UNIVERSIDAD DE ALMERÍA

TESIS DOCTORAL

Beneficial Insects in Greenhouses: A Study of Some Aspect of Cannibalism and Pollination

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Insectos beneficiosos en invernaderos: Estudio de algunos aspectos del canibalismo y la polinización

Beneficial Insects in Greenhouses: A Study of Some Aspects of Cannibalism and Pollination

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Tesis Doctoral presentada para optar al Grado de Doctor por la Universidad de Almería. Programa de Doctorado en Ciencias Aplicadas al Medio Ambiente.

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Resumen

Los cultivos de invernadero en Almería, España, presentan una gran importancia económica, tanto en términos de superficie como de producción, especialmente para la exportación. Los insectos beneficiosos, principalmente entomófagos (depredadores y parasitoides) y polinizadores, proporcionan importantes servicios ecológicos en los agroecosistemas. Sin embargo, las condiciones de los invernaderos, debido a su mayor o menor confinamiento con respecto al entorno, hacen que estos insectos no puedan acceder a los cultivos. Por ello, son producidos por industrias auxiliares de la agricultura (denominadas bio-factorías) y son liberados artificialmente dentro de los invernaderos.

En esta tesis doctoral se han utilizado dos especies de insectos beneficiosos: el depredador *Nabis pseudoferus* Remane (Hem.: Nabidae) y el polinizador *Eristalix tenax* (L.) (Dip.: Syrphidae). El objetivo, para la primera especie, ha sido determinar experimentalmente las bases biológicas del canibalismo, para posteriormente construir un modelo matemático que pueda representar el efecto del canibalismo; tanto en los procesos de producción, en las bio-fábricas, como posteriormente, cuando se realicen las sueltas en los programas de control biológico en invernaderos. Para la segunda especie, se ha trabajado a la inversa, desarrollando un modelo matemático teórico que pueda representar el uso y optimización del polinizador en el invernadero para el cultivo de tomate, que luego deberá ser validado mediante ensayos experimentales.

Los efectos del canibalismo se han llevado a cabo en dos grupos de ensayos. En el primero, se evaluaron los efectos del régimen de alimentación del depredador y de la ontogenia post embrionaria sobre el canibalismo; esto también se comparó con la depredación intragremial utilizando la especie depredadora *Nesidiocoris tenuis* (Reuter) (Hem.: Miridae). Los ensayos se llevaron a cabo en condiciones de laboratorio, incluyendo ensayos en microcosmos. En el segundo grupo de ensayos, en relación con el canibalismo, se estudió la aceptación de presas comparando dos especies: la especie plaga *Spodoptera exigua* Hübner (Lep.: Noctuidae) como presa heteroespecífica y *N. pseudoferus* como presa coespecífica. Asimismo, se ha determinado la respuesta funcional del depredador en relación con la presa coespecífica.

Los resultados muestran que el canibalismo en *N. pseudoferus* era predominante en todos los estadios de desarrollo estudiados, mientras que en *N. tenuis*, el canibalismo es escaso, estaba restringido principalmente a los tres primeros estadios de las ninfas. Esto parece estar causado por el diferente régimen de alimentación de cada especie: zoófago estricto en la primera y fitozoófago en la segunda. Asimismo, los resultados muestran que tanto el canibalismo como la depredación intragremial no guardan una relación lineal con las diferentes relaciones de tamaño caníbal-presa, evaluadas por las tasas de mortalidad y los tiempos de supervivencia.

Los resultados del segundo conjunto de ensayos muestran que la tasa de depredación en la presa plaga, *S. exigua*, se redujo al 59,09±7,08% en presencia de presas coespecíficas. El comportamiento de captura de presas de las hembras adultas difiere cuando captura presas coespecíficas frente a las heteroespecíficas. Ello queda demostrado por el tiempo medio de manipulación, que fue de 23,3±3,3 min en el primer caso (conespecífica) frente a 16,6±2,5 min en el segundo (heteroespecífica). Además, los valores aumentaron en el primer caso y disminuyeron en el segundo según el orden de captura de las presas. Estos resultados indican probablemente una aversión al riesgo y un temor a las represalias entre los congéneres. También, se presentan y discuten las implicaciones para el desarrollo de un modelo matemático.

Se ha comprobado que el modelo matemático teórico, desarrollado para el caso del insecto polinizador, es capaz de representar el comportamiento del mismo y sus efectos sobre el cultivo en función de determinados parámetros biológicos. En concreto, se han utilizado los siguientes parámetros: comportamiento de alimentación (tiempos de búsqueda y manipulación), longevidad de los adultos, fecundidad de las hembras adultas, funciones de alimentación, etc.; junto con el aporte de alimento suplementario.

Aunque el modelo experimental debe ser validado experimentalmente, presenta varias ventajas: (1) El modelo es lo suficientemente flexible como para permitir su extensión a otros insectos polinizadores y cultivos en invernaderos. (2) Permite ahorrar costes de experimentación, ya que los parámetros biológicos que se utilizan pueden determinarse mediante ensayos de laboratorio y/o semi-campo, con un coste mucho menor que los ensayos en invernaderos comerciales. (3) Con la introducción de la optimización en el modelo, podría utilizarse para calcular los insectos polinizadores necesarios, en función del desarrollo de la floración y de la producción. Ello conjuntamente con la estimación de los costes de los insectos liberados.

Abstract

Greenhouse crops in Almeria, Spain, are of great economic importance, both in terms of surface area and production, especially for export. Beneficial insects, mainly entomophagous (predators and parasitoids) and pollinators, provide important ecological services in agroecosystems. However, the conditions of greenhouses, due to their greater or lesser confinement with respect to the surrounding environment, mean that these insects cannot access the crops. Therefore, they are produced by auxiliary industries of agriculture (called bio-factories) and they are released artificially inside the greenhouses.

In this doctoral thesis, two beneficial insect species have been utilized: the predator *Nabis pseudoferus* Remane (Hem.: Nabidae) and the pollinator *Eristalix tenax* (L.) (Dip.: Syrphidae). The objective, for the first species, was to experimentally determine the biological basis of cannibalism, and subsequently build a mathematical model that can represent the effect of cannibalism; both in the production processes, in the bio-factories, and later, when the releases are carried out, in the biological control programmes in greenhouses. For the second species, we have worked the other way round, developing a theoretical mathematical model that can represent the use and optimisation of the pollinator in the greenhouse for tomato crop, which must then be validated by means of experimental trials.

The effects of cannibalism have been carried out in two groups of trials. In the first one, the effects of predator feeding regime and post-embryonic ontogeny on cannibalism were evaluated; this was also compared with intraguild predation using the predatory species *Nesidiocoris tenuis* (Reuter) (Hem.: Miridae). Trials were carried out under laboratory conditions, including microcosm assays. In the second group of trials, in relation to cannibalism, prey acceptance was studied by comparing two species: the pest species *Spodoptera exigua* Hübner (Lep.: Noctuidae) as heterospecific prey and *N. pseudoferus* as conspecific prey. Likewise, the functional response of the predator has been determined in relation to the conspecific prey.

The results showed that cannibalism in *N. pseudoferus* was prevalent in all the developmental stages studied, whereas in *N. tenuis*, cannibalism was rarely observed, and it was restricted mainly to the first three nymphal stages. This seems to be caused by the different feeding regime of each species: strict zoophagous in the former and phyto-zoophagous in the latter species. Likewise, the results show that both cannibalism and intraguild predation no linear relationship with the different cannibal–prey size ratios, as evaluated by the mortality rates and survival times.

The results of the second trial set show that the rate of predation for pest prey, *S. exigua*, was reduced to 59.09±7.08% in the presence of conspecific prey. The prey-capture behaviour of adult females differed when they hunted conspecific versus heterospecific prey. This was shown in the average handling time, which was 23.3±3.3 min in the first case (conspecific) versus 16.6±2.5 min in the second (heterospecific) one. Furthermore, the values increased in the former case and declined in the latter according to the order in which the prey were captured. We argue that these results likely indicate a risk aversion and a fear of reprisal among conspecifics. The mathematical model's implications are presented and discussed.

The theoretical mathematical model, developed for the case of the insect pollinator, has been found to be able to represent the behaviour of the pollinator and its effects on the crop based on certain biological parameters. In concrete terms, the following parameters were used: feeding behaviour (searching and handling times), adult longevity, adult female fecundity, feeding functions, etc.; in conjunction with the addition of supplementary feed.

Although the experimental model needs to be validated experimentally, it has several advantages: (1) The model is sufficiently flexible to allow its extension to other pollinating insects and crops for greenhouses. (2) It allows savings in experimental costs, as the biological parameters it uses can be determined by laboratory and/or semi-field trials, at a much lower cost than trials in commercial greenhouses. (3) With the introduction of optimisation in the model, it could be used to calculate the pollinating insects to be used, depending on the development of flowering and production, together with the costs of the released insects.

Capítulo I

Introduction and Objectives

Chapter I

Introduction and Objectives

I.1. Introduction

I.1.1. Importance of the greenhouse horticultural crops in Almeria

The importance of greenhouse horticultural crops in Almeria can be assessed in different ways. On the one hand, by the surface area dedicated to them; thus, according to cartographic methods, the overwintered surface area represents 31,034 ha (Junta de Andalucía, 2017). This area has increased to 32,368 ha in the 2019/20 season (CAJAMAR, 2021).

The main greenhouse crops in Almería, from a larger to a smaller area, are pepper, tomato, watermelon, melon, bean, zucchini, cucumber and aubergine (Valera-Martínez et al. (1999). In the 2019/20 season, the increase in area of aubergine (+10.5%), pepper (+7.3%), which maintains the upward trend that began ten years ago, as well as the leadership that has taken away from tomato and cucumber, stand out; thus, in this season, the tomato crop decreases (-9.6%) and the bean crop (-34.5%), which continues its decline year after year, reflecting its behaviour as a residual crop (CAJAMAR, 2021).

On the other hand, regarding the importance of the sector, it is worth mentioning that the fruit and vegetable production in Almeria, in the 2019/20 season, has registered a total volume of 3,721,118 metric tons, of which 3,488,510 metric tons corresponds to the greenhouse horticultural crops sector (CAJAMAR, 2021).

Finally, the economic importance of the sector is given by its economic value, especially from the point of view of exports. With an export rate of 80% of the production volume, this continues to be one of the strong points of the sector. The crops that have recorded the best performance in terms of exported volume have been aubergine, pepper, watermelon, melon and cucumber (CAJAMAR, 2021).

Another important aspect, in relation to the importance of fruit and vegetable production in Almeria, is the auxiliary industry sector. This will have a turnover of 1,274,500 euros in

2019, generating employment for 5,477 people employed in this industry. By subsectors: biotechnology represents 378 million euros, followed by containers and packaging (190 million euros), irrigation and climate (68 million euros) and IPM together with agricultural machinery (31.4 million euros) (CAJAMAR, 2021). This sector includes companies producing and/or marketing natural enemies, as well as pollinating insects, as discussed below.

I.1.2. Beneficial insects and their types

In the broad sense, the term "beneficial insect" (sometimes also called "auxiliary insect") can be applied to any insect from which humans derive a direct or indirect benefit. More selectively and frequently, however, the term is reserved for pollinating insects and for natural enemies of organisms considered harmful to humans or their interests (Alford, 2019).

Beneficial insects provide a wide range of ecosystem services essential to human life (Schowalter *et al.*, 2018; Redhead et al., 2020) (**Figure I.1**). In agricultural systems two of the most economically important services are pollination and biological control of crop pests (Jones & Snyder, 2018; Redhead et al., 2020). Pollination services have been valued at $235-577 billion (Lautenbach *et al.*, 2012) and affect the yield or quality of most of the world's major crop types (Klein *et al.*, 2007; Gallai *et al.*, 2009), while biological control of crop pests has been valued at over $400 billion (Costanza *et al.*, 1997) and is vital for many crops of high economic value (e.g., Colloff *et al.*, 2013; Classen *et al.*, 2014; Vila & Cabello, 2014).

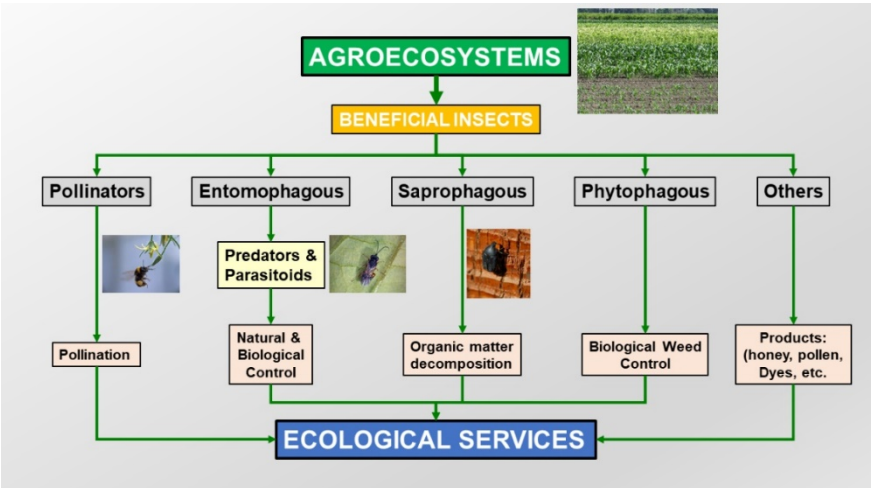

Figure I.1. Main ecological services provided by beneficial insects in agroecosystems (Source: Jones & Snyder, 2018; Alford, 2019; Readhead et al., 2020).

We currently consider that beneficial insects play an especially important role in the management of horticultural crops in greenhouses. However, this is done in a forced way, by the hand of the grower. The reason for this is the greater or lesser closing of the greenhouses, which makes the exchange of insects in and out of the greenhouses difficult and/or impossible. Therefore, all pollination and biological pest control activities must be carried out through the industrial production of pollinating and entomophagous arthropods, which are then released into the greenhouse crops.

I.1.3. Entomophagous arthropods and their use in biological pest control

The importance of the industrial sector of biological pest control, worldwide, can be deduced from the fact that more than 500 companies (with more than 30 employees) produce and/or market biological control agents (entomophages and entomopathogens), of which more than 20 are in Europe (Bolckmans, 2008). Also, worldwide the market for these commercialised natural enemies is estimated to be worth 300 million euros (at end-user value) (Cock *et al.*, 2010).

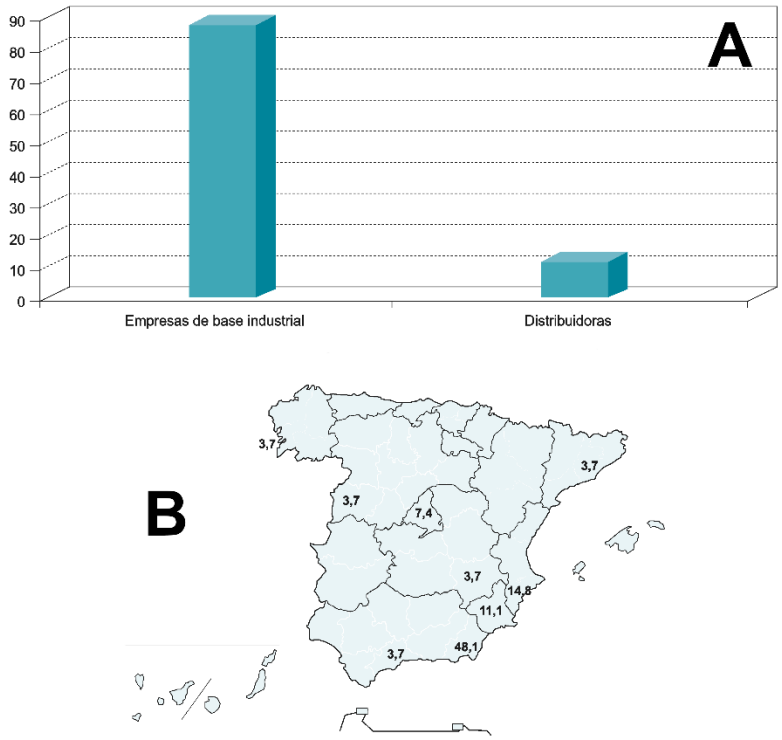

Figure I.2. Main ecological services provided by beneficial insects in agroecosystems (Source: Jones & Snyder, 2018; Alford, 2019; Readhead et al., 2020)

The biological control sector in Spain is made up of about 30 companies that are classified into two types: Industrial-based companies (they develop the industrial activity by themselves or through a third company belonging to the same business group) and Distributors (they commercialise biological control organisms produced by other industrial-based companies) (Teruel-Moreno, 2011). **Figures I.2 a,b** show, on the one hand, the distribution of the two previous groups; on the other hand, it shows their geographical location. It is noted that the highest percentage corresponds to industrially based companies and, also, the highest percentage is in Almeria.

Table I.1. Main entomophagous natural enemies used in greenhouse crops in Spain, feeding strategy, main pest species controlled and classification according to type of use in biological control programmes: (**A**) predatory mites and insects; (**B**) parasitoid insects (Source: Vila & Cabello, 2014).

A

Type	Natural enemy	Feeding strategy	Main pests controlled	Biological control method			Notes
				Preventive	Curative		
					Inoculative	Inundative	
Predatory mites	*Amblyseius swirskii*	True omnivorous (also feeding on pollen)	Whiteflies, thrips	✔ (1)	✔	✔	(1) Releases with factitious prey and pollen
	Neoseiulus cucumeris	True omnivorous (also feeding on pollen)	Thrips	✔ (1)	✔	✔	
	N. californicus	Zoophagous-oliphagous	Spider mites	✔ (1)	✔	✔	
	Phytoseius persimilis	Zoophagous-oliphagous	Spider mites	–	✔	✔	–
Predatory insects	*Aphidoletes aphidimiza*	Zoophagous-oliphagous	Aphids	–	✔	✔	–
	Adalia bipunctata	Zoophagous-oliphagous	Aphids	–	–	✔	–
	Chrysoperla carnea	Zoophagous-oliphagous	Aphids	–	✔	✔	–
	Orius laevigatus	True omnivorous (also feeding on pollen)	Thrips	✔ (1) (2)	✔	✔	(2) Intragremial predation on *A. swirskii*
	Nesidicocoris tenuis	True omnivorous (also feeding plant tissue)	Whiteflies, thrips	✔ (3)	✔	–	(3) Releases on seedlings with factitious preys
	Macrolophus pygmaeus	True omnivorous (also feeding plant tissue)	Whiteflies, thrips	✔ (3)	✔	–	
	Nabis pseudoferus	Zoophagous-polyphagous	Aphids, lepidoptera	–	–	✔ (4)	(4) Releases in hot-spots
	Feltiella acarisuga	Zoophagous-oliphagous	Spider mites	–	✔	✔	–

(continued)

B

Type	Natural enemy	Feeding strategy	Main pests controlled	Biological control method			Notes
				Preventive	Curative		
					Inoculative	Inundative	
Parasitoids	*Aphidius colemani*	Parasitism	Aphids	✔ (5)	✔	✔	(5) Banker plants
	A. ervi		Aphids	✔ (5)	✔	✔	
	A. matricariae		Aphids	✔ (5)	✔	✔	
	Dacnusa sibirica		Leaf miners	–	–	✔	–
	Diglyphus issaea		Leaf miners	–	✔	✔	–
	Encarsia formosa		Whiteflies	–	✔	✔	–
	Eretmocerus mundus		Whiteflies	–	✔	✔	–
	E. eremicus		Whiteflies	–	✔	✔	–
	T. achaeae		Lepidoptera	–	✔	✔	–

The main commercially available entomophagous species in Spain are listed in **Table I.1** (**A**: predatory arthropods, mites and insects; and **B**: parasitoid insects). The different

methods of biological control with these mites and insects are also indicated. A total of 22 species of entomophagous insects are indicated, distributed in 13 species of predators and 9 species of parasitoids.

The current rearing systems for entomophages (predators and parasitoids), listed above, and used in Spain are shown in **Figure I.3**. Different combinations can occur in the overall production systems as also indicated in the figure.

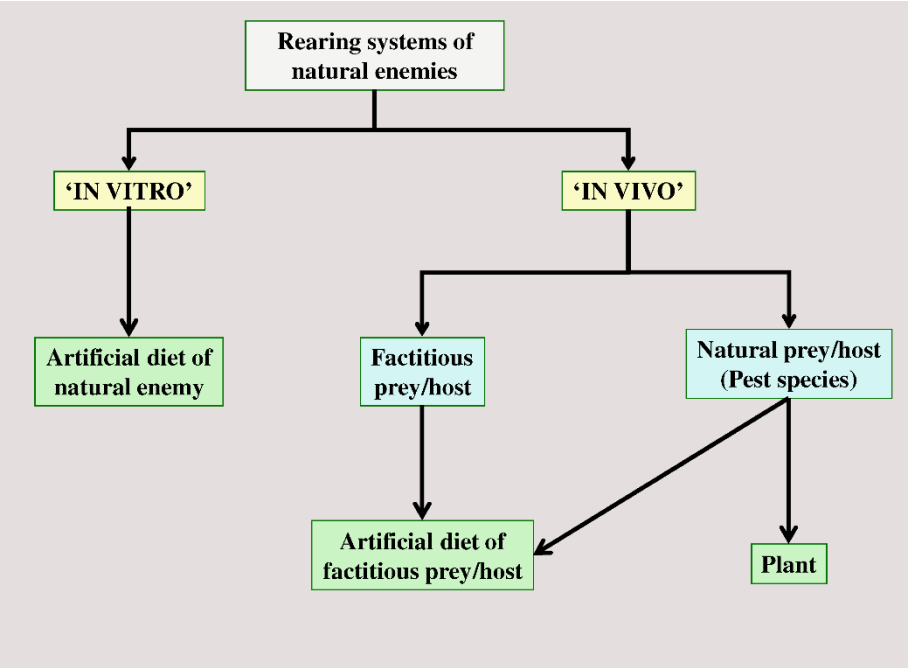

Figure I.3. General *in vitro* and *in vivo* rearing systems for entomophagous arthropods produced and commercialised in Spain.

In more detail, for each entomophagous species, the industrial entomophagous rearing systems in Spain are detailed in the **Table I.2**.

A little studied aspect, both in rearing systems (in bio factories) and in the use of biological control in greenhouses (especially through inoculative releases) is the effect of cannibalism.

Table I.2. Main characteristics in the industrial production of entomophages (predators and parasitoids) used in Spain, according to species (Source: Vila & Cabello, 2014).

production of the main currently released natural enemies in greenhouses of Almeria, Spain

Type	Natural enemy	Factitious prey/hosts	Whole plant-free	Climatic controlled rooms	Climatic rooms pests on artificial diets	Pests on plants
Predatory mites	*A. swirskii*	*Carpoglyphus lactis* *Thyreophagus entomophagous* *Suidasia medanensis*	✔	✔	–	–
	N. cucumeris	*T. putrescentiae Tyreophagus entomophagus*	✔	✔	–	–
	N. californicus	*Glycyphagus sp.*	✔	✔	–	✔[a]
	P. persimilis	–	–	–	–	✔
Predatory insects	*A. aphidimiza*	–	–	–	–	✔
	A. bipunctata	*E. kuehniella*	✔	✔	–	✔
	C. carnea	*E. kuehniella*	✔	✔	–	✔[a]
	O. laevigatus	*E. kuehniella. C. capitata*	✔	✔	–	✔
	N. tenuis	*E. kuehniella, Artemia sp.*	✔	✔	–	✔[a]
	M. pygmaeus	*E. kuehniella, Artemia sp.*	✔	✔	–	✔[a]
	N. pseudoferus	*E. kuehniella*	✔	✔	–	✔[a]
Parasitoids	*A. colemani*	–	✔	✔	✔	✔[a]
	A. ervi	–	✔	✔	✔	✔[a]
	A.matricariae	–	✔	✔	✔	✔[a]
	D. issaea	–	–	✔	–	✔
	E. formosa	–	–	–	–	✔
	E. mundus	–	–	–	–	✔
	E. eremicus					✔
	T. achaeae	*Ephestia kuheniella*	✔	✔	–	–

[a] Several companies are still producing this natural enemies using pests reared on whole plants

I.1.4. Insect pollinators

Insects are the most common and abundant pollinators of flowering plants; other insects (anthophiles) visit flowers, but do not necessarily pollinate them. The history of relationships between insects (both pollinators and anthophiles) and flowers is broad and varied (*e.g.*, Real, 1983; Patiny, 2012). Today, their importance in the functioning of ecosystems in agricultural production has been the subject of numerous scientific studies (*e.g.*, Kevan, 2008; Abrol, 2012).

Although the best-known insect pollinators are the honeybees and bumble bees. Insect species that are also pollinators and flower visitors are very abundant; mainly belonging to the orders: Hymenoptera, Diptera and Coleoptera, (Kevan, 2008).

From a strictly economic point of view, the importance of pollinators in greenhouse crops is fundamental; it is estimated that approximately 45 % of the value of production can be attributed to pollinating insects (Guerra-Sanz, 2008).

Many books and scientific papers have been published on pollination biology in the case of honeybees (*e.g.*, Delaplane *et al.*, 2000; James & Pitts-Singer, 2008); as well as in the case of bumblebees (*e.g.*, Goulson, 2003; Heinrich, 2004). However, in insects of other groups, studies related to crop pollination are less studied, as is the case for Diptera species.

Diptera are one of the three largest and most diverse groups of animals in the world. As a group of pollinators, their study has often been rather neglected, but they are considered to play an important role in agrobiodiversity and plant biodiversity worldwide. Recently, from an agricultural point of view, their importance in seed production in cultivated varieties has been pointed out (Ssymank *et al.*, 2011).

In the order Diptera, species of the family Syrphidae appear to provide a dual ecological service, both as pollination and biological pest control (Dunn *et al.*, 2020) (**Figure I.4**).

Reviews of the biology of species in the family Syrphidae are available in Gilbert (1986), Schmid (1996), and Rotheray & Gilbert (2011).

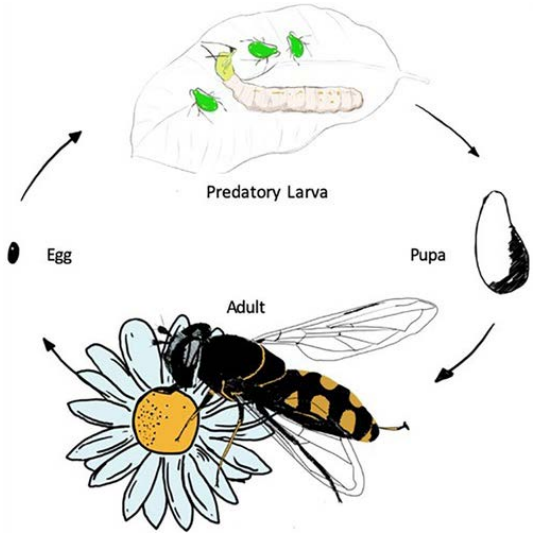

Figure I.4: Life cycle of syrphids whose larvae are entomophagous (Source: Dunn et al., 2020).

I.2. Objectives

I.2.1. General objective

The general objectives of this doctoral thesis are to address the issue of beneficial insects and their importance in greenhouse horticultural crops. For this purpose, two insects have been chosen: *Nabis pseudoferus* Remane (Hemiptera: Nabidae) and *Eristalis tenax* (L.) (Diptera: Syrphidae). The former among the entomophagous insects and the latter among the pollinating insects.

I.2.2. Specific objectives

These are defined in the following two points:

(a) In the entomophagous species, the specific objective has been to carry out experimental studies on cannibalism and intraguild predation (IGP) that can later be used as basis for the development of mathematical models.

(b) In turn, for the insect pollinator, the specific objective has been to carry out theoretical studies of pollination in greenhouse crops, without experimental support, in order to subsequently validate and extend it to other cases.

I.3. Future trends

As indicated in the previous section. This will be continued in studies after this doctoral thesis, in order to complete the mathematical models of cannibalism and its effects on biological control in greenhouses. These models are being developed in a new doctoral thesis currently in progress.

It is also expected to address the validation of the mathematical model, non-experimental, developed in the case of pollinating insects, using experimental data, both for the selected species and crop, but especially its extension to other insect pollinators and other horticultural crops in greenhouses.

I.4. References

Abrol, D.P. (2012). Pollination biology: biodiversity conservation and agricultural production. Springer, Dordrecht, NL: 792 pp.

Alford, D.V. (2019). Beneficial insects. CRC Press. Boca Raton, Fl, USA: 401 pp.

Bolckmans, K. (2008) De insectenfabriek. In: Osse, J.; Schoonhoven, L.; Dicke, M.; Buiter, R. (eds.). Natuur als bondgenoot: biologische bestrijding van ziekten en plagen. Bio-Wetenschappen en Maatschappij, Den Hagg: 51 pp.

CAJAMAR (2021). Informe de campaña 2019/2020. [https://www.cajamar.es/es/agroalimentario/innovacion/noticias-agro/ano-2020/cajamar-destaca-un-incremento-del-valor-de-la-produccion-en-su-informe-de-campana-20192020/] (last Access: 10/04/2021)

Classen, A.; Peters, M.K.; Ferger, S.W.; Helbig-Bonitz, M.; Schmack, J.M.; Maassen, G.;

Steffan-Dewenter, I. (2014). Complementary ecosystem services provided by pest predators and pollinators increase quantity and quality of coffee yields. Proc. Biol. Sci. 281: 2013.3148. DOI: doi.org/10.1098/rspb.2013.3148

Cock, M.J.W.; van Lenteren, J.C.; Brodeur, J.; Barratt, B.I.P. et al (2010) Do new access and benefit sharing procedures under the convention on biological diversity threaten the future of biological control? Biocontrol, 55: 199-218.

Colloff, M.J.; Lindsay, E.A.; Cook, D.C. (2013). Natural pest control in citrus as an ecosystem service: integrating ecology, economics and management at the farm scale. Biol. Contr., 67: 170-177.

Costanza, R.; d'Arge, R.; De Groot, R.; Farber, S.; Grasso, M.; Hannon, B.; Paruelo, J. (1997). The value of the world's ecosystem services and natural capital. Nature, 387: 253-260.

Delaplante, K.S.; Mayer, D.R.; Mayer, D.F. (2000). Crop pollination by bees. CABI Publishing, Wellingford, UK.

Dunn, L.; Lequerica, M.; Reid, C.R.; Latty, T. (2020). Dual ecosystem servicies syrphid flies (Diptera: Syrphidae): pollinators and biological control agents. Pest Mang. Sc., 76: 1973-1979.

Gallai, N.; Salles, J.M.; Settele, J.; Vaissiere, B.E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol. Econ., 68: 810-821.

Gilbert, F.S. (1986): Hoverflies. Naturalists' Handbooks. - Cambridge, 66 pp.

Goulson, D. (2003). Bumblebees: their behaviour and ecology. Oxford University Press. Oxford, UK.

Guerra-Sanz, J.M (2008). Crop pollination in greenhouses. In: James, R.R.; Pitts-Singer, T.L. (eds.). Bee pollination in agricultural ecosystems. Oxford University Press, NY, USA: 27-47.

Heinrich, B. (2004). Bumblebee economics. 2nd edition. Harvard University Press, Cambridge, USA.

James, R.R.; Pitts-Singer, T.L. (2008). Bee pollination in agricultural ecosystems. Oxford University Press, NY, USA: 27-47.

Jones, M.S.; Snyder, W.E. (2018) Beneficial insects in agriculture: enhancement of biodiversity and ecosystem services. In: Foottit, R.G.; Adler, P.H. (eds.). Insect biodiversity. Vol. 2. John Wiley & Sons Ltd., Hoboken, NJ, USA: 105-122 pp.

Junta de Andalucía (2017). Cartografía de invernaderos en Almería, Granada y Málaga. Conserjería de Agricultura, Pesca y Desarrollo Rural. Sevilla: 24 pp.

Kevan P.G. (2008). Pollination and flower visitation. In: Capinera, J.L. (ed.). Encyclopedia of entomology. Springer, 2nd edition: 2960-2971.

Klein, A.M.; Vaissiere, B.E.; Cane, J.H.; Steffan-Dewenter, I.; Cunningham, S.A.; Kremen, C.; Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. Lond. B Biol. Sci., 274: 303-313.

Lautenbach, S.; Seppelt, R.; Liebscher, J.; Dormann, C.F. (2012). Spatial and temporal trends of global pollination benefit. PloS One, 7: e35954.

Teruel-Moreno, M. (2011). El sector del control biologico en España. Informes y Monografías. Fundación Cajamar. Almería: 77 pp.

Ministerio de Agricultura, Pesca y Alimentación (2020). Encuesta sobre superficies y rendimiento de cultivos: resultados nacionales y autonómicos. Subsecretaría de Agricultura, Pesca y Alimentación. Madrid [https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/totalespanayccaa2020_tcm30-553610.pdf] (last access: 10/04/2021).

Patiny S. (2012). Evolution of plant-pollinator relationships. Cambridge University Press, Cambridge, UK: 477 pp.

Pérez-Parra, J. (2017). Principales retos de la horticultura protegida española. Madrid, 18/04/2017. [https://www.mapa.gob.es/es/agricultura/temas/producciones-agricolas/3-visiondelainvestigacionespanaijeronimoperez_tcm30-379494.pdf] (last access: 10/04/2021).

Real, L. (1983). Pollination biology. Academic Press Inc, Orlando, FL, USA: 338 pp.

Redhead, J.W.; Powney, G.D.; Woodcock, B.A.; Pywell, R.F. (2020). Effects of future agricultural chan ge scenarios on beneficial insects. J. Environ. Manag., 265: 110550. DOI: 10.1016/j.jenvman.2020.110550

Rotheray, G.E.; Gilbert, F.S. (2011). The natural history of hoverflies. Forrest Text. USA: 334 pp.

Schmid, U. (1996): Auf gläsernen Schwingen. Stuttgarter Beiträge zur Naturkunde, Serie C 40: 1-81, Stuttgart.

Schowalter, T.D.; Noriega, J.A.; Tscharntke, T. (2018). Insect effects on ecosystem services—Introduction. Basic Appl. Ecol., 26: 1-7.

Ssymank, A.; Kearns, C.A.; Pape, T.; Thompson, F.C. (2011). Pollinating flies (Diptera): a major contribution to plant diversity and agricultural production. Biodiversity, 9: 86-89. DOI: 10.1080/14888386.2008.9712892

Valera-Martínez, D.L.; Molina-Aiz, F.D.; Gil-Ribes, J.A. (1999). Los invernaderos de Almería: tipología y mecanización de la gestión del clima. Universidad de Almería, Servicio de publicaciones del Instituto de Estudio Almerienses. Almería, ES: 284 pp.

Vila, E.; Cabello, T. (2014). Biosystems engineering applied to greenhouse pest control. In: Torres, I.; Guevara, R. (eds.) Biosystems engineering: biofactories for food production in the XXI Century. Springer, Cham, Switzerland: 99-128.

Do development and diet determine the degree of cannibalism in insects? To eat or not to eat conspecifics

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Chapter II

Do development and diet determine the degree of cannibalism in insects? To eat or not to eat conspecifics

Abstract: Cannibalism in insects plays an important role in ecological relationships. Nonetheless, it has not been studied as extensively as in other arthropod groups (*e.g.*, Arachnida). From a theoretical point of view, cannibalism has an impact on the development of more realistic stage-structure mathematical models. Additionally, it has a practical application for biological pest control, both in mass-rearing and out in the field through inoculative releases. In this paper, the cannibalistic behavior of two species of predatory bugs was studied under laboratory conditions - one of them a generalist predator (strictly carnivorous), *Nabis pseudoferus*, and the other a true omnivore (zoophytophagous), *Nesidiocoris tenuis* - and compared with the intraguild predation (IGP) behavior. The results showed that cannibalism in *N. pseudoferus* was prevalent in all the developmental stages studied, whereas in *N. tenuis*, cannibalism was rarely observed, and it was restricted mainly to the first three nymphal stages. Cannibalism and intraguild predation had no linear relationship to different cannibal-prey size ratios, as evaluated by the mortality rates and survival times, although there were variations in cannibalism between stages, especially for *N. pseudoferus*. The mathematical model implications are presented and discussed.

Keywords: *Nabis pseudoferus*; *Nesidiocoris tenuis*; predatory insect; generalist predator; true omnivore; intraguild predation; ontogeny; biological control; mathematical model.

II.1. Introduction

Omnivores can be classified according to their diet or their role in ecological food webs [27]. Omnivory may be opportunistic, obligatory or facultative, based on the relative importance of plant and prey materials in the insect's diet. However, according to their ecological role in food webs, an omnivore that feeds on more than one trophic level is commonly termed a 'trophic omnivore' [27]. Intraguild predation is an example of trophic omnivory in which a predator consumes other predators with whom it shares a common herbivore prey [27-29]. 'True omnivory', therefore, is a particular case of trophic omnivory in which the consumer feeds

on both plants and prey [27]. Therefore, *N. tenuis* is a "true omnivore" and *N. pseudoferus* is a "generalist predator". According to Hurd [30], generalist arthropod predators are typically bi-trophic: they simultaneously occupy the third and fourth trophic levels by virtue of feeding both on herbivores and each other, *i.e.*, they engage in intraguild predation (IGP).

At the same time, predation can be either between species or among individuals within the same species, since most generalist predators are cannibals [30]. Cannibalism occurs very frequently in nature and has been documented in more than 1,300 species [31,32]. For many arthropods, cannibalism is a normal phenomenon, not an anomaly. Cannibalism has been documented in many insect orders including Odonata, Orthoptera, Thysanoptera, Hemiptera, Trichoptera, Lepidoptera, Diptera, Neuroptera, Coleoptera and Hymenoptera. It occurs among predatory species and herbivores, involving predation by the mobile adults and larvae or nymphs on each other, and on immobile eggs and pupae [33,34]. There are many types of cannibalism, e.g., filial cannibalism as an energetic benefit [35], sibling cannibalism [36,37] and intrauterine cannibalism in parasitoid insects [38], in which the cannibalism can increase the survival rate when food is scarce [39], sexual cannibalism, in which a female insect cannibalizes her male mate during copulation [40], cannibalism as competition [41] or the parasitizing of offspring [34] etc.

In many systems, IGP and cannibalism occur together [28], and IGP is often associated with cannibalism [42,43]. Omnivory can be viewed as a strategy to reduce intraguild predation levels (and cannibalism) as it may allow omnivores to change locations and feed on plants under threat of predation [27].

From a practical point of view, the effects of IGP and cannibalism in biological pest control have received unequal attention. Many previous studies have been dedicated to the effects of IGP on the efficacy of natural enemies [56]. Most frequently, IGP is reported to be damaging or antagonistic [43,57-62] although it may sometimes have a neutral [62] or beneficial (synergistic) effect [63,64]. The effect of cannibalism has received less modelling attention, which is curious since it is often associated with IGP. Moreover, it is an important impediment to efficiency in the mass production of biological control agents [65-70]. In addition, in augmentative biological control, releases can result in high densities of natural enemies at low pest

levels, or before the pest appears on the crop [16]. Thus, cannibalism could exert an important influence on biological control outcomes [72-75]. Finally, because cannibalism is ubiquitous in food webs and frequent in systems where predator and prey share a common resource (IGP), its impacts on interspecific interactions and community dynamics and structure need to be better understood [54,76].

Biological pest control systems are very complex, especially in greenhouse crops where various beneficial organisms may be employed at the same time (predators, parasitoids and entomopathogens) in the same crop cycle to control different phytophagous species [16]. In such systems, it becomes more important, and sometimes fundamental, to recognize all the ecological relationships because the success of the system may depend on this knowledge [64]. Information is limited on the effects of cannibalism regarding the efficacy of biological pest control in these systems when, for example, high densities of natural enemies are released in augmentative biological programs [15] or when the pest population is not present, or present only at a low density.

At the same time, agroecosystems are usually modified to be simpler than natural ecosystems [77], involving fewer factors and fewer interactions, which could facilitate the interpretation of ecological relationships. The aim of this work was to study the importance of cannibalism in two species of predatory bugs with different feeding behavior that are often used in biological control programs.

Nesidiocoris tenuis (Reuter) (Hem.: Miridae), an omnivorous species [1], was introduced into Europe [2,3] from an originally paleotropical distribution. The species feeds both phytophagously and zoophagously, and has been considered a crop pest [4,5]. Its prey range includes aphids, whitefly, and eggs and larvae of small lepidopterans [6-9]. Conversely, *Nabis pseudoferus* Remane (Hem.: Nabidae) can be considered a generalist predator (a non-omnivorous species) [10]. The majority of the Nabidae studied also practice plant feeding but they are not able to develop in the absence of prey [11-13]. Plant feedings are believed to be for the purpose of water acquisition [14] and do little or no damage to the plant. This practice seems to help the predator survive during prey scarcity [13]. *N. tenuis* is currently used as a biological control agent in tomato greenhouses to control whitefly *Bemisia tabaci* (Gennadius) (Hem.: Aleyrodidae) and the tomato leafminer *Tuta absoluta* (Meyrick) (Lep.: Gelechiidae) [15,16].

The other species, *N. pseudoferus* (Hem.: Nabidae), has a wide prey range and is considered to be an important predator of aphids [17,18], but also a voracious predator of lepidopterans and other groups of arthropods, including hemipterans and spider mites [19-22]. *N. pseudoferus* is also currently used as a biological pest control agent of lepidopterans in greenhouse crops [16].

The cannibalism performed by each species was studied, both in the presence and absence of prey, in relation to their ontogeny under laboratory conditions, after which the IGP between both species was assayed under similar conditions. Cannibalism by the generalist predator was also studied under microcosm conditions.

II.2. Materials and methods

II.2.1 Ethics stamen

The *N. pseudoferus* specimens were collected using a sweep net from alfalfa grown on private land after obtaining the owners' permission. The sampling methods, the collection of the experimental *N. pseudoferus*, the rearing under controlled conditions, and the design and development of the experiments, etc. (for this species and the other insect species used in this work) were in accordance with the Spanish and European legislation on the protection of animals used for scientific purposes, which exclude invertebrates/insects.

II.2.2. Biological material

A colony of *N. pseudoferus* was established from a population collected in Pinos Puente (37.248258 N, 3.765974 W) and Atarfe (37.218402 N, 3.713381 W), Granada, Spain, and reared under laboratory conditions for 25 generations (3 years; 1.5 months/generation) before being used in the experiments. Every year new individuals collected at the same locations were added to the laboratory population to avoid inbreeding and loss of genetic variability. A colony of *N. tenuis* was established from material purchased from a commercial producer (Nesidiocontrol ®, Agrobio S.L., La Mojonera, Almeria, Spain) and reared in the lab for two generations before being used in the assays. Frozen eggs of *Ephestia kuehniella* Zeller (Lep.: Pyralidae), which

were also used in the experiments, were purchased from a commercial supplier (Ephescontrol®, Agrobio S.L., La Mojonera, Almeria, Spain) and stored at -40ºC until use.

II.2.3. Laboratory trial

Four laboratory assays, adapted from the methodology of Walzer and Shausberger [78], and Schausberger and Croft [79], were conducted under the physical conditions of 25±1ºC, 60–80% relative humidity (R.H.) and a 16 h:8 h light:dark photoperiod.

II.2.3.1. Experimental design and methodology

Seven-day-old adult females collected from the stock colony were assumed to be sexually mature and mated. Newly molted nymphal stages with hardened exoskeletons were selected to avoid presenting conspecifics during a vulnerable period of ecdysis [32]. All the individuals were isolated in plastic containers (500 ml) with a sponge (2.0 x 2.0 x 0.2 cm) moistened with distilled water and starved for 24 hours prior to use in the assays. Individuals were then transferred to a new container in pairs, depending on the treatments described below, without refuge, water or food. The trials were performed over three days.

The treatments were as follows: (a) Cannibalism assay for *N. pseudoferus*: all 21 pairwise mathematical combinations, with repetitions and non-order from the following life stage/instar: adult female, V-, IV-, III-, II- and I-instar nymph (**Table II.A.1**); (b) Cannibalism assay for *N. tenuis*: the treatments consisted of the same 21 combinations as above (**Table II.A.1**); (c) *N. pseudoferus-N. tenuis* IGP-assay 1: in the hypergeometric distribution in which there are two subpopulations (or subgroups) that do not have elements in common: subpopulation 1 (6 elements): adult female, V-, IV-, III-, II- and I-instar nymphs of *N. pseudoferus*, and subpopulation 2 (6 elements): adult female, V-, IV-, III-, II- and I-instars of *N. tenuis*; twenty-one pairs were chosen, as shown in **Table II.A.2**, in which the two species were at the same or lower stage class; and (d) *N. pseudoferus-N. tenuis* IGP-assay 2: in the hypergeometric distribution in which there are two subpopulations (or subgroups) that do not have elements in common: subpopulation 1 (5 elements): V-, IV-, III-, II- and I-instar nymphs of *N. pseudoferus*, and subpopulation 2 (5 elements): adult female, V-, IV-, III- and II-instars of *N. tenuis*. Fifteen pairs were chosen, as shown in **Table II.A.3**, in which *N. pseudoferus* was always in a lower stage class than *N. tenuis*.

Twenty repetitions were performed for each assay and treatment. All the assays were conducted identically on different days until all the treatments and repetitions were completed.

We used the instantaneous sampling method [80,81] to analyze the survival times of individuals. Each container was observed for one minute (the sample point) every 30 minutes (the sample interval) until the sixth hour of the first day. If no individuals died, the procedure was performed again on the second day, and if there was still no reaction, the procedure was repeated on the third day.

Additionally, 20 adult females and 20 nymphs from each developmental stage (instars) were selected from the rearing populations, placed under the same conditions as before, mounted in alcohol and measured (length and width) using a micrometer under a binocular microscope.

II.2.3.2. Statistical analysis

The cumulative survival times of the nymphal instars or adults, caged with either a conspecific or heterospecific, were analyzed using the Kaplan–Meier procedure [82,83]. This procedure is a method of estimating time-to-event models in the presence of censored cases. Within the Kaplan–Meier procedure, the equality of survival functions was compared with Breslow tests [84] using IBM SPSS version 25 software [85].

The mortality data were expressed as percentages, and survival times in hours. The data obtained in the cannibalism assay of *N. pseudoferus* and *N. tenuis*, as well as the values corresponding to IGP *N. pseudoferus-N. tenuis*, were adjusted to follow the non-linear (quadratic) regression:

$$Y = a + b \cdot x + c \cdot x^2 \quad (1)$$

where Y = the mortality or survival time, and x is the size ratio (the difference of the product of length x width of the predator minus the product of length x width of the prey), expressed in mm^2. The size difference parameter was used because, for many species, cannibalism and IGP are more related to size disparity than to absolute size [32]. The previous equation was adjusted

by non-linear regression using the Statgraphics Centurion version 18 statistical software package [86].

II.2.4. Microcosmos trials

Regarding the high level of *N. pseudoferus* cannibalism found in previous trials, the starting hypothesis was to check whether the presence of alternative prey and refuge could significantly reduce such cannibalism. To test this hypothesis, two trials were conducted under microcosm conditions to evaluate filial and sibling cannibalism as a function of the predator developmental stage and prey density.

II.2.4.1. Experimental design and procedures

The two trials were performed with individuals selected from the lab stock colony of *N. pseudoferus*, using the same procedure as described above. Individuals were isolated in 500 ml plastic containers (as above) and starved for 24 hours prior to the assays, after which the individuals were transferred to a new plastic container (40.0 x 30.0 x 21.0 cm.; used as a microcosm). The containers had two holes on the top (5.0 cm. in diameter) covered with mesh. One tomato plant, cv Vernal®, Enza Zadem (ca. 23 cm high, with 7–8 leaves), was included in each container. *E. kuehniella* eggs were used as prey and they were always provided in the same way to avoid prey search problems and conflicts between conspecifics, as well as to ensure uniform distribution, as described below. The *E. kuehniella* eggs were adhered with water to a 15 cm-long portion of sisal rope. To ensure the correct prey weight, all the ropes were weighed with precision scales before and after the trial. The rope pieces with the *E. kuehniella* eggs attached were then entwined around the plant stem to eliminate any predator concentration in one location. *Nabis* adults are known to prefer the upper parts of the plant (inside the plant canopy) while the immature stages tend to stay lower down on the plant, outside the canopy [87,88].

The factorial design used a single factor at two levels: (1) the presence or absence of adult females, and (2) the *E. kuehniella* prey density. Each treatment was repeated four times.

In the first trial, 10 I-instar nymphs were placed in each container whereas in the second assay, there were five III-instar nymphs per container. The same prey densities were used as in

the first assay: 0, 0.006, 0.011 and 0.040 g/day; and in the second assay: 0, 0.011, 0.040 and 0.080 g/day.

Both trials were performed at 25±2 ºC, 60–80% R.H. and a 16:8 L:D photoperiod. The containers were examined daily for *E. kuehniella* prey replenishment and the developmental stage of the predators was checked. The first assay was terminated when 50% of the nymphs molted to the III-instar. Similarly, the second assay ended when 50% of the nymphs reached the adult stage. The number of individuals surviving to the end of the assay was then recorded. In addition, the females used in the second microcosm assay were previously marked [89]: A dot of 0.4 pigment liner (art. no.: 308 04-9, Staedtler®) was applied to each quadrant of the pronotum.

II.2.4.2. Statistical analysis

The survival percentages were subjected to a generalized linear model (GZLM) analysis using IBM SPSS version 25 software [85]. The models were fitted by maximum quasi-likelihood estimation using the GenLin procedure with normal errors and the identity function. In each trial, the significance of the model was assessed with an Omnibus test (to test whether the explained variance in a data set is significantly greater overall than the unexplained variance). For each regression effect specified in the model, a Wald statistical test was carried out, which is based on the linearly independent pairwise comparisons among the estimated marginal means. Then, the mean values were compared pairwise, with significance indicated at $P = 0.05$.

To estimate the nymph mortality specifically due to cannibalism by adult females, the Henderson-Tilton equation [90] was applied:

$$M_C = \frac{M_t - M'_t}{100 - M'_t} * 100 \qquad ((2)$$

where M_C is the corrected percentage of mortality due to adult females, M_t is the percentage of nymphal mortality in the presence of adult females at the end of the assay, and M'_t is the percentage of nymphal mortality in the absence of adult females at the end of the assay.

II.3. Results

II.3.1. Stage structure

The size of *N. pseudoferus*, especially the length, increased from 1.84 ± 0.04 mm for the first instars to 7.11 ± 0.06 mm in adult females, while in *N. tenuis*, it increased from 0.96 ± 0.03 mm to 3.12 ± 0.02 mm (**Figure II.1**). In contrast, only the last nymph instars (IV- and I-instars) and adult females of *N. tenuis* had the same or greater size than the first nymphal instars of *N. pseudoferus* (I- and II-instars).

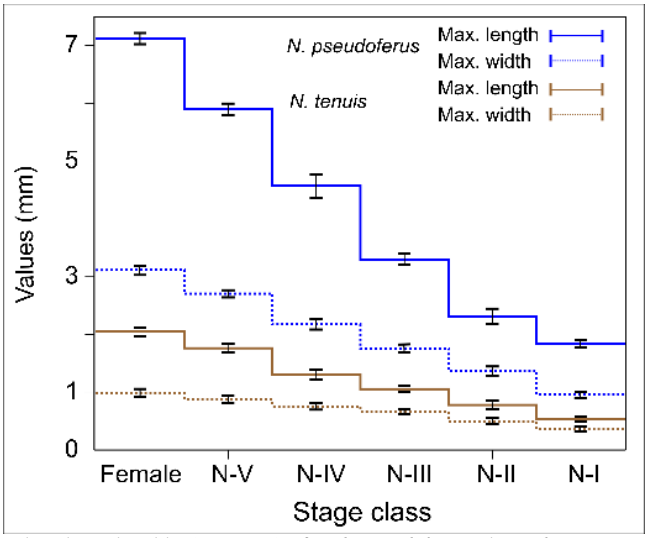


Figure II.1. Average maximum lengths and widths (mm, ±SE) of *Nabis pseudoferus* and *Nesidiocoris tenuis* according to post-embryonic development.

II.3.2. Laboratory trial 1: *N. pseudoferus* cannibalism

Cannibalism by *N. pseudoferus* in the absence of prey was very high in all nymphal instars and adult females (**Figure II.2**). The average survival of all the bugs was quite low (41.7%). Higher values were only observed when both conspecifics were in the same developmental stage. Average survival increased from 11.7% for first instars to 65.0 % for adult females.

The Kaplan-Meier procedure revealed significant differences within the treatments (Breslow test, generalized Wilcoxon χ^2 = 68.925, df = 5, P < 0.0001) (**Table II.A.4**). Except for one case, the differences in the survival time were all significant, indicating high levels of cannibalism, but with different values. For all stages, the average survival time was 38.05 ± 1.38 h, just over half the experimental time limit (72 h). The survival time increased from 23.17 ± 2.02 h for the I-instars to 63.00 ± 2.91 h for adult females. The highest survival times were observed

when conspecifics from the same developmental stage were paired. Lower survival times were observed when first and second instar nymphs were paired with later developmental stages.

The mortality and survival time adjusted for the size ratio are shown in **Figures II.A1a, b**. The values for the *a*, *b* and *c* parameters were 34.32 ± 7.51, 12.29 ± 3.36 and -0.60 ± 0.27, and 57.75 ± 4.66, -6.70±2.08 and 0.28±0.17 for the mortality and survival time, respectively. Both models were highly significant (F = 15.03, df = 2, P = 0.001; and F = 15.32, df = 2, P = 0.001, respectively). One could observe that prey mortality (in smaller sizes) increased with increasing size difference between conspecifics. In other words, the survival time decreased with increasing size disparity.

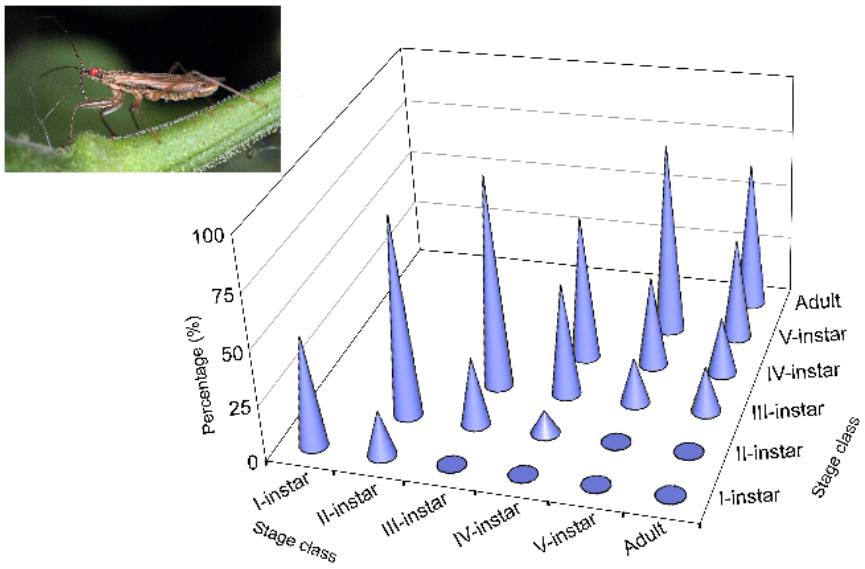


Figure II.2. Survival (%) from *Nabis pseudoferus* cannibalism by life stage when caged with other conspecifics of the same or lower stage class, over 72 hours under laboratory conditions (25±1ºC and 60–80% R.H.) without prey.

II.3.3. Laboratory trial 2: *N. tenuis* cannibalism

In contrast to *N. pseudoferus*, *N. tenuis* showed a lower level of cannibalism (**Figure II.3**). The average survival time for all stages was 82.6%, almost double that of *N. pseudoferus*. The survival percentage was lower for the I-instars (61.0 %) and it increased up to 100% for adult females. The lowest survival percentages were lower than the average of the I- to III-instar nymphs from the same developmental stages (Figure 3). Additionally, unlike the other species, the most developed nymphal stages and the adult females exhibited little or no more cannibalism than first instars.

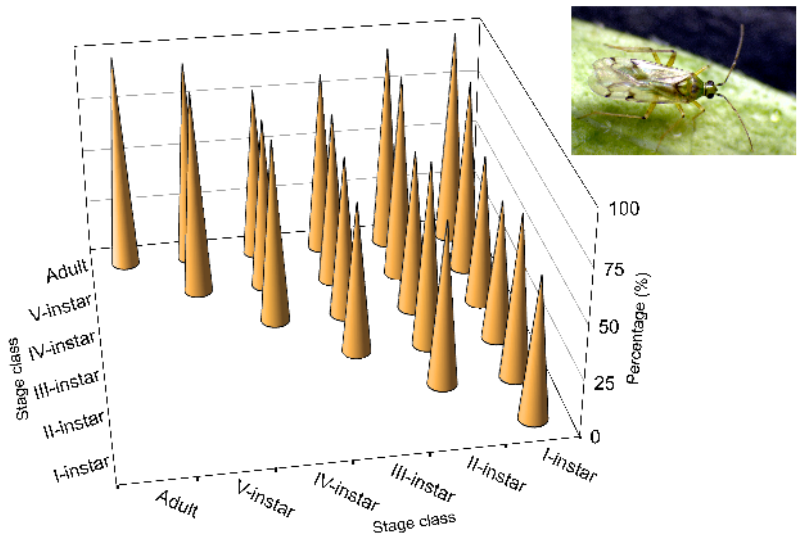


Figure II.3. Survival (%) from *Nesidiocoris tenuis* cannibalism by life stage when caged with other conspecific of the same or lower stage class, over 72 hours under laboratory conditions (25±1ºC and 60–80% R.H.) without prey.

The aggressiveness of *N. tenuis* in relation to its conspecifics, measured as survival time (**Table II.A.5**) was also very low, with an average value of 63.19±0.95 h over the 72h trial time. Despite the overall comparison in the Kaplan-Meier procedure, significant differences between treatments (Breslow test, generalized Wilcoxon χ^2 = 11.443, df = 5, P < 0.043) for the adult females were found by comparing strata or pair-only differences. The data found in the *N. tenuis* trial in the absence of prey or a food source (*e.g.*, the plant) demonstrated very low cannibalistic behavior.

Figures II.A.2 a,b shows the nonlinear adjustments of mortality and victim survival in response to *N. tenuis* cannibalism. The values for the *a*, *b* and *c* parameters were: 16.16±3.53, 18.89±9.16 and -10.54±4.15; and 63.42±1.77, -8.85±4.57 and 5.26±2.07, respectively. In this case, the model's adjustments to mortality and survival time were significant (F = 4.16, df = 2, P = 0.0328; and F = 4.87, df = 2, P = 0.0204, respectively).

II.3.4. Laboratory trial 3: IGP *N. pseudoferus-N. tenuis* assay 1

From the overall comparison in the Kaplan-Meier procedure, it was determined that there were significant differences between treatments (Breslow test, generalized Wilcoxon χ^2 = 74.582, df = 5, P < 0.0001).

Table II.A.6 shows the aggressiveness of *N. pseudoferus* when paired with *N. tenuis* individuals at the same developmental stage. The average survival time values were very short

compared with those observed for *N. pseudoferus* in the cannibalism assay (**Table II.A.4**). The average time of the trials was 16.04±0.60 h, which was less than one-quarter of the exposure time (72 h). The low survivorship time of *N. tenuis* adults in the presence of *N. pseudoferus* adult females was notable (**Table II.A.6**), with a value of 3.53 ± 1.43 h, which was very significant compared to other values. This might be because the higher prey mobility (due to wings) encourages more intensive predation by *N. pseudoferus* females. However, the survival time for *N. tenuis* I-instars seems to be very similar to that observed for *N. pseudoferus* I-instars (**Table II.A.4**), indicating that adult females of both species were equally aggressive toward first instars.

The failure of any *N. tenuis* stages to survive 72 h indicates that *N. tenuis* is prey for *N. pseudoferus*. There was no mortality of any *N. pseudoferus* stage as a result of *N. tenuis* predation. Therefore, another IGP trial was carried out, as described in the following section, to evaluate the IGP when *N. pseudoferus* was always in a lower stage class than *N. tenuis*.

II.3.5. Laboratory trial 4: IGP *N. pseudoferus*-*N. tenuis* assay 2

Due to the very low survival of *N. tenuis* as IGP-prey in relation to the actions of *N. pseudoferus* as an IGP-predator (as indicated in the previous section), a second trial was carried out in which the size differences between the two species were smaller. There were significant result differences between treatments (Breslow test, generalized Wilcoxon χ^2 = 280.776, df = 1, P < 0.0001) for the overall comparison in the Kaplan-Meier procedure (**Table II.A.7**). The mean survival time of the different *N. pseudoferus* stages was 68.76±1.15 h compared to 35.23±0.74 h for *N. tenuis*. This last value is much higher than that found for this species in the previous trial.

The survival of *N. pseudoferus* in the V- and IV-instars was 100%, but it was slightly lower in the earlier stages (70 - 100%) (**Figure II.4**). Conversely, the survival of *N. tenuis* was low (**Figure II.4**), but higher than in the previous trial, in which no individuals survived to the end of the assay. The results as a whole lead us to say that, in terms of IGP, size differences are very important, as indicated in the cannibalism trials.

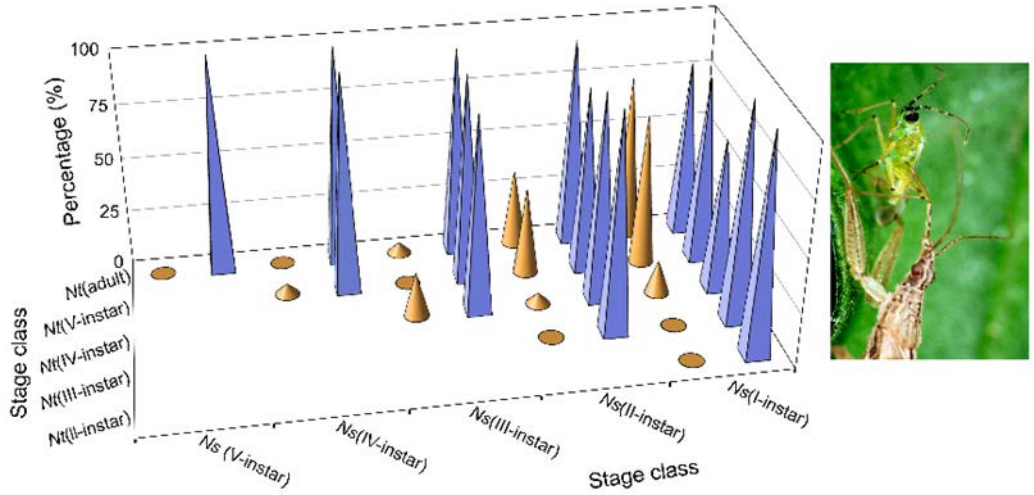


Figure II.4. Stage-specific survival (%) of *Nesidiocoris tenuis* (Brown cone, Nt) and *Nabis pseudoferus* (Blue pyramid, Ns) in intraguild predation (IGP) when caged singly with the other species for 72 hours under laboratory conditions (25±1ºC and 60–80% R.H.) without prey, and when *N. pseudoferus* was always in a lower stage class than *N. tenuis*.

It has to be pointed out that *N. pseudoferus* I-instar aggressiveness towards *N. tenuis* II-instars (19.38±1.81 h) (**Table II.A.7**) is similar to that of *N. pseudoferus* I-instars towards *N. tenuis* I-instars (20.60±1.60 h) (**Table II.A.3**), although both of the values were substantially lower than that found in I-instar cannibalism for *N. pseudoferus* (43.60±6.58 h) (**Table II.A.4**). This seems to indicate the cannibalistic intensity, which was lower in this stage than in the non-conspecific.

The model adjusted to the *N. tenuis* mortality percentage and survival time, as the data of the two former assays (IGP), is shown in **Figure II.A.3a, b**. At the same time, the parameter values *a*, *b* and *c* were 80.04±3.37, 7.25±1.47 and -0.46±0.12, and 34.61±2.04, -4.95±0.90 and 0.26±0.07 for the mortality and the survival time, respectively. The same was true for *N. pseudoferus* cannibalism, as the IG predation produced an increase in mortality and a decrease in the survival time for the IG prey, with an increase in the size differences between them in both cases.

II.3.6. Microcosm trials: Effects of the prey density

II.3.6.1. N. pseudoferus *cannibalism in the I- to III-instars*

Figure II.5a shows the survival percentage for the *N. pseudoferus* nymphs (I- to III-instars), depending on prey density, and the presence or absence of adult females. The GZLM analysis showed that the model was highly significant (likelihood ratio χ^2 = 45.431, d.f.= 7, P <

0.0001). The presence of adult females (likelihood ratio χ^2 = 20.998, df = 1, P < 0.0001), prey density (likelihood ratio χ^2 = 33.356, df = 3, P < 0.0001) and interactions (likelihood ratio χ^2 = 10.134, df = 3, P = 0.0170) had significant effects on nymphal survival. Survival was zero in the absence of prey and increased with prey density. Similarly, the survival of nymphs was lower in the presence of adult females than in their absence for prey densities 1, 2 and 3, respectively (**Figure II.5a**). However, there were no significant differences in nymphal survival at densities 1, 2 and 3 in the absence of adult females, nor in the presence of adult females at high prey densities (**Figure II.5a**).

II.3.6.2. N. pseudoferus *cannibalism in the III-instar to adult stages*

For nymphs in a more advanced developmental stage (III-instar to adult), the GZLM analysis showed the model was highly significant (likelihood ratio χ^2 =80.823, d.f.= 7, P < 0.0001). The presence of adult females (likelihood ratio χ^2 = 11.811, df = 1, P < 0.001) and prey density (likelihood ratio χ^2 = 79.230, d.f. = 3, P < 0.0001) had significant effects on survival, with no significant interaction between both factors (likelihood ratio χ^2 = 10.134, d.f. = 3, P = 0.190).

The nymphal survival for prey densities 1, 2 and 3 (the III-instar to adult trial) in the absence of adult females was 50.0±5.8, 75.0±5.0 and 90.0±5.8 %, respectively, and this was lower in the presence of females, 35.0±5.0, 55.0±5.0 and 75.0±9.6 %, respectively (**Figure II.5b**). There were no significant differences in nymphal survival for each prey dose evaluated (1, 2 and 3) in the presence or absence of adult females. Therefore, compared to the previous trials, these values seem to indicate that, at the initial developmental stages of *N. pseudoferus* (the I- to III-instar trial) in the presence of different prey doses, there is a higher incidence of adult female cannibalism than in the later nymphal developmental stages, as shown in the previous cannibalism trials. Using the Henderson-Tilton equation, the mortality values for adult female cannibalism were 79.0, 81.1 and 41.7% in the first microcosm assay, and 30.0, 26.7 and 16.7% in the second, for prey densities 1, 2 and 3, respectively. Thus, in the presence of plant and prey, most cannibalism is carried out by adult females.

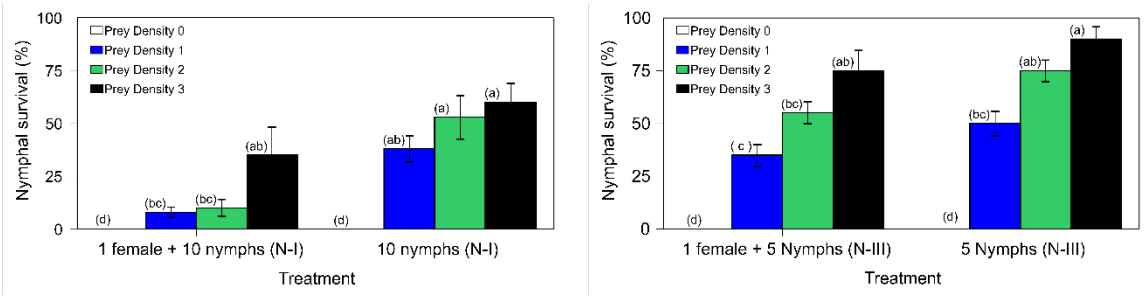


Figure II.5. Survival (%) (±SE) of the later *Nabis pseudoferus* instars: (**a**) N-I to N-III) or (**b**) N-III to adult in the presence or absence of an adult female, according to the prey density. The microcosm trial was performed using tomato plants under laboratory conditions (25±2ºC and 60–80% R.H.).

II.4. Discussion

The predatory species *N. pseudoferus*, which feeds on food sources from more than one trophic level, may be considered "trophic omnivores", according to Coll and Guershon [91]. It is considered a generalist predator; however, in regard to the diversity of the taxonomical groups attacked, it is more specialist than other generalist predators, for instance, spiders, which are able to feed on several trophic levels [10]. *N. pseudoferus* was strongly cannibalistic when prey was absent. Later development stages performed more acts of cannibalism, especially adult females. The results are within the general rule for cannibalism [32]. In contrast, the cannibalism rate for the omnivorous species *N. tenuis* was substantially lower; this is a "true omnivore", following the terminology of Coll and Guershon [91], a particular case of trophic omnivory in which the consumer feeds on both plants and prey. For this species, the same nymphal instars (I, II and, to a lesser extent, III) were cannibalized by conspecifics of the same developmental stage (**Figure II.3** and **Table II.A.4**). All of this serves to differentiate the two species. It also means that cannibalism in *N. tenuis* is an exception to the general rule of cannibalism, in which the largest (and older) individuals commit more acts of cannibalism than smaller (and younger) individuals. This is similar to other exceptions cited in other species, such as certain species of fish, dragonfly larvae and parasitoid larvae that are more cannibalistic when smaller (younger) [32].

There are few studies published on cannibalism in *Nabis* species, with the exception of the studies performed on the American species *N. alternatus* Parshley [92,93], as well as on the European species *Himacerus apterus* F. [94]. The observed results regarding the incidence of cannibalism in *N. pseudoferus* are larger than those cited for *N. alternatus*.

On the other hand, the cannibalism rate for *N. pseudoferus* in the absence of prey is comparable to those cited in spiderlings of several wolf spider species [31,95,96]. However, in other species of this spider group, the cannibalism rate is lower [97,101].

In relation to other arthropod groups, the values found for *N. pseudoferus* were similar to those cited for larvae preyed upon by adult females in some species of predatory mites (Acari: Phytoseiidae) [79].

Cannibalism in *N. pseudoferus*, as commented on before, is very important and seems to be closely related to the absence of prey, as well as to size differences between the victim and the predator, as can be observed in the mortality percentages (**Figure II.A.1a**) and survival times for the different developmental stages studied (**Figure II.A.1b**). The importance of size differences in cannibalism has been cited and widely documented for scorpions [32, 107], spiders [36,96,107,108] and predatory coccinellids [109,110], as well as for other invertebrate and vertebrate species [106, 111]; however, it has seldom been studied in insects, with the exception of the work by Laycock *et al.* [112].

Analyzing the importance of size differences in *N. pseudoferus* cannibalism in more detail, for some species (*e.g.*, fish or wolf spiders), it was reported that there is a predator-prey size difference threshold at which cannibalism takes place [32, 108]; this does not seem to be the case in *N. pseudoferus*. However, only the papers by Polis [32,106] have studied size and cannibalism in detail. Polis [106] found that the relationship between size (size ratio: larger/smaller) and cannibalism was linear in desert scorpions. However, for *N. pseudoferus*, the relationship between size and cannibalism, whether the mortality percentage or the survival time, are not linear. The differences could be due, at least in part, to differences in development and life-cycle duration, as in the scorpion species studied, *e.g.*, *Paruroctonus mesaensis* (Stahnke), which has a life cycle > 60 months, compared to the short developmental period of *N. pseudoferus* (30 days; unpublished data), and in part due to different predation behavior.

A nonlinear relationship between the mortality, or the survival time, and the size ratio was found in *N. pseudoferus* (**Figures II.A1a, b**), which seems to be because of two effects: size differences and developmental stages, given that size differences are fundamental for cannibalism

to occur. However, cannibalism is also influenced by the developmental stage of the predator, as can be observed from the survival percentages and survival times (**Figure II.A1a,b**). Therefore, cannibalism varies with the different developmental stages of *N. pseudoferus*, as measured in the survival percentage and survival time.

The cannibalism results for *N. tenuis* show very low rates in the absence of prey or other food sources (*e.g.*, plant), and in the absence of water. These results accord with those of Moreno-Ripoll *et al.* [113] for I- and II-instars of the same species in the absence of prey, although these authors reported different cannibalism results for adult females. In our work, there was no case of cannibalism between adult females whereas these authors did cite adult female cannibalism. The differences could be explained by the higher densities of female used by these authors. High density increases the number of encounters, and thus more cannibalism occurs [32, 107]. Additionally, the survival values for *N. tenuis* in the presence of conspecifics are similar to those found in *Macrolophus pygmaeus* Wagner (Hem.: Miridae).

Higher values of *N. tenuis* cannibalism were observed (**Figure II.3**) in the first three nymphal instars, performed by conspecifics at the same stages, as mentioned above. It should be noted that these *N. tenuis* nymphal instars have a higher degree of phytophagy. Thus, the I-instars of the species are able to survive until they become III-instars by feeding only on plant material [117,118, 119]. In contrast to the III-instars, the species shows greater zoophagy [117]; this is contrary to the results that showed reduced cannibalism at that stage and at subsequent stages. Perhaps in the first three instars, cannibalism is not caused by the need to eliminate potential competitors, as cited for other species [28,31,32,110,120].

The results showing low-level cannibalism in omnivorous *N. tenuis* suggest that omnivores sustain themselves on plant sources in the absence of prey without the need to resort to cannibalism, as stated by Leon-Beck and Coll [121]. Moreover, their potential as a phytophagous species, as observed in our results, is opposite to that reported by Bernays [122] for cannibalism in phytophagous insects, suggesting that in this group of insects, cannibalism is more common among generalist than specialist herbivores.

For *N. tenuis*, despite finding weak cannibalism, we also established a nonlinear relationship between the mortality percentage or survival time, and the size differences between the predator and the victim (**Figures II.A.2 a,b**); this contrasts with *N. pseudoferus*, where we found cannibalism differences occurring at low to intermediate sizes. This would suggest that cannibalism is more influenced by behavior, mainly of the early instars, than the size differences of the conspecifics.

In this work, the differences between *N. pseudoferus* and *N. tenuis* in the cannibalism rate and the attack stage, when both species share the same ecological niche [123], could be due to their different diets: *N. pseudoferus* is a "non-omnivorous predator", and *N. tenuis* is a "true omnivore". Thus, in the absence of prey, *N. pseudoferus* cannot opt for any other food source than cannibalism whereas, under the same circumstances, *N. tenuis* can choose to feed phytophagously. In contrast, omnivory could be a strategy to reduce IGP levels (and cannibalism) as it allows omnivores to change their location and to feed on plants in the presence of other predators [27].

From the IGP trial results, we can see that *N. pseudoferus* acts as an IG predator and kills *N. tenuis* as IG prey (**Figure II.4**). It is an asymmetrical relationship in favor of the former species, depending on the size differences between the species (**Figure II.1**). This confirms the work of Polis *et al.* [28], which states that relative body size and the degree of trophic specialization are the two most important factors influencing IGP frequency and direction. Most IGP occurs in systems with size-structured populations and is carried out by generalist predators who are usually larger than their intraguild prey. Many of these IG predators also cannibalize smaller conspecifics.

By studying *N. tenuis* predation at different developmental stages by *N. pseudoferus* in the two IGP tests performed, we can observe that both the survival percentage and the survival time are strongly influenced by size differences between the predator and prey (**Figures II.A.3 a,b**; **Tables II.A.6** and **II.A.7**) – this is known to happen in cannibalism, and has been shown, not only in our results, but also in numerous studies [28,32,93,124,125].

However, in the present study, we found exceptions to the above relationship of size and IGP. In general, the different *N. tenuis* developmental states are smaller in size than those of *N. pseudoferus*, except for the adult stage of *N. tenuis*, which is very similar to the III-instar state of *N. pseudoferus* (and larger than the I- and II-instars), whereas the V-instars of *N. tenuis* are larger than the I- and II-instars of *N. pseudoferus* (**Figure II.1**). Despite these size differences, similar survival percentages and survival times were observed for *N. tenuis* adult females paired with I-instars of *N. pseudoferus* (**Figure II.3** and **Table II.A.7**). In the other cases where there was an equal or smaller size, *N. pseudoferus* predated *N. tenuis*. This may be motivated (in the absence of molting individuals) by the fact that, for smaller or same-sized individuals, *N. tenuis* exhibits better-suited predatory behavior for capturing and killing than does *N. pseudoferus*. We know that *Nabis* species inject venom into their prey [126,127] and/or have better morphological adaptations (raptorial forelegs) [128] (**Figure II.2**), characteristics that are not present in the other species. Such behavior in predatory *Nabis* species was observed when they were attacking larger, phytophagous species (*e.g. Spodoptera exigua* (Hübner), Lep.: Noctuidae) [129].

The importance of the presence of prey and refuge (the plant) in *N. pseudoferus* cannibalism has been underscored in both microcosm assays (**Figures II.5 a, b**). This had already been cited in numerous studies on the presence and density of prey [37,73,109] and refuge [31,58]. However, in such circumstances, the cannibalism level of *N. pseudoferus* is still very high, especially by adult females on the first instars (I- to III-instars) (**Figure II.5a**), more so than on the later stages (III to V-instars) (**Figure II.5b**). This could explain the location of individuals within the plant. In the absence of other predatory species, the females lay eggs mostly on the leaf petioles spread out equally over the height of the plant [130]. Moreover, *Nabis* adults prefer to sit on the top or slightly lower in the plant canopy, while immature individuals are found lower down in the plant [87,88].

Insects and other arthropods, unlike vertebrate species, have complex life cycles in which the successive stages may differ more dramatically, both in physical appearance and in their ecological role [132-135]. The findings from the two species studied indicate that cannibalism depends not only on the species, but also on their stage-structure. Most ecological models in contemporary ecological theory ignore the implications of the age and size variation, particularly

within populations. This is also true for empirical studies, both experimental and non-experimental [76]. However, recent studies show that stage-structure can modify the dynamics of consumer-resource communities owing to stage-related shifts in the nature and strength of interactions that occur within and between populations [135]. Consequently, these results can help to develop mathematical models based on stage structure, by considering a more realistic species ontogeny. Furthermore, and from the applied standpoint, the results of this study also highlight the importance of cannibalism, and its repercussions, in current biological control systems.

II.5. Conclusions

1.- The diet, whether strictly carnivorous or omnivorous, seems to have a marked effect on the cannibalism of the two species studied. This could be extended to other insect species.

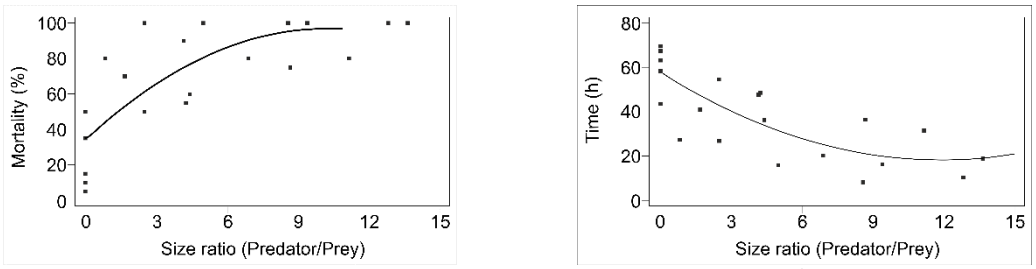
2.- Ontogenetic development in insects with a stage structure doubly influences the cannibalism and the intra-guild predation (IGP) by affecting both the individual prey and the predator.

3.- The ratio of predator-prey size in relation to the rate of cannibalism and intra-guild predation (IGP) is not a linear relationship, as has already been pointed out in the literature on arthropods.

4.- These findings can help to develop mathematical models based on stage structure, by more realistically considering species with this type of ontogeny.

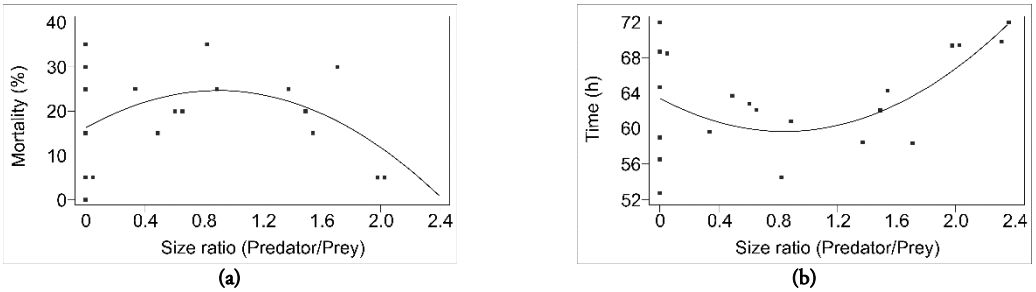
5- From an applied standpoint, these study results also highlight the importance of cannibalism, and its repercussions, in current biological control systems for pest species.

II. 6. Appendix A



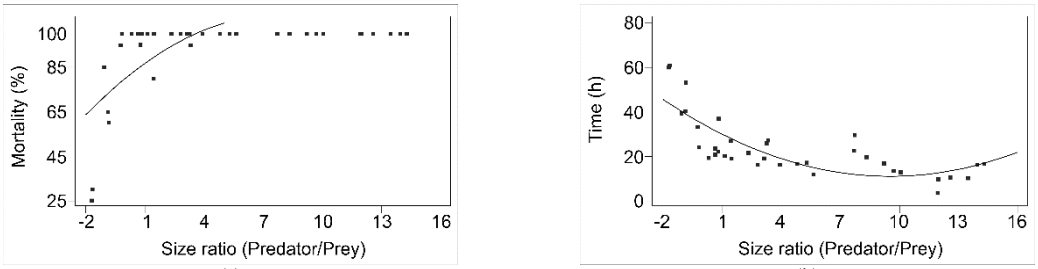
(a) **(b)**

Figure II.A.1. Relationship between mortality (%) **(a)** and survival time (h) **(b)** of *Nabis pseudoferus* as a function of the size ratio between cannibal and prey.



(a) **(b)**

Figure II.A.2. Relationship between mortality (%) **(a)** and survival time (h) **(b)** of *Nesidiocoris tenuis* as a function of the size ratio between cannibal and prey.



(a) **(b)**

Figure II.A.3. Relationship between mortality (%) **(a)** and survival time (h) **(b)** in the predation of *Nesidiocoris tenuis* by *Nabis pseudoferus*, depending on the size ratios between prey-predator, when *N. pseudoferus* was always in a lower stage class than *N. tenuis*.

Table II.A.1. Combinations used in cannibalism assays for *N. pseudoferus* and *N. tenuis* under laboratory conditions and without alternative prey.

	Combinations (*)				
Adult-Adult	–	–	–	–	–
Adult-NV	NV-NV	–	–	–	–
Adult-NIV	NV-NIV	NIV-NIV	–	–	–
Adult-NIII	NV-NIII	NV-NIII	NIII-NIII	–	–
Adult-NII	NV-NII	NV-NII	NIII-NII	NII-NII	–
Adult-NI	NV-NI	NV-NI	NIII-NI	NII-NI	NI-NI

(*) NV, NIV, NIII, NII and NI: instars

Table II.A.2. Combinations used in the IGP *N. pseudoferus-N. tenuis*, assay 1, under laboratory conditions and without alternative prey, when both predatory species are in the same or lower stage class.

	Combinations (*)				
N.s.-N.t.	N.s.-N.t.	N.s.-N.t.	N.s.-N.t.	N.s.-N.t.	N.s.-N.t.
Adult-Adult	–	–	–	–	–
Adult-NV	NV-NV	–	–	–	–
Adult-NIV	NV-NIV	NIV-NIV	–	–	–
Adult-NIII	NV-NIII	NV-NIII	NIII-NIII	–	–
Adult-NII	NV-NII	NV-NII	NIII-NII	NII-NII	–
Adult-NI	NV-NI	NV-NI	NIII-NI	NII-NI	NI-NI

(*) NV, NIV, NIII, NII and NI: instars. N.s. = *Nabis pseudoferus*, N.t. = *Nesidicoris tenuis*.

Table II.A.3. Combinations used in the IGP *N. pseudoferus-N. tenuis*, assay 2, under laboratory conditions and without alternative prey, when *N. pseudoferus* was always in a lower stage class than *N. tenuis*.

Combinations (*)				
N.s.-N.t.	**N.s.-N.t.**	**N.s.-N.t.**	**N.s.-N.t.**	**N.s.-N.t.**
NI-Adult	NII-Adult	NIII-Adult	NIV-Adult	NV-Adult
NI-NV	NII-NV	NIII-NV	NIV-NV	–
NI-NIV	NII-NIV	NIII-NIV	–	–
NI-NIII	NII-NIII	–	–	–
NI-NII	–	–	–	–

(*) NV, NIV, NIII, NII and NI: instars. N.s. = *Nabis pseudoferus*, N.t. = *Nesidicoris tenuis*.

Table II.A.4. Aggressiveness of *Nabis pseudoferus* cannibalism on different stage class, measured as survival time (hours) (±SE) when caged singly with other conspecifics for 72 hours under laboratory conditions (25±1ºC and 60–80% R.H.) and without prey.

Stage	Significance level (*P*) (Breslow test)														
	Adult		N-V		N-IV		N-III		N-II		N-I		$\chi 2$	df	P^2
	Value	P^1	Value	P^1	Value	P^1	Value	P^1	Value	P^1	Value	P^1			
Adult	63.0±2.9	–	48.5±5.7	0.065	36.5±5.8	0.001	31.5±5.6	0.0001	10.3±2.5	0.0001	19.08±2.4	0.0001	59.614	5	0.001
N-V			67.2±2.8	–	36.2±6.8	0.001	20.0±6.2	0.0001	8.0±2.3	0.0001	16.2±2.1	0.0001	49.626	4	0.001
N-IV					58.3±4.4	–	54.5±4.3	0.6520	47.9±3.6	0.0290	16.0±2.7	0.0001	68.335	3	0.001
N-III							69.7±2.3	–	41.01±5.8	0.0001	26.9±3.8	0.0001	30.974	2	0.001
N-II									67.5±3.0	–	27.4±5.7	0.0001	21.375	1	0.001
N-I											43.6±6.6	–	–	–	–
Average	63.0±2.9		57.8±3.5		43.7±3.6		43.9±3.3		34.9±2.8		23.2±2.0	Total avg.		38.05±1.38	

[1]Significance level (*P*) of pairwise comparisons between high stage of development and the others (Breslow test). [2]Significance level (*P*) of Breslow test equality of survival distributions for different levels of stage factor.

Table II.A.5. Aggressiveness of *Nesidiocoris tenuis* cannibalism on different stage class, measured as survival time (hours) (±SE) when caged singly with other conspecifics for 72 hours under laboratory conditions (25±1ºC and 60–80% R.H.) and without prey.

Stage	Significance level (*P*) (Breslow test)														
	Adult		N-V		N-IV		N-III		N-II		N-I		$\chi 2$	df	P^2
	Value	P^1	Value	P^1	Value	P^1	Value	P^1	Value	P^1	Value	P^1			
Adult	72.0±0.0	–	68.5±3.5	0.317	62.1±4.5	0.037	64.3±4.2	0.076	69.5±2.5	1.000	72.0±0.0	1.000	11.969	5	0.035
N-V			68.7±3.3	–	62.8±4.2	0.187	62.0±4.5	0.187	69.4±2.6	0.971	69.9±3.5	0.594	3.537	4	0.472
N-IV					64.7±4.0	–	60.9±4.4	0.628	58.5±5.4	0.285	58.4±478	0.435	1.082	3	0.781
N-III							56.6±5.5	–	63.7±4.6	0.394	54.5±5.5	0.431	2.510	2	0.285
N-II									59.0±5.2	–	59.6±4.9	0.789	0.720	1	0.789
N-I											52.8±6.1	–	–	–	–
Average	72.0±0.0		68.6±2.4		63.2±2.4		61.0±2.3		64.0±1.9		60.7±1.9	Total avg.		63.2±1.0	

[1]Significance level (*P*) of pairwise comparisons between high stage of development and the others (Breslow test). [2]Significance level (*P*) of Breslow test equality of survival distributions for different levels of stage factor.

Table II.A.6. Aggressiveness of *Nabis pseudoferus* predation (intraguild competition) on *Nesidiocoris tenuis* (both predatory species are in the same or lower stage class), measured as survival time (hours) (±SE), when caged one individual of each species during 72 hours under laboratory conditions (25±1ºC and 60–80% R.H.) and without prey.

Species/ stage	Significance level (*P*) (Breslow test)														
	Nesidiocoris tenuis														
Nabis pseudoferus	Adult		N-V		N-IV		N-III		N-II		N-I		$\chi 2$	df	P^2
	Value	P^1	Value	P^1	Value	P^1	Value	P^1	Value	P^1	Value	P^1			
Adult	3.5±1.4	–	9.7±2.4	0.23	10.5±2.8	0.0020	10.2±2.2	0.0050	16.1±2.2	0.0001	16.7±2.0	0.0001	47.912	5	0.001
N-V			29.5±3.7	–	19.6±4.5	0.0570	16.7±3.5	0.2190	13.4±2.9	0.0040	12.9±2.2	0.0001	12.282	4	0.015
N-IV					16.1±3.5	–	16.7±2.7	0.7040	17.2±2.1	0.0580	11.8±2.4	0.9460	5.560	3	0.135
N-III							21.6±1.6	–	16.1±2.1	0.0001	19.0±1.9	0.0001	30.993	2	0.001
N-II									20.1±1.8	–	19.0±1.8	0.1440	2.137	1	0.144
N-I											20.6±1.6	–	–	–	–
Average	3.5±1.4		19.6±2.7		15.4±2.2		16.3±1.4		16.6±1.0		16.7±0.9	Total avg.		16.0±0.6	

[1]Significance level (*P*) of pairwise comparisons between high stage of development and the others (Breslow test). [2]Significance level (*P*) of Breslow test equality of survival distributions for different levels of stage factor.

Table II.A.7. Aggressiveness of *Nabis pseudoferus* predation (intraguild competition) on *Nesidiocoris tenuis* (*N. pseudoferus* was always in a lower stage class than *N. tenuis*), measured as survival time (hours) (±SE), when caged one individual of each species during 72 hours under laboratory conditions (25±1ºC and 60–80% R.H.) and without prey.

N.t.	Significance level (*P*) (Breslow test)																			*N.t.*
	Value			Value			Value			Value			Value							
	N. t.	N. s.	P^1	*N. t.*	N. s.	P^1	*N. t.*	N. s.	P^1	*N. t.*	N. s.	P^1	*N. t.*	N. s.	P^1	$\chi 2$	df	P^2	
Stage	-	N-V		-	N-IV		-	N-III		-	N-II		-	N-I						**Avg.**
Adult	22.6 ±1.0	72.0 ±0.0	0.001	26.1 ±2.7	72.0 ±0.0	0.001	22.4 ±3.1	68.5 ±3.4	0.001	40.5 ±5.3	69.6 ±2.5	0.001	60.2 ±4.6	60.2 ±5.5	0.910	20.372	4	0.000		33.8 ±2.2
N-V				27.1 ±3.2	72.0 ±0.0	0.001	36.9 ±3.1	70.7 ±1.3	0.001	53.2 ±4.3	62.2 ±4.8	0.670	60.9 ±4.2	64.8 ±4.3	0.300	12.446	3	0.000		45.3 ±2.3
N-IV							27.1 ±3.2	72.0 ±0.0	0.001	33.3 ±3.1	69.5 ±2.5	0.001	39.2 ±3.5	62.0 ±4.5	0.000	2.745	2	0.250		37.0 ±2.2
N-III										23.5 ±2.0	72.0 ±0.0	0.001	24.1 ±0.5	72.0	0.001	1.516	1	0.210		23.8 ±1.0
N-II													19.4 ±1.8	72.0	0.001	–	–	–		19.4 ±1.8
Avg.		72.0 ±0.0			72.0 ±0.0			69.6± 1.5			68.9 ±1.4			66.2 ±1.7						

[1]Significance level (*P*) of pairwise comparisons between high stage of development and the others (Breslow test). [2]Significance level (*P*) of Breslow test equality of survival distributions for different levels of stage factor. *N.t.* = *Nesidiocoris tenuis*; *N.s.* = *Nabis pseudoferus*.

II.7. References

1. Sanchez, J.A. Zoophytophagy in the plantbug *Nesidiocoris tenuis*. *Agric. For. Entomol.* **2008**, *10*, 75-80. doi: 10.1111/j.1461-9563.2007.00357.x.

2. Wheeler, A.G.; Henry, T.J. *A synthesis of the holarctic Miridae (Het.): distribution, biology, and origin, with emphasis on North America*; Entomological Society of America: Lanham, MD, USA 1992.

3. Rabitsch, W. Alien true bugs of Europe (Insecta: Hem.: Het.). *Zootax*. 2008, *1827*, 1-44. doi: 10.11646/zootaxa.1827.1.1.

4. Wheeler, A.G. Predacious plant bugs (Miridae). In *Heteroptera of economic importance*; Schaefer, C.W.; Panizzi, A.R., Eds.; CRC Press: Boca Raton, FL, USA, 2000; pp. 657-693.

5. Sanchez, J.A.; Lacasa, A. Impact of the zoophytophagous plant bug *Nesidiocoris tenuis* (Het.: Miridae) on tomato yield. *J. Econ. Entomol.* **2008**, *101*, 1864-1870. doi: 10.1603/0022-0493-101.6.1864.

6. Wheeler, A.G. *Biology of the plant bugs (Hem.: Miridae): Pests, predators, opportunists*; Cornell University Press: Ithaca, NY, USA, 2001.

7. Valderrama, K.; Granobles, J.; Valencia, E.; Sanchez, M. *Nesidiocoris tenuis* (Hem.: Miridae) predator in tobacco crops (*Nicotiana tabacum*). *Rev. Colomb. Entomol*. **2007,** *33*, 141-145. doi:10.13140/RG.2.2.22183.19361.

8. Li, L.R.; Xue, R.F.; Wang, X. J.; Zeng, F.R. Predation of *Nesidiocoris tenuis* to *Trialeurodes vaporariorum* and *Plutella xylostella*. *J. Agr. Univ. Hebei* **2008**,1.

9. Perez-Hedo, M.; Urbaneja, A. Prospects for predatory mirid bugs as biocontrol agents of aphids in sweet peppers. *J. Pest Sc.* **2015**, *88*, 65-73. doi: 10.1007/s10340-014-0587-1.

10. Fagan, W.F. Omnivory as a stabilizing feature of natural communities. *Am. Nat.* **1997**, *150*, 554-567. doi: 10.1086/286081.

11. Pericart, J. *Hemipteres Nabidae d'Europe Occidentale et du Maghreb*; Federation Française des Societes de Sciences Naturelles: Paris, FR 1987.

12. Lattin, J.D. Bionomics of the Nabidae. *Annu. Rev. Entomol.* 1989, *34*: 383-400. doi: 10.1146/annurev.en.34.010189.002123.

13. Braman, S.K. Damsel bugs (Nabidae). In Heteroptera of economic importance; Schaefer, C.W.; Panizzi, A.R., Eds.; CRC Press: Boca Raton, FL, USA, 2000; pp. 639-656.

14. Hagen, K.S.; Mills, N.J.; Gordh, T.G.; McMurtry, J.A. Terrestrial arthropod predators of insect and mite pests. In *Handbook of biological control: Principles and applications of biological control*; Bellows, T.S.; Fisher, T.W.; Caltagirone, D.L.; Dahlsten, D.L.; Gordh, G.; Huffaker, C.B., Eds.; Academic Press: San Diego, CA, USA, 1999; pp. 383-503.

15. Cabello, T.; Gallego, J.R.; Fernandez, F.J.; Gamez, M.; Vila, E.; Pino, M. del; Hernandez-Suarez, E. Biological control strategies for the South American tomato moth (Lep.: Gelechiidae) in greenhouse tomatoes. *J. Econ. Entomol.* **2012**, *105*: 2085-2096. doi: 10.1603/EC12221.

16. Vila, E.; Cabello, T. Biosystems engineering applied to greenhouse pest control. In *Biosystems engineering: Biofactories for food production in the XXI century*; Torres, I.; Guevara, R., Eds.; Springer: Berlin, DE, 2014; pp. 99-128.

17. Obrtel, R. Variation in abundance and dominance of insects inhabiting lucerne fields. *Acta Entomol. Bohemos.* **1970**, *67*: 175-187.

18. Puchkov, A.V. Particulars of the biology of predacious *Nabis* spp. *Zash. Rast.* **1980**, *8*: 1-44.

19. Cabello, T. Natural enemies of noctuid pests in alfalfa, corn, cotton and soybean crops in Southern Spain. *J. Appl. Entomol.* **1988**, *108*: 80-88. doi: 10.1111/j.1439-0418.1989.tb00436.x

20. Cabello, T. Control biológico de noctuidos y otros lepidópteros. In *Control biológico de plagas*; Jacas, J.; Urbaneja, A.; Phytoma España: Valencia, ES, 2009; pp. 279-306.

21. Aydemir, M.; Toros. S. Natural enemies of *Tetranychus urticae* (Acari: Tetranychidae) on bean plants in Erzincan. In Proceedings of the Second Turkish National Congress of Biological Control, Ankara, TR, 1990, 261-271.

22. Ulusoy, M.R.; Ulgenturk, S. The natural enemies of whiteflies (Hem.: Aleyrodidae) in southern Anatolia. *Zool. Middle East* **2003**, *28*: 119-124. doi: 10.1080/09397140.2003.10637961

23. Pimm, S.L.; Lawton, J.R. On feeding on more than one trophic level. *Nature* **1978**, *275*: 542-544. doi: 10.1038/275542a0

24. Schowalter, T.D. *Insect ecology: An ecosystem approach*; Academic Press: Amsterdam, NL 2011.

25. Chapman, J.L.; Reiss, M.J. *Ecology: Principles and applications*; Cambridge University Press: Cambridge, UK 1992.

26. Krimmel, B. A. Omnivorous insects: Evolution and ecology in natural and agricultural ecosystems. *Nat. Educ. Knowl.* **2011**, *2*: 2.

27. Coll, M.; Guershon, M. Omnivory in terrestrial arthropods: mixing plant and prey diets. *Annu. Rev. Entomol*. **2002**, **47**: 267-297. doi: /10.1146/annurev.ento.47.091201.145209

28. Polis, G.A.; Myers, C.A.; Holt, R.D. The ecology and evolution of intraguild predation: Potential competitors that eat each other. *Annu. Rev. Ecol. Syst.* **1989**, *20*: 297-330. doi: 10.1146/annurev.es.20.110189.001501.

29. Polis, G.A.; Holt, R.D. Intraguild predation: the dynamics of complex trophic interactions. *Trends Ecol. Evol.* **1992**, *7*: 151-54. doi: 10.1016/0169-5347(92)90208-S

30. Hurd, L.E. Predation: The Role of generalist predators in biodiversity and biological control. In *Encyclopedia of Entomology*; Capinera, J.L., Ed.; Springer: Dordrecht, NL, 2008; pp. 3038-3042.

31. Fox, L.R. Cannibalism in natural populations. *Annu. Rev. Ecol. Syst.* **1975**, *6*: 87-106. doi: 10.1146/annurev.es.06.110175.000511

32. Richardson, M.L.; Mitchell, R.F.; Reagel, P.F.; Hanks, L.M. Causes and consequences of cannibalism in noncarnivorous insects. *Annu. Rev. Entomol.* **2010**, *55*: 39-53. doi: 10.1146/annurev-ento-112408-085314

33. Capinera, J.L. Cannibalism. In *Encyclopedia of entomology*; Capinera, J.L., Ed.; Springer: Dordrecht, NL 2008; pp. 710-714.

34. Richardson, M.L.; Mitchell, R.F.; Reagel, P.F.; Hanks, L.M. Causes and consequences of cannibalism in noncarnivorous insects. *Annu. Rev. Entomol*. **2010**, *55*: 39-53. doi: 10.1146/annurev-ento-112408-085314

35. Lawa, Y.H.; Rosenheim, J.A. Presence of conspecific females motivates egg cannibalism owing to lower risk of filial cannibalism. *Anim. Behav*. **2013**, *85*: 403-409. doi: 10.1016/j.anbehav.2012.11.015

36. Lida, H. Small within-clutch variance in spiderling body size as a mechanism for avoiding sibling cannibalism in the wolf spider *Pardosa pseudoannulata* (Aran.: Lycosidae). *Popul. Ecol*. **2003**, *45*: 1-6. doi:10.1007/s10144-002-0133-5

37. Santana, A.F.; Roselino, A.C.; Cappelari, F.A.; Zucoloto, F.S. Cannibalism in insects: In *Insect bioecology and nutrition for integrated pest management*; Panizzi, A.R.; Parra, J.R.P., Eds.; CRC Press: Boca Raton, FL, USA 2012; pp. 177-194.

38. De Vries, T.; Lakes-Harlan, R. Prenatal cannibalism in an insect. *Naturwissenschaften* **2007**, *94*: 477-482. doi: 10.1007/s00114-006-0213-z

39. White, T.C.R. *Why does the world stay green?: Nutrition and survival of plant-eaters*; CSIRO Pub.: Sydney, AU 2005. doi: 10.1071/9780643093157

40. Buskirk, R.E.; Frohlich, C.; Ross, K.G. The natural selection of sexual cannibalism. *Am. Nat*. **1984**, *123*: 612-625. doi: 10.1086/284227.

41. Claessen, D.; de Roos, A.M.; Persson, L. Population dynamic theory of size–dependent cannibalism. *Proc. R. Soc. Lond. B* **2004**, *271*: 333-340. doi: 10.1098/rspb.2003.2555.

42. Dick, J.T.A.; Montgomery, I.; Elwood, R.W. Replacement of the indigenous amphipod *Gammarus duebeni celticus* by the introduced *G. pulex*: differential cannibalism and mutual predation. *J. Anim. Ecol*. **1993**, *62*: 79-88. doi: 10.2307/5484.

43. Rosenheim, J.A. Intraguild predation: new theoretical and empirical perspectives. *Ecology* **2007**, *88*: 2679-2680. doi: 10.1890/07-0790.1.

44. Holt, R.D.; Polis, G.A.A. theoretical framework for intraguild predation. *Am. Nat*. **1997**, *149*: 745-764. doi: 10.1086/286018.

45. Holt, R.D.; Huxel, G.R. Alternative prey and the dynamics of intraguild predation: Theoretical perspectives. *Ecology* **2007**, *88*: 2706-2712. doi: 10.1890/06-1525.1.

46. Dennis, B.; Desharnais, R.A.; Cushing, J.M., Henson, S.M.; Costantino, R.F. Estimating chaos and complex dynamics in an insect population. *Ecol. Monog*. **2001**, *71*: 277-303. doi: 10.17537/2017.12.385.

47. Klug, H.; Bonsall, B. When to care for, abandon, or eat your offspring: The evolution of parental care and filial cannibalism. *Am. Nat*. **2007**, *170*: 886-901. doi: 10.1086/522936.

48. Bonsal, M.B.; Klug, H. Effects of among-offspring relatedness on the origins and evolution of parental care and filial cannibalism. *J. Evol. Biol.* **2011**, *24*: 1335-1350. doi: 10.1111/j.1420-9101.2011.02269.x.

49. Skurdal, J.; Bleken, E.; Stenseth, N.C. Cannibalism in whitefish (*Coregonus lavaretus*). *Oecologia* **1985**, *67*: 566-571. doi: 10.1007/BF00790028.

50. Stenseth, N.C. On the evolution of cannibalism. *J. Theor. Biol.* **1985**, *115*: 161-177. doi: 10.1016/S0022-5193(85)80093-X.

51. Kohlmeier, C.; Ebenhoh, W. The stabilizing role of cannibalism in a predator prey system. *Bull. Math. Biol.* **1995**, *57*: 401-411. doi: 10.1016/S0092-8240(05)81775-6.

52. Rault, J., Benoit, E. & Gouze, J. L. Stabilizing effect of cannibalism in a two stages population model. Acta Biotheor. 2013, 61:119-139. doi: 10.1007/s10441-013-9172-x.

53. Borer, E.T.; Briggs, C.J.; Holt, R.D. Predators, parasitoids, and pathogens: A cross-cutting examination of intraguild predation theory. *Ecology* **2007**, *88*: 2681-2688. doi:10.1890/06-1707.1.

54. Rudolf, V.H.W. The interaction of cannibalism and omnivory: Consequences for community dynamics. *Ecology* **2007**, *88*: 2697-2705. doi: 10.1890/06-1266.1.

55. Hatcher, M.J.; Dick, J.T.A.; Dunn, A.M. A keystone effect for parasites in intraguild predation? *Biol. Lett.* **2008**, *4*: 534-537. doi: 10.1098/rsbl.2008.0178.

56. Brodeur, J.; Boivin, G. *Trophic and guild interactions in biological control*; Springer: Dordrecht, NL 2006.

57. Snyder, W.E.; Ives, A.R. Generalist predators disrupt biological control by a specialist parasitoid. *Ecology* **2001**, *82*: 705-716. doi: 10.1890/0012-9658.

58. Denno, R.F.; Finkle, D.L.; Langellotto, G.A. Direct and indirect effects of vegetation structure and habitat complexity on predator-prey and predator-predator interactions: In *Ecology of predator-prey interaction*; Barbosa, P.; Castellanos, I., Eds.; Oxford University Press: Oxford, UK, 2005; pp. 211-236.

59. Erbilgin, N.; Dahlsten, D.L.; Chen, P.Y. Intraguild interactions between generalist predators and an introduced parasitoid of *Glycaspis brimblecombei* (Hom.: Psylloidae). *Biol. Control* **2004**, *31*: 329-337. doi: 10.1016/j

60. Perez-Lachaud, G.; Bachelor, L.; Hardy, I.C.W. Wasp cat wasp: facultative hyperparasitism and intra-guild predation by bethylid wasps. *Biol. Control* **2004**, *30*: 149-155. doi: 10.1016/j.biocontrol.2004.03.003

61. Rosenheim, J.A.; Harmon, J.P. The influence of intraguild predation on the suppression of a shared prey population: An empirical reassessment. In *Trophic and guild interactions in biological control*; Brodeur, J.; Boivin, G., Eds.; Springer: Dordrecht, NL, 2006; pp. 1-20.

62. Liman, A.S. Intra- and interspecific interactions between heteropteran generalist predators - the effect of behavioural differences and consequences for biological control. M.Sc. Thesis, Swedish University of Agricultural Sciences, Uppsala, SE, 2003.

63. Cardinale, B.J.; Harvey, C.T.; Gross, K.; Ives, A.R. Biodiversity and biocontrol: emergent impacts of a multienemy assemblage on pest suppression and crop yield in an agroecosystem. *Ecol. Lett.* **2003**, *6*: 857-865. doi: 10.1046/j.

64. Cabello, T.; Bonfil, F.; Gallego, J.R.; Fernandez, F.J., Gamez, M.; Garay, J. Can interactions between an omnivorous hemipteran and an egg parasitoid limit the level of biological control for the tomato pinworm? *Environ. Entomol.* **2014**, *44*: 12-26. doi: 10.1093/ee/nvu017 (2014). DOI: 10.1093/ee/nvu017.

65. Schmidt, J. M.; Taylor, J.R.; Rosenheim J.A. Cannibalism and intraguild predation: In *Predatory Heteroptera: Their ecology and use in biological control*; Coll, M.; Ruberson, J.R., Eds.; Entomological Society of America: Lanham, MD, USA, 1998); pp. 131-170.

66. Etzel, L.K.; Legner, E.F. Culture and colonization. In *Handbook of biological control: Principles and applications of biological control*; Bellows, T.S.; Fisher, T.W.; Caltagirone, D.L.; Dahlsten, D.L.; Gordh, G.; Huffaker, C.B., Eds.; Academic Press: San Diego, CA, USA, 1999; pp. 125-197.

67. Ito, K. A simple mass-rearing method for predaceous *Orius* bugs in the laboratory. *Appl. Entomol. Zool.* **2007**, *42*: 573-577. doi: 10.1303/aez.2007.573.

68. Hodek, I.; Evans, E.W. Food relationships. In *Ecology and behaviour of the ladybird beetles (Coccinellidae)*; Hodek, I.; van Emden, H.F.; Honek, A., Eds; John Wiley & Sons, Ltd.: Chichester, UK, 2012; pp. 141-274.

69. Clercq, P. de; Coudron, T.A.; Riddick, E.W. Production of heteropteran predators. In *Mass production of beneficial organisms: Invertebrates and entomopathogens*; Morales-Ramos, J. A.; Rojas, G.; Shapiro-Ilan, D.I., Eds.; Academic Press: Amsterdam, NL, 2014; pp. 57-100.

70. Riddick, E.W.; Chen, H. Production of coleopteran predators. In *Mass production of beneficial organisms: Invertebrates and entomopathogens*; Morales-Ramos, J. A.; Rojas, G.; Shapiro-Ilan, D.I., Eds.; Academic Press: Amsterdam, NL, 2014; pp. 17-56.

71. Carayon, J. Quelques remarques sur les Hemipteres-Heteropteres: leur importance comme insectes auxiliaires et les possibilites de leur utilisation dans la lutte biologique. *Entomophaga* **1961**, *6*: 133-141. doi: 10.1007/BF02373218.

72. Mills, N. J. Voracity, cannibalism and coccinellid predation. *Ann. Appl. Biol.* **1982**, *101*: 144-148. doi: 10.1111/j.1744-7348.1982.tb00809.x

73. Chang, G.C.; Kareiva, P. The case for indigenous generalists in biological control. In *Theoretical approaches to biological control*; Hawkins, B.A.; Cornell, H.V., Eds.; Cambridge University Press: Cambridge, UK, 2004; pp. 103-115.

74. Pires, E.M.; Zanuncio, J.C.; Serrao, J.C. Cannibalism of *Brontocoris tabidus* and *Podisus nigrispinus* during periods of pre-release without food or fed with *Eucalyptus cloeziana* plants. *Phytoparasitica* **2011**, *39*: 27-34. doi: 10.1007/s12600-010-0132-6.

75. Moerkens, R.; Berckmoes, E.; van Damme, V.; van den Broecke, S.; Wittemans, L.; Casteels, H.; Tirry, L., de Clercq, P.; de Vis, R. Optimization of pest control by the predatory bug *Macrolophus pygmaeus* in greenhouse tomato production. *IOBC WPRS Bull.* **2014**, *102*: 157-162.

76. Persson, L. Dynamics of age- and stage-structured populations and communities. In *Oxford Bibliographies in Ecology* (2014). Available at: https://www.oxfordbibliographies.com/view/document/obo-9780199830060/obo-9780199830060-0036.xml?rskey=Oo4p3m&result=1&q=Dynamics+of+age-+and+stage-structured+populations+and+communities#firstMatch (accessed on 11/02/2020). doi: 10.1093/OBO/9780199830060-0036.

77. Vandermeer, J. H. *The ecology of agroecosystems*; Jones and Bartlett Publishers: Sudbury, UK, 2011.

78. Walzer, A.; Shausbergerb, P. Cannibalism and interspecific predation in the phytoseiid mites *Phytoseiulus persimilis* and *Neoseiulus californicus*: predation rates and effects on reproduction and juvenile development. *BioControl* **1999**, *43*: 457–468. doi: 10.1023/A:1009980401662.

79. Schausberger, P.; Croft, B.A. Cannibalism and intraguild predation among phytoseiid mites: Are aggressiveness and prey preference related to diet specialization? *Exp. Appl. Acarol.* **2000**, *24*: 709-725. doi: 10.1023/A:1010747208519.

80. Altmann, J. Observational sampling methods for insect behavioral ecology. *Fla. Entomol.* **1984**, *67*: 50-56. doi: 10.2307/3494104.

81. Martin, P.; Bateson, P. *Measuring behavior: An introductory guide*; Cambridge University Press: Cambridge, UK, 2004.

82. Kaplan, E.L.; Meier, P. Nonparametric estimations from incomplete observation. *J. Am. Stat. Assoc.* **1958**, *53*: 457-481. doi: 10.1007/978-1-4612-4380-9_25.

83. Borgan, O. Kaplan–Meier Estimator. In *Encyclopedia of Biostatistics*; Armitage, P.; Colton, T.) (2005) Available at: https://onlinelibrary.wiley.com/doi/abs/10.1002/0470011815.b2a11042. (Accessed 11/02/2020). doi: 10.1002/0470011815.b2a11042

84. Breslow, N. E. Analysis of survival data under the proportional hazards model. *Int. Stat. Rev.* **1975**, *43*: 45-58. doi: 10.1016/0197-2456(81)90005-2.

85. IBM. *IBM SPSS Advanced Statistics* 25; IBM Corp.: Armonk, NY, USA, 2017.

86. STATGRAPHICS Centurion user manual. The Plains, VA, USA, 2009.

87. Braman, S.K.; Yeargan, K.V. Intraplant distribution of three *Nabis* species (Hem.: Nabidae), and impact of *N. roseipennis* on green cloverworm populations in soybean. *Environ. Entomol.* **1989**, *18*: 240-244. doi: 10.1093/ee/18.2.240.

88. Wilson, L.T.; Gutierrez, A.P. Within-plant distribution of predators on cotton: comments on sampling and predator efficiencies. *Hilgardia* **1980**, *48*: 3-11. doi: 10.3733/hilg.v48n02p009.

89. Wineriter, S.A.; Walker, T.J. Insect marking techniques: Durability of materials. *Ent. News* **1984**, *95*: 117-123.

90. Carreño, R.; Andujar, A.; Cabello, T. Evaluación de la eficacia de pesticidas agricolas. In Actas V Conf. Esp. Biom. 1995. Valencia, ES, pp. 139-142. doi: 10.13140/2.1.4816.6723.

91. Coll, M.; Guershon, M. Omnivory in terrestrial arthropods: mixing plant and prey diets. *Annu. Rev. Entomol.* **2002**, *47*: 267-297. doi: 10.1146/annurev.ento.47.091201.145209

92. Perkins, P.V.; Watson, T.F. Biology of *Nabis alternatus* (Hem.: Nabidae). *Ann. Entomol. Soc. Am.* **1972**, *65*: 54-57. doi: 10.1093/aesa/65.1.54.

93. Takizawa, T.; Snyder, W.E. Predator biodiversity increases the survival of juvenile predators. *Oecologia* **2011**, *166*: 723-730. doi: 10.1007/s00442-010-1891-y.

94. Koschel, H. Zur kenntnis der raubwanze *Himacerus apterus* F. (Het.: Nabidae). *J. Appl. Entomol.* **1971**, *68*: 113-137. doi: 10.1111/j.1439-0418.1971.tb03132.x.

95. Wagner, J.D.; Wise, D.H. Cannibalism regulates densities of young wolf spiders: Evidence from field and laboratory experiments. *Ecology* **1996**, *77*: 639-52. doi: 10.2307/2265637.

96. Wise, D.H. Cannibalism, food limitation, intraspecific competition, and the regulation of spider populations. *Annu. Rev. Entomol.* **2006**, *51*: 441-465. doi: 10.2307/2265637.

97. Edgar, W.D. Prey and predators of the wolf spider *Lycosa lugubris*. *Entomol. Mon. Mag.* **1970**, *106*: 71-73. doi: 10.1111/j.1469-7998.1969.tb03897.x.

98. Hallander, H. Prey, cannibalism, and microhabitat selection in the wolf spiders *Pardosa chelata* and *P. pullato*. *Oikos* **1970**, *21*: 337-340. doi: 10.2307/3543691.

99. Samu, F.; Toft, S.; Kiss, B. Factors influencing cannibalism in the wolf spider *Pardosa agrestis* (Aran.: Lycosidae). *Behav. Ecol. Sociobiol.* **1999**, *45*: 349-354. doi: 10.1007/s002650050.

100.Yasuda, H.; Kikuchi, T.; Kindlmann, P.; Sato, S. Relationships between attack and escape rates, cannibalism, and intraguild predation in larvae of two predatory ladybirds. *J. Insect Behav.* **2001**, *14*: 373-384. doi: 10.1023/A:1011175430247.

101. Buddle, C.M.; Walker, S.E.; Rypstra, A.L. Cannibalism and density-dependent mortality in the wolf spider *Pardosa milvina* (Aran.: Lycosidae). *Can. J. Zool.* **2003**, *81*: 1293-1297. doi: 10.1139/z03-124.

102. McMurtry, J.A.; Croft, B.A. Lifestyles of phytoseiid mites and their roles in biological control. *Annu. Rev. Entomol.* **1997**, *42*: 291-321. doi: 10.1146/annurev.ento.42.1.291.

103. Cottrell, T.E. Predation and cannibalism of lady beetle eggs by adult lady beetles. *Biol. Control* **2005**, *34*: 159-164. doi: 10.1016/j.biocontrol.2005.04.008.

104. Pervez, A.; Gupta, A.K.; Omkar. Kin recognition and avoidance of kin cannibalism by the larvae of co-occurring ladybirds: a laboratory study. *Eur. J. Entomol.* **2005**, *102*: 513-518. doi:10.14411/eje.2005.073.

105. Pervez, A.; Gupta, A.K.; Omkar. Larval cannibalism in aphidophagous ladybirds: Influencing factors, benefits and costs. *Biol. Control* **2006**, *38*: 307-313. doi: 10.14411/eje.2005.073.

106. Polis, G.A. Exploitation competition and the evolution of interference, cannibalism, and intraguild predation in age/size-structured populations: In *Size-structured populations: Ecology and evolution*; Ebenman, B.; Persson, L., Eds; Springer-Verlag, Berlin, DE, 1988; pp. 185-202.

107. Joyner, K.; Gould, F. Conspecific tissues and secretions as sources of nutrition. In *Nutritional ecology of insects, mites, spiders, and related invertebrates*; Slansky, F.; Rodriguez, J.G., Eds.; Wiley: New York, NY, USA, 1987; pp. 697-719.

108. Mayntz, D.; Toft, S. Nutritional value of cannibalism and the role of starvation and nutrient imbalance for cannibalistic tendencies in a generalist predator. *J. Anim. Ecol.* **2006**, *75*: 288-297. doi: 10.1111/j.1365-2656.2006.01046.x.

109. Agarwala, B.K.; Dixon, A.F.G. Laboratory study of cannibalism and interspecific predation in ladybirds. *Ecol. Entomol.* **1992**, *17*: 303-309. doi: 10.1111/j.1365-2311.1992.tb01062.x.

110. Michaud, J.P.A comparative study of larval cannibalism in three species of ladybird. *Ecol. Entom.* **2003**, *28*: 92-101. doi: 10.1046/j.1365-2311.2002.00481.x.

111. Claessen, D.; de Roos, A.M.; Persson, L. Dwarfs and giants: Cannibalism and competition in size-structured populations. *Am. Nat.* **2000**, *155*: 219-237. doi: 10.1086/303315.

112. Laycock, A.; Camm, E.; van Laerhoven, S.; Gillespie, D. Cannibalism in a zoophytophagous omnivore is mediated by prey availability and plant substrate. *Insect Beha.* **2006**, *19*: 219-229. doi: 10.1007/s10905-006-9020-9.

113. Moreno-Ripoll, R.; Agustin N.; Berruezo, R.; Gabarra, R. Conspecific and heterospecific interactions between two omnivorous predators on tomato. *Biol. Control* **2012**, *62*: 189-196. doi: 10.1016/j.biocontrol.2012.04.005.

114. Hamdi, F.; Chadoeuf, J.; Chermiti, B.; Bonato, O. Evidence of cannibalism in *Macrolophus pygmaeus*, a natural enemy of whiteflies. *J. Insect Behav.* **2013**, *26*: 614-621. doi: 10.1007/s10905-013-9379-3.

115. Collyer, E. Cannibalism as a factor affecting mortality of *Blepharidopterus angulatus* (Het.: Miridae). *Rep. East Malling Res. Stn.* **1965**, *52*: 177-179.

116. Lucas, E.; Alomar, O. *Macrolophus caliginosus* as an intraguild prey for the zoophytophagous *Dicyphus tamaninii* (Het.: Miridae). *Biol. Control* **2001**, *20*: 147-152. doi: 10.1006/bcon.2000.0890.

117. Urbaneja, A.; Tabia, G.; Stansly, P. Influence of host plant and prey availability on developmental time and survival of *Nesidiocoris tenuis* (Het.: Miridae). *Biocontrol Sci. Techn.* **2005**, *15*: 513-518. doi: 10.1080/09583150500088777.

118. Puysseleyr, V.; de Man, S.; Hofte, M.; de Clercq, P. Plantless rearing of the zoophytophagous bug *Nesidiocoris tenuis*. *Biocontrol* **2013**, *58*: 205-213. doi: 10.1007/s10526-012-9486-7.

119. Nakaishi, K.; Fukui, Y.; Arakawa, R. Reproduction of *Nesidiocoris tenuis* on sesame. *J. Appl. Entomol. Zool.* **2011**, *55*: 199-205. doi: 10.1303/jjaez.2011.199.

120. Polis G.A. Intraspecific predation and "infant killing" among invertebrates. In *Infanticide: Comparative and evolutionary perspectives*; Hausfater, G.; Hrdy, S.B., Eds.; Alsinde: New York, NY, USA, 1984); pp. 87-104.

121. Leon-Beck, M.; Coll, M. Plant and prey consumption cause a similar reduction in cannibalism by an omnivorous bug. *J. Insect Behav.* **2007**, *20*: 67-77. doi: 10.1007/s10905-006-9063-y.

122. Bernays, E.A. Evolution of feeding behavior in insect herbivores. *Bioscience* **1998**, *48*: 35-44. doi: 10.2307/1313226.

123. Snyder, W.E.; Snyde, G.B.; Finke, D.L.; Straub, C.S. Predator biodiversity strengthens herbivore suppression. *Ecol. Lett.* **2006**, *9*: 789-796. doi: 10.1111/j.1461-0248.2006.00922.x.

124. Rosenheim, J.A.; Kaya, H.K.; Ehler, L.E.; Marois, J.J.; Jaffee, B.A. Intraguild predation among biological-control agents: Theory and evidence. *Biol. Control* **1995**, *5*: 303-335. doi:10.1006/bcon.1995.1038.

125. Mochizuki, A.; Naka, H.; Hamasaki, K.; Mitsunaga, T. Larval cannibalism and intraguild predation between the introduced green lacewing, *Chrysoperla carnea*, and the indigenous trash carrying green lacewing, *Mallada desjardinsi* (Neur.: Chrysopidae), as a case study of potential nontarget effect assessment. *Environ. Entomol.* **2006**, *35*: 1298-1303. doi: 10.1603/0046-225X(2006)35[1298:LCAIPB]2.0.CO;2.

126. Cohen, A.C. How carnivorous bugs feed. In *Heteroptera of economic importance*; Schaefer, C.W.; Panizzi, A.R., Eds.; CRC Press: Boca Raton, FL, USA, 2000); pp. 563-570.

127. Torres, J.B.; Boyd, D.W. Zoophytophagy in predatory Hemiptera. *Braz. Arch. Biol. Technol.* **2009**, *52*: 1199-1208. doi: 10.1590/S1516-89132009000500018.

128. Schuh, R.T.; Slater, J.A. *True bugs of the world (Hem.: Heteroptera): Classification and natural history*; Cornell University Press, Ithaca, NY, USA, 1995.

129. Fernandez, F.J.; Gallego, J.R.; Valencia, A.; Gamez, M.; Varga, Z.; Garay, J.; Cabello, T. Cannibalism: Do risksof fighting and reprisal reduce predatory rates? *Community Ecol.* **2017**, *18*: 87-96. doi: 10.1556/168.2017.18.1.10

130. Pumarino, L.; Alomar, O.; Lundgren, J.G. The influence of intraguild competitors on reproductive decisions by two predatory Heteroptera, *Orius insidiosus* (Anthocoridae) and *Nabis americoferus* (Nabidae). *Biocontrol Sci. Techn.* **2011**, *21*: 1321-1330. doi: 10.1080/09583157.2011.622837.

131. Montserrat, M.; Magalhaes, S.; Sabelis, M.W.; de Roos, A.M.; Janssen, A. Patterns of exclusion in an intraguild predator: Prey system depends on initial conditions. *J. Anim. Ecol.* **2008**, *77*: 624-630. doi: 10.1111/j.1365-2656.2008.01363.x.

132. Polis, G.A. Age structure component of niche width and intraspecific resource partitioning: Can age groups function as ecological species? *Am. Nat.* **1984**, *123*: 541-564. doi: 10.1086/284221.

133. May R.M. Prologue. In *Size-structured populations: Ecology and evolution*; Ebenman, B.; Persson, L, Eds.; Springer-Verlag, Berlin, DE, 1988; pp. 1-2.

134. Miller, T.E.X.; Rudolf, V.H.W. Thinking inside the box: Community-level consequences of stage-structured populations. *Trends Ecol. Evolut.* **2011**, *26*: 457-466. doi: 10.1016/j.tree.2011.05.005.

135. Rudolf, V.H.W. Seasonal shifts in predator body size diversity and trophic interactions in size-structured predator–prey systems. *J. Anim. Ecol.* **2012**, *81*: 524-532. doi: 10.1111/j.1365-2656.2011.01935.x.

Cannibalism: Do risks of fighting and reprisal reduce predatory rate?

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Chapter III

Cannibalism: Do risks of fighting and reprisal reduce predatory rate?

Abstract: Cannibalism is a common phenomenon among insects. It has raised considerable interest both from a theoretical approach and for its importance in population dynamics in natural ecosystems. From an applied perspective, especially in the case of using predatory species in biological control programs, it could also play an important role. The present paper aims to study the cannibalistic behaviour of *Nabis pseudoferus* Remane, and the functional response of adult females. In a non-choice experiment, adult females show a clear acceptance of immature conspecifics as prey, with relatively high mortality values (51.89 ± 2.69 %). These values are lower than those occurring in a heterospecific prey, *Spodoptera exigua* Hübner, under the same conditions (80.00 ± 2.82 %). However, the main result was that the predation rate of heterospecific prey was reduced to 59.09 ± 7.08 % in the presence of conspecific prey. The prey-capture behaviour of adult females differed when they hunted conspecific versus heterospecific prey. This was shown in the average handling time, which was 23.3 ± 3.3 m in the first case (conspecific) versus 16.6 ± 2.5 m in the second (heterospecifics). Furthermore, values increased in the former case and reduced in the latter, according to the order in which the prey was captured. The difference in handling time is not significant when adjusting adult females' functional response to conspecific nymphs. We argue that these results likely indicate risk aversion and fear of reprisal between conspecifics.

Keywords: Insect, *Nabis pseudoferus*, *Spodoptera exigua*, Prey-Predator Relationship, Heterospecific and Conspecific prey, Predatory Behaviour, Functional Response.

III.1. Introduction

Cannibalism, defined as an intraspecific predation, occurs very frequently in nature. It has been observed in more than 1,300 species, including protozoa, turbellaria, rotifers, snails, copepods, centipedes, spider mites, scorpions, spiders, insects, fishes, frogs, birds and mammals (Fox 1975, Polis 1981, Joyner and Gould 1987). In insects, cannibalism is a common behaviour, which has been well-documented in many orders, including Odonata, Orthoptera,

Thysanoptera, Hemiptera, Trichoptera, Lepidoptera, Diptera, Neuroptera, Coleoptera and Hymenoptera (Capinera, 2010). It occurs in both terrestrial and aquatic habitats, and among carnivorous and herbivorous species (Joyner and Gould 1987). Cannibalism involves predation by adults and other mobile life stages (larvae or nymphs) of on less developed or weaker stages; as well as on non-mobile stages (*e.g.*, eggs) (Joyner and Gould 1987).

There are many types of cannibalism. These can be defined according to the damage caused to the cannibalised individual: destructive (when the prey undergoes injuries or death) or non-destructive cannibalism (that does not cause serious damage to the prey individual) (Joyner and Gould 1987). Cannibalism can also be classified in relation to the degree of genetic relatedness between cannibal and prey (Santana *et al*. 2012). This is important since the potential loss of inclusive fitness will depend clearly on the degree of relatedness (Dixon 2000). Thus cannibalism can also be subdivided into categories such as heterocannibalism (cannibal and prey are unrelated), filial cannibalism (parents prey upon their own offspring), or sibling cannibalism (siblings prey upon each other) (Dixon 2000).

The occurrence of cannibalism in insects may be due to different causes, as recently reviewed by Santana *et al*. (2012), such as: genetic characteristics, food availability and quality, population density, availability of victims, etc. cannibalism is often increased by overcrowded conditions at high population density (Polis 1981) and also influenced by other stressors, such as the lack of food (Joyner and Gould 1987). However, Santana *et al*. (2012) have pointed out that cannibalism at a high population density has been wrongly attributed to the lack of food, since there are examples of cannibalism at high densities even when food is sufficient or plentiful.

When food is sufficient, there are two possible ways to explain the cannibalism-density association (Polis 1981). First, changes in cannibalism rates may co-vary with conspecific density for the same reasons why predators show different responses depending on the density of heterospecific prey. Second, many species do not tolerate the presence of conspecifics in their own territory. Thus overpopulation may increases the frequency of conspecific encounters, and hence, increase cannibalism in high-density populations.

The Damsel bug *Nabis pseudoferus* Remane (Hemiptera: Nabidae) is a generalist predator, rather than omnivorous (*e.g.*, Fagan 1997). Although most Nabidae species that have been studied conduct feeding trials in plants (Braman 2000), they are unable to complete their developmental cycle in the absence of prey. Feeding trials are performed to obtain water (Hagen *et al.* 1999) and seem to do little or no harm to plants, while it can help predators survive during periods of food scarcity (Braman 2000). *N. pseudoferus* has a wide range of arthropod prey (insects and mites, mainly) (Puchkov 1980, Cabello 1988, Ulusoy and Ulgenturk 2003). It is worth mentioning that *N. pseudoferus* is often used as a biological control agent against Lepidopteran pests in greenhouse crops (Vila and Cabello 2014).

Adults and nymphs of *N. pseudoferus* show a type II functional response to the larvae of Beet Armyworm or Small Mottled Willow Moth *Spodoptera exigua* Hübner (Fernandez-Maldonado unpublished data). Other *Nabis* species also present, almost generally, type II responses in their nymph and adult stages. This is the case with *Nabis americoferus* Carayon (Propp, 1982), *Nabis kinbergii* Reuter (Siddique *et al.* 1987), *Nabis capsiformis* Germar (Fathipour and Jafari 2003), and other unidentified *Nabis* species (Stasek 2009).

Cannibalism in *N. pseudoferus* has not been documented either. Actually, there are only a few studies on cannibalism in species of this genus, except for the American species *Nabis alternatus* Parshley and *Nabis roseipennis* Reuter (Perkins and Watson 1972).

Current of biological control systems are quite complex, especially in greenhouse crops, where different species of natural enemies (predators, parasitoids and entomopathogens) are often used at the same time, within the same crop cycle to control different phytophagous species (Vila and Cabello 2014). It has become increasingly important, sometimes even crucial, to understand ecological relationships among them (*e.g.*, Cabello *et al.* 2015). Regarding ecological relationships, little thought has been given to the effects of cannibalism, although it could erode effectiveness of biological control. Indeed, high densities of natural enemies are often used, especially in augmentative biological control programmes (*e.g.*, Cabello *et al.* 2012), even as a preemptive measure in the absence of the phytophagous target pest species.

Interestingly, the effect of cannibalism has received little or no attention in biological pest control programmes. On the one hand, it may suppress production performance in mass breeding procedures (Clercq *et al.* 2014). On the other hand, as mentioned before, the release rates of natural enemies can lead to high densities when prey pest levels are very low, or before the prey infests the crop (*e.g.*, Vila and Cabello 2014). All of this suggests that cannibalism may influence the efficiency of biological control methods (*e.g.*, Mills 1982).

The present paper aims to study the importance of cannibalism in *N. pseudoferus,* a predator often used in biological pest control programmes. The study was carried out under laboratory conditions to evaluate adult females' preference for heterospecific versus conspecific prey by means of choice and non-choice trials; and also to reveal the functional response of adult females, as cannibalism may be dependent on conspecific density.

III.2. Materials and methods

III.2.1. Biological material and experimental conditions

A colony of *N. pseudoferus* was established from wild populations at different locations (Granada and Almeria, Spain) and reared in laboratory conditions. We used plastic containers (12 l) equipped with a cardboard panel to act as a shelter material, bean pods as oviposition substrate and water source, and Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs as prey. The eggs were supplied frozen (Ephescontrol®, Agrobio S.L., La Mojonera, Almeria, Spain) and kept at -40 °C until use. The *N. pseudoferus* specimens used in trials were kept in the laboratory for only two generations before performing the trial. The *S. exigua* larvae used as prey were provided as eggs by the company Entomotech S.L. (Almeria, Spain). After hatching, larvae were reared on artificial diet, following the methodology described by Cabello *et al.* (1984) up to the second larval stage. The conditions for rearing and conducting the two tests were: 25±1 ºC, 60-80 % HR and a photoperiod of 16:8 light:darkness ratio.

The laboratory arena method was used in all trials. Despite the fact that this method may underestimate the daily consumption of prey in relation to field cage, it is still considered appropriate to estimate such values (Latham and Mills 2009). Arenas were Petri dishes (Ø=9 cm, h=1.5 cm) sealed with Parafilm © to prevent the escape of *S. exigua* larvae.

III.2.2. Prey-preference trial

III.2.2.1. Experimental design and methodology

The preference trial was conducted with adult females and second-instar nymphs of the predator (*N. pseudoferus)*, as second-instar nymphs have proven to be most susceptible to cannibalism by adult females (Fernandez-Maldonado unpublished data). The test design was completely random. A single treatment was used at three levels: non-choice of heterospecific prey (second-instar larvae of *S. exigua*), non-choice of conspecific (second-instar nymphs of *N. pseudoferus*) and choice (heterospecific larvae and conspecific nymphs together). 24 replications were carried out for each different treatment.

The trial methodology was adapted from Cabello *et al.* (2015). *N. pseudoferus* adult mated females were used less than one week after final nymphal ecdysis. They were individually isolated in Petri dishes and subjected to a starving period of 24 hours prior to testing. They were only given a piece of sponge, moistured with distilled water.

Twelve specimens of a single species (second-instar larvae of *S. exigua* as heterospecific or second-instar nymphs as conspecific) were introduced in non-choice treatments; whereas 6-6 specimens of heterospecific and conspecific prey species were introduced in the choice treatment. Each adult female predator was left to prey on them for a period of 4 hours.

III.2.2.2. Recorded data

Recorded data were twofold: a) the number of killed prey was annotated at the end of the trial (4 h) for each treatment and replication and b) the prey-capture behaviour of adult females was recorded. Since direct human observation may cause interference in the predation behaviour of *Nabis* species (Wade *et al.* (2005), we photographed the trial arena every 10 seconds using an Eos 550D (Canon®) digital camera, EFS 18-55 lens with macro function (Canon®), connected by cable to a computer. The software used was Communication Software for the Camera EOS Utility, version 2.14 (Canon 2014). Due to the type of lens and pixel resolution, only the images of six arenas could be captured simultaneously, thus it took 12 days to complete all treatments and replications. Different treatments and replications were randomly assigned to

photo shoots. For the above reasons, these replications are actually pseudo replications, and were treated as such in the statistical analysis, as indicated below.

Photographs were set in time-lapse, using the Image Processing and Analysis in Java –ImageJ– software, version 1.49 (Schneider *et al.* 2012), which recorded the behaviour, the time need of a predation event, the identity of the killed prey, and the sequence of predation events.

The time need of predation events was quantified as handling time (T_h) (Holling 1959). This comprises time spent in quelling, killing and eating a prey, as well as time spent on cleaning the predator's body and appendages, and resting time. The time spent exclusively in searching for the prey (T_s) was also recorded. The sum of both is equals the total available time (T), 4 h in this case.

III.2.2.3. Statistical analysis

Since the trials had to be performed on different days, replications of treatments do not represent real replications in a strictly randomised design. This is common in insect behavioural studies, for example, when using olfactometers (of which only one or very few are available), as indicated by Ramirez *et al.* (2000). Under these circumstances, traditional statistical analysis of variance (ANOVA) does not apply, nor does the general linear model (GLM); but generalized linear models (GZLM) (Ricard 2008) can be applied. Additionally, statistical analyses assessed the effects of 'treatments'. The variable 'day' was included as an explanatory variable to avoid pseudo replications. In the case of preference studies on organisms, when there is a single count variable (with Poisson distribution), generalized linear models (GZLM) are the most powerful statistical methods from a statistical point of view (Mangeaud and Videla 2005).

Therefore, data corresponding to the number of killed prey were analysed using the GENLIN procedure (GZLM). In the analysis of these data, a single factor was used at three levels: non-choice with heterospecific, non-choice with conspecific and choice of both. The analysis used Poisson distribution and log-linear link function. The analysis of measured times, *i.e.* handling time (T_h) and searching time (T_s), used two factors: Factor 1, Factor 2 and Factor 1 x Factor 2 (Factor 1 as before and Factor 2 as the handling time of prey by predator or

searching time). All analyses were carried out using the statistical programme SPSS, version 21 (IBM 2012).

Additionally, in the choice treatment, adult predators' preference towards different offered prey was studied by using the Manly Preference Index (μ) (Manly *et al*. 1972). As established by Cock (1978), the Manly Index is the only method that takes into account the reduction in prey density that occurs during the course of the trial. This has been corroborated in the review by Sherratt and Harvey (1993). The index equation is as follows:

$$\mu = \frac{\frac{r_i}{N_i}}{\frac{r_i}{N_i} + \frac{r_j}{N_j}}$$

where r_i = number of consumed prey *i*; r_j = number of consumed prey *j*; N_i = number of offered prey *i*; N_j = number of offered prey *j*.

III.2.3. Fuctional response

III.2.3.1. Experimental design and methodology

The trial to establish the type of functional response was conducted with female adults as predator and dead, second-instar nymphs, as prey. The nymphs were previously collected from laboratory cultures, and stored at -40 °C until use. By applying dead prey items we aim to eliminate the "fight" factor from handling times (T_h). The experimental design was completely random, with a single treatment (conspecific prey density) at seven levels. The number of replications per treatment was 10. All treatments and replications were carried out simultaneously in a single trial.

All *N. pseudoferus* adult females were the same age and had been handled the same way as in trial 1. They were placed in Petri dishes for 24 h without food, and only with water supply. After that time, dead conspecific second-instar nymphs were introduced in the dishes. The densities of the used prey were 1, 3, 6, 9, 12, 15 or 18. After 24 hours, adult females were removed and the number of nymphs consumed was evaluated (based on the exoskeletons remained).

III.2.3.2. Statistical analysis

Two types of analysis were conducted using data from consumed conspecific prey. Firstly, a logistic regression was performed between proportion of consumed prey and density of offered prey, according to the polynomial function used by Juliano (2001) by means of the following equation:

$$\frac{N_e}{N_0} = \frac{EXP(P_0 + P_1 \cdot N_0 + P_2 \cdot N_0^2 + P_3 \cdot N_0^3}{1 + EXP(P_0 + P_1 \cdot N_0 + P_2 \cdot N_o^2 + P_3 \cdot N_0^3)}$$

where N_e = number of consumed prey; N_0 = initial value of available prey; P_0, P_1, P_2 and P_3 stand for cut-off, linear, quadratic and cubic coefficients, respectively, estimated according to the method of maximum likelihood. P_0-P_3 parameters were obtained by logistic regression.

The logistic regression procedure and the method of maximum likelihood estimation were carried out using statistical software package Statgraphic Centurion XVI version 16.1.18 (Statgraphics 2010).

Secondly, the data were adjusted to the three types of functional response according to equations suggested by Cabello *et al.* (2007), as follows:

- Type I functional response:

$$N_a = N\left[1 - \exp(-a' \cdot T \cdot P\right]$$

where N_a = number of consumed prey; N = number of available prey; a' = instantaneous search rate (1/day); T = total time available for search (d); P = number of predators. In this study, P = 1 (predator) and T = 1 (day).

- Type II functional response:

$$N_a = N\left\{1 - \exp\left[a' \cdot P\left(T - T_h \cdot \frac{N_a}{P}\right)\right]\right\}$$

where, T_h is the handling time (days) and the rest as before. Similarly, P = 1 and T = 1 (day).

- Type III functional response:

$$N_a = N\left\{1-\exp\left[-\frac{\alpha\cdot N\cdot P}{1+T_h(\exp(-\alpha)-1)\cdot N}\cdot\left(T-T_h\cdot\frac{N_a}{P}\right)\right]\right\}$$

where α measures the predation potential (value between 0 and 1) and the remaining variables are like those in previous responses; also, P = 1 (predator) and T = 1 (day).

The adjustments to the previous equations were completed by non-linear regression using the statistical software package Tablecurve 2D, version 5.0 (Jandel Scientific, 1994).

In order to select the best adjustment type, Akaike Information Criterion Corrected (AIC_c) was conducted, as it has proven to be more accurate for statistically comparing models than the regression coefficient (R^2) (Motulsky and Christopoulus 2003). However, R^2 was used to establish the goodness of non-linear regression adjustments.

III.3. Results

III.3.1. Prey-preference trial

Table III.1 shows the mean number of killed specimens in the prey-preference trial by *N. pseudoferus* female adults, according to prey species and the non-choice and choice treatments. **Figure III.1** shows such values in percentages.

Table III.1: Mean (±SE) number of prey killed by *N. pseudoferus* adult females when exposed to *S. exigua* second-instar larvae versus or conspecific second-instar nymphs in non-selection and selection options (means with the same letter are not significantly different from each other at P = 0.05).

Treatment	Number of dead prey	
	Mean	SE
Non-choice: heterospecific prey	8.71 a	1.18
Non-choice: conspecific prey	6.23 b	1.05
Choice: both	5.41 b	0.97

The statistical analysis performed by GZML found that, in the Omnibus test, when the adjusted model was compared with the model including only the intersect, the model-explained variance exceeded the unexplained variance (likelihood ratio χ^2 = 19.609, df = 2, P < 0.0001).

Likewise, in the model-effect testing, a highly significant effect was found in the type of prey (only natural prey, only conspecific or both) available for predatory females (Wald χ^2 = 19.749, df = 2, P < 0.0001).

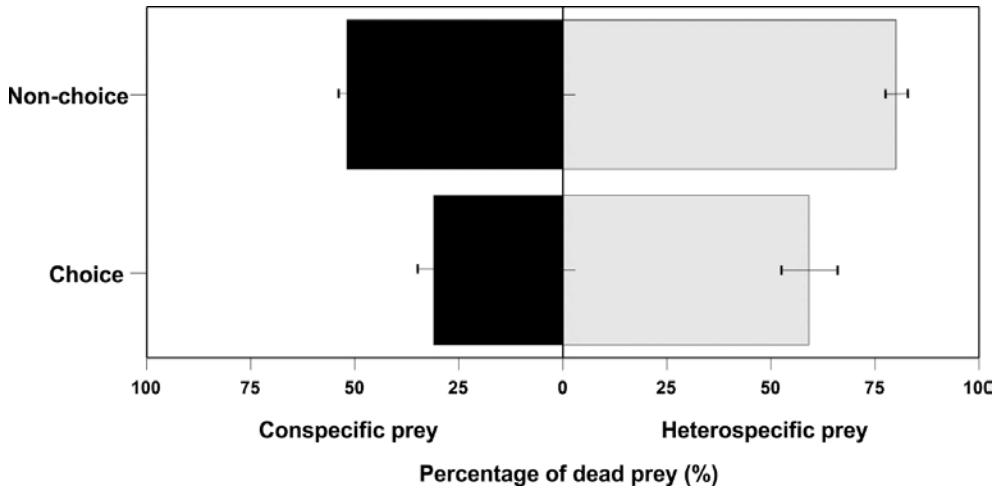

Figure III.1. Mean percentages (±SE) of dead prey in a preference trial using *N. pseudoferus* adult females exposed to *S. exigua* second-instar larvae or versus conspecific second-instar nymphs in non-election and election options.

In the choice treatment, in the presence of conspecific and heterospecific prey items, only a single nymph was observed to kill and eat a *S. exigua* larva; a fact not considered in the data analysis. Also, no second-instar nymph of *N. pseudoferus* was observed to kill a conspecific nymph.

Adult females accepted both *S. exigua* and their conspecifics as prey (**Table III.1**). However, the number of prey killed by predatory females was significantly higher in *S. exigua* non-choice treatment than in the other two treatments. Manly Preference Index (μ) supports these results. μ value indicates preference when exceeding 0.5, rejection when lower than 0.5 and indifference when the value is exactly 0.5. In our trials predators showed a clear preference for *S. exigua* larvae (μ_1= 0.74 ± 0.06, n= 24) and rejection of conspecific nymphs (μ_2= 0.26 ± 0.06, n= 24). On the other hand, the presence of conspecific (whose body size is like that of heterospecific prey) reduced females' predation activity in non-choice and choice treatments (**Figure III.1**).

Figures III.2 and **III.3** show handling times (T_h) for adult females and their actual searching time (T_s). Both values correspond to the sequence of prey captures. The Omnibus test also showed high significance in the model-explained variance (likelihood ratio χ^2 = 17.901, *df* = 2, $P < 0.0001$). Additionally, a highly significant effect was found on the handling time of the prey (T_h) for treatment (Wald χ^2 = 17.901, *df* = 2, $P < 0.0001$), but not for the sequence of prey captures (Wald χ^2 = 5.219, *df* = 11, $P = 0.920$).

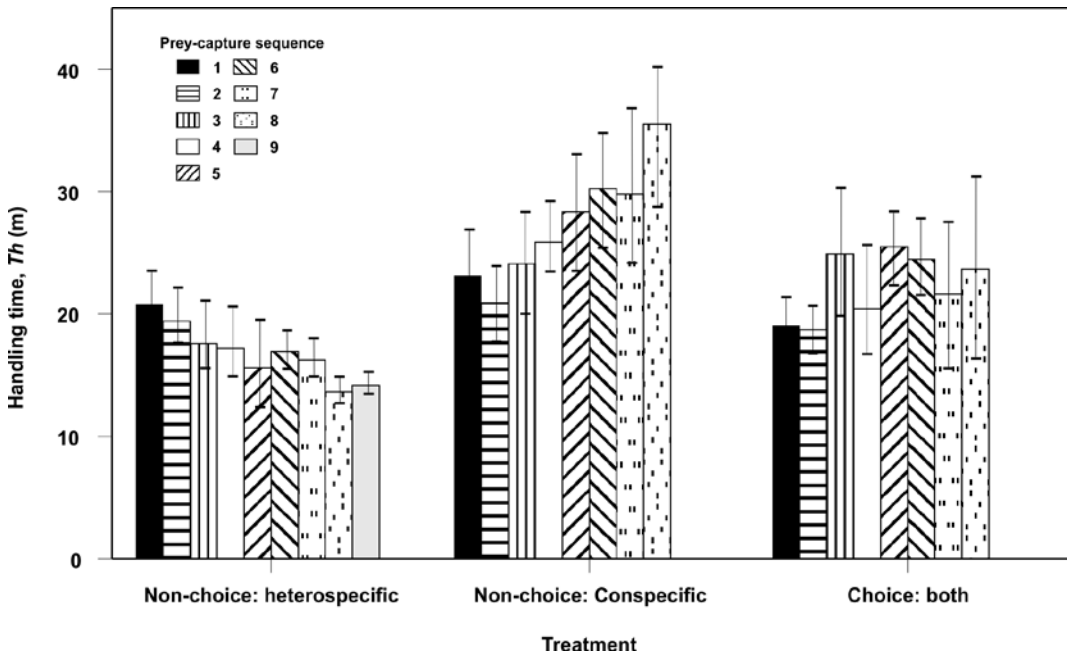

Figure III.2. Mean of handling times (± SE) of *N. pseudoferus* adult females in the prey preference trials (heterospecific or conspecific prey: *S. exigua* second-instar larvae or *N. pseudoferus* second-instar nymphs).

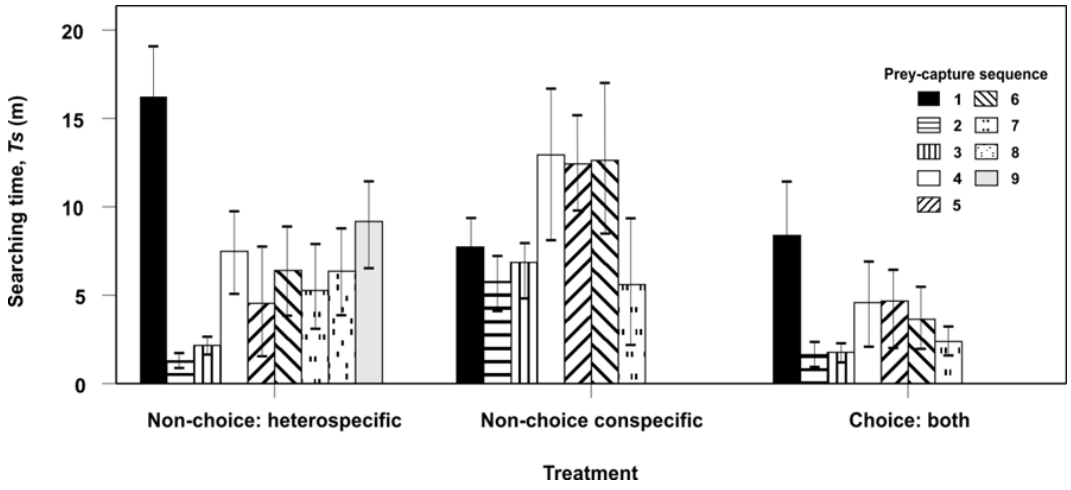

Figure III.3. Mean of searching times (± SE) of *N. pseudoferus* adult females in the prey preference trial (heterospecific or conspecific prey: *S. exigua* second-instar larvae or *N. pseudoferus* second-instar nymphs).

As for the searching time (T_s), the Omnibus contrast was also highly significant (likelihood ratio $\chi^2 = 39.352$, $df = 13$, $P < 0.0001$). Both the treatment and the capture sequence had significant effects on research time (Wald $\chi^2 = 15.516$, $df = 2$, $P < 0.0001$ and Wald $\chi^2 = 26.285$, $df = 11$, $P = 0.006$, respectively).

Likewise, the searching time (T_s) for the first heterospecific prey capture was high, probably due to the lack of learning of adult females (**Figure III.3**). In general terms, searching times were erratic, and shorter than handling times. **Table III.2** shows the mean values of both handling and search times. Average handling time (T_h) was significantly higher in the presence of conspecifics (choice and non-choice treatments). By contrast, the average searching time (T_s) was significantly higher when only conspecifics were present (non-choice treatment).

Table III.2. Mean times (± SE) of the predatory activity (searching and handling time) of the *N. pseudoferus* adult females when exposed to *S. exigua* second-instar larvae versus conspecific second-instar nymphs in non-selection and selection options (means with the same letter are not significantly different from each other at $P = 0.05$).

Times	Treatment	Time (m)	
		Mean	SE
Handing (T_h)	Non-choice: heterospecific prey	16.6 a	2.5
	Non-choice: conspecific prey	23.3 b	3.3
	Choice: both	21.9 b	3.4
Searching (T_s)	Non-choice: heterospecific prey	5.3 a	1.9
	Non-choice: conspecific prey	8.4 b	2.5
	Choice: both	3.2 a	2.6

III.3.2. Functional response

As a previous adjustment, a logistic regression between the proportion of consumed prey and density of offered prey was performed to estimate the type of functional response. The values obtained are shown in **Table III.3.** According to Juliano (2001), P_1 coefficient is not significantly different from zero (it was considered different from zero when the latter was not included

in its confidence interval) in type I; P_1 coefficient is significantly negative in type II; and positive in type III.

Table III.3. Maximum likelihood estimates from logistic regression of proportion of conspecific prey consumed by *N. pseudoferus* adult females as a function of initial prey densities.

Parameters	Values	SE	Confident limits (95%)		Predicted function
P_0 (Intercept)	5.3479	1.4103	0.8595	9.8363	Type I
P_1 (Lineal)	-1.3926	0.4228	-2.7381	0.0470	

This was confirmed by adjusting the data to the three types of functional response, using equations of Cabello *et al.* (2007), and then comparing them through the Akaike Information Criterion Corrected (AIC_c). Type I functional response presented the lowest value of AIC_c (**Table III.4**), and it best represents the behaviour of adult female predators as the density of dead conspecific varies (**Figure III.4**).

Table III.4. Parameters and statistical significance for functional response equations for numbers of conspecific nymphs consumed by *N. pseudoferus* adult females.

Functional response	Biological parameters			Statistical parameters		
	a' (day^{-1})	α	T_h (day)	*df*	R^2	AIC_C
Type I	0.6807	—	—	5	0.9802	-6.5151
Type II	1.8654	—	0.1105	7	0.9742	-5.2716
Type III	—	0.4281	0.1480	7	0.9493	-1.9275

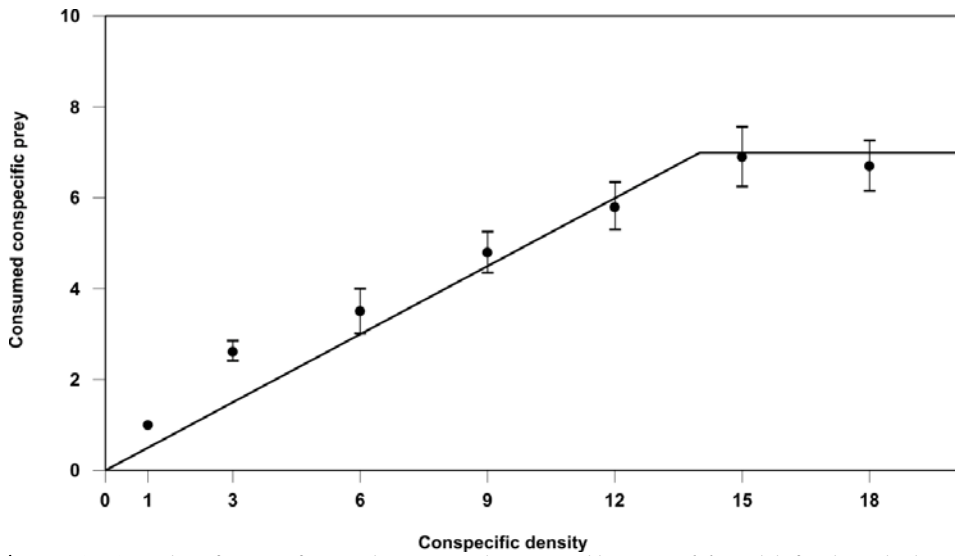

Figure III.4. Mean (±SE) number of conspecific second-instar nymphs consumed by *N. pseudoferus* adult females and values predicted by a type-I functional response model at different density levels.

III.4. Discussion

Nabis pseudoferus is a generalist predator (Fagan 1997), and shows a sit-and-wait behaviour (Schmitz 2007). Hurd (2008) noted that generalist arthropod predators are typically bitrophic (occupying the 3nd and 4rd levels simultaneously), as they feed on herbivores and on other predators, the latter involving what is called 'intraguild predation' (IGP). Due to the non-omnivorous nature of *N. pseudoferus,* the absence of prey (both phytophagous and/or other predatory species) involves the need of intraspecific predation or cannibalism, as shown by the results of our study.

Our preference trials showed that *N. pseudoferus* adult females have a clear preference for preying on immature conspecifics, causing relatively high mortality values (51.89 ± 2.69%) (**Figure III.1**). This cannibalistic behaviour has been found previously in this species (Fernandez-Maldonado unpublished data) and other species of the genus, such as *N. alternatus* (Perkins and Watson 1972), *Nabis ferus* (L.) and *Nabis punctatus* A. Costa, (Puchkov 1980). This is consistent with Hurd (2008), who reported most generalist predatory arthropods to be cannibalistic.

However, cannibalism values found in non-choice treatments for *N. pseudoferus* adult females were significantly lower than values of predation on the heterospecific prey (*S. exigua*) (80.0 ± 2.82 %) (**Figure III.1**). This indicates a lower preference for conspecific than for phytophagous prey, a fact that was also confirmed by the preference index (μ) in the choice treatment. This is a logical result, as previous studies have proven that the density of heterospecific prey reduces cannibalism rates in Hemipteran species (*e.g.,* Leon-Beck and Coll 2007, Hamdi *et al.* 2013), as well as in other animal groups (*e.g.*, Fox 1975).

At first sight, these differences may seem to be caused by the different nutritional values in of conspecific *versus* heterospecific prey. Following this line, it has been suggested that some carnivores can balance their diet based on prey composition (Mayntz *et al.* 2005). However, the reduced rate of cannibalism may signify an anti-cannibalism effect, or the energetic costs of fighting a with conspecifics, as has been noted in other arthropods (*e.g.*, Riechert 1988, Hack 1997). Polis (1981) indicated that in some species, cannibalism may not be frequent because of

the risks of reprisals or reprisal costs. This will be discussed below along with the results of our functional response trials.

The percentage of cannibalism in non-choice treatment (51.89 ± 2.69%) decreased in the presence of heterospecific prey in choice treatment (31.06 ± 4.43) (**Figure III.1**). This seems logical and has already been reported in cases where the increase in heterospecific density reduced the cannibalism rate (*e.g.*, Wagner and Wise 1996, Rudolf 2008).

On the contrary, the same phenomenon is surprising to occur in case of heterospecific prey; the percentage of killed specimens in the non-choice trial was 80%, which decreased to 59.09 ± 7.08% in choice treatment (**Figure III.1**). Overall, *N. pseudoferus* females kill significantly less prey in the presence of conspecifics, regardless of whether they are alone or with heterospecifics (**Table III.1**). In some predatory arthropods, the mortality of heterospecific prey is similar in the presence or absence of conspecifics, as is the case with adult female coccinellids (Aleosfoor *et al.* 2014). In vertebrates, it has also been found that cannibalism rates can be higher than the predation rates of heterospecific prey, due to their better escape behaviour (Rudolf 2008) or defence (Kishida *et al.* 2009). Therefore, according to the literature consulted, it is the first case cited in predatory insects; although it has been mentioned in other animal groups with a novelty (Rudolf 2008). The same implies that there is a lower predation rate on heterospecific, in the presence of conspecifics. The reason could be found in the previously mentioned risks of fighting between conspecifics.

Adult females' prey-capturing behaviour was different in case of conspecific versus and heterospecific prey, as reflected in their handling time (T_h) (**Figure III.2**). In the sequence of prey items, handling times increased for conspecific preys, but decreased for heterospecific ones. Overall, handling time was significantly longer when capturing conspecific prey (**Table III.2**). The increase in conspecific handling time may indicate an aversion to capture them. This has been noted in some species of spiders regarding toxic prey (Toft and Wise 1999). Likewise, the handling time of the prey is important to determine prey profitability, and should decrease as the prey resistance diminishes (Dong and Polis 1992). This is consistent with the two strategies usually described in the foraging behaviour of predators: active and passive selection. While active selection occurs when predators select prey with a high-energy content and that require

minimal energy to be captured, passive selection should do with predator-prey encounter rates and capture success (Weber *et al.* 2010).

The decreased number of heterospecific prey killed in the presence of conspecifics (choice treatment, **Table III.1** and **Figure III.1**) may possibly be explained by the longer handling time for conspecific prey (**Figure III.2**). The time spent by handling prey affects the response by decreasing the time available for active searching (Holling 1961).

N. pseudoferus adult females exhibit type II functional response for *S. exigua* second-instar larvae (Fernandez-Maldonado unpublished data), just like other *Nabis* species (Propp 1982, Siddique and Chapman 1987, Fathipour and Jafari 2003, Stasek 2009). Contrarily, however, *N. pseudoferus* adult females exhibited a type I functional response in our trials when conspecific prey was offered dead (**Figure III.4**).

According to nutritional ecology theory, the type of predators' functional response may vary because predators must adjust prey capture and consumption rates to the nutritional composition of the prey and other factors causes (Bressendorff and Toff 2011).

The same effect (change in the functional response from type II to I) was also observed with an increase in the predation rate for *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae), in IGP with the egg-parasitoid *Trichogramma achaeae* Nagaraja and Nagarkatti (Hymenoptera: Trichogrammatidae) (Cabello *et al*. 2015), and in the interspecific competition between two parasitoid species: *Chelonus oculator* (F.) (Hymenoptera: Chelonidae) and *Trichogramma brassicae* Bezdenko parasitoids (Cabello *et al*. 2011).

The type I functional response found for *N. pseudoferus* adult females, under the above conditions (**Figure III.4**), implies a linear increase in the rate of prey consumption, until reaching a treshold value of maximal consumption. This type of functional response indicates that the predators have a negligibly small handling time (Jeschke *et al*. 2004). From a theoretical point of view, when there is no handling time, the total opportunism provides maximal gain of energy for the predator (Garay and Mori 2010).

Our results also indicate the lack of an apparent adverse effect of prey due to nutritional properties, rather the cannibalism of this species is likely motivated by the risk (associated to conspecific fight) aversion and the energy costs of starvation. Killing a conspecific may reduce the cannibal's own inclusive fitness (Pfennig *et al.* 1993), while also expose it to the threat of reprisal from a prey having similar predatory skills (Elgar and Crespi 1992).

Furthermore, our results seem to support Schenk *et al.* (2005), who studied the *Polistes dominulus* (Christ) (Hymenoptera: Vespidae) – *Cassida rubiginosa* Müller (Coleoptera: Chrysomelidae) system and noted that the functional response depended both on the abundance of prey and predator. Therefore, the system is affected by both direct (*e.g.*, aggression) and indirect mechanisms (depletion of easy-to-find prey). This view can be extended to cannibalism in our present study.

These findings suggest the importance of fighting costs in the context of intraspecific predation. From a theoretical approach, our results support the three-individual encounter model developed by Garay *et al.* (2015). This model stresses the importance of the struggle between individual predators, which results in a low mortality rate. The ecological implications are clear: when growth and conversion rates are low enough, the balance of predator abundance is shown in the three-individual encounter model rather than in classical models. This can be explained by the results of the present study.

Likewise, from a practical point of view, the results found in this work would be important in explaining the efficacy in biological control of pests by augmentation (the periodic release of natural enemies), predatory in this case with a high level of cannibalism. Thus, on the one hand, it could imply a reduction in the effectiveness of the predator when biological control methods are carried out by inundative releases of predator (at high rates); due to the combined effect of cannibalism and the reduction in the predatory rates in heterospecific species (pest species in this case). On the other hand, the observed phenomenon can also reduce the effectiveness in the biological control by inoculative releases (at low rate). In this case, this corroborates the theoretical results found from the aforementioned model by Garay *et al.* (2015). All this leads us to take into account the role of cannibalism in such biological pest control programs. So that it will be assessed in field trials as well.

III.5. References

Aleosfoor, M., N. Mortazavi and M. Poorkashkooli. 2014. Comparison cannibalistic behavior between two ladybirds, *Coccinella septempunctata* and *Hippodamia variegata* under laboratory experiments. *Mun. Ent. Zool*. 9: 645–650.

Braman, S.K. 2000. Damsel bugs (Nabidae). In: Shaefer C.W. and A.R. Panizzi (eds.). *Heteroptera of Economic Importance*. CRC Press. Boca Raton, FL., pp. 639–656

Bressendorff B.B. and S. Toff. 2011. Dome-shaped functional response induced by nutrient imbalance of the prey. *Biol. Lett.* 7: 517–520.

Cabello, T. 1988. Natural enemies of noctuid pests in alfalfa, corn, cotton and soybean crops in Southern Spain. *J. Appl. Entomol.* 108: 80–88.

Cabello, T., F. Bonfil, J.R. Gallego, F.J. Fernandez-Maldonado, M. Gamez and J. Garay. 2015. Can interactions between an omnivorous hemipteran and an egg parasitoid limit the level of biological control for the tomato pinworm? *Environ. Entomol.* 44: 12–26.

Cabello, T., J.R. Gallego, F.J. Fernandez-Maldonado, M. Gamez, E. Vila, M. Pino and E. Hernandez-Suarez. 2012. Biological control strategies for the South American tomato moth (Lep.: Gelechiidae) in greenhouse tomatoes. *J. Econ. Entomol*. 105: 2085–2096.

Cabello, T., M. Gamez, A. Torres and Garay J. 2011. Possible effects of inter–specific competition on the coexistence of two parasitoid species: *Trichogramma brassicae* and *Chelonus oculator* (Hym.: Trichogrammatidae, Braconidae). *Community Ecol*. 12: 78–88.

Cabello, T., M. Gamez and Z. Varga. 2007. An improvement of the Holling type III functional response in entomophagous species model. *J. Biol. Syst*. 15: 515–524.

Cabello, T., H. Rodriguez and P. Vargas. 1984. Development, longevity and fecundity of *Sopodoptera littoralis* (Lep.: Noctuidae) reared on eight artificial diets. *J. Appl. Entomol*. 97: 494–499.

Canon, 2014. *Communication Software for the Camera EOS Utility*, Version 2.14. Canon Inc.

Capinera, J.L. 2010. *Insects and Wildlife*. Wiley–Blackwell, Singapore, SG.

Clercq, P. de, T.A. Coudron and E.W. Riddick. 2014. Production of heteropteran predators. In: Morales-Ramos, J.A., M.G. Rojas and D.I. Shapiro-Ilan (eds.) *Mass Production of Beneficial Organisms: Invertebrates and Entomopathogens*. Academic Press, Amsterdam, NL, pp. 57–100.

Cock, M. 1978. The assessment of preference. *J. Anim. Ecol*. 47: 805–816.

Dixon, A.F.G. 2000. *Insect Predator-Prey Dynamics: Ladybird Beetles and Biological Control*. Cambridge University Press, Cambridge, UK.

Dong, Q. and G.A. Polis. 1992. The dynamics of cannibalistic populations: a foraging perspective. In: Elgar, M.A. and B.J. Crespi (eds.). *Cannibalism: Ecology and Evolution among Diverse Taxa*. Oxford University Press, Oxford, UK, pp. 13–37.

Elgar, M.A. and B.J. Crespi. 1992. Ecology and evolution of cannibalism. In: Elgar, M.A. and B.J. Crespi (eds.). *Cannibalism: Ecology and Evolution among Diverse Taxa*. Oxford University Press, Oxford, UK, pp. 1–12.

Fagan, W.F. 1997. Omnivory as a stabilizing feature of natural communities. *Am. Nat*. 150: 554–567.

Fathipour Y. and A.A.F. Jafari. 2003. Functional response of predators *Nabis capsiformis* and *Chrysoperla carnea* to different densities of *Creontiades pallidus* nymphs. *J. Agric. Sci. Nat. Resour*. 10: 125–133.

Fox, L.R. 1975. Cannibalism in natural populations. *Annu. Rev. Ecol. Evol. S.* 6: 87–106.

Garay, J. and F.T. Mori. 2010. When is the opportunism remunerative? *Community Ecol*. 11: 160–170.

Garay, J., Z. Varga, M. Gamez and T. Cabello. 2015. Functional response and population dynamics for fighting predator, based on activity distribution. *J. Theor. Biol*. 368: 74–82.

Hack, M.A. 1997. The energetic costs of fighting in the house cricket, *Acheta domesticus* L. *Behav. Ecol*. 8: 28–36.

Hagen, K.S., N.J. Mills, G. Gordh and J.A. McMurtry. 1999. Terrestrial arthropod predators of insect and mite pests. In: Bellows, T.S., T.W. Fisher, L.E. Caltagirone, D.L. Dahlsten, G. Gordh and C.B. Huffaker (eds.). *Handbook of Biological Control: Principles and Applications of Biological Control*. Academic Press, London, UK, pp. 383–503.

Hamdi, F., J. Chadoeuf, B. Chermiti and O. Bonato. 2013. Evidence of cannibalism in *Macrolophus pygmaeus*, a natural enemy of whiteflies. *J. Insect Behav*. 26: 614–621.

Holling, C.S. 1959. The components of predation as revealed by a study of small–mammal predation of the European pine sawfly. *Can. Entomol.* 91: 293–320.

Holling, C.S. 1961. Principles of insect predation. *Annu. Rev. Entomol*. 6: 163–182.

Hurd, L.E. 2008. Predation: The role of generalist predators in biodiversity and biological control. In: Capinera, J.L. (ed.). *Encyclopedia of Entomology*. Springer, Dordrecht, NL, pp. 3038–3042.

IBM. 2012. *IBM SPSS Statistics for Windows*, Version 21.0. IBM Corp. Armonk, NY.

Jandel Scientific. 1994. *Table Curve 2D User's Manual*. Version 2.0. Jandel Scientific. San Rafael, CA.

Jeschke J.M., M. Kopp and R. Tollrian. 2004. Consumer-food systems: why type I functional responses are exclusive to filter feeders. *Biol. Rev*. 79: 337–349.

Joyner, K. and F. Gould. 1987. Conspecific tissues and secretions as sources of nutrition. In: Slansky, F. and J.G. Rodriguez (eds.). *Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates*. Wiley, NY, pp 697–719.

Juliano, S.A. 2001. Nonlinear curve fitting. Predation and functional response curves. In: Scheiner, S.M. and J. Gurevitch (eds.). *Design and Analysis of Ecological Experiments*. Oxford University Press, Oxford, UK, pp. 178–196.

Kishida, O., G.C. Trussell, K. Nishimura and T. Ohgushi. 2009. Inducible defenses in prey intensify predator cannibalism. *Ecology* 90: 3150–3158.

Latham, D.R. and N.J. Mills. 2009. Quantifying insect predation: a comparison of three methods for estimating daily per capita consumption of two aphidophagous predators. *Environ. Entomol*. 38: 1117–1125.

Leon–Beck, M. and M. Coll. 2007. Plant and prey consumption cause a similar reductions in cannibalism by an omnivorous bug. *J. Insect Behav*. 20: 67–76.

Mangeaud, A. and M. Videla. 2005. En busca de la independencia perdida: la utilización de modelos lineales generalizados mixtos en pruebas de preferencia. *Ecol. Austral* 15: 199–206.

Manly, B.F.J., P. Miller and L. Cook. 1972. Analysis of a selective predation experiment. *Am. Nat*. 106: 719–736.

Mayntz, D., D. Raubenheimer, M. Salomon, S. Toft and S.J. Simpson. 2005. Nutrient–specific foraging in invertebrate predators. *Science* 307: 111–112.

Mills, N.J. 1982. Voracity, cannibalism and coccinellid predation. *Ann. Appl. Biol*. 101: 144–148.

Motulsky, H. and A. Christopoulos. 2003. *Fitting Models to Biological Data Using Linear and Nonlinear Regression: A Practical Guide to Curve Fitting*. GraphPad Software, Inc., San Diego, CA.

Perkins, P.V. and T.F. Watson. 1972. Biology of *Nabis alternatus* (Hem.: Nabidae). *Ann. Entomol. Soc. Am*. 65: 54–57.

Pfennig, D.W., H.K. Reeve and P.W. Sherman. 1993. Kin recognition and cannibalism in spadefoot toad tadpoles. *Anim. Behav*. 46: 87–94.

Polis, G.A. 1981. The evolution and dynamics of intraspecific predation. *Annu. Rev.Ecol. Evol. S.*, 12: 225–251.

Propp, G.D. 1982. Functional response of *Nabis americoferus* to two of its prey, *Spodoptera exigua* and *Lygus hesperus*. *Environ. Entomol*. 11: 670–674.

Puchkov, A.V. 1980. Particulars of the biology of predacious *Nabis* spp. *Zashch. Rast*. 8: 44.

Ramirez, C.C., E. Fuentes-Contreras, L.C. Rodríguez and H.M. Niemeyer. 2000. Pseudoreplication and its frequency in olfactometric laboratory studies. *J. Chem. Ecol*. 26: 1423–1431.

Ricard, I. 2008. *Statistical Methods for Insect Choice Experiments*. École Polytechnique Fédérale de Lausanne. PhD Dissertation. Lausanne, CH.

Riechert, S.E. 1988. The energetic costs of fighting. *Integr. Comp. Biol*. 28: 877–884.

Rudolf, H.W. 2008. Impact of cannibalism on predator–prey dynamics: size-structured interactions and apparent mutualism. *Ecology* 89: 1650–1660.

Samu, S., S. Toft and B. Kiss. 1999. Factors influencing cannibalism in the wolf spider *Pardosa agrestis* (Araneae, Lycosidae). *Behav. Ecol. Sociobiol*. 45: 349–354.

Santana, A.F., A.C. Roselino, F.A. Cappelari and F.S. Zucoloto. 2012. Cannibalism in insects. In: Panizzi, A.R. and J.R.P. Parra (eds.). *Insect Bioecology and Nutrition for Integrated Pest Management*. CRC Press and Taylor and Francis Group, Boca Raton, FL, pp. 177–194.

Schenk, D., L.F. Bersier and S. Bacher. 2005. An experimental test of the nature of predation: neither prey- nor ratio-dependent. *J. Anim. Ecol*. 74: 86–91.

Schmitz, O.J. 2007. Predator diversity and trophic interactions. *Ecology* 88: 2415–2426.

Schneider, C.A., W.S. Rasband and K.W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9: 671–675.

Sherratt, T. and I. Harvey. 1993. Frequency-dependent food selection by arthropods: a review. *Biol. J. Linn. Soc*. 48: 167–186.

Siddique, A.B. and R.B. Chapman. 1987. Functional response of Pacific damsel bug, *Nabis kinbergii* (Hem.: Nabidae). *Entomophaga* 32: 303–309.

Statgraphics. 2010. *Statgraphics Centurion XVI User Manual*. StatPoint Technologies, Inc., Warrenton, VA.

Stasek, D.J. 2009. *Population Responses of a Generalist Insect Predator and its Prey to Patch Characteristics in Forage Crops.* PhD Dissertation. Faculty of Miami University, FL.

Toft, S. and D.H. Wise. 1999. Behavioral and ecophysiological responses of a generalist predator to single- and mixed-species diets of dfferent quality. *Oecologia* 119: 198–207.

Ulusoy, M.R. and S. Ulgenturk. 2003. The natural enemies of whiteflies (Hem.: Aleyrodidae) in southern Anatolia. *Zool. Middle East* 28: 119–124.

Vila, E. and T. Cabello. 2014. Biosystems engineering applied to greenhouse pest control. In: Torres I. and Guevara R. (eds.). *Biosystems Engineering: Biofactories for Food Production in the XXI Century*. Springer, Berlin, DE, pp. 99–128.

Wade M.R., M.P. Zalucki and B.A. Franzmann. 2005. Influence of observer presence on Pacific damsel bug behavior: who is watching whom? *J. Insect Behav*. 18: 651–667.

Wagner, J.D. and D.H. Wise. 1996. Cannibalism regulates densities of young wolf spiders: evidence from field and laboratory experiments. *Ecology* 77: 639–652.

Weber, M.J., J.M. Dettmers, D.H. Wahl and S.J. Czesny. 2010. Effects of predator-prey interactions and benthic habitat complexity on selectivity of a foraging generalist. *T. Am. Fish. Soc*. 139: 1004–1013.

Theoretical foundation of the control of pollination by hoverflies in a greenhouse

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Chapter IV

Theoretical foundation of the control of pollination by hoverflies in a greenhouse

Abstract: We propose a conceptual model for pollination and fertilization of tomato flowers in greenhouse crops by hoverflies, when the maximal number of adult pollinators maintained by the crops, is less than what is needed for an economically successful pollination in greenhouses. The model consists of a two-stage process for additional feeding of hoverfly to maintain the pollinator density at the economically desired level. First, with a stochastic model we calculate the density of flies necessary for the economically successful pollination, determined according to the economically expected yield. Second, by using a deterministic optimal control model, we find a minimum cost supplementary feeding strategy. In summary, we theoretically demonstrate, at the present stage of the research without validations in case studies, that optimal supplementary feeding can maintain the economically desired hoverfly density.

Keywords: *Tomato; Eristalis tenax; feeding of larvae; adult feeding; Markov model; population dynamics; pollen; nectar; supplementary food; optimal control*

IV.1. Introduction

Pollination is one of the most important issues concerning flowering plants, both from the point of view of conservation biology [1] and agriculture [2]. A practical problem in greenhouses is that the maximal number of adult pollinators maintained by greenhouse crops, is less than what is needed for an economically level of fructification [3]. For instance, a problem in the tomato flower is the absence of nectar [4]. Thus, tomato plants are apparently of little or no value in attracting bees [2]. [5,6] stated that the blossom "contains little or no nectar." It is reported that bumble bees "gathered chiefly pollen'' from tomato flowers [7]. Thus, if nectar is produced, a question that should be settled, it is of little significance in the relation of insect pollination of tomatoes. The pollen is more attractive to wild bees than honeybees. There are two possible solutions: a) Release of pollinators other than honeybees and bumblebees, such as syrphids [8], since the flowers of tomato are widely visited by hoverfly species [9,10]. b)

Supplying of additional food to pollinators in order to maintain more adult insects than what is maintained by greenhouse crops [11,12].

Syrphids, commonly known as hoverflies or flower flies, make up one of the largest families of Diptera. To date, the family has 6674 species in 284 genera, with most species distributed in the Neotropical, Nearctic and Palaeotropical regions of the world [13]. The adults of hoverflies, as their name implies, are usually found at flowers or hovering in the bright sunlight. They require pollen, nectar and honeydew during the adult stage [14-16]. Haslett [14] studied the role of flower nectar and pollen, by dissecting the adult females of *Rhyngia campestris* Meigen (Diptera: Syrphidae) captured in the field. Thus, this author found that nectar seems to be necessary at the beginning and at the end of the females' ovarian development. On the contrary, pollen is essential for such ovarian development and subsequent oviposition. The same has been observed for adult females of *Eristalis tenax* (L.) (Diptera: Syrphidae) under laboratory conditions; thus, after several unsuccessful tries, artificial rearing of *E. tenax* was successfully carried out for three years. For this reason, a pollen-base diet for adults was essential for the longevity and fertility of the females [17]. This has been corroborated more recently for this species and other species of the same genus [18]. All the above discussion seems to indicate that pollen is a fundamental feeding factor for adult longevity and fecundity. On the contrary, the immature stages of Syrphidae species present different behaviour: there are species in which the larvae are predatory, others present a phytophagous or mycophagous diet; finally, there is a wide group of Syrphidae species that are saprophagous. Therefore, this last group need a specific food substrate [18]. Also, several species of this family of insects have demonstrated their effectiveness as pollen vectors in the main horticultural crops in greenhouses [19-21].

The present study is motivated by the pollination of tomato by *Eristalis tenax*. The adults of this species feed on flowers, while their immatures are saprophagous. In this paper we will use a theoretical approach, namely mathematical models, and will start out from the models of theoretical ecology (*e.g.*, [22-24]). In fact, to make crop pollination economic and efficient, a dynamic optimization approach is necessary, which on its turn is necessarily based on a dynamic mathematical model (differential equations) available in theoretical ecology. In this way the optimal control of pollination can be applied to quantify the number of released agents necessary

for a sufficient pollination. Since the classical theoretical models (*e.g.*, [22]) do not take account of some important factors of our problem, we must develop models that, as far as possible, involve such details of this practical problem (*e.g.*, longevity of adults, different habitat for larvae and adults). Our method addresses the above practical problem by developing models that, as far as possible, involve the details of the crop pollination situation. We address two basic questions.

The first one is: How many flies are needed for the desired level of pollination? (Given an expected economic yield, the necessary pollination level can be experimentally determined.) To answer this question, we will apply a Markov model, which has been already used to study pollination (*e.g.*, [25,26]). The Markov model is one of the simplest mathematical tools to handle the general pollination process, when during a day the flowers are emptied. (The number of non-visited flowers decreases, but due to the repeated visits, new pollination takes place with smaller and smaller probability.)

The second question is: In which way can we maintain this desired pollinator density (*e.g.*, [27, 23])? Here, instead of the extremely inefficient continuous release of adult flies in the greenhouse, we propose another way: Initially we release adult flies. Then we maintain the hoverfly population at the desired density by supplementary feeding (sugar and pollen), ensuring the reproduction of hoverfly. We will study the case when in the greenhouse we also release larvae supplied with special larval substrate provided in containers distributed throughout the greenhouse. We suppose the amount of food for the juveniles is sufficient both for the juveniles released and for the offspring of the adult released in the greenhouse. Hence, we will need a two-stage model, including the juvenile stage. On the other hand, the supplementary food supply can increase both fecundity and longevity of adult flies, but it does not increase the survival of the hoverfly larvae. Furthermore, in our case supplementary food increases their longevity. The longevity already plays a crucial role in the models of host-parasite systems (e.g., [28,29]), which is quite different from pollination. We introduce a non-additive model to consider the increased longevity due to supplementary food [17] (see Section 2.3).

In our approach, the effect of feeding on the population dynamics of the hoverfly, will be described in terms of an optimal control model. Such models have already been applied to

the optimization of release strategies in biological pest control (see *e.g.*, [30,31]). In the present case, the optimal control model will be used to keep the adult hoverfly density above, but close to the desired level.

The main aim of this paper is to face at least two basic questions, as we already mentioned: 1. How many hoverflies are necessary for an economically suitable level of pollination of a given amount of crop plants? 2. In which way can we maintain a required pollinator density higher than what is maintained by the greenhouse? Now, in the present paper we deal with the first phase of our research project, building up a *conceptual model* based on mathematical methods, addressing these questions.

Finally, we emphasize that this is a theoretical work, and literature data are used only to show the potentialities of the model (and may be helpful for a sensitivity analysis too).

IV.2. Materials and methods

At this first stage we develop a conceptual theoretical model that will help the design of appropriate experiments, and only at the second stage of the research we will carry out experiments. Therefore, in the present paper for the simulations we will use only illustrative data, based on general experience, in part on data of the literature.

The species considered for this work was *Eristalis tenax* (L.) (Diptera, Syrphidae: Eristalinae). Data on its biology and ecology have been collected from the available literature on this species and other nearby species in the same genus (*e.g.*, [14,27], [32-36]).

Our aim is to maintain the hoverfly population at a density necessary for a sufficient pollination, at minimum cost. To this end, we must go through the following three steps. A) We introduce a stochastic model to calculate the pollinator density needed for the economically successful pollination of a given number of plants, during a fixed time interval (see Section 2.1). B) We set up a stage-specific deterministic population dynamic for the hoverfly (see Section 2.2). C) In these dynamics, we introduce the effect of time-dependent quantity of supplementary food intended for the stable maintenance of the economically desired pollinator density. This leads to a deterministic optimal control model for the hoverfly with added supplementary food

(See section 2.3). These three models are built upon each other in the sense that the desired density of pollinator is calculated by a stochastic model and then, based on a deterministic population dynamic, an optimal control model is set up.

IV.2.1 How many pollinators are needed for an economically successful pollination of crop plants?

In our simplified model there are M flowers in a greenhouse waiting for pollinations. For our conceptual stochastic model, we use some simplifying assumptions about the flowers. We suppose that there is no intra-flower variability in the probability of being visited (in reality, some flowers could be exposed at the top of the plant, and others could be hidden behind leaves). Moreover, we also assume that M is fixed during flowering period.

In our model, pollination agents are insects (for example, pollinators of a single species), there are N of them ($0 \ll N \ll M$). Pollinators are continuously searching flowers during their daily active period (when pollinators feed intensively), denoted by T. During this active period, we consider the following events, since the pollinator does not know if a flower is already exhausted by another pollinator: (a) Searching: a pollinator stops feeding and starts searching. When a pollinator finds a flower, it faces the following possibilities: (b) Pollinate an unfertilized flower. A simplifying assumption of our conceptual stochastic model on pollination is that a single visit by a pollinator is enough for the pollination of a flower. (c) Find an unoccupied but pollinated flower, and still spend a certain amount of time in it. (d) Find a flower occupied by another adult and start a new search. In other words, there is no interference between pollinators during feeding (i.e., there is no fighting between pollinators). We emphasize that our model considers that at the beginning of the activity period T more pollination is carried out than at the end of T, since the number of pollinated flowers increases with time, consequently more and more times, the pollinators find flowers that are already pollinated. In other words, during each day the pollinators find less and less virgin flowers, because they have already visited a part of the flowers before, repeatedly. We also assume that during T, all flies are active. This simplifying assumption based on the fact that for the desired pollination rate we need more flies than the maximal number of pollinators maintained by the tomato, thus the overwhelming majority of flies have not fed (are not full), thus they will not stop visiting flowers. For the simplicity, we

also do not consider other activities during the feeding period of a day, such as mating, fighting and displaying.

Both searching and handling/pollinating take time. Searching times are supposed to be independent and exponentially distributed random variables with expectation τ_s. The same holds for handling times with mean τ_h. For the sake of simplicity, we suppose that the flower found at the end of the searching time is random with uniform distribution, though, in fact, closer flowers are found with greater probability. We also suppose that the mean time a pollinator spends in an exhausted flower, and that in a flower waiting for pollination are the same, though feeding presumably requires more time. We note, our technical assumption on exponential distribution makes our investigation easier, but we note that [37] fitted gamma distribution to the handling time for the herbivore. Observe, that at this starting stage of our model, we only concentrate on the pollination, so we do not consider other activities of the pollinator (like rest on a flower during feeding, interaction between pollinators, e.g., mating and fighting, or egg laying etc.), which also take time, so these other activities can decrease the efficiency of pollination.

Our object is to find an approximate answer to the question "Given a fixed, relatively long-time interval T (a day, say), and a small positive number ε, how many pollinators are needed so that the proportion of unpollinated flowers would fall below ε by the end of the given time period?" We assume τ_s and τ_h are dwarfed by T.

A naïve estimation can easily be derived. Since the number of pollinators is much smaller than the number of flowers, it is of small probability that a pollinator finds an occupied flower. Neglecting this unlikely possibility, we can say that N pollinators perform approximately $\frac{NT}{\tau_s+\tau_h}$ turns of searching and handling during time T. Considering them independent, the chance that a given flower has not been visited by the end of the monitoring period T is

$$\left(\frac{M-1}{M}\right)^{\frac{NT}{\tau_s+\tau_h}} \approx \exp\left(-\frac{N}{M}\cdot\frac{T}{\tau_s+\tau_h}\right).$$

This is equal to ε if

$$N \approx \frac{\tau_s + \tau_h}{T} M \ln \frac{1}{\varepsilon}.$$

The justification of this naïve estimation in a more elaborated stochastic model is presented in **Appendix IV.A.1**.

The temperature has not been included among these parameters. Although insects are ectotherms, we have considered that environmental factors in greenhouse crops allow us to simplify the model.

IV.2.2. A population dynamic model for pollinator

As in Section 2, we suppose that in the greenhouse the density of mono-cultural crops does not change. We deal with mono-cultural crops, so we suppose that both the number of flowers and the quantity of nectar and pollen produced in unit time are constant during the flowering period of the plant. Clearly, this simplifying assumption does not consider that the proportion of plants and flowers on plants flowering, and hence nectar and pollen availability, will vary through the lifetime of a crop.

This assumption simplifies the modelling, because we do not have to take into consideration how fertility, due to the pollinators, can affect the plant density throughout generations. In mathematical terms, this also means that the plant dynamics can be neglected, since we concentrate on greenhouses. Another simplifying consequence of the constant plant density is that, in the dynamics of pollinator density, the numerical response (i.e., α in our model below) can be considered constant. Therefore, here we will consider the following deterministic model for the density change of hoverflies.

Let $x_1$and x_2 be the density of juvenile and adult pollinators, respectively, α the number of juveniles produced by an adult hoverfly in unit time (egg number), β the rate of leaving juvenile stage (by either death or development), d_1 and d_2 the death rates of juvenile and adult, respectively ($0 < d_i < 1$) and K the coefficient of the density dependent competition. Then the dynamic model is

$$\frac{dx_1}{dt} = \alpha x_2 - \beta x_1 \tag{1}$$

$$\frac{dx_2}{dt} = \beta(1-d_1)x_1 - x_2\left[1-\left(1-\frac{x_2}{K}\right)(1-d_2)\right] \tag{2}$$

Observe that in the above model, there is no competition for food between juveniles and adults.

Explanation of the first equation: What happens to the juveniles in unit time? Using the above introduced parameters, the juvenile population is increased by the egg laying of adults (i.e., by αx_2) and decreased by leaving the juvenile stage (i.e., by βx_1). The food competition between larvae does not appear in equation (1) since larvae are supplied with sufficient food.

Explanation of the second equation: What happens to an adult in unit time? We suppose two types of issues:

a) The survival rate of a "newcomer" adult is $(1-d_1)$, they are not sensitive to either the quality or the quantity of food, since they have some reserve from their larva stage, collected from the ground (see the first term of the right-hand side of (2)).

b) An adult emerged (see the second term of the right-hand side of (2)) may die for two reasons:

1. by competition for food among them, implying a density-dependent death rate: $\frac{x_2}{K}$;

2. by ageing, this is not density-dependent, it is characterized just by a death rate d_2. Here the individuals are supposed to be "ever-young" (i.e., the imago's death rate does not depend on age), e.g., there is an exponentially distributed survival. In the latter case the life span expected at emergence (shortly also often called longevity) is $\frac{1}{d_2}$.

In order to survive, an individual must survive both death causes in unit time, which occurs with probability $\left(1-\frac{x_2}{K}\right)(1-d_2)$, thus the survival probability of an individual in unit time is

$$\left(1-\frac{x_2}{K}\right)(1-d_2),$$

and hence the resulting death rate (the proportion of individuals dying in unit time) is

$$1-\left(1-\frac{x_2}{K}\right)(1-d_2).$$

Therefore, the number of individuals died in unit time is

$$x_2\left[1-\left(1-\frac{x_2}{K}\right)(1-d_2)\right].$$

Remark 1. The death rate of the adult can also be written as

$$1-\left(1-\frac{x_2}{K}\right)(1-d_2)=d_2+\frac{x_2}{K}-\frac{x_2}{K}d_2.$$

An explanation to the latter may be the following: Suppose that an individual can face death risk either by starving, or by aging or both. If for example 10 individuals died only by starving, 10 only by aging, 5 faced both death risks, then 15 individuals died by starving, 15 by aging, but in this way, those having faced both death causes, are counted twice, so the latter should be subtracted to obtain the actual number of individuals that died. Consequently, if there are two death causes then the sum of the two death rates is an upper estimate of the actual death rate.

In **Appendix IV.A.2**, we show that under a simple condition on the model parameters, an asymptotically stable equilibrium of dynamics (1)-(2) is guaranteed. Namely, equilibrium x^* with

$$x_1^*=\frac{\alpha}{\beta}K\frac{(1-d_1)\alpha-d_2}{(1-d_2)},\ x_2^*=K\frac{(1-d_1)\alpha-d_2}{(1-d_2)}$$

is positive, if

$$(1 - d_1)\alpha > d_2 \tag{3}$$

Remark 2. Condition (3) is obviously fulfilled if the fertility rate α is high enough. Furthermore, this condition also implies that x^* is locally asymptotically stable (see **Appendix IV.A.2**). If the convergence is quick enough, the insect population reaches its stable density during a short time respect to the crop season. If not, the asymptotically stable equilibrium would not help to reach the desired number of adults.

IV.2.3. A deterministic optimal control model for the hoverfly with added supplementary food

A first idea might be a continuous release of adult flies in the greenhouse, considering the dynamics of the adult without reproduction, obtained from system (1)-(2):

$$\frac{dx_2}{dt} = -x_2\left[1 - \left(1 - \frac{x_2}{K}\right)(1 - d_2)\right],$$

the purely adult population would die out very quickly, therefore a control by the release of adults according to the control equation

$$\frac{dx_2}{dt} = -x_2\left[1 - \left(1 - \frac{x_2}{K}\right)(1 - d_2)\right] + u(t)$$

would be extremely inefficient.

IV.2.4. Controlling pollination level by supplementary food

A common practice in both pollinating and entomophagous insects is the application of nutritional supplements to increase their activity in crop pollination or application of biological agents in crops and/or greenhouses (e.g., [38]). In the case of pollinating insects, applications of food and nutritional supplements would be applied in water by spraying on plants (e.g., [39]) or better placing feeders in different sites inside the greenhouse. Evidently, solar radiation, air oxygen and other factors can degrade the food supplement. Therefore, it will be necessary to re-

spray the food supplement repeatedly. Remember, the juveniles need different supplementary food.

The supplementary food added by spray has three possible effects:

Decreases the density dependent food competition between adults (e.g., [40]): Let us denote by $y_1 \geq 0$ the increment of the coefficient *K*-nek due to the supplementary food for the hoverfly given in unit time by the farmer.

Increase longevity (expected life span) of the adult (e.g., [16]): Let y_2 denote the effect of supplementary food decreasing the death rate of adult hoverfly, $0< 1 + y_2 - d_2 < 1$. The pollinator mainly needs nectar for food ([17]).

Increase fecundity (number of eggs of a female) (e.g., [14]): Let $y_3 \geq 0$ be the effect of supplementary food increasing fecundity.

We can consider a "feeding function" depending on the added sugar and pollen (u_1, u_2),

$$y(u_1, u_2) = (y_1(u_1, u_2), y_2(u_1, u_2), y_3(u_1, u_2)).$$

This function is based on the physiology of the insect [17]. The exact forms of functions $y_1(u_1, u_2), y_2(u_1, u_2), y_3(u_1, u_2)$ are unknown, in our dynamic model. We can consider the following simplest theoretical feeding functions based on biological intuition and experimental practice.

a) Density dependent food competition function: The pollinators mainly need nectar for survival; thus, the supplementary sugar decreases the starvation of adults. We assume that this function is linear.

$$y_1(u_1, u_2) = a_1 u_1,$$

where a_1 is a positive constant.

b) The longevity function displays a saturation with respect to u_1, since the death rate cannot decrease arbitrarily.

$$y_2(u_1, u_2) = \frac{b_0 u_1}{1+b_1 u_1},$$

where b_0 and b_1 are positive constants. Since the latter function is increasing and is saturated at value $\frac{b_0}{b_1}$, if condition $\frac{b_0}{b_1} < d_2$ holds.

c) The fertility function may be

$$y_3(u_1, u_2) = \frac{c_0 u_2}{1+c_1 u_1+c_2 u_2},$$

where c_1 and c_2 are positive constants. This function, for each fixed u_1, is a saturation function of u_2.

Since now the pollinator population has two types of food at disposal, the optimal foraging strategy of the pollinator could also be taken into consideration (e.g., [41]). For the sake of simplicity, we will not consider the optimal foraging behaviour in our present conceptual model (4) and (5) below. This simplifying condition can be a good approximation, if the quantity of food sprayed on the plants can be considered constant between two actions (e.g., the added food does not oxidize or deteriorate in the meanwhile). Then, even if the hoverfly consumes the added food and also visits the flowers, being the densities of both resources fixed, the optimal foraging is also fixed, hence in the model describing the dynamics of pollinator density, the food-dependent parameters are also fixed (and the optimal foraging preference is included in the interaction parameters).

Now inserting the above functions y_i into dynamics (1)-(2), we have the following control dynamics:

$$\frac{dx_1}{dt} = (\alpha + \frac{c_0 u_2}{1 + c_1 u_1 + c_2 u_2})x_2 - \beta x_1 \quad (4)$$

$$\frac{dx_2}{dt} = \beta(1 - d_1)x_1 - x_2\left[1 - \left(1 - \frac{x_2}{K + a_1 u_1}\right)\left(1 + \frac{b_0 u_1}{1 + b_1 u_1} - d_2\right)\right] \quad (5)$$

Hence, the optimal control problem to be solved is the following: We start with initial release of juvenile and adult flies $x(0) = \big(x_1(0), x_2(0)\big)$, with $x_2(0) = N^*$, where N^* is the number of adult hoverfly necessary for the satisfactory pollination, calculated according to the reasoning of Section 2.1. Then, with time-dependent food supply $u_1\,(t), u_2\,(t)$ as control in a time interval $[0, T]$, with control dynamics (4)-(5), we will solve the problem of minimization of the total costs of the food supply under the condition that $x_2(t) \geq N^*$. For mathematical details see **Appendix IV.A.3**.

The above optimal control problem has been solved with a toolbox developed for MatLab in [42] and [43]. The results are plotted in the Results section.

IV.3. Result

In our simulations of this section, we work with illustrative data as explained in the Materials and Methods section, in part based on data from the literature.

IV.3.1. Result on how many pollinators are needed for an economically successful pollination of crop plants

Here we use our general result of Section 2.1. In this model simulation, we use some data from the literature on hoverfly [44,45] such as searching time (interplant movement duration): 16.2 – 32.4 sec and handling time (residence duration): 11.7 – 120.0 sec. So, we roughly chose the arithmetic mean of these time durations, so let $\tau_s = 24$ sec and $\tau_h = 60$ sec in each figure. This hoverfly activity occurs for 6 hours a day, so let $T = 6$ hours. In tomato crops there are 60-80 open flowers/square meter, so we set M=70. Considering a 10000 m^2 greenhouse (which is the average size in the area of Almeria, Spain) we have M = 700000 open flowers waiting for pollinators.

Firstly, in **Figure IV.1**, for a given plant species and pollinator species (i.e., for fixed τ_s and τ_h) we illustrate how the percentage of visited flowers depend on the density of the pollinator.

Remark 1. We emphasize that, in spite that the searching and handling times are random variables, and we roughly use the arithmetic means of these parameters, our simple model

gives a "rather realistic" prediction. Indeed, the recommended dose under commercial conditions is 10-15 flies / m^2.

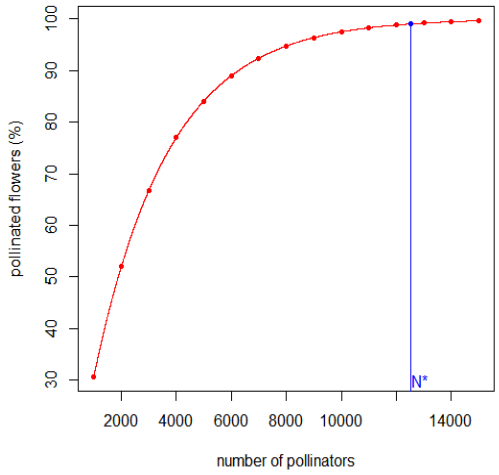


Figure IV.1. 99% of 700 000 open flowers are visited by pollinator during 6 hours in a 10 000 m^2 greenhouse, if $\tau_s = 24$ sec, $\tau_h = 60$ sec, and there are N^*=12 536 pollinators, i.e. 1.2536/m^2. Observe that, for an 80% pollination 6000 adult pollinators are needed, but for a 95% pollination already 12000 adult pollinators are necessary, since during the day the pollinators find a virgin flower with lower and lower probability.

Secondly, for given fixed number of open flowers and pollinator density, in **Figure IV.2** we illustrate how the number of visited plants depends on the time duration of searching and handling. These parameters depend on the anatomy of the flower and the pollinator at the same time. As expected, the minimal handling and searching time determines the best, i.e., the most efficient pollination.

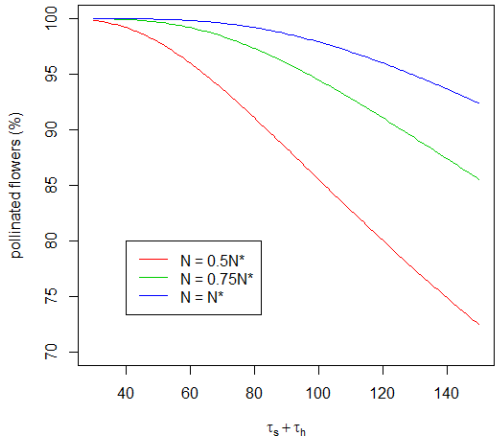


Figure IV.2. The time duration $\tau_s + \tau_h$ of the pollination of a flower in nonlinear way determines the pollination success. The parameters are the following: T = 6x60x60 sec, M = 700000, N=cN^*, where c=0.5 (red), 0.75 (green), 1 (blue).

Thirdly, not only searching and handling times determine the efficiency of the pollinator, but the activity time duration also has effect on the pollination success of the crops (see **Figure IV.3**).

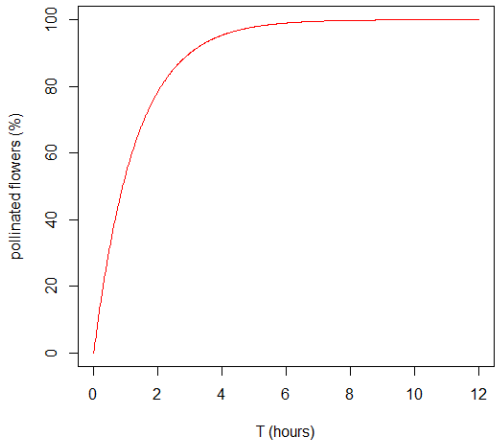


Figure IV.3. Here we can see how the activity time duration of pollinator also determines the success of pollination in nonlinear way, with parameters $\tau_s = 24$ sec, $\tau_h = 60$ sec, $M = 700000$.

The above **Figures IV.1-3** illustrate what kind of practical information is predicted by our stochastic model.

IV.3.2. Result on the deterministic dynamic model for hoverflies

First, in Example 1, we demonstrate that without supplementary feeding, the density of hover fly is not enough for the successful pollination.

Example 1. We consider the following parameters α=5; β=0.03; d_1=0.2; d_2=0.03; K=1697. With them, for the equilibrium of dynamics (1)-(2), we have $x_1^* = 1.156\ 10^6$; $x_2^* = 0.693\ 10^4$, and the simulation results can be seen in **Figure IV.4**.

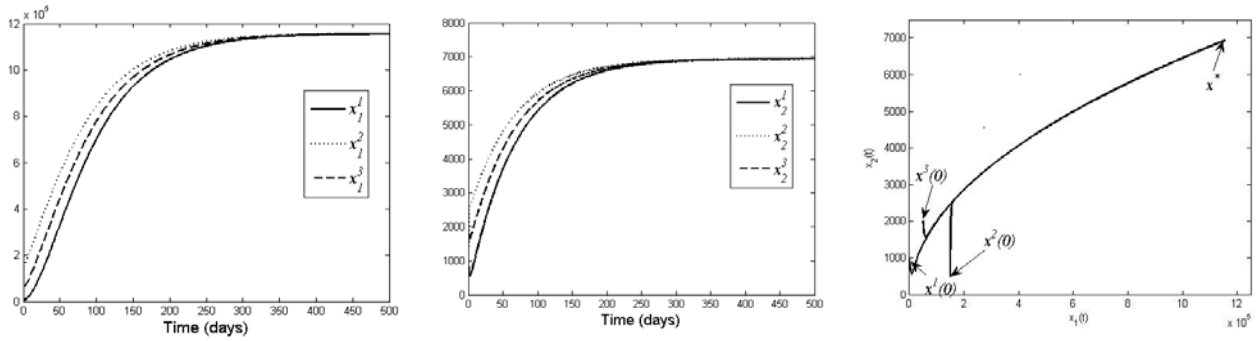


Figure IV.4. Solution of (1)-(2) with different initial values x1(0) = (0, 1000); x2(0) = (150000, 500) and x3(0) = (50000, 2000).

Remark 2. Figure IV.4 illustrates the asymptotic stability of equilibrium x* (implying the convergence of the solutions from different initial values to x*). Although in all cases the density of adults is getting somewhat closer to the required value, but this convergence to x_2^* is too slow, with respect to the 75 days of operational period. Therefore, uncontrolled dynamics (1)-(2) cannot help us to get closer to the desired value adult flies.

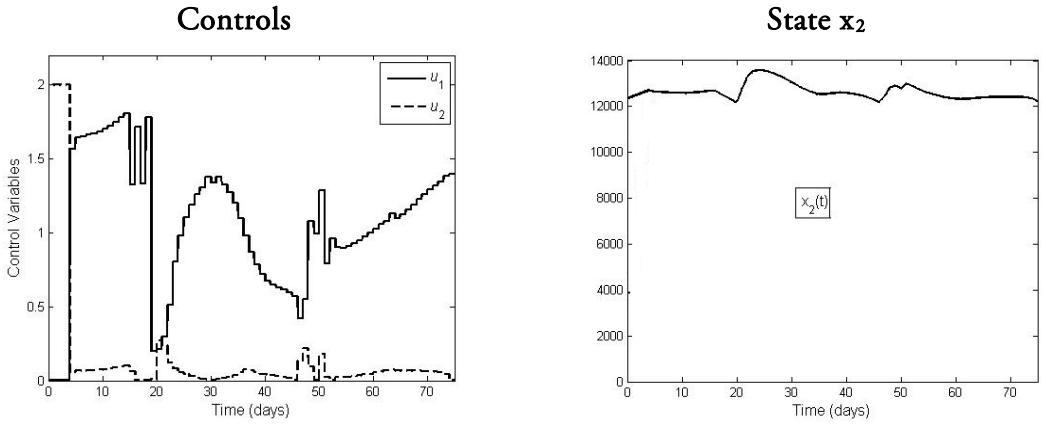
IV.3.3. Results on the deterministic optimal control model for the hoverfly with added supplementary food

Now we are the position to set up a model for the optimal control of pollination by supplementary feeding, for the maintenance of the desired hoverfly density.

Example 2. Let us consider the same illustrative parameters of the zero control dynamics as in Example 1: α=5; β=0.03; d_1=0.2; d_2=0.03; K=1697, completed with the following illustrative parameter values of the feeding functions: a=1; b_0=2, b_1=80 (they satisfy $b_0/b_1 < d_2$); c_0= 400, c_1=0.7, c_2=1.

We assume 90 days for our cultivation and that we have flowers from the 15th day on, so we have 75 days with control: T=75, and $u_1(t), u_2(t) \in [0,2]$ ($t \in [0,75]$). The unit prices of sugar and pollen are p_1=1€/kg and p_2= 40 €/kg, respectively.

In **Figure IV.5** (**a**)-(**d**), the solutions (optimal controls and the corresponding adult population density) of the optimal control problems are plotted four different initial conditions for juveniles.



(a)

Controls State x_2

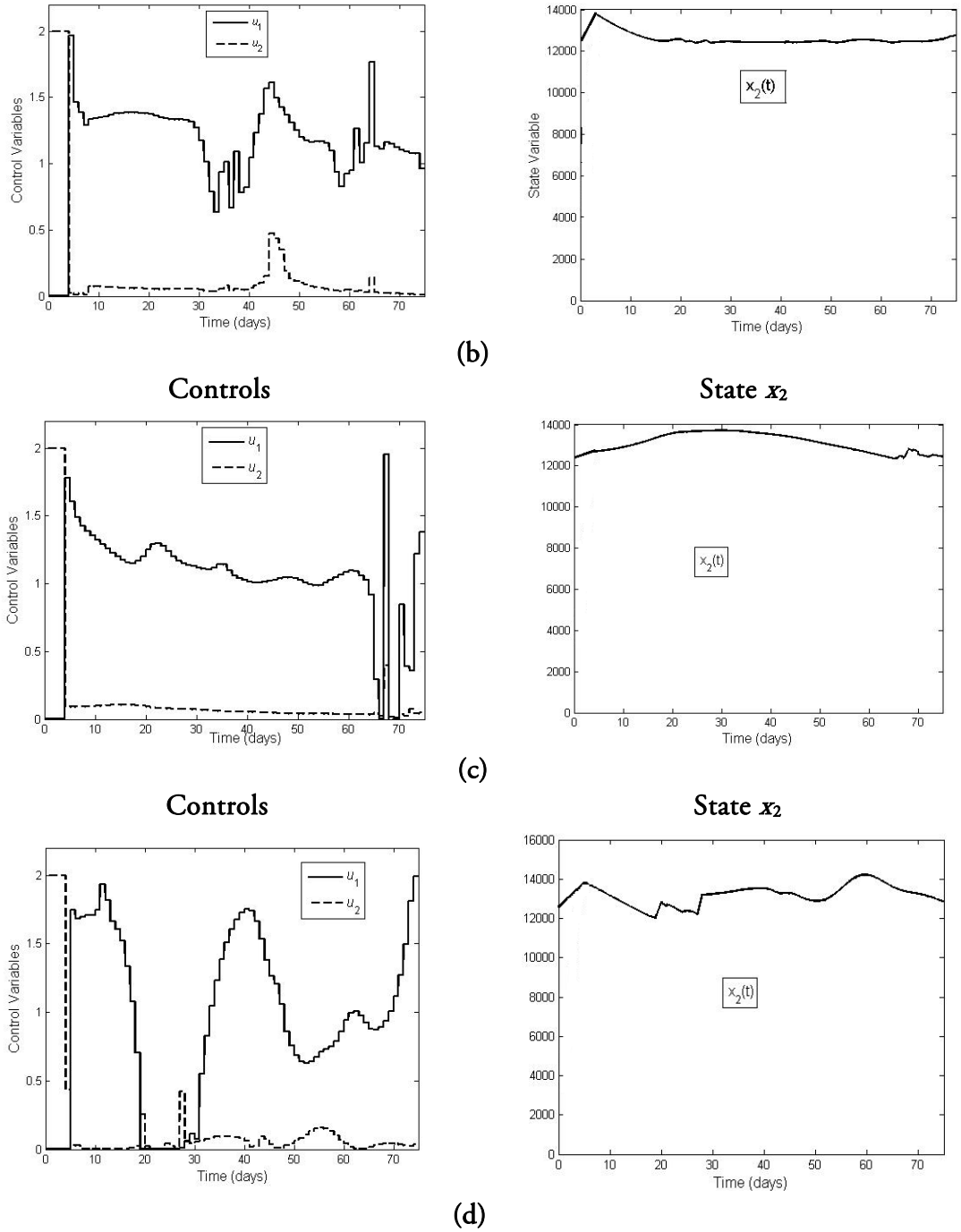


Figure IV.5. (a) Case 1: $x_1(0) = 12000$ and $x_2(0) = N^* = 12500$; **(b)** Case 2: $x_1(0) = 13000$ and $x_2(0) = N^* = 12500$; **(c)** Case 3: $x_1(0) = 14000$ and $x_2(0) = N^* = 12500$; **(d)** Case 4: $x_1(0) = 15000$ and $x_2(0) = N^* = 12500$.

In these cases, the values of the total cost are shown in **Table IV.1**.

Table IV.1. Total costs for different initial values.

Initial values (No. per ha)	Total cost
$x_1(0) = 12000$ and $x_2(0) = 12500$	Flies 28.15€ + supplementary food 662 €
$x_1(0) = 13000$ and $x_2(0) = 12500$	Flies 29.33€ + supplementary food 608 €
$x_1(0) = 14000$ and $x_2(0) = 12500$	Flies 30.48€ + supplementary food 584 €
$x_1(0) = 15000$ and $x_2(0) = 12500$	Flies 31.63€ + supplementary food 556 €

This optimal control model, on the one hand, makes it possible to design strategies consisting in release of flies and supply of food sustaining the desired pollination level. On the other hand, by the estimation of costs of the interventions, it also provides information on the economically most reasonable intervention strategy. In our Example, as it is seen from **Table IV.1**, the most economic pollination strategy corresponds to the initial release of 15000 juveniles (or eggs) and 12500 adults per ha.

IV.4. Discussion

In this paper our main objective is to show that with the aid of a pilot theoretical study, a deeper and more detailed insight can be gained into our concrete problem on pollination. However, in general, we suggest the introduction of a new approach, where in the first step, based on previous knowledge, a mathematical model is set up, the behaviour of which would be the basis for certain experimental design. Then in the light of the future experimental results we may refine the model, and design further experiments, and so forth. In this sense the present paper is a modelling-methodological development, to be followed by the next stage when the necessary parameters will be experimentally measured, and the model itself might be also refined.

IV.4.1. Discussion of operational issues

Our conceptual model provides the following predictions:

Figure IV.1 calls the attention to the fact that with increasing number of pollinators, the proportion of pollinated flowers saturates. For example, from 65% to reach 92%, the number of pollinators must be doubled. In practice, this means that the number of pollinators necessary for a high percentage pollination may exceed the maximum number of adult pollinators maintained by the crops. This nonlinear dependence will also remain in more sophisticated models because the pollinators visit the already pollinated flowers again and again, thus for a higher pollination, substantially more pollinators are needed. Therefore, food supply may facilitate pollination, since it helps to maintain a substantially higher adult pollinator population. Our optimal control model also makes it possible to calculate the necessary food supply, minimizing its cost once we have obtained the real model parameters in the second stage of our project. Further model calculations should be interpreted similarly.

Our model has also shown to what extend the decrease of searching and handling time (see Fig 2) and the increase of activity time (see Fig 3) would increase the efficiency of the pollinators. This knowledge may facilitate the choice of new pollinator agents.

Hoverflies, like *E. tenax* and species with similar size and behaviour, are already being applied for pollination in greenhouses. According to the protocol in practice, adult flies are released twice. Larvae feed on substrate placed on the ground, implying no hygienic risk. Therefore, we would suggest releasing also juvenile at the beginning, with mould added.

The results of our optimal control model show that increasing initial release of juvenile, the necessary supplementary food can be reduced, implying the decrease of costs. Concerning the control model, we also notice that for the application of the corresponding toolbox, we had to apply piecewise constant control functions, changing the added food on daily basis (see Fig 5.). If for technical convenience we want to intervene less frequently, we can apply a less fine model, applying food supply with longer time intervals of constancy for the control functions.

IV.4.2. Discussion of theoretical issues

In this paper we start out from a practical problem of beneficial insects in greenhouses, in particular insect pollinators. Observe, that for the presented conceptual model, a combination of different mathematical methods was necessary. We emphasize, we use Markov model to handle the general pollination process. After the mathematical study of our model, we were also surprised that when we used some data on hoverfly from the literature [44,45], the prediction obtained from the Markov model matched surprisingly well with the density suggested by the agent producing company. That is why we rely on Markov processes as a good approximation in this context. Moreover, the investigation of our practical problem can raise some interesting questions from the point of view of theoretical population ecology, as well. Namely, in our population dynamic model, we must take account the fact that the density does not only depend on the carrying capacity, but also on the life span of the insect.

The theoretical foundations of applied sciences using mathematical models have the following advantages:

First advantage: during the model building, the modeler must list the assumptions of the model in question. These assumptions can be considered as starting hypotheses for the corresponding experiments and are either validated or confuted by the experiments. In the latter case we need a new model, and so on. For instance, in our model, we formulated concrete biological hypotheses, that can be tested by trials also appropriate to estimate the model parameters, including those of the feeding functions (see, a, b and c) functions in Section 2.4. With these trials we may also point out whether certain changes in the model are necessary. In this way, from the original conceptual model we will obtain an executable model. The final result of our project should be the optimal control of the economically successful pollination by appropriate maintenance of the pollinator population.

Moreover, from the modelling point of view, the efficiency of adding food to enhance pollination of plants, as a matter of fact, depends on the optimal foraging of the pollinator. On the one hand, one of the well-known results of optimal foraging theory is the zero-one rule which claims that: If the more valuable food is abundant enough then the foragers accept only this type, while if the more valuable food is not abundant enough, the foragers accept both types of food [46]. Therefore, if the added abundant food is more attractive for the pollinator than the plant flowers, then the additional food can decrease the economically success of pollination. Another issue is that we have no information on the searching and handling times of the additional food. In order to build up a well-detailed density dependent optimal foraging model and dynamics, these data would also be a prerequisite since the possible optimal foraging strategy dependent functional response needs these parameters [47]. On the other hand, if the crop plants offer no sufficient food for the pollinator, then a small quantity of added food (e.g., some vitamin) can increase the pollinator density, so Liebig's law of the minimum may play an important role (see e.g., [48]). Obviously, the numerical response of pollinators will depend, at the same time, on the nectar and pollen produced by the plant, and on the additional food. Clearly, the numerical response can be measured by experiment.

Second advantage: When we have a model with the right assumptions, then the model parameters will be estimated by trials (e.g., searching and handling times, all feeding functions

in our case). We emphasize these experiments can be carried out in laboratory, which are less expensive than field experiments.

Third advantage: A theoretical model, with appropriate assumptions and estimated parameters, can also give some hints to the design of field experiments, as a result, the number of field experiments, and hence their costs can be reduced.

Furthermore, the present conceptual model raises not only experimental problems, but also theoretical ones. For example, we have shown how pollination of flowers by insects can be described by appropriate stochastic models. In the future, this kind of model can predict how many flowers are visited by the pollinator only once, twice and so on. The number of visits influences the successful pollination (*e.g.*, [49]). Furthermore, since the nectar of a flower can be depleted by the insect, the stochastic model can predict the nectar and pollen collected by the insect, so this kind of model provides the numerical response of the insect, as well.

Moreover, appropriate deterministic population models can predict the density dynamics of the pollinator. We note that, in order to maintain the desired pollination level, an option could have been a control model for tracking the constant desired value of adult hoverfly as reference trajectory. In our optimal control model instead, dealing with an inequality constraint for the adult hoverfly density, the minimization of the feeding costs also keeps the adult density near (and above) the required level.

Of course, more realistic population dynamics may also consider the optimal foraging behavior of the pollinator (when the searching and handling times are measured in a trial). Furthermore, the optimal control model used here can calculate the optimal regime of added food for more complicated population dynamics, as well.

According to the results found for the mathematical model developed even without the support of an experimental part, first we can calculate the hypothetical density of adult hover flies needed for a successful pollination of tomato crop and second, the deterministic control model allows establishing the strategy of supplementary food supply for an optimal achievement of pollination of the crop. Of course, for different parameter values, the result of the model calculation would be different. However, at the present stage we only wanted to show how the

optimal strategy can be calculated, and not the concrete illustrative numerical result is the achievement. We can consider, on the one hand, the need to carry out experimental trials that allow us to validate the two stages of the models and achieve a better adjustment to the real pollination data in tomato crops in greenhouses. On the other hand, these experimental trials should also be extended to the other 7 most important crops in Mediterranean greenhouses, in addition to tomato: pepper, eggplant, cucumber, zucchini, melon, watermelon and beans. Its relevance derives from the total area of these crops (201,000 ha), which represents 10.6% of the overall area of greenhouses worldwide [50]. It is considered that this can be extremely useful as the theoretical models developed can be flexible enough to be extended to these crops. This is because not all plant species are equally good for pollinators. Some of them supply both nectar and pollen abundantly when in bloom, and these are often called honey plants, because they are best suited for honey production. Plants producing nectar but little or no pollen are also considered to be honey plants. Other plants, however, may yield pollen but little or no nectar [51]. This can be extended to the previously described greenhouse crops [4].

IV.5. Appendices

IV.A.1. A stochastic model for pollination

In what follows we set up and analyze a stochastic Markov model of the pollination process.

In addition to the conditions of Section 2 we suppose that the searching periods are independent and exponentially distributed random variables with expectation τ_s. The same holds for handling periods with mean τ_h.

Consider the moments when one of the following events occurs: (a) a pollinator stops feeding and starts searching, (b) a pollinator founds an unpollinated flower, (c) a pollinator founds an unoccupied but pollinated flower, (d) a pollinator founds an occupied flower. Denote these moments by $0 < t_1 < t_2 < \cdots$, and let $t_0 = 0$. Let X_i be the number of pollinated flowers and Y_i the number of victualling pollinators just after time t_i (more precisely, between t_i and t_{i+1}), thus $X_0 = Y_0 = 0$. Then the conditional distribution of the time period $t_{i+1} - t_i$ given the whole history up to t_i is exponential with parameter (reciprocal of the mean) $\varrho_i =$

$\lambda(N - Y_i) + \mu Y_i$, where $\lambda = 1/\tau_s$ and $\mu = 1/\tau_h$. (This is a simple consequence of the constant hazard rate property of the exponential distribution.)

Consider now the process (X_i, Y_i). It is Markov with the following transition probabilities.

$$\boldsymbol{P(X_{i+1} = X_i, Y_{i+1} = Y_i - 1 \,|Y_1, \dots, Y_i) = \frac{\mu Y_i}{\varrho_i}} \qquad \text{case (a)},$$

$$\boldsymbol{P(X_{i+1} = X_i + 1, Y_{i+1} = Y_i + 1 \,|Y_1, \dots, Y_i) = \frac{\lambda(N - Y_i)}{\varrho_i}\left(1 - \frac{X_i}{M}\right)} \qquad \text{case (b)},$$

$$\boldsymbol{P(X_{i+1} = X_i, Y_{i+1} = Y_i + 1 \,|Y_1, \dots, Y_i) = \frac{\lambda(N - Y_i)}{\varrho_i} \cdot \frac{X_i - Y_i}{M}} \qquad \text{case (c)},$$

$$\boldsymbol{P(X_{i+1} = X_i, Y_{i+1} = Y_i|Y_1, \dots, Y_i) = \frac{\lambda(N - Y_i)}{\varrho_i} \cdot \frac{Y_i}{M}} \qquad \text{case (d)}.$$

If we only concentrate on the process (Y_i), it is also a homogeneous Markov process.

$$\boldsymbol{P(Y_{i+1} = Y_i - 1 \,|Y_1, \dots, Y_i) = \frac{\mu Y_i}{\varrho_i}},$$

$$\boldsymbol{P(Y_{i+1} = Y_i + 1 \,|Y_1, \dots, Y_i) = \frac{\lambda(N - Y_i)(M - Y_i)}{\varrho_i M}},$$

$$\boldsymbol{P(Y_{i+1} = Y_i|Y_1, \dots, Y_i) = \frac{\lambda(N - Y_i)Y_i}{\varrho_i M}}.$$

Thus the state space is $\{0,1, \dots, N\}$, and the transition probabilities are

$$p_{k,k-1} = \frac{k\gamma}{N - k + k\gamma}, \qquad p_{k,k} = \frac{(N - k)k}{(N - k + k\gamma)M}, \qquad p_{k,k+1} = \frac{(N - k)(M - k)}{(N - k + k\gamma)M},$$

and $p_{k,j} = 0$ if $|k - j| > 1$, where $\gamma = \mu/\lambda = \tau_s/\tau_h$. This process is an aperiodic, irreducible Markov chain, so there exists a unique stationary distribution $(u_k, 0 \leq k \leq N)$, which satisfies the following equations:

$$u_0 = \frac{\gamma}{N - 1 + \gamma} u_1,$$

$$u_k = \frac{(N - k + 1)(M - k + 1)}{(N - k + 1 + (k - 1)\gamma)M} u_{k-1} + \frac{(N - k)k}{(N - k + k\gamma)M} u_k + \frac{(k + 1)\gamma}{N - k - 1 + (k + 1)\gamma} u_{k+1},$$

$$u_N = \frac{M - N + 1}{(1 + (N - 1)\gamma)M} u_{N-1}\,.$$

$X_i$can increase only if $\mathrm{Y_i}$ does not decrease, and then $\mathrm{X_i}$increases by 1 with conditional probability $1 - \frac{\mathrm{X_i}}{\mathrm{M}}$, and does not change with conditional probability $\frac{\mathrm{X_i}}{\mathrm{M}}$. Let $(\mathrm{Z_i})$ denote the rarefied version of the process $(\mathrm{X_i})$, i.e., $\mathrm{Z_i}$ is the valueof X at the $\mathrm{i^{th}}$of such moments when Y does not decrease. Then

$$\left(\frac{M}{M - 1}\right)^i (M - Z_i)$$

is a martingale. Therefore,

$$EZ_i = M\left[1 - \left(\frac{M}{M - 1}\right)^i\right].$$

Equating it with $(1 - \varepsilon)M$ we obtain that $\approx M \ln\frac{1}{\varepsilon}$.

In the long run the proportion of steps without motion in the sequence (Y_i) is asymptotically

$$\varkappa = \sum_{k=1}^{N-1} u_k p_{k,k}\,.$$

Since in the long run the proportion of positive steps must coincide that of the negative steps, otherwise the sequence could not stay bounded, we get that the asymptotic proportion of

nonnegative steps is $\frac{1+\varkappa}{2}$. Thus, in the original Markov chain $n \approx \frac{2M}{1+\varkappa}\ln\frac{1}{\varepsilon}$ steps are needed to achieve $X_n = (1-\varepsilon)M$. The ratio $\frac{1+\varkappa}{2}$ can be expressed in the following way.

$$\frac{(\gamma-1)(1+\varkappa)}{2} = \sum_{k=1}^{N} u_k(1-p_{k,k-1})(\gamma-1) = \sum_{k=0}^{N-1} u_k \frac{(N-k)(\gamma-1)}{N-k+k\gamma}$$
$$= \sum_{k=0}^{N-1} u_k\left(\frac{N\gamma}{N-k+k\gamma}-1\right) = N\gamma\sum_{k=0}^{N}\frac{u_k}{N-k+k\gamma} - 1.$$

From this we have

$$\sum_{k=0}^{N}\frac{u_k}{N-k+k\gamma} = \frac{\gamma(1+\varkappa)+(1-\varkappa)}{2N\gamma}.$$

Therefore, by using the approximation $n \approx \frac{2M}{1+\varkappa}\ln\frac{1}{\varepsilon}$ we obtain

$$t_n \approx \sum_{i=0}^{n-1}\frac{1}{\varrho_i} = \sum_{i=0}^{n-1}\frac{1}{\lambda(N-Y_i)+\mu Y_i} \approx \frac{n}{\lambda}\sum_{k=0}^{N}\frac{u_k}{N-k+k\gamma} = \frac{M}{\lambda N}\left(1+\frac{1}{\gamma}\cdot\frac{1-\varkappa}{1+\varkappa}\right)\ln\frac{1}{\varepsilon}.$$

That is, the time needed until all but εM flowers get pollinated is approximately

$$\frac{M}{N}\left(\tau_s + \frac{1-\varkappa}{1+\varkappa}\tau_h\right)\ln\frac{1}{\varepsilon}.$$

If we want to achieve this goal by time T, we shall need

$$N \approx \frac{M}{T}\left(\tau_s + \frac{1-\varkappa}{1+\varkappa}\tau_h\right)\ln\frac{1}{\varepsilon}$$

pollinators.

Let us estimate $\varkappa$. Maximizing the probability $p_{k,k}$ we get

$$\varkappa \le \max p_{k,k} \le \frac{1}{(1+\sqrt{\gamma})^2}\frac{N}{M} \le \frac{\tau_s+\tau_h}{T}\ln\frac{1}{\varepsilon}.$$

If this is sufficiently small, we can take $\varkappa \approx 0$, and so we arrive at the naïve estimator

$$N \approx \frac{\tau_s + \tau_h}{T} M \ln \frac{1}{\varepsilon}$$

IV.A.2. Local stability of the deterministic dynamic model (1)-(2)

An equilibrium of dynamics (1)-(2) is easily obtained

$$x_1^* = \frac{\alpha}{\beta} K \frac{(1-d_1)\alpha - d_2}{(1-d_2)} \qquad x_2^* = K \frac{(1-d_1)\alpha - d_2}{(1-d_2)}$$

and it is positive, if

$$(1-d_1)\alpha > d_2.$$

This is fulfilled if the fertility rate α is high enough.

The Jacobian matrix at x^* is:

$$J^* = \begin{pmatrix} -\beta & \alpha \\ \beta(1-d_1) & -\left(d_2 + 2(1-d_2)\frac{x_2^*}{K}\right) \end{pmatrix}.$$

An easy calculation shows that, under the same condition of the existence of a positive equilibrium, we have

$$\mathrm{tr}J^* = -\left(d_2 + 2(1-d_2)\frac{x_2^*}{K}\right) - \beta < 0,$$

and

$$\det J^* = \beta\left(d_2 + 2(1-d_2)\frac{x_2^*}{K}\right) - \alpha\beta(1-d_1) > 0,$$

implying the required asymptotic stability (in particular, convergence to the equilibrium) For the Routh- Hurwitz stability criterion applied here, see e.g. [52].

Observe that trJ^* is always negative since our parameters are positive. Furthermore, the condition that guarantees the existence of a positive equilibrium of the dynamics (1)-(2), i.e., $(1-d_1)\alpha > d_2$, implies the positivity of detJ^*.

IV.A.3. Optimal control by additional feeding

The control dynamics is given by the system of differential equations (4)-(5) from section 2.3:

$$\frac{dx_1}{dt} = (\alpha + \frac{c_0 u_2}{1 + c_1 u_1 + c_2 u_2})x_2 - \beta x_1 \tag{4}$$

$$\frac{dx_2}{dt} = \beta(1-d_1)x_1 - x_2\left[1 - \left(1 - \frac{x_2}{K + a_1 u_1}\right)\left(1 + \frac{b_0 u_1}{1 + b_1 u_1} - d_2\right)\right] \tag{5}$$

Here function

$$u(t) = (u_1(t), u_2(t)) \quad (t \in [0,T])$$

describes the time-dependent feeding as the control function. In our control model for i =1,2, there is an upper limit M_i for the feeding rate u_i (t). So, the admissible controls on a time interval $[0,T]$ are defined as follows: Controls $u_i(t)$ are piece-wise constant functions corresponding to a fixed uniform division of interval $[0,\mathrm{T}]$, with $0 \le u_i(t) \le M_i (t \in [0,T])$. The set of all admissible controls $u = (u_1, u_2)$ will be denoted by $U[0,T]$.

Suppose now that with appropriate constants (prices)$p_1, p_2 > 0$, for any $u \in U[0,T]$, the total cost of the additional feeding, over the time interval $[0,T]$, is

$$\int_0^T (p_1 u_1(t) + p_2 u_2(t))dt.$$

In what follows, dynamics (4)-(5) will be shortly denoted by

$$\frac{dx}{dt} = F(x, u(t)).$$

The initial value for the state process is $x(0) = (x_1(0), N^*)$, where N^* is the required adult density. To keep the latter above N^* during time T we prescribe constraint $x_2(t) \geq N^*$ $(t \in [0, T])$. If in the control process we minimize the total cost of additional feeding, this objective function will also help us to keep $x_2(t)$ close to N^*.

Now the optimal control problem to be solved is the following

$$\int_0^T (p_1 u_1(t) + p_2 u_2(t))dt \rightarrow min,$$

under conditions

$$u \in U[0, T]$$

$$\frac{dx}{dt} = F(x, u(t))(t \in 0, T]),$$

$$x(0) = (x_1(0), N^*),$$

$$x_2(t) \geq \bar{x}_2 \ (t \in [T_1, T_0]).$$

IV.6. References

1. Sáez, A.; di Virgilio, A.; Tiribelli, F.; Geslin, B. Simulation models to predict pollination success in apple orchards: a useful tool to test management practices. Apidologie 2018, 49, 551–561. DOI:10.1007/s13592-018-0582-2

2. McGregor, S.E. Insect pollination of cultivated crop plants. Agricultura Research Service-United States Department of Agriculture: Washington DC, USA, 1976; 411 pp.

3. Winter, K.; Adams, L.; Thorp, R.; Inouye, D.; Day, L.; Ascher, J.; Buchmann, S. Importation of non-native bumble bees for greenhouse crop pollination in Canada, Mexico, and the United States. North American Pollinator Protection Campaign (NAPPC): San Francisco, CA, USA, 2006; 33 pp.

4. Guerra-Sanz, J.M. Crop pollination in greenhouses. In: James R.R., Pitts-Singer T.L. (eds.). Bee pollination in agricultural ecosystems. Oxford University Press: Oxford, UK, 2008; 27-46.

5. Neiswander, R.B. Honeybees as pollinators of greenhouse tomatoes. Glean. Bee Cult.1954, 82, (10), 610-613.

6. Neiswander, R.B. Pollination of greenhouse tomatoes by honeybees. J. Econ. Entomol. 1956, 49, (4), 436-437.

7. Fink, B. Spermatophyta of the flora of Fayette. Proc. Iowa Acad. Sci, Iowa, United States, 1898, 4, 81-107.

8. Dunn, L.; Lequerica, M.; Reid, C.R.; Latty, T. Dual ecosystem service of syrphid flies (Dip.: Syrphidae): pollinators and biological control agents. Pest Manag. Sci. 2019, 76, 1973-1979.

9. Westerkamp, C.; Gottsberger, G. Diversity pays in crop pollination. Crop Science 2000, 40, 1209–1222.

10. Teppner, H. Pollinators of tomato, *Solanum lycopersicum* (Solanaceae), in Central Europe. Phyton, 2005, 45, 217-235.

11. Steen, J. van der. Effect of a home-made pollen substitute on honeybee colony development. J. Apic. Research, 2015, 46, 114-119.

12. Requier, F.; Loanhardt, S.D. Beyond flowers: including non-floral resources in bee conservation schemes. J. Insect Conserv., 2020, 24, 5-16.

13. Rotheray, G.E.; Gilbert, F.S. The natural history of Hoverflies. Forrest Text, Ceredigion: Wales, UK, 2011; 333 pp.

14. Haslett, J.R. Adult feeding by holometabolous insects: pollen and nectar as complementary nutrient sources for *Rhyngia campestris* (Dip.: Syrphidae). Oecologia, 1989, 81, 361-363. DOI:10.1007/BF00377084

15. Lundgren, J.G. Relationships of natural enemies and non-prey foods. 2009, Springer: 453 pp.

16. Pinheiro, L.A.; Torres, L.M.; Raimundo, J.; Santos, S.A.P. Effects of pollen, sugars and honeydew on lifespan and nutrient levels of *Ephisyrphus balteatus*. BioControl, 2015, 60, 47-57. DOI:10.1007/s10526-014-9621-8

17. Dolley, W.L.; Hasset, C.C.; Bowen, W.B.; Philies, G. Culture of the drone fly, *Eristalis tenax*. Science, 1933, 78, 313-314. DOI: 10.1126/science.78.2023.313

18. Hurtado-Asencio, P. Estudio del ciclo de vida de sírfidos cristalinos (Diptera: Syrphidae) y bases para su cría artificial. Doctoral dissertation. Universidad de Alicante, Spain, 2013, 290 pp. [http://hdl.handle.net/10045/45586]

19. Jarlan, A.; Oliveira, D. de; Gingras, J. Pollination by *Eristalis tenax* (Dip.: Syrphidae) and seed set of greenhouse sweet pepper. J. Econ. Entomol. 1997 a, 90, 1646-1649. DOI:10.1093/jee/90.6.1646

20. Jarlan, A.; Oliveira, D. de; Gingras, J. Effects of *Eristalis tenax* (Dip.: Syrphidae) pollination on characteristics of greenhouse sweet pepper fruit. J. Econ. Entomol. 1997 b, 90, 1650-1654. DOI:10.1093/jee/90.6.1650

21. Ssymank, A.; Kearns, C.A.; Pape, T.; Thompson, F.C. Pollinating flies (Diptera): A major contribution to plant diversity and agricultural production. Biodiversity, 2012, 9: 86-89.DOI:10.1080/14888386.2008.9712892

22. Fagan, W.F.; Lewis, M.A.; Neubert, M.G.; Van Den Driessche, P. Invasion theory and biological control. Ecol. Lett. 2002, 5, 148-157. DOI:10.1046/j.1461-0248.2002.0_285.x

23. Hawkins, B.A.; Cornell, H.V. Theoretical approaches to biological control. Cambridge University Press, 2008.

24. Murdoch, W.W.; Briggs C.J. Theory for biological control: Recent developments. Ecology 1996, 77, 2001-2013. DOI:10.2307/2265696

25. Straw, R.M. A Markov model for pollinator constancy and competition. Am. Nat.1972, 106, 597-620. DOI:10.1086/282800

26. Leebens-Mack, J.; Milligan, B.G. Pollination biology in hybridizing *Baptisia* (Fabaceae) populations. Am. J. Botany 1998, 85, 500–507. DOI:10.2307/2446433

27. Garay, J.; Sebestyén, Z.; Varga, Z.; Gámez, M.; Torres, A.; Belda, J.E.; Cabello, T. A new multistage dynamic model for biological control exemplified by the host-parasitoid system *Spodoptera exigua-Chelonus oculator*. J. Pest Sci. 2014, 88, 343–358. DOI:10.1007/s10340-014-0609-z

28. Hancock, P.A.; Sinkins, S.P.; Charles H.; Godfray, J. Population dynamic models of the spread of *Wolbachia*. Am. Nat. 2011, 177, 323-333. DOI:10.1086/658121

29. Souto-Maior, C.; Lopes, J.S.; Gjini, E.; Struchiner, C.J.; Teixeira, L.; Gomes M.G.M. Heterogeneity in symbiotic effects facilitates *Wolbachia* establishment in insect populations. Theor. Ecol. 2015, 8, 53–65. DOI:10.1007/s12080-014-0235-7

30. Rafikov, M.; Balthazar, J.M.; Von Bremen, H.F. Mathematical modeling and control of population systems: applications in biological pest control. Appl. Math. Comput. 2008: 200, 557-573. DOI:10.1016/j.amc.2007.11.036

31. Molnár, S.; López, I.; Gámez, M.; Garay, J. A two-agent model applied to the biological control of the sugarcane borer (*Diatraea saccharalis*) by the egg parasitoid *Trichogramma galloi* and the larvae parasitoid *Cotesia flavipes*. Biosystems 2016, 141, 45-54. DOI:10.1016/j.biosystems.2016.02.002

32. Llandres, A.L.; De Mas, E.; Rodriguez-Girones, M.A. Response of pollinator to the tradeoff between resource acquisition and predator avoidance. Oikos 2011, 121, 687-696. DOI: 10.1111/j.1600-0706.2011.19910.x

33. Campos-Jiménez, J.; Martinez, A.J; Golubov, J.; Garcia-Franco, J.; Ruiz-Montiel, C. Foraging behavior of Apis mellifera (Hym.: Apidae) and *Lycaste rhynchanitens* (Dip.: Syrphidae) on *Pontederia sagittate* (Commlinales: Pontederiaceae) on a disturbed site. Florida Entomol. 2014, 97, 217-223. DOI: 10.1653/024.097.0128

34. Inouye, D.W.; Larson, B.M.H.; Ssymank, A.; Kevan, P.G. Flies and flowers III: Ecology of foraging and pollination. J. Pollinat. Ecol. 2015, 16, 115-133. DOI: 10.26786/1920-7603%282015%2915

35. Emtia, C.; Ohno, K. Foraging behaviour of an aphidophagous horverfly, *Sphaerophoria macrogaster* on insectary plants. Pak. J. Biol. Sci. 2018, 21, 323-330. DOI: 10.3923/pjbs.2018.323.330

36. Singh, P.; Thakur, M.; Sharma, H.K.; Sharma, K.C. Study of foraging behaviour of syrphids viz. *Episyrphus* (Dip.: Syrphidae) on mustard bloom (*Brassicae juncea*, Cruciferae) under mid hill conditions. J. Entomol. Zool. Stud. 2018, 6, 1167-1171.

37. Kohlmann, S.G.; Matis, J.H.; Risenhoover, K.L. Estimating handling times for herbivore diets: a statistical method using the gamma distribution. J. Anim. Ecol. 1999, 68, 638–645. DOI: 10.1046/j.1365-2656.1999.00303.x

38. Lundgren, J.G. Relationships of natural enemies and non-prey foods. Springer: 2009, 453 pp.

39. Evans, E.W.; Swallow, J.G. Numerical responses of natural enemies to artificial honeydew in Utah alfalfa. Environ. Entomol. 1993, 22, 1392-1401. DOI:10.1093/ee/22.6.1392

40. Benadi, G. Requirements for plant coexistence through pollination niche partitioning. Proc. Biol. Sci. 2015, 282 (1810): 20130117. DOI: 10.1098/rspb.2015.0117

41. Garay, J.; Cressman, R.; Varga, Z. Optimal behaviour of honeybees based on imitation at fixed densities. Com. Ecol. 2003, 4, 219–224. DOI:10.1556/ComEc.4.2003.2.8

42. Banga, J.R.; Balsa-Canto, E.; Moles, C.G.; Alonso, A.A. Dynamic optimization of bioprocesses: efficient and robust numerical strategies. J. Biotechnol. 2005, 117, 407–419. DOI:10.1016/j.jbiotec.2005.02.013

43. Hirmajer, T.; Balsa-Canto, E.; Banga, J. DOTcvpSB, a software toolbox for dynamic optimization in systems biology. BMC Bioinform. 2009, 10, 199.

44. Emtia, C.; Ohno, K. Diurnal foraging behaviour of an aphidophagous hoverfly in an insectary plant patch. J. Biopestic. 2017, 10, 154-162.

45. Golding, Y.C.; Ennos, A.R.; Edmunds, M. Similarity in flight behaviour between the honeybee *Apis mellifera* (Hymenoptera: Aphidae) and its presumed mimic, the dronefly *Eristalis tenax* (Diptera: Syrphidae). J. Exp. Biol. 2001, 204, 139-145. PMID: 11104717

46. Charnov, E.L. Optimal foraging: attack strategy of a mantid. Am. Nat. 1976, 110, 141–151. DOI:10.1086/283054

47. Garay, J. Technical review on derivation methods for behavior dependent functional responses. Com. Ecol. 2019, 20, 28–44. DOI:10.1556/168.2019.20.1.4

48. Garay, J.; Varga, Z.; Cabello, T.; Gamez, M. Optimal nutrient foraging strategy of an omnivore: Liebig's law determining numerical response. J. Theor. Biol. 2012, 310, 31-42. DOI:10.1016/j.jtbi.2012.06.021

49. Wang, H.; Cao, G.X.; Wang, L.L.; Yang, Y.P.; Zhang, Z.Q.; Duan, Y.W. Evaluationof pollinator effectiveness based on pollen deposition and seed production in a gynodioecious alpine plant, *Cyananthus delavayi*. Ecol. Evol. 2017, 7, 8156-8160. DOI: 10.1002/ece3.3391

50. Castilla N. Greenhouse Technology and Management. 2nd edition. CABI International. Wallingford, UK, 2012; 360 pp.

51. Akratankul P. Beekeeping in Asia. FAO Agriculture Organisation of the United Nations. Rome, Italia, 1990; 73 pp.

52. Porter, B. Stability criteria for linear dynamical systems. Academic Press, New York, USA, 1968; 195 pp.

Conclusions

Capítulo V

Conclusiones

Capítulo II: ¿Determinan el desarrollo y la dieta el grado de canibalismo en los insectos? Comer o no comer a los congéneres

1.- La dieta, ya sea estrictamente carnívora u omnívora, parece tener un marcado efecto en el canibalismo de las dos especies estudiadas. Esto podría extenderse a otras especies de insectos.

2.- El desarrollo ontogenético en insectos con estructura de estadios influye doblemente en el canibalismo y en la depredación intragremial (IGP) al afectar tanto a la presa individual como al depredador.

3.- La relación entre el tamaño del depredador y de la presa (medido por la diferencia del producto de la longitud por la anchura del depredador menos el producto de la longitud por la anchura de la presa, expresada en mm^2) en relación con la tasa de canibalismo y de depredación intragremial (IGP) no es una relación lineal, como ya se ha señalado en la literatura sobre artrópodos.

4.- La importancia de la presencia de presa y refugio (la planta) en el canibalismo de *N. pseudoferus* ha sido subrayada en ambos ensayos de microcosmos. Sin embargo, en estas circunstancias, el nivel de canibalismo de esta especie zoófaga estricta sigue siendo muy elevado, especialmente por parte de las hembras adultas sobre los primeros estadios de ninfas (Ninfa-I a Ninfa-III), más que en los estadios posteriores (Ninfa-III a Ninfa-V). Los porcentajes de canibalismo oscilan entre el 16,7% y el 81,1%.

5.- Estos resultados pueden ayudar a desarrollar modelos matemáticos basados en la estructura de los estadios, al considerar de forma más realista especies con este tipo de ontogenia.

6.- Desde un punto de vista aplicado, los resultados de este estudio también ponen de manifiesto la importancia del canibalismo, y sus repercusiones, en los actuales sistemas de control biológico de especies plaga.

Capítulo III: Canibalismo: ¿Los riesgos de lucha y represalia reducen la tasa de depredación?

7.- Las hembras adultas de *Nabis pseudoferus* muestran una clara aceptación de los congéneres inmaduros como presa, con valores de mortalidad relativamente altos (51,89 ± 2,69 %). Esto corrobora los resultados encontrados en el capítulo anterior.

8.-Estos valores son inferiores a los que se dan en una presa heteroespecífica, la especie plaga *Spodoptera exigua*, en las mismas condiciones (80,00 ± 2,82 %).

9.- Sin embargo, el principal resultado fue que la tasa de depredación de las presas heteroespecíficas de la especie plaga se redujo al 59,09 ± 7,08 % en presencia de presas coespecíficas.

10.-Sostenemos que estos resultados probablemente indican aversión al riesgo y miedo a las represalias entre congéneres.

11.-El comportamiento de captura de presas de las hembras adultas difiere cuando cazan presas conespecíficas frente a las heteroespecíficas. Así:

A) El tiempo medio de manipulación (tiempo dedicado a capturar, matar y comer una presa, así como el tiempo dedicado a la limpieza del cuerpo y apéndices del depredador, y el tiempo de descanso) del depredador, fue de 23,3 ± 3,3 minutos en el primer caso (coespecíficos) significativamente mayor que los 16,6 ± 2,5 minutos en el segundo (heteroespecíficos).

B) A su vez, el tiempo medio de búsqueda (tiempo de búsqueda de una nueva presa dentro del parche) fue también significativamente mayor para las presas coespecíficas (8,4 ± 2,5 minutos) que para las heteroespecíficas (5,3 ± 1,9 minutos).

12.- La respuesta funcional de la hembra adulta de *Nabis pseudoferus* cambia de tipo II cuando la presa es la especie plaga a tipo I cuando la presa es coespecífica. Esto corrobora el diferente

comportamiento de las hembras cuando atacan a los congéneres en comparación con las especies plaga.

Cap. IV: Fundamentación teórica del control de la polinización por Sírfidos en un invernadero

13.- Desde un punto de vista teórico, se ha desarrollado un modelo estocástico para calcular la densidad de sírfidos necesaria para la polinización económicamente exitosa de un número determinado de plantas, con un coste total mínimo, durante un intervalo de tiempo fijo. En el caso de cultivo de tomate en invernadero.

14.- El modelo matemático desarrollado presenta una serie de ventajas:

A) El modelo es lo suficientemente flexible como para permitir su extensión a otros insectos polinizadores y cultivos en invernaderos.

B) El mismo, permite ahorrar costes de experimentación, ya que los parámetros biológicos que utiliza pueden determinarse mediante ensayos de laboratorio y/o semicampo, con un coste muy inferior al de los ensayos en invernaderos comerciales.

C) Con la introducción de la optimización en el modelo, podría utilizarse para calcular los insectos polinizadores a emplear, en función del desarrollo de la floración y de la producción, junto con los costes de los insectos liberados.

Chapter V

Conclusions

Chapter II: Do development and diet determine the degree of cannibalism in insects? To eat or not to eat conspecifics

1.- The diet, whether strictly carnivorous or omnivorous, seems to have a marked effect on the cannibalism of the two species studied. This could be extended to other insect species.

2.- Ontogenetic development in insects with a stage structure doubly influences the cannibalism and the intra-guild predation (IGP) by affecting both the individual prey and the predator.

3.- The ratio of predator-prey size (the difference of the product of length x width of the predator minus the product of length x width of the prey, expressed in mm^2) in relation to the rate of cannibalism and intra-guild predation (IGP) is not a linear relationship, as has already been pointed out in the literature on arthropods.

4.- The importance of the presence of prey and refuge (the plant) in *N. pseudoferus* cannibalism has been underscored in both microcosm assays. However, in such circumstances, the cannibalism level of *N. pseudoferus* is still very high, especially by adult females on the first instars (I- to III-instars), more so than on the later stages (III to V-instars). With cannibalism percentages ranging from 16.7% to 81.1%.

5.- These findings can help to develop mathematical models based on stage structure, by more realistically considering species with this type of ontogeny.

6.- From an applied standpoint, these study results also highlight the importance of cannibalism, and its repercussions in current biological control systems for pest species.

Chapter III: Cannibalism: Do risks of fighting and reprisal reduce predatory rate?

7.- Adult females show a clear acceptance of immature conspecifics as prey, with relatively high mortality values (51.89 ± 2.69 %). This corroborates the results found in Chapter II.

8.-These values are lower than those occurring in a heterospecific prey, the pest species *Spodoptera exigua*, under the same conditions (80.00 ± 2.82 %).

9.- However, the main result was that the predation rate of heterospecific the pest species prey was reduced to 59.09 ± 7.08 % in the presence of conspecific prey.

12.- We argue that these results likely indicate risk aversion and fear of reprisal between conspecifics.

11.-The prey-capture behaviour of adult females differed when they hunted conspecific versus heterospecific prey. Thus:

A) The average handling time (time spent in quelling, killing and eating a prey, as well as time spent on cleaning the predator's body and appendages, and resting time) of the predator was 23.3 ± 3.3 minutes in the first case (conspecific), significantly higher than the 16.6 ± 2.5 minutes in the second (heterospecific) case.

B) In turn, the average searching time (time spent searching for a new prey within the patch) was also significantly longer for conspecific prey (8.4 ± 2.5 minutes) than for heterospecific prey (5.3 ± 1.9 minutes).

12.- The functional response of *Nabis pseudoferus* adult female changes from type II when the prey is the pest species to type I, when the prey is conspecific. This corroborates the different behaviour of females when attacking conspecifics compared to pest species.

Cap. IV: Theoretical foundation of the control of pollination by hoverflies in a greenhouse

13.- From a theoretical point of view, a stochastic model has been developed to calculate the pollinator density needed for the economically successful pollination of a given number of plants, at minimum total cost, during a fixed time interval.

14.- The mathematical model has a number of advantages:

A) The model is sufficiently flexible to allow its extension to other pollinating insects and crops for greenhouses.

B) It allows savings in experimental costs, as the biological parameters it uses can be determined by laboratory and/or semi-field trials, at a much lower cost than trials in commercial greenhouses.

C) With the introduction of optimisation in the model, it could be used to calculate the pollinating insects to be used, depending on the development of flowering and production, together with the costs of the released insects.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