °C (2.1 nymphs/day) was lower than at 25 °C (3.7 nymphs/day), which was lower than *N. tenuis* (4.3 nymphs/day) but similar to *M. pygmaeus* (3.1 nymphs/day) and *D. maroccanus* (3.6 nymphs/day) (Abbas et al. 2014; Mollá et al. 2014). At 30 °C, very few nymphs were obtained (3 nymphs from 21 couples). Despite this, in the embryonic development experiment, eggs (laid at 25 °C) still hatched when placed at 30 °C and even 32.5 °C. This suggests that *D. cerastii* adults obtained from nymphs reared at 30 °C suffered a negative impact on their reproductive capacity. By contrast, *N. tenuis* can reproduce at 30 °C and even 35 °C (Sanchez et al., 2009).

Temperature had a clear influence on the demographic parameters of *D. cerastii*. The net reproductive rate, generation, and doubling time were all higher at 20 °C, whereas the intrinsic and finite rate of increase were higher at 25 °C. At 20 °C, *D. cerastii* had a net reproductive rate of 88.96 female nymphs per female, which was lower than *M. pygmaeus* (97.05) at the same temperature (Perdikis and Lykouressis, 2002a). At 25 °C, the net reproductive rate of *D. cerastii* was lower (44.24) than at 20 °C.

However, it was higher than *D. maroccanus* (34.52), *M. pygmaeus* (20.03) and *N. tenuis* (32.21) but similar to *Tupiocoris cucurbitaceus* (Spinola) (46.89) (Abbas et al. 2014; Mollá et al. 2014; Cagnotti et al. 2021).

The intrinsic rate of increase of *D. cerastii* increased from 0.067 to 0.091 females per female per day at 20 °C and 25 °C, respectively. *Dicyphus cerastii* increases its population at a similar rate than *M. pygmaeus* at 20 °C (r_m=0.065), and at 25 °C it may be similar, if not faster, than *M. pygmaeus*, depending on the diet of this predator (r_m =0.072-0.097) (Mollá et al., 2014; Perdikis and Lykouressis, 2002a). At 25 °C *D. cerastii* increases faster than *D. maroccanus* (r_m =0.087) but slower than *N. tenuis* (r_m =0.112) (Abbas et al., 2014; Mollá et al., 2014). In fact, even in absence of prey and reared on sesame (*Sesamum indicum* L.), *N. tenuis* can display a similar intrinsic rate of increase (r_m = 0.094) to what we found for *D. cerastii* at this temperature (Nakano et al. 2021). At 20 °C, *D. cerastii* takes 68.85 days between two generations whereas *M. pygmaeus* needs 84.50 days (Perdikis and Lykouressis 2002a). At 25 °C the generation time of *D. cerastii* is 39.03 days, whereas *N. tenuis* needs only 31.77 days, and on sesame without prey it still takes just 37.80 days (Mollá et al., 2014; Nakano et al., 2021). *Dicyphus maroccanus* and *M. pygmaeus* take 40.48 and 40.31 days, respectively, which is slightly more than *D. cerastii* (Abbas et al., 2014; Mollá et al., 2014). Besides temperature, the demographic parameters of dicyphines also depend on the diet (Cagnotti et al. 2021; Owashi et al. 2020), plant host (Nakano et al., 2021), or a combination of both (Perdikis and Lykouressis 2002b). Therefore, their impact should also be considered in future research on *D. cerastii*, as this may influence both field performance and mass rearing of this predator.

To our knowledge this is the first study to explore the life history parameters of *D. cerastii*. Our results showed that, despite significant differences on some biological traits, the host species we compared did not differ notably when prey is available. Possibly because all the studied hosts belong to the family Solanaceae. Our results also demonstrate that, in general, *D. cerastii* is outperformed by *N. tenuis* on most of the parameters we observed. This underperformance may be driven, primarily, by its slower development and higher mortality at higher temperatures (above 20 °C). We also acknowledge that the diet used in this study (a mix of *E. kuehniella* eggs and *Artemia* sp. cysts) may be considered less optimal than a diet consisting purely of *E. kuehniella* eggs. This could negatively influence the performance of *D. cerastii*. However, the high fertility observed in comparison to other dicyphines suggests otherwise.

Our results indicate that *D. cerastii* is less thermophilous than *N. tenuis*, and could be more adapted to temperate climates, as proposed for other *Dicyphus* species, such as *D. errans* and *D. eckerleini* which have relatively similar development rates as *D. cerastii* (Ingegno et al., 2021). Therefore, *D. cerastii* may be an interesting BCA for cooler conditions, such as those found in early spring in Mediterranean protected tomato crops, when *N. tenuis* does not perform well.

Direct interactions between *D. cerastii* and *N. tenuis* have been demonstrated to favor *D. cerastii* (Abraços-Duarte et al. 2021; Mouratidis et al. 2022). Despite this, *D. cerastii*, having a slower population increase rate, may be negatively affected from the outcome of indirect interactions with *N. tenuis*, such as competition for food sources or space. In fact, *N. tenuis* is reported as being able to outcompete other dicyphines like *D. maroccanus* and *M. pygmaeus* (Michaelides et al. 2017; Salas Gervassio et al. 2017).

Conservation strategies could be key to compensate for the slower population growth of *D. cerastii* and its likely lower capacity to compete with *N. tenuis*. It has been demonstrated that providing additional refuge and food sources between crop cycles allows local production, increased biodiversity, and better establishment of dicyphines on horticultural crops (Aviron et al. 2016; Balzan 2017; Perrin et al. 2019). Therefore, future research on *D. cerastii* should also focus on its conservation considering a wider range of host species.

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2.8. Supplementary material

Table S.1. Modelling mathematical equations used to fit temperature (T) and developmental rate (1/D) relationship.

Model	**Equation**	**Calculated parameters**
Linear	$1/D = a + bT$	a,b = constants
Lactin-2	$1/D = e^{(\varrho T)} - e^{(\varrho TL - (TL-T)/\Delta T)} + \lambda$	ϱ = constant LT = lethal maximum temperature ΔT = temperature range over which physiological breakdown becomes the overriding influence λ = constant
Brière-1	$1/D = aT\,(T-T_0)\,(T_L-T)^{1/2}$	a = constant T_0 = lower developmental threshold T_L = lethal threshold

Table S.2. Log-Rank test comparison of *Dicyphus cerastii* survival curves on tomato, tobacco, and Cape gooseberry at different temperatures, without factitious prey.

Comparison	χ^2	**d.f.**	p
15 °C			
tomato - tobacco	46.8	1	<0.001
tomato – C. gooseberry	62.3	1	<0.001
tobacco – C. gooseberry	0.2	1	0.7
20.0 °C			
tomato - tobacco	29.4	1	<0.001
tomato – C. gooseberry	31.8	1	<0.001
tobacco – C. gooseberry	0.1	1	0.8
25.0 °C			
tomato - tobacco	14.5	1	<0.001
tomato – C. gooseberry	4.9	1	0.003
tobacco – C. gooseberry	3.9	1	0.05
Tomato			
15-20 °C	2.4	1	0.1
15-25 °C	24.9	1	<0.001
20-25 °C	8.1	1	0.004
Tobacco			
15-20 °C	5	1	0.03
15-25 °C	16.9	1	<0.001
20-25 °C	4.5	1	0.03
Cape gooseberry			
15-20 °C	8.5	1	0.003
15-25 °C	5.8	1	0.02
20-25 °C	1	1	0.3

Table S.3. Log-Rank test comparison of *Dicyphus cerastii* survival curves on tomato, tobacco, and Cape gooseberry at different temperatures, with factitious prey.

Comparison	χ^2	**d.f.**	*p*
15 °C			
tomato - tobacco	3.9	1	0.05
tomato – C. gooseberry	0.9	1	0.3
tobacco – C. gooseberry	7.5	1	0.006
20.0 °C			
tomato - tobacco	2	1	0.2
tomato – C. gooseberry	0.1	1	0.8
tobacco – C. gooseberry	7.8	1	0.005
25.0 °C			
tomato - tobacco	10.9	1	0.001
tomato – C. gooseberry	3.7	1	0.05
tobacco – C. gooseberry	1.6	1	0.2
Tomato			
15-20 °C	39.3	1	<0.001
15-25 °C	73.7	1	<0.001
20-25 °C	30.9	1	<0.001
Tobacco			
15-20 °C	0.4	1	0.5
15-25 °C	4.2	1	0.04
20-25 °C	39.2	1	<0.001
Cape gooseberry			
15-20 °C	53.4	1	<0.001
15-25 °C	68.2	1	<0.001
20-25 °C	33.9	1	<0.001

Table S.4. Generalized linear models (GLM) analysis of the effect of the explanatory variables "host", "temperature" and "sex" on the response variables "Post-embryonic development" and "adult longevity", of *Dicyphus cerastii* reared on tomato, Cape gooseberry and tobacco at 15, 20 and 25ºC, with factitious prey.

Explanatory variable	**d.f.**	**F**	***p***
Post-embryonic development			
Host	2	32.71	<0.001
Temperature	2	2411.38	<0.001
Sex	1	1,58	0.210
Host x Temperature	4	17.40	<0.001
Host x Sex	2	2,85	0.060
Adult longevity			
Host	2	1.14	0.322
Temperature	2	135.89	<0.001
Sex	1	13.53	<0.001
Host x Temperature	4	2.62	0.035
Temperature x Sex	2	4.54	0.011

Table S.5. Longevity in days (mean ± SE) of male and female *Dicyphus cerastii* at three different temperatures (15, 20, 25 °C) with factitious prey

	Temperature		
Sex	**15 °C**	**20 °C**	**25 °C**
Male	183.3±11.2Aa	90.2±7.1Ba	50.3±4.3Ca
Female	136.7±9.1Ab	78.0±5.2Ba	39.6±2.8Cb

Means followed by the same uppercase letter within rows, or lowercase within columns, correspond to groups among which means are not significantly different for Tukey HSD test (p<0.05).

Table S.6. Generalized linear models (GLM) analysis of the effect of the explanatory variables "temperature" and "sex" on the response variables "embryonic development", "Nymph instar", "Post-embryonic development" and "adult longevity" of *Dicyphus cerastii* reared on tomato, at 15.0, 20.0, 25.0, 27.5, 30.0 and 32.5 °C, with factitious prey.

Explanatory variable	**d.f.**	**F**	***p***
Embryonic development			
Temperature	5	4619.7	<0.001
1st Instar			
Temperature	4	212.97	<0.001
2nd Instar			
Temperature	4	241.06	<0.001
3rd Instar			
Temperature	4	118.60	<0.001
Sex	4	7.36	0.007
4th Instar			
Temperature	4	189.22	<0.001
5th Instar			
Temperature	4	483.69	<0.001
Sex	1	4.43	0.037
Post-embryonic development			
Temperature	4	735.84	<0.001
Adult longevity			
Temperature	4	64.45	<0.001

Table S.7. Log-Rank test comparison of *Dicyphus cerastii* nymph survival curves on tomato at different temperatures, with factitious prey.

Comparison	χ^2	d.f.	*p*
15.0 °C – 20.0 °C	0.6	1	0.4
15.0 °C – 25.0 °C	7.6	1	0.006
15.0 ºC – 27.5 °C	3.8	1	0.05
15.0 °C – 30.0 °C	18.5	1	<0.001
20.0 °C – 25.0 °C	10.7	1	0.001
20.0 ºC – 27.5 °C	7.5	1	0.006
20.0 ºC – 30.0 °C	25.9	1	<0.001
25.0 °C – 27.5 °C	0.9	1	0.4
25.0 °C – 30.0 °C	2.2	1	0.1
27.5 ºC – 30.0 °C	6.1	1	0.01

Table S.8. Log-Rank test comparison of *Dicyphus cerastii* survival curves (from egg hatch to adult death) on tomato at different temperatures, with factitious prey.

Comparison	χ^2	d.f.	*p*
15.0 °C – 20.0 °C	30.5	1	<0.001
15.0 °C – 25.0 °C	63.9	1	<0.001
15.0 °C - 27.5 °C	71.2	1	<0.001
15.0 °C – 30.0 °C	74.2	1	<0.001
20.0 ºC – 25.0 °C	26.9	1	<0.001
20.0 °C - 27.5 °C	65.1	1	<0.001
20.0 ºC – 30.0 °C	71.7	1	<0.001
25.0 °C - 27.5 °C	20.1	1	<0.001
25.0 ºC – 30.0 °C	23.8	1	<0.001
27.5 °C – 30.0 °C	0.8	1	0.4

Table S.9. Mean values (± SE) of parameters of three models describing the developmental rate of *Dicyphus cerastii*.

Model	Parameters	Egg	N1	N2	N3	N4	N5	Post-embryonic	Total
Linear	a	-0.0265±0.0150	-0.0133±0.0455	-0.0086±0.0930	-0.0589±0,0631	-0.0310±0.0367	-0.0597±0.0210	-0.0120±0,0068	-0.0107±0,0038
	b	0.0043±0.0006	0.0110±0.0018	0.0136±0.0036	0.0157±0,0026	0.0119±0.0015	0.0091±0.0009	0.0025±0,0003	0.0017±0,0002
	K	230.36	91.17	73.80	63.60	84.13	109.47	393.98	584.76
	t_{min}	6.1	1.2	0.6	3.7	2.6	6.5	4.7	6.3
	R^2	0.9328	0.9056	0.7780	0.9232	0.9530	0.9733	0.9639	0.9749
	RSS	0.000289	0.002666	0.011140	0.002982	0.001009	0.000331	0.000035	0.000011
	AIC	-36.6256	-23.2861	-14.7078	-16.9332	-22.3496	-27.9205	-39.1676	-44.9791
Lactin-2	ϱ	0.0048±0.0003	0.0101±0.0015	0.0153±0.0023	0.0043±0.0015	0.0113±0.0005	0.0079±0.0005	0.0027±0.0003	0.0018±0.0001
	T_L (T_{max})	38.2±0.4	36.5±0.5	37.3±0.6	34.0±0.4	34.2±0.2	33.5±0.6	35.2±0.7	37.7±0.5
	ΔT	1.5±0.2	1.2±0.4	2.2±0.6	1.3±0.3	1.3±0.1	0.6±0.4	1.0±0.3	1.0±0.2
	λ	-1.04±0.01	-1.02±0.05	-1.09±0.07	-1.07±0.04	-1.05±0.01	-1.05±0.01	-1.02±0.01	-1.01±0.00
	t_{min}	8.4	1.8	5.9	5.0	4.0	5.9	6.0	7.5
	t_{opt}	30.8	31.2	29.6	28.6	28.7	30.3	29.2	29.2
	R^2	0.9957	0.9778	0.9787	0.9928	0.9987	0.9972	0.9943	0.9980
	RSS	0.000043	0.001926	0.003054	0.000855	0.000093	0.000092	0.000016	0.000002
	AIC	-54.1302	-27.5214	-24.2942	-26.1072	-39.4525	-39.5058	-49.84	-61.48
Brière-1	a	0.00006±0.00000	0.00009±0.00006	0.00023±0.00004	0.00020±0.00008	0.00015±0.00001	0.00003±0.00004	0.00002±0.00001	0.00002±0.00001
	t_{min} (T_0)	6.9±0.6	-4.7±12.9	4.8±2.8	3.3±5.3	1.6±0.7	-7.8±16.0	-1.8±7.0	3.9±3.6
	t_{max} (T_L)	37.4±0.4	40.8±5.8	35.8±0.9	35.8±2.9	36.0±0.3	61.3±46.4	40.8±5.8	38.1±3.3
	t_{opt}	30.6	32.2	29.2	29.0	29.0	48.4	32.4	30.8
	R^2	0.9986	0.9223	0.9223	0.9668	0.9996	0.9922	0.9875	0.9888
	RSS	0.000006	0.001835	0.001479	0.001290	0.000008	0.000097	0.000011	0.000005
	AIC	-58.0480	-23.5260	-24.8233	-19.1219	-44.3218	-32.0656	-42.8202	-47.0284

Dicyphus cerastii adult feeding on *Nesidiocoris tenuis* nymph, author's original

Section III - Predatory interactions of *Dicyphus cerastii* Wagner (Hemiptera: Miridae)

Chapter 3. Functional response and predation rate of *Dicyphus cerastii* Wagner (Hemiptera: Miridae)

Abraços-Duarte G, Ramos S, Valente F, Silva EB, Figueiredo E (2021) Functional response and predation rate of *Dicyphus cerastii* Wagner (Hemiptera: Miridae). Insects, 12: 530. https://doi.org/ 10.3390/insects12060530

Chapter 4. Intraguild predation and cannibalism among Dicyphini: *Dicyphus cerastii* vs. two commercialized species

Abraços Duarte G, Caldas F, Pechirra A, Silva EB, Figueiredo E (2021) Intraguild predation and cannibalism among Dicyphini: *Dicyphus cerastii* vs. two commercialized species. Entomol Exp Appl, 169: 90-96. https://doi.org/10.1111/eea.12943

Chapter 3. Functional response and predation rate of *Dicyphus cerastii* Wagner (Hemiptera: Miridae)

Gonçalo Abraços-Duarte, Susana Ramos, Fernanda Valente, Elsa Borges da Silva and Elisabete Figueiredo

3.1. Abstract

Dicyphine mirids are important biological control agents (BCAs) in horticultural crops. *Dicyphus cerastii* Wagner can be found in protected tomato crops in Portugal and has been observed feeding on several tomato pests. However, the predation capacity of this species is poorly studied. In order to investigate the predation capacity of *D. cerastii*, and how it is affected by prey size and mobility, we evaluated the functional response (FR) and predation rate of female predators on different densities of four prey species: *Myzus persicae* 1st instar nymphs (large mobile prey), *Bemisia tabaci* 4th instar nymphs, *Ephestia kuehniella* eggs (large immobile prey) and *Tuta absoluta*[1] eggs (small immobile prey). Experiments were performed on tomato leaflets in Petri dish arenas for 24 h. *Dicyphus cerastii* exhibited type II FR for all prey tested. The predator effectively preyed upon all prey, consuming an average of 88.8 *B. tabaci* nymphs, 134.4 *E. kuehniella* eggs, 37.3 *M. persicae* nymphs and 172.3 *T. absoluta* eggs. Differences in the FR parameters, attack rate and handling time, suggested that prey size and mobility affected predation capacity. Considering the very high predation rates found for all prey species, *D. cerastii* proved to be an interesting candidate BCA for tomato crops.

Keywords: Biological control, dicyphini, *Bemisia tabaci*, *Ephestia kuehniella*, *Myzus persicae*, *Tuta absoluta*, protected crops, tomato

3.2. Introduction

Tomato is an economically important crop in the Mediterranean region, in both protected and open field conditions. It is affected by several pests such as aphids (Hemiptera: Aphididae), leafminers (Diptera: Agromyzidae), mites (Acari: Tetranychidae and Eriophyidae), whiteflies (Hemiptera: Aleyrodidae), thrips (Thysanoptera), and with great importance, since its arrival in Spain in 2006, by *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Urbaneja et al. 2007).

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[1] The designation *Tuta absoluta*, instead of *Phthorimaea absoluta*, is used in this chapter since it was the adopted scientific name at the date this study was published

The biological control of pests has been used for a long time in tomato crops. For instance, the whitefly parasitoid Encarsia formosa Gahan (Hymenoptera: Aphelinidae) is mass-produced and released since the 1920s (van Lenteren 1988). More recently, tomato crops have benefited from the use of other biological control agents (BCAs) like dicyphine mirids (Heteroptera: Miridae: Bryocorinae: Dicyphini). The wide use of dicyphines is due to the fact that some of these species are zoophytophagous, which allows them to endure periods of prey scarcity by feeding on host plants and are particularly well adapted to plants with glandular trichomes like tomato (Wheeler and Krimmel 2015; Voigt 2019).

In the Mediterranean region, several dicyphine species in the genera *Dicyphus*, *Macrolophus*, and *Nesidiocoris* naturally occur on tomato crops (Lykouressis et al. 2000; Alomar et al. 2002; Castañé et al. 2004; Ferracini et al. 2012), and their role against pests is widely recognized (Lykouressis et al. 2000, 2009; Calvo et al. 2009; Ingegno et al. 2013). *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* (Rambur) are currently mass-produced and commercialized for augmentation, whereas European *Dicyphus* species provide biological control services, mostly through conservation strategies (Castañé et al. 2004; Ingegno et al. 2017b). Despite their importance and broad use, currently commercialized dicyphines can present unfavorable aspects that limit their usefulness to biological control. Plant feeding by *Nesidiocoris tenuis*, in particular, can cause severe damage to tomato (Arnó et al. 2010; Castañé et al. 2011; Pérez-Hedo and Urbaneja 2016), while *M. pygmaeus* may take a long time to establish plentiful populations on crops (De Backer et al. 2014; Sanchez et al. 2021). Because of these limitations, it is important to evaluate other European dicyphines as candidate BCAs of tomato pests, as is demonstrated by the increasing research interest in species of the genus *Dicyphus* (Bouagga et al. 2018; Sanchez and Cassis 2018; Ingegno et al. 2019; Madeira et al. 2019).

In Europe, the genus *Dicyphus* has 14 known species. Among them, *Dicyphus cerastii* Wagner is distributed along the Mediterranean region (Kerzhner and Josifov 1999; Aukema et al. 2013; Sanchez and Cassis 2018) and, in Portugal, it is commonly found in low pesticide pressure tomato greenhouses (Carvalho and Mexia 2000; Figueiredo et al. 2012, 2016). Like other dicyphines, *D. cerastii* can feed on different prey species and has been observed preying upon pests like leafminers (Carvalho and Mexia 2000), whiteflies (Figueiredo et al. 2012, 2016), *T. absoluta* (Figueiredo et al. 2016), mealybugs (Hemiptera: Pseudococcidae) and aphids (Francisco 2019). However, the extent to which this predator contributes to biological control on tomato crops is not fully understood, particularly on key indicators like prey preference, predation rate, numerical and functional response.

The functional response (FR) describes how the individual predation rate changes with increasing prey availability, and it is a major element when it comes to assessing the predatory efficacy of a BCA (Solomon 1949). Three types of FR were proposed by Holling (1959a): type I describes a linear increase of prey consumption with increasing prey density until a maximum is reached, and is mostly associated to predators like filter feeders (Jeschke et al. 2004); type II expresses a negatively density-dependent relation in which the predation rate decreases with increasing prey density and is represented by a hyperbolic curve; in type III FR, a positively density-dependent is described, in which the predation rate first increases at lower prey density and then decreases at higher prey density resulting in a sigmoidal curve. Despite some records of type III FR (van Lenteren et al. 2016; Michaelides et al. 2017; Ziaei Madbouni et al. 2017), dicyphine predators are more commonly reported to have type II FR (Alvarado et al. 1997; Maselou et al. 2015; Sharifian et al. 2015; Hassanpour et al. 2016; van Lenteren et al. 2016; Ingegno et al. 2017a; Ziaei Madbouni et al. 2017; Ingegno et al. 2019).

Type II FR is associated with unstable predator–prey dynamics (Murdoch and Oaten 1975; Van lenteren and Bakker 1975), since at lower densities, there is a risk of prey extinction, as predators are able to consume most prey available. Differently, at higher densities, predators may not consume enough prey, and thus provide limited control over prey populations. This unstable dynamic leads to associating predators exhibiting type II FR to inundative biological control programs for direct pest population reduction (van Lenteren 2012), such as mirid releases in seasonal crops like tomato, rather than long-term biological control.

Functional response type and its parameters, attack rate (a) and handling time (h), are influenced by abiotic factors including spatial complexity (Alexander et al. 2012) and temperature (Ziaei Madbouni et al. 2017), and also biotic factors like the presence of alternative prey (Murdoch 1969), prey distribution (Feng et al. 2018), and prey type and size (Kalinoski and DeLong 2016).

The aim of this study was to further understand the predation capacity of *D. cerastii*, by evaluating the influence of prey size and mobility on the shape and magnitude of its FR, and on predation rate. In laboratory bioassays, *D. cerastii* females were exposed to different densities of immobile *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) nymphs and *T. absoluta* eggs, given the economic importance of these species as pests (Desneux et al. 2010; Orfanidou et al. 2016). We also evaluated predation on, also immobile, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, as these are widely used as factitious prey in mirid mass rearing. And finally, predation was also evaluated for a mobile prey species, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae).

3.3. Materials and Methods

3.3.1 Insects

3.3.1.1 Predator

Dicyphus cerastii was originally collected from different geographical sites in Portugal. Fataca, in the south (collected from *Physalis peruviana* and *Pelargonium* sp. in gardens), Ferreira do Zêzere in central Portugal (collected on *P. peruviana* and tomato in gardens), Lisbon area (collected on tomato and *P. peruviana* in gardens), Mafra and Silveira in the Oeste region (collected on tomato greenhouses), and Póvoa de Varzim in the north (collected on tomato and tobacco in greenhouses). A mixed population colony was started from these original populations, which is frequently refreshed with wild individuals, mainly from the Oeste region. The insects used in these experiments came from this mixed population rearing kept at Instituto Superior de Agronomia (ISA). Rearing was performed in mesh cages 60 × 40 × 40 cm (Entosphinx, Pardubice, Czech Republic) set with tobacco plants about 20 cm high. To obtain young adult females (aged between 2 and 8 days after emergence) large nymphs were regularly collected from rearing cages and placed in separate cages where they were allowed to reach adulthood. The adults emerging from these cages were regularly removed and placed on a separate 35 × 35 × 35 cm cage (Entosphinx, Pardubice, Czech Republic), with access to food on tomato plants cv Montfavet (Vilmorin Iberica S.A., Alicante, Spain).

All rearing cages were kept at 25 ± 2 °C, 50 ± 20% R.H. and 14 h photoperiod, and fed a mix of eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), and *Artemia* sp. (Anostraca: Artemiidae) cysts (Entofood®, Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands) as factitious prey. Commercial bee pollen grains (Serramel®, Euromel Apicultores, Penamacor, Portugal) were also provided ad libitum (sprinkled on the leaves).

3.3.1.2 Prey

All prey, with the exception of *E. kuehniella*, were reared at ISA's Insectary, at room temperature (25 ± 2 ◦C), 50 ± 20% R.H. and a 14 h photoperiod. *Tuta absoluta* was reared from individuals collected in tomato crops from the Oeste and Alentejo regions in Portugal. Rearing units consisted of 60 × 40 × 40 cm mesh cages (Entosphinx, Pardubice, Czech Republic). In order to obtain *T. absoluta* eggs, a bouquet of fresh tomato leaves cv Montfavet (Vilmorin Iberica S.A., Alicante, Spain) was placed in plastic cups (125 mL) with water. Leaves were offered the day before bioassays to avoid egg hatching during the experimental period. Using a thin brush, fresh *T. absoluta* eggs were carefully placed on tomato leaflets to be used in experiments the same day.

Bemisia tabaci individuals were originally collected on *Gerbera* sp. crops in Montijo, Portugal. Colonies were kept in 60 × 40 × 40 cm mesh cages on cabbage *Brassica oleracea* L. cv Acephala. Every two weeks, new plants were placed inside rearing cages. To prevent the emergence of adults during the experimental period, only early 4th instar *B. tabaci* nymphs (Naranjo and Ellsworth 2017) were used. For this, after selection under a stereoscopic microscope (SMZ-2B, Nikon, Tokyo, Japan), nymphs were carefully detached and transferred onto tomato leaflets using a small brush or needle, to be used in experiments the same day.

Myzus persicae individuals, collected from rose plants in Lisbon, were mixed with others provided by Koppert España SL. and were reared in 60 × 40 × 40 cm mesh cages on pepper *Capsicum annuum* L. *cv* Piccante di Cayenna. Small nymphs (1st instar) were collected from pepper leaves with a fine brush and allowed to settle on tomato leaflets in the day of experiments.

Defrosted *E. kuehniella* eggs were obtained from the commercial product Entofood® (Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands) and only intact, undamaged eggs, were selected and placed on the tomato leaflets using a brush.

In order to consider prey dimensions, prey mass was obtained by weighing three groups of 50 individuals for each prey, using an AE200 scale (Mettler Toledo GmbH, Greiffensee, Switzerland), with a precision of 0.1 g. Prey body size was obtained from published literature.

3.3.2. Functional response Bioassays

In this study, female predators were used, as predatory heteropteran females have to mate and feed in order to mature and produce eggs (Legaspi and Legaspi 2008), and their predation is often higher than that of males (Ingegno et al. 2013; Hassanpour et al. 2016). Young *D. cerastii* females, between 2 and 8 days after emergence, collected from the adult cage, were individually starved in 15 mL plastic vials capped with moist cotton wool, for 24 h, at 25 ± 2 °C, to reduce differences related to varying hunger level.

Experimental arenas consisted of vertically inverted plastic Petri dishes (90 mm Ø, 15 mm high). A hole (5 mm Ø) was drilled on the top half of the dish and sealed with loose cotton wool to allow ventilation during experiments. The Petri dish was lined with one piece of filter paper (90 mm Ø) that was moistened with ca. 1 mL of water. This amount of water allowed sufficient leaf turgor during experiments. One or two (in higher prey densities) tomato leaflets cv Montfavet (Vilmorin Iberica S.A., Alicante, Spain) about 6–7 cm in length, were placed at the center of the dish, abaxial side up. During the bioassays, the arenas were sealed with Parafilm M®.

Each prey species was offered according to the densities in **Table 1**. Prey density was determined by preliminary tests performed to assess the upper predation limit for each prey, and to identify the signs of predation by mirids. Consumed prey was recognized as fully sucked prey items, when the predator left only a transparent empty chorion, in the case of eggs, or exoskeleton in the case of nymphs.

Table 1. Number of replicates for each prey density offered to *Dicyphus cerastii* females

Prey Species	**Density**								
	5	**10**	**20**	**30**	**50**	**100**	**150**	**200**	**300**
Bemisia tabaci	20	20	20	20	20	17	17	17	-
Ephestia kuehniella	20	20	20	20	21	17	15	15	15
Myzus persicae	20	20	20	20	20	15	-	-	-
Tuta absoluta	20	20	20	20	20	15	-	15	10

A single *D. cerastii* female was introduced into each arena and the number of consumed prey was counted after 24 h, under a stereoscopic microscope at a magnification of 50×. Consumed prey was not replaced during the experiments. The bioassays were performed in a climatic chamber (Fitoclima S600; Aralab, Rio de Mouro, Portugal) at 25 ± 1 °C, 60 ± 10% R.H., and a 14 h photoperiod.

3.3.3. Data Analysis

All FR data analyses were done in R (R Core Team 2020) with the RStudio software (RStudio Team 2020) using the package "FRAIR" (Pritchard et al. 2017). First, data were visually inspected resulting in rejection of type I functional response. In order to determine which functional response model (between type II and III) best represented the data, we fitted candidate models applying the frair_fit function that uses maximum likelihood estimation (with a binomial likelihood function) to obtain parameter estimates of the non-linear models.

For the type II functional response model, and considering that prey depletion occurred during the experiment, Rogers' random predator equation (Rogers 1972) (Equation (1)) was used:

$$N_e = N_0(1 - \exp(a(N_e h - T))) \tag{1}$$

where N_e is the number of consumed prey, N_0 is the original prey density, a is the attack rate, h is handling time and T is experimental period (days).

For the type III functional response model, and also considering prey depletion, we used the Hassel's type III extension to Rogers' random predator equation (Hassell et al. 1977). The number of consumed prey (N_e) follows the same relationship defined for Rogers' type II model, but the attack rate (a) is assumed to vary with prey density in the following hyperbolic function (Equation (2)):

$$a = bN_0/(1 + cN_0) \tag{2}$$

where b and c are coefficients to be fitted and N_0 is the original prey density.

To select the best model between type II and III FR, we followed the general approach proposed by Okuyama (2013) that suggests model selection by the application of a model selection index. In our case, the fitted models were compared using the Akaike information criterion (AIC), considering that the best model has the lowest AIC, and that a $\Delta AIC \leq 2$ between two fits indicates that both corresponding models fit the data well (Burnham and Anderson 2004).

In addition to the AIC approach, we used the method proposed by Juliano (2001) to distinguish the overall shape of FR curves, using the frair_test function. This method consists of fitting a polynomial logistic function of the proportion of prey consumed (N_a/N_0) (Equation (3)) that, at lower prey densities, is more suitable to detect slight differences in curve shape between type II and III, than a non-linear curve (Juliano 2001).

$$\frac{N_e}{N_0} = \frac{\exp\left(P_0 + P_1 N_0 + P_2 {N_0}^2\right)}{1 + \exp\left(P_0 + P_1 N_0 + P_2 {N_0}^2\right)} \tag{3}$$

where N_e is the number of prey consumed, N_0 is the initial prey density, and P_0, P_1, and P_2 are the constant, linear, and quadratic coefficients.

The sign and significance of these coefficients determine the type of functional response: significant negative linear coefficient indicates a type II functional response (declining proportional prey consumption with increasing prey density), and significant positive linear and negative quadratic coefficients suggest a type III functional response (initial increase and subsequent decrease in proportional prey consumption) (Juliano 2001; Pritchard et al. 2017). Finally, to compare the fitted coefficients, 95% confidence intervals (CIs) were generated by nonparametric bootstrapping using the frair_boot function; parameters with non-overlapping 95% CIs are considered significantly different (Pritchard et al. 2017). Functional response curves were plotted with their respective 95% CIs using the drawpoly function. In order to compare predation rates among the same prey density, we used the R package "FSA" (Dinno 2017; Ogle et al. 2021) to perform a Kruskall–Wallis test, followed by Dunn's multiple comparisons with p-values adjusted with the Holm method.

3.4. Results

Prey mass was directly related to its body size. *Tuta absoluta* eggs were the lightest prey (**Table 2**) as, on average, each egg is 0.36 mm long and 0.22 mm in diameter (2005). The remaining three prey species had similar masses (**Table 2**) and body sizes, as *E. kuehniella* eggs are 0.58 mm long and 0.33 mm wide

(Brindley 1930), *Bemisia tabaci* 4th instar nymphs are approximately 0.63 mm long and 0.39 mm wide (Thompson 2000), and *Myzus persicae* 1st instar nymphs are 0.78 mm long and 0.33 mm wide (Sylvester 1954).

Table 2. Weight (mean ± standard error) of groups of 50 prey individuals (*Bemisia tabaci* 4th instar nymphs, *Ephestia kuehniella* eggs, *Myzus persicae* 1st instar nymphs or *Tuta absoluta* eggs).

Prey Species	Weight (mg)
Bemisia tabaci	1.13 ± 0.03
Ephestia kuehniella	1.27 ± 0.03
Myzus persicae	1.33 ± 0.03
Tuta absoluta	0.67 ± 0.03

Dicyphus cerastii readily accepted all prey species tested. Average consumption increased with prey availability, reaching a maximum of 88.8 *B. tabaci* 4th instar nymphs at a density of 200, 134.4 *E. kuehniella* eggs at a density of 300, 37.3 *M. persicae* 1st instar nymphs at a density of 100 and 172.3 *T. absoluta* eggs at a density of 200 (**Table 3**).

Table 3. Number (mean ± standard error) of prey (*Bemisia tabaci* 4th instar nymphs, *Ephestia kuehniella* eggs, *Myzus persicae* 1st instar nymphs or *Tuta absoluta* eggs) consumed by *Dicyphus cerastii* females at each density in 24 hours*.

Prey	Density								
	5	10	20	30	50	100	150	200	300
B. tabaci	5.0 ± 0.0a	9.8 ± 0.1a	19.5 ± 0.2a	29.3 ± 0.4a	44.8 ± 1.1a	77.5 ± 2.6a	83.4 ± 6.1a	88.8 ± 6.7a	-
E. kuehniella	5.0 ± 0.0a	10.0 ± 0.0a	18.5 ± 0.8a	29.9 ± 0.1a	47.9 ± 0.9ab	85.1 ± 3.1ab	122.1 ± 5.0b	125.8 ± 10.6b	134.4 ± 5.8a
M. persicae	4.9 ± 0.1a	8.8 ± 0.3b	17.7 ± 0.4b	24.0 ± 1.0b	34.0 ± 2.1c	37.3 ± 2.7c	-	-	-
T. absoluta	5.0 ± 0.0a	9.9 ± 0.1a	19.9 ± 0.1a	29.9 ± 0.1a	49.4 ± 0.4b	95.1 ± 1.8b	-	172.3 ± 7.4c	171.4 ± 12.6b

* Means followed by the same letter within columns correspond to groups among which values are not significantly different for Dunn's multiple comparison test (Holm $p > 0.05$).

The results of fitting Rogers' random predator equation (type II FR) and Hassel's extension for type III are presented in **Tables 4** and **5**, respectively. The type II FR model, showed lower AIC values than type III, for each of the four prey. However, ΔAIC values indicate that both models could describe the data well (**Table 6**). The highest ΔAIC was found for B. tabaci, and coherently, a plot of attack rate, as fitted by Hassel's extension for type III FR, reveals that the attack rate of B. tabaci quickly tends to the asymptote of the hyperbolic function (b/c) that is very close to the value of attack rate obtained from fitting Rogers' random predator equation (**Figure 1**, **Table 4**). In the remaining prey species (with lower ΔAIC values), this tendency is also observed, although not as fast as in B. tabaci (**Figure 1**). Despite this, the logistic regression of the proportion of prey consumed derived significant linear coefficients for all prey species (**Table 7**), which indicates that a type II FR should be preferable in our case, as presented in the fitted curves (**Figure 2**).

Table 4. Parameters *a* (attack rate) and *h* (handling time), standard error (S.E.) estimated by maximum likelihood using Rogers' random predator equation (Type II functional response) for *Dicyphus cerastii* females feeding on different prey (*Bemisia tabaci* 4th instar nymphs, *Ephestia kuehniella* eggs, *Myzus persicae* 1st instar nymphs or *Tuta absoluta* eggs).

Prey	Parameter	Estimate	S.E.	Z *	*p*-Value
Bemisia tabaci	*a*	4.57	1.72×10^{-1}	26.49	<0.001
	h	9.60×10^{-3}	1.73×10^{-4}	55.48	<0.001
Ephestia kuehniella	*a*	4.42	1.34×10^{-1}	32.90	<0.001
	h	6.06×10^{-3}	9.76×10^{-5}	62.06	<0.001
Myzus persicae	*a*	3.28	1.77×10^{-1}	18.57	<0.001
	h	2.13×10^{-2}	7.66×10^{-4}	27.73	<0.001
Tuta absoluta	*a*	5.93	2.11×10^{-1}	28.18	<0.001
	h	4.43×10^{-3}	7.71×10^{-5}	57.40	<0.001

* z-statistics value to the test of the parameter difference from zero and the corresponding *p*-value.

Table 5. Parameters *b* and c, and *h* (handling time), and respective standard error (S.E.) estimated by maximum likelihood using Hassel's extension to Rogers's random predator equation (Type III functional response) for *Dicyphus cerastii* females feeding on different prey (*Bemisia tabaci* 4th instar nymphs, *Ephestia kuehniella* eggs, *Myzus persicae* 1st instar nymphs or *Tuta absoluta* eggs).

Prey	Parameter	Estimate	S.E.	Z *	*p*-Value
Bemisia tabaci	*b*	801.24	4.89×10^{-10}	1.64×10^{12}	<0.001
	c	175.45	2.23×10^{-9}	7.85×10^{10}	<0.001
	h	9.60×10^{-3}	1.31×10^{-4}	73.41	<0.001
Ephestia kuehniella	*b*	13.89	2.06×10^{-2}	675.55	<0.001
	c	3.11	9.08×10^{-2}	34.29	<0.001
	h	6.07×10^{-3}	9.77×10^{-5}	62.19	<0.001
Myzus persicae	*b*	3.25	3.86	8.40×10^{-1}	4.01×10^{-1}
	c	0.91	1.18	7.73×10^{-1}	4.40×10^{-1}
	h	2.17×10^{-2}	$9.65 \text{ x}10^{-4}$	22.53	<0.001
Tuta absoluta	*b*	4.28	4.14×10^{-3}	1033.38	<0.001
	c	6.98×10^{-1}	2.46×10^{-2}	28.38	<0.001
	h	4.46×10^{-3}	7.68×10^{-5}	58.13	<0.001

* z-statistics value to the test of the parameter difference from zero and the corresponding *p*-value.

Table 6. Akaike information criterion (AIC) for the two candidate functional response models for each prey (*Bemisia tabaci* 4th instar nymphs, *Ephestia kuehniella* eggs, *Myzus persicae* 1st instar nymphs or *Tuta absoluta* eggs).

Prey	Type II	Type III *
Bemisia tabaci	1252.89	1254.89 (2.00)
Ephestia kuehniella	1844.38	1846.29 (1.92)
Myzus persicae	698.53	699.77 (1.25)
Tuta absoluta	1164.98	1165.90 (0.92)

* Values in brackets represent ΔAIC: difference between AIC value for the current model and the lowest AIC value for each prey.

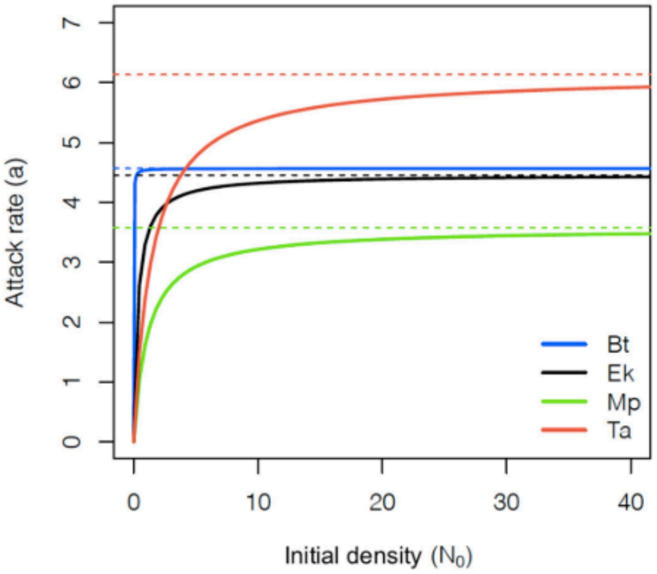

Figure 1. Attack rate as a function of initial prey density estimated by the Hassel's extension for type III functional response (Equation (2)) of *Dicyphus cerastii* females preying on *Bemisia tabaci* 4th instar nymphs (Bt), *Ephestia kuehniella* eggs (Ek), *Myzus persicae* 1st instar nymphs (Mp) and *Tuta absoluta* eggs (Ta). Dotted lines represent the asymptote of the hyperbolic function (b/c) for each prey**.**

Table 7. Estimates and respective standard error (S.E.) of the linear coefficient of logistic regression analysis of the proportion of prey (*Bemisia tabaci* 4th instar nymphs, *Ephestia kuehniella* eggs, *Myzus persicae* 1st instar nymphs or *Tuta absoluta* eggs) consumed by *Dicyphus cerastii* females in 24 hours.

Prey	Estimate	S.E.	Z *	*p*-Value
Bemisia tabaci	-1.69×10^{-2}	4.49×10^{-4}	−37.73	<0.001
Ephestia kuehniella	-1.12×10^{-2}	2.45×10^{-4}	−45.63	<0.001
Myzus persicae	-2.84×10^{-2}	1.13×10^{-3}	−25.18	<0.001
Tuta absoluta	$-1.52 \text{ x}10^{-2}$	4.36×10^{-4}	−34.75	<0.001

* z-statistics value to the test of the parameter difference from zero and the corresponding *p*-value.

Estimated parameters for the Rogers' random equation (**Table 4**) reveal that attack rate (a) was highest on *T. absoluta* eggs, followed by *B. tabaci* nymphs, *E. kuehniella* eggs and *M. persicae* nymphs. Handling time (h) was highest on *M. persicae* nymphs, followed by *B. tabaci* nymphs, *E. kuehniella* eggs, and *T. absoluta* eggs. From handling time, it was possible to calculate the theoretical maximum predation rate (1/h), which was 225.9 *T. absoluta* eggs day−1, 165.1 *E. kuehniella* eggs day−1, 104.2 *B. tabaci* nymphs day−1, and 47.1 *M. persicae* nymphs day−1. Fitting Hassel's extension for type III also resulted in similar estimates for handling time compared to type II (**Table 5**). According to the bootstrapped 95% CIs of parameter estimates (**Figure 3**, **Table S1**), attack rate did not differ between *B. tabaci* nymphs, *E. kuehniella* eggs and *T. absoluta* eggs as the correspondent 95% CIs overlapped, and the attack rate of *M. persicae* only overlapped with that of *E. kuehniella*. Handling times were different for all prey, except for *E. kuehniella* and *T. absoluta* eggs, in which 95% CIs briefly meet at 0.005 (**Table S1**).

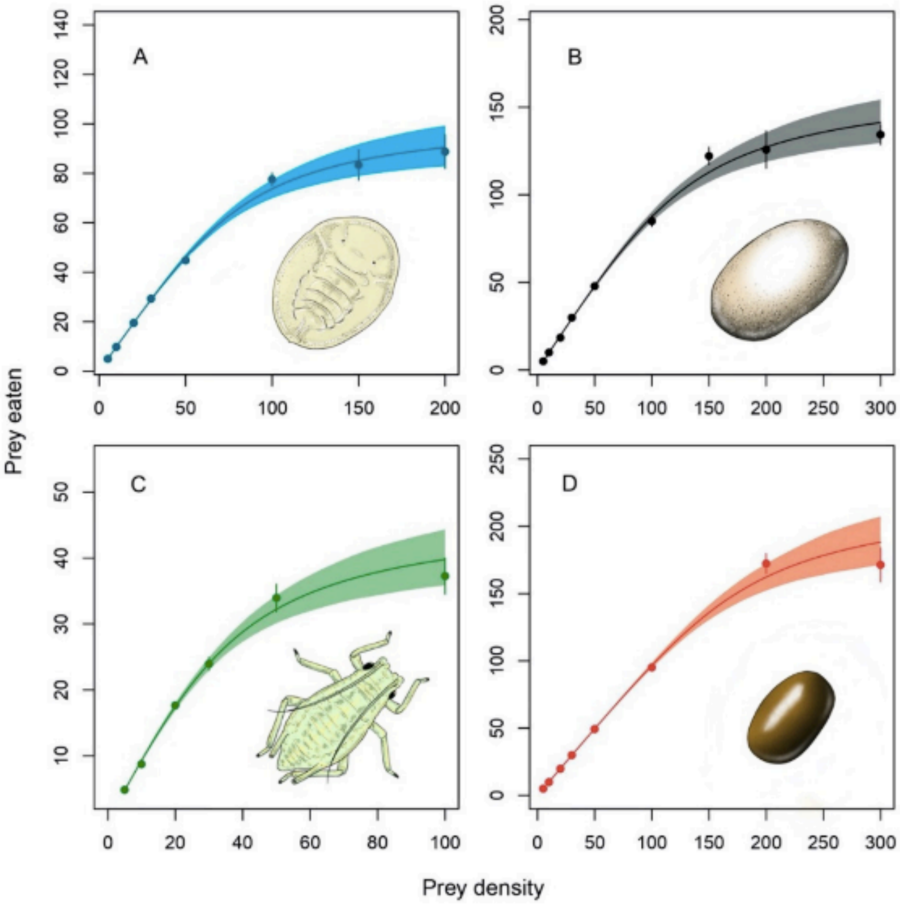

Figure 2. Functional response curves of *Dicyphus cerastii* females when preying on *Bemisia tabaci* 4th instar nymphs (A), *Ephestia kuehniella* eggs (B), *Myzus persicae* 1st instar nymphs (C) and *Tuta absoluta* eggs (D). Dots represent the average consumption and bars the respective standard error. Shaded areas represent bootstrapped 95% confidence intervals. Note that vertical and horizontal axis scales are not the same among prey species.

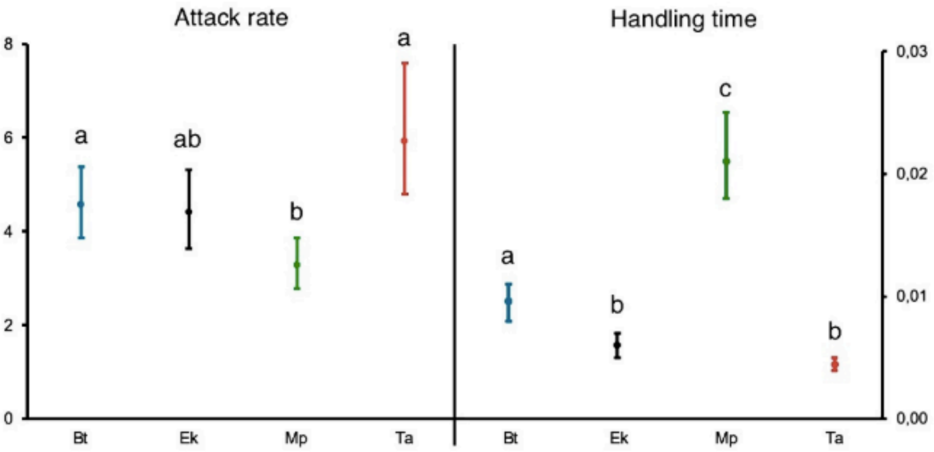

Figure 3. Estimates of functional response parameters attack rate, and handling time for *Dicyphus cerastii* females feeding on *Bemisia tabaci* 4th instar nymphs (Bt), *Ephestia kuehniella* eggs (Ek), *Myzus persicae* 1st instar nymphs (Mp) and *Tuta absoluta* eggs (Ta). Error bars represent bootstrapped 95% confidence intervals (CIs). In each parameter, overlapping 95% CIs are represented with the same letter. Note the different vertical axis scale.

3.5. Discussion

In this study, *D. cerastii* females were offered prey belonging to different species, with different characteristics of mobility and size. We found that *D. cerastii* females were able to consume the majority of prey individuals at lower densities, but predation rate decelerated as prey density increased, thus showing a type II FR for all prey tested (**Table 3**, **Figure 2**). This agrees with previous records of other dicyphine species feeding on *E. kuehniella* (Sharifian et al. 2015) and *T. absoluta* eggs (Sharifian et al. 2015; van Lenteren et al. 2016; Ingegno et al. 2019), whitefly nymphs (Hassanpour et al. 2016; Ingegno et al. 2017a; Ziaei Madbouni et al. 2017) and aphids (Alvarado et al. 1997; Fantinou et al. 2008; Maselou et al. 2015).

The FR parameters, attack rate (a) and handling time (h), determine the slope and the height of the FR curve, respectively (Holling 1959b) The attack rate is a measure of a predator's efficiency in finding prey at low prey densities, whereas handling time is defined as the time a predator stops searching for prey after a capture (Holling 1959b). In this study, prey mobility greater than prey size may have negatively affected the predator's efficiency at lower densities, as the mobile prey, *M. persicae*, had the lowest attack rate and the values of 95% CIs for attack rate overlapped among immobile prey (*E. kuehniella*, *B. tabaci*, *T. absoluta*). However, mobility could not explain why the attack rate of *M. persicae* marginally overlapped with that of *E. kuehniella* (**Figure 3**, **Table S.1**).

Prey size influences handling time, as bigger prey may require more time for manipulation and feeding (Milonas et al. 2011). Accordingly, the lowest handling time was found on the smaller prey that we offered: *T. absoluta* eggs. Despite the size difference, and similarly to attack rate, the 95% CI estimate for handling time of *T. absoluta* overlapped with that of *E. kuehniella* (**Figure 3**, **Table S1**). However, even when 95% CIs of parameter estimates overlap, parameters may still combine to produce differences in predicted consumption as a function of prey density (Pritchard et al. 2017), as was observed for these two prey species (**Figure S.1**). Handling time was different among *B. tabaci*, *E. kuehniella* and *M. persicae*, despite their similarities in size and mass (**Figure 3**, **Table S.1**). Other factors, besides prey size, can determine the feeding capacity of a predator (Kalinoski and DeLong 2016) and, in our case, prey mobility could also explain the lower predation found on M. persicae nymphs. We observed that, at higher densities, aphid dispersion in the arenas at the end of experiments was also higher. Even though the majority was found on the leaflets, consumed aphids were found dispersed throughout the arena at higher densities. In this case, predators could have spent more time searching for prey compared to the other immobile prey offered. In addition to mobility, aphids can also present defense behaviours, such as exudate secretion, which can affect predation (Butler and O'Neil 2006) and thus increase predator handling time. Despite both being immobile and size equivalent, *E. kuehniella* eggs and *B. tabaci* nymphs also had different maximum predation rates. This could be due to other prey features, that may limit predatory capacity, such as integument hardness (Kalinoski and DeLong 2016), nutritional content (Schmidt et al. 2012) and prey digestion (Papanikolaou et al. 2014).

Type II FR is associated with unstable predator–prey dynamics (van Lenteren and Bakker 1975; Murdoch and Oaten 1975). However, in more natural setups, predators displaying type II FR may be under the pressure of stabilizing elements such as temperature (Ziaei Madbouni et al. 2017), host plant species (Messina and Hanks 1998), presence of alternative prey (Murdoch 1969), prey distribution (Feng et al. 2018), prey species (Sarmento et al. 2007), prey size (Hassanzadeh-Avval et al. 2019), and spatial complexity (Alexander et al. 2012), which may approach their predatory activity to a type III FR. In our case, although the combination of the AIC method with the logistic regression indicated that a type II FR model was preferable, the low ΔAIC between type II and III models suggests that the type III model could also fit well, particularly on *E. kuehniella*, *M. persicae* and *T. absoluta* (**Table 6**, **Figure 1**). This could mean that our setup may have hampered a more evident distinction between type II and III FR, as either due to its small size, or low spatial complexity, predators could have found prey unrealistically easily, particularly at low densities.

Although traditionally more associated to vertebrates, type III FR has been increasingly reported for arthropods including dicyphine predators such as *M. pygmaeus* and *N. tenuis* (Michaelides et al. 2017), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stål) females (van Lenteren et al. 2016) feeding on *T. absoluta* eggs, and for *N. tenuis* on *B. tabaci* (Ziaei Madbouni et al. 2017). In more natural setups, like greenhouse crops, it is possible that the presence of stabilizing elements could drive dicyphine predators to display an FR closer to type III. One of such effects could be that, in more complex habitats, predation may be reduced at lower prey densities, since the ability to find prey can be affected by the availability of refuge (Alexander et al. 2012; Barrios-O'Neill et al. 2015). Additionally, horticultural crops are often colonized by different arthropods that may be preyed upon by dicyphine predators. This abundance of alternative prey can also stabilize predator–prey systems if the predator is able to switch between available prey (Murdoch 1969), which dicyphines, in general, do (Enkegaard et al. 2001). Furthermore, glandular trichome bearing plants, such as tomato, provide abundant entrapped arthropod cadavers, which may serve as lower effort prey on which these predators also feed (Carvalho and Mexia 2000). Phytophagy may also stabilize predator-prey dynamics by helping dicyphines avoid bottom-up effects of prey scarcity. However, this effect may be more important on adult predators, as plant feeding alone may affect immature development in some species (Ingegno et al. 2011; Perdikis and Arvaniti 2016). Additionally, some plant resources may reduce excessive top-down effects on prey and stabilize predator-prey dynamics, as was demonstrated for *M. pygmaeus*, which reduced its predation rate on *M. persicae* when eggplant flowers or pollen were available (Maselou et al. 2014).

Functional response outcomes, although important in understanding predator–prey dynamics, can be difficult to interpret, and mostly give a theoretical contribution to the assessment of the biological control potential of a natural enemy. Predation rate, however, provides a concrete measure of the feeding capacity of a BCA and allows for direct comparison with other predators.

We observed that, for all prey, *Dicyphus cerastii* females were highly voracious and predation often started when Petri dishes were still being sealed. *Dicyphus cerastii* was able to consume an average of 172.3 *T. absoluta* eggs, and we estimated a maximum predation rate of 225.9 eggs day^{-1}. These are higher values than those found for similar sized dicyphines like *D. bolivari* and *D. errans* that can feed on more than 130 *T. absoluta* eggs when exposed to 350 prey items but have estimated maximum predation rates of 188.52 and 197.24 eggs for *D. bolivari* and *D. errans* females, respectively (Ingegno et al. 2019). *Dicyphus cerastii* also showed higher predation than *M. pygmaeus* and *N. tenuis* which are reported to consume approximately 50 *T. absoluta* eggs daily (Urbaneja et al. 2009). Our results also indicate higher predation compared to neotropical mirid species: *Tupiocoris cucurbitaceus* (Spinola) could prey on an average of 147.45 eggs day−1 (López et al. 2019); *Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stål) females consumed an average of 51.0, 91.1 and 100.8 *T. absoluta* eggs, respectively, although these experiments were carried on tomato seedlings (van Lenteren et al. 2016).

Predation on aphids was also high, as *D. cerastii* females could consume up to an average of 37.3 *M. persicae* 1st instar nymphs day^{-1} whereas *D. tamaninii* and *M. pygmaeus* can feed on 22.8 and 21.7 1st instar nymphs of *M. persicae*, respectively (Messelink et al. 2015). *D. cerastii* also surpassed *T. cucurbitaceus* that preyed on 19.75 *M. persicae* nymphs (López et al. 2019), although these authors used mixed nymphal instars of the aphid (1st–3rd). In another study, *D. maroccanus* (syn. *D. bolivari* (Sanchez and Cassis 2018)) and *N. tenuis* females only preyed on approximately 15 1st instar nymphs of *M. persicae*, whereas *M. pygmaeus* fed on roughly 10 (Pérez-Hedo and Urbaneja 2015); however, only 20 aphids were offered initially. Despite the previously mentioned lower predation rates, in experiments with different aphid species, *D. tamaninii* females fed on 46.2 young nymphs of *Aphis gossypii* Glover on cucumber, and 43.6 *Macrosiphum euphorbiae* (Thomas) on tomato (Alvarado et al. 1997), which suggests that predation rate may depend on aphid species.

Dicyphus cerastii females could prey upon an average of 88.8 *B. tabaci* 4th instar nymphs when 200 individuals were offered, which is a much higher predation rate than that found for most other dicyphine species. *Dicyphus tamaninii* is reported to prey on an average of 12 *B. tabaci* 4th instar nymphs day−1, whereas *M. pygmaeus* could consume 5 (Barnadas et al. 1998). *Tupiocoris cucurbitaceus* females can prey on an average of 38.2 *B. tabaci* nymphs (3rd–4th instar) (López et al. 2019), and *N. tenuis* on up to 42.1 and 45.1 *B. tabaci* 4th instars day−1 at 25 ◦C and 35◦C respectively (Ziaei Madbouni et al. 2017). Finally, in a study with the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood), *D. errans* females were reported to have an estimated maximum predation rate of 114 4th instar prey (Ingegno et al. 2017a), which is similar to what we found here for *D. cerastii* on *B. tabaci* (104.2 nymphs day−1).

The predation rate on *E. kuehniella* eggs is important for mass rearing dicyphine predators, as it is widely used as factitious prey. We found that *D. cerastii* females fed on an average of 134.4 *E. kuehniella* eggs which agrees with the predation rate previously reported for *D. hesperus* that can consume approximately 139 *E. kuehniella* eggs in 24h (VanLaerhoven et al. 2003). A lower predation has been reported for *N. tenuis*, which is able to consume 58 *E. kuehniella* eggs day−1 (Malkeshi et al. 2017).

As mentioned above, our setup may have been too simple, as Petri dishes represent very simplified versions of what predators may encounter in nature. In the future, FR should be evaluated under more complex arenas, with multiple prey in order to assess the effect of stabilizing elements like spatial complexity and prey switching.

Besides FR and predation rate, there are other factors that may be important to address in future research regarding *D. cerastii*. Among these, the numerical response, or how the predator population changes with prey density (Solomon 1949), is of major importance to fully understand the potential of *D. cerastii* to regulate pests. In the case of type II predators, population size is crucial for the success of biological control, particularly at higher prey densities, when individual predation capacity may be limited. Moreover, numerical response is influenced by biological parameters that drive population dynamics such as reproductive and developmental thermal thresholds, and further information about these parameters is required for *D. cerastii*. Prey suitability also impacts predator populations, since different prey may have distinct impact on predator performance, as shown for *M. pygmaeus* (Mollá et al. 2014; Sylla et al. 2016) and *N. tenuis* (Mollá et al. 2014) females that have lower fertility when feeding on *T. absoluta* eggs compared to those of *E. kuehniella*. Determining prey preference is also essential in the case of generalist predators like dicyphines, which may disperse their predation through prey switching behaviour (Enkegaard et al. 2001). Therefore, an insight into predator preferences may help to understand and predict the efficacy of *D. cerastii* in multiple prey situations. Dicyphines can also present cannibalistic and intraguild predatory interactions (Arvaniti et al. 2019; Abraços Duarte et al. 2021). Both these direct, and indirect interactions such as competitive displacement (Michaelides et al. 2017), may also affect the success of biological control programs with these predators. Finally, it is important to note that despite their services as BCAs, dicyphines can also damage crops through plant feeding (Arnó et al. 2010; Castañé et al. 2011; Pérez-Hedo and Urbaneja 2016), and the severity of plant damage is related to mirid species (Castañé et al. 2011). So far, the phytophagy of *D. cerastii* has been studied on tomato plantlets, on which it produced necrotic spots on leaves (Figueiredo et al. 2016). Therefore, a larger assessment on the impact of phytophagy of this species should also be considered in the future.

3.6. Conclusion

This work presents the first data on the functional response and predation rate of *D. cerastii* on four different prey species. *Dicyphus cerastii* exhibited type II FR for all prey tested. There were differences in the FR parameters, attack rate and handling time, suggesting that prey characteristics such as size and mobility, had an impact on predation capacity. Overall, *D. cerastii* was quite voracious, as higher predation rates were found for all prey compared to other predatory mirids, suggesting that this species may be relevant among dicyphine BCAs. Although our experiments were carried in small arenas, these results provide a valuable insight into the predatory capacity of *D. cerastii* on different prey, particularly on important tomato pests like *T. absoluta* and *B. tabaci*, encouraging further research on the BCA potential of this predator.

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3.9. Supplementary material

Table S.1. Parameters *a* (attack rate) and *h* (handling time), standard error (S.E.) and respective bootstrapped 95% confidence intervals estimated by Rogers's random predator equation (Type II functional response) for *Dicyphus cerastii* females feeding on different prey species (*Bemisia tabaci* 4th instar nymphs, *Ephestia kuehniella* eggs, *Myzus persicae* 1st instar nymphs or *Tuta absoluta* eggs)

Prey	Parameter	Estimate	S.E.	95% CI Lower	95%CI Upper	z *	*p*-Value
Bemisia tabaci	*a*	4.566	1.724×10^{-1}	3.864	5.372	26.486	<0.001
	h	9.596×10^{-3}	1.730×10^{-4}	0.008	0.011	55.481	<0.001
Ephestia kuehniella	*a*	4.416	1.342×10^{-1}	3.632	5.307	32.900	<0.001
	h	6.057×10^{-3}	9.761×10^{-5}	0.005	0.007	62.058	<0.001
Myzus persicae	*a*	3.280	1.767×10^{-1}	2.781	3.863	18.567	<0.001
	h	2.125×10^{-2}	7.662×10^{-4}	0.018	0.025	27.732	<0.001
Tuta absoluta	*a*	5.931	2.105×10^{-1}	4.789	7.597	28.179	<0.001
	h	4.427×10^{-3}	7.711×10^{-5}	0.004	0.005	57.401	<0.001

* z-statistics value to the test of the parameter difference from zero and the corresponding *p*-value

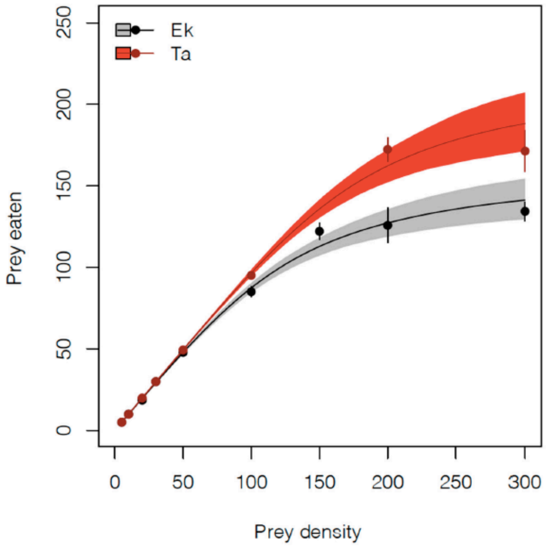

Figure S.1. Functional response curves of *Dicyphus cerastii* females when preying on *Ephestia kuehniella* eggs (Ek) and *Tuta absoluta* eggs (Ta). Black dots represent the average consumption and bars the respective standard error. Shaded areas represent bootstrapped 95% confidence intervals.

Chapter 4. Intraguild predation and cannibalism among Dicyphini: *Dicyphus cerastii* vs. two commercialized species

Gonçalo Abraços-Duarte, Filipa Caldas, Ariadna Pechirra, Elsa Borges da Silva and Elisabete Figueiredo

4.1. Abstract

Dicyphine mirids are one of the most important groups of predators on tomato. In the Mediterranean region, several species in the genera *Dicyphus*, *Macrolophus*, and *Nesidiocoris* (Hemiptera: Miridae, Bryocorinae, Dicyphini) colonize protected horticultural crops. In Portugal, *Nesidiocoris tenuis* (Reuter) is increasingly abundant in the mirid species complex of tomato crops and appears to be displac- ing the native *Dicyphus cerastii* Wagner. In order to know whether intraguild predation (IGP) can explain the decreasing abundance of *D. cerastii*, we evaluated predatory interactions between adult females and first instars of *D. cerastii* vs. *N. tenuis* but also *D. cerastii* vs. *Macrolophus pygmaeus* (Rambur), as this species is also naturally present in horticultural crops in Portugal. Cannibalistic interactions were also tested for the same three species. All experiments were performed under laboratory conditions, in Petri dish arenas, in the presence or absence of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs as alternative prey. Predation on both heterospecific and conspecific nymphs occurred only in the absence of alternative food. Intraguild predation was mutual and symmetrical between *D. cerastii* and *M. pygmaeus*. However, IGP was asymmetrical between *D. cerastii* and *N. tenuis*, favouring the first. Cannibalism was not significantly different among these mirid species. Our results show that *D. cerastii* has a greater capacity to feed on intraguild prey than *N. tenuis*. Therefore, IGP on small nymphs does not explain the abundance shift between *D. cerastii* and *N. tenuis*.

Keywords: biological control, competition, conservation, *Macrolophus pygmaeus*, Mediterranean crops, Hemiptera, Miridae, alternative prey, intraguild predation, cannibalism, tomato, *Ephestia kuehniella*, *Dicyphus cerastii*

4.2. Introduction

Mirid bugs (Hemiptera: Miridae) are important predators in tomato (*Solanum lycopersicum* L., Solanaceae). In the Mediterranean region, several mirid species colonize protected and field horticultural crops and prey on important pests (Alomar et al. 2002; Castañé et al. 2004; Ingegno et al. 2019) The genera *Dicyphus*, *Macrolophus*, and *Nesidiocoris* (Miridae, Dicyphini) are well known for their relevant role against protected crop pests (Lykouressis et al. 2000; Calvo et al. 2009; Urbaneja et al. 2009; Ingegno et al. 2013; Bouagga et al. 2018a).

Dicyphines are zoophytophagous and despite their services as predators, their phytophagous behaviour can cause economically important damage to crops under some circumstances (Castañé et al. 2011). Because of its predatory activity and wide range of prey, *Nesidiocoris tenuis* (Reuter) is commonly released in Mediterranean greenhouses. However, this species is widely recognized as capable of causing damage to tomato crops (Raman and Sanjayan 1984; Sanchez 2008; Arnó et al. 2010; Castañé et al. 2011; Pérez-Hedo and Urbaneja 2016).

Dicyphus cerastii Wagner can be found along the Mediterranean from Portugal to Turkey (Sanchez and Cassis 2018). It is able to feed on several prey species such as leafminers (Carvalho and Mexia 2000), whiteflies, *Tuta absoluta* (Meyrick), mealybugs, and aphids (Henriques, 2019). In Portugal, *D. cerastii* used to be the predominant mirid species in tomato greenhouses of the Oeste Region (Carvalho and Mexia 2000; Figueiredo et al. 2016), one of the main production zones in the country. However, in recent years, following the beginning of commercial releases, *N. tenuis* became more abundant (Figueiredo et al. 2016). *Nesidiocoris tenuis* often reaches high densities in summer crops. Growers resort to pesticides to manage these populations, resulting in disturbances to biological control services provided by other natural enemies (Figueiredo et al. 2016). Unlike *N. tenuis*, plant feeding by *D. cerastii* does not cause necrotic rings. Some lesions (necrotic spots) have been observed on leaves and stems, in cases of very high populations (Figueiredo et al. 2016). Portuguese growers tend to tolerate this type of phytophagy, as it is rarely observed and does not cause plant stems to break like that of *N. tenuis*.

Dicyphus cerastii could be an alternative to *N. tenuis* as it is a polyphagous predator and shows more acceptable phytophagous behaviour. The efficacy of *D. cerastii* on tomato crops depends not only on its predatory capacity, but also on other factors such as the outcome of both direct and indirect interactions with other mirid species that share the same ecological niche.

Intraguild predation (IGP) is a direct interaction between organisms of the same trophic guild. It can be characterized by its intensity (as the number of IGP interactions between opponents) and direction (unidirectional or mutual, if the two predators feed on each other). Intraguild predation is symmetrical if similar levels of predation occur between the two species, or asymmetrical if one tends to prey more than the other (Lucas et al. 1998; Lucas 2005). Intraguild predation is pervasive among generalist predators (Polis et al. 1989; Lucas 2005) and has been reported for Dicyphini species that share the same plant host (Moreno-Ripoll et al. 2012; Perdikis et al. 2014; Salas Gervassio et al. 2017).

In the present study, we tried to understand whether IGP is behind the abundance shift between *D. cerastii* and *N. tenuis* where the two species coexist. Under laboratory conditions, we assessed the occurrence of predation between adult females of *D. cerastii* and first instars of *N. tenuis* and vice versa. The same procedure was repeated between *D. cerastii* and *Macrolophus pygmaeus* (Rambur), as this species is also present, although less frequently, on Portuguese tomato crops (SG Seabra, cE3c – Centre for Ecology Evolution and Environmental Changes, Lisbon, pers. com.) Besides IGP, cannibalistic interactions were also evaluated for each species.

4.3. Materials and Methods

4.3.1. Insect rearing

Dicyphus cerastii and *N. tenuis* were originally collected from tomato crops in different locations in Portugal and kept in rearing cages in the laboratory. Individuals used in bioassays were sourced from mixed population rearing cages. *Macrolophus pygmaeus* was collected from tomato at Instituto Superior de Agronomia in Lisbon. All species were kept at room temperature (25±2 °C), 50–70% r.h., and L14:D10 photoperiod, in 60 x 40 x 40 cm mesh cages (Entosphinx, Pardubice, Czech Republic) with two tobacco plants of approximately 30 cm high. Once a week, a mix of eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and *Artemia* sp. cysts (Entofood; Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands) was provided ad libitum as factitious prey. Pollen collected by honeybees, *Apis mellifera* L. (Serramel, Euromel- Apicultores, Penamacor, Portugal), was also provided weekly ad libitum.

In order to obtain young nymphs, 5–8 adult pairs were placed in a 35 x 35 x 35 cm mesh cage with two tomato plants *cv*. Montfavet (Vilmorin Iberica, Ali- cante, Spain). Once a week, the plants were changed and new food was provided. Dead insects were replaced by new ones of the same sex. The old plants were placed in separate cages for egg hatching. Emerging first instars were collected daily into a ventilated 150-ml plastic cup with tomato leaflets and factitious prey ad libitum (Entofood sprinkled on the leaflets). These first instars were used in bioassays the same day of collection. When they were not needed, the nymphs were released into juvenile rearing cages, and allowed to reach adulthood.

4.3.2. Arenas

The bioassays were performed in plastic Petri dish arenas (90 mm diameter, 15 mm high). The dish was inverted vertically, and a hole (5 mm diameter) was drilled on the top half. During experiments, this hole was sealed with cotton wool to reduce excessive condensation.

In preliminary tests, the leaf petiole was inserted into an Eppendorf vial filled with water and sealed with cotton wool. However, it was observed that some nymphs would hide in the cotton, which could reduce predation opportunities. We altered the initial design for one in which the Petri dish was lined with one piece of 90-mm-diameter filter paper that was moistened with 1 ml of water. This amount of water was enough to keep leaf turgor during experiments, while keeping the arena sufficiently dry for the insects to walk under good conditions. A tomato leaflet (*cv*. Montfavet) of about 4–5 cm long was placed in the centre of the dish, abaxial side up. The leaflets had a natural concave shape that allowed adult predators to fully inspect both sides of its surface. During the bioassays, the arenas were sealed with Parafilm M laboratory film.

4.3.3. Intraguild predation and cannibalism bioassays

For each of the three species, adult females of between 1 and 7 days old were collected from rearing cages and placed for 24 h in a 35 x 35 x 35 cm cage with access to water, food, and a tobacco plant. After this, to reduce differences related to varying hunger levels, females were starved individually for 24 h in 15-ml plastic vials capped with moist cotton wool, at 25±2 °C.

We tested the predation of adult *D. cerastii* females on first instar (≤24 h old) of *M. pygmaeus* and *N. tenuis* and vice versa; cannibalism was also evaluated for each species. We opted for first instars, as they should be more susceptible to predation given their size. Controls were set for each species and consisted of one first instar alone in the Petri dish, to record non-predation mortality.

All treatments were replicated at least 30x with and without *E. kuehniella* eggs as alternative prey. The experiments were performed in the Insectary of Instituto Superior de Agronomia in climatic chamber (Fitoclima S600; Aralab, Rio de Mouro, Portugal) at 25±1 °C, 60±10% r.h., and L14:D10 photoperiod.

Observations were made at 2, 4, 6, 8, 22, and 24 h in order to produce the survival curve of each treatment. At each observation, Petri dishes were checked, and mortality or predation was recorded. The bioassays ended when nymphs were found dead, preyed on, or survived after 24 h.

4.3.4. Statistical analysis

Pearson's χ^2 and two-tailed Fisher's exact test were used to compare differences in cannibalism between species and in intraguild predation when *D. cerastii* was the predator or the prey. Survival was compared with Kaplan-Meier survivorship analysis, and survival time was compared with Log-Rank (Mantel-Cox) tests. All statistical analyses were performed with IBM SPSS statistics v.25 (IBM, Armonk, NY, USA).

4.4. Results

4.4.1. Mortality

Nymphal mortality in controls was low, approximately 3 and 1% in bioassays with (n = 93) and without (n = 91) alternative food, respectively. In the other IGP treatments, non-predation mortality (events when nymphs were found dead but not entirely consumed in the presence of a predator) were always equal or superior to mortality in controls (approximately 4 and 5% in experiments with and without alternative food, respectively). These partially consumed prey were included as predation events in our analysis.

4.4.2. Cannibalism

Cannibalism did not occur in any of the tested combinations when alternative food was present. In the assay without alternative prey, some females of all three predator species preyed on conspecific first instars (**Table 1**) but there was no difference among species (Fisher's exact test: P = 0.72). Nymph survival was also not different among the three species (v2 = 1.154, d.f. = 2, P = 0.56; n = 93; **Figure 1**).

4.4.3. Intraguild predation

Dicyphus cerastii as predator; no IGP predation was recorded in the experiments with alternative food. In treatments without alternative food, the predation of *D. cerastii* females differed among prey nymph species (χ^2 = 20.670, d.f. = 2, P<0.001; n = 93). Predation on *N. tenuis* occurred more often than on *M. pygmaeus* and conspecific nymphs (**Table 1**). Survival of nymphs also differed among species (χ^2 = 25.693, d.f. = 2, P<0.001; n = 93); the mean survival of *N. tenuis* nymphs was 12.9±1.9 h, whereas *D. cerastii* nymphs survived 22.1±1.0 h and *M. pygmaeus* 22.3±1.0 h (**Figure 2**).

Dicyphus cerastii as prey; no IGP predation was recorded in the experiments with alternative food. Predation on *D. cerastii* nymphs did not differ among adult predator species (χ^2 = 2.033, d.f. = 2, P = 0.40; n = 98). *Dicyphus cerastii* nymph survival was not different among adult predators (χ^2 = 1.834, d.f. = 2, P = 0.40; n = 98), and showed similar survival curves for both cannibalism and heterospecific predation (**Figure 3**).

Table 1. Number of replicates in which predation occurred when adult females of *Dicyphus cerastii*, *Macrolophus pygmaeus*, and *Nesidiocoris tenuis* were paired with first instars of the same or different species for 24 h, in the absence of alternative prey.

Adult predator species	Nymph prey species		
	D. cerastii	*M. pygmaeus*	*N. tenuis*
D. cerastii	4 (n = 31)	5 (n = 32)	18 (n = 30)
M. pygmaeus	7 (n = 34)	2 (n = 31)	-
N. tenuis	9 (n = 33)	-	2 (n = 31)

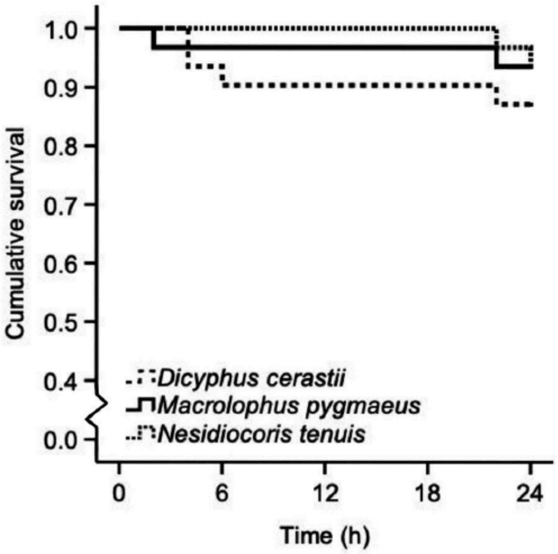

Figure 1. Cumulative survival of first instars of *Dicyphus cerastii*, *Macrolophus pygmaeus*, and *Nesidiocoris tenuis* in cannibalism experiments without alternative prey.

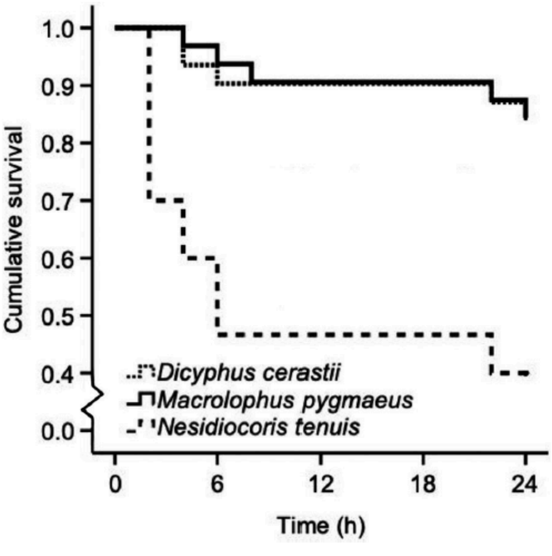

Figure 2. Cumulative survival of first instars of *Dicyphus cerastii*, *Macrolophus pygmaeus*, and *Nesidiocoris tenuis* under predation by *D. cerastii* females without alternative prey.

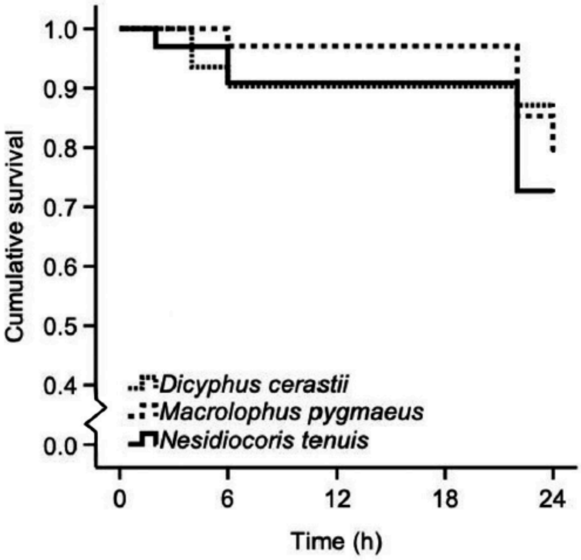

Figure 3. Cumulative survival of first instars of *Dicyphus cerastii* under predation by adult females of *D. cerastii*, *Macrolophus pygmaeus*, and *Nesidiocoris tenuis* without alternative prey.

4.5. Discussion

Optimal foraging theory predicts that predators should focus primarily on consuming prey that maximizes gains in nutrients and energy (Stephens and Krebs, 1986). Therefore, generalist predators like Dicyphini mirids should preferably feed on easy-to-handle and abundant prey. Several studies relate the occurrence of predatory interactions between mirids with the availability of alternative prey (Lucas et al. 2009; Moreno-Ripoll et al. 2012; Hamdi et al. 2013a; Perdikis et al. 2014). Abundance, quality and suitability of alternative food resources impact IGP among mirid species too (Lucas et al. 2009). In the present study, this influence was also demonstrated, as predatory interactions occurred exclusively in the absence of *E. kuehniella* eggs.

Cannibalism has been documented previously for Dicyphini species. *Macrolophus pygmaeus* (Hamdi et al. 2013b), *N. tenuis* (Moreno-Ripoll et al. 2012; Salas Gervassio et al. 2017), *Dicyphus tamaninii* Wagner (Castañé et al. 2002), *Dicyphus errans* (Wolff) (Arvaniti et al. 2019), and *Dicyphus hesperus* Knight (Laycock et al. 2006) all resort to cannibalism when alternative prey is scarce. In our study, cannibalism was also observed for all species in the absence of *E. kuehniella* eggs.

Intraguild predation was mutual in all tested species. Nevertheless, in the present study, only first instars were presented to predators as this stage should be the most susceptible to predation. Intraguild predation between *D. cerastii* and *M. pygmaeus* was symmetrical and each species preyed on the other in 10% of cases. Lucas and Alomar (2000) found that young nymphs of *M. pygmaeus* were more susceptible to predation by *D. tamaninii* than vice versa. In our study, interactions between *D. cerastii* and *N. tenuis* were asymmetrical and *D. cerastii* preyed on *N. tenuis* nymphs in 60% of cases. However, *N. tenuis* females still killed *D. cerastii* nymphs in 20% of cases. Furthermore, survival time of nymphs preyed upon by *D. cerastii* also differed significantly. The mean survival of *N. tenuis* was lower than that of *D. cerastii* or *M. pygmaeus* nymphs. Salas Gervassio et al. (2017) found a different result in the *Dicyphus maroccanus* Wagner–*N. tenuis* system; *D. maroccanus* females preyed on *N. tenuis* first instars in fewer than 5% of cases, but *N. tenuis* females killed *D. maroccanus* first instars in 30% of cases.

The outcome of predatory interactions can depend on the relative body size of adversaries (Rosenheim et al. 1995; Lucas 2018). In fact, *D. cerastii* is comparatively larger than *N. tenuis* and *M. pygmaeus* (Sanchez et al. 2006; Sanchez and Cassis 2018) and may be able to subdue the same prey more easily. This can also explain the difference between our results and those of Salas Gervassio et al. (2017) with *D. maroccanus*, as this species is smaller than *D. cerastii* (Sanchez & Cassis, 2018). However, this cannot explain why more *N. tenuis* nymphs were attacked, compared to those of *M. pygmaeus*, as they have similar body sizes.

The occurrence of IGP, although common among generalist predators (Polis et al., 1989; Lucas, 2005), may not necessarily impact the success of biological control (Lucas and Alomar 2002; Taylor et al. 2005; Janssen et al. 2006; Messelink and Janssen 2014; Michaelides et al. 2017). Despite the higher predation on *N. tenuis*, *D. cerastii* females only preyed on nymphs when no alternative food was provided.

Laboratory tests in Petri dishes represent an oversimplified two-dimensional arena for insect interactions. In preliminary tests, we used cotton discs rather than filter paper in our setup, and we observed that first instars could hide and avoid predation regularly. Therefore, it is possible that our experiment may be making young nymphs unrealistically vulnerable compared to the natural situation on plants with 3D architecture.

Plant structure can influence the occurrence of IGP, as Dicyphini species may not prefer the same plant strata. Arnó et al. (2010) reported that *N. tenuis* concentrates on the top part of the plant; Perdikis et al. (2014) reported this as well, and also noted that *M. pygmaeus* prefers mid-level parts of tomato plants. Similarly, Figueiredo et al. (2016) found that *D. cerastii* nymphs and adults are more abundant on the middle section of tomato plants, and *N. tenuis* on the upper parts. Therefore, the presence of *D. cerastii* in greenhouses where *N. tenuis* or *M. pygmaeus* are augmented for biological control may not necessarily impact their efficacy as biological control agents.

Indirect competition can be more important than direct interactions between mirid species. Salas Gervassio et al. (2017) reported that *N. tenuis* can be a better competitor than other species of mirids, describing its greater capacity to obtain food and reach larger population sizes compared to other mirids in eastern Spain tomato crops. Michaelides et al. (2017) also found that *N. tenuis* is a better competitor than *M. pygmaeus* when feeding on *T. absoluta* eggs, so it is possible that *D. cerastii* may also be outcompeted by *N. tenuis*.

Biological traits may also explain the competitive advantage of *N. tenuis*. Among them, thermal development requirements may be particularly important. Nymphal development time of *N. tenuis* was shorter than that of *D. errans* at 25 °C when feeding on *E. kuehniella* eggs on tomato plants (Perdikis and Arvaniti 2016). *Nesidiocoris tenuis* is more thermophilic than other Mediterranean Dicyphini species (Sanchez et al. 2009). Reproductive capacity may also represent an advantage to *N. tenuis*. At 25 °C, the fertility of *N. tenuis* (83.7 nymphs per female) was significantly higher than that of *M. pygmaeus* (48.1 nymphs per female) (Mollá et al. 2014). Abbas et al. (2014) reported a fertility of 50.8 nymphs per female for *D. maroccanus*, and Saleh (2002) observed a fecundity of 68.1 eggs per female for *D. tamanini* at 25 °C, which were also lower than that of *N. tenuis*.

Recent studies have shown that mirids induce physiological defensive responses in plants such as the abscisic (ABA) and jasmonic (JA) acid pathways (Pappas et al. 2015; Pérez-Hedo et al. 2015b, a). Mirids can also activate direct plant defenses normally associated with herbivory, as shown by Pappas et al. (2015), who demonstrated a worse performance of *Tetranychus urticae* Koch on plants previously exposed to *M. pygmaeus* due to an increase of proteinase inhibitors induced by the mirid. The activation of plant defenses can also start the production of herbivore-induced plant volatile compounds (HIPVs) (Bouagga et al. 2018b; Pérez-Hedo et al. 2018), and these compounds can differ among mirid species as demonstrated by Pérez-Hedo et al. (2015a,b): plants that were previously colonized by *N. tenuis* were less attractive to herbivores such as *Bemisia tabaci* (Gennadius) and *T. absoluta*, whereas *M. pygmaeus* and *D. maroccanus* did not induce the same response. The response to mirid HIPVs may also differ among mirid species, as Lins et al. (2014) reported that *M. pygmaeus* avoided tomato plants previously damaged by conspecifics whereas *N. tenuis* was attracted to them. Therefore, it is possible that phytophagy by *N. tenuis* could induce plant defense responses that may reduce its quality or attractiveness to *D. cerastii.*

4.6. Conclusion

To our knowledge, this study represents the first record of cannibalism and intraguild predation in *D. cerastii.* Overall, *D. cerastii* was more aggressive towards *N. tenuis* than vice versa. These results contrast with field observations that suggest that *N. tenuis* may be displacing *D. cerastii* populations. Intraguild predation is therefore an unlikely explanation for the increasing abundance of *N. tenuis*, as IGP interactions were found to favour *D. cerastii*. Indirect competition could be the main driving force behind the changing dynamics of these species. Further research is needed, both in the field and semi-field level, in order to fully understand how competitive displacement may influence the populations of these mirid predators and what measures may effectively contribute to the conservation of *D. cerastii* in protected crops.

4.7. Acknowledgments

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Dicyphus cerastii adult feeding on tomato leaf, author's original

Section IV – Phytophagy in *Dicyphus cerastii* Wagner

Chapter 5. Half friend, half enemy? Comparative phytophagy between two Dicyphini species (Hemiptera: Miridae)

Souto P, Abraços-Duarte G, Silva EB, Figueiredo E (2022) Half friend, half enemy? Comparative phytophagy between two Dicyphini species (Hemiptera: Miridae). Insects, 13: 175. https://doi.org/10.3390/insects13020175

Chapter 5. Half friend, half enemy? Comparative phytophagy between two Dicyphini species (Hemiptera: Miridae)

Paula Souto, Gonçalo Abraços-Duarte, Elsa Borges da Silva and Elisabete Figueiredo

5.1. Abstract

Despite their importance as biological control agents, zoophytophagous dicyphine mirids can produce economically important damage. We evaluated the phytophagy and potential impact on tomato plants of *Dicyphus cerastii* and *Nesidiocoris tenuis*. We developed a study in three parts: (i) a semi-field trial to characterize the type of plant damage produced by these species on caged tomato plants; (ii) a laboratory experiment to assess the effect of fruit ripeness, mirid age, and prey availability on feeding injuries on fruit; and (iii) a laboratory assay to compare the position of both species on either fruit or plants, over time. Both species produced plant damage, however, although both species produced scar punctures on leaves and necrotic patches on petioles, only *N. tenuis* produced necrotic rings. Both species caused flower abortion at a similar level. Overall, *N. tenuis* females produced more damage to tomato fruit than *D. cerastii*. There was an increased frequency of *D. cerastii* females found on the plants over time, which did not happen with *N. tenuis*. Our results suggested that, although *D. cerastii* caused less damage to fruit than *N. tenuis*, it still fed on them and could cause floral abortion, which requires field evaluation and caution in its use in biological control strategies.

Keywords: biological control, *Dicyphus cerastii,* fruit injury, omnivorous predator, *Nesidiocoris tenuis*, plant damage, protected crops, tomato, zoophytophagy

5.2. Introduction

Zoophytophagous mirid species (Hemiptera: Miridae) are important biological control agents in several crops. Dicyphine (Miridae: Bryocorinae: Dicyphini) species, such as *Nesidiocoris tenuis* (Reuter), and several species of the genera *Macrolophus* Fieber and *Dicyphus* Fieber, are used worldwide as generalist predators (van Lenteren, 2012) on several vegetable crops, both in conservation and augmentative biological control strategies.

Dicyphus cerastii Wagner is a Palearctic mirid, reported in the Mediterranean Basin, which spontaneously colonizes Portuguese greenhouses (Figueiredo et al., 2012). It is currently being evaluated as a candidate biological control agent (BCA), since it can feed on several horticultural pests (Abraços-Duarte et al., 2021). *Nesidiocoris tenuis* is currently commercialized and released to control whiteflies and *Tuta absoluta* (Meyrick)[2] in Mediterranean greenhouses (Figueiredo et al., 2016; Urbaneja-Bernat et al., 2015; van Lenteren, 2012).

Dicyphine mirids may resort to phytophagy in periods of prey scarcity (Castañé et al., 2011; Gillespie et al., 2012), and to obtain water (Sinia et al., 2004) and nutrients (Gillespie and Mcgregor, 2000) from plants. Despite being advantageous as a feeding strategy, phytophagy can have negative effects in an agronomical context. Plant feeding may lead to a decrease in predation activity (Gillespie and Mcgregor, 2000; Han et al., 2015), and, more importantly, phytophagy can cause damage of economic importance, such as necrotic rings in stems and leaf petioles, as well as flower or fruit abortion, and punctures in the fruit (Castañé et al., 2011; Moerkens et al., 2020, 2016; Puentes et al., 2018; Sanchez, 2009). This is particularly evident with *N. tenuis*, which is often the target of pesticide sprays that are used to control its populations when there is a risk of plant damage occurring, a practice that negatively impacts other natural enemies present on crops.

The increasing demand for food products without pesticide residues, combined with the need to control pests, highlights the urgency for sustainable alternatives that reduce negative effects on both the consumer and the environment's health.

The use of predatory mirids in biological control has been very successful in protected tomato crops (Moerkens et al., 2017; Urbaneja-Bernat et al., 2015; Urbaneja et al., 2012), despite the damage produced by some species. Therefore, to enhance biological control in tomato crops, research should focus on less phytophagous yet efficient dicyphine predators.

Plant damage can greatly vary with host plant species (Castañé et al., 2011), mirid species (Castañé et al., 2011; Pérez-hedo et al., 2021; Roda et al., 2020), and even among populations of the same species (Chinchilla-Ramírez et al., 2020; Dumont et al., 2017). Therefore, understanding the risk that phytophagy represents to crops is a key point in the evaluation of a new candidate BCA (Puentes et al., 2018), such as *D. cerastii*, that will help decision making while choosing the best suited mirid species. Although damage caused by *N. tenuis* has been studied (e.g., Calvo et al., 2009; Castañé et al., 2011; Fantinou et al., 2009; Sanchez, 2009), little is known about those induced by *D. cerastii*. The latter has been observed producing chlorotic punctures on leaves (Figueiredo et al., 2016) and also necrotic damage on tomato stems and leaf petioles, as well as feeding punctures on fruit (our pers. obs.). However, the influence of its damage on plant development and possible economic impacts has never been evaluated or compared with other mirid species.

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[2] The designation *Tuta absoluta*, instead of *Phthorimaea absoluta*, is used in this chapter since it was the adopted scientific name at the date this study was published

We hypothesized that *D. cerastii* and *N. tenuis* display different phytophagous behaviour. Within this context, the aim of this study was to compare the phytophagy and potential impact on tomato production of these two Dicyphini species. For this, we characterized the type of plant damage produced by these species on tomato plants. Then, we assessed the effect of fruit ripeness, mirid age, and prey availability on feeding injuries on tomato fruit. Finally, we compared the location of both species on either tomato fruit or plants.

5.3. Materials and Methods

5.3.1. Rearing of mirid predators

Stock colonies of both species (*D. cerastii* and *N. tenuis*) are maintained at the Instituto Superior de Agronomia (ISA), Lisbon, Portugal, on tobacco plants (*Nicotiana tabacum* L.). The colony of *D. cerastii* was started with individuals from different geographical sites in Portugal (Fataca, Ferreira do Zêzere, Lisbon, and Póvoa de Varzim) and is frequently refreshed with individuals, mostly from the Oeste region (Mafra and Silveira). The colony of N. tenuis was started with individuals from the Oeste region (Silveira) and from Koppert Biological Systems (The Netherlands). For rearing details, see Abraços-Duarte et al. (2021). Young adult females (between 1 and 7 days old), for all three bioassays, were obtained from the regular collection of large nymphs from breeding cages that were transferred to separate cages, where they could reach adulthood. For nymph experiments (see Section 5.3.3, Fruit damage), 4th/5th instar nymphs were collected from immature rearing cages.

5.3.2. Phytophagy in semi-field conditions

Phytophagy was observed for *N. tenuis* and *D. cerastii* in semi-field conditions in a greenhouse at ISA's campus. For this, mesh cages (1.5 m high and 1.0 m wide) were used. In each cage, there were two tomato plants (cv. Vayana), each one in a 15 L pot. Plants were fertilized and watered as needed, using an organic fertilizer solution (Húmus Líquido Horta®, SIRO, Mira, Portugal). When plants had 4 to 5 developed leaves (ca. 30–40 cm high), six couples of *D. cerastii* or *N. tenuis* were released. Control cages were set without any insects. Each treatment (12 *D. cerastii*, 12 *N. tenuis* and a control) had five replicates. To simulate the natural presence of prey, a teaspoon (ca. 4 g) of a mix of *Artemia* spp. (Anostraca: Artemiidae) and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (Entofood®, Koppert Biological Systems) was sprinkled evenly on the plants at the time of insect release, and every two weeks after that. Six weeks after release, all insects in the cages were captured into vials containing 96% ethanol and counted. Plant damage, expressed as the total number of necrotic rings or necrotic patches, was recorded for every cage. Flower abortion was counted as the proportion of missing flowers/total number of flowers in the flower rachis, for every cage. When trusses already had small fruit, these were counted as flowers. The mirids were released on 28 April 2021, and the assay ended on 11 June 2021.

5.3.3. Fruit damage

The following factors were considered to compare the puncture level on fruit: mirid species (*D. cerastii* and *N. tenuis*), mirid age (nymph and adult female), tomato ripeness (unripe and fully ripe), and availability of food and/or water. For each species, three adult females or three nymphs were placed in plastic cups (8 cm high and 6 cm diameter) with one tomato fruit that was approximately 4–5 cm long. The lid of the cups had a hole (3 cm Ø) covered with fabric to allow ventilation. Four treatments were considered (15 replications), for each species, mirid age, and tomato ripeness: (a) fruit only (N); (b) fruit with water (W) (supplied through an Eppendorf vial with moist cotton wool); (c) fruit with water and alternative food (FW) (*Ephestia kuehniella* eggs and *Artemia* spp. cysts Entofood®, Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands), supplied on a sticky paper strip (2.0 cm × 0.8 cm); (d) fruit with alternative food (F) (Entofood®) but no water. Tomato fruit (**Figure 1**, cv. Suntasty) were obtained from an organic commercial tomato greenhouse. Before the experiment, the fruit were washed with abundant water and individually inspected for any marks or possible feeding punctures. Fruit with any defect were discarded. The unripe condition was considered as fully grown green fruit and fully ripe was considered as fully red fruit. For each bioassay, a control (i.e., only fruit) was made to ensure that punctures were caused by mirids. Insects were allowed to feed for 24 h, after which punctures (injuries resulting from fruit feeding) were counted under a stereomicroscope with a magnification of 50×. Injury was considered as a puncture surrounded by a small whitish or yellowish halo (Moerkens et al., 2016; Silva et al., 2017). Replicates in which death occurred, or nymphs molted into adults, were discarded.

5.3.4. Location on tomato plant vs fruit

The tomato fruit were the same cultivar as those in the fruit-damage bioassay (see Section 5.3.3, above). Females of both species were placed individually in 600 mL transparent plastic cups covered with fabric to allow ventilation. The cups contained a young tomato plant (cv. San Pedro) held in water in a 10 mL glass bottle, and an unripe (green) tomato fruit. The unripe tomato was elected after analyzing the data from the tomato-damage bioassay, in which the most injured tomato was unripe. Females were placed individually for 24 h before the experiment in empty test tubes (starved). After release into the plastic cups, the position of the females was recorded at 1 h, 2 h, 6 h, and 24 h as being on the young plant, on the fruit, or elsewhere in the cup (as proposed by McGregor et al. (2000)). Observations were conducted in a controlled chamber (Fitoclima CP500, Aralab Lda., Albarraque, Sintra, Portugal) at a temperature of 25 ◦C and photoperiod of 14 h. At the end, fruit were inspected for feeding punctures. Females were used only once. In total, 20 replicates were made for each species.

5.3.3. Data analysis

Phytophagy in semi-field conditions. Both *D. cerastii* and *N. tenuis* populations at the end of the experiment and plant damage (necrotic rings or necrotic patches) were compared between species using one-way ANOVAwith species as an independent variable. Differences in flower abortion among treatments were comparedwith Pearson's $\chi 2$ tests. These statistical analyses were performed with IBMSPSS statistics v.26 (IBM, Armonk, NY, USA).

Fruit damage bioassay. Classification tree methods were used to understand the relative importance of the variables used (i.e., the absence or presence of water and/or food, developmental stage of the mirid, tomato ripeness, and species) on the number of feeding punctures. Statistical analyses were performed using R software version 4.1.0 implemented in RStudio version 1.4.1106. Conditional inference trees were made using the "ctree" function (R package party, http://cran.r-project.org/web/packages/party/index.html, accessed on 9 June 2021), which bases node splitting on statistical tests, providing a p-value for the significance of splitting (Hothorn et al., 2006). The importance of the variables was measured using the random forest algorithm (Breiman, 2019) and computations were performed in the randomForest package with 1001 trees (ntree = 1001). The random forest algorithm combines many classification trees to produce more accurate classifications and has measures of variable importance and measures of similarity of data points as by-products of its calculations (Cutler et al., 2007). Data were analysed together (i.e., considering counts for both species) and separately for each species. All preliminary analysis considered the modalities in two groups: (i) without food (N and W); and (ii) with food (F and FW). We grouped them into absence (A) and presence (P) of food and/or water, respectively, in the presented output.

Position on tomato plant versus fruit. The position of insects was compared between species for each observation time using the Fisher's exact test and z-test with Bonferroni correction method. For the comparison among locations within each species and each observation time, the non-parametric $\chi 2$ test for one sample was used. These statistical analyses were performed with IBM SPSS statistics v.26 (IBM, Armonk, NY, USA).

5.4. Results

5.4.1. Phytophagy in semi-field conditions

The average temperature during the assay was 24.3 °C, with a minimum of 10.4 °C and a maximum of 45.4 °C. The relative humidity was ca. 50%. At the end of the experimental period, *N. tenuis* had a larger average population (137.4 ± 30.3 individuals/cage) than *D. cerastii* (68.4 ± 14.4 individuals/cage); this difference, however, was not significant (F = 4.182; df = 1; p = 0.075). *Nesidiocoris tenuis* produced both necrotic patches (ca. 10% of total damage) on leaves and stems and necrotic rings (ca. 90% of total damage) (**Figure 1**), whereas *D. cerastii* only produced necrotic patches (**Figure 2**). Necrotic rings caused by *N. tenuis* occasionally led to withering of young shoots or leaves (**Figure 1b**), while this was not observed with *D. cerastii* patches. Plant damage numbers were significantly different between species (F = 17.114; df = 1; p = 0.003), and *N. tenuis* produced more necrotic injuries on the plants than *D. cerastii* (**Figure 3**).

Figure 1. Plant damage by *Nesidiocoris tenuis* on tomato plants: **(a)** necrotic rings on leaves and shoots; **(b)** shoot wilting**;** **(c,d)** detail of necrotic rings on leaves

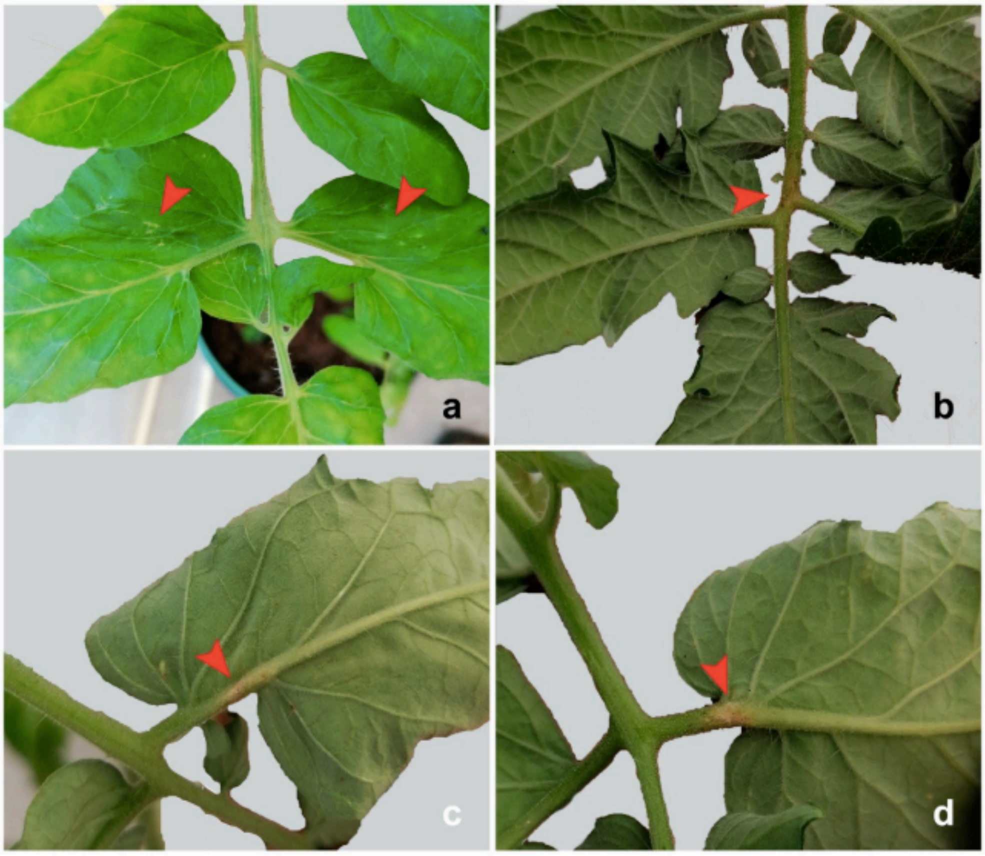

Figure 2. Plant damage by *Dicyphus cerastii* on tomato plants: (**a**) puncture scars on expanded leaves, (**b–d**) detail of necrotic patches on leaves.

Both species also produced puncture scars on leaves as a result of feeding on young stem/leaf tissues (**Figure 2a**). Control cages had significantly lower flower abortion than both *D. cerastii* ($\chi2 = 12.047$; df = 1; $p = 0.001$) and *N. tenuis* cages ($\chi2 = 16.395$; df = 1; $p < 0.001$), whereas the two mirid species displayed similar levels of flower abortion ($\chi2 = 0.670$; df = 1; $p = 0.413$) (**Figure 4**).

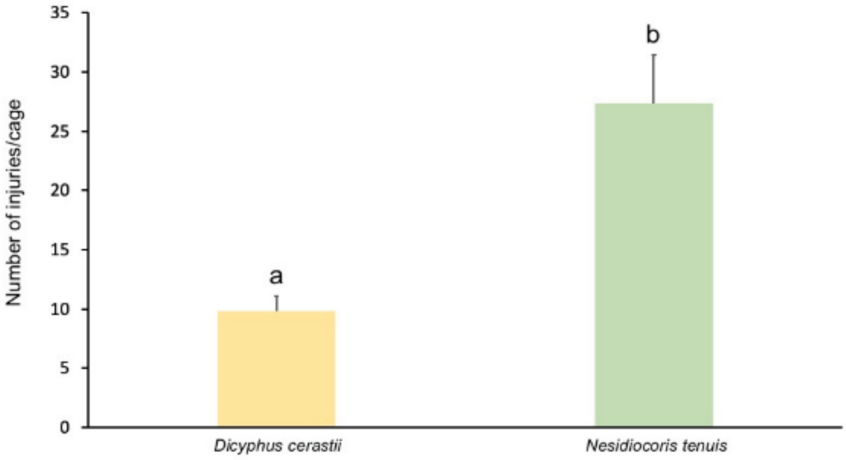

Figure 3. Number of plant injuries (necrotic rings or patches) per cage, by *Dicyphus cerastii* and *Nesidiocoris tenuis* on tomato plants (two plants/cage). Bars topped by different letters represent means that are significantly different (ANOVA, $p < 0.05$).

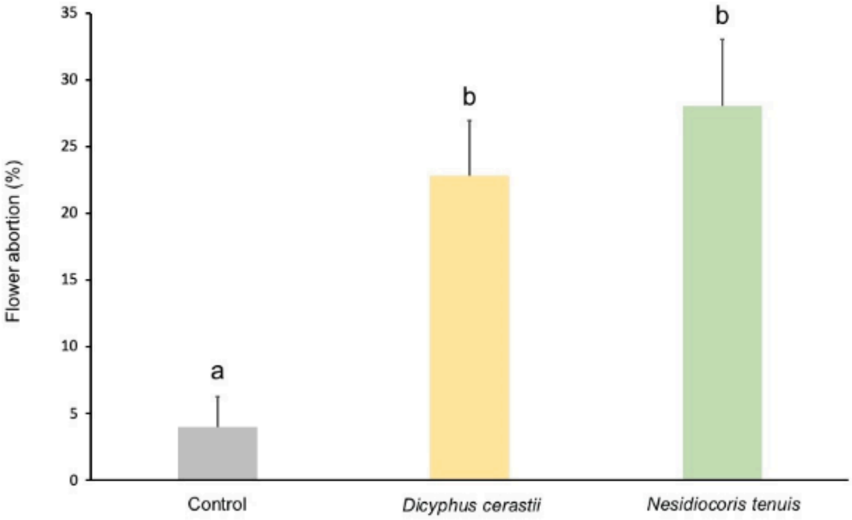

Figure 4. Percentage (+SE) of flower abortion (missing flowers/total number of flowers × 100) on control, *Dicyphus cerastii*, and *Nesidiocoris tenuis* tomato plants. Bars topped by different letters represent significantly different percentages ($\chi2$, $p < 0.05$).

5.4.2. Fruit damage

Both species fed on tomato fruit and produced punctures that appeared as damaged epicarp/mesocarp cells. It was often possible to observe that the damaged area extended beyond the puncture point following stylet movement inside the fruit. Punctures were structurally similar (**Figure 5**), but the pattern of each species was different. *Dicyphus cerastii* punctures tended to be aggregated, forming clearly visible patches in cases of highly damaged fruit. *Nesidiocoris tenuis* punctures appeared less aggregated compared to *D. cerastii*. Punctures on fruit did not heal, as punctures on green fruit did not disappear even when fruit changed color during maturation. Punctures produced by females and nymphs appeared similar, for both species. We observed that, occasionally, females of both species laid eggs on fruit. It is possible that an amount of the punctures may have been egg laying attempts or the result of probing.

A high variability was observed in the number of punctures inflicted in all treatments and in both species, with low numbers or even no punctures to high numbers of punctures in the same treatment, which generated great variance in the data for both species studied. When analyzing the dataset considering both species or for each species separately, there was no difference between the two treatments without food (N and W) or between the two treatments with food (F and FW). Considering both species, the most important variable was the tomato ripening stage (tomato_age), with the unripe (green) tomato being the one with the highest number of feeding punctures, followed by the presence/absence of food. Food presence (F, FW) or absence (N, W) was only significant in the case of green fruit, and species was only significant for females in the presence of food in the case of green fruit, and for females in the ripe fruit. While the most important factor for *D. cerastii* was also the tomato ripeness, for N. tenuis the most important factor was the individual's stage of development (i.e., whether it is a nymph or an adult). Food was the second factor for *D. cerastii* but only the third, and more distant, for *N. tenuis* (**Figures 6** and **7**).

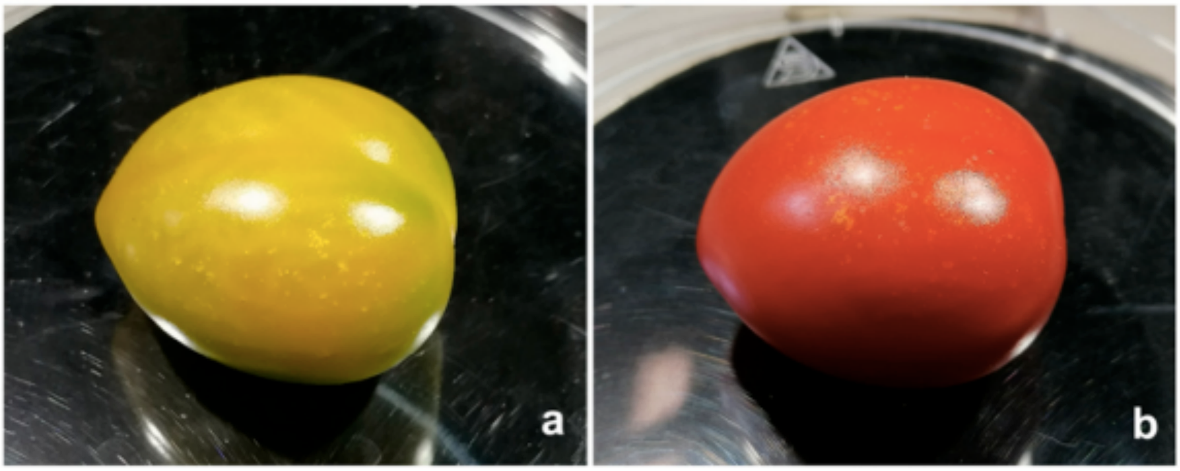

Figure 5. Feeding punctures in tomato fruit: (**a**) unripe fruit; (**b**) ripe fruit.

5.4.3. Location on tomato plant vs. fruit

The locations of *N. tenuis* and *D. cerastii* were only significantly different at the first observation (1 h) (Fisher's exact test value = 6.423, p = 0.033), with the former more present on the young plant than the latter and the inverse regarding the cup ($\alpha = 0.05$) (**Figure 8**). Both *N. tenuis* and *D. cerastii* were mainly found on the young plant. However, in the case of *D. cerastii*, differences among locations were only verified at 2 h, 6 h, and 24 h, with females more present on the young plant (or on the young plant or on the cup walls) than on the fruit.

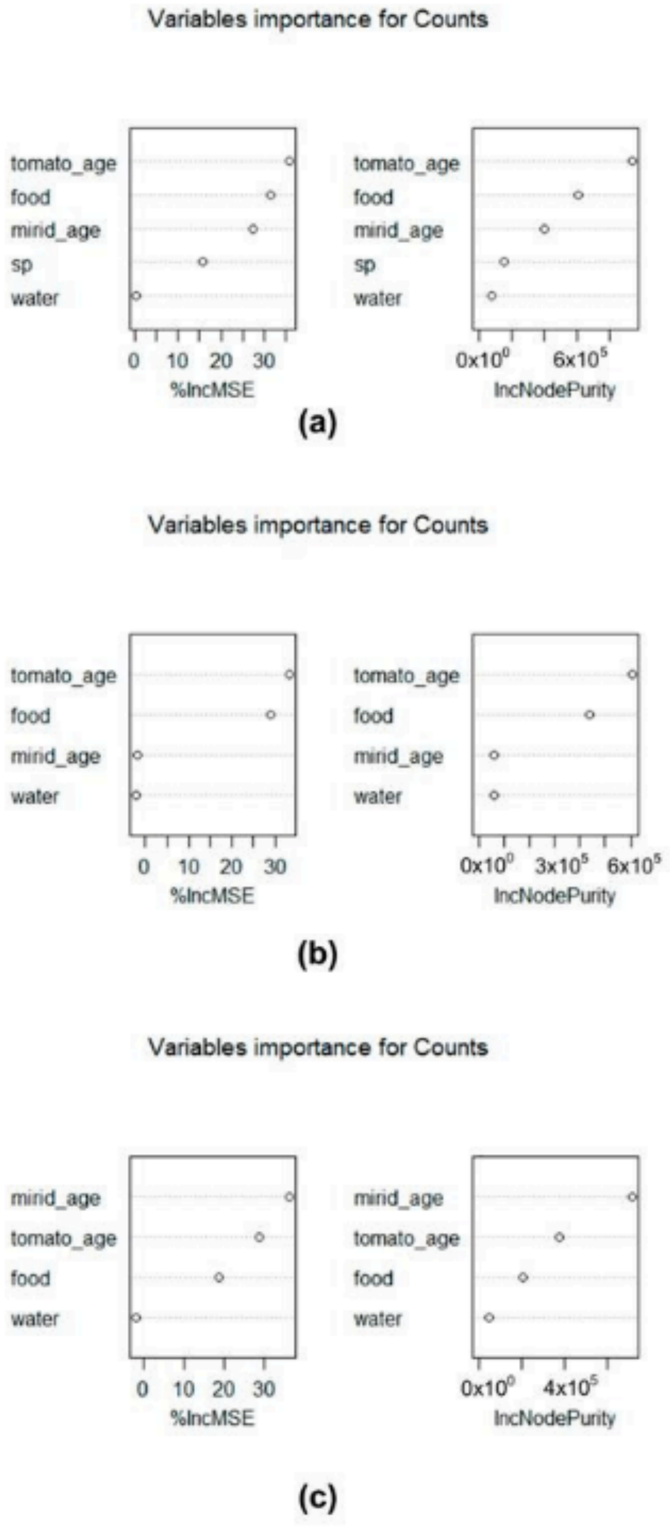

Figure 6. Variable importance plot from the random forest model (randomForest). The variables are ordered top-to-bottom as most-to-least important for an increase in feeding punctures (counts) on tomato fruit. (a) Using all datasets with data from both species, *Dicyphus cerastii* and *Nesidiocoris tenuis*; (b) database containing data collected only for *D. cerastii*; (c) database containing data collected only for *N. tenuis*. %incMSE: increase in mean square error of predictions as a result of the variable being permuted; inNodePurity: importance of each predictor variable**.**

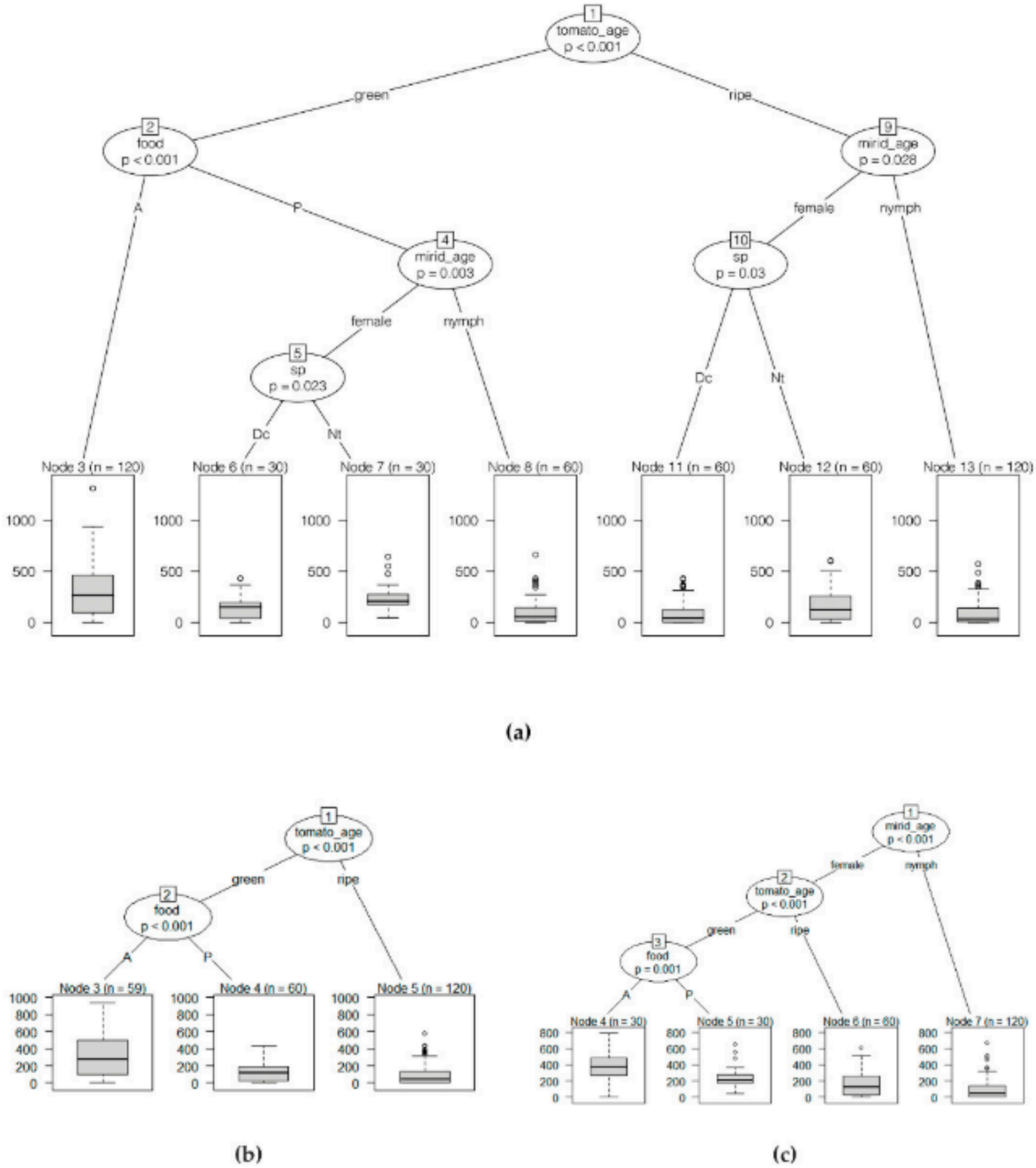

Figure 7. Classification trees from the conditional inference trees (ctree) model. For each internal node, input variable and P values are provided, the boxplot of the number of feed punctures is displayed for each end node. Numbers in boxes above the variable indicate the node number. Number above boxes (n) indicates number of fruit. (a) Using all datasets with data from both species, *Dicyphus cerastii* and *Nesidiocoris tenuis*; (b) database containing data collected only for *D. cerastii*; (c) database containing data collected only for *N. tenuis*. Dc: *Dicyphus cerastii*; Nt: *Nesidiocoris tenuis*; A: absence of food; P: presence of food.

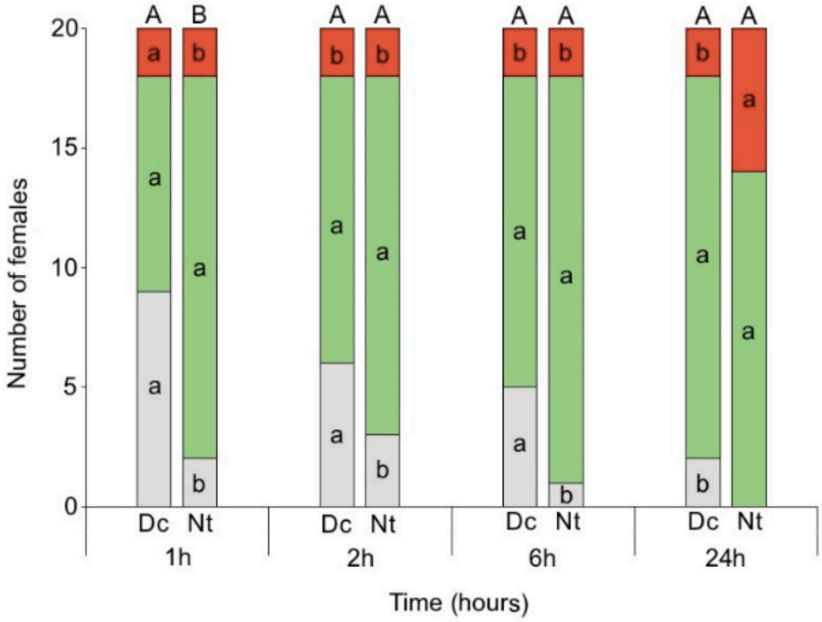

Figure 8. Number of *Dicyphus cerastii* (Dc) and *Nesidiocoris tenuis* (Nt) females on the young tomato plant (green), tomato fruit (red), or cup wall (grey) after 1 h, 2 h, 6 h, and 24 h. Bars topped by different letters for each observation time represent significant differences between species (Fisher's exact test, $p < 0.05$); different letters within the same column indicate differences among location for each species ($\chi2$, $p < 0.05$).

In the case of *N. tenuis*, the females were more often found on the young plant than on fruit or cup walls, except at 24 h; at this time there were no differences between the young plant and fruit and no female was observed on cup walls. In this bioassay, damage on plants was not quantified since, in some treatments, they would not be identified, especially with *D. cerastii*. Feeding punctures on fruit were found (although they were not counted) in all cases when the females of both species were sighted on the fruit. Furthermore, in both species, at least one fruit with feeding punctures was found, although the female was never observed in that same fruit during the bioassay (one case in *N. tenuis* and two cases in *D. cerastii*).

5.5. Discussion

The occurrence of plant damage production by zoophytophagous mirids is influenced by factors such as prey scarcity (Arnó et al., 2006; Calvo et al., 2009; Sanchez, 2009), water stress (Sinia et al., 2004), and also host plant and mirid species (Castañé et al., 2011).

Phytophagous behaviour in *N. tenuis* is regarded as more severe than in other dicyphine mirids (Castañé et al., 2011; Pérez-hedo et al., 2021; Roda et al., 2020) since this species has the particularity to feed on vascular tissues and to aggregate on feeding sites (Raman and Sanjayan, 1984). Our study corroborates this, as plants with *N. tenuis* suffered more damage than those with *D. cerastii*. Moreover, the higher number of necrotic rings produced by *N. tenuis* were also more severe for the plant compared to the necrotic patches observed for *D. cerastii*. We observed that, in some *N. tenuis* infested plants, the apical shoots or leaflets withered because of necrotic rings in stems, whereas this was not observed in *D. cerastii* plants.

Besides the damage to vegetative parts of the plant, dicyphines are also reported to damage reproductive organs, such as flowers and fruit (Castañé et al., 2011). *N. tenuis* is also recognized for causing flower and fruit abortion on tomato plants (Calvo et al., 2012), and in a study by Sanchez et al. (2006) the percentage of flower abortion by *N. tenuis* reached up to 50% during population peaks. Flower and fruit abortion on tomato plants has also been reported for *M. pygmaeus* (Sanchez et al., 2018). However, to our knowledge, this type of damage has not been previously described for *Dicyphus* spp. In our experimental conditions, we found that the percentage of flower abortion was not different between both mirid species. As flower abortion is particularly important on cluster tomato cultivars, *D. cerastii* may have a similar impact to *N. tenuis* on such cultivars, despite the lower damage to vegetative tissues produced by *D. cerastii*.

In this study, the presence of water did not influence fruit puncture level. Therefore, both mirid species and both development stages could obtain the water they needed from green or ripe tomato fruit, at least when water was not provided. Water provision has been reported as one reason for phytophagy on heteropteran predators (e.g., (Gillespie and Mcgregor, 2000)). However, as puncture numbers did not differ when water was supplied for both *N. tenuis* and *D. cerastii*, these mirids may have looked for other resources when they fed on the fruit.

Among the nutrients obtained from phytophagy, carbohydrates may have a particular ecological function since they have been reported to influence both predation and reproduction in dicyphines. This was demonstrated for *N. tenuis*, which was able to reduce the amount of prey feeding needed to establish itself on tomato plants (Urbaneja-Bernat et al., 2015), and increased its progeny (Urbaneja-Bernat et al., 2013), in the presence of sucrose dispensers. In another study, *N. tenuis* reduced its phytophagy when provisioned with sucrose dispensers (Urbaneja-Bernat et al., 2019).

In our study, when considering both species combined, tomato ripeness was the most important factor, with green fruit suffering more punctures than mature ones. This difference may be due to distinct nutritional profiles between unripe and ripe fruit. Sugar concentration, among other nutrients, may be higher in ripe tomato fruit (Duma et al., 2015; Opara et al., 2012), so it is possible that mirids may obtain more nutritional value per feeding puncture on ripe fruit than on green ones. On green fruit, the main effect was the presence of prey, which reduced fruit damage. There were differences between the species as *N. tenuis* females produced more damage than those of *D. cerastii*. On ripe fruit the most important factor was mirid age, with females producing most damage and, in these fruit, food did not significantly reduce damage. However, and once again, females of *N. tenuis* produced more damage than those of *D. cerastii*.

Considering *N. tenuis*, the most important factor on fruit damage was age, with females damaging more fruit than nymphs. Differently, in studies with whole plants, *N. tenuis* nymphs showed higher carbohydrate content (Urbaneja-Bernat et al., 2019) and spent more time feeding on the apical part of the plant (Chinchilla-Ramírez et al., 2020), compared to adults. A similar trend was found for nymphs of the neotropical mirids, such as *Macrolophus basicornis* (Stål), *Engytatus varians* (Distant) and *Campyloneuropsis infumatus* (Carvalho), that also produced fruit punctures, whereas females did not (Silva et al., 2017). Even though we observed less fruit damage by *N. tenuis* nymphs than females, our results indicated that nymphs were less influenced by factors such as fruit ripeness or presence of prey, suggesting that *N. tenuis* nymphs may be less prone to change their phytophagous behavior than adults. Following mirid age, fruit ripeness was the next important factor for *N. tenuis*, with green fruit sustaining more damage. The presence of prey reduced the amount of damage on green fruit, whereas on ripe fruit it did not produce differences. This further suggests that green fruit may be a less valuable nutritional source for *N. tenuis*.

Tomato ripeness was the most relevant factor to explain fruit punctures by *D. cerastii*. The presence of prey was also important for fruit damage reduction in green fruit. Differently to *N. tenuis*, mirid age was not important in this species, which may suggest that *D. cerastii* may not be as dissimilar in phytophagy between adults and nymphs as *N. tenuis*.

Plant damage by zoophytophagous mirids has been associated with prey scarcity (Calvo et al., 2009; Sanchez, 2009). However, in our study the presence of food did not affect puncture level on ripe or green fruit with *N. tenuis* nymphs. Similarly, McGregor et al. (2000) reported that the presence of food did not influence the level of fruit feeding by *Dicyphus hesperus* on mature tomato fruit, and Lucas and Alomar (2002) reported that in whole caged plants the presence of *E. kuehniella* eggs did not prevent fruit injury by *D. tamaninii*.

Plant damage by zoophytophagous mirids may be determined by a complex combination of factors, besides prey abundance. Different species may have distinct preference or behaviour that produce different types and levels of damage. Under the same conditions *M. caliginosus* (in fact, *M. pygmaeus*, C. Castañé, pers. comm.) did not produce fruit damage, whereas *D. tamaninii* did (Lucas and Alomar, 2002). A different dicyphine, the nearctic *D. hesperus* preferred to feed on tomato leaves producing negligible damage on fruit (McGregor et al., 2000). Host plant and cultivar may also determine phytophagy, as was demonstrated for *N. tenuis*, which varied its phytophagy among different tomato cultivars (Siscaro et al., 2019). The health of the host plant may also shape phytophagy by dicyphines. *Macrolophus pygmaeus* was reported to increase the number and produce more evident fruit damage on tomato plants infected with Pepino mosaic virus (PepMV) (Moerkens et al., 2016), but the same did not occur with *N. tenuis* (Moerkens et al., 2020). Defence-activated plants may also be less susceptible to mirid phytophagous behaviour. This was demonstrated for *N. tenuis*, which produced less plant damage on tomato plants inoculated with the endophytic *Fusarium solani* K strain, a fungal isolate that confers tomato resistance to foliar and root fungal pathogens (Garantonakis et al., 2018).

Other factors may explain differences in phytophagy, such as genetic variation within species (Chinchilla-Ramírez et al., 2020; Dumont et al., 2017). In fact, for the same treatments, we observed high variability in puncture numbers inflicted on fruit. As the large majority of the individuals used to initiate, and all the ones used to refresh the rearings, came from nearby locations (less than 45 km of linear distance), it is likely that the geographic origin was not a key determining factor in the high variability in feeding puncture number. This suggests that other factors, other than those considered in our study, may be driving fruit feeding in both *N. tenuis* and *D. cerastii*, and genetically determined behaviours should probably be considered in future research.

The fact that most *N. tenuis* and *D. cerastii* females were found on the young plants rather than elsewhere in the cup, and that there was an increased frequency of *D. cerastii* females found on young plant over time, may be related to the search for a better oviposition site (McGregor et al., 2000). Despite this, we could observe a slight increase over time of *N. tenuis* females occurring on fruit, which became the same as that for young plants at 24 h, suggesting a potential risk to fruit by this species. Furthermore, although few females of both species were observed on fruit compared to plant parts, feeding punctures were observed on fruit where females were not seen throughout the observations, showing that the female was at some moment on the fruit and fed on it. Finally, as *D. cerastii* preferred to be on young tomato plants than tomato fruit for plant feeding over time, the potential for damage to tomato fruit by this zoophytophagous mirid may be lower when compared to *N. tenuis*.

In the field, in commercial protected tomato crops, necrotic rings, shoot, and flower cluster withering, and also punctures on fruit, are common when *N. tenuis* is present at high densities. This has repercussions on tomato production, leading growers to use a tolerance threshold and apply control measures. In the case of *D. cerastii*, necrotic tissues and punctures on fruit have been observed in the field by our team in commercial greenhouses when this species is present in high population densities. In order to fully assess how *D. cerastii* may affect tomato production (both in quantity and quality), further research is needed in semi-field conditions and commercial greenhouses, to establish safe population density thresholds. Since the damage caused by *D. cerastii* was apparently different from the necrotic rings of *N. tenuis*, histological studies are needed to characterize the necrotic patch damage reported here. Furthermore, it is important to understand if the feeding behaviour of *D. cerastii* induces the production of volatile defence compounds in the damaged plant, as reported for *N. tenuis*, *M. pygmaeus* and *Dicyphus maroccanus*, Wagner (syn. *D. bolivari* Lindberg) (Pérez-Hedo et al., 2015, 2021), with the consequent attraction of other biological control agents (Pérez-Hedo et al. 2021).

5.6. Conclusion

Overall *D. cerastii* damage was less severe than *N. tenuis*, as it did not cause necrotic rings and was more likely to seek out parts of the plant than the fruit. Despite this, it fed on fruit and caused flower abortion. Therefore, as was already known for *N. tenuis* and *M pygmaeus*, *D. cerastii* has the potential to cause an economic impact on tomato fruit production, particularly for cluster tomato cultivars, since its damage is related to the parts of the plant responsible for fruit production. We suggest that decision making regarding its use as a biological control agent should be made through field evaluation considering different cultivars. We also found that fruit damage was highly variable within treatments, indicating that there may be differences in phytophagy on both species and individual levels. Therefore, in the future, selection of less phytophagous populations/strains combined with adequate management strategies may also benefit from the predatory behaviour of dicyphine mirids with lower negative impact on tomato production.

5.7. Acknowledgments

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Ecological infrastructures in protected crops in the Oeste region, author's original

Section V – Discussion and conclusions

Chapter 6 – Discussion

Chapter 7 – Conclusions

Chapter 6. Discussion

6.1. Discussion

The reduction of pesticide use is essential for more sustainable agricultural production, and biological control is emerging as an ever more important crop protection method. Dicyphines are important biological control agents, especially in protected crops, but only two species (*Macrolophus pygmaeus* and *Nesidiocoris tenuis*) are currently commercialy available in Europe, each with their advantages and drawbacks.

The interest in dicyphine predators, as biological control agents, has been increasing in the last two decades. Globally, several research groups are actively studying the potential of dicyphine mirids. In Europe, there has been a particular interest in the *Dicyphus* genus, with research focusing on native species that may be an alternative or or complementary to dicyphines curreny commercially available.

Dicyphus cerastii is a common species in Portuguese greenhouses, and during the period of this thesis we were able to expand the knowledge of its distribution in Portugal (**Figure 1**). However, there was a lack of knowledge on both the services and diservices it provides. Therefore, research was needed to better understand its contributions and limitations, and to assess its role within the Portuguese tomato mirid complex. For this we focused on understanding biological traits of *D. cerastii*, particularly looking at the influence of host plant and temperature on development, survival and longevity, but also on its reproductive capacity and thermal thresholds (Chapter 2), its predatory interactions with pests (Chapter 3) and other dicyphines (Chapter 4), and finally we also investigated the phytophagous behaviour of *D. cerastii* (Chapter 5).

In this chapter we discuss the main findings of this work interpreting them in a comprehensive context.

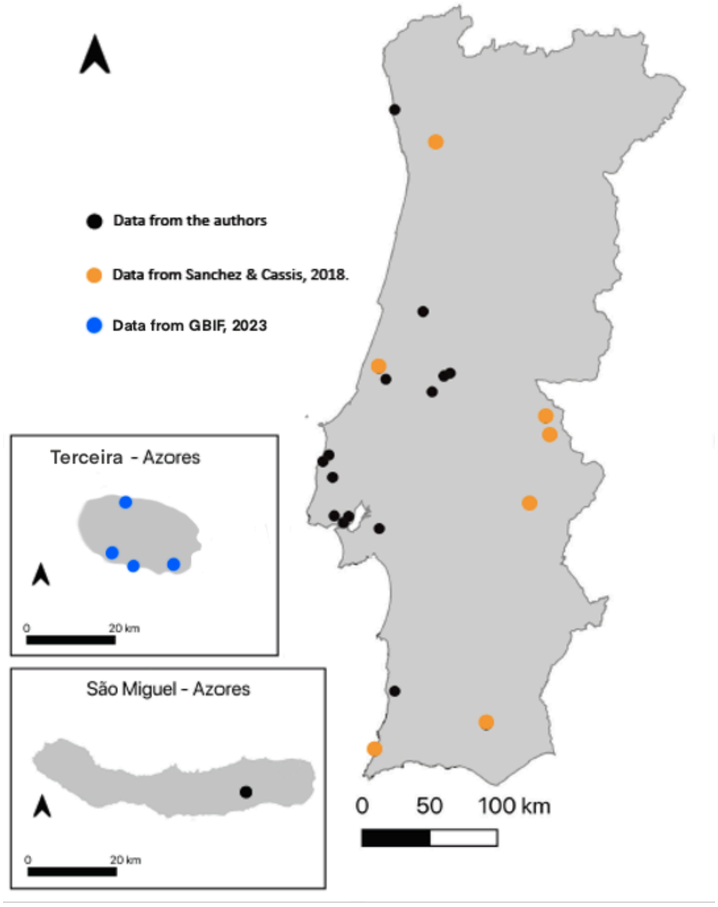

Figure 1. Distribution of *Dicyphus cerastii* in Portugal, adapted from Duarte et al. (2018) and GBIF.org.

6.1.1. Biological traits of *Dicyphus cerastii*

For a thorough evaluation of a candidate biological control agent, it is determinant to understand its basic life history traits and how they may be affected by abiotic factors like temperature and host plant. To our knowledge, this work (Chapter 2) provides the first detailed information on the biological traits of *D. cerastii*.

As expected, the development time, survival, and adult longevity of *D. cerastii* decreased, as temperature increased. Despite significant differences on some traits, the three host species (tobacco, tomato, and Cape gooseberry) were relatively similar, especially when prey was available. This similitude could be due to all the hosts tested belonging to the family Solanaceae.

In general, we observed that *D. cerastii* performs worse at higher temperatures compared to what is reported for *N. tenuis*. While the nymphs of *D. cerastii* develop faster than those of *N. tenuis* at 15 °C, they are already surpassed by *N. tenuis* from 20 °C onwards. At 30 °C, *D. cerastii* had high mortality during nymphal development, and did not reproduce, whereas *N. tenuis* is still able to complete development and reproduce at 35 °C (Sanchez et al. 2009).

Our data showed that *D. cerastii* displays lower thermal development thresholds for development, but also a lower intrinsic rate of increase and higher generation time compared to what has been reported before for *N. tenuis* (Sanchez et al. 2009; Mollá et al. 2014). Therefore, our results further corroborate previous observations of *N. tenuis* as being a more thermophilous species within the Mediterranean protected tomato dicyphine complex (Sanchez et al. 2009; Ingegno et al. 2021).

Considering its thermal performance, *D. cerastii* could possibly be a relevant biological control agent in Portuguese early spring protected crops by filling a gap before *N. tenuis* populations build up sufficiently. In fact, in Portugal, growers struggle to establish *N. tenuis* in early season as its populations increase slower than required. Because of this, *N. tenuis* is now often introduced in tomato nurseries to obtain a faster establishment in the greenhouse (Calvo et al. 2012b). This type of approach could be tested with *D. cerastii* too, since it develops faster than *N. tenuis* under 20 °C. In fact, this has been investigated previously with other *Dicyphus* species with promising results, though still not used in practice since these species are commercially unavailable (Madeira et al. 2018).

Our results also suggest than for different scenarios forecasted of global warming, with increasing temperatures in the Iberian Peninsula in future decades (Pereira et al. 2021), *D. cerastii* may be in a disadvantageous position compared to the more thermophilous *N. tenuis*, and therefore, may be at risk of becoming less abundant in the Portuguese mirid tomato complex.

6.1.2. Predatory capacity

Dicyphine mirids are generally versatile predators able to exploit different prey types. This is a key feature for a generalist predator biological control agent for crops like tomato, on which many other predator groups can't perform adequately due to the glandular trichomes.

In this work (Chapter 3), we assessed the predatory capacity of *D. cerastii* on different prey and how it responds to increasing prey density. *Dicyphus cerastii* readily fed on the four different prey we tested, thus further denoting the generalist nature of dicyphine predators. This corroborates field observations that found this species preying on leafminers and whiteflies (Carvalho and Mexia 2000), aphids, mealybugs and *Phthorimaea absoluta* (Abraços-Duarte pers. obs) (**Figure 2**).

We observed that *D. cerastii* exhibited a type II functional response to all the prey tested, as is usually reported for dicyphine predators (Maselou et al. 2015; Sharifian et al. 2015; Hassanpour et al. 2016; van Lenteren et al. 2016; Ingegno et al. 2019). However, a type III functional response could not be totally dismissed for some of the prey tested, even though our results indicated it as less preferable. A clearer distinction was possibly limited by our simple experimental setup.

Even tough type II functional response is associated with unstable predator-prey dynamics (Murdoch and Oaten 1975; van Lenteren and Bakker 1975), it is likely that, under more natural conditions, dicyphine predators are influenced by stabilizing factors that may near its functional response to type III. Such factors are often temperature, host plant, presence of alternative prey, prey species/size and spatial complexity (Murdoch 1969; Messina and Hanks 1998; Sarmento et al. 2007; Alexander et al. 2012; Ziaei Madbouni et al. 2017; Hassanzadeh-Avval et al. 2019). In fact, type III functional response has been already reported for dicyphine predators (van Lenteren et al. 2016; Michaelides et al. 2017; Ziaei Madbouni et al. 2017).

In general, the predation rate of *D. cerastii* was higher than what is reported for the commercialized species, *M. pygmaeus* and *N. tenuis*, on the same or similar prey (Urbaneja et al. 2009; Messelink et al. 2015; Pérez-Hedo and Urbaneja 2015; Malkeshi et al. 2017; Ziaei Madbouni et al. 2017). This was particularly interesting on relevant tomato pests such as *P. absoluta* eggs (172.3 day^{-1}), of which *M. pygmaeus* and *N. tenuis* can consume only 50 (Urbaneja et al. 2009). The same trend was observed for *Bemisia tabaci* 4th instar nymphs (88.8 day^{-1}) whereas *N. tenuis* can feed on only 45.1 day^{-1} (Ziaei Madbouni et al. 2017). A recent work also demonstrated that *D. cerastii* maintains similar predation rates between the day and night periods, and that during the day it is more active than *N. tenuis* (Rodrigues 2022).

Dicyphus cerastii predation capacity was influenced by prey size since the highest predation rate was found on the smaller prey (*P. absoluta* eggs). However, there were differences between similar sized sessile prey like *B. tabaci* nymphs and *Ephestia kuehniella* eggs, which could mean that other prey traits like integument hardness (Kalinoski and DeLong 2016), nutritional value (Schmidt et al. 2012) and digestion (Papanikolaou et al. 2014) may have influenced the predation rate. In our case, prey mobility also had an influence on predation which was lowest on the only mobile prey tested (*M. persicae*). Prey defense mechanisms also impact the predation rate and preference as observed by Rodrigues (2022), that reported that *D. cerastii* struggled to prey on *Planococcus citri* Risso (Hemiptera: Pseudoccidae), because the mealybug's defensive secretions disturbed the predator by coating its rostrum.

Our results indicate that *D. cerastii* may have a valuable contribution to biological control on tomato crops, especially when compared with other already used dicyphines. However, in future research, the numeric response of this predator should also be further investigated in more realistic conditions such as field or semi-field, and on different pest-season combinations. This is particularly important since there may be a chance that, despite having a higher individual predation rate, this may not compensate for the fact that it has a lower population increase capacity compared to the commonly introduced *N. tenuis.* In fact, in a semi-field experiment under the Umbert-Eco project (PTDC/ASPPLA/29110/2017), we observed that, in a five-week semi-field experiment on tomato infested with *B. tabaci*, both *D. cerastii* and *N. tenuis* significantly reduced pest populations. Pest reduction was stronger on *N. tenuis* modalities, probably because populations of *N. tenuis* grew faster than *D. cerastii*. However, *N. tenuis* produced plant damage whereas this was not the case with *D. cerastii* (Abraços-Duarte et al. 2023; Abraços-Duarte et al., in prep).

Besides the numerical response, prey preference and switching should also be further studied, especially considering how biological control may be affected by necrophagy, given that dead prey tend to be abundant on sticky plants like tomato (Carvalho and Mexia 2000; Wheeler and Krimmel 2015).

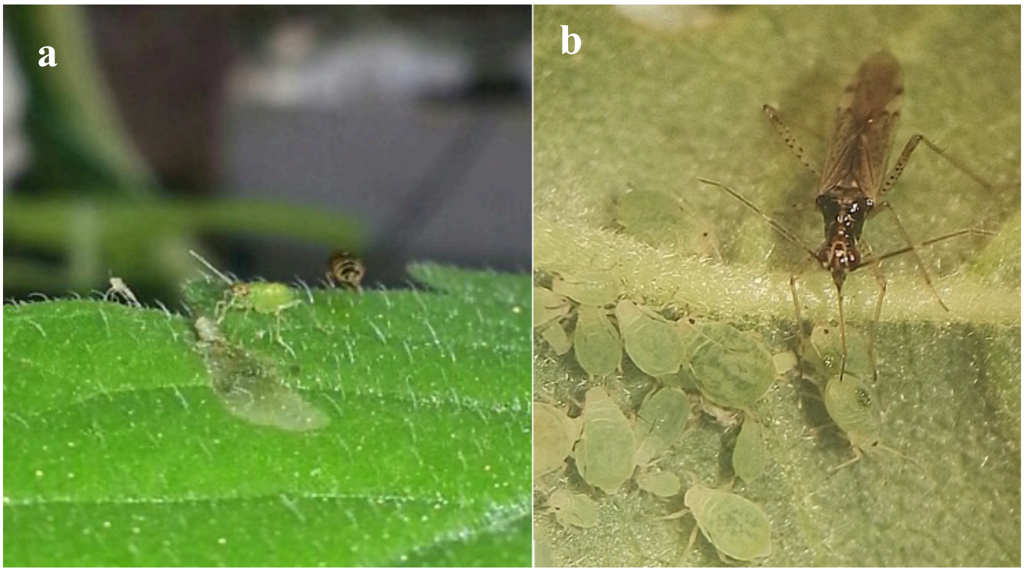

Figure 2. *Dicyphus cerastii* nymph feeding on larva of *Phthorimaea absoluta* (**a**) and adult feeding on aphids (**b**) (author's original).

6.1.3. Interactions between *Dicyphus cerastii* and other dicyphines

As generalists, dicyphines engage in both direct and indirect interactions among them which may also affect the success of biological control with these predators. To assess how direct interations with other naturally present, but also commonly released, dicyphines may affect *D. cerastii* we studied the predation of adult predators on hetero and conspecific nymphs (Chapter 4).

Low abundance or quality of alternative food is often related to the occurrence of predatory interactions among dicyphine predators (Lucas et al. 2009; Moreno-Ripoll et al. 2012; Perdikis et al. 2014; Mouratidis et al. 2022). Accordingly, in our study we found that cannibalism and intraguild predation only occurred in the absence of alternative prey.

We observed symmetrical interactions between *D. cerastii* and *M. pygmaeus*. However, those between *D. cerastii* and *N. tenuis* were asymmetrical and favoured the former. We acknowledge that these experiments represent a very simplified two-dimensional arena that possibly overestimates the outcome of these direct interactions, especially since they would naturally occur in more complex environments like plants, where *D. cerastii* nymphs would likely find more refuge. However, this already provides an indication that predation by *N. tenuis* on *D. cerastii* may not sufficiently explain the seasonal abundance shift observed in the Portuguese Oeste region.

Competitive displacement can be more important than direct interactions among dicyphine species. By having a higher reproduction capacity, shorter developmental period and survival at high temperatures, *N. tenuis* can have higher population increase, and consequent higher resource exploitation capacity. This has been proposed as a possible explanation for *N. tenuis* competitive advantage over other Mediterranean dicyphine species such as *D. maroccanus* (syn. *D. bolivari*), and *M. pygmaeus* (Salas Gervassio et al. 2017). Correspondingly, in this work (Chapter 2) we found that, like other European dicyphines, *D. cerastii* has a lower daily fertility and intrinsic rate of increase compared to *N. tenuis*. Moreover, *D. cerastii* performs worse at higher temperatures when *N. tenuis* is still able to reproduce and complete development.

Mouratidis et al. (2022) reported that previously established populations of different *Dicyphus* species (including *D. cerastii*) were able to suppress *N. tenuis* populations by 90% on tomato. However, it must be noted that factitious prey was not provided after the last introduction of *N. tenuis*. This could have forced the already high *D. cerastii* population to excessively prey on *N. tenuis* compared to more natural situations like commercial greenhouses, where initial density would likely be lower. However, these results suggest that spontaneous populations of *D. cerastii* might have a better competing chance against *N. tenuis* if they are provided with additional factitious prey.

Dicyphines can activate plant defense responses and elicit the emission of herbivore induced plant volatiles (Bouagga et al. 2018; Pérez-Hedo et al. 2018) and these compounds may have implications in the interactions between species occupying the same ecological niche. Mouratidis et al. (2022) reported that, in laboratory, *N. tenuis* was attracted to volatiles emited by tomato plants previously damaged by *M. pygmaeus* and *D. cerastii*. Differently, Morais et al. (2023) reported that neither *D. cerastii* or *N. tenuis* discriminate plants previously infested by either con- or heterospecific individuals. Therefore, further research on this topic may be necessary to fully understand the implications of these chemical interactions and their relevance at field level conditions.

The effect of augmentation by commercial releases on the mirid complex must also be considered. The commercial releases of *N. tenuis* in tomato crops of the Oeste region started in 2003 (Figueiredo et al. 2012). Since then, every year, there is an influx of new individuals into the established population, provenient from different insectaries. Moreover, the introduction of *N. tenuis* in the Oeste region has been increasing in the last years, which is not the case with *D. cerastii* that is only present through conservation biological control and has an intrinsic lower population increase capacity than *N. tenuis*. Moreover, most greenhouses in the Oeste region does not promote conservation biological control yet.

6.1.4. Phytophagy of *Dicyphus cerastii*

Dicyphines are zoophytophagous and, despite being important predators, the phytophagous behaviour of some species can produce economically important damage to crops (Castañé et al. 2011). Because of this, it is particularly important to know what type of damage a new dicyphine candidate biological control agent may originate. In Chapter 5 of this work, we investigated the type of damage produced by *D. cerastii* on tomato plants and fruits and tried to understand which factors may influence its phytophagous behaviour, compared to *N. tenuis*.

Prior to this work, *D. cerastii* was already known to produce plant damage on tomato. Particularly chlorotic punctures on leaves (Figueiredo et al. 2016), and necrotic damage on tomato stems and leaf petioles, as well as punctures on fruits (our pers. observation) (**Figure 3**).

The results from our semi-field condition experiments, corroborated previous field observations of *D. cerastii* damage on tomato, but also demonstrated that *D. cerastii* produced significantly less damage to vegetative parts of tomato plants compared to *N. tenuis*. We observed that while *D. cerastii* did not produce necrotic rings like *N. tenuis*, it still produced necrotic patches on leaf petioles and stems. In this experiment, *N. tenuis* produced its characteristic necrotic rings that often lead to breaking of stems and shoot wilting. On the other hand, the necrotic patches produced by *D. cerastii* never caused leaves or shoots to wilt.

A different trend was observed on reproductive organs as it produced flower abortion in similar level as *N. tenuis*. Tomato flower abortion was already known for other dicyphines such as *M. pygmaeus* and *N. tenuis* (Calvo et al. 2012a; Sanchez et al. 2018) and, even though there is a possibility that experimental conditions forced a higher flower abortion than what would normally happen, our results with *D. cerastii* advice for caution in case this predator reaches high population densities in tomato crops. Flower abortion is concerning since it directly compromises production and is particularly important on higher value tomato produce such as cultivars sold in trusses. This type of plant damage has been a high concern among growers using *N. tenuis* as a biological control agent in their crops and constitutes one of the main reasons why these growers are eager for alternative, less phytophagous dicyphine species (R Silva, Hortipor, pers. com.).

Dicyphines like *D. hesperus*, *D. tamaninii* and *M. pygmaeus* are known to produce damage to tomato fruit (Alomar and Albajes 1996; Sampson and Jacobson 1999; Shipp and Wang 2006). We observed that both *D. cerastii* and *N. tenuis* produced fruit damage in the form of punctures surrounded by whitish halos.

The number of punctures on tomato fruits produced by *N. tenuis* was higher than *D. cerastii*. However, those of *D. cerastii* tended to be more aggregated, forming patches, whereas those of *N. tenuis* were more disperse. This may have economical importance if these aggregated patches are more conspicuous and depreciate the aesthetic value of fruits.

Supplying a water source did not influence the number of punctures on fruits, for both species, which may indicate that these mirids may have searched for other resources when feeding on fruits. Both species punctured unripe fruits more often, which could mean that more nutrition could be obtained per puncture on ripe fruits. This is further supported by the fact that the presence of animal prey did not change the number of punctures on ripe fruits, which has also been described for *D. hesperus* (McGregor et al. 2000). Predators may have benefited from sugars as these are present in higher concentrations in ripe tomato fruits (Opara et al. 2012; Duma et al. 2015) and some, like sucrose, can reduce prey feeding and phytophagy in *N. tenuis* (Urbaneja-Bernat et al. 2015; Urbaneja-Bernat et al. 2019).

This raises the concern that, even when prey is present, there could be a risk of damage to fruits in case of high populations of these mirids. Lucas and Alomar (2002) demonstrated that, in caged plants, *D. tamaninii* damaged tomato fruits even when *E. kuehniella* eggs were available. However, the level of damage may also depend on the cultivar. So far, in field conditions, we only observed damage by high populations of *D. cerastii* on small fruits like cherry or mini plum cultivars. Whereas in past observations in the Oeste region both Carvalho (1999) and Carvalho and Mexia (2000) did not report any type of fruit damage, despite high populations of *D. cerastii*, likely on larger tomato cultivars.

Our results suggest that, overall, *D. cerastii* may be safer than *N. tenuis* since it produces less severe damage to tomato plants and fruits. Even though it produced similar levels of flower abortion, the risk may not be as high as with *N. tenuis* since *D. cerastii* has a slower populational growth and does not significantly damage the vegetative parts of the plant as *N. tenuis*. Future research should also try to determine safe population density thresholds for *D. cerastii* on tomato.

Figure 3. Cherry tomato with punctures from *Dicyphus cerastii* (author's original).

6.1.5. Perspectives

During the period of this thesis, rearings of *D. cerastii*, *M. pygmaeus* and *N. tenuis* were maintained, continuously, in Instituto Superior de Agronomia's insectary (University of Lisbon). In our system, using tobacco plants was easier as new plants can be easily obtained from cuttings and, thus avoiding the need of frequent acquisition of other normally used rearing substrates such as green bean pods.

The use of green bean pods is standard practice in mass rearing of dicyphine and anthocorid predators, although some approaches also aim to exclude the need of plant material by using artificial oviposition substrates (Vandekerkhove et al. 2011; De Puysseleyr et al. 2013, 2014). Even though the tobacco system worked for our needs, we also did some exploratory tests on rearing these mirids on green bean pods. Among the three species, *D. cerastii* was, in general, the most difficult species to rear (even in the tobacco system), and it was particularly worse than *M. pygmaeus* and *N. tenuis* when using green bean pods, as much less eggs and nymphs were obtained.

The same poor performance on green bean pods was also reported for *D. errans* that frequently has to be reared on other hosts like tomato or tobacco (F Madeira, CIMO - Centro de Investigação de Montanha, Bragança, pers. com.) (Messelink et al. 2015; Madeira et al. 2018; Ingegno et al. 2021).

Therefore, it could be possible that some *Dicyphus* species are more selective than other dicyphines regarding oviposition substrate. This could be a bottleneck to the large-scale production of *D. cerastii* as living plants require more space and energy than readily available, easy to use, plant parts like green bean pods. This limitation and possible implications for the cost-effectiveness of *D. cerastii* mass production should be further explored in future research, especially since other dicyphines are already efficiently commercially mass produced in large scale.

In this work we did not study the commercial potential of *D. cerastii* as an augmentation biological control agent. However, given the limitations we observed during its laboratory rearing, it is possible to assume that its production costs could be higher than those of species like *M. pygmaeus* and *N. tenuis*, that are currently produced in large scale. Another possible drawback is the amount of food needed by *D. cerastii*, as we observed (in Chapter 3) that the females of this species can feed on more *E. kuehniella* eggs than what is reported for *N. tenuis* for example, which may indicate higher needs of this expensive food source. Future research should also investigate effective cheaper alternative diet possibilities that would help make dicyphines mass rearing in general, and *D. cerastii*, in particular, more economic.

Conservation biological control may be a more immediate and economic way for growers to benefit from *D. cerastii* services, as this species is relatively common in Portuguese greenhouses. The use of selected alternative host plants close to the crop may recruit naturally occurring *D. cerastii* and allow the establishment and mantenaince of its populations in periods of prey scarcity.

This type of approach has already been demonstrated with other dicyphines like *D. hesperus* that builds higher populations on Canadian tomato crops when the host plant *Verbascum thapsus* L. is available (Sanchez et al. 2003a). Perrin et al. (2019) and Ardanuy et al. (2022) also showed that alternative plants like *Calendula officinalis* L. can be used to improve *M. pygmaeus* establishment in tomato crops in France and Spain, repectivelly. *Sesamum indicum* (L.) is reported as a good alternative host plant for *N. tenuis* on which it can even complete development without prey (Biondi et al. 2016). Lately, we have also observed *D. cerastii* on strips of sesame and in *C. officinalis* flower strips planted in tomato greenhouses. We also found that, in tomato greenhouses, *D. cerastii* nymphs and adults were found abundantly on strips of *Phacelia tanacetifolia* Bentham, which suggests that this plant should also be further studied as a companion plant in tomato crops (**Figure 4**).

In this study (Chapter 2) we only compared two alternative hosts to tomato (tobacco and Cape gooseberry) on which *D. cerastii* is commonly found. These three hosts did not differ notably on the biological traits observed, possibly since they all belong to Solanaceae. However, during the period of this work, through the Umbert-Eco project, we found *D. cerastii* on more plant species than those we initially knew, which expands the list of hosts for *D. cerastii* as presented in **Table 1** (Souto et al. 2021).

This expanded list, particularly the species on which *D. cerastii* is found in high numbers should be further investigated as candidate companion plants integration in protected tomato crops. These plants should be carefully selected for possible risks (pests and diseases), invasiveness, and agronomical requirements. When aiming at promoting *D. cerastii* populations, companion plants should also be, as much as possible, relatively selective so that they do not also excessively favour populations of competitor species like *N. tenuis*.

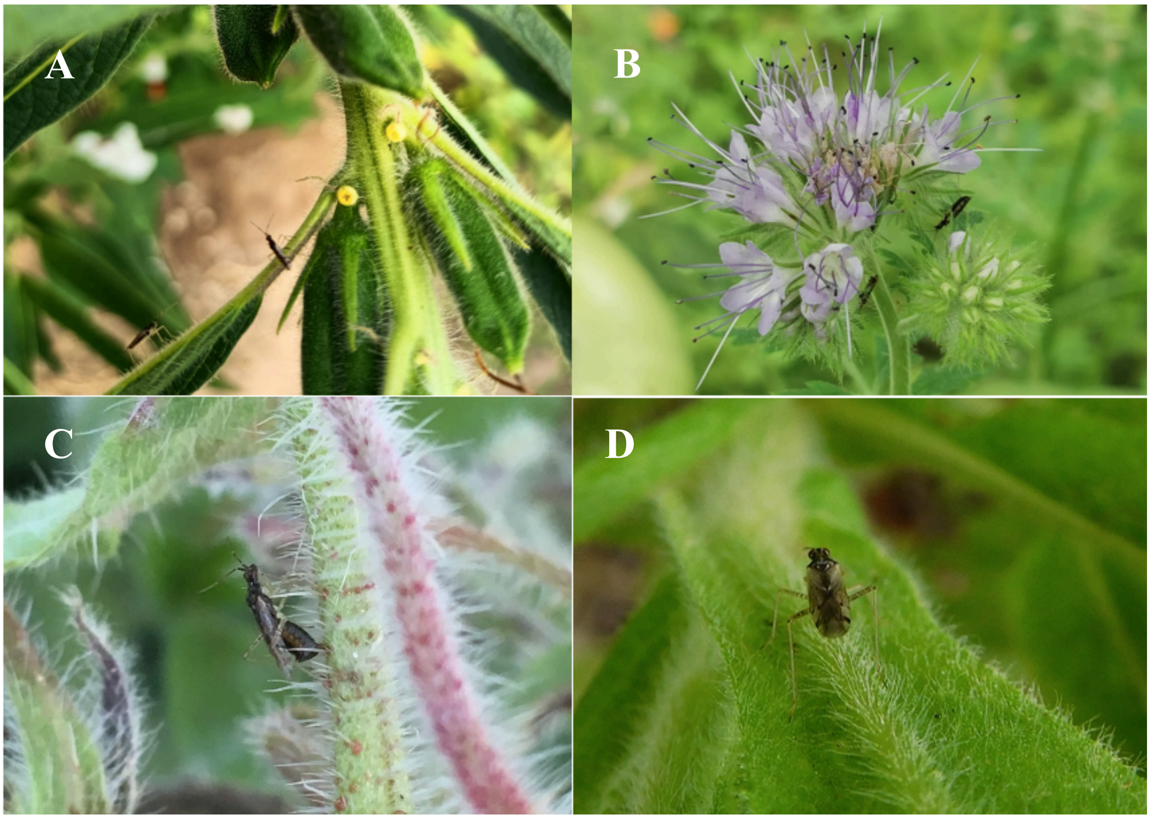

Figure 4. *Dicyphus cerastii* on several host plants in tomato greenhouses, *Sesamum indicum* (A), *Phacelia tanacetifolia* (B), *Borago officinalis* (C) and *Dittrichia viscosa* (D) (author's original).

Besides companion plants, other resources may be used to promote *D. cerastii* populations, including offering factitious prey to natural populations. In fact, feeding natural enemies' populations with factitious prey is becoming common practice under biological control programs. In different crops, growers use commercial formulations of *E. kuehniella* and/or *Artemia* sp. to maintain or boost mirid predator populations in periods of natural prey scarcity (Moerkens et al. 2017; Labbé et al. 2018; Brenard et al. 2019; Owashi et al. 2020). We observed that *D. cerastii* takes advantage of the factitous prey growers use in Portuguese greenhouses to support *N. tenuis* commercial releases (**Figure 5**). This food supplementation may be particularly important in the case of *D. cerastii* since, in Chapter 2, we observed that the nymphs of this species could not complete development in the absence of animal prey, on different host plants.

Unlike *N. tenuis*, that is mostly concentrated in the top (Arnó et al. 2010; Figueiredo et al. 2016), *D. cerastii* is more evenly distributed between the top and middle section of tomato plants (Carvalho 1999; Figueiredo et al. 2016). This different behaviour could be used by growers aiming to primarily increase *D. cerastii* populations rather than *N. tenuis*, by avoiding distributing factitious prey on plant tops, and by spraying only the plant tops when they need to reduce *N. tenuis* populations using pesticides or aspiration equipment.

This thesis opens new research questions that should be addressed in future work with *D. cerastii* and other dicyphines.

Understanding the role of different types of communication (chemical, mechanical, visual) could bring answers on ways to manipulate the behaviour of these insects in a favorable way to growers. The role of olfaction in *D. cerastii* may need to be further studied to know what cues trigger responses, allowing to attract it to crops and/or pests, for example, or manage its populations and those of other dicyphines.

It would be interesting to know if the phytophagous behavior of *D. cerastii* also induces plant defense responses, and what metabolic pathways are activated in the plant, as well as if *D. cerastii* is also able to elicit the emission of herbivore induced plant volatiles like it happens with other dicyphines.

On a broader scope, the dietary and behavioral differences among Dicyphini may also be interesting to study from different perspectives. It may be interesting to investigate the possibility of artificial selection of dicyphine mirids for more tolerance to different environmental conditions and crop combinations, and for lower phytophagy. For this, it may also be important to strengthen the knowledge on the background of phytophagy, by understanding how plant damage of dicyphine species differ histologically, and to what extent symbiont organisms have an influence on dietary needs.

From a fundamental research perspective, it may also be pertinent to continue the exploration of how the feeding strategy evolved within the Miridae. Different studies indicate that the ancestors of mirids were phytophagous, and that during the family's speciation, multiple subsequent reversals to zoophagy and zoophytophagy occurred (Jung and Lee, 2012; Weirauch et al. 2019). Within the Miridae, the Dicyphini may be a particularly interesting group to study from an evolutionary perspective, especially since species in this same tribe display differences in their phytophagous behaviour. Therefore, it could be interesting to establish if and what feeding strategy shifts and specializations may be occurring within the Dicyphini.

Table 1. Updated list of host plants of *Dicyphus cerastii*.

Family	Species	Reference
Asteraceae	*Calendula arvensis* L.	Luz (2001)
	Calendula officinalis L.	Souto et al. (2021)
	Dittrichia viscosa L.	Souto et al. (2021)
	Lactuca sativa L.	Souto et al. (2021)
Boraginaceae	*Phacelia tanacetifolia* Benth.	Souto et al. (2021)
	Borago officinalis L.	Souto et al. (2021)
Caryophyllaceae	*Cerastium arvense* L.	Wagner (1951)
	Cerastium virescens L.	Wagner (1951)
Chenopodiaceae	*Chenopodium murale* L.	Carvalho (1999)
Cucurbitaceae	*Cucumis sativus* L.	Figueiredo et al. (2012)
	Cucurbita maxima Duchesne	Sanchez et al 2006
	Cucurbita moschata Duchesne.	Figueiredo et al. (2012)
	Cucurbita pepo L.	Sanchez et al (2003b)
	Lagenaria siceraria (Molina) Stanl.	Sanchez et al 2006
Fabaceae	*Ononis natrix* L.	Sanchez et al (2003b)
	Phaseolus vulgaris L.	Figueiredo et al. (2012)
Geraniaceae	*Erodium petraeum* (Gouan) Willd	Sanchez et al (2003b)
	Pelargonium cucullatum (L.) L'Hér	Souto et al. (2021)
	Pelargonium x hortorum Bailey	Souto et al. (2021)
Lamiaceae	*Salvia glutinosa* L.	Ingegno et al. 2008
Malvaceae	*Malva parviflora* L.	Luz (2001)
Pedaliaceae	*Sesamum indicum* L.	(G Abraços-Duarte pers. ob.)
Plantaginaceae	*Digitalis grandiflora* Mill.	Ingengo et al. 2008
	Plantago lanceolata L.	Carvalho (1999)
	Antirhinum majus L.	Souto et al. (2021)
Solanaceae	*Hyoscyamus albus* L.	Sanchez et al 2006
	Nicotiana tabacum L.	Souto et al. (2021)
	Physalis peruviana L.	Souto et al. (2021)
	Solanum lycopersicum L.	Carvalho (1999)
	Solanum melongena L.	Souto et al. (2021)
	Solanum nigrum L.	Figueiredo et al. (2012)
	Withania frutescens (L.) Paquy	Sanchez et al (2003b)

Figure 5. *Dicyphus cerastii* adults on a tomato leaflet that was sprinkled with a mix of *Ephestia kuehniella* eggs and *Artemia* sp. cysts to support the establishment of introduced *Nesidiocoris tenuis* (author's original).

6.1.6. References

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Chapter 7. Conclusions

7.1. Conclusions

Our results indicate that *D. cerastii* may give a valuable contribution to biological control in tomato crops, as it showed high voracity on important tomato pests even when compared to other dicyphines. Despite being able to damage fruit and cause flower abortion, *D. cerastii* has a much less severe impact on the whole plant than *N. tenuis*, which is a considerable advantage.

Despite this advantages, *D. cerastii* has a longer developmental period, higher mortality, and lower reproductive rate than *N. tenuis*, particularly at higher temperatures. This may be a disadvantage for its populations when the two species co occur at warmer temperatures. This may explain the seasonal abundance shift in the Oeste region and suggests a higher vulnerability of *D. cerastii* in a scenario of global warming with more episodes of extreme temperatures in summer and increase in the average temperature of the region.

Even though growers often need to reduce its populations, *N. tenuis* is generally still considered to be more beneficial than detrimental, and its use is increasing, as it establishes well and is readily available. However, *N. tenuis* often struggles to establish in the beginning of the season due to low temperatures. During this period, growers could rely on *D. cerastii* by promoting its populations through conservation practices, like providing alternative hosts or feeding natural populations. In fact, promoting its populations may also help to regulate those of *N. tenuis*, since *D. cerastii* is usually the predator in direct interactions with *N. tenuis*, therefore further reducing the need for pesticide use in tomato greenhouses.

This thesis has contributed to understand the role of *D. cerastii* in tomato crops by:

- Providing the first data on *D. cerastii* biological traits such as development and survival on different hosts and temperatures, reproductive capacity, and thermal thresholds;
- Providing an evaluation of predation capacity on different prey, contributing to the assessment of its possible contribution to biological control of tomato pests;
- Contributing to understanding the role of direct interactions like intraguild predation between dicyphines in the context of a seasonal abundance shift between *D. cerastii* and *N. tenuis*;
- Characterizing the plant damage of *D. cerastii* and comparing it to that of *N. tenuis*.

In summary, we found that:

- *Dicyphus cerastii* could not complete development in the absence of animal prey;
- *Dicyphus cerastii* had a worse performance than *N. tenuis* at temperatures above 20 °C, mainly due to increased mortality and longer development. Consequently, *D. cerastii* displays a lower intrinsic rate of increase compared to *N. tenuis*;
- *Dicyphus cerastii* showed high predation rate on key tomato pests, being more voracious than currently used dicyphines like *M. pygmaeus* and *N. tenuis*;
- *Dicyphus cerastii* displayed a type II to all prey tested, however a type III could also be possible for some of the prey tested;
- Predation was mainly influenced by prey size and mobility;
- Dicyphine mirids only engaged in intraguild predation or cannibalism in absence of animal prey;
- In the absence of animal prey, intraguild predation interactions between *D. cerastii* and *N. tenuis* were asymmetrical and favorable to *D. cerastii*;
- Compared to *N. tenuis*, *D. cerastii* produced less damage to the vegetative parts of tomato plants. However, it produced a similar level of flower abortion;
- Both species punctured tomato fruits, but *N. tenuis* produced more damage;
- Both species produced more damage on unripe tomato fruit, water did not influence the damage on fruits, and availibilty of food did not influence damage on ripe fruits;
- For *D. cerastii*, the most important factor for fruit damage was fruit ripeness, and after that, the presence of prey reduced damage in unripe fruit, and insect stage was not important;
- For *N. tenuis*, the most important factor for fruit damage was insect stage, followed by fruit ripeness, and availability of prey.