TESIS DOCTORAL

Effects of abiotic conditions on the structure and dynamics of agricultural communities:

Avocado agro-ecosystems as study model to evaluate the implications of climate change for biological pest control

Inmaculada del Rocío Torres Campos

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Directora: Marta Montserrat Larrosa

AUTOR: Inmaculada del Rocío Torres Campos

http://orcid.org/0000-0002-8775-7537

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Portada: Fotos realizadas por I. Torres Campos. De izquierda a derecha: 1. Nidos construidos por *Oligonychus perseae* con individuos y huevos en su interior; 2. Hembra de *Neoseiulus californicus* en el interior de un nido de *O. perseae*; 3. Hembra de *Euseius stipulatus* depredando a una hembra de *O. perseae* que se encontraba en el exterior de un nido; 4. Hembra de *E. stipulatus* depredando a un juvenil de *N. californicus*; 5. Dos juveniles de *N. californicus* depredando a una hembra de *O. perseae* en el interior de un nido; 6. Juvenil de *N. californicus* depredando a huevos de *O. perseae* en el interior de un nido. Diseño de portada realizado por MayPrint. Francisco José Díaz Cobos

UNIVERSIDAD DE MÁLAGA

FACULTAD DE CIENCIAS

PROGRAMA DE DOCTORADO DE BIOLOGÍA CELULAR Y MOLECULAR

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Memoria de Tesis Doctoral presentada por la Licenciada en Biología

Inmaculada del Rocío Torres Campos para optar al grado de Doctor.

Directora: **Dra. Marta Montserrat Larrosa**

Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora"

(IHSM-UMA-CSIC)

Málaga, Enero 2017

Marta Montserrat Larrosa, Científica Titular del Consejo Superior de Investigaciones Científicas en el Instituto de Hortofruticultura Subtropical y Mediterránea “La Mayora” (IHSM-UMA-CSIC),

CERTIFICA:

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Y para que así conste, y tenga los efectos que corresponda extiendo el presente informe.

Málaga, Enero de 2017.

Fdo.: Marta Montserrat Larrosa

Facultad de Ciencias
Departamento de Microbiología

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Fdo.: Antonio de Vicente Moreno

Facultad de Ciencias
Departamento de Microbiología

Dr. Juan José Borrego García, Director del Departamento de Microbiología de la Facultad de Ciencias de la Universidad de Málaga,

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Que la Tesis Doctoral titulada **"Effects of abiotic conditions on the structure and dynamics of agricultural communities: Avocado agro-ecosystems as study model to evaluate the implications of climate change for biological pest control"** que presenta Dña. Inmaculada del Rocío Torres Campos para optar al grado de Doctor, ha sido realizada bajo la dirección de la Dra. Marta Montserrat Larrosa, científica titular del Consejo Superior de Investigaciones Científicas.

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Málaga, Enero de 2017.

Fdo.: Juan José Borrego García

Los trabajos realizados en la presente tesis doctoral han sido realizados en las instalaciones del Instituto de Hortofruticultura Subtropical y Mediterránea “La Mayora” y fueron financiados con una beca JAE-PREDOC del Consejo Superior de Investigaciones Científicas (CSIC), y por el proyecto del Ministerio de Ciencia e Innovación, AGL2011-30538-C03-03, titulado “Ingeniería de redes tróficas en aguacate y cítricos: mejora del control biológico y efectos de las condiciones ambientales en interacciones tri-tróficas de relevancia”.

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Congresos nacionales e internacionales

- I. Torres-Campos, R.M. Sahún, M. Montserrat. Abiotic conditions modify the trophic structure in the predator-prey avocado mite community. Póster. 4th International Entomophagous Insects Conference. Torre del Mar, Málaga (España), octubre 2015.

- I. Torres-Campos, R.M. Sahún, M. Montserrat. Abiotic conditions modify the trophic structure in the predator-prey avocado mite community. Póster. 5th Meeting of the Working Group "Integrate Control of Mite Pest". Castellón (España), septiembre 2015.

- I. Torres-Campos, R.M. Sahún, M. Montserrat. Las condiciones ambientales afectan a la depredación intragremial: implicaciones en el control biológico del cultivo del aguacate. Comunicación oral. Ponente: I. Torres-Campos. VIII Congreso Nacional de Entomología Aplicada y XIV Jornadas científicas de la SEEA. Mataró (España), octubre 2013.

- I. Torres-Campos, R.M. Sahún, M. Montserrat. Environmental conditions affect intraguild predation: implications for pest biological control in avocado orchards. Póster. 4th International Symposium on Biological Control of Arthropods. Pucón (Chile), marzo 2013.

- C. Guzmán, E. Aguilar-Fenollosa, I. Torres-Campos, R.M. Sahún, J.A. Jacas, M. Montserrat. Environmental conditions affect competitive and predatory interactions among natural enemies. Implications for pest biological control in a changing climate. Comunicación oral invitada. Ponente: M. Montserrat. 4th International Symposium on Biological Control of Arthropods. Pucón (Chile), marzo, 2013.

Otros méritos

- Premio a la mejor comunicación póster presentada por estudiantes de doctorado en el Congreso "4th International Entomophagous Insects Conference", con el título "Abiotic conditions modify the trophic structure in the predator-prey avocado mite community". Torre del Mar, Málaga (España), octubre 2015.

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SUMMARY

Climate change might severely disrupt the effective maintenance of agricultural communities under biocontrol management. Most of pests and natural enemies used to control them are arthropods and, as ectothermic organisms, many of their physiological functions, and the features of many of the biotic interactions individuals will establish with other individuals during their life span, are climate-dependent. The future success of biological pest control, thus, might depend on the capacity of predicting how each agricultural community will respond to a changing climate. The main goal of this thesis was to generate knowledge on the effects of changes in abiotic conditions on the way how biotic interactions occur among species that inhabit agricultural communities under biological pest control, and how these effects determine the trophic structure and dynamics of these communities. To achieve this goal, in the present thesis I studied a mite community present in avocado agro-ecosystems in South-eastern Spain. Because this community is composed by a low number of species, it is an optimal study model to understand the effects of abiotic conditions on interactions between pests and their natural enemies. The avocado mite community is composed of the herbivore pest *Oligonychus perseae* (Tuttle, Baker & Abatiello) (Acari: Tetranychidae) and two species of phytoseiid mites: *Neoseiulus californicus* (McGregor), which is specialized in tetranychid mites, and *Euseius stipulatus* (Athias-Henriot), which is preferentially pollen feeder.

The work presented in this thesis was addressed from two different approaches. On the one hand, the effects of abiotic conditions on the strength and direction of biotic interactions in the avocado mite community were evaluated at the individual level, and at three combinations of temperature and relative humidity: mild (M), hot and dry (HD) and very hot and dry (VHD). On the other hand, it was studied the impact of abiotic conditions on the community dynamics at M and HD conditions, i.e., how the observed effects on species interactions at the individual

level were rendered at the population level, determining the trophic structure and dynamics of the community.

The work started evaluating how abiotic conditions affected the strength of predator-prey interactions between either *E. stipulatus* or *N. californicus*, and their herbivore prey *O. perseae*, in the presence and the absence of alternative food (i.e. pollen). Results revealed that changes in abiotic conditions had different effects on predator-prey interactions depending on the species of predator. At M conditions both species of predatory mite preyed on *O. perseae* females, whereas at HD and VHD conditions only *N. californicus* fed on the prey. Furthermore, the strength of interaction between *N. californicus* and *O. perseae* varied among abiotic conditions, being the highest one at M conditions. The presence of alternative food (i.e. pollen) influenced in predator-prey interaction strength between *E. stipulatus* and *O. perseae*, but not between *N. californicus* and *O. perseae*. *Euseius stipulatus* preyed on *O. perseae* females in the absence of pollen at M conditions, but clearly preferred to forage on pollen when was available. Also, oviposition rates of *E. stipulatus* were increased in the presence of pollen at the three abiotic conditions. Therefore, the addition of pollen as alternative food for *E. stipulatus* might promote the growth of its populations and favour pest control through apparent competition between the pest and pollen.

Next, I studied the effects of abiotic conditions and presence of alternative food on intraguild interactions between the two predatory mites, *E. stipulatus* and *N. californicus*, with *O. perseae* as shared prey. Results showed that the trophic structure of the avocado mite community changed with changes in abiotic conditions, resulting in community configurations that would be detrimental to pest control. At M conditions, results revealed that the community resembled a trophic chain, with juveniles of *N. californicus* preyed on *O. perseae*, and females of *E. stipulatus* preyed on juveniles of *N. californicus*. On the other hand, juveniles of *E. stipulatus* fed on *O. perseae* and induced antipredator behaviour in females of *N. californicus*, which did not interact with the pest at all. Increasing unfavourable abiotic conditions shifted the structure of the community to one dominated by exploitative competition between the two species of predators. However, results also revealed that adding pollen to the system would likely enhance pest population control, because trophic interactions between predators ceased due to *E. stipulatus* preference for pollen, what strengthened the predator-prey interaction between *N. californicus* and *O. perseae*, at the three abiotic conditions.

Finally, mite predator/prey population dynamics were evaluated in the presence and the absence of alternative food at two different abiotic conditions, M and HD. Contrary to expectations, results revealed that the addition of pollen did not reduce negative trophic interactions occurring between predator species; instead, *E. stipulatus* excluded *N. californicus* at both abiotic conditions, independently of the presence or absence of pollen. Also, in spite that the addition of pollen to the system favoured a numerical response of *E. stipulatus* populations at M conditions, population increase did not translate into a better *O. perseae* population control. Furthermore, at HD conditions, the growth of *E. stipulatus* populations was negatively affected by high temperatures and drought.

Therefore, results from this thesis evidence that abiotic conditions modify the way in which species interact, affecting trophic structure and dynamics of communities. This implies that in a changing climate the successful use of biocontrol agents will need to take into account the influence that abiotic factors exert on interactions occurring among the components of each specific agricultural community.

1. INTRODUCTION

1. INTRODUCTION

1.1. Climate change

Climate change is defined as "a change in the state of the climate that can be identified (e.g., by using statistical tests) by changes in the mean and/or the variability of its properties, and that persists for an extended period, typically decades or longer" (IPCC 2014). Changes in climate may be attributed to natural causes such as modulations of the solar cycles and occurrence of volcanic eruptions in the Earth, or external forcings such as human activities that alter the composition of the atmosphere, or both (IPCC 2007). According to the Fifth Assessment Report (AR5) of the Intergovernmental Panel on Climate Change (IPCC), warming of the climate system is unequivocal, and causation due to human activity is undeniable (IPCC 2014).

Over the last century the temperature of the atmosphere and the oceans has increased, the amount of water reserve in the form of snow and ice has decreased, and the sea level has risen. The Earth surface temperature (averaging land and ocean surface' temperature) has increased 0.85ºC over the period 1880 to 2012 (Figure 1.1.1a). Anthropogenic greenhouse gas (GHG) emissions have contributed to increase the atmospheric concentrations of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_20) since the pre-industrial era (Figure 1.1.1b), and it is very likely (between 99 – 100 %) that they have been the dominant causes of the observed warming from 1951 to 2010 (IPCC 2014) (Figure 1.1.1a). Over this period the contribution of GHGs to the global mean surface temperature ranges between 0.5°C and 1.3°C (Figure 1.1.2; see green bar), and together with other anthropogenic (i.e. the cooling effect of aerosols) (Figure 1.1.2; see orange bar) and natural forcings, and natural internal variability, explains the observed warming of approximately 0.6°C to 0.7°C over this period (IPCC 2014) (Figure 1.1.2; see black bar).

The AR5 also revealed that there have been changes in many extreme weather and climate events since 1950, and that some of these changes are influenced by past and present human activities. The number of extreme cold days and nights has decreased, and the number of extreme warm days and nights has increased. The frequency of heat waves has increased in large parts of Europe, Asia and Australia, and there are more land regions where the number of heavy precipitation events has increased. In addition, extreme sea levels (i.e. as experienced in storm surges) have increased since 1970, being mainly a consequence of the rising mean sea level.

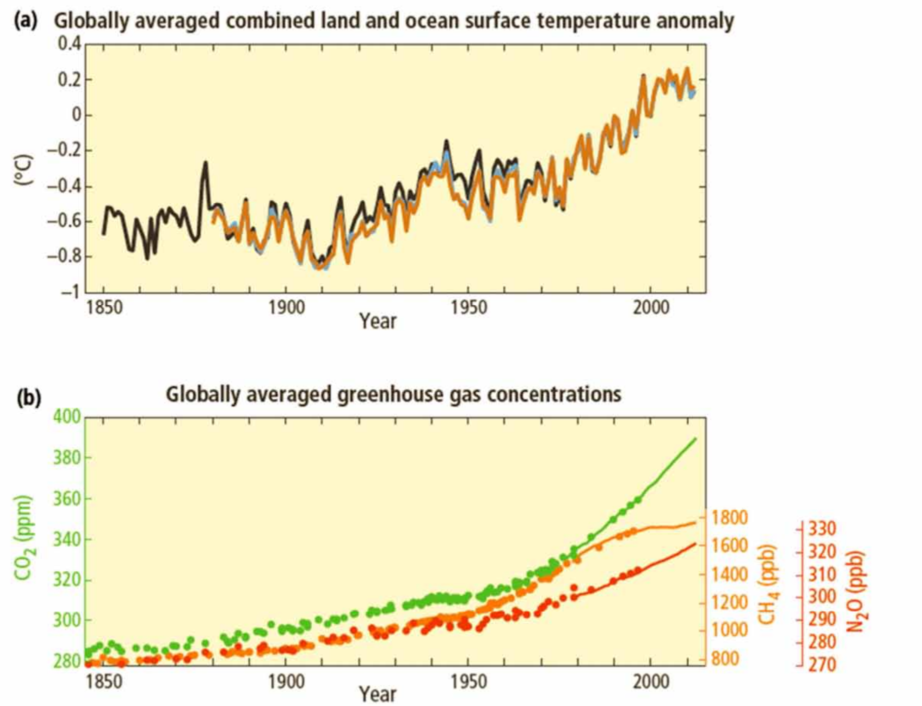

Figure 1.1.1. (a) Annual global average temperature (land and ocean surface) anomalies relative to the average temperature over the period 1986 to 2005. Colours indicate different data sets. (b) Atmospheric concentrations of the greenhouse gases carbon dioxide (CO_2, green), methane (CH_4, orange) and nitrous oxide (N_2O, red) determined from ice core data (dots) and from direct atmospheric measurements (lines). Figure reproduced from IPCC (2014).

Continued emission of greenhouse gases will further drive warming and long-lasting changes in all the components of the climate system (IPCC 2014).

Future climate will depend on the warming caused by past anthropogenic emissions, as well as on future anthropogenic emissions and natural climate variability. In accordance with the AR5, the global mean surface temperature is projected to increase over the 21st century under all the assessed emission scenarios, and it is likely that an increase in the frequency and intensity of heat waves and extreme precipitations events will occur in many regions. Additionally, warming and acidification of the ocean will continue, as will the global sea level rise.

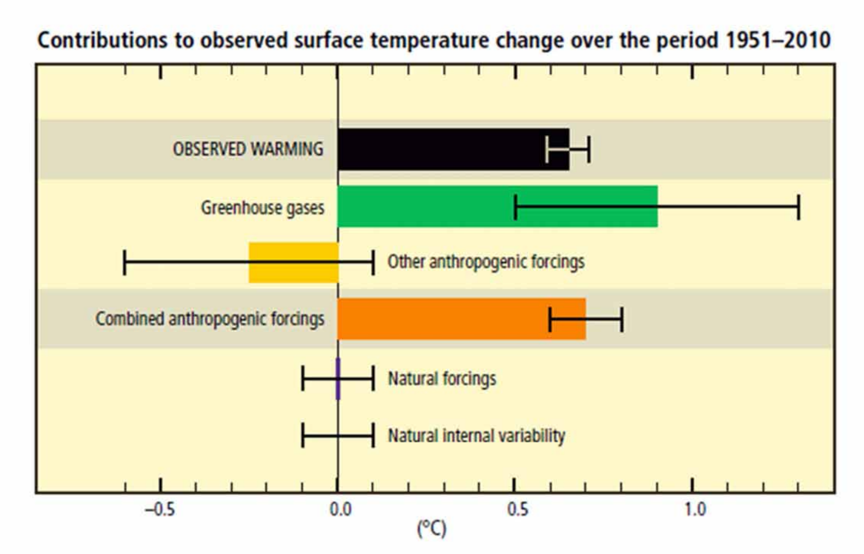

Figure 1.1.2. Ranges (whiskers) and mid-points (bars) for contributions to warming trends over the 1951–2010 period explained by well-mixed greenhouse gases, other anthropogenic forcings (including cooling effects of aerosols and effects of land use change), combined anthropogenic forcings, natural forcings, and natural internal climate variability (which is the element of climate variability that arises spontaneously within the climate system in the absence of forcings). The observed surface temperature change is shown in black, with the 5 to 95% uncertainty range due to observational uncertainty. The attributed warming ranges (colours) are based on observations combined with climate model simulations, in order to estimate the contribution of an individual external forcing to the observed warming. Note the the contribution of the combined anthropogenic forcings to warming is similar to the observed warming. Figure reproduced from IPCC (2014).

1.2. Impacts of climate change

1.2.1. Natural systems

Because of the speed of climate change in nature many species will not be able to endure rapid evolution to adapt to the new environment. At present, impacts in terrestrial and aquatic ecosystems attributed to climate change have already been documented (Walther et al. 2002; Parmesan 2006; Warren et al. 2011; Bellard et al. 2012). As examples, degradation of tropical coral reefs is widespread because these systems are highly vulnerable to the increase of oceanic temperature and acidification (Hoegh-Guldberg et al. 2007; IPCC 2014); warming is also causing phenological shifts in different taxonomic groups, what might have profound ecological consequences when the timing of change is not synchronous between groups that depend on each other. This is the case of flowering plants and their pollinators; if warming causes phenological mismatching between both groups, the consequences could lead to local/global extinctions (Memmott et al. 2007; Kiers et al. 2010). Responses of individuals to changes in abiotic factors (i.e. altering their phenology, individual fitness or geographic ranges) may modify the way they interact with other individuals from the same or different species, and alter the composition and structure of ecological communities (Dunson and Travis 1991; Gilman et al. 2010; Woodward et al. 2010). Indeed, Tylianakis et al. (2008) analysed data of 688 publications and showed that climate change is affecting the strength and/or direction of virtually every type of biotic interactions including competition, mutualism, parasitism, predation, herbivory. Recent works have focused on predicting how ecological communities will respond to the change in climate (e.g. Gilman et al. 2010; Lavergne et al. 2010), a question that is primordial in communities proving relevant ecological services, such as biological pest control.

1.2.2. Agricultural systems

Biological pest control

Biological control involves the use of natural enemy populations by man to reduce pest populations to lower densities (Van Driesche et al. 2007). Biological control incorporates the "top-down" concept (Hairston et al. 1960; Oksanen et al. 1981), which states that population increase in the third trophic level (i.e. natural enemies) will indirectly increase the productivity in the first trophic level (i.e. crops) by reducing herbivore populations (i.e. pest). This phenomenon is known as "trophic cascade". Therefore, in biological pest control herbivores are regulated rather than eradicated, as both pests and natural enemies remain in the agroecosystems at low densities (Bale et al. 2008).

In agricultural systems the vast majority of pests belong to the phylum Arthopoda, and the natural enemies that are used against them are predators and parasitoids, most of them being arthropods as well, and pathogens (De Bach 1964). The definition and classification of the different types of biological pest control differ depending on the literature consulted (e.g. Eilenberg et al. 2001; Van Driesche et al. 2007; Bale et al. 2008). In the present thesis, I will follow the types and definitions described in Bale et al. (2008). According to these authors there are three main techniques of biological control: (i) C*lassical*: the origin of pests is exotic and natural enemies that are used to regulate them are collected in the region of origin of the pest. Therefore, exotic natural enemies are introduced in a new environment and are allowed to stablish for long-term pest control; (ii) *Augmentative*: this strategy of control involves the release of biocontrol agents, which must be available commercially. Releases of natural enemies can be done by either 'seasonal inoculation' or 'inundation'. In seasonal inoculation, natural enemies are periodically released in crops where pests can have many generations in each growing season, with the expectation that natural enemies will reproduce and control the pest for a long period, but not permanently. In inundative releases, the introduction of biocontrol agents is massive, and the mode of action would be analogous to that of pesticides. Pest control is expected to be achieved with the individuals that have been released, not with their offspring or next generations; (iii) *Conservation*: this method implies the modification of the environment with the aim of protecting and increasing the abundance of indigenous natural enemies. Conservation practices include the manipulation of the microclimate, the creation of refuges against adverse conditions, the supply of alternative food (e.g. pollen) and hosts (Landis et al. 2000), etc. The aims of supplying alternative food are to promote the establishment of natural enemies when pests are at low densities in the crops, and, when pest densities are high, to promote pest control through apparent competition (Holt 1977) (mechanism described in section 1.3).

Consequences of climate change for agricultural communities under biological pest control

Among others, increases in atmospheric CO_2, shifts in precipitation, land-use changes and, particularly, increases in temperature, all potential causes/ consequences of climate change, can have various effects on the interactions among plants, pests and their natural enemies (Aguilar-Fenollosa and Jacas 2014).

Increases in CO_2

Changes in plant physiology as response to changes in concentration of CO_2 in the air (i.e. increasing the C:N ratio in foliage and decreasing foliar N (Lindroth et al. 1995)) can indirectly affect the efficiency of natural enemies. On the one hand such effects can be positive; elevated levels of CO_2 reduce nutritional quality of plants, and, consequently, herbivores grow less, slowlier, and their nutritional quality decreases. Smaller prey items that are easier to handle by predators and changes in prey quality may lead to an increase in prey consumption rates, enhancing biological pest control (Chen et al. 2005; Coll and Hughes 2008). Also, herbivores that develop slower are in their most vulnerable stages for a longer time (Sequeira and Mackauer 1994). On the other hand, effects can be negative; under higher atmospheric CO_2 levels, plant growth rates increase (Rogers et al. 1983), and the time spent by predators searching for prey also increases because of extra plant foliage (Thomson et al. 2010), which might negatively affect pest control.

Shifts in precipitation

The increase of periods of drought predicted in models of climate change can have a negative impact on predator's ability to control pests (Stireman et al. 2005). For instance, dry conditions were the cause of spider mite population outbreaks because water stress caused an unfavourable leaf microclimate (i.e. with an increase of leaf temperature and a decrease of relative humidity) that affected the efficiency of its main predator, the phytoseiid *Phytoseiulus persimilis* (English-Loeb 1990).

Land-use changes

The distribution of crops is predicted to change over time because growers will select species that optimize productivity under new climate conditions. Herbivores are expected to track these changes and the effectiveness of natural enemies for pest control will depend on their ability to concurrently expand their range (Thomson et al. 2010; Cock et al. 2013).

Increases in temperature

Most of pests and their natural enemies are arthropods, and, as ectothermic organisms, many key processes related with their fitness - such as development, reproduction, activity, interactions with other organisms (e.g. predation rate or competitive ability) -are climate-dependent (Beveridge et al. 2010; Gilman et al. 2010). Consequently, it is expected warming will have an important effect on agricultural communities under biological pest control. Because higher trophic levels are more vulnerable to temperature increases than lower trophic levels (Voigt et al. 2003; Schweiger et al. 2008), the loss of top consumers associated with warming will favour herbivory in systems regulated with top-down control, such as agricultural communities (e.g. Montserrat et al. 2013b). Added to this, temperature increase will likely widen the geographic distribution of cold-limited species in the future, favouring the movement of some pest species (Cannon 1998; Thomson et al. 2010).

1.3. Studying the effects of climate change on agricultural systems: *Community modules*

Biotic interactions, which structure ecological communities, are climate-dependent (Gilman et al. 2010). The capacity to evaluate the effect of climate change in agricultural communities depends on the identification of those interactions that are key to the well-functioning of the community, and are vulnerable to rapid changes in abiotic conditions (Bascompte and Stouffer 2009). A good tool to study interactions among species is to use the so-called *community modules* (Holt 1997), which simplify food webs into much more manageable subunits (2-6 interacting species) to help to understand mechanistic responses in entire complex communities (Gilman et al. 2010). There are five community modules commonly found in agricultural systems:

(1) *Trophic chain*

Species are rarely connected forming linear trophic chains, where each component defines a trophic level; instead, they usually interact with each other forming food webs where the limits of trophic levels become diffused (Gallopin 1972; Polis and Strong 1996). Nonetheless, the study of trophic chains can provide a starting point for the understanding of shifts happening in the community when more species and/or interactions are added. (Figure 1.3.1a)

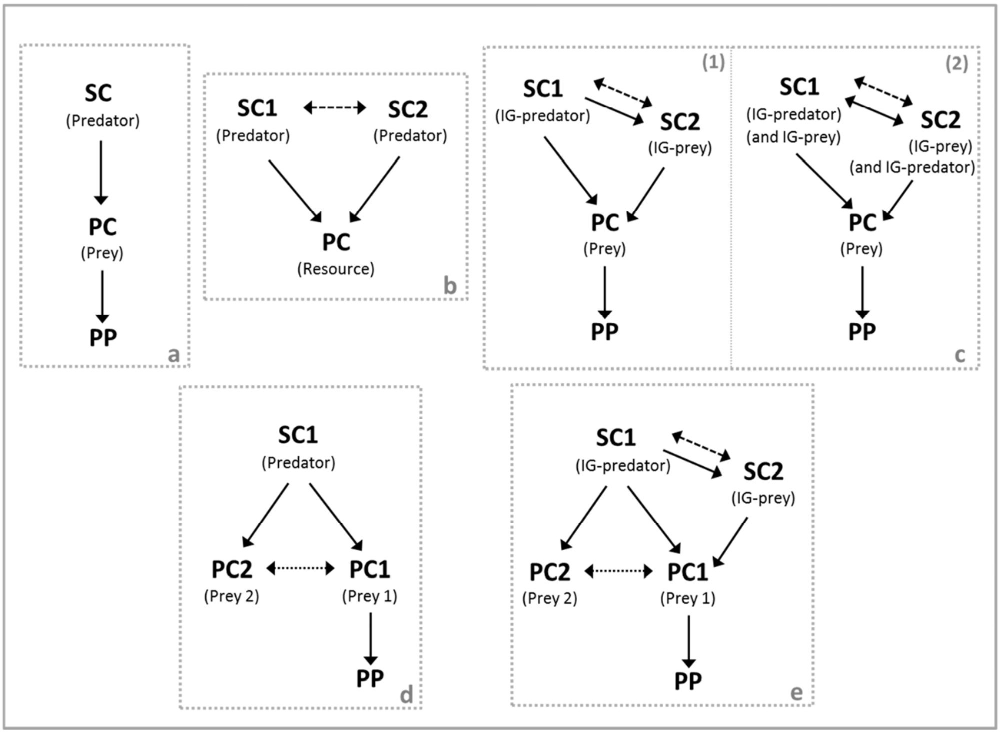

Figure 1.3.1. Community modules: a. Trophic chain; b. Resource competition; c. Intraguild predation: basic (1) and reciprocal (2); d. Apparent competition; and e. Intraguild predation-Apparent competition. Solid arrows: trophic interactions; Dashed arrows: predator-predator negative interactions (competition); Dotted arrows: indirect prey-alternative prey negative interactions (competition). PP: primary producer; PC: primary consumer; SC: secondary consumer.

(2) *Resource competition*

Interspecific competition (Figure 1.3.1b) is one of the most important interaction influencing the distribution and abundance of species in communities. The “competitive exclusion principle” or “Gause´s law” states that two species that are competing for the same resource cannot coexist in a limiting resource environment, when the other ecological factors remain constant. The competitor that exploits the resource more efficiently will exert a pressure to the other competitor, leading to either its extinction or to its evolutionary or behavioural adaptation toward a different ecological niche (Gause 1934). However, the dominance of one competitor over another can vary with abiotic conditions (Park 1954; Dunson and Travis 1991; Guzmán et al. 2016a). Guzmán et al. (2016a) showed that the outcome of competitive interactions between two sister species of predatory mites, *Euseius* *stipulatus* and *Euseius sculatis*, which are present in the avocado agro-ecosystem in south-eastern Spain, is strongly affected by abiotic conditions, leading to temperature-dependent species dominance. Thus, in agricultural communities under biocontrol management, the effect of changes in abiotic conditions on exploitative competition superiority may depend on which species is favoured; for example, the outcome could be detrimental to biological control if the most efficient natural enemy is excluded by a lesser efficient predator during episodes of extreme climate.

(3) *Intraguild predation*

Intraguild predation (IGP, hereafter) is the simplest form of omnivory and occurs when two competing predator species also engage in direct predator-prey interactions (Polis et al. 1989). Therefore, IGP is a community module that combines competition and predation, the two interactions most determinant of the structure of ecological communities (Chase et al. 2002).

IGP is now considered widespread in natural and managed ecosystems (Polis et al. 1989; Holt and Polis 1997; Arim and Marquet 2004; Gagnon et al. 2011), although initially it was thought to be not ubiquitous in nature due to theoretical studies that suggested that omnivory was an important destabilizer of food web dynamics (Pimm and Lawton 1978). IGP has received considerable attention in the theoretical literature (Polis and Holt 1992; Holt and Polis 1997; Mylius et al. 2001; Kuijper et al. 2003). Most of models consider three species: a top predator (IG-predator), an intermediate consumer (IG-prey), and a shared resource. Both predators (IG-predator and IG-prey) share a prey, and one of them (the IG-predator) also feeds on the other (the IG-prey) (Figure 1.3.1, c1). IGP can also be reciprocal when the IG-predator and the IG-prey are predators of one another (Figure 1.3.1, c2). An important component of these models is the effect of productivity of the environment on the coexistence of competing predators; at low productivity, only the IG-prey should persist since it is able to exploit the shared resource more efficiently than the IG-predator. Therefore, a necessary condition is that the IG-prey has to be the superior competitor; at high productivity, the IG-predator should drive the IG-prey to extinction through a combined effect of competition and predation. Hence, IGP theory predicts that the coexistence of the IG-predator and the IG-prey is only possible at intermediate productivity levels, and depends on the existence of differences in the efficiency of resource exploitation between competing predators (Polis and Holt 1992; Holt and Polis 1997; Diehl and Feißel 2000; Mylius et al. 2001; Borer et al. 2003; Borer et al. 2007). Therefore, the range of productivity that allows coexistence is narrow, a theoretical results that disagrees with the observation that IGP is common in natural communities (Arim and Marquet 2004). This mismatch between model predictions and observation has led to a large number of theoretical and empirical studies studying additional factors present in real systems that could explain long-term coexistence of IG-predator and IG-prey in nature. In this sense, Mylius et al. (2001) studied the influence of stage structure in the persistence of IGP. Models with either a stage of IG-prey being invulnerable to IG-predator (i.e. large adults), or a stage of IG-predator being incapable to prey on IG-prey (i.e. juveniles) only slightly increased the region of coexistence along the productivity gradient. Other works have included the effect of spatial heterogeneity (Finke and Denno 2002, 2006; Janssen et al. 2007; Okuyama 2008; Schmitt et al. 2009; Liu and Zhang 2013) and temporal refuges for the IG-prey (Amarasekare 2008), which may increase the possibilities for coexistence by weakening the strength of the interactions between IG-predator and IG-prey. Also, other mechanisms such as adaptive foraging behaviour by IG-predator (Křivan 2000; Křivan and Diehl 2005), changes in the IG-predator diet during ontogeny (Van de Wolfshaar et al. 2006), antipredator behaviour of IG-prey (Heithaus 2001), spatial segregation of IG-predator and IG-prey when they are engaged in reciprocal IGP (Montserrat et al. 2012), and cannibalism (Rudolf 2007), are thought to increase persistence of communities with IGP. However, in spite of extensive literature addressing this topic, it is still a challenge to explain the factors that fully resolve the discrepancy between theory and observation.

In agricultural systems IGP has been traditionally considered a possible threat to successful biological pest control (Rosenheim et al. 1995; Rosenheim 1998), although 10 years after the seminal paper of Rosenheim et al. (1995) the relevance of IGP as disruptive factor of biological pest control was alleviated (Janssen et al. 2006). The theoretical impact of IGP on agricultural communities may decrease through mechanisms weakening trophic interactions between the two predator species, such as spatial heterogeneity (Janssen et al. 2007) or antipredator behaviour (Magalhães et al. 2005b; Finke and Denno 2006). However, the effect of abiotic conditions modulating negative interactions between predators remains almost unexplored (Guzmán et al. 2016b)

(4) *Apparent competition*

Apparent competition is a type of indirect interaction defined as a reduction in the population density of one prey species when the population density of another prey species increases, mediated by a numerical increase of a shared natural enemy (Holt 1977) (Figure 1.3.1c). Apparent competition is considered a process that structures ecological communities in manners similar to those in communities driven by exploitation competition (Holt 1977). The time scale over which apparent competition operates is, however, different than that of resource competition. In the short-term, in which responses of communities occur within a single generation and, therefore, predator numbers are fixed, satiation of predators can lead to an increase in numbers of the two prey populations. Such positive indirect effect between prey species is known as “apparent mutualism” (Abrams 1987; Abrams and Matsuda 1996; Abrams et al. 1996). In the long-term, in which responses of communities operate over more than one generation of predators, the numerical response of predators due to the higher availability of prey leads to an increase of the total predation rate on both prey species, despite the per capita predation rate being the same. This is the effect known as “apparent competition”. Over time, the prey that is able to sustain the shared predator at higher densities will exclude the more vulnerable prey (Holt and Lawton 1994).

From the viewpoint of biological control, the presence of an alternative prey or food can decrease pest populations through induction of apparent competition between the pest and the alternative food source. Some studies have shown that the addition of other prey species (Karban et al. 1994; Liu et al. 2006; Messelink et al.

2008; Messelink et al. 2010; Bompard et al. 2013), or the supply of non-prey food such pollen (van Rijn et al. 2002; González-Fernández et al. 2009; Nomikou et al. 2010; Aguilar-Fenollosa et al. 2011; Maoz et al. 2011) as additional food sources for natural enemies enhances pest control.

(5) *Intraguild predation-Apparent competition*
Community modules with IGP are generally embedded in more complex food webs (Polis 1991) within which a large number of potential interactions outside the IGP module can affect its dynamic. Such interactions can include foraging of either IG-prey or IG-predators, or both of them, on alternative food sources outside the IGP module. Few authors have explored theoretically how alternative food sources affect the persistence and stability of communities with IGP (Heithaus 2001; Daugherty et al. 2007; Holt and Huxel 2007), and predictions show that it can increase the chance for coexistence of IG-prey and IG-predator.

In agricultural systems where IGP occurs, the presence of alternative food sources for IG-predators could reduce the strength of predator-prey interactions between predators, increase predator populations through apparent competition between the alternative food and the pest (Figure 1.3.1e), and improve, therefore, pest control (González-Fernández et al 2009).

1.4. The experimental agro-ecosystem

1.4.1. Primary producer: Avocado

Avocado (*Persea Americana* Mill., Lauraceae) (Figure 1.4.1.1) is a fruit tree species originating from Central America, which was introduced in Spain during the sixteenth century. The first plantations of avocado were stablished in 1955 along the Mediterranean area, in the Andalusia region, from Malaga to Granada (Díaz Robledo 1997), and since then it has become an economically important crop in south-eastern Spain. Indeed, Spain is presently the largest European avocado producer, with a production of 77.401 tonnes in 2014 (MAGRAMA 2016), and the leading exporting country in Europe, with 65.273 out of the 69.400 tonnes produced in 2013 being exported (FAOSTAT 2016). The Spanish area destined to this crop occupied 14.937 hectares in 2015, 13.392 hectares of which were located in the coastal areas of Malaga and Granada (MAGRAMA 2016)

The excellent phytosanitary status of the avocado cultivation in Andalusia might be one of the reasons by which it takes an important place in the European market. In fact, avocado trees in Spain had been exempted from important pests until the arrival of the persea mite in 2004. A possible reason could be that avocado leaves, seeds, roots (Armstrong 1964) and fruits (Platt-Aloia et al. 1983) contain specialized oil cells, so-called idioblast cells, which accumulate alkaloids and terpenes (Platt and Thomson 1992) with insecticidal, antifeedant and growth inhibitory activities (Rodriguez-Saona et al. 1997; Rodriguez-Saona et al. 1998; Rodriguez-Saona and Trumble 2000), and that probably have protected the crop from most, if not all, of the local pests (González-Fernández et al. 2009).

Figure 1.4.1.1. a) Avocado tree var. Hass located in the experimental station of the IHSM "La Mayora", where the work presented in this thesis was realized; and b) avocado fruits var. Hass.

1.4.2. Primary consumer: *Oligonychus perseae*, the herbivore pest

The persea mite, *Oligonychus perseae* (Tuttle, Baker & Abbatiello) (Acari: Tetranychidae), was first detected in avocado orchards of south-eastern Spain (provinces of Malaga and Granada) in 2004 (Vela et al. 2007). This pest is native from Mexico, and it has been also described as avocado pest in other regions of

Spain, Canary Island and Valencia, and in other countries such as USA (California), Costa Rica, Israel and Portugal (Vela et al. 2007). *O. perseae* builds dense silken nests on the underside of avocado leaves, mainly along the midrib and main nerves (Aponte and McMurtry 1997a) (Figure 1.4.2.1a). Nests are circular-shaped and are constructed with one or more small marginal openings used as entrances or exits for the mites (Aponte and McMurtry 1997a) (Figure 1.4.2.1b). Inside the nests adults feed and reproduce, and juveniles undergo development (Figure 1.4.2.1 b and c). Nests also protect the persea mite against attack from some species of natural enemies, and against adverse abiotic conditions (Mori et al. 1999; Montserrat et al. 2008a). The damage caused by feeding of individuals is initially confined to the cells of the lower epidermis and the outer spongy parenchyma tissue, followed later by widespread damage to the mesophyll cells and some damage to the palisade parenchyma (Aponte and McMurtry 1997a). Such damage causes characteristic circular necrotic spots that can occupy up to 90% of the leaf area (Aponte and McMurtry 1997a) (Figure 1.4.2.1a), affecting the photosynthesis efficiency of the tree. When mite densities are high (ca. 500 mites per leaf (Bender 1993)) partial or total defoliation of trees may occur (Bender 1993; Aponte and McMurtry 1997a), and, consequently, young fruits and tree trunks may become exposed to the risk of sunburn (Bender 1993).

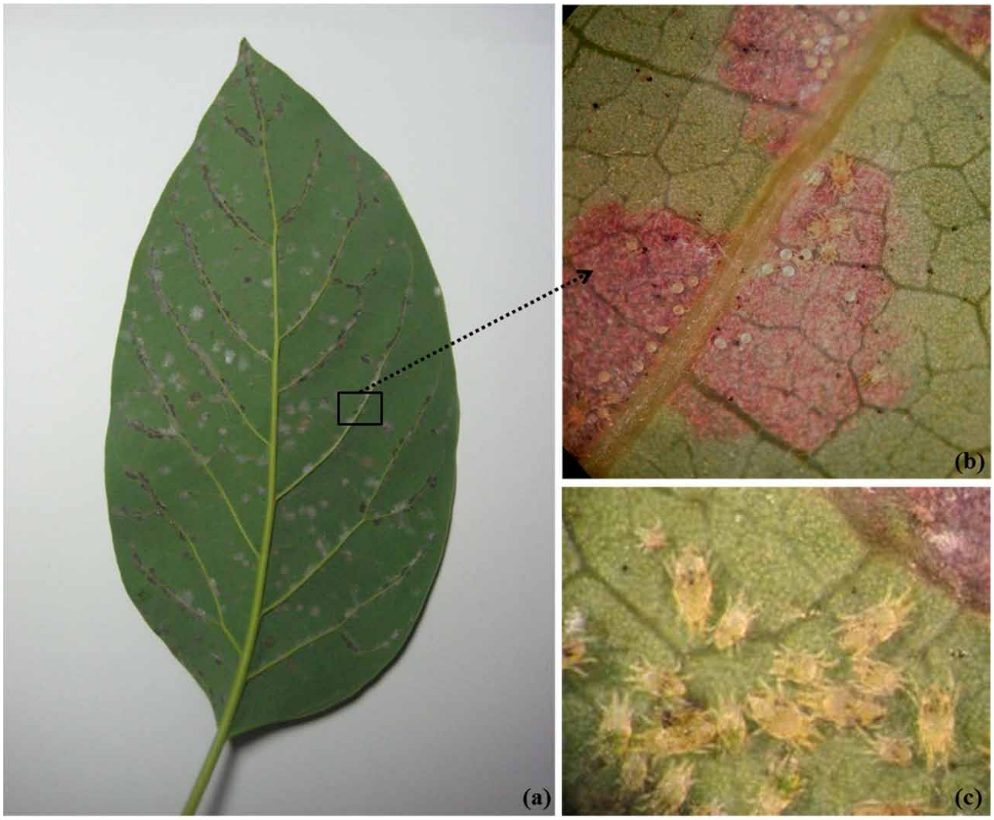

Figure 1.4.2.1. a)Avocado leaf infested by *O. perseae*; b) Nest build by *O. perseae* with females, juveniles and eggs inside; and c) Individuals of *O. perseae* in different stages of development inside the same nest.

Oligonychus perseae has five developmental stages: egg, larva, protonymph, duetonymph and adult (Figure 1.4.2.2). The time required to undergo development to adult depends on temperature: 34.89, 16.90, 13.87 and 9.81 days, at 15, 20, 25 and 30°C, respectively (Aponte and McMurtry 1997b). The number of nests built per female varies between 6 and 12 during her life-span, and it is also influenced by temperature; females build more nests at 20ºC. Females can lay up to 15 eggs per nest, although the number of eggs/nest decreases as temperature increases (Aponte and McMurtry 1997a). Other life-history parameters such as female longevity, oviposition rate and mortality also vary with temperature (determined at 15, 20, 25 and 30°C): the highest fecundity (45.80 eggs/female) and highest oviposition rate (1.84 eggs/female/day) is reached at 25°C and 30°C, respectively, and the highest mortality during development is 36.92% for the combined stages at 30°C (Aponte and McMurtry 1997b).

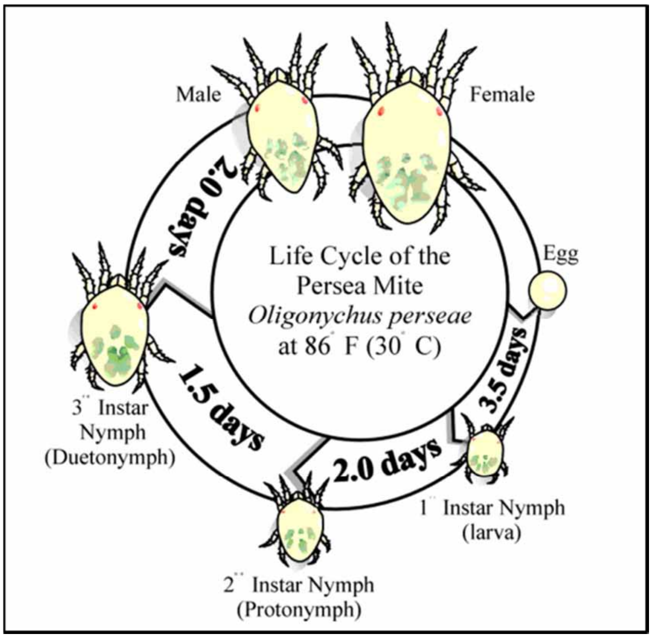

Figure 1.4.2.2. Developmental stages of *O. perseae* (egg, larva, protonymph, duetonymph and adult), and duration of each stage at 30 ºC. Figure reproduced from Hoddle (1998).

1.4.3. Secondary consumers: phytoseiid mites

The main natural enemies of the persea mite are predatory mites of the family Phytoseiidae (Hoddle et al. 1999). This is a worldwide family with more than 2000 identified species belonging to 67 genera (De Moraes et al. 2004). Phytoseiid mites have received increasing attention during the last four decades because of their potential as biocontrol agents of phytophagous mites. At present, they are the first taxonomic group providing mass reared predators, and 3 species are among the 10 (I quote) "most important invertebrate biological control agents used in augmentative biological control" (Van Lenteren 2012). Phytoseiid mites are popular because they are small, easy to rear, easy to release in the crops, and, because many of them are generalists, they can potentially control several pest species at the same time (Van Lenteren 2012).

In the Andalusian avocado crops three species of phytoseiid mites co-occurr with the persea mite: *Euseius stipulatus* (Athias-Henriot), *Euseius scutalis* (Athias-Henriot) and *Neoseilus californicus* (McGregor) (Figure 1.4.3.1 a, b and c, respectively) (González-Fernández et al. 2009; Guzmán et al. 2016a). Despite both species of *Euseius* are categorized as specialized pollen feeders (Ferragut and Escudero 1997; McMurtry and Croft 1997), they can prey on soft bodies arthropods and are known to contribute to the control of pest species in citrus (McMurtry 1977; Garcia-Mari et al. 1985; Ferragut et al. 1988; Abad-Moyano et al. 2009) and avocado (González-Fernández et al. 2009; Maoz et al. 2011) orchards. The two species cannot penetrate inside the persea mite nests and they attack only the individuals wandering outside the nests (González-Fernández et al. 2009). Based on the capacity of both *Euseius* species to use pollen as alternative feeding resource, they are currently being managed through conservational biological control methods for the regulation of *O. perseae* populations (González-Fernández et al. 2009; Maoz et al. 2011).

Neoseilus californicus is a polyphagous predator specialized in tetranychid mites (McMurtry and Croft 1997). Unlike the two species of *Euseius*, females of this species are able to enter inside the nests of persea mites using their first pair of legs to rip the dense web (Montserrat et al. 2008a). However, whether smaller stages of *N. californicus* can penetrate inside the *O. perseae* nests, and whether they benefit from this ability, still remains to be explored (Sections 3.2 and 3.3 of this thesis). *N. californicus* is commercially available, and it is a successful biocontrol agent in augmentative field release experiments on avocado trees in California (Hoddle et al. 1999; Kerguelen and Hoddle 1999) and in Israel (Maoz et al. 2011). Therefore, this species is a good candidate to be considered in biological control programs against the persea mite in the Andalusian avocado crops.

Figure 1.4.3.1. a) Female of *Euseius stipulatus* preying on a female of *O. perseae* outside the nest; b) Female of *Euseius scutalis*; and c) Female of *N. californicus* inside the nest.

Although the three species colonize avocado agro-ecosystems in south-eastern Spain, their distribution depend on the location of avocado orchards (Guzmán et al. 2016a). In coastal areas, with relatively mild abiotic conditions, the phytoseiid mite species that dominate are *E. stipulatu*s and *N. californicus*, whereas inland, where abiotic conditions are hotter and dryer, *E. scutalis* is the most abundant species as it is better adapted to heat stress (Kasap and Şekeroğlu 2004).

1.5. The area of study

The work presented in this thesis was developed in the IHSM "La Mayora". The IHSM "La Mayora" is a unique European research centre as it is the only one in Europe that investigates subtropical agronomic species. The experimental station of the IHSM, located in Algarrobo-Costa in the province of Malaga, has an area of 50 ha, 20 of which occupied by experimental plots with avocado, mango and cherimoya. Because it is located next to the sea the two dominant species in avocado orchards are *E. stipulatus* and *N. californicus*.

1.5.1. Climate change in the area of study

The area of study is located in the Mediterranean basin in south Europe, where models of climate change project an increase of temperature in spring and summer, and a decrease of precipitations (IPCC 2007, 2014).

An analysis of climate data recorded in the experimental station of the IHSM "La Mayora" from 1980 to 2008 showed a trend toward an increase in the average temperature in spring and summer (Figure 1.5.1.1), which is in accordance with the IPCC predictions (Montserrat et al. 2013b).

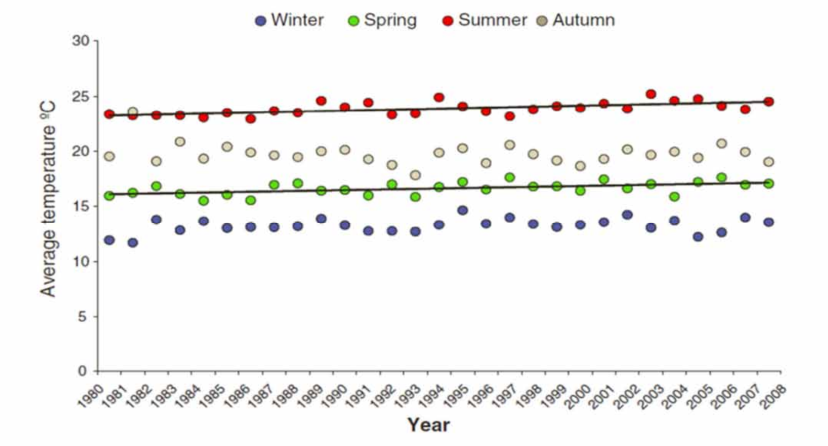

Figure 1.5.1.1. Average temperature of each season (winter, spring, summer and autumn) from 1980 to 2008. Statistically significant regression lines are shown in the figure. The slopes of the lineal regression functions for spring and summer data indicated that annually temperature increased, on average, 0.0375 and 0.0445 ºC, respectively. Figure in Montserrat et al. (2013b).

1.5.2. The mite predator/prey community dynamics in the area of study

The population dynamics of the *O. perseae* and the two predatory mites was studied from March to September (spring and summer) during four consecutive years (2006-2009) in an experimental avocado orchard located in the experimental station of the IHSM "La Mayora" (Montserrat et al. 2013b). The population dynamics of *O. perseae* was characterized by an exponential increase starting in early summer (July), with a maximum in August-September, and a dramatic exponential decrease at the end of summer (Figure 1.5.2.1a). The population dynamics of phytoseiid mites showed two maxima: (i) the first maximum was observed in spring when the persea mite population were low (Figure 1.5.2.1a). A previous work carried out by González-Fernández et al. (2009) revealed that phytoseiid populations responded to the abundance of pollen in the atmosphere in spring (Figure 1.5.2.1b), being that of

Olea europea (i.e. olive) the most abundant in avocado orchards of this area (77% of the total identified pollen in May). The identification of the phytoseiid species from samples taken during this first peak revealed that *E. stipulatus* was the dominant species (81% of the identified individuals), and that *N. californicus* was poorly represented (2% of the identified individuals); (ii) the second maximum was detected in summer, corresponding with a numerical response of predators to the persea mite abundance (Figure 1.5.2.1a). The most abundant specie in summer was *N. californicus* (50% of the identified individuals) followed by *E. stipulatus* (34% of the identified individuals) (González-Fernández et al. 2009). This second peak was observed in 2006 and 2007, but not in 2008 and 2009 (Figure 1.5.2.1a). The inspection of daily records of temperature and relative humidity during these sampling periods revealed that summers in 2008 and 2009 were hotter and dryer than those in 2006 and 2007 (Figure 1.5.2.1c), affecting negatively the phytoseiid populations. Harsh abiotic conditions were also a direct cause of biological control disruption detected in a field experiment carried out in the area of study during summers of 2009 and 2010 (Montserrat et al. 2013a).

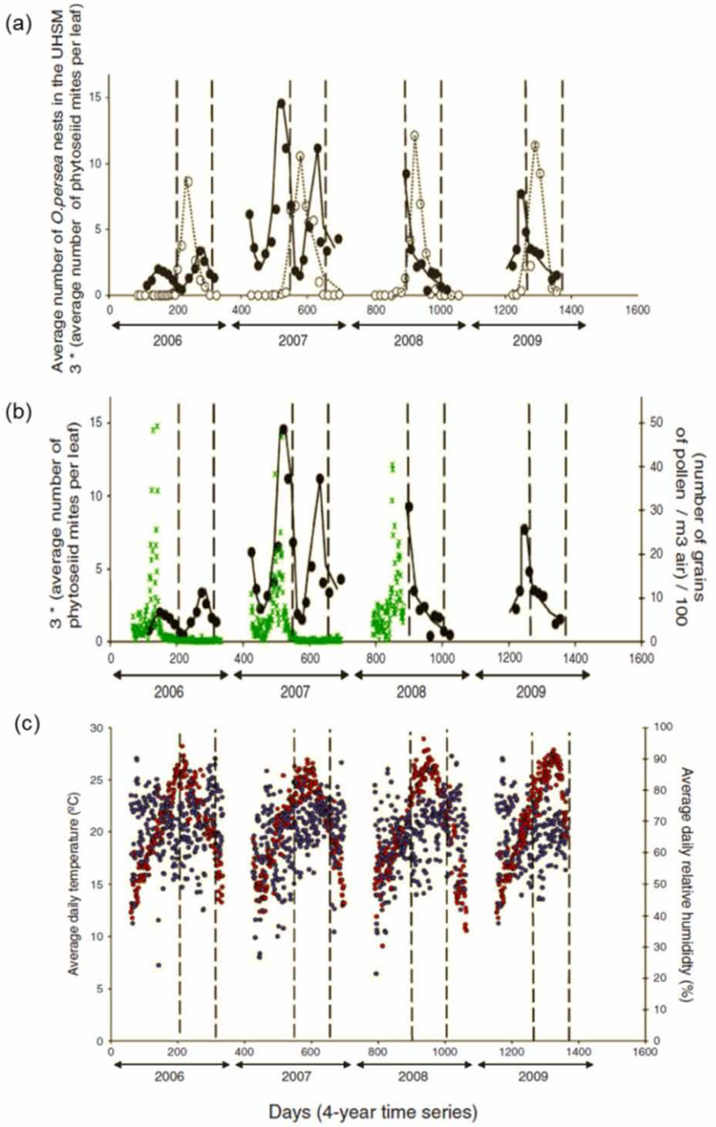

Figure 1.5.2.1. a) Population dynamics of the phytoseiid mites (black dots) and the persea mite (white dots) during four consecutive years (2006-2009); b) Population dynamics of phytoseiid mites (black dots) and grains of pollen in the atmosphere (green stars), during the same period as in a; and c) Daily average temperature (red dots) and relative humidity (blue dots) registered from early spring to late summer, during the same period as in a. Vertical lines delimit the time period with presence of *Oligonychus perseae* in the avocado trees. Note the empty spaces inside cluster of points in 2008 and 2009 that indicate days with high temperature and low relative humidity. Figure reproduced from Montserrat et al. (2013b).

1.6. Objectives

The well-functioning of arthropod communities providing important ecological services, such as biological pest control, might be jeopardised by changes in biotic interactions caused by shifts in abiotic conditions in the present scenario of climate change. Because the avocado mite community is composed by a low number of species, it is an optimal study model to study the effects of abiotic conditions on interactions between pests and their natural enemies. The use of community modules described in the section 1.3 can help to identify key interactions for an effective biological pest control that might be negatively affected by changes in the abiotic conditions.

The main goal of this thesis was to evaluate the effects of abiotic conditions on the strength and direction of biotic interactions occurring among mite species inhabiting avocado agro-ecosystems, and how these effects determine the trophic structure and dynamics of the community. This main goal was divided into several specific objectives from two different approaches:

1. *At the individual level*

1.a. Determine the influence of abiotic conditions in predator-prey interactions between the predatory mites *E. stipulatus* and *N. californicus*, and the herbivore pest *O. perseae*, in the presence and the absence of alternative food. Two community modules were used to achieve this objective: *Trophic chain* and *Apparent competition.*

1.b. Evaluate the effects of abiotic conditions and presence of alternative food on intraguild interactions between the two predatory mites, *E. stipulatus* and *N. californicus*, with *O. perseae* as shared prey. Interactions were analysed using two community modules: *Intraguild predation* and *Intraguild predation-Apparent competition.*

2. *At the population level*

2.a. Evaluate the impact of abiotic conditions on the community dynamics, i.e., how the observed effects on species interactions at the individual level are rendered at the population level. For this purpose, mite predator/prey population dynamics were examined in the presence and the absence of alternative food at two different abiotic conditions.

2. MATERIAL AND METHODS

2. MATERIAL AND METHODS

2.1. Effects of abiotic conditions and presence of alternative food on predator-prey interactions between predatory mites (*E. stipulatus* or *N. californicus*) and their herbivore prey (*O. perseae*)

2.1.0. Experimental details

Mites cultures

Predatory mites were cultured in a climate chamber at 25±1ºC, 65±5% RH and 16:8h L:D (Light:Dark). Cultures of *E. stipulatus* were started in 2007 from ca. 300 individuals collected from avocado trees located in the experimental station of the IHSM "La Mayora". Rearing units consisted of three bean (*Phaseolus vulgaris* L.) plants (6-10 leaves old) positioned vertically, with the stems in contact with sponges (30 x 20 x 5 cm, approx.) covered with cotton wool and a plastic sheet (27 x 17 cm) on top, and placed inside of water-containing trays (8 L, 42.5 x 26 x 7.5 cm). The roots of the plants were in contact with the water of the trays, and the aerial parts were touching each other forming a tent-like three-dimensional structure, where individuals could easily walk from one plant to the other (Figure 2.1.0.1a). Some cotton threads were placed on leaves serving as oviposition sites for mite females. Mites were fed *ad libitum* twice a week with pollen of *Carpobrotus edulis* (cat´s claw) that was spread on leaves with a fine brush. *Euseius stipulatus* is able to develop and reproduce when fed on pollen of *C. edulis* (Ferragut et al. 1987). Every three weeks, new rearings were made by transferring, from old rearings to a new unit, either leaves with mites and the cotton threads filled with eggs, or adult females (ca. 300) if old units were contaminated. Pollen of *C. edulis* was obtained from flower stamens that were dried in a stove at 37ºC for 48h, and then sieved (350 µm) (see Appendix 1 for further details). Individuals of *N. californicus* were obtained from Koppert Biological Systems S.L. in bottles of 1000 individuals (Spical®). Colonies were kept on detached bean leaves infested with *Tetranychus urticae* that were placed on top of inverted flower-pots (20 cm Ø) inside water-containing trays (Figure 2.1.0.1b).

Females of *O. perseae* were obtained from detached infested avocado leaves taken from avocado orchards located in the experimental station of the IHSM "La Mayora".

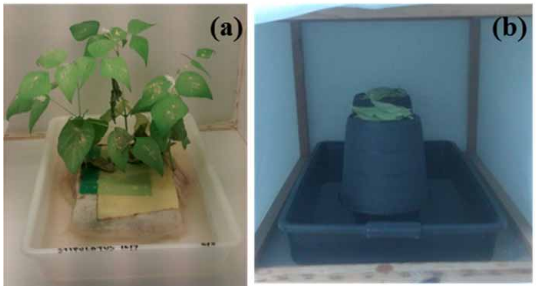

Figure 2.1.0.1. Rearing units for predatory mites. a) *Euseius stipulatus*; b) *Neoseiulus californicus*.

Experimental arenas

Experimental arenas were constructed according to the methodology described in Guzmán et al. (2016a). These experimental arenas were designed to create two abiotic environments: the upper environment, where interacting individuals experience the conditions of treatments, and the lower environment, which is kept humid to maintain the avocado leaf-discs turgor.

Experimental arenas were built as follows (Figure 2.1.0.2): a circular hole (6.5 cm Ø) was made in the middle of the base of Petri dishes (9 cm Ø) using a welder. The hole was covered with a plasticine ring along the perimeter. The plasticine was flattened on the inner margin of the hole. An avocado leaf disc (7.5 cm Ø) was placed on the base of each Petri dish with the leaf underside facing out through the hole of the Petri dish, and the borders glued to the plasticine. A disc of foam of 8 cm Ø was wrapped with wet cotton wool and was placed inside the base of Petri dishes in contact with the leaf discs. Petri dish lids were then placed upon the foam, and both the lid and the base were wrapped with parafilm®. Arenas were then turned upside down, so that lids were now the base of the arena and the underside of leaf was displayed. Finally, to refrain individuals from escaping, a ring of Tanglefoot® was applied along the outer margin of the leaf disc, in contact with the plasticine.

Figure 2.1.0.2. Experimental arena used in laboratory experiments where interactions between species were evaluated at the individual level.

Abiotic conditions

Effects of abiotic conditions on predator–prey interactions were evaluated at three combinations of temperature and relative humidity (Table 2.1.0.1): "mild" (M, hereafter), "hot and dry" (HD, hereafter) and "very hot and dry" (VHD, hereafter). Both abiotic factors, temperature and relative humidity, explain the population dynamic of phytoseiids to a high extent (Montserrat et al. 2013b).

The range of temperature (T) and relative humidity (RH) combinations tested in this study (Table 2.1.0.1) was obtained from daily T and RH averages registered during the day and night in July and August over five consecutive years (2006-2010) in the study area. To obtain the mild condition (M), 4 ºC were subtracted from daily averages T, and 10% of RH was added to daily averages RH to mimic springtime (March to May), when environmental conditions are relatively mild. To obtain HD and VHD conditions, T and RH were modified to reflect an increasingly arid environment, as it is predicted in the models of climate change.

Experiments were done at a photoperiod of 16:8h L:D (Light:Dark) in high-performance climate chambers (Fitoclima S 600, Aralab) that allow the fine-tuning of temperature and relative humidity.

Table 2.1.0.1. Combinations of temperature (T) and relative humidity (RH) set for each abiotic condition during day and night time.

	Day		Night	
Abiotic condition	**T**	**RH**	**T**	**RH**
Mild (M)	25 ºC	70 %	22 ºC	85 %
Hot and dry (HD)	30 ºC	50 %	27 ºC	50 %
Very hot and dry (VHD)	33 ºC	50 %	30 ºC	50 %

2.1.1. Community module: Trophic chain

The aim of this experiment was to determine how changes in abiotic conditions affect predator-prey interaction strength between either *E. stipulatus* or *N. californicus*, and the herbivore *O. perseae*.

All the predator females used in this experiment were 10-14 days old since egg hatching. Cohorts of *E. stipulatus* were made by transferring with a fine brush 400 eggs from the rearings to 2-3 bean leaves placed on top of sponges (30 x 20 x 5 cm, approx.) covered with cotton wool, inside water-containing trays (3.5 L), and with pollen of *C. edulis* as food. Because it was difficult to obtain eggs of *N. californicus* from the rearings, cohorts of this species were made by transferring 100 females to 2-3 bean leaves infested with *Tetranychus urticae* placed on containers similar to those explained above. After allowing *N. califonicus* females to feed and oviposit for 48 h, they were removed with a fine brush. Cohorts were maintained at 25±1ºC, 65±5% RH and 16:8h L:D (Light:Dark). Before the experiment, gravid females were randomly taken from the cohorts, and they were starved for 16 h on experimental containers and conditions similar to those above. Starvation was done to standardize hunger among individuals, and to ensure that egg production in tested females was not obtained from food ingested prior to the experiment.

Ten females of *O. perseae* were let on experimental arenas (see section 2.1.0) to build nests and lay eggs in a climate chamber at 25±1ºC, 65±5% RH and 16:8h L:D (Light:Dark) during 4 days. The number of nests and of eggs laid per nest on each experimental arena was counted at the onset of the experiment. Then, either a) one starved *E. stipulatus* or b) *N. californicus* female was introduced into the experimental arena (Table 2.1.1.1). After 24 h, the number of *O. perseae* dead females (predation/mortality rate), the number of eggs laid by predators (oviposition rate), and the fate of predators (whether alive or dead) were recorded.

Females of *N. californicus* are able to penetrate inside the persea mite nests (Montserrat et al. 2008a; González-Fernández et al. 2009). Therefore, the number of *O. perseae* eggs eaten was also recorded in the replicates with this predator-prey combination.

Experimental arenas containing c) 10 *O. perseae* females without predators, and either d) one *E. stipulatus* or e) one *N. californicus* female, without prey (Table 2.1.1.1) were done as control treatments for prey natural mortality and predator oviposition rate, respectively. All treatments (Table 2.1.1.1) were done at the three abiotic conditions. There were between 10 and 18 replicates per treatment.

Table 2.1.1.1. Combination of species present in each treatment studied in the community module *trophic chain.*

Treatments
1. Predator + Prey + avocado a) *E. stipulatus* + *O. perseae* + avocado b) *N. californicus* + *O. perseae* + avocado
2. Prey + avocado c) *O. perseae* + avocado
3. Predator + avocado d) *E. stipulatus* + avocado e) *N. californicus*+ avocado

Data analysis

Data was analysed using Generalized Lineal Models (GLM), which allow for non-linearity and heteroscedasticity in data.

Herbivore prey: Mortality of *O. perseae* females was analysed assuming a Poisson distribution of errors, typically used when the dependent variable consists of count data, with Log as the Link function. The model included "predator" (*E. stipulatus*, *N. californicus* or none), and "abiotic condition" (M, HD or VHD), as explanatory variables.

Due to results showed that *N. californicus* females preyed on *O. perseae* females at the three abiotic conditions, predation rates were compared among abiotic conditions. A new data set was created in which the actual number of *O. perseae* females killed per day was corrected for natural mortality. Data were analysed assuming a Normal distribution of errors and Identity as the Link function.

Egg predation inside nests was analysed only with *N. californicus* as predator. Analyses were done assuming a Negative Binomial distribution of errors, which is also typically used with count data sets, using Log as the Link function. The model included "abiotic condition" as explanatory variable.

Predators: Data of oviposition rates of predators needed correction. Despite predatory females were subjected to starvation to allow them to lay the eggs that had been produced from food obtained prior to the experiment, few females still laid eggs in experimental treatments with no food. Scavenging or cannibalism occurring during the starvation period probably caused this unexpected result, as tested females were starved together. To correct this error, the average oviposition rate of either predator in the control treatment with no food was subtracted from the oviposition value of each replicate in all the treatments, and this new set of data was used in the analyses.

Oviposition rates of each predator were analysed assuming a Normal distribution of errors and using Identity as the Link function, and models included "presence of prey" (yes or no) and "abiotic condition", as explanatory variables.

Additionally, specific analyses to compare oviposition rates of *N. californicus* and *E. stipulatus* in the presence of prey at M conditions, and oviposition rates of *N. californicus* in the presence of prey between M and HD conditions were performed. Data were analysed assuming a Normal distribution of errors with Identity as the Link function.

The ratio between daily oviposition and daily predation was estimated for each predator species at the abiotic conditions where predation on *O. perseae* females and oviposition was observed, i.e. at M conditions for *E. stipulatus*, and at M and HD conditions for *N. californicus*. A new data set was created in which the actual number of *O. perseae* females killed per day was corrected for natural mortality. Similarly, oviposition rates of predators were also corrected by the rates of oviposition observed in the control treatment with no food.

The survival of predators (alive or dead) was analysed in each species separately. Models assumed a binomial distribution of errors with a Logistical function as the Link function, and included "presence of prey" and "abiotic condition", as explanatory variables.

When necessary, analyses to evaluate the effect of explanatory variables on dependent variables within each abiotic condition were made separately. Post-hoc comparisons were made with the least significant difference (LSD) test. Analyses were done using IBM SPSS statistics v.23.

2.1.2. Community module: Apparent competition

The goal of this experiment was to determine whether the presence of alternative food (i.e. pollen) influences predator-prey interaction strength between either predatory species and *O. perseae*, and whether such influence is in turn affected by abiotic conditions.

The experimental methodology for this experiment was similar to that of the previous section. All the treatments and respective controls are summarized in Table 2.1.2.1. They consisted of: one female of either a) *E. stipulatus* or b) *N. californicus* with 10 females of *O. perseae* and pollen supplied *ad libitum*; as in above but without pollen [treatments c) and d)]; e) 10 females of *O. perseae* only, as control for natural mortality; and either of the two predator species with [treatments f) and g)] or without [treatments h) and i)] pollen supplied *ad libitum*, as controls for oviposition. All the treatments (Table 2.1.2.1) were done at the three abiotic conditions. The number of replicates per treatment varied between 10 and 18.

Table 2.1.2.1. Combination of species present in each treatment studied in the community module *apparent competition*.

Treatments
1. Predator + Prey + pollen + avocado a) *E. stipulatus* + *O. perseae* + pollen + avocado b) *N. californicus* + *O. perseae* + pollen + avocado
2. Predator + Prey + avocado c) *E. stipulatus* + *O. perseae* + avocado d) *N. californicus* + *O. perseae* + avocado
3. Prey + avocado e) *O. perseae* + avocado leaf
4. Predator + pollen + avocado f) *E. stipulatus* + pollen + avocado g) *N. californicus*+ pollen+ avocado
5. Predator + avocado h) *E. stipulatus* + avocado i) *N. californicus*+ avocado

Data analysis

Data was also analysed using Generalized Lineal Models (GLM).

Herbivore prey: Mortality of *O. perseae* females was analysed assuming a Poisson distribution of errors with Log as the Link function. Model included "experimental treatment" (with pollen, without pollen or control) and "abiotic condition" (M, HD or VHD), as explanatory variables.

Egg predation by *N. californicus* inside nests was analysed assuming a Negative Binomial distribution of errors, and using Log as the Link function. The explanatory variables included were "abiotic condition" and "presence of pollen" (yes or no).

Predators*:* Data correction on oviposition rates was done as well (see section 2.1.1). Oviposition rates of each predator were analysed assuming a Normal distribution of errors using Identity as the Link function, and models included "presence of prey" (yes or no), "abiotic condition" and "presence of pollen", as explanatory variables.

The survival of predators (alive or dead) was analysed in each species separately. Models assumed a binomial distribution of errors with a Logistical function as the Link function, and included "presence of prey", "abiotic condition" and "presence of pollen", as explanatory variables.

All models included only interactions among explanatory variables containing the main factor "presence of pollen", to assess the effect of supply of pollen on the different dependent variables.

When necessary, analyses to evaluate the effect of explanatory variables on dependent variables within each abiotic condition were made separately. Post-hoc comparisons were made with the least significant difference (LSD) test. Analyses were done using IBM SPSS statistics v.23.

2.2. Effects of abiotic conditions and presence of alternative food on intraguild interactions between two predatory mites (*E. stipulatus* or *N. californicus*) sharing an herbivore prey (*O. perseae*)

2.2.0. Mite cultures, experimental arenas and abiotic conditions

Mite cultures and experimental arenas were similar to those described in the section 2.1.0 (p. 53). Similarly, trophic interactions among species were evaluated at the three abiotic conditions detailed in the Table 2.1.0.1 (Section 2.1.0).

2.2.1. Community module: Intraguild predation

This experiment was designed to determine whether intraguild predation (IGP, hereafter) occurs between *E. stipulatus* and *N. californicus*, and whether IGP strength and direction is in turn affected by abiotic conditions.

All predator females used as IG-predators in this experiment were 10-14 days old since egg hatching and were subjected to starvation before the experiment, as explained in section 2.1.1. Heterospecific juveniles, of 2-3 days old since egg hatching, were used as IG-prey.

After letting 10 *O. perseae* females on each experimental arena in a climate chamber (25±1ºC, 65±5% RH and 16:8h L:D) during 4 days, the number of nests built and of eggs laid per nest by females were counted. Then, either one starved a) *E. stipulatus* or b) *N. californicus* female acting as IG-predator, and 10 heterospecific juveniles acting as IG-prey were introduced into the experimental arenas (Table 2.2.1.1). The number of *O. perseae* dead females (predation/mortality rate), the number of IG-prey juveniles dead (predation/mortality rate), alive, or escaped (found in the tanglefoot), the number of eggs laid by IG-predators (oviposition rate), and the fate of IG-predators (alive or dead), were recorded after 24h.

Additionally, several control treatments (Table 2.2.1.1) were done to evaluate: (i) the predation/mortality rate of *O. perseae* in the presence of IG-predator but not of IG-prey [treatments c) and d)], in the presence of IG-prey but not of IG-predator [treatments e) and f)], and in the absence of both IG-predator and IG-prey (i.e. natural mortality) [treatment k]; (ii) the predation/mortality rate of IG-prey in the presence of IG-predator but not of prey [treatments g) and h)], in the presence of prey but not of IG-predator [treatments e) and f)], and in the absence of both IG-predator and prey (i.e. natural mortality) [treatments i) and j)]; and (iii) the oviposition rate of IG-predators in the presence of prey but not of IG-prey [treatments c) and d)], in the presence of IG-prey but not of prey [treatments g) and h)], and in the absence of both prey and IG-prey [treatments l) and m)].

Table 2.2.1.1. Combination of species present in each treatment studied in the community module *intraguild predation*.

Treatments
1. IG-predator + IG-prey + Prey + avocado
a) *E. stipulatus* + *N. californicus* + *O. perseae* + avocado
b) *N. californicus* + *E. stipulatus* + *O. perseae* + avocado
2. IG-predator + Prey + avocado
c) *E. stipulatus* + *O. perseae* + avocado
d) *N. californicus* + *O. perseae* + avocado
3. IG-prey + Prey + avocado
e) *E. stipulatus* + *O. perseae* + avocado
f) *N. californicus* + *O. perseae* + avocado
4. IG-predator + IG-prey + avocado
g) *E. stipulatus* + *N. californicus* + avocado
h) *N. californicus* + *E. stipulatus* + avocado
5. IG-prey + avocado
i) *E. stipulatus* + avocado
j) *N. californicus* + avocado
6. Prey + avocado
k) *O. perseae* + avocado
7. IG-predator + avocado
l) *E. stipulatus* + avocado
m) *N. californicus* + avocado

While it is known that females of *N. californicus* can penetrate inside the *O. persea* nests and females of *E. stipulatus* cannot, whether juveniles of both species are able to penetrate and forage inside the nests remains to be explored. I, thus, also recorded the number of *O. perseae* eaten eggs.

The 13 treatments (Table 2.2.1.1) were carried out at the three abiotic conditions (Table 2.1.0.1). There were between 10 and 18 replicates per treatment.

Data analysis

Data was also analysed using Generalized Lineal Models (GLM).

Shared prey: The analysis of *O. perseae* female mortality was done assuming a Poisson distribution of errors and Log as the Link function. The model included "presence of IG-predator" (yes or no), "presence of IG-prey" (yes or no) and "abiotic condition" (M, HD or VHD), as explanatory variables.

Predation on *O. perseae* eggs was analysed assuming a Negative Binomial distribution of errors and using Log as the Link function. The model to analyse predation rates of the IG-predator (*N. californicus*) on *O. perseae* eggs included "presence of IG-prey" and "abiotic condition", as explanatory variables; and the model to analyse predation rates of the two IG-prey (*N. californicus* and *E. stipulatus*) on *O. perseae* eggs included "presence of IG-predator" and "abiotic condition", as explanatory variables.

IG-prey: IG-prey mortality was analysed assuming a Poisson distribution of errors using Log as the Link function. The model included as explanatory variables "presence of IG-predator", "presence of prey" (yes or no), and "abiotic condition".

IG-predators: Data correction on oviposition rates was done as well (see section 2.1.1). Oviposition rates of each IG-predator were analysed assuming a Normal distribution of errors with Identity as the Link function, and the models included "presence of prey", "presence of IG-prey" and "abiotic condition", as explanatory variables.

The survival of IG-predator females (alive or dead) was analysed assuming a binomial distribution of errors using a Logistical function as the Link function, and included as explanatory variables "presence of prey", "presence of IG-prey" and "abiotic condition".

When necessary, analyses to evaluate the effect of explanatory variables on the different dependent variables within each abiotic condition were made separately in both community modules. Post-hoc comparisons were made with the least significant difference (LSD) test. Analyses were done using IBM SPSS statistics v.23.

2.2.2. Community module: Intraguild predation - Apparent competition

This experiment aimed at testing whether IGP interactions, presumably occurring between *E. stipulatus* and *N. californicus*, could be reduced by providing an alternative food (i.e. pollen), and whether influences are in turn affected by abiotic conditions.

Methodologies were analogous to those in the previous section. Table 2.2.2.1 summarizes all the treatments included in this section. Because *O. perseae* forages on leaf tissue and does not include pollen in its diet, a control treatment with prey and pollen was not needed. All treatments were carried out at the three abiotic conditions (Table 2.1.0.1). Each treatment was replicated between 10 to 18 times.

Table 2.2.2.1. Combination of species present in each treatment studied in the community module *intraguild predation-apparent competition.*

Treatments
1. IG-predator + IG-prey + Prey + pollen + avocado
a) *E. stipulatus* + *N. californicus* + *O. perseae* + pollen + avocado
b) *N. californicus* + *E. stipulatus* + *O. perseae* + pollen + avocado
2. IG-predator + IG-prey + Prey + avocado
c) *E. stipulatus* + *N. californicus* + *O. perseae* + avocado
d) *N. californicus* + *E. stipulatus* + *O. perseae* + avocado
3. IG-predator + Prey + pollen + avocado
e) *E. stipulatus* + *O. perseae* + pollen + avocado
f) *N. californicus* + *O. perseae* + pollen + avocado
4. IG-predator + Prey + avocado
g) *E. stipulatus* + *O. perseae* + avocado
h) *N. californicus* + *O. perseae* + avocado
5. IG-prey + Prey + pollen + avocado
i) *E. stipulatus* + *O. perseae* + pollen + avocado
j) *N. californicus* + *O. perseae* + pollen + avocado
6. IG-prey + Prey + avocado
k) *E. stipulatus* + *O. perseae* + avocado
l) *N. californicus* + *O. perseae* + avocado
7. IG-predator + IG-prey + pollen + avocado
m) *E. stipulatus* + *N. californicus* + pollen + avocado
n) *N. californicus* + *E. stipulatus* + pollen + avocado
8. IG-predator + IG-prey + avocado
o) *E. stipulatus* + *N. californicus* + avocado
p) *N. californicus* + *E. stipulatus* + avocado
9. IG-prey + pollen + avocado
q) *E. stipulatus* + pollen + avocado
r) *N. californicus* + pollen + avocado
10. IG-prey + avocado
s) *E. stipulatus* + avocado
t) *N. californicus* + avocado
11. Prey + avocado
u) *O. perseae* + avocado
12. IG-predator + pollen + avocado
v) *E. stipulatus* + pollen + avocado
w) *N. californicus* + pollen + avocado
13. IG-predator + avocado
x) *E. stipulatus* + avocado
y) *N. californicus* + avocado

Data analysis

Data was also analysed using Generalized Lineal Models (GLM).

Shared prey: The mortality of *O. perseae* females was analysed assuming a Poisson distribution of errors and Log as the Link function. The model included "experimental treatment" (IG-predator with pollen, IG-predator without pollen or control (i.e. only prey)), "presence of IG-prey" (yes or no), and "abiotic condition" (M, HD or VHD), as explanatory variables.

Predation on *O. perseae* eggs was analysed assuming a Negative Binomial distribution of errors, and using Log as the Link function. Predation rates of *O. perseae* eggs were only analysed for *N. californicus* (acting as IG-predator and as IG-prey), since results from the community module *intraguild predation* revealed that *E. stipulatus* juveniles did not prey on *O. perseae* eggs. Explanatory variables were "presence of IG-prey", "abiotic condition" and "presence of pollen" (yes or no) -data with *N. californicus* females, i.e. IG-predators-, and "presence of IG-predator" (yes or no), "abiotic condition" and "presence of pollen" - data with *N. californicus* juveniles, i.e. IG-prey-.

IG-prey: IG-prey mortality was analysed assuming a Poisson distribution of errors using Log as the Link function. The model included "presence of IG-predator", "presence of prey" (yes or no), "abiotic condition" and "presence of pollen", as explanatory variables

IG-predators: Data correction on oviposition rates was done as well (see section 2.1.1). Oviposition rates of each IG-predator were analysed assuming a Normal distribution of errors with Identity as the Link function, and the model included "presence of prey", "presence of IG-prey", "abiotic condition" and "presence of pollen", as explanatory variables.

The survival of IG-predator females (dead or alive) was analysed assuming a binomial distribution of errors using a Logistical function as the Link function, and included as explanatory variables “presence of prey”, “presence of IG-prey”, “abiotic condition” and “presence of pollen”.

All models included only interactions among explanatory variables containing the main factor “presence of pollen”, to assess the effect of supply of pollen on the different dependent variables.

When necessary, analyses to evaluate the effect of explanatory variables on the different dependent variables within each abiotic condition were made separately in both community modules. Post-hoc comparisons were made with the least significant difference (LSD) test. Analyses were done using IBM SPSS statistics v.23.

2.3. Unravelling behavioural/environmental effects: fine-tuning of results

2.3.1. IG-prey role reversals triggered by IG-predators diet

Results from other section in this thesis revealed that some juveniles of *N. californicus* (i.e. the IG-prey) preyed on *E. stipulatus* eggs (i.e. the IG-predator), but only when pollen was available. It is known that IG-prey behaviour may be determined by the diet of IG-predators (Magalhães et al. 2005b). Given that females of *E. stipulatus* preferably foraged on pollen when it was available (see Results 3.2.2, p. 109), I decided to test whether *N. californicus* juveniles were able to discriminate between IG-predator eggs produced from mothers having different diets (i.e. pollen or eggs of *O. perseae*).

Cultures of *E. stipulatus* and *N. californicus* were described in the section 2.1.0. (p. 53). Eggs of *O. perseae* were obtained from infested avocado leaves taken from avocado trees located in the experimental station of the IHSM "La Mayora". Experimental arenas used in this experiment were similar to those described in the section 2.1.0, but smaller (inner diameter 4.5 cm instead of 7.5 cm).

Females of *E. stipulatus* (10-14 days old since egg hatching) were starved individually during 16 h on bean leaf discs (2 cm Ø) placed on top of sponges (30 x 20 x 5 cm, approx.) covered with cotton wool, inside water-containing trays (3.5 L), and maintained at 25±1ºC, 65±5% RH and 16:8h L:D. Starved females were individually allocated to two different treatments: (i) arenas with pollen (*Carpobrotus edulis*) supplied *ad libitum*, and (ii) arenas with eggs of *O. perseae* supplied *ad libitum* (30 eggs). Females were allowed to eat and lay eggs during 24 h. Next, five juveniles of *N. californicus* (2-3 days old since egg hatching), and six *E. stipulatus* eggs either produced by females from arenas (i) or arenas (ii) were introduced into new experimental arenas. Because some leaf structures may facilitate [e.g. leaf nerves functioning as a hold for eggs (Schausberger and Croft 1999), or leaf domatia, which are typically used as oviposition place by phytoseiid mites (Walter 1996)] or hamper (e.g. oval-shaped phytoseidd eggs may roll in the limbo of leaves when phytoseiid try to grasp them) predation on eggs by juveniles, *E. stipulatus* eggs were placed on three different locations within each avocado leaf disc: (1) two eggs in the domatia; (2) two eggs next to the midrib; and (3) two eggs on the leaf limbo (Figure 2.3.1.1). After 24 h, the number of predated eggs and their location (domatia, midrib or limbo) was assessed. Each treatment was replicated 10 times at M conditions.

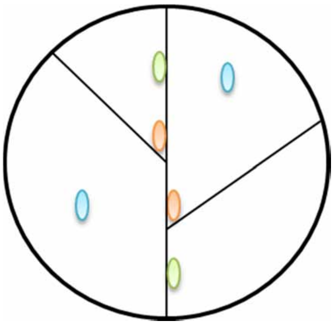

Figure 2.3.1.1. Location of *E. stipulatus* eggs on experimental arenas: two eggs in the domatia (orange), two eggs next to the midrib (green), and two eggs at the leaf limbo (blue).

Data analysis

Differences in predation on *E. stipulatus* eggs by *N. californicus* juveniles depending on the diet of mothers were analysed with MANOVA using number of eggs eaten in Domatia, Midrib and Limbo as dependent variables, and type of food (pollen or eggs of *O. perseae*) as explanatory variable. Univariate ANOVA's were subsequently done for each of the dependent variables, to detect potential egg predation differences within specific locations. Analyses were conducted with IBM SPSS statistics v.23.

2.3.2. Nests of *O. perseae* as refuges for *N. californicus* juveniles against adverse abiotic conditions

Results from section 3.2.1 (p. 96) indicated that *N. californicus* juveniles were able to go inside the nests. Furthermore, that nests were never observed damaged suggested that juveniles accessed nests using their entrances, an ability that I later observed directly. Based on this, I hypothesized that *N. californicus* juveniles could use the nests of *O. perseae* as refuges against adverse abiotic conditions. To test that, I evaluated the mortality of *N. californicus* juveniles at three abiotic conditions (M, HD and VHD) in the presence and the absence of *O. perseae*' nests.

Cultures of *N. californicus* were described in the section 2.1.0 (p. 53), and females of *O. perseae* were obtained from infested avocado leaves taken from avocado trees located in the experimental station of the IHSM "La Mayora". Experimental arenas used in this experiment were similar to those described above (inner diameter 4.5 cm).

Groups of ten females of *O. perseae* were let to build nests and lay eggs in experimental arenas during 2 days, inside a climate chamber at 25±1ºC, 65±5% RH and 16:8h L:D (Light:Dark). Then, pairs of experimental arenas were allocated to either of the following two treatments: a) *With nests*: Females of *O. perseae* inside nests were removed by touching them through the wall nests with a fine brush, being very careful to not damage the nests, until they reached one of the entrances and exited. Next, 5 nests with eggs were left untouched and the rest were removed from the arenas. The total number of eggs inside nests was then counted; b) *Without nests*: Females of *O. perseae* and the nests were removed, and only the same number of eggs as in the corresponding arena with nests was left in the arenas. Then, one juvenile of *N. californicus* (2-4 days old since egg hatching) was introduced in all the arenas. The number of eggs per pair of arenas varied between 10 and 20. After 24 h, the number of *O. perseae* predated eggs, and the fate of the *N. californicus* juvenile (alive or dead) were recorded. The number of paired replicates per abiotic condition (Section 2.1.0; Table 2.1.0.1) varied between 10-14.

Data analysis

The effect of the presence of *O. perseae* nests on *N. californicus* juvenile mortality depending on abiotic conditions was analysed with Generalized Lineal Models (GLM), assuming a Binomial distribution of errors and using a Logistical function as a Link function. The model included “Fate” (‘0’ if the juvenile was alive, ‘1’ if it was dead) as the dependent variable, and “nests” (with or without) and “abiotic condition” (M, HD or VHD), and their interaction, as explanatory variables. The total number of eggs inside nests was added to the model as co-variable. If the effect of the co-variable was not significant, it was removed from the model and data was re-analysed.

The effect of presence of nests and abiotic conditions on *O. perseae* egg predation by *N. californicus* juveniles was analysed with GLM, assuming Normal distribution of errors, and Identity as the link function. Because replicates were paired, the dependent variable included in the model was the difference in the number of eaten eggs between replicates with and replicates without nests. Hence, if the difference was significantly < ‘0’ would indicate a higher number of eaten eggs in the absence of nests; whereas if this values was > ‘0’ would indicate a higher number of eaten eggs in the presence of nests. The model included “abiotic condition” as explanatory variable. Analyses were conducted with IBM SPSS statistics v.23.

2.4. Effects of abiotic conditions and presence of alternative food on mite predator/prey population dynamics in avocado

2.4.0. Mite cultures and abiotic conditions

Mite cultures

The rearing methods of *N. californicus* and *E. stipulatus* were described in the section 2.1.0 (p. 53). Females of *O. perseae* were collected from detached infested avocado leaves taken from avocado orchards located in the experimental station of the IHSM "La Mayora".

Abiotic conditions

The mite predator/prey population dynamics in avocado were evaluated at two abiotic conditions defined as "mild" (M, hereafter) and "hot and dry" (HD, hereafter). Experiments were carried out in a walk-in high-performance climate chamber (Fitoclima 27000 EHHF, Aralab) that, by fine-tuning of temperature, relative humidity and light each 30 minutes, allows reproducing daily cycles.

The temperature (T) - relative humidity (RH) combinations for each abiotic condition simulated daily fluctuating conditions. Daily cycles were obtained from averages of 30 minutes records of T and RH registered on July and August during 5 consecutive years (2006 to 2010) in the study area (Figure 2.4.0.1). Firstly, to obtain M conditions, 4ºC were subtracted from the average T and 10% was added to the average RH in each of the 30-minute records, to mimic a spring day when environmental conditions are relatively mild; and to obtain HD conditions, 4ºC were added to the average T and 10% was subtracted from the average RH in each of the 30 minute records, to mimic extreme abiotic conditions predicted in models of climate change (Figure 2.4.0.1). Secondly, to reduce the number of intervals to program, successive values of T and RH that were very similar were averaged (see Figure 2.4.0.1). This way we obtained 19 segments of different duration that simulated conditions of an entire day (i.e. sunrise, morning-noon-afternoon, sunset and night) for each abiotic condition (Table 2.4.0.1). The photoperiod was 16:8h L:D (Light:Dark), imitating both the sunrise, with a progressive increase of light, and the sunset, with a progressive decrease of light.

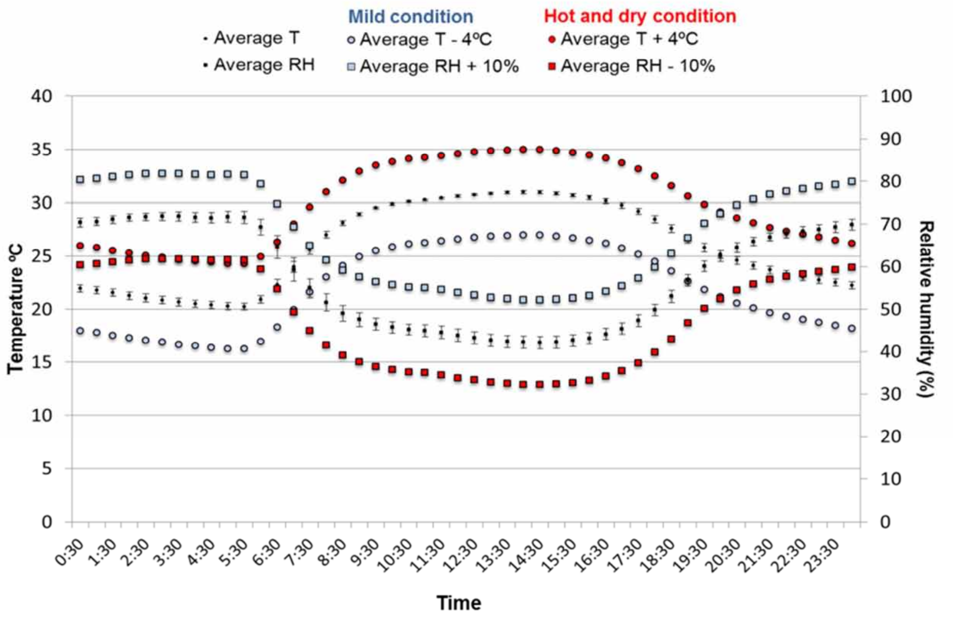

Figure 2.4.0.1. Thirty minute records of temperature (T) and relative humidity (RH) (average ± SE) registered on July and August during 5 consecutive years (2006-2010) in the study area (black circular and square symbols, respectively). To obtain "mild" conditions, 4ºC were subtracted from the average T and 10% was added to the average RH in each of the 30-minute records (blue circular and square symbols, respectively); and to obtain "hot and dry" conditions, 4ºC were added to the average T and 10% was subtracted from the average RH in each of the 30 minute records (red circular and square symbols, respectively).

Table 2.4.0.1. Combinations of temperature (T), relative humidity (RH) and Light set for each abiotic condition, "mild" (blue) and "hot and dry" (red) pooled in 19 segments of different time duration that simulated conditions of an entire day (i.e. sunrise, morning-noon-afternoon, sunset and night).

Part of day	**Time**	**Duration**	**T (ºC):**		**RH (%):**		**Ligh (%)**
			Mild	**Hot and dry**	**Mild**	**Hot and dry**	
Sunrise	06:00-06:30 h	30 min	17.6	25.6	76.9	56.9	20%
	06:30-07:00 h	30 min	19.1	27.1	72.0	52.0	40%
	07:00-07:30 h	30 min	20.8	28.8	67.1	47.1	60%
	07:30-08:00 h	30 min	22.3	30.3	63.2	43.2	70%
	08:00-08:30 h	30 min	23.5	31.5	60.3	40.3	80%
	08:30-09:00 h	30 min	24.5	32.5	58.3	38.3	90%
Morning-noon-afternoon	09:00-17:30 h	8h 30 min	26.3	34.3	54.2	34.2	100%
	17:30-18:00 h	30 min	24.8	32.8	58.5	38.5	100%
	18:00-18:30 h	30 min	24.0	32.0	61.4	41.4	100%
	18:30-19:00 h	30 min	23.1	31.1	64.9	44.9	100%
Sunset	19:00-19:30 h	30 min	22.2	30.2	68.4	48.4	90%
	19:30-20:00 h	30 min	21.5	29.5	71.3	51.3	80%
	20:00-20:30 h	30 min	20.9	28.9	73.5	53.5	70%
	20:30-21:00 h	30 min	20.3	28.3	75.1	55.1	60%
	21:00-21:30 h	30 min	19.8	27.8	76.4	56.4	40%
	21:30-22:00 h	30 min	19.5	27.5	77.3	57.3	20%
Night	22:00-00:00 h	2 h	18.8	26.8	78.7	58.7	0%
	00:00-03:00 h	3 h	17.5	25.5	81.0	61.0	0%
	03:00-06:00 h	3 h	16.5	24.5	81.3	61.3	0%

2.4.1. Mite predator/prey population dynamics in avocado seedlings under two abiotic conditions, and in the presence or absence of pollen

Population dynamic experiments were designed to determine whether the effects of abiotic conditions and presence of pollen observed at the individual level (i.e. community modules) are rendered at the population level.

Experiments were performed using avocado seedlings (*Persea Americana* var. Hass) of 1 m high (Figure 2.4.1.1a) as experimental units. Plants were introduced inside insect-proof mesh cages to isolate them from each other (Figure 2.4.1.1b). Ten days before the start of the experiment, each plant was infested with 50 *O. perseae* females to allow herbivore populations to establish and grow at optimal conditions (25 ºC/ 70% RH and 16:8h L:D (Light:Dark)). The first day of the experiment, the number of occupied nests (those with mobile stages and/or eggs inside) built by the herbivore on the upper margin of the second leaf vein of the underside of the leaf (UML2, hereafter) of 10 randomly selected leaves was recorded. As González-Fernández et al. (2009) described, the number of occupied nests on the UML2 is a good estimate of the total number of individuals (mobiles and eggs) on the leaf: the number of occupied nests relates to the total number of nests on the leaf ($y=11.84x+3.28$, $R^2=0.80$, $P<<0.001$, $N=422$), and the total number of nests on the leaf relates to the total number of individuals ($y=2.98x$, $R^2=0.84$, $P<<0.001$, $N=431$). Next, 10 gravid females of both *E. stipulatus* and *N. californicus* were introduced in each plant. Plants were then allocated to two of the following treatments: (i) with pollen, and (ii) without pollen. Each treatment was replicated 4 times (i.e. 4 plants) at each abiotic condition (i.e. M and HD). Plants that received pollen were supplied with 20 mg of pollen (*Carpobrotus edulis*) in a plastic vial (0.9 mm diameter and 0.5 mm high) glued on the top of a single leaf using a strip of VELCRO® (Figure 2.4.1.1c). Once a week, plants received new vials with fresh pollen. Vials that were removed from the plant were checked for predators being inside with a binocular microscope, and individuals were transferred back to the leaf. Twice a week, the number of occupied nests on the UML2 and the total number of mobile stages (i.e. adults and juveniles) of predatory mites were recorded in 10 randomly chosen leaves using a field magnifying glass (Ruper x8). The two predatory mite species were not identified at the species level because *E. stipulatus* and *N. calfornicus* are morphologically very similar and they can only be differentiated at a microscopic level. Experiments lasted until either the phytoseiid mite or the prey populations crashed, or until population stability was detected (~38 days). At the end of the experiment, each plant was subjected to destructive sampling, where the total number of occupied nests, the total number of necrotic spots (that estimate the damage produced by this pest in avocado leaves), and the total number of mobile stages (i.e. adults and juveniles) and eggs of phytoseiid mites were recorded in all the leaves under the binocular. All adult phytoseiids were collected and prepared for identification at the species level under the microscope, and the juveniles and eggs were isolated to allow them to develop into adults. They were left on two bean leaf discs together (2 cm Ø) placed on wet cotton wool inside small containers (4.5 cm Ø), and provided with pollen and eggs and larvae of *Tetranychus urticae* (Figure 2.4.1.1d) in a climate chamber at 25±1ºC, 65±5% RH and 16:8h L:D. Once immatures reached adulthood, they were identified at the species level under the microscope.

The taxonomic identification of phytoseiid mites was performed according to the protocol described in Gutiérrez (1985). First, adults were stored in 70% ethanol with 5% glycerine to protect the specimens until the clearing. Then, phytoseiid mites were cleared in 65% lactic acid (48h, 50ºC), which attacks soft tissues, but not chitinized cuticles, and inflates the body and extends the legs. Last, they were slide-mounted in Hoyer's medium. Identification to species level, using a phase contrast microscope, was based on the morphology, number, length and position of setae on ventral plates (Figure 2.4.1.2), and on the shape of spermathecas and spermatodactyls of females and males, respectively (Figure 2.4.1.3) (Çakmak and Çobanoğlu 2006; Ferragut et al. 2010).

Figure 2.4.1.1. a) Avocado plant used in the population dynamics experiments; b) Avocado plants were isolated inside cages placed in a walk-in high-performance climate chamber where population dynamic experiments were performed; c) Plastic vials with pollen, attached to the upper surface of leaves in plants under the treatment “with pollen”; d) Small containers where immatures of predatory mites reached adulthood after destructive sampling.

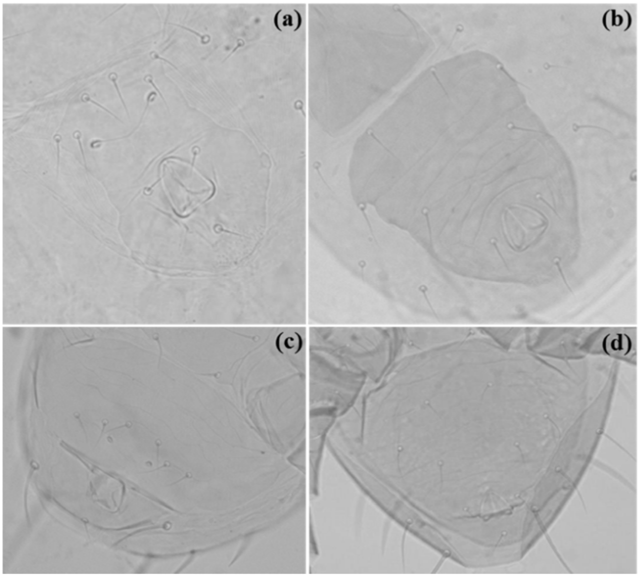

Figure 2.4.1.2. Ventral plate of a) *E. stipulatus* female, b) *N. californicus* female, c) *E. stipulatus* male, and d) *N. californicus* male.

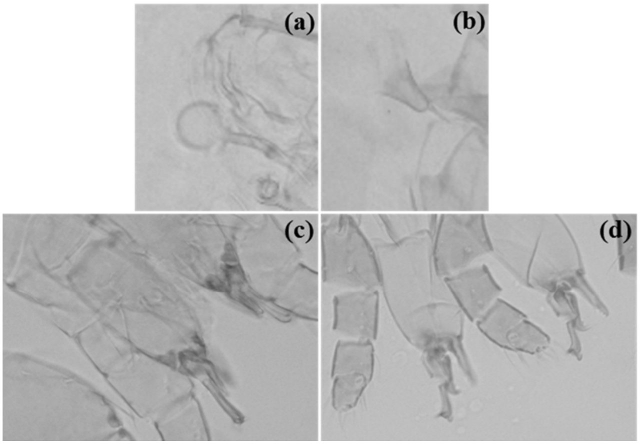

Figure 2.4.1.3. Spermatheca of a) *E. stipulatus* female and b) *N. californicus* female. Spermatodactyl of c) *E. stipulatus* male and d) *N. californicus* male.

Data analysis

Destructive sampling data

The effect of abiotic conditions and presence of pollen was analysed using data from the final destructive sampling. The dependent variables were total number of predators, occupied *O. perseae* nests, and necrotic spots, per leaf. The three dependent variables were analysed separately with a 3-factor full-nested generalized lineal mixed model (GLMM) to account for dependence of data to each plant, assuming negative binomial distribution of error, with "abiotic condition" (M or HD), "treatment" (with pollen or without pollen) nested to abiotic condition, and "plant" (16 plants) nested to treatment and to abiotic condition, as main factors. Post-hoc comparisons were made with the least significant difference (LSD) test. Analyses were conducted with IBM SPSS statistics v.23.

Taxonomic identification of phytoseiid mites

With regard to the estimates of abundances of each predator species, because most immatures and eggs collected in the destructive sampling did not survive until adulthood, the percentage of each predatory mite species in each replicate (i.e. plant) was estimated using only the adults that were collected and identified at the end of the experiment.

3. RESULTS

3. RESULTS

3.1. Effects of abiotic conditions and presence of alternative food on predator-prey interactions between predatory mites (*E. stipulatus* or *N. californicus*) and their herbivore prey (*O. perseae*)

3.1.1. Community module: Trophic chain

(i) Predation rate on females and eggs of O. perseae

Results showed that *O. perseae* female mortality rates were mainly affected by the presence of predators (Wald statistic for "predator"=54.29, *df*=2, *P*<0.001). Mortality of *O. perseae* females was in overall significantly higher in the presence of *N. californicus* than in the presence of *E. stipulatus* (Figure 3.1.1.1). The effect of predators on *O. perseae* mortality was marginally different among abiotic conditions (Wald statistic for "predator*abiotic condition"=9.30, *df*=4, *P*=0.054). Whereas *N. californicus* preyed on *O. perseae* females at the three abiotic conditions, *E. stipulatus* only did it at M conditions (Figure 3.1.1.1).

Predation rates of *N. californicus* on *O. perseae* females were unaffected by abiotic conditions (Wald statistic for "abiotic condition"=0.209, *df*=2, *P*=0.901). However, predation rates of *N. californicus* on *O. perseae* eggs inside the nests were affected by abiotic conditions (Wald statistic for "abiotic condition"=6.84, *df*=2, *P*=0.033), being significantly higher at M than at VHD conditions (Figure 3.1.1.2).

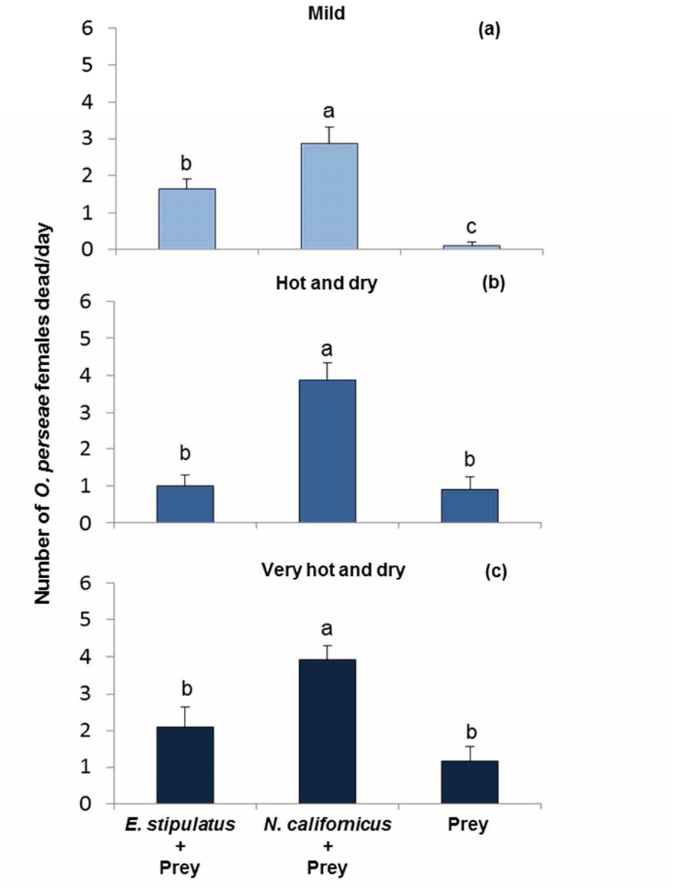

Figure 3.1.1.1. Predation/mortality rate (average ± SE) of *O. perseae* females at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry") in the presence of either *E. stipulatus* or *N. californicus*, and in the absence of both predators. Letters above bars are from LSD post-hoc tests and indicate significant differences (*P*≤0.05) among treatments within abiotic conditions.

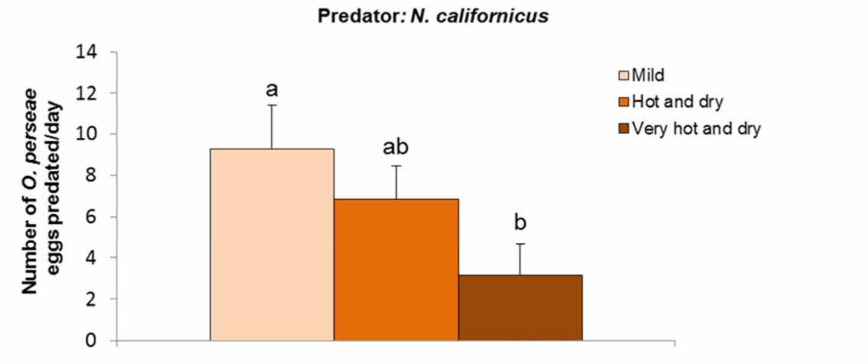

Figure 3.1.1.2. Predation rate (average ± SE) of *N. californicus* on *O. perseae* eggs at the three abiotic conditions (see legend). Letters above bars are from LSD post-hoc test and indicate significant differences ($P \leq 0.05$).

(ii) Oviposition rate of predators

Oviposition rates of *E. stipulatus* were mainly affected by the presence of prey (Wald statistic for "presence of prey"=4.25, *df*=1, P=0.039), although such effect differed among abiotic conditions (Wald statistic for "presence of prey*abiotic condition"=14.96, *df*=2, *P*=0.001): only at M conditions the number of eggs were significantly higher in the presence of prey than in its absence (Figure 3.1.1.3, 1a). In the case of *N. californicus*, the presence of prey contributed to increase its oviposition rates (Wald statistic for "presence of prey"=23.64, *df*=1, $P \ll 0.001$), independently of abiotic conditions (Wald statistic for "presence of prey*abiotic condition"=2.11, *df*=2, *P*=0.349). However, results from LSD post-hoc tests revealed that at VHD conditions the number of eggs laid by females were unaffected by the presence of prey (Figure 3.1.1.3, 2c). Further analysis revealed that at M conditions and in the presence of prey there were no significant differences in oviposition rates between species of predators (Wald statistic for "species"=0.138, *df*=1, *P*=0.711); and that the number of eggs laid by *N. californicus* in the presence of prey did not vary significantly between M and HD conditions (Wald statistic for "abiotic condition"=0.278, *df*=1, *P*=0.598).

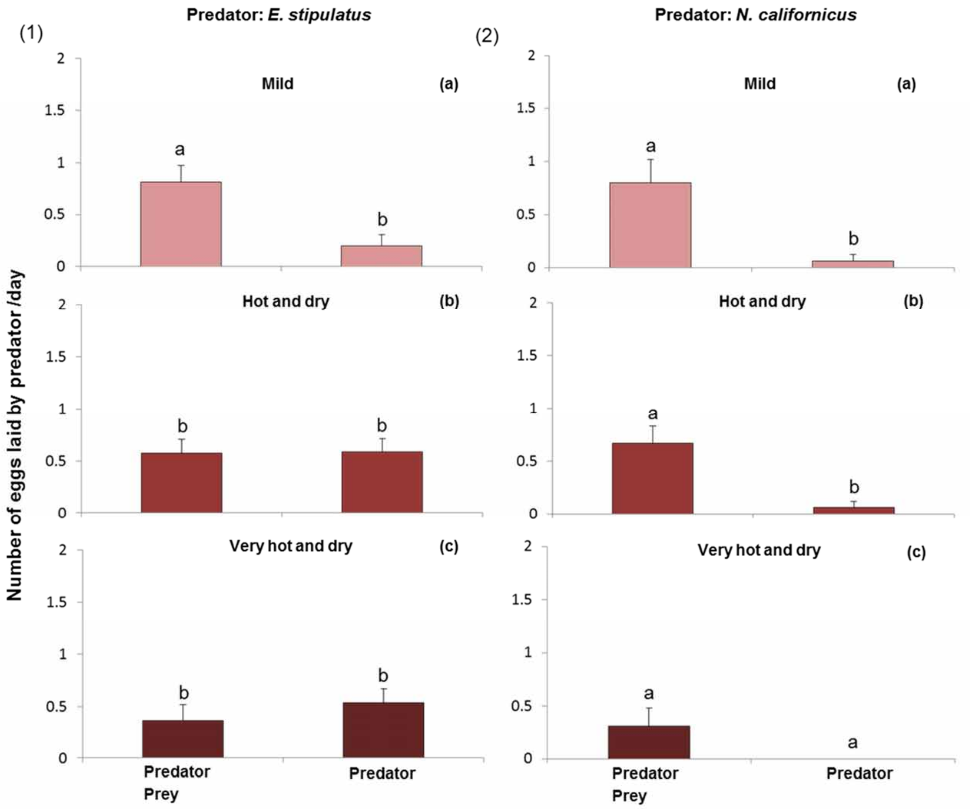

Figure 3.1.1.3. Oviposition rates (average ± SE) of (1) *E. stipulatus* and (2) *N. californicus* at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry"), in the presence or in the absence of prey. Letters above bars are from LSD post-hoc tests and indicate significant differences ($P \leq 0.05$) among treatments within abiotic conditions. The figure shows original values, without the correction applied for the analyses (see M&M 2.1.1, p. 58).

(iii) Food-to-egg conversion efficiency

At M conditions, the ratio daily oviposition/daily predation showed that *E. stipulatus* was more efficient than *N. californicus* at converting prey into eggs (Table 3.1.1.1). In *N. californicus*, prey conversion rate into eggs slightly decreased as temperature and drought increased (Table 3.1.1.1).

Table 3.1.1.1. Predation rates (average ± SE) of *E. stipulatus* or *N. californicus* on *O. perseae* females, predator's oviposition rates (average ± SE), and the ratio daily oviposition/daily predation at different abiotic conditions.

Predator species	**Abiotic conditions**	**Number of *O. perseae* predated females /day**	**Number of eggs laid by predators /day**	**Ratio of oviposition/ predation**
E. stipulatus	Mild	1.53±0.30	0.68±0.14	0.44
N. californicus	Mild	2.77±0.45	0.77± 0.22	0.28
	Hot and dry	2.99±0.45	0.63± 0.16	0.21

(iv) Survival of predators

The survival of *E. stipulatus* females was not affected by abiotic conditions (Wald statistic for "abiotic condition"=2.31, *df*=2, *P*=0.316), the presence of prey (Wald statistic for "presence of prey"=0.67, *df*=1, *P*=0.412), or an interaction between both factors (Wald statistic for "abiotic condition*presence of prey"=0.36, *df*=2, *P*=0.835) (Figure 3.1.1.4, 1). However, the survival of *N. californicus* females depended on abiotic conditions (Wald statistic for "abiotic condition"=12.16, *df*=2, *P*=0.002), and on the presence of prey (Wald statistic for "presence of prey"=7.62, *df*=1, *P*=0.006). Female survival was negatively influenced by high temperatures and low relative humidity in the absence of food, both at HD and VHD conditions (Figure 3.1.1.4, 2).

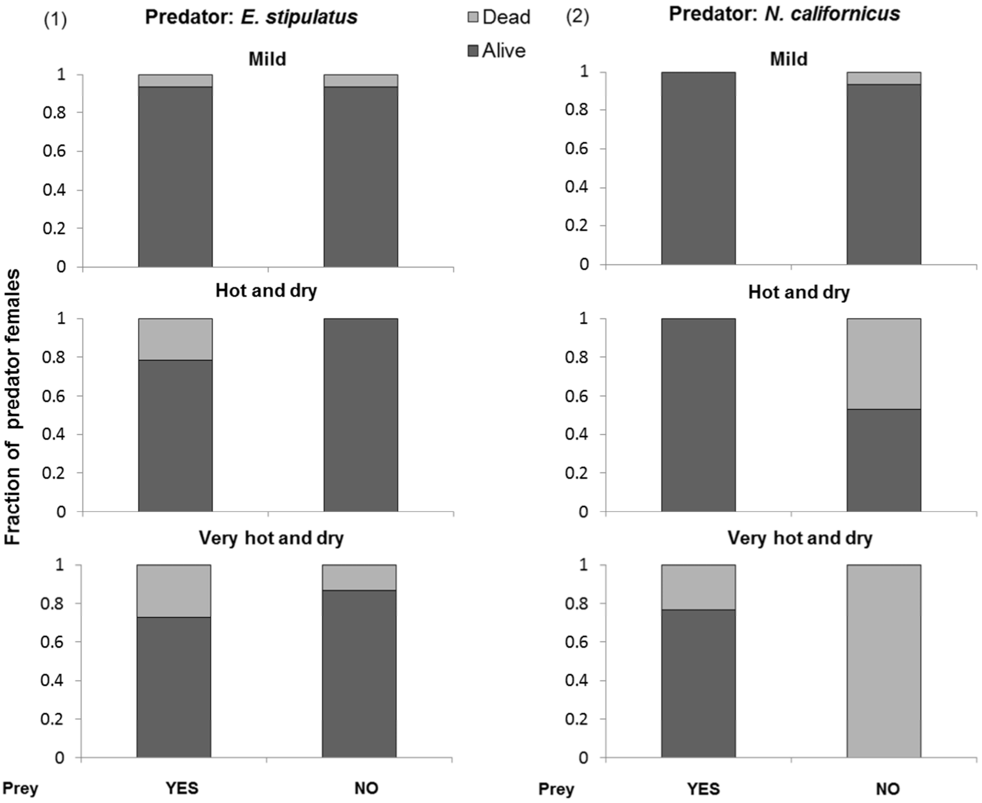

Figure 3.1.1.4. Fraction of predatory (1) *E. stipulatus* and (2) *N. californicus* females that were alive (dark grey) or dead (light grey) after 24 h at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry"), in the presence or in the absence of the prey.

3.1.2. Community module: Apparent competition

(i) Predation rate on females and eggs of O. perseae

When the predator was *E. stipulatus*, *O. perseae* female mortality was influenced by the interaction between experimental treatment and abiotic conditions (Wald statistic for "experimental treatment*abiotic condition"=12.27, *df*=4, *P*=0.015). Indeed, *E. stipulatus* did not prey on *O. perseae* females in the treatment in which pollen was available at any of the three abiotic conditions (Figure 3.1.2.1, 1).

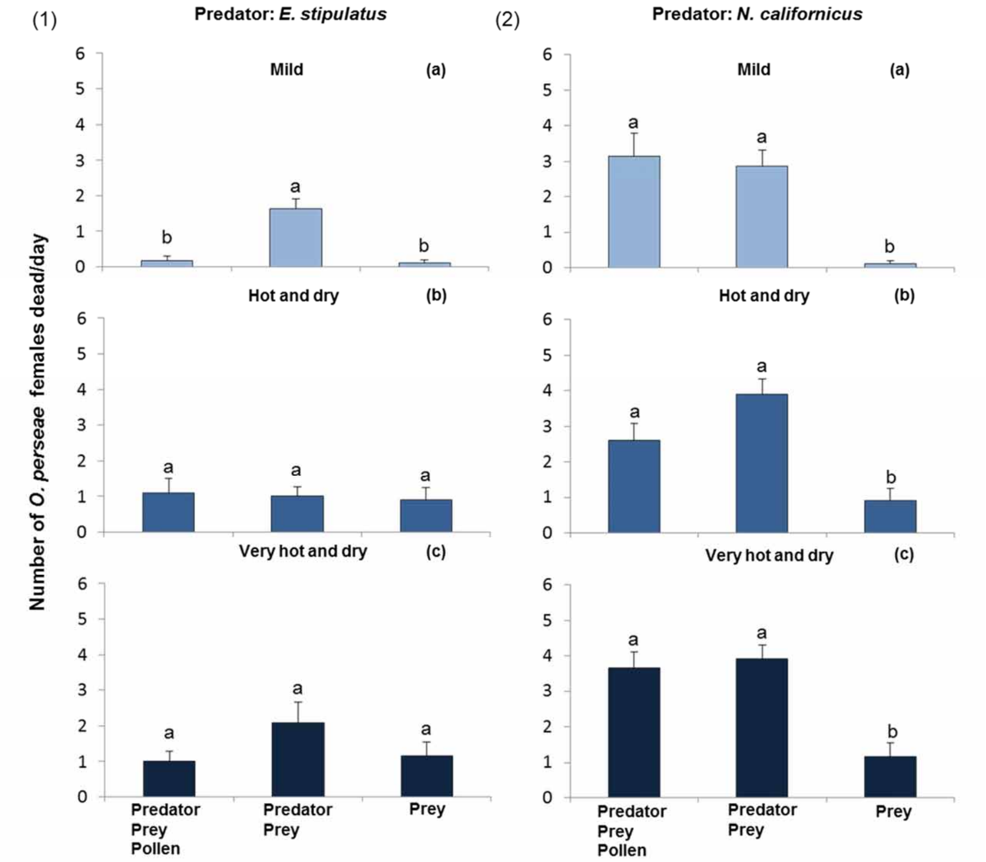

Figure 3.1.2.1. Predation/mortality rate (average ± SE) of *O. perseae* females at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry") in the presence or in the absence of (1) *E. stipulatus* or (2) *N. californicus*, or pollen. Letters above bars are from LSD post-hoc tests and indicate significant differences ($P \leq 0.05$) among treatments within abiotic conditions.

When the predator was *N. californicus*, *O. perseae* female mortality was significantly influenced by experimental treatment (Wald statistic for "experimental treatment"=29.42, *df*=2, $P << 0.001$), and such influence was unaffected by abiotic conditions (Wald statistic for "experimental treatment*abiotic condition"=7.08, *df*=4, $P = 0.132$). Mortality of *O. perseae* females was significantly higher in the presence of *N. californicus*, independent of the availability of pollen (Figure 3.1.2.1, 2; compare first and second bars with third bar). Therefore, the supply of pollen had no effect on the rates of predation in *N. californicus*.

The predation rate of *N. californicus* on *O. perseae* eggs inside the nests did not differ between treatments with or without pollen at any of the three abiotic conditions (Wald statistic for “presence of pollen*abiotic condition”=2.45, *df*=2, *P*=0.294) (Figure 3.1.2.2).

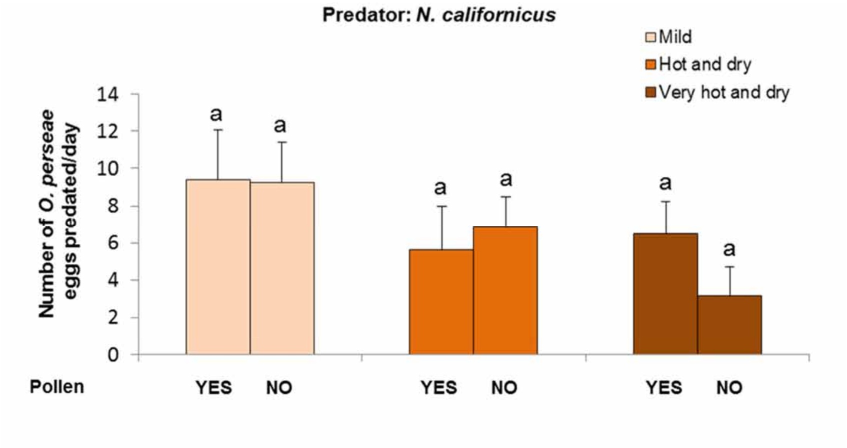

Figure 3.1.2.2. Predation rate (average ± SE) of *N. californicus* on *O. perseae* eggs at the three abiotic conditions (see legend), in the presence or in the absence of pollen. Letters above bars are from LSD post-hoc tests and indicate significant differences (*P*≤0.05) among treatments within abiotic conditions.

(ii) Oviposition rate of predators

The effect of the presence of pollen on oviposition rates of *E. stipulatus* depended on abiotic conditions (Wald statistic for “presence of pollen*abiotic conditions”=7.64, *df*=2, *P*=0.022). A subsequent analysis at M conditions exposed that the supply of pollen contributed to increase the oviposition rates (Wald statistic for “presence of pollen”=26.67, *df*=1, *P*<0.001), independently of the presence or the absence of prey (Wald statistic for “presence of pollen*presence of prey”=2.16, *df*=1, *P*=0.142) (Figure 3.1.2.3, 1a). At HD conditions, there was a significant interaction between the presence of pollen and the presence of prey (Wald statistic for “presence of pollen*presence of prey”=4.14, *df*=1, *P*=0.042), being the oviposition rates significantly higher in the presence of both pollen and prey (Figure 3.1.2.3, 1b). However, at VHD conditions, *E. stipulatus* oviposition rates were only affected by the presence of pollen (Wald statistic for “presence of pollen”=6.67, *df*=1, *P*=0.010). There was a tendency toward a higher number of eggs laid by females in those treatments in which pollen was available (Figure 3.1.2.3, 1c).

On the other hand, the presence of pollen did not affect oviposition rates of *N. californicus*, neither in the presence nor in the absence of prey at any of the three abiotic conditions (Wald statistic for "presence of pollen*presence of prey*abiotic conditions"=4.25, *df*=4, *P*=0.373) (Figure 3.1.2.3, 2).

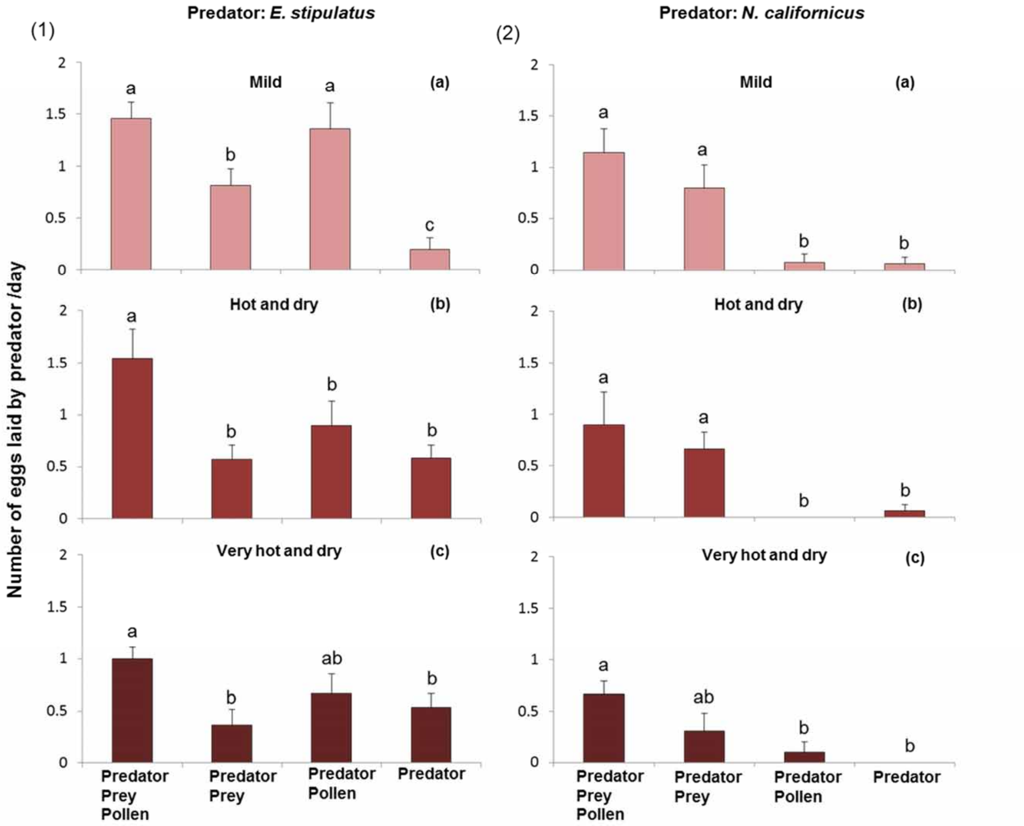

Figure 3.1.2.3. Oviposition rates (average ± SE) of (1) *E. stipulatus* and (2) *N. californicus* at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry") in the presence or in the absence of the prey, or pollen. Letters above bars are from LSD post-hoc tests and indicate significant differences (*P*≤0.05) among treatments within abiotic conditions. The figure shows original values, without the correction applied for the analyses (see M&M 2.1.1, p. 58).

(iii) Survival of predators

Neither the presence of pollen (Wald statistic for "presence of pollen"=0.25, *df*=1, *P*=0.614) nor its interaction with the other factors (i.e. "presence of prey" and "abiotic condition") affected significantly the survival of *E. stipulatus* (Wald statistic for "presence of pollen*presence of prey*abiotic condition"=0.70, *df*=4, *P*=0.952) (Figure 3.1.2.4, 1). Similarly, the presence of pollen did not influence the survival of *N. californicus* females (Wald statistic for "presence of pollen"=1.53, *df*=1, *P*=0.217), either in the presence or in the absence of prey, at any of the three abiotic conditions (Wald statistic for "presence of pollen*presence of prey*abiotic condition"=0.54, *df*=4, *P*=0.245) (Figure 3.1.2.4, 2). Therefore, the supply of pollen did not increase the survival of *N. californicus* and *E. stipulatus*.

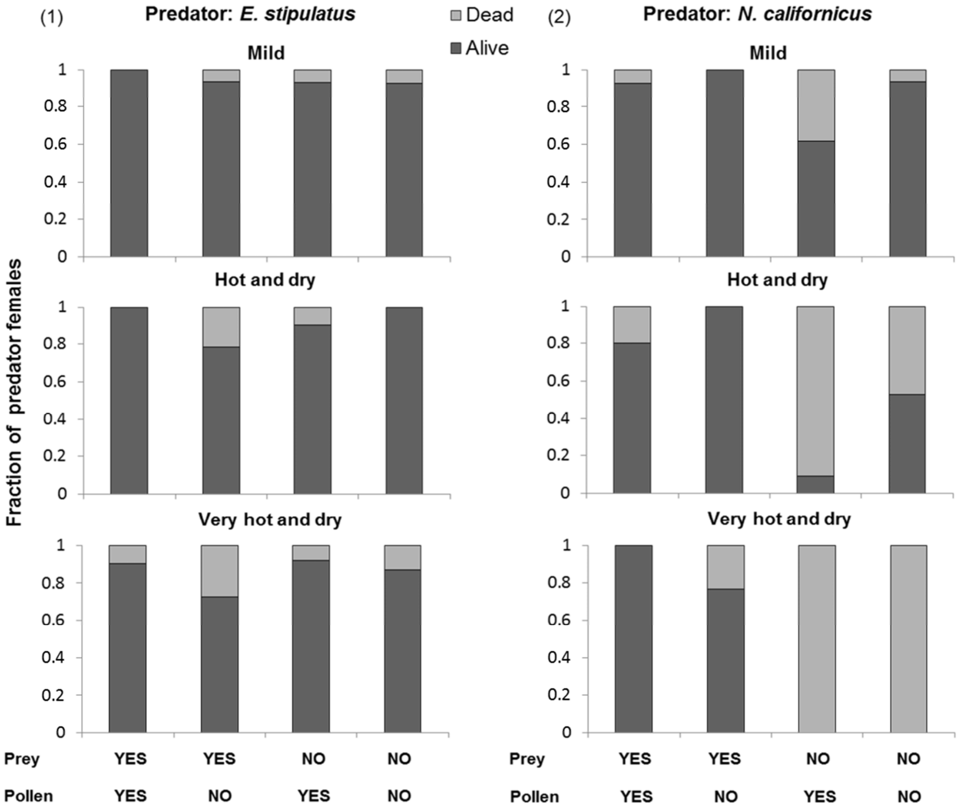

Figure 3.1.2.4. Fraction of predatory (1) *E. stipulatus* and (2) *N. californicus* females that were alive (dark grey) or dead (light grey) after 24 h at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry") in the presence or in the absence of the prey, or pollen.

3.2. Effects of abiotic conditions and presence of alternative food on intraguild interactions between two predatory mites (*E. stipulatus* or *N. californicus*) sharing an herbivore prey (*O. perseae*)

3.2.1. Community module: Intraguild predation

When the IG-predator was *E. stipulatus*

(i) Predation on the shared prey (O. perseae females and eggs)

O. perseae female mortality rates were significantly influenced by the presence of both the IG-predator (*E. stipulatus*) and the IG-prey (*N. californicus*), and such influence varied among abiotic conditions (Wald statistic for "presence of IG-predator*presence of IG-prey*abiotic condition"=8.55, *df*=2, *P*=0.014). When the effect of both factors was analysed at each abiotic condition separately, results showed that, at M conditions, *O. perseae* mortality was affected by a significant interaction between the presence of IG-predator and the presence of IG-prey (Wald statistic for "presence of IG-predator*presence of IG-prey"=7.73, *df*=1, *P*=0.005). In the presence of IG-predator, prey mortality was higher when the IG-predator and the IG-prey were together (Figure 3.2.1.1a; compare first and second bars). Additionally, LSD post-hoc tests revealed that there were not significant differences in the number of *O. perseae* dead females between the treatment in which both the IG-predator and the IG-prey were present and the treatment in which the IG-prey was alone (Figure 3.2.1.1a; compare first and third bars). This result indicated that only juveniles of *N. californicus* could have preyed on *O. perseae* females in the treatment in which both predator species were together. At HD conditions, prey female mortality rates only were significantly influenced by the presence of IG-prey (Wald statistic for "presence of IG-prey=42.94, *df*=1, *P*<0.001). However, the number of *O. perseae* dead females in the treatment in which both the IG-predator and the IG-prey were present was significantly higher than in the treatment in which the IG-prey was alone (Figure 3.2.1.1b; compare first and third bars), indicating that both predator species might have preyed on *O. perseae* females. At VHD conditions, the result was similar that of HD conditions (Figure 3.2.1.1; compare b and c).

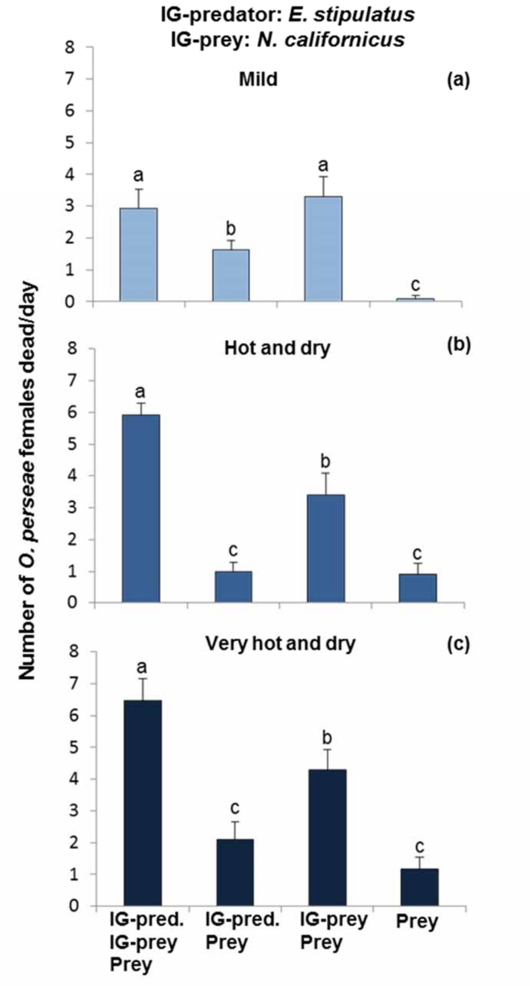

Figure 3.2.1.1. Predation/mortality rate (average ± SE) of *O. perseae* females at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry") in the presence or in the absence of the IG-predator (IG-pred.; *E. stipulatus*), or the IG-prey (*N. californicus*). Letters above bars are from LSD post-hoc tests and indicate significant differences ($P \leq 0.05$) among treatments within abiotic conditions.

With regard to predation on *O. perseae* eggs, results revealed that juveniles of *N. californicus* preyed on *O. perseae* eggs at the three abiotic conditions, showing that juveniles are able to go inside the nests (Figure 3.2.1.2). Because nests were undamaged, *N. californicus* juveniles probably penetrated through the entrances built by *O. perseae* individuals. Predation rates on *O. perseae* eggs were affected by abiotic conditions (Wald statistic for "abiotic condition"=6.51, *df*=2, *P*=0.039), independently of the presence or the absence of the IG-predator (Wald statistic for "presence of predator*abiotic condition"=0.96, *df*=2, *P*=0.953). Rates of predation on eggs were higher at M conditions (Figure 3.2.1.2).

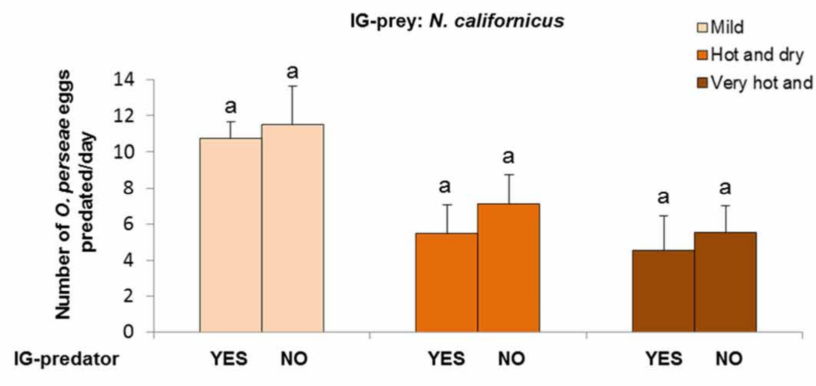

Figure 3.2.1.2. Predation rate (average ± SE) of *O. perseae* eggs by *N. californicus* juveniles (IG-prey) at the three abiotic conditions (see legend), in the presence and in the absence of the IG-predator (*E. stipulatus* females). Letters above bars are from LSD post-hoc tests and indicate significant differences (*P*≤0.05) among treatments within abiotic conditions.

(ii) Predation on the IG-prey (N. californicus juveniles)

IG-prey (*N. californicus*) mortality rates were significantly affected by the presence of the IG-predator (*E. stipulatus*) and by the presence of prey (*O. perseae*), although such effects varied among abiotic conditions (Wald statistic for "presence of IG-predator*abiotic condition"=15.93, *df*=2, *P*<0.001; "presence of prey*abiotic condition"=8.21, *df*=2, *P*=0.016). The analysis at M conditions revealed that the presence of prey reduced juvenile mortality in the absence of IG-predator, but not in its presence (Wald statistic for "presence of IG-predator*presence of prey"=7.92, *df*=1, *P*=0.005) (Figure 3.2.1.3a; compare first and third bars (green tone)). In fact, juvenile mortality was significantly higher in those treatments in the presence of IG-predator when compared to the treatment in the absence of IG-predator and prey (i.e. natural mortality) (Figure 3.2.1.3a (green tone)), indicating that females of *E. stipulatus* probably preyed on juveniles of *N. californicus*. However, at HD conditions the analysis showed that only the presence of prey influenced on IG-prey mortality rates (Wald statistic for "presence of prey"=5.28, *df*=1, *P*=0.022) (Figure 3.2.1.3b (green tone)). Lastly, results from the analysis at VHD conditions exposed that none of the factors (i.e. presence of IG-predator and presence of prey) or their interaction was statistically significant (Figure 3.2.1.3c (green tone)).

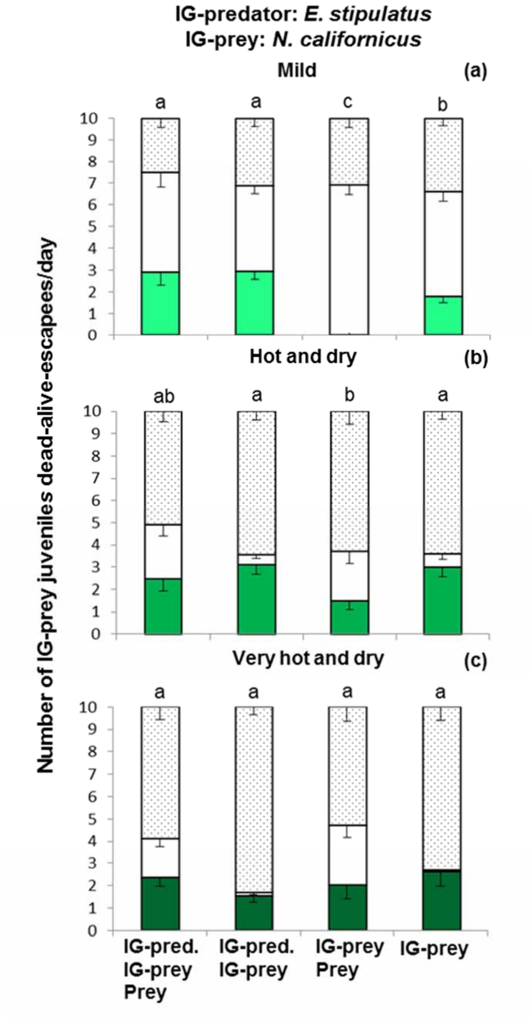

Figure 3.2.1.3. Number (average ± SE) of IG-prey juveniles (*N. californicus*) dead (green tones), alive (white) and escapees from experimental arena to the tanglefoot (dotted) at the three abiotic conditions ("mild", "hot and dry"and "very hot and dry") in the presence or in the absence of the IG-predator (IG-pred.; *E. stipulatus*), or the prey (*O. perseae* females). Letters above bars are from LSD post-hoc tests and indicate significant differences (*P*≤0.05) in the number of IG-prey dead juveniles (predation/mortality rate) among treatments within abiotic conditions.

(iii) Oviposition rates

The analysis of the effect of presence of prey (*O. perseae*), presence of IG-prey (*N. californicu*s) and abiotic conditions on oviposition rates of *E. stipulatus* females showed a significant interaction among the three factors (Wald statistic for “presence of prey*presence of IG-prey*abiotic condition”=12.76, *df*=2, *P*=0.002). A subsequent analysis at M conditions indicated that the presence of prey significantly increased the oviposition rate of the IG-predator, but only in the absence of IG-prey (Wald statistic for “presence of prey*presence of IG-prey”=9.08, *df*=1, *P*=0.003) (Figure 3.2.1.4a; compare first and second bars). However, the analyses both at HD, and at VHD conditions exposed that none of factors affected oviposition rates of the IG-predator (Figure 3.2.1.4, b and c).

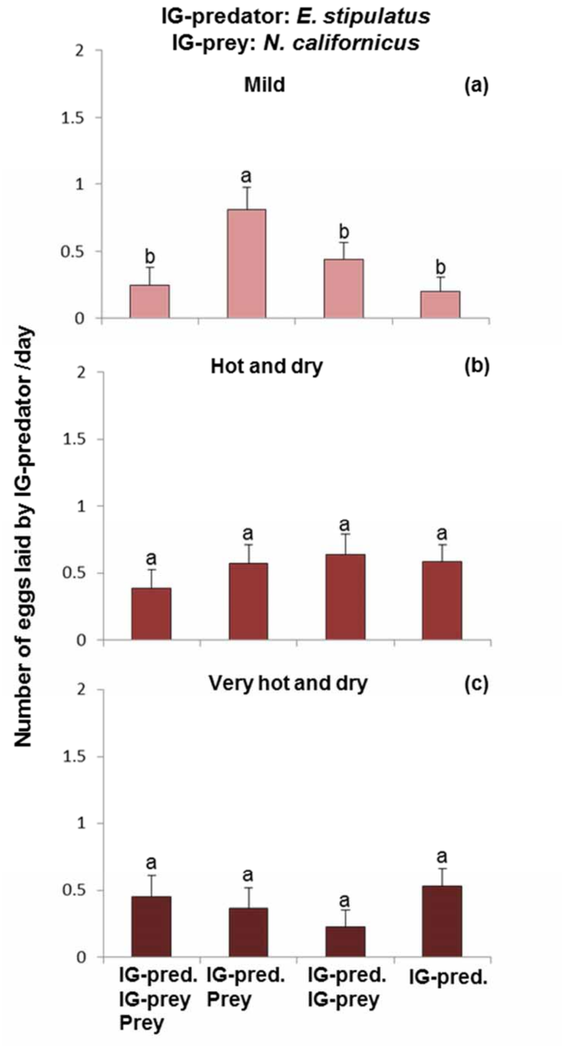

Figure 3.2.1.4. Oviposition rates (average ± SE) of *E. stipulatus* females at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry"), in the presence or in the absence of the prey (*O. perseae* females) or the IG-prey (*N. californicus*). Letters above bars are from LSD post-hoc tests and indicate significant differences ($P \leq 0.05$) among treatments within abiotic conditions. The figure shows original values, without the correction applied for the analyses (see M&M 2.2.1, p. 58).

When the IG-predator was *N. californicus*

(i) Predation on the shared prey (O. perseae females and eggs)

The effect of the presence of both IG-predator (*N. californicus*) and IG-prey (*E. stipulatus*) on *O. perseae* mortality rates was different depending on abiotic conditions (Wald statistic for "presence of IG-predator*presence of IG-prey*abiotic condition"=9.57, *df*=2, *P*=0.008). A subsequent analysis at M conditions showed that the number of *O. perseae* dead females was significantly influenced by the presence of the IG-predator (Wald statistic for "presence of IG-predator"=10.55, *df*=1, *P*=0.001), although this number was reduced to half when the IG-prey was also present (Wald statistic for "presence of IG-predator*presence of IG-prey"=9.14, *df*=1, *P*=0.003) (Figure 3.2.1.5a; compare first and second bars). In addition, there were not significant differences between the number of *O. perseae* dead females in the treatment in which the IG-predator and the IG-prey were together and the treatment in which only the IG-prey was present (Figure 3.2.1.5a). This result indicated that only juveniles of *E. stipulatus* could have preyed on *O. perseae* females in the presence of both predatory mite species. At HD conditions, the analysis exposed that *O. perseae* mortality rates only were influenced by the presence of IG-predator (Wald statistic for "presence of IG-predator"=36.8, *df*=1, *P*<0.001): IG-predator predation rates on *O. perseae* were not affected by the presence or the absence of IG-prey (Wald statistic for "presence of IG-predator*presence of IG-prey"=0.74, *df*=1, *P*=0.391) (Figure 3.2.1.5b; compare first and second bars). Finally, results from the analysis at VHD conditions showed that O. *perseae* mortality rates were significantly influenced by the presence of both IG-predator and IG-prey (Wald statistic for "presence of IG-predator"=24.20, *df*=1, *P*<0.001; "presence of IG-prey"=4.14, *df*=1, *P*=0.042), but similar to HD conditions, the presence of IG-prey had no effect on IG-predator predation rates on *O. perseae* females (Wald statistic for "presence of IG-predator*presence of IG-prey"=1.89, *df*=1, *P*=0.169) (Figure 3.2.1.5c; compare first and second bars). Therefore, results suggested that only females of *N. californicus* might have preyed on *O. perseae* females when IG-predator and IG-prey were together, both at HD and VHD conditions.

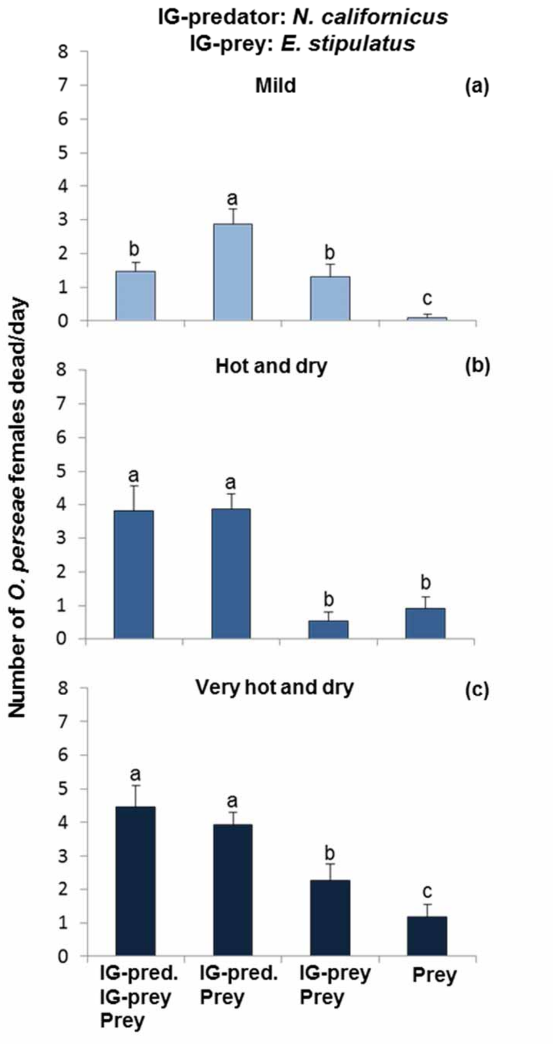

Figure 3.2.1.5. Predation/mortality rate (average ± SE) of *O. perseae* females at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry") in the presence or in the absence of the IG-predator (IG-pred.; *N. californicus*), or the IG-prey (*E. stipulatus*). Letters above bars are from LSD post-hoc tests and indicate significant differences ($P \leq 0.05$) among treatments within abiotic conditions.

Predation rates of *N. californicus* females on *O. perseae* eggs inside the nests depended on both the presence of IG-prey and abiotic conditions (Wald statistic for "presence of IG-prey*abiotic condition"=13.6, *df*=2, *P*=0.001). At M conditions, the number of eggs eaten by females was significantly higher in the absence of IG-prey (Figure 3.2.1.6a). However, at HD and VHD conditions, the presence of IG-prey had no effect on egg predation rates. On the other hand, results exposed that juveniles of *E. stipulatus* were not able to penetrate inside the nests, since there was no *O. perseae* eaten eggs in the treatment in which the IG-prey was alone (Figure 3.2.1.6b).

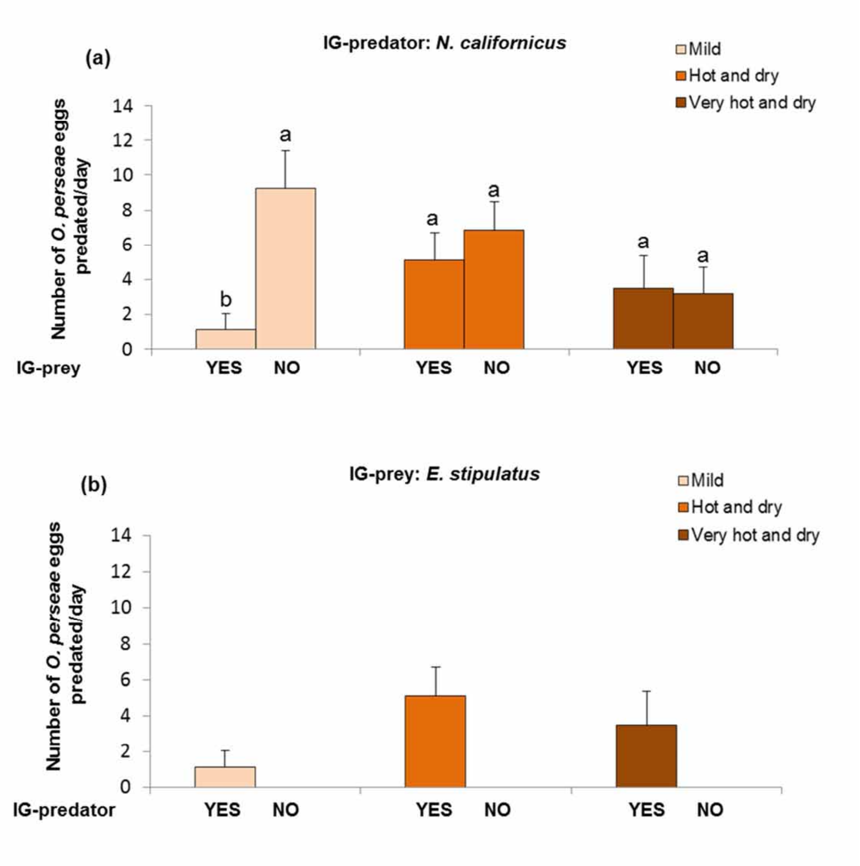

Figure 3.2.1.6. Predation rate (average ± SE) of *O. perseae* eggs by (a) *N. californicus* females (IG-predator) and (b) *E. stipulatus* juveniles (IG-prey), at the three abiotic conditions (see legend), in the presence or in the absence of the IG-prey (*E. stipulatus* juveniles), or the IG-predator (*N. californicus* females), respectively. Letters above bars are from LSD post-hoc tests and indicate significant differences ($P \leq 0.05$) among treatments within abiotic conditions.

(ii) Predation on the IG-prey (E. stipulatus juveniles)

IG-prey mortality rates were significantly affected by the presence of IG-predators (*N. californicus*) and by the presence of prey (*O. perseae*), but such effects were different among abiotic conditions (Wald statistic for "presence of IG-predator*abiotic condition"=11.33, *df*=2, *P*=0.003; "presence of prey*abiotic condition"=14.6, *df*=2, *P*=0.001). The analysis at M conditions showed that neither the presence of predator nor the presence of prey nor their interaction influenced significantly in IG-prey mortality rates (Wald statistic for "presence of IG-predator"=2.42, *df*=1, *P*=0.120; "presence of prey"=0.57, *df*=1, *P*=0.452; "presence of IG-predator*presence of prey"=0.06, *df*=1, *P*=0.808) (Figure 3.2.1.7a (green tone)). At HD conditions, results indicated that the presence of prey significantly reduced juvenile mortality (Wald statistic for "presence of prey"=18.53, *df*=1, *P*<0.001) (Figure 3.2.1.7b; compare first and third bars with second and fourth bars (green tone)). However, the presence of prey did not temper the effect of extreme abiotic conditions on juvenile mortality at VHD conditions (Wald statistic for "presence of prey"=0.42, *df*=1, *P*=0.838) (Figure 3.2.1.7c (green tone)).

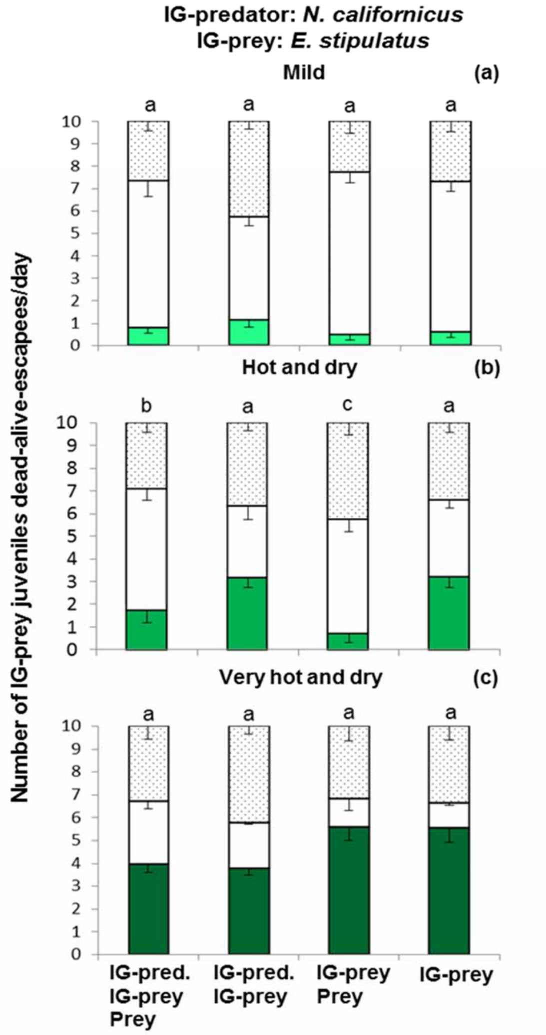

Figure 3.2.1.7. Number (average ± SE) of IG-prey juveniles (*E. stipulatus*) dead (green tones), alive (white) and escapees from experimental arena to the tanglefoot (dotted) at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry") in the presence or in the absence of the IG-predator (IG-pred.; *N. californicus*), or the prey (*O. perseae* females). Letters above bars are from LSD post-hoc tests and indicate significant differences ($P \leq 0.05$) in the number of IG-prey dead juveniles (predation/mortality rate) among treatments within abiotic conditions.

(iii) Oviposition rates

Oviposition rates of *N. californicus* females were not affected by any significant interaction among the presence of prey, the presence of IG-prey and abiotic conditions (Wald statistic for “presence of prey*abiotic condition”= 4.68, *df*=2, *P*=0.097; “presence of IG-prey*abiotic condition”= 3.31, *df*=2, *P*=0.191; “presence of prey*presence of IG-prey*abiotic condition”=2.80, *df*=2, *P*=0.246). At the three abiotic conditions, the number of eggs laid by the IG-predator in the presence of prey was lower when the IG-prey was present than when the IG-prey was absent (Wald statistic for “presence of prey*presence of IG-prey”= 8.12, *df*=1, *P*=0.004) (Figure 3.2.1.8; compare a, b and c). However, LSD post-hoc tests revealed that, at HD conditions, the oviposition rate of IG-predator in the treatment in which the prey and the IG-prey were together was not significantly different from the treatment in which only the prey was present (Figure 3.2.1.8b; compare first and second bars). This result suggested the presence of IG-prey did not affect oviposition rates of *N. californicus* females at these conditions.

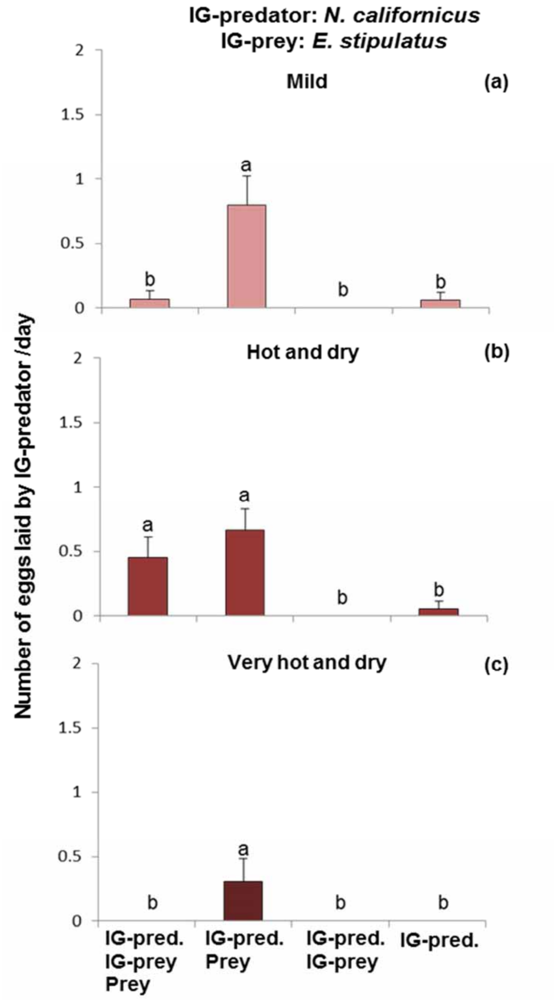

Figure 3.2.1.8. Oviposition rates (average ± SE) of *N. californicus* females at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry"), in the presence or in the absence of the prey (*O. perseae* females) or the IG-prey (*E. stipulatus*). Letters above bars are from LSD post-hoc tests and indicate significant differences ($P \leq 0.05$) among treatments within abiotic conditions. The figure shows original values, without the correction applied for the analyses (see M&M 2.2.1, p. 58).

Survival of IG-predators

The survival of *E. stipulatus* females was affected by abiotic conditions (Wald statistic for "abiotic condition"=6.29, *df*=2, *P*=0.043), independently of the presence of prey and the presence of IG-prey (Wald statistic for "presence of prey*presence of IG-prey*abiotic condition"=1.62, *df*=2, *P*=0.444). Mortality of *E. stipulatus* females increased at VHD conditions (Figure 3.2.1.9, 1). In *N. californicus*, survival of females depended on abiotic conditions (Wald statistic for "abiotic condition"=21.1, *df*=2, *P*<0.001), and on the presence of prey (Wald statistic for "presence of prey"=16.03, *df*=1, *P*<0.001), but not on the presence of IG-prey (Wald statistic for "presence of IG-prey"=0.135, *df*=2, *P*=0.713). Mortality of females was higher at HD, and at VHD conditions, although the presence of prey contributed to reduce it. (Figure 3.2.1.9, 2; compare treatments with the prey and treatments without the prey).

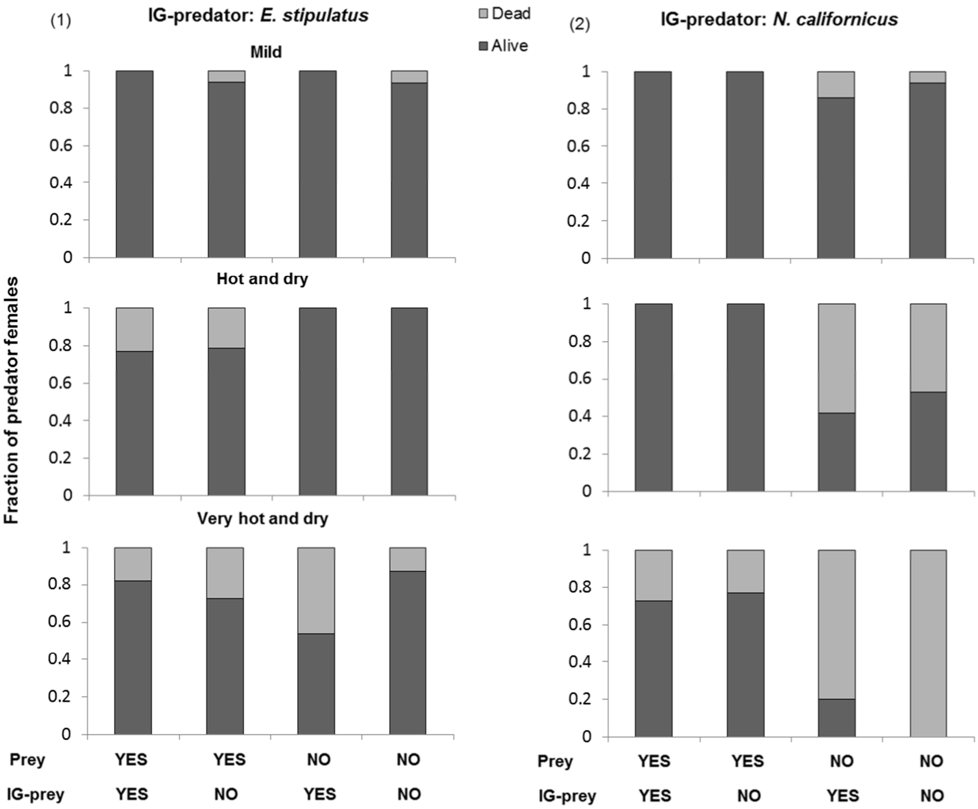

Figure 3.2.1.9. Fraction of (1) *E. stipulatus* and (2) *N. californicus* females that were alive (dark grey) or dead (light grey) after 24 h at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry") in the presence or in the absence of the prey (*O. perseae* females) or the IG-prey (*N. californicus* and *E. stipulatus* juveniles, respectively).

3.2.2. Community module: Intraguild predation - Apparent competition

<u>When the IG-predator was *E. stipulatus*</u>

(i) Predation on the shared prey (O. perseae females and eggs)

O. perseae mortality rates were differently influenced by the presence of IG-predator (*E. stipulatus*) -with and without pollen- (i.e. experimental treatment: IG-predator with pollen, IG-predator without pollen or control (i.e. only prey)), and by the presence of IG-prey (*N. californicus*) depending on abiotic conditions (Wald statistic for "experimental treatment*presence of IG-prey*abiotic condition"=16.01, *df*=6, *P*=0.013). The influence of both factors (i.e. experimental treatment and presence of IG-prey) was separately analysed at each abiotic condition. At M conditions, prey mortality depended on the experimental treatment and on the presence of IG-prey (Wald statistic for "experimental treatment*presence of IG-prey"=12.92, *df*=2, *P*=0.002). The supply of pollen contributed to reduce the number of *O. perseae* dead females in the presence of IG-predator, but not in the presence of both IG-predator and IG-prey (Figure 3.2.2.1a; compare first and second bars, and third and fourth bars). In addition, *O. perseae* mortality rates were not significantly different among treatments in which the IG-prey was present, both in the presence and in the absence of the pollen (Figure 3.2.2.1a). This result suggested that the presence of pollen did not influence on the number of *O. perseae* females eaten by juveniles of *N. californicus*. At HD conditions, the analysis revealed that *O. perseae* mortality rates only were affected by the presence of IG-prey (Wald statistic for "presence of IG-prey"=59.8, *df*=1, *P*<0.001). In the community module *intraguild predation*, results from HD conditions revealed that both the IG-predator and the IG-prey might have preyed on *O. perseae* females in the treatment in which both predatory mites were together (see section 3.2.1). However, the availability of pollen seemed to have reduced the number of *O. perseae* females predated by the IG-predator, since there were no significant differences between the treatment in which the IG-predator and the IG-prey were together in the presence of pollen, and the treatment in which the IG-prey was alone (Figure 3.2.2.1b). At VHD conditions, results from the analysis were similar that of the HD conditions (Wald statistic for "presence of IG-prey"=54.3, *df*=1, *P*<0.001) (Figure 3.2.2.1, compare b and c).

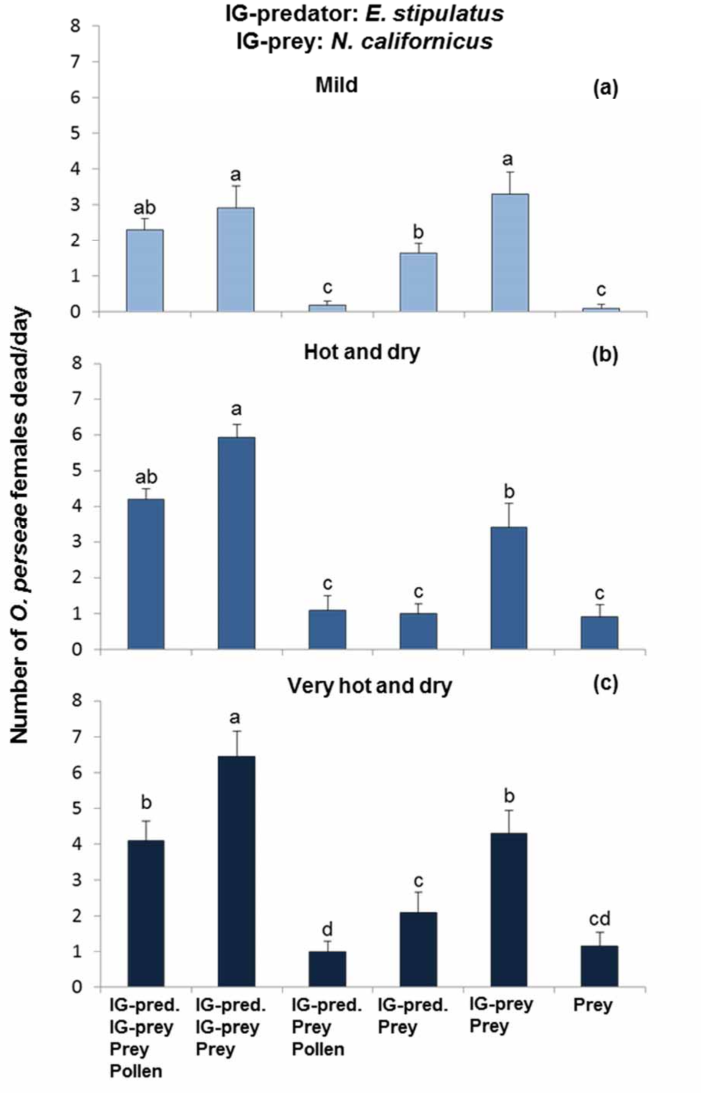

Figure 3.2.2.1. Predation/mortality rate (average ± SE) of *O. perseae* females at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry") in the presence or in the absence of the IG-predator (IG-pred.; *E. stipulatus*), the IG-prey (*N. californicus*), or pollen. Letters above bars are from LSD post-hoc tests and indicate significant differences ($P \leq 0.05$) among treatments within abiotic conditions.

Predation rates of *N. californicus* juveniles on *O. perseae* eggs inside the nests were differently affected by the presence of pollen depending on abiotic conditions (Wald statistic for “presence of pollen*abiotic condition”=7.36, *df*=2, *P*=0.025). The analysis at M conditions showed that, in overall, the presence of pollen contributed to reduce the number of *O. perseae* eggs eaten by juveniles (Wald statistic for “presence of pollen”=5.54, *df*=1, *P*=0.019), although LSD post-hoc tests revealed that there were not significant differences in the number of eggs eaten between treatments in which the pollen was present and treatment in which the pollen was absent (Figure 3.2.2.2). At HD, and at VHD conditions, the supply of pollen did not influence in predation rates of *N. californicus* juveniles on *O. perseae* eggs (Wald statistic for “presence of pollen”=2.07, *df*=1, *P*=0.151; “presence of pollen”=0.001, *df*=1, *P*=0.978, respectively)

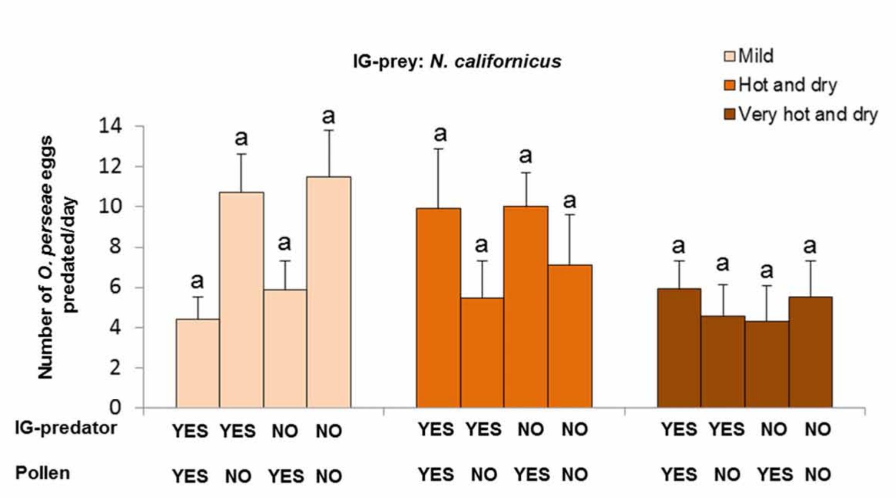

Figure 3.2.2.2. Predation rate (average ± SE) of *O. perseae* eggs by *N. californicus* juveniles (IG-prey) at the three abiotic conditions (see legend), in the presence or in the absence of the IG-predator (*E. stipulatus* females), or pollen. Letters above bars are from LSD post-hoc tests and indicate significant differences ($P \leq 0.05$) among treatments within abiotic conditions.

(ii) Predation on the IG-prey (N. californicus juveniles)

IG-prey (*N. californicus*) mortality rates were influenced by a significant interaction among the presence of IG-predator (*E. stipulatus*), the presence of pollen and abiotic conditions (Wald statistic for “presence of IG-predator*presence of pollen*abiotic condition”=22.04, *df*=4, *P*<0.001). The presence of prey (*O. perseae*) and the presence of pollen also influenced on IG-prey mortality rates (Wald statistic for “presence of prey*presence of pollen”=4.2, *df*=1, *P*=0.04), and such influence was marginally different among abiotic conditions (Wald statistic for “presence of prey*presence of pollen*abiotic condition”=9.17, *df*=4, *P*=0.057). The analyses to evaluate the effect of the three factors (i.e. presence of IG-predator, presence of prey and presence of pollen) at each abiotic condition revealed that, at M conditions, IG-prey mortality rates were significantly lower in the presence of IG-predator when pollen was available (Figure 3.2.2.3a; compare first and second bars, and third and fourth bars (green tone)), but not in the presence of prey and in the absence the IG-predator (Figure 3.2.2.3a; compare fifth and sixth bars, and seventh and eighth bars (green tone)) (Wald statistic for “presence of IG-predator*presence of prey*presence of pollen”=8.68, *df*=2, *P*=0.013). Therefore, *E. stipulatus* females did not feed on juveniles when the pollen was present, unlike what was observed in the community module *intraguild predation* in the absence of pollen (see section 3.2.1). At HD conditions, the analysis showed that the presence of pollen reduced in overall IG-prey mortality (Wald statistic for “presence of pollen”=5.09, *df*=1, *P*=0.024), independently of the presence of IG-predator and prey (Wald statistic for “presence of IG-predator*presence of prey*presence of pollen”=1.46, *df*=2, *P*=0.481) (Figure 3.2.2.3b; see treatments with pollen (green tone)). Finally, at VHD conditions, the presence of pollen did not significantly influence on IG-prey mortality rates (Wald statistic for “presence of pollen”=0.08, *df*=1, *P*=0.777).

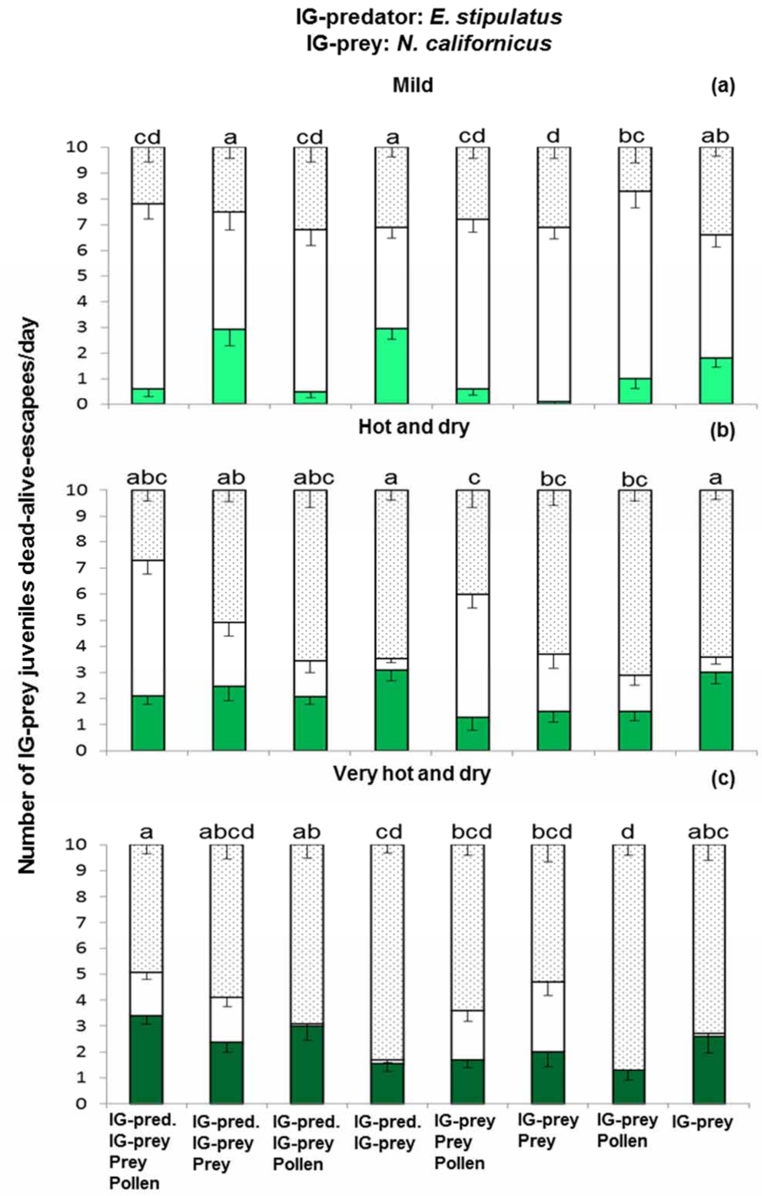

Figure 3.2.2.3. Number (average ± SE) of IG-prey juveniles (*N. californicus*) dead (green tones), alive (white) and escapees from experimental arena to the tanglefoot (dotted) at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry") in the presence or in the absence of the IG-predator (IG-pred.; *E. stipulatus*), the prey (*O. perseae* females), or pollen. Letters above bars are from LSD post-hoc tests and indicate significant differences (*P*≤0.05) in the number of IG-prey dead juveniles (predation/mortality rate) among treatments within abiotic conditions.

(iii) Oviposition rates

The availability of pollen contributed to increase oviposition rates of *E. stipulatus* females (Wald statistic for “presence of pollen”=101.72, *df*=1, *P*<0.001), independently of the presence or the absence of prey (*O. perseae*) and IG-prey (*N. californicus*) at the three abiotic conditions (Wald statistic for “presence of prey*presence of IG-prey*presence of pollen*abiotic condition”=7.01, *df*=4, *P*=0.135); although such increase was higher at M and HD than at VHD conditions (Wald statistic for “presence of pollen*abiotic condition”=16.86, *df*=2, *P*<0.001) (Figure 3.2.2.4; compare treatments with pollen and treatments without pollen at the three abiotic conditions).

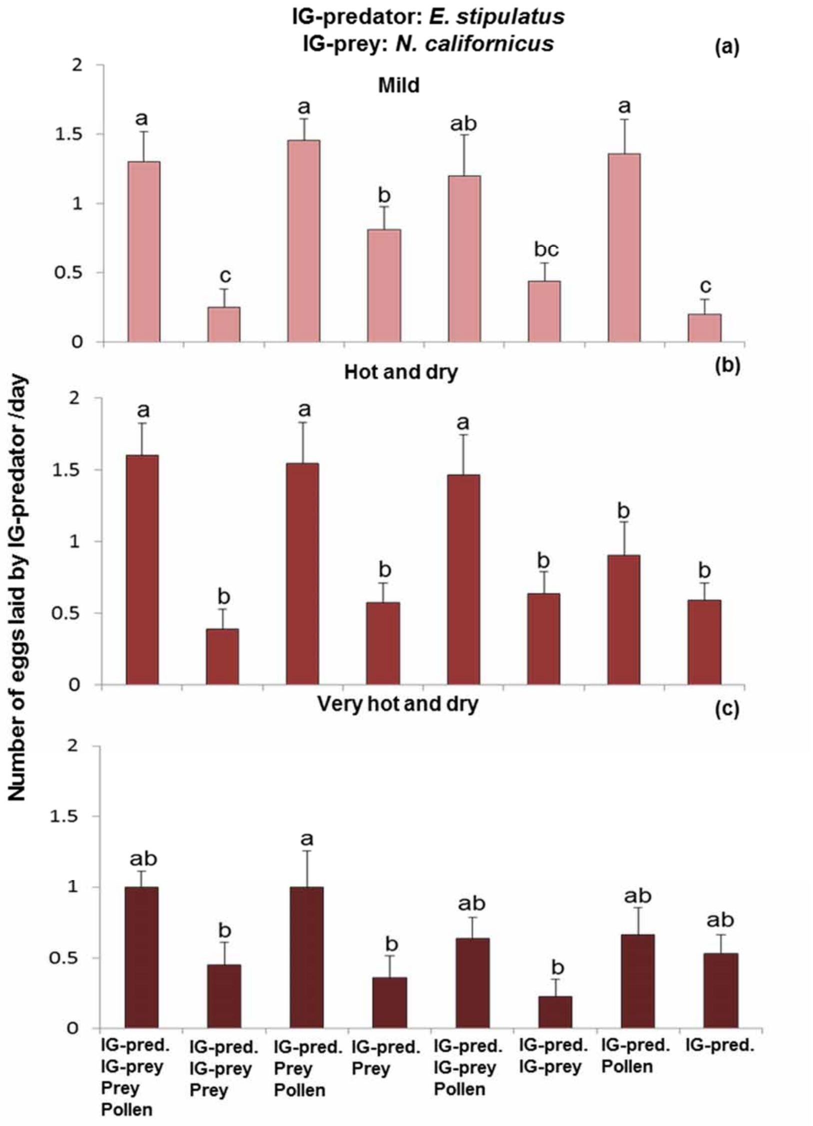

Figure 3.2.2.4. Oviposition rates (average ± SE) of *E. stipulatus* females at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry"), in the presence or in the absence of the prey (*O. perseae* females), the IG-prey (*N. californicus*), or pollen. Letters above bars are from LSD post-hoc tests and indicate significant differences ($P \leq 0.05$) among treatments within abiotic conditions. The figure shows original values, without the correction applied for the analyses (see M&M 2.2.1, p. 58).

When the IG-predator was *N. californicus*

(i) Predation on the shared prey (O. perseae females and eggs)

The analysis of the effect of the experimental treatment (i.e. IG-predator with pollen, IG-predator without pollen or control (i.e. only prey)), the presence of IG-prey (*E. stipulatus*) and abiotic conditions on *O. perseae* mortality rates showed a significant interaction among these three factors (Wald statistic for "experimental treatment*presence of IG-prey*abiotic condition"=14.48, *df*=6, *P*=0.025). The analysis at M conditions exposed that prey mortality was significantly affected by the presence of IG-predator-with and without pollen- (i.e. experimental treatment) and by the presence of IG-prey (Wald statistic for "experimental treatment*presence of IG-prey"=13.02, *df*=2, *P*=0.001). The availability of pollen did not affect the number of *O. perseae* dead females when the IG-predator was alone (Figure 3.2.2.5a; compare third and fourth bars), whereas in the treatment in which the IG-predator and the IG-prey were together, the presence of pollen contributed to increase *O. perseae* mortality rates (Figure 3.2.2.5a; compare first and second bars). In the community module *intraguild predation*, results indicated that only juveniles of *E. stipulatus* could have preyed on *O. perseae* females in the presence of both predatory mite species (see section 3.2.1). However, in this community module, results suggested that only *N. californicus* females preyed on *O. perseae* females in the presence of pollen, since there were no significant differences among the treatment in which the IG-predator and the IG-prey were together with pollen, and treatments in which the IG-predator was alone (Figure 3.2.2.5a; compare first bar with third and fourth bars). Results from the analysis at HD conditions exposed that *O. perseae* mortality rates only were influenced by the experimental treatment (Wald statistic for "experimental treatment"=36.86, *df*=2, *P*<0.001): there were significant differences between treatments in which the IG-predator was present and treatments in which the IG-predator was absent (Figure 3.2.2.5b). In addition, prey mortality rates were similar among treatments in which the IG-predator was present, independent of the presence or the absence of IG-prey and pollen (Figure 3.2.2.5b; compare the first four bars). Finally, results from the analysis at VHD conditions were similar that of the analysis at HD conditions (Wald statistic for "experimental treatment"=25.45, *df*=2, *P*<0.001) (Figure 3.2.2.5, compare b and c).

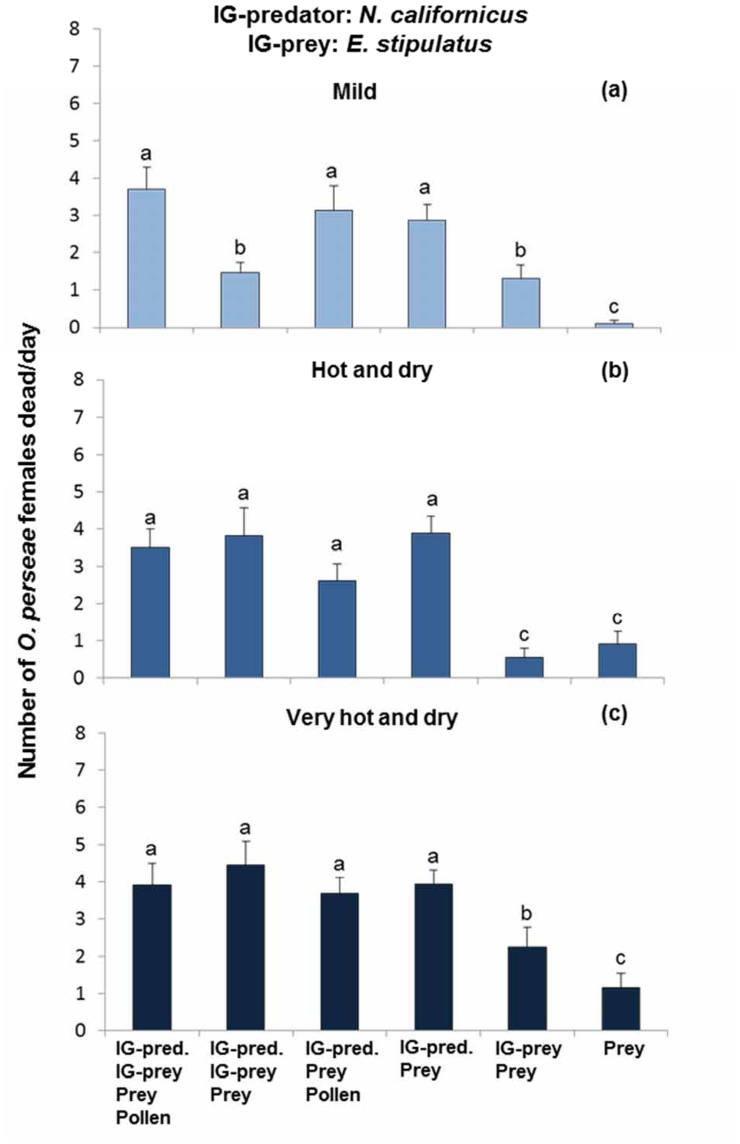

Figure 3.2.2.5. Predation/mortality rate (average ± SE) of *O. perseae* females at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry") in the presence or in the absence of the IG-predator (IG-pred.; *N. californicus*), the IG-prey (*E. stipulatus*), or pollen. Letters above bars are from LSD post-hoc tests and indicate significant differences ($P \leq 0.05$) among treatments within abiotic conditions.

The effect of the presence of IG-prey and the presence of pollen on predation rates of *N. californicus* females on *O. perseae* eggs inside the nests varied among abiotic conditions (Wald statistic for "presence of IG-prey*presence of pollen*abiotic condition"=18.5, *df*=4, *P*=0.001). At M conditions, the availability of pollen contributed to increase egg predation rates in the presence of IG-prey (Wald statistic for "presence of IG-prey*presence of pollen"=3.83, *df*=1, *P*=0.05) (Figure 3.2.2.6; compare first and second bars at mild conditions). However, at HD conditions the presence of pollen had no effect on egg predation rates, neither in the presence nor the absence of the IG-prey (Wald statistic for "presence of IG-prey*presence of pollen"=0.631, *df*=1, *P*=0.427) (Figure 3.2.2.6; see HD conditions). On the other hand, the analysis at VHD conditions showed that, in overall, the presence of pollen significantly increased the number of *O. perseae* eggs eaten by *N. californicus* females (Wald statistic for "presence of pollen"=6.36, *df*=1, *P*=0.012), although LSD post-hoc tests exposed that there were no significant differences in the number of eggs eaten between treatments in which the pollen was present and treatments in which the pollen was absent, independently of the presence or the absence of IG-prey (Wald statistic for "presence of IG-prey*presence of pollen"=0.094, *df*=1, *P*=0.759) (Figure 3.2.2.6; see VHD conditions).

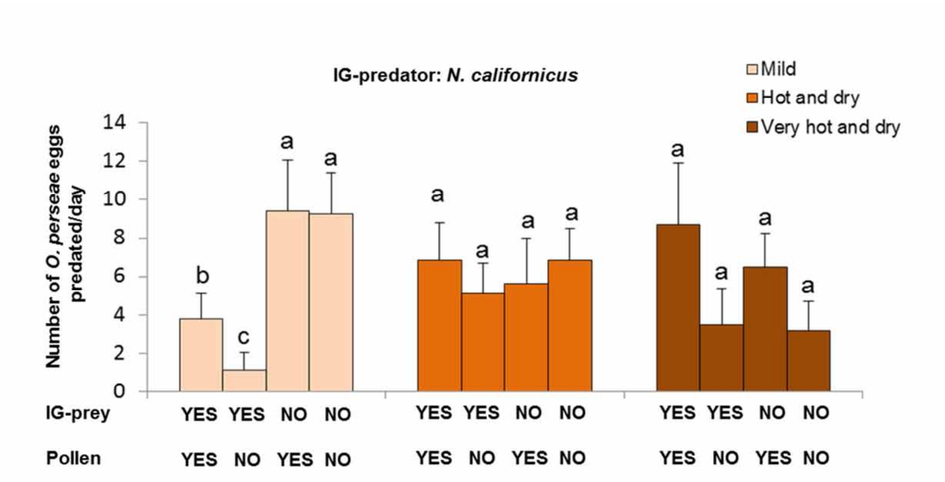

Figure 3.2.2.6. Predation rate (average ± SE) of *O. perseae* eggs by *N. californicus* females (IG-predator) at the three abiotic conditions (see legend), in the presence or in the absence of the IG-prey (*E. stipulatus* juveniles), or pollen. Letters above bars are from LSD tests and indicate significant differences ($P \leq 0.05$) among treatments within abiotic conditions.

(ii) Predation on the IG-prey (E. stipulatus juveniles)

The effect of presence of the IG-predator (*N. californicus*) and pollen on IG-prey (*E. stipulatus*) mortality rates was different depending on abiotic conditions (Wald statistic for "presence of IG-predator*presence of pollen*abiotic condition"=12.24, *df*=4, *P*=0.016). Additionally, the effect of presence of prey and pollen also depended on abiotic conditions (Wald statistic for "presence of prey*presence of pollen*abiotic condition"=15.67, *df*=4, *P*=0.003). The analysis at M conditions exposed that IG-prey mortality rates were lower in the presence of pollen (Wald statistic for "presence of pollen"=9.61, *df*=1, *P*=0.02), independently on whether the IG-predator and the prey were present or absent (Wald statistic for "presence of IG-predator*presence of prey* presence of pollen"=1.09 *df*=2, *P*=0.581) (Figure 3.2.2.7a; compare treatments with pollen and treatments without pollen (green tone)). At HD conditions, the supply of pollen contributed to reduce IG-prey mortality in the presence of IG-predator, but not whether the prey was also present (Wald statistic for "presence of IG-predator*presence of prey*presence of pollen"=0.21 *df*=2, *P*=0.045) (Figure 3.2.2.7b (green tone)). Finally, at VHD conditions, IG-prey mortality rates were lower in the presence of pollen (Wald statistic for “presence of pollen”=67.74, *df*=1, *P*<0.001), both in the presence of IG-predator and in the presence of prey (Wald statistic for “presence of IG-predator*presence of prey* presence of pollen”=0.41 *df*=2, *P*=0.813) (Figure 3.2.2.7c; compare treatments with pollen and treatments without pollen (green tone)).

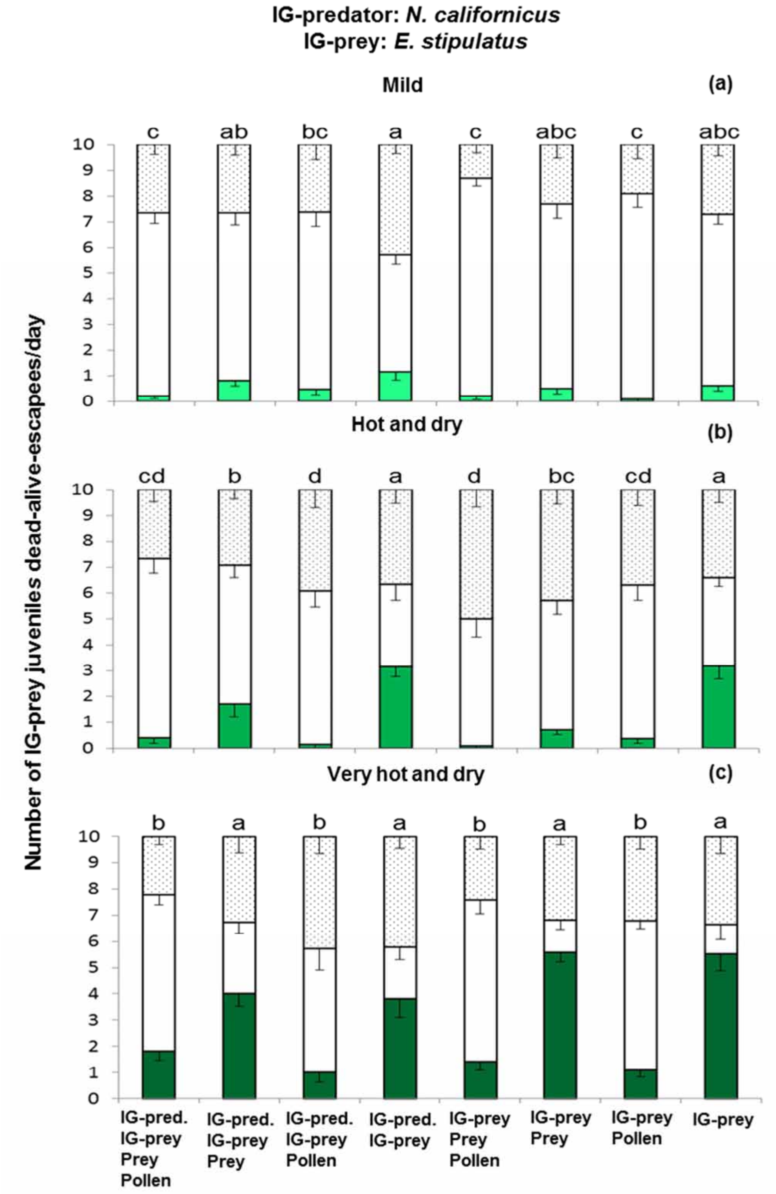

Figure 3.2.2.7. Number (average ± SE) of IG-prey juveniles (*E. stipulatus*) dead (green tones), alive (white) and escapees from experimental arena to the tanglefoot (dotted) at the three abiotic conditions (“mild”, “hot and dry” and “very hot and dry”) in the presence or in the absence of the IG-predator (IG-pred.; *N. californicus*), the prey (*O. perseae* females), or pollen. Letters above bars are from LSD post-hoc tests and indicate significant differences (*P*≤0.05) in the number of IG-prey dead juveniles (predation/mortality rate) among treatments within abiotic conditions.

(iii) Oviposition rates

Oviposition rates of *N. californicus* females were affected by the presence of pollen and the presence of prey (Wald statistic for "presence of pollen*presence of prey"=19.34, *df*=1, *P*<0.001), independently of abiotic conditions (Wald statistic for "presence of pollen*presence of prey*abiotic condition"=3.52, *df*=4, *P*=0.474): oviposition rates were higher in the presence of pollen when the prey was also present. On the other hand, the overall number of eggs laid by *N. californicus* females in the presence of IG-prey was significantly higher when the pollen was supply (Wald statistic for "presence of pollen*presence of IG-prey"=4.1, *df*=1, *P*=0.043), at the three abiotic conditions (Wald statistic for "presence of pollen*presence of IG-prey*abiotic condition"=6.26, *df*=4, *P*=0.181) (Figure 3.2.2.8).

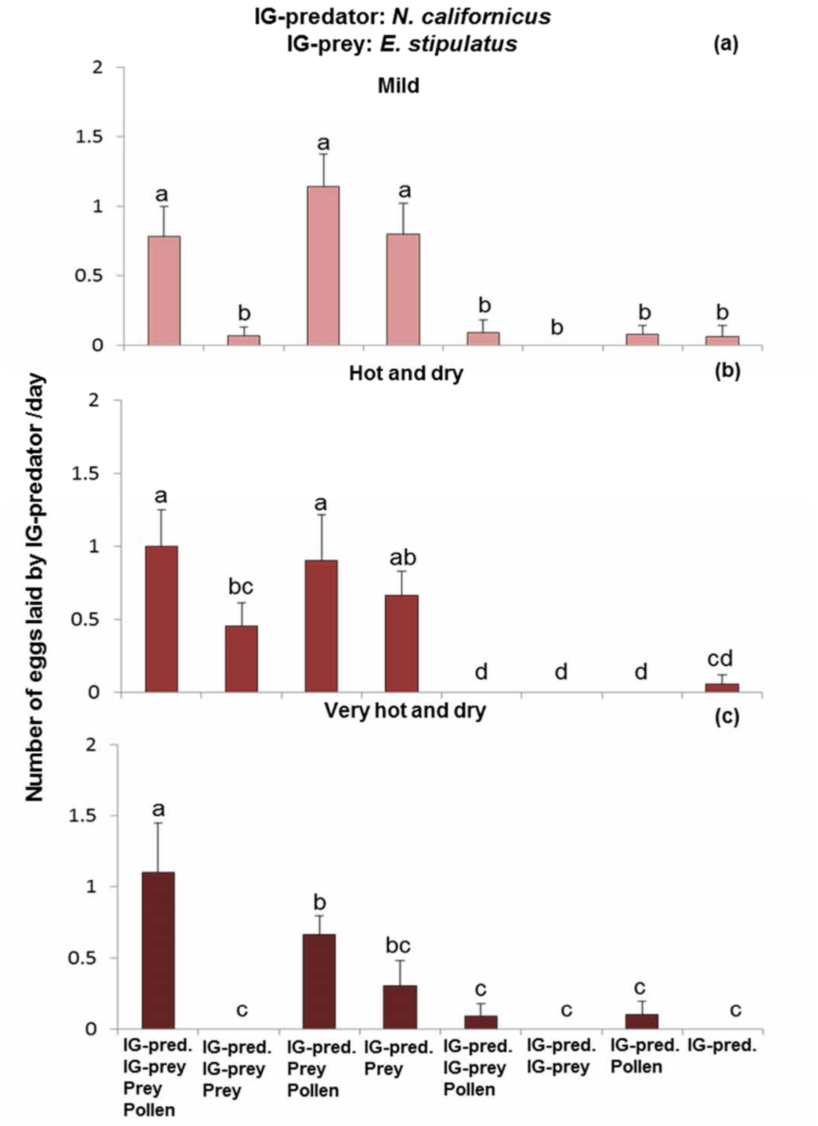

Figure 3.2.2.8. Oviposition rates (average ± SE) of *N. californicus* females at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry"), in the presence or in the absence of the prey (*O. perseae* females), the IG-prey (*E. stipulatus*), or pollen. Letters above bars are from LSD post-hoc tests and indicate significant differences ($P \leq 0.05$) among treatments within abiotic conditions. The figure shows original values, without the correction applied for the analyses (see M&M 2.2.1, p. 58).

Survival of IG-predators

The availability of pollen did not affect the survival of *E. stipulatus* females (Wald statistic for "presence of pollen"=1.08, *df*=1, *P*=0.298), neither in the presence nor in the absence of prey and IG-prey at any of abiotic conditions (Wald statistic for “presence of prey* presence of IG-prey*presence of pollen*abiotic conditions”=1.74, *df*=4, *P*=0.784) (Figure 3.2.2.9, 1). Similarly, the presence of pollen did not increase *N. californicus* female survival (Wald statistic for “presence of pollen”=1.08, *df*=1, *P*=0.300), neither in the presence nor in the absence of prey, or IG-prey, at any of the abiotic conditions (Wald statistic for “presence of prey* presence of IG-prey*presence of pollen*abiotic conditions”=1.74, *df*=4, *P*=0.784) (Figure 3.2.2.9, 2).

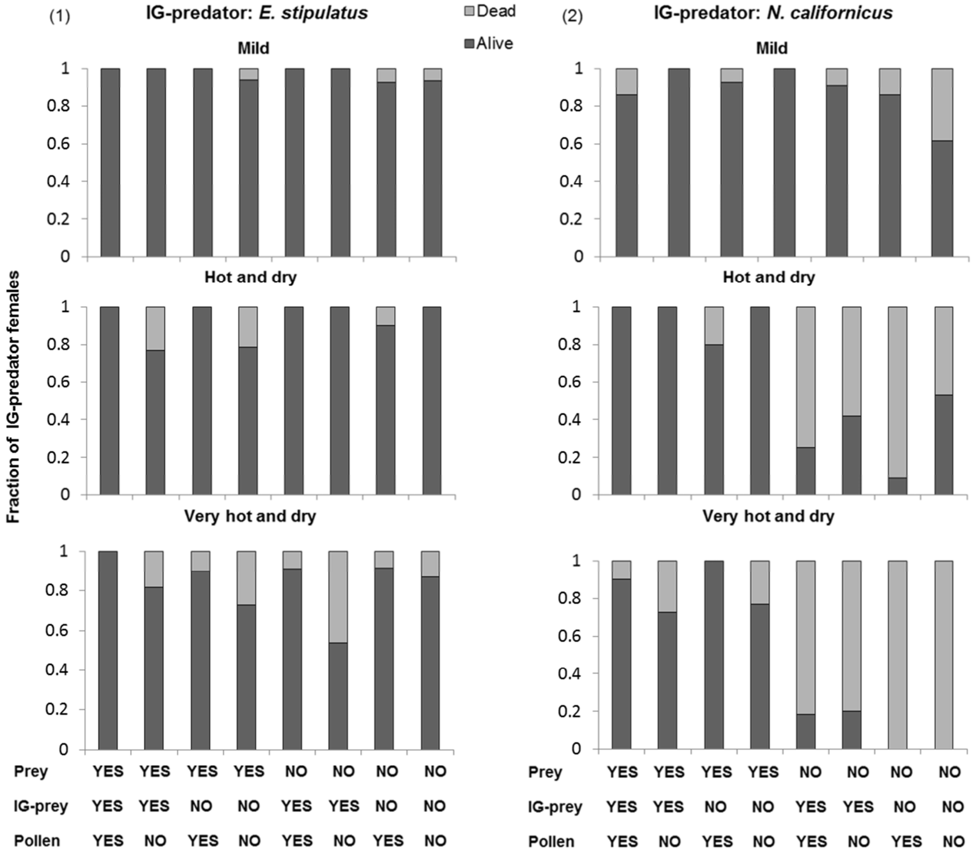

Figure 3.2.2.9. Fraction of predatory (1) *E. stipulatus* and (2) *N. californicus* females that were alive (dark grey) or dead (light grey) after 24 h at the three abiotic conditions (“mild”, “hot and dry” and “very hot and dry”) in the presence or in the absence of the prey (*O. perseae* females), the IG-prey (*N. californicus* and *E. stipulatus* juveniles, respectively), or pollen.

3.3. Unravelling behavioural/environmental effects: fine-tuning of results

3.3.1. IG-prey role reversals triggered by IG-predators diet

The overall MANOVA did not bring significant differences between rates of predation of *N. californicus* juveniles on *E. stipulatus* eggs produced from mothers fed with either pollen or eggs of *O. perseae* (Table 3.3.1.1).

Table 3.3.1.1. Results of MANOVA with domatia, midrib and limbo, as dependent variables, and type of food (pollen or *O. perseae* eggs), as explanatory variable.

Effect	Wilks λ	*F*	*d.f.*	*P*
Intercept	0.352	9.819	3;16	0.001
Type of food	0.766	1.625	3;16	0.223

However, subsequent univariate ANOVA's revealed that predation of *N. californicus* juveniles on eggs of *E. stipulatus* located in domatia tended to be higher when eggs were produced from mothers feeding on pollen (Table 3.3.1.2., Figure 3.3.1.1.).

Table 3.3.1.2. Results of three univariante ANOVA with domatia, midrib and limbo, as dependent variables, and type of food (pollen or *O. perseae* eggs), as explanatory variable.

Dependent variables	Explanatory variables	*d.f.*	Mean square	*F*	*P*
Domatia	Intercept	1	18.050	30.943	<0.0001
	Type of food	1	2.450	4.200	0.055
	Error	18	0.583		
Midrib	Intercept	1	14.450	18.447	<0.001
	Type of food	1	0.450	0.575	0.458
	Error	18	0.783		
Limbo	Intercept	1	5.000	8.333	0.010
	Type of food	1	0.200	0.333	0.571
	Error	18	0.600		

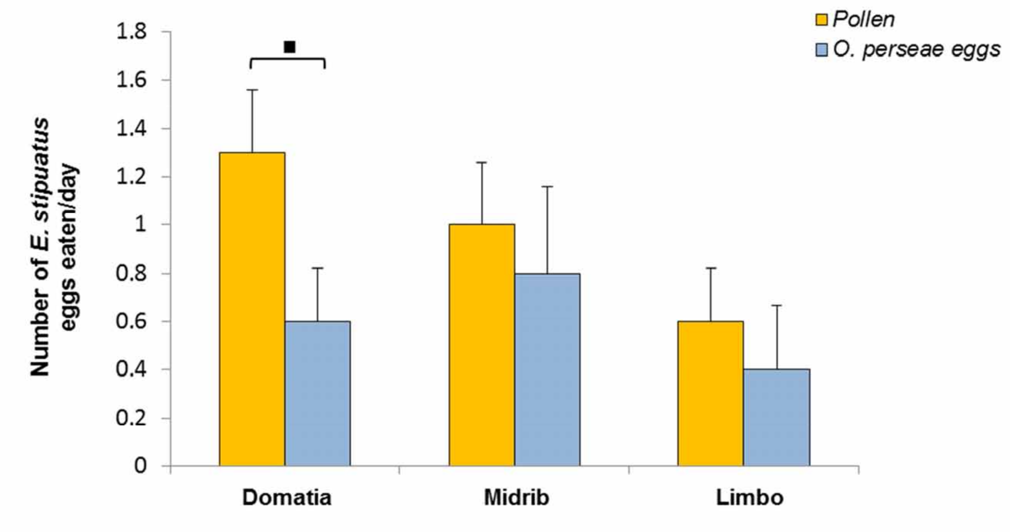

Figure 3.3.1.1. Predation rate (average ± SE) of *E. stipulatus* eggs by *N. californicus* juveniles depending on the type of food (i.e. pollen or *O. perseae* eggs) ingested by *E. stipulatus* females before oviposition in the three different locations within avocado leaf discs (i.e. domatia, midrib and limbo). The symbol (■) indicates marginal significant differences (*P*=0.055).

3.3.2. Nests of *O. perseae* as refuges for *N. californicus* juveniles against adverse abiotic conditions

N. californicus juvenile mortality

The mortality of *N. californicus* juveniles was affected by abiotic conditions (Wald statistic for "abiotic condition"=6.08, *df*=2, *P*=0.048): juvenile mortality at VHD conditions was significantly higher than at M and HD conditions (Figure 3.3.2.1). Results from the analysis showed that the number of dead *N. californicus* did not vary between the treatment with nests and the treatment without nests within each abiotic condition (Wald statistic for "abiotic condition*nests"=9.98, *df*=2, *P*=0.613). However, results from LSD post-hoc test exposed that juvenile mortality at VHD conditions was significantly higher in the treatment with nests (Figure 3.3.2.1).

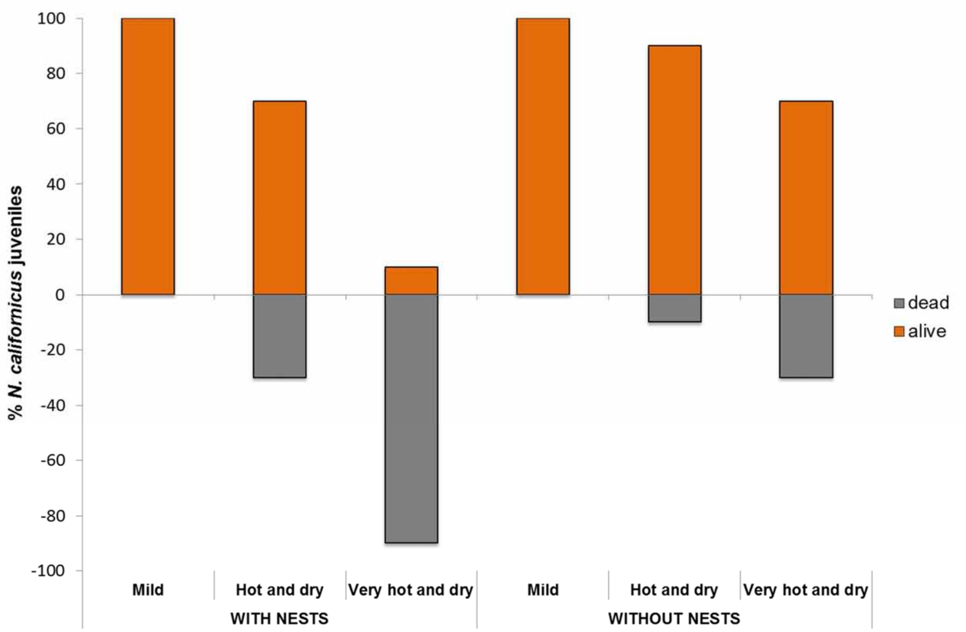

Figure 3.3.2.1. Percentage of juveniles of *N. californicus* that were alive or dead (see legend) after 24 h at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry") in the treatment with nests and in the treatment without nests.

Predation on eggs of O. perseae

Differences in the number of *O. perseae* predated eggs by *N. californicus* juveniles between paired replicates (i.e. replicates with nests and replicates without nests) were influenced by abiotic conditions (Wald statistic for “abiotic condition”=19.32, *df*=2, *P*<0.001). Such differences significantly varied between M and HD conditions, and VHD conditions, but not between M and HD conditions (Figure 3.3.2.2). At the three abiotic conditions, *N. californicus* juveniles significantly predated a higher number of *O. perseae* eggs in the absence of nests (Table 3.3.2.1), since the three mean differences were less than ‘0’ (Figure 3.3.2.2), and none of 95% Wald confident intervals included 0 (M: (-6.95, -2.62); HD: (-7.27, -2.13); VHD: (-14.17, -9.03)).

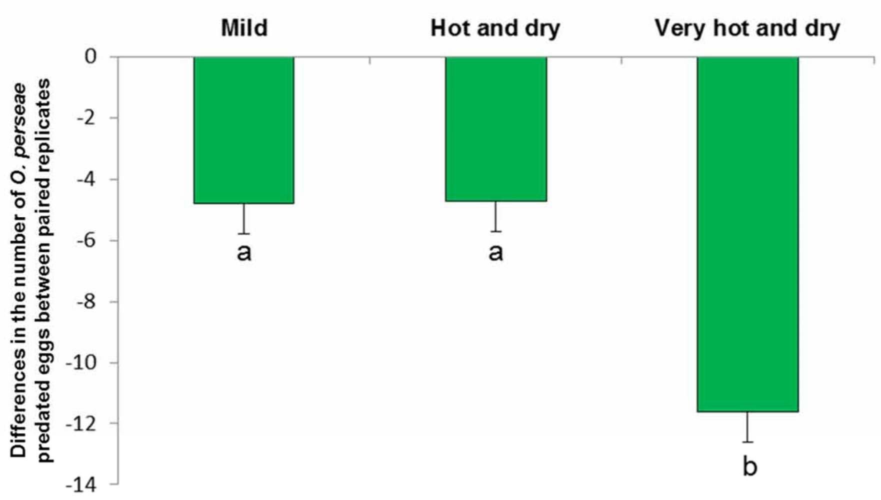

Figure 3.3.2.2. Differences (average ± SE) in the number of *O. perseae* predated eggs by *N. californicus* juveniles between paired replicates at the three abiotic conditions (“mild”, “hot and dry” and “very hot and dry”). Differences in the number of eaten eggs between paired replicates were estimated by subtracting the number of eaten eggs in replicates without nests from the number of eaten eggs in replicates with nests. Differences that were < ‘0’ indicated a higher number of eaten eggs in the absence of nests; whereas differences that were > ‘0’ indicated a higher number of eaten eggs in the presence of nests. Letters below bars are from LSD post-hoc test and indicate significant differences (*P*≤0.05).

Table 3.3.2.1. Predation rates (average ± SE) of *N. californicus* juveniles on *O. perseae* eggs at the three abiotic conditions ("mild", "hot and dry" and "very hot and dry") depending on nest treatment.

Abiotic condition	**Nest treatment**	
	With nests	**Without nests**
Mild	4.93±0.77	9.71± 1.08
Hot and dry	3.70±0.60	8.40± 0.70
Very hot and dry	0.80±0.59	12.40±1.03

3.4. Effects of abiotic conditions and presence of alternative food on mite predator/prey population dynamics in avocado

3.4.1. Mite predator/prey population dynamics in avocado seedlings under two abiotic conditions, and in the presence or absence of pollen

Destructive sampling

The population abundance of the two predatory mites, *E. stipulatus* and *N. californicus* (predators, hereafter), per leaf at the end of the experiment was significantly affected by abiotic conditions (Table 3.4.1.1): there were higher numbers of predators at M conditions than at HD conditions (Figure 3.4.1.1a). In addition, at M conditions the final number of predators per leaf was marginally different between treatments (Wald statistic for "treatment (M conditions)"=3.362, *df*=1, *P*=0.057), but not at HD conditions (Wald statistic for "treatment (HD conditions)"=0.314, *df*=1, *P*=0.576) (Figure 3.4.1.1a).

Table 3.4.1.1 Results of the 3-factor full-nested GLMMs analysis, with total number of predatory mites (eggs, juveniles and adults), occupied *O. perseae* nests, and necrotic spots per leaf, as dependent variables, and "abiotic condition" (mild or hot and dry), "treatment" (with pollen or without pollen) nested to abiotic condition, and "plant" nested to treatment and to abiotic condition, as main factors.

Dependent variables	**Sources of variation**	***F***	***d.f.***	***P***
#Predators per leaf	Abiotic cond.	53.691	1	<0.0001
	Treatment (Abiotic cond.)	1.981	2	0.139
	Plant (Treatment (Abiotic cond.))	7.453	12	<0.0001
#Nests per leaf	Abiotic cond.	498.045	1	<0.0001
	Treatment (Abiotic cond.)	39.199	2	<0.0001
	Plant (Treatment (Abiotic cond.))	47.761	12	<0.0001
# Necrotic spots per leaf	Abiotic cond.	0.009	1	0.926
	Treatment (Abiotic cond.)	8.025	2	<0.0001
	Plant (Treatment (Abiotic cond.))	27.799	12	0.001

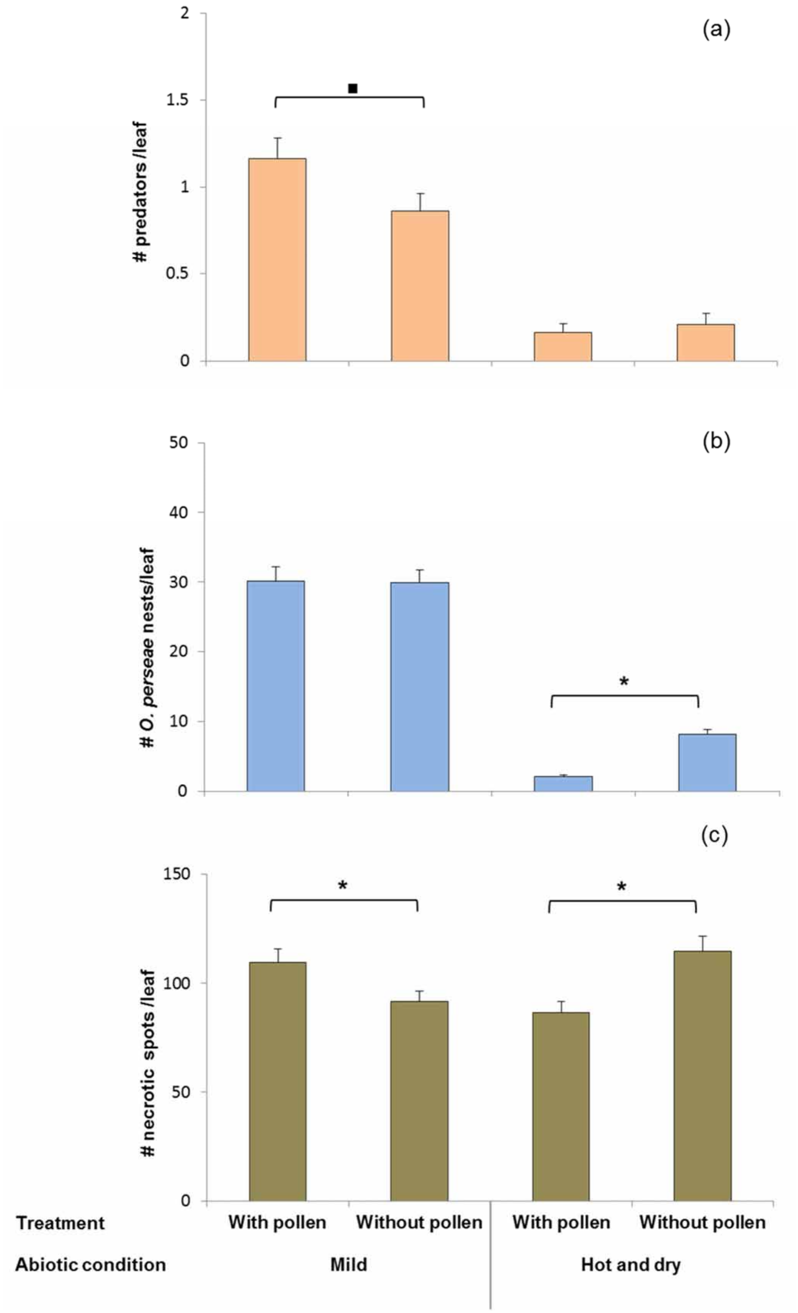

Figure 3.4.1.1. Averages (± SE) of total number of predatory mites (eggs, juveniles and adults), occupied *O. perseae* nests, and necrotic spots per leaf that were counted in the destructive sampling at the two abiotic conditions (“mild” and “hot and dry”), and in the presence or in the absence of pollen. Significant differences (p ≤ 0.05) between treatments are showed as (*). The symbol (■) indicates marginal significant differences (*P*=0.057).

Significantly more occupied *O. perseae* nests per leaf were present at the end of the experiment at M than at HD conditions (Table 3.4.1.1; Figure 3.4.1.1b). At M conditions the presence or the absence of pollen did not affect the number of nests on leaves (Wald statistic for "treatment (M condition)"=0.004, *df*=1, *P*=0.948) (Figure 3.4.1.1b). In contrast, at HD conditions the final population size of prey per leaf was significantly lower in the treatment with pollen (Wald statistic for "treatment (HD conditions)"=66.517, *df*=1, *P*<0.0001) (Figure 3.4.1.1b).

Abiotic conditions had no effect on the final number of necrotic spots per leaf (Table 3.4.1.1; Figure 3.4.1.1c). However, the number of necrotic spots per leaf was higher in the treatment with pollen, at M conditions (Wald statistic for "treatment (M condition)"=5.010, *df*=1, *P*=0.026), and lower at HD conditions (Wald statistic for "treatment (HD conditions)"=10.499, *df*=1, *P*=0.0001) (Figure 3.4.1.1c). Therefore, the presence of pollen at HD conditions resulted in lower plant damage.

Taxonomic identification of phytoseiid mites

The identification of phytoseiid adults collected in each plant at the end of the experiment revealed that 100% of the adults were *E. stipulatus* in all plants, at the two abiotic conditions and in the two treatments, except in one of them, in the treatment with pollen at HD conditions (plant 12) (Figure 3.4.1.2), where 50% of the adults were *N. californicus*. However, it needs to be taken into account that these percentages represent only part of the collected individuals in each plant at the end of the experiment, as a great number of juveniles and eggs could not be identified (Figure 3.4.1.2).

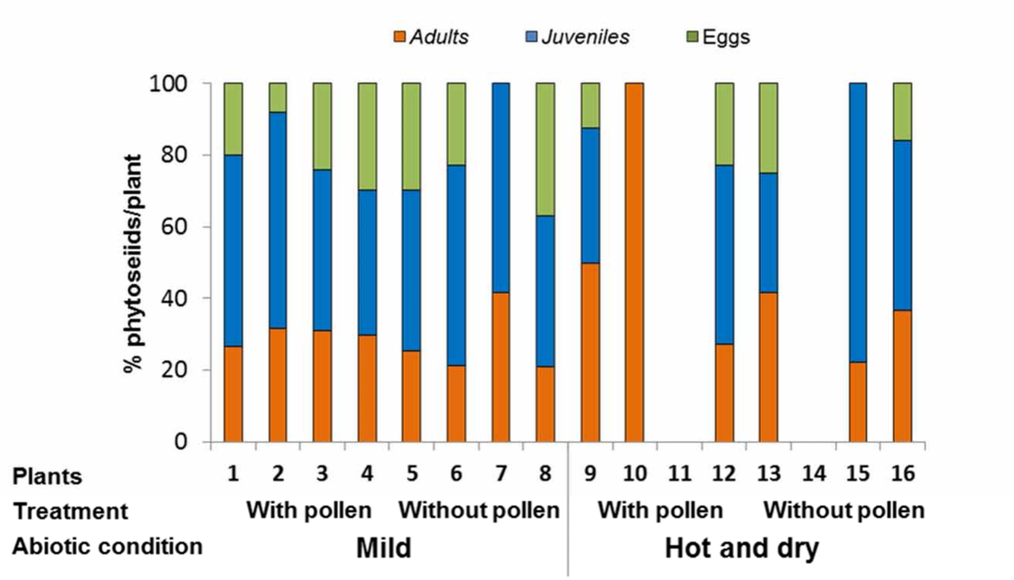

Figure 3.4.1.2. Percentage of adults, juveniles and eggs of predatory mites of each plant that were collected in the destructive sampling depending on the two abiotic conditions ("mild" and "hot and dry"), and treatment (with pollen and without pollen).

Population dynamics

Population dynamics of *O. perseae* and the two predatory mites, *E. stipulatus* and *N. californicus*, for each replicate at the two abiotic conditions and in the presence or absence of pollen are shown in the appendices (Figure A2 - Appendix 2).

4. DISCUSSION

4. DISCUSSION

Community module: Trophic chain

Changes in abiotic conditions (temperature and relative humidity) had different effects on predator-prey interactions depending on the species of predator. Results revealed that at M conditions both species of predatory mite preyed on *O. perseae* females (Figure 4.1; 1a and 2a), whereas at HD and VHD conditions only *N. californicus* fed on the prey (Figure 4.1; 1b and c, and 2b and c).

Predation rate of *N. californicus* on *O. perseae* females (after correcting for *O. perseae* natural mortality) was higher than that of *E. stipulatus* (2.77± 0.45 and 1.53±0.30, respectively) at M conditions. Although *N. californicus* killed more *O. perseae* females per day, oviposition rates did not vary between predators. This indicates that *E. stipulatus* was likely the most efficient predator converting prey into eggs. Indeed, the ratio between daily oviposition and daily predation -which is informative on the food utilisation efficiency of phytoseiids (Sabelis 1985) because they allocate a remarkably large fraction (about 70% in *Phytoseiulus persimilis*) of food ingested to egg production (Sabelis and Janssen 1994)- was twice higher in *E. stipulatus* than in *N. californicus* (0.44 vs. 0.28, respectively). Such difference could likely be even higher considering that *N. californicus* also preyed on *O. perseae* eggs (on average 9.27±2.15 eggs/day) and *E. stipulatus* did not. Differences in conversion rate efficiencies between predators could be explained if food intake per prey item differed between predators. Oviposition/predation ratios in phytoseiids vary with prey availability: some species of phytoseiids are more efficient at converting prey eaten into eggs when prey availability is low (Friese and Gilstrap 1982; Cuellar et al. 2001; Saber 2013). This might be because at high prey densities phytoseiids may kill a higher number of prey items due to increasing encounter rates having a stimulatory effect in predators that result in higher predation rates, an effect known as 'stimulation-interference'(Sandness and McMurtry 1970). Consequently, the time spent feeding on each prey item, and thus the amount of food ingested per prey item, may decrease. In my experiment the level of prey availability could in fact have been higher for *N. californicus* than for *E. stipulatus* because *E. stipulatus* had no access to *O. perseae* eggs or females located inside the nests (Montserrat et al. 2008a; González-Fernández et al. 2009). Therefore, if 'stimulation-interference' had occurred it would have more likely in *N. californicus*. Indeed, Saber (2013) found that *N. californicus* females converted prey into eggs more efficiently at lower prey densities (5 individuals/day of *Tetranychus urticae* nymphs, *Trips tabaci* larvae and *Bemisia tabaci* nymphs) than at higher prey levels (10, 15 and 30 individuals, respectively). This behaviour is considered beneficial to control pest populations, because results in higher killing of prey at high prey densities (Ferragut et al. 1992).

Results showed that *E. stipulatus* did not prey on *O. perseae* at HD and VHD conditions. However, it is known that when climate is hot and dry the production of webbing in spider mites increases to rise the humidity inside nests and better protect the immature stages, in particular the eggs, against desiccation (Hazan et al. 1974, 1975). Hence, *O. perseae* females might have been spending more time outside the nests weaving new layers of webbing. That being true would not explain why predation rates of *E. stipulatus* on *O. perseae* did not increase as temperature increases, simply because encounter rates between predators and prey would be higher than at M conditions with lower number of *O. perseae* females outside the nests. It could be that unfavourable abiotic conditions affected *E. stipulatus* performance as predator, reducing its activity and movement. Other species of phytoseiids have been found to reduce mobility and predation rates at hot temperatures, such as *P. persimilis* preying on *Tetranychus urticae* (Skirvin and Fenlon 2003), and *Amblyseius cucumeris* preying on *Frankliniella occidentalis* (Shipp et al. 1996). In any case, further studies examining searching activities of *E. stipulatus* at extreme abiotic conditions would confirm whether the performance of this species is affected by adverse abiotic conditions.

Predation rates of *N. californicus* on *O. perseae* females were not affected by abiotic conditions (M: 2.77± 0.45; HD: 2.99± 0.45; VHD: 2.75± 0.38). However, predation rates of *N. californicus* on *O. perseae* eggs decreased as abiotic conditions were becoming more extreme (M: 9.27±2.15; HD: 6.83±1.64; VHD: 3.16±1.53), suggesting that *N. californicus* spent less time inside the nests likely due to *O. perseae* females spending more time outside the nests, when abiotic conditions were harsh. Contrary to *E. stipulatus*, it has been reported that high temperatures (from 25 to 35 ºC) and deprivation of food can elicit dispersal behaviour and increase walking speed in *N. californicus* (Auger et al. 1999). Therefore, higher temperatures may translate into greater energy demands due to such increase in movement, reducing, consequently, the amount of resources allocated to reproduction. This could explain the difference in oviposition/predation ratios between abiotic conditions (M: 0.28 vs. HD: 0.21). Furthermore, at VHD conditions (33 ºC) *N. californicus* females did not oviposit, perhaps because resource allocation shifted from reproduction to somatic maintenance. Indeed, whereas no female of *N. californicus* survived at VHD conditions in the absence of food, almost 80 % did when food was available. In the case of *E. stipulatus*, survival of females deprived of food was high at each abiotic condition (more than 70%), suggesting that reduction of the activity perhaps contributes to increase survival. Therefore, it seems that differences in the strategy adopted when facing high temperatures could explain differences in survival between the two predators.

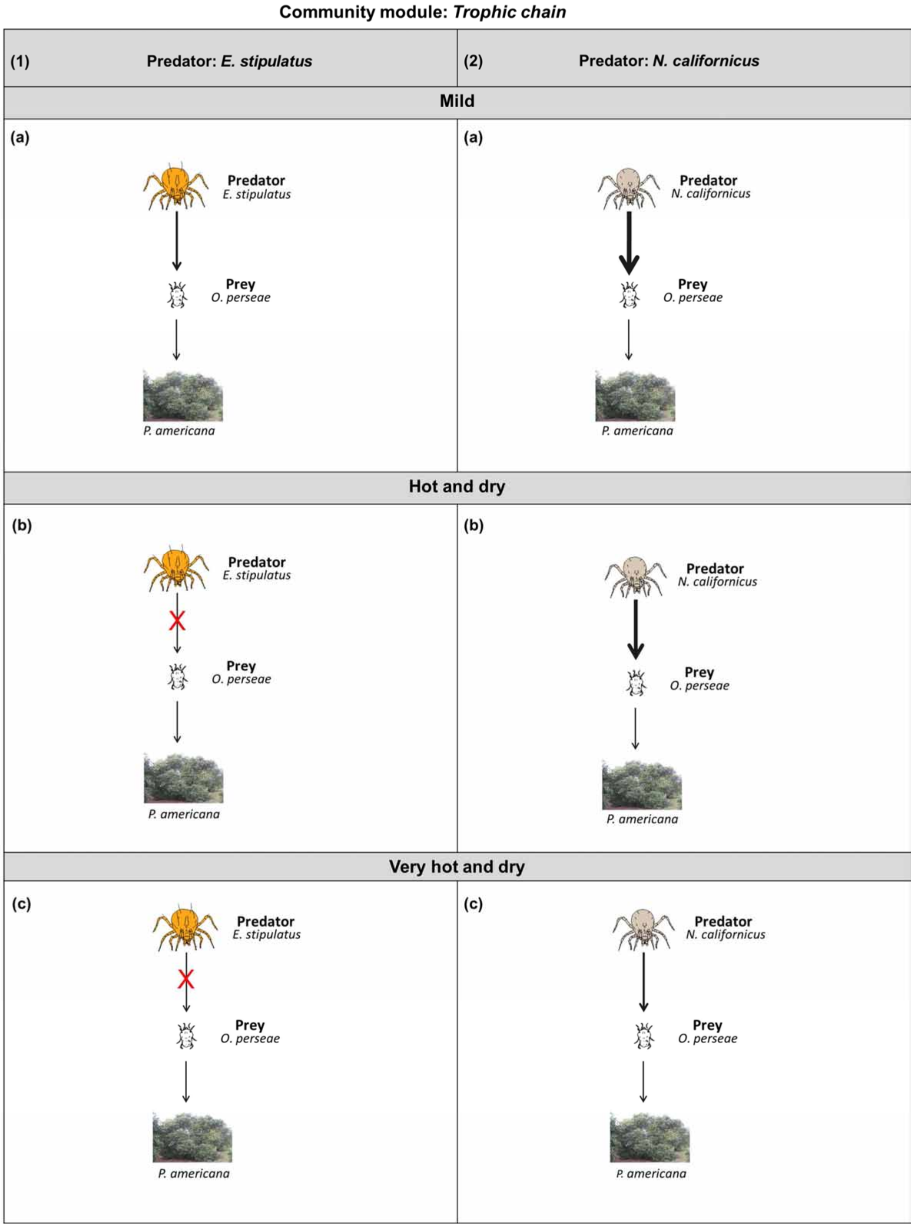

Figure 4.1. Diagrams showing predator-prey interactions in the community module *trophic chain* at the three abiotic conditions ('mild', 'hot and dry' and 'very hot and dry') when predators were females of either (1) *E. stipulatus* or (2) *N. californicus*. Solid arrows indicate trophic interactions and red crosses indicate absence of trophic interactions. In (2): shifts in thickness of arrows indicate changes in the strength of predator-prey interaction between *N. californicus* and *O. perseae* depending on abiotic conditions. To assign the thickness of arrows, relative values of shifts in the strength of interactions among abiotic conditions taking into account predation rates of *N. californicus* both on females and eggs of *O. perseae* were estimated.

Community module: Apparent competition

The presence of alternative food (i.e. pollen) influenced the strength by which *E. stipulatus* and *O. perseae* interacted, and such influence was in turn affected by abiotic conditions. *Euseius stipulatus* preyed on *O. perseae* females in the absence of pollen at M conditions but clearly preferred to forage on the alternative food when pollen was available (Figure 4.2; 1a, b and c). Pollen is considered an optimal food source in *Euseius* sp. as all the species typically reach their highest reproductive potential feeding on this type of food (Ferragut et al. 1987; McMurtry and Croft 1997; Bouras and Papadoulis 2005; González-Fernández et al. 2009). Indeed, rates of oviposition were always higher when *E. stipulatus* females were provided with pollen. Furthermore, egg-laying in females enduring harsh abiotic conditions only occurred when pollen was available, suggesting that availability of an easily accessible and nutritive food source, such as pollen, might temper the negative effects that extreme abiotic conditions cause in this species. Yet, although *E. stipulatus* would be able to oviposit under unfavourable abiotic conditions if pollen were available, it is known that the rate of egg-hatching in this species is very low when the abiotic environment is dry (Ferragut et al. 1987; Guzmán 2014). Therefore, it is probable that extended periods of high temperatures and low relative humidities would result in a decline of *E. stipulatus* populations, even in the presence of pollen as alternative food.

The availability of pollen had no effect on the predator-prey interactions between *N. californicus* and *O. perseae* at any of the abiotic conditions. My results suggest that *N. californicus* females that were provided with pollen and *O. perseae* fed only on the herbivore, at the three abiotic conditions (Figure 4.2; 2a, b and c). Indeed, neither predation nor oviposition rates differed between treatments with or without pollen. Despite some studies claim that *N. californicus* is able to feed and reproduce on pollen (Castagnoli and Simoni 1999; Castagnoli et al. 1999; Sazo et al. 2006), my results and my experience do not support this statement. On the one hand, in my experiments females never oviposited when pollen was the only available food, neither did pollen contribute to increase their survival. On the other hand, during my PhD my colleagues and I attempted to rear this species using pollen as food source, and we never succeeded. Wild strains of *N. californicus* need several generations to optimize development and reproduction when diet is shifted from an optimal to a suboptimal food, such as pollen (Castagnoli and Liguori 1994; Castagnoli et al. 1999). However, mass-reared *N. californicus* strains fed on pollen only needed a short acclimatization period (5 days) to recover their ability to prey and convert prey into eggs after a dietary change from pollen to an elective prey (Castagnoli and Simoni 1999). Therefore, pollen could be used as alternative food only with strains of *N. californicus* previously adapted to feed on this resource. Otherwise such supply would be useless.

In short, my results suggest that the addition of pollen as alternative food could promote a numerical response in *E. stipulatus* and favour pest control through the induction of apparent competition (Holt 1977) between the pest and pollen, but only when field abiotic conditions are mild. However, populations exposed to harsh abiotic conditions will likely decrease, or crash, because vulnerability of eggs and juveniles to heat and dryness (Ferragut et al. 1987; Guzmán 2014) will reduce population turnover to a great extent. Last, my results also suggest that the supply of alternative food would have no effect on the populations of *N. californicus*.

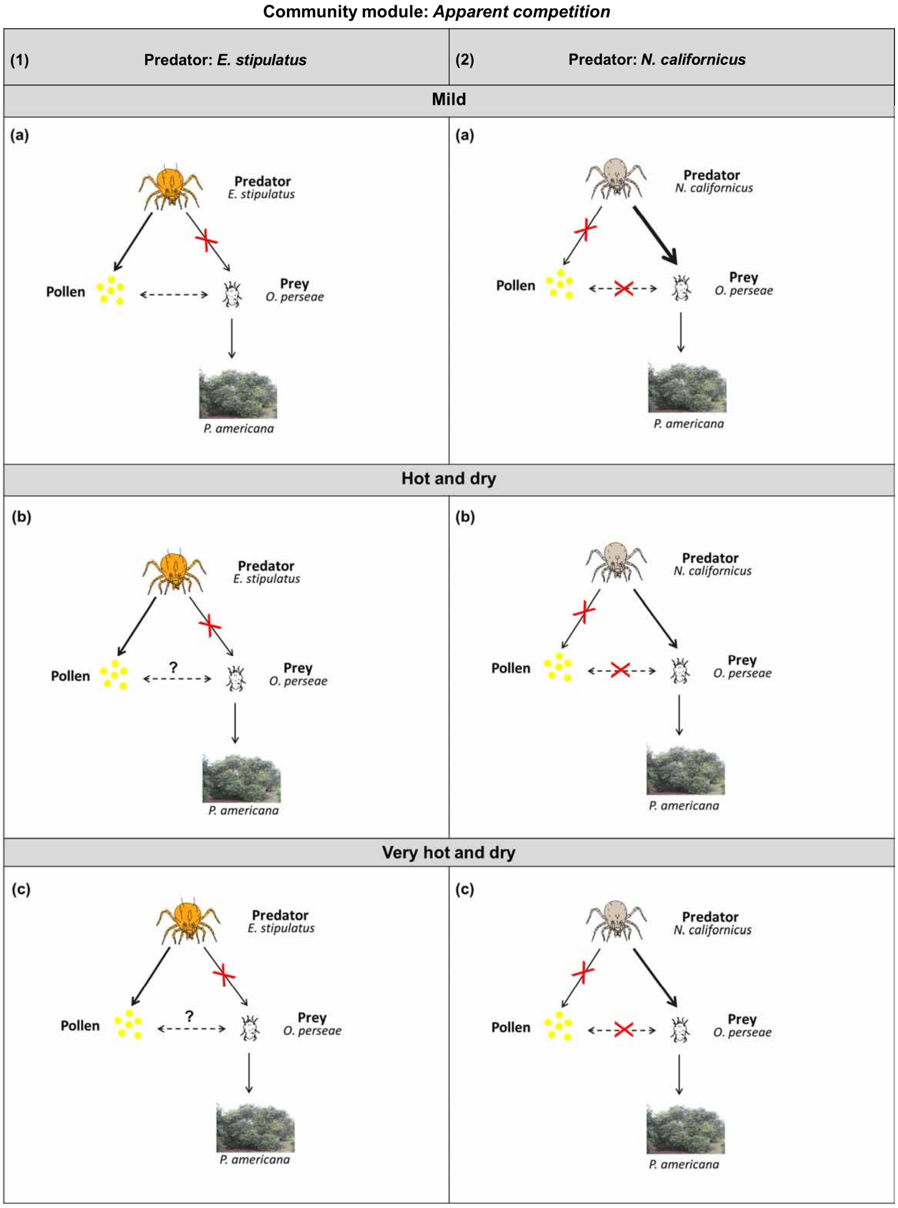

Figure 4.2. Diagrams showing predator-prey interactions in the community module *apparent competition* at the three abiotic conditions ('mild', 'hot and dry' and 'very hot and dry') when predators were females of either (1) *E. stipulatus* or (2) *N. californicus*. Solid arrows indicate trophic interactions, dashed arrows indicate indirect prey-alternative prey (i.e. pollen) negative interactions (apparent competition), and red crosses indicate absence of trophic interactions. In (2): shifts in thickness of arrows indicate changes in the strength of predator-prey interaction between *N. californicus* and *O. perseae* depending on abiotic conditions. To assign the thickness of arrows, relative values of shifts in the strength of interactions among abiotic conditions taking into account predation rates of *N. californicus* both on females and eggs of *O. perseae* were estimated.

Community module: Intraguild predation

The study of trophic interactions in the avocado mite community when mimicking a community module with IGP evidenced the significant role of abiotic conditions in shaping the trophic structure of such communities.

At mild conditions

When the IG-predator was E. stipulatus

When *E. stipulatus* acted as the IG-predator the number of *O. perseae* dead females was not significantly different between treatments where the IG-prey, *N. californicus*, was present, either alone or with the IG-predator, and oviposition rates of *E. stipulatus* did not differ in treatments with presence of IG-prey, but they were lower than those of the treatment in which only the prey was present. Furthermore, mortality of *N. californicus* juveniles was significantly higher in treatments with presence of the IG-predator, compared to the control without them. This suggested that mortality in the herbivore was mainly inflicted by juveniles of *N. californicus*, and that *E. stipulatus* females preyed preferentially on juveniles of *N. californicus*, when they were present. Altogether suggests that the structure of this community resembled more a trophic chain, with *E. stipulatus* females preying on juveniles of *N. californicus*, and juveniles of *N. californicus* preying on females of *O. perseae* (Figure 4.3; 1a), than a community with IGP.

Predation of IG-predators on IG-prey often occurs when densities of the shared prey, usually from another guild, are low (Polis et al. 1989; Lucas et al. 1998), suggesting that when both types of food are available IG-predators prefer foraging on extraguild prey, probably because the quality of IG-prey is typically low relative to the quality of extraguild prey (Polis et al. 1989). In my experiments the IG-predator foraged only on the IG-prey when the three species were together, suggesting that the availability of herbivore prey might not have been high. The presence of eaten *O. perseae* eggs and females inside the nests indicated that juveniles of *N. californicus* could forage inside the nests. Therefore, the number of females of *O. perseae* that were susceptible to be preyed upon by *E. stipulatus* probably was lower in the presence of IG-prey, what would increase the relative rate of encounters between *E. stipulatus* and *N. californicus*.

E. stipulatus females did not convert eaten *N. californicus* juveniles (1.12±0.62 on average) into eggs, but they did when only *O. perseae* females were available, supporting the idea that nutrition provided was higher when IG-predators fed on the second. Differences in nutritional benefits between the two types of prey could be explained by (i) the costs associated with attacking another predator, i.e. running the risk of being injured by the victim, may be higher than the costs of attacking the shared prey, usually defenceless (Polis 1981); and (ii) given that prey sizes are generally correlated with nutrient gain (Schoener 1969; Roger et al. 2000), the larger prey (i.e. *O. perseae* females) probably provides higher energetic gain than smaller *N. californicus* juveniles.

When the IG-predator was N. californicus

When *N. californicus* acted as the IG-predator the mortality rate of *O. perseae* females was twice lower when the three species were together than when the IG-predator was alone with the shared prey, probably due to IG-predator females reducing its activity in the presence of IG-prey (see below). Additionally, mortality of *O. perseae* females was similar in treatments where the IG-prey was present, independently of presence or absence of IG-predators. These results suggested that only juveniles of *E. stipulatus* preyed on *O. perseae* females. Furthermore, mortality of IG-prey did not differ between treatments with and without the IG-predator, indicating that *N. californicus* females did not forage on *E. stipulatus* juveniles. These results suggests that the structure of the community resembled a trophic chain as well, with juveniles of *E. stipulatus* preying on the herbivore (Figure 4.3; 2a) and females of *N. californicus* not interacting at all.

Behavioural changes in females of *N. californicus* induced by *E. stipulatus* could have been the cause of the inactivity in *N. californicus* females, if, for example, females perceived juvenile IG-prey as predators and, consequently, displayed anti-predator behaviour. Abad-Moyano et al. (2010b) reported that the presence of *E. stipulatus* immatures exerted non-lethal IG-effects on *N. californicus* females, causing daily oviposition to decrease over time despite the availability of the shared prey was kept constant. In line with this, in my experiments the oviposition rate of *N. californicus* was nil in the presence of the IG-prey.

With regard to pest control, my results point to conclude that at M conditions the trophic configurations of avocado community would always favour an increase in herbivore populations through density-mediated and trait-mediated indirect effects (Abrams 1995; Werner and Peacor 2003; Preisser et al. 2005) of *E. stipulatus* on *O. perseae*. Predators can lessen prey populations through direct consumption (i.e. density-mediated interaction), or by inducing changes in behavioural, morphological or life history traits of prey (i.e. trait-mediated interaction). In the two types of interactions predators may exert indirect effects on the resources of its prey, affecting community dynamics (Schmitz et al. 1997; Werner and Peacor 2003; Schmitz et al. 2004; Preisser et al. 2005; Abad-Moyano et al. 2010a). In the avocado community, predation of *E. stipulatus* females on *N. californicus* juveniles would lead to an increase of *O. perseae* abundances through density-mediated indirect effects of *E. stipulatus* on *O. perseae*. On another hand, the effect of *E. stipulatus* juveniles on the behaviour of *N. californicus* females, reducing their foraging activity on the persea mite and, therefore, leading to a growth of its populations, is a trait-mediated indirect effect of *E. stipulatus* on *O. perseae*.

At hot and dry and very hot and dry conditions

When the IG-predator was E. stipulatus

When *E. stipulatus* was the IG-predator the mortality of *O. perseae* females was higher when both IG-predator and IG-prey were present, suggesting that both species preyed on the pest. In addition, the mortality of *N. californicus* juveniles did not differ depending on the presence or absence of IG-predators. These results suggest that increasing unfavourable abiotic conditions shifted the structure of the community from a trophic chain to one dominated by exploitative competition between both species of predators.

At M conditions females of *E. stipulatus* foraged only on juveniles of *N. californicus* while at harsh abiotic conditions they foraged only on *O. perseae* females (Figure 4.3; 1a, b and c). This raises the question of why IG-predators would consume different prey types depending on abiotic conditions. A potential explanation is that the availability of both prey species might have changed depending on abiotic conditions. Availability of *N. californicus* juveniles could have been lesser at the two unfavourable abiotic conditions due to two possible causes, which are not mutually exclusive: (i) to the tendency of *N. californicus* juveniles to escape from adverse abiotic conditions, reflected in finding more juveniles in the glue surrounding the experimental arenas at harsh than at M abiotic conditions (see figure 3.2.1.3; compare 1a with 1b and c; p. 98); and (ii) to the ability of juveniles of *N. californicus* to penetrate inside the nests built by *O. perseae* without damaging them, using the entrances of the nests (personal observation). Juveniles penetrating the nests could, therefore, have been protected against adverse abiotic conditions, as it is known nests protect the persea mites (Aponte and McMurtry 1997a). This hypothesis has been partially supported in an additional experiment, which will be discussed further in this section.

The presence of *N. californicus* juveniles in the nests could have refrained *O. perseae* females from entering them, prolonging the risk of falling victims of *E. stipulatus* females. That is, both predator species could have interacted synergistically in a phenomenon termed ‘predator facilitation’ (Charnov et al. 1976), i.e. when the presence of a predator species alters the behaviour of a prey making it more susceptible to attack by another predator species. In synergistic or facilitative interactions, the complex of species of predator kill more prey in combination than the sum of impacts from each individual species (Soluk 1993), as it is observed in the results showed in figure 3.2.1.1; 1b and c; p. 96. Nevertheless, long-term effects of positive predator-predator interactions will only be expected if both predator species inhabit different habitats, and one of the predators drives the prey from one to other habitats where the other predator will forage on the displaced prey (Losey and Denno 1998). In my experimental system, although juveniles of *N. californicus* may ‘facilitate’ *O. perseae* female predation by *E. stipulatus* females at harsh abiotic conditions, female oviposition rates were nil at these conditions, suggesting that the consumed resources by *E. stipulatus* were probably allocated to somatic maintenance to help to increase survival. Therefore, facilitative interactions between *N. californicus* and *E. stipulatus* would affect only *E. stipulatus* survival. In addition, given that both predator species share the same habitat, they also engage in competitive interactions that might lead to the exclusion of less efficient predator species in the long term.

When the IG-predator was N. californicus

When *N. californicus* was the IG-predator there were no differences in the mortality of *O. perseae* females between treatments with or without the IG-prey. Added to this, the predation rates of *N. californicus* on *O. perseae* eggs, as well as oviposition rates, were not affected by the presence of IG-prey. These results indicated that *N. californicus,* but not *E. stipulatus* juveniles, foraged on the herbivore. On the other hand, mortality of *E. stipulatus* juveniles was not affected by the presence of the IG-predator, indicating that *N. californicus* did not attack juveniles. Therefore, at harsh abiotic conditions the community configuration resembled again a trophic chain, but this time with females of *N. californicus* preying on the herbivore and juveniles of *E. stipulatus* not interacting at all (Figure 4.3; 2b and c). The shift in the structure of the community respect to that at M conditions led me to hypothesize that extreme abiotic conditions might have impacted the performance of juveniles of *E. stipulatus* to a great extent. That being true, weakened juveniles would have induced less interference in *N. californicus*, encouraging females to resume their foraging activity.

In short, the strength and direction of predatory interactions among the components of the community were strongly affected by abiotic conditions. Individual responses of predatory species to shifts in abiotic conditions modified the way individuals interacted with each other. It is relevant to notice that all the results suggest that the two predator species might not engage in IGP because none of them preyed on both IG-prey and shared prey when the two prey types were available. Instead, they engaged in competition or predator–prey interactions. Although it is widely assumed that IGP occurs between predatory mites belonging to the family Phytoseiidae (Schausberger and Croft 2000), a recent literature search carried out by Guzmán et al. (2016b) showed that only few works have used experimental set-ups with the adequate array of treatments allowing to assess the existence or not of IGP in phytoseiid mite communities. Accordingly, my experiments included the set of species combinations and control treatments allowing measuring the occurrence of IGP.

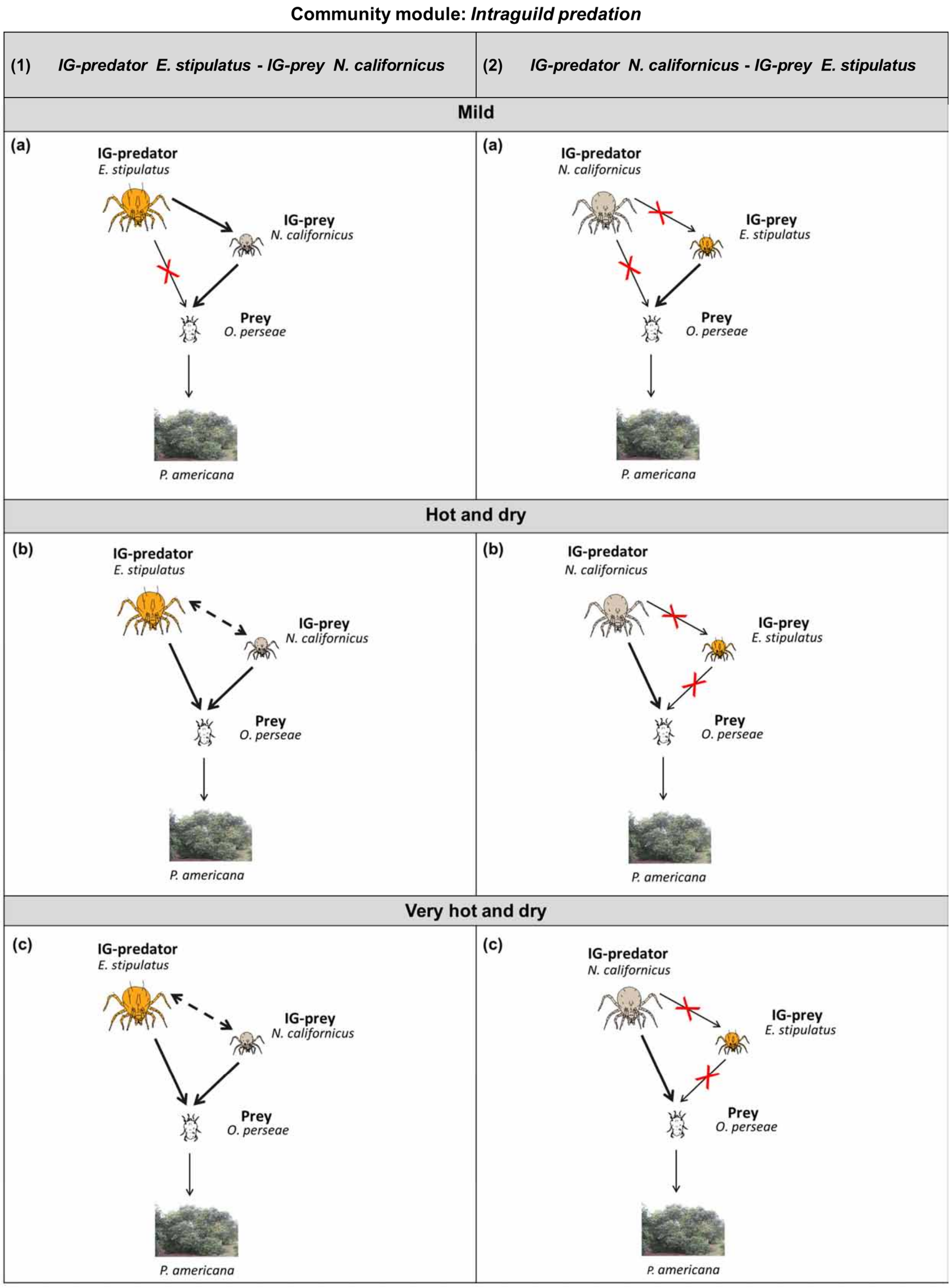

Figure 4.3. Diagrams showing predator-prey interactions in the community module *intraguild predation* at the three abiotic conditions ('mild', 'hot and dry' and 'very hot and dry') when females of either (1) *E. stipulatus* or (2) *N. californicus* acted as IG-predator, and juveniles of either (1) *N. californicus* or (2) *E. stipulatus* acted as IG-prey. Solid arrows indicate trophic interactions, dashed arrows indicate predator-predator negative interactions (competition), and red crosses indicate absence of trophic interactions.

Community module: Intraguild predation-Apparent competition

The presence of alternative food (i.e. pollen) contributed to reduce trophic interactions between predator species resulting in community configurations that could enhance pest control at the three abiotic conditions.

<u>At mild conditions</u>

When the IG-predator was E. stipulatus

When *E. stipulatus* acted as the IG-predator there were no significant differences in the mortality of *O. perseae* females among treatments where the IG-prey was present, independently of the presence or absence of IG-predator, or of pollen. This suggested that only juveniles of *N. californicus* preyed on *O. perseae* females, and that the supply of pollen did not influence the rates of predation of juveniles on the prey. The presence of pollen, however, contributed to reduce significantly IG-prey mortality rates when the IG-predator was present. Therefore, when pollen was available females of *E. stipulatus* preferred to forage on pollen over juveniles of *N. californicus*. In accordance with this, *E. stipulatus* females laid significantly more eggs when pollen was present.

These results showed that predator-prey interactions between predators ceased in the presence of pollen, what divided the structure of the community into two trophic chains, one with females of *E. stipulatus* feeding exclusively on pollen, and the other with juveniles of *N. californicus* feeding only on the pest (Figure 4.4; 1a). Hence, supplying alternative and preferred food to the IG-predator would be detrimental to populations of *O. perseae*, as it would eliminate density-mediated indirect effects of *E. stipulatus* on *O. perseae* that were predicted in the absence of pollen (see p.146).

When the IG-predator was N. californicus

When *N. californicus* was the IG-predator the availability of pollen contributed to increase mortality in *O. perseae* females when the IG-prey was present.

Furthermore, mortality of prey was not significantly different from treatments in which the IG-predator was alone, either with or without pollen. Added to this, the oviposition rates of the IG-predator were similar among these treatments. Results suggested, therefore, that only the females of *N. californicus* foraged on the prey when the three species were together, independently of the presence of alternative food. On the other hand, IG-prey mortality rates were lower when pollen was available, independently of the presence or absence of IG-predators, indicating that *E. stipulatus* juveniles probably foraged on pollen, and that females of *N. californicus* did not attack them.

Altogether, results exposed that the structure of the community shifted again into one with two trophic chains, the first with females of *N. californicus* feeding on *O. perseae* and the second with juveniles of *E. stipulatus* feeding on pollen (Figure 4.4; 2a). In other words, that the effects of *E. stipulatus* juveniles on the behaviour of *N. californicus* females were diluted when pollen was added. It is likely that the presence of a preferred food source minimized the attempts of attacks, or the interference, of juveniles on females of *N. californicus*, what allowed the latest to resume its foraging activities. Indeed, even the predation on *O. perseae* eggs by females of *N. californicus* was significantly higher in the presence of IG-prey when pollen was also present, indicating that females were more active.

At hot and dry and very hot and dry conditions

When the IG-predator was E. stipulatus

When *E. stipulatus* was the IG-predator mortality rates of *O. perseae* were only affected by the presence of IG-prey. In the absence of pollen, results showed that IG-predator and IG-prey only engaged in competition for the shared prey. However, the addition of pollen ceased predation of *E. stipulatus* females on the prey (there were no significant differences in the mortality of *O. perseae* females between the treatment where IG-predator and IG-prey were together with pollen and the treatment where IG-prey were alone). In addition, oviposition rates of IG-predators increased in the presence of pollen.

In short, the addition of pollen ceased competitive interactions between predatory mites and the structure of the community shifted again into one with two trophic chains, the first with females of *E. stipulatus* feeding exclusively on pollen, and the second with juveniles of *N. californicus* feeding on the herbivore (Figure 4.4; 1b and c).

When the IG-predator was N. californicus

When *N. californicus* was the IG-predator the mortality of *O. perseae* females was similar among treatments, independently of the presence or absence of IG-prey and pollen. This result suggested that only females of *N. californicus* fed on the prey when the three species were together. Also, the mortality of IG-prey was significantly lower in all the treatments with pollen, compared to the treatment measuring natural mortality. This suggested that IG-predators did not attack juvenile IG-prey in the presence of pollen, and that juveniles foraged on pollen when it was available, indicating, once again, that the community structure consisted in two trophic chains, one with females of *N. californicus* feeding on *O. perseae*, and another with juveniles of *E. stipulatus* feeding on pollen (Figure 4.4; 2b and c). Furthermore, the availability of pollen also buffered the negative effects that extreme abiotic conditions had on juveniles of *E. stipulatus*.

Concluding, the supply of pollen reduced trophic interactions between predators and divided the avocado mite community into two trophic chains with *E. stipulatus* feeding on pollen and *N. californicus* on the pest, independently of abiotic conditions. As mentioned before, *E. stipulatus* are described as being preferentially pollen-feeders (McMurtry and Croft 1997). Thus, in the presence of this food source one would expect *E. stipulatus* to reduce attacks on all the other food sources and forage preferentially on pollen. This resulting community configuration would be beneficial for pest control because (i) it would increase the strength of predator-prey interactions between *N. californicus* and *O. perseae*, and (ii) it could induce apparent competition between alternative food and pest, favouring the increase of the populations of *E. stipulatus* in the long-term, mostly when abiotic conditions are favourable, that could add an extra impact on the populations of the pest.

In summary, results from the community module *intraguild predation* showed that abiotic conditions modified the trophic structure of the avocado mite community in a way that would often be detrimental to biological pest control. On the other hand, results from the community module *intraguild predation-apparent competition* evidenced that adding pollen to the system would likely improve pest suppression, because it weakens trophic interactions between predators.

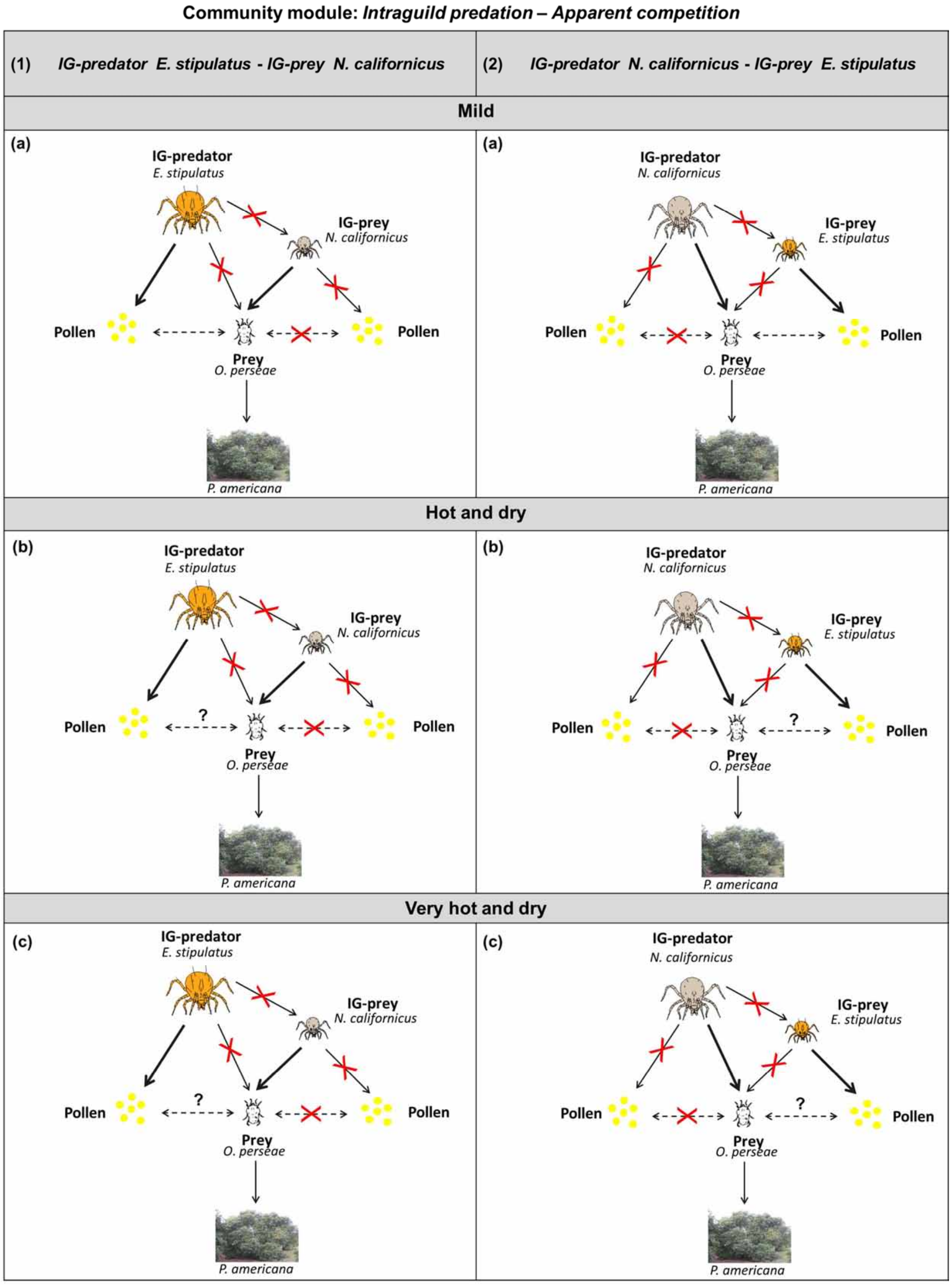

Figure 4.4. Diagrams showing predator-prey interactions in the community module *intraguild predation-apparent competition* at the three abiotic conditions ('mild', 'hot and dry' and 'very hot and dry') when females of either (1) *E. stipulatus* or (2) *N. californicus* acted as IG-predator, and juveniles of either (1) *N. californicus* or (2) *E. stipulatus* acted as IG-prey. Solid arrows indicate trophic interactions, dashed arrows indicate indirect prey-alternative prey (i.e. pollen) negative interactions (apparent competition), and red crosses indicate absence of trophic interactions.

Unravelling behavioural/environmental effects: fine-tuning of results

(i) IG-prey role reversals triggered by IG-predators diet

Counterattack on vulnerable predator stages could be considered anti-predator behaviour if it results in predators being scared away from patches occupied by IG-prey (Janssen et al. 2002). Yet, it could also result in counter-counter-attack of predators if the attacked vulnerable predator individuals are the offspring of mothers patrolling those patches (Magalhães et al. 2005a).Whatever is the case in my experimental system, the display of anti-predator behaviour in the IG-prey (i.e. juveniles of *N. californicus*) might have been determined by the diet of IG-predators (Magalhães et al. 2005a). Results showed that predation events were similar between eggs placed next to the midrib and on the limbo, independently of diet of the females. However, predation on eggs inside domatia was higher when eggs were produced from mothers that had eaten pollen.

Leaf domatia are refuges for predatory mites that often occupy them to oviposit and moult (Walter 1996). Indeed, these are structures that protect vulnerable stages, such as eggs (Faraji et al. 2002b, a) and immatures (Ferreira et al. 2011), from predation. Furthermore, leaf domatia can have hair-tufts which can act as pollen traps that provide food to mites (Kreiter et al. 2003; Romero and Benson 2005). Because *E. stipulatus* is preferentially pollen-feeder (McMurtry and Croft 1997), it could be that females use avocado leaf domatia as oviposition sites more often when those provide as well pollen for their offspring. Thus, *N. californicus* juveniles might associate the presence of pollen in the system with the presence of *E. stipulatus* eggs inside leaf domatia. Although pollen was not present during the experiment, *E. stipulatus* eggs produced from mothers feeding on pollen came from experimental arenas containing pollen. Therefore, eggs could have had few grains of pollen attached to their surfaces that could have been detected by *N. californicus* juveniles. *Neoseiulus californicus* juveniles might have adaptively learnt to recognize predator eggs through cues provided by pollen, and attack them if they are inside domatia as a counter-attack to scare bigger predators away from them; a strategy, true, that would only be beneficial if mothers do not display parental care (Magalhães et al. 2005a).

(ii) Nests of *O. perseae* as refuges for *N. californicus* juveniles against adverse abiotic conditions

N. californicus juveniles have the ability to penetrate inside the persea mite nests using their entrances (personal observation, this thesis). Therefore, I hypothesized that juveniles could benefit from one of the functions attributed to the *O. perseae* nests: protection against adverse abiotic conditions (Aponte and McMurtry 1997a). My results, however, only partially supported this idea.

At M and HD conditions the presence of nests had no effect on *N. californicus* juvenile mortality. At VHD conditions the mortality of juveniles was unexpectedly higher in the treatment with nests, a result that clearly contradicts the initial hypothesis. However, if we look at egg predation we can see that despite at the three abiotic conditions predation on eggs was significantly higher in the absence of nests, this difference was significantly more pronounced at VHD conditions (see Figure 3.3.2.2; p. 128). This indicates that juveniles may have had problems to get inside the nests when conditions were the harshest. Very extreme abiotic conditions may have desiccated the web and deformed the entrances of the nests. Aponte and McMurtry (1997a) reported that during summer *O. perseae* spins several layers of strands over the original layer. By doing this the humidity inside nests increases, protecting immature stages, in particular the eggs, against desiccation (Hazan et al. 1974, 1975), and the structure of nests is reinforced. In my experiments *O. perseae* females built the nests under M conditions. Hence, nests could have been deformed later when arenas were exposed to very high temperatures and low relative humidity, hampering juveniles from going inside and forage on the eggs.

Based on my results, it seems that mortality of juveniles of *N. californicus* at the three abiotic conditions was determined by accessibility to food rather than to the presence or absence of nests. At HD conditions juveniles could still get access to the nests as egg predation in the presence of nests, despite being lower, still occurred. However, when abiotic conditions were extremely harsh, there was almost no predation on eggs in the presence of nests indicating that juveniles were hampered to get inside. It is interesting to notice that accessibility to eggs influenced the survival of juveniles, buffering adverse effects of high temperatures and extreme dryness, likely because feeding on the eggs afforded water and protected juveniles from desiccation.

Another function attributed to nests is protection against attack from some species of natural enemies (Mori et al. 1999). Given that *N. californicus* juveniles can easily go in and out of nests, they could also use them as refuges against their own predators. In a similar manner, *N. californicus* uses the web of another species of spider mite, *Tetranychus urticae*, to get protection against *E. stipulatus* (Ferragut et al. 1987; Abad-Moyano et al. 2010b). This, however, is a function that in the avocado mite community still remains to be studied.

Effects of abiotic conditions and presence of alternative food on mite predator/prey population dynamics in avocado

At the individual level the addition of pollen changed the avocado mite community into two trophic chains, one with *E. stipulatus* foraging only on pollen and another with *N. californicus* preying only on *O. perseae*, independently of abiotic conditions (Figure 4.4), i.e. the presence of alternative food ceased predator-prey interactions between the two phytoseiid species. Hence, based on these results, I hypothesized that, at the population level, supply of an alternative food (i.e. pollen) for *E. stipulatus* would enhance pest population control by (i) weakening negative predator-prey interactions occurring between *E. stipulatus* and *N. californicus*, both at optimal and extreme abiotic conditions, and by (ii) promoting a numerical response in *E. stipulatus*, probably only at optimal conditions, that could induce apparent competition between the herbivore and pollen.

Contrary to expectations, at M conditions the final population size of *O. perseae* per leaf did not differ between treatments with and without pollen, although the presence of alternative food promoted a higher growth rate in the predator population. At HD conditions, however, the addition of pollen resulted in a higher reduction of the final abundance of prey per leaf, although the number of predators per leaf that were present at the end of the experiment did not vary between treatments. Results from taxonomic identification of predatory mite adults collected from plants in the final destructive sampling revealed that, at M conditions, *N. californicus* was excluded by *E. stipulatus*, independently of the presence or absence of pollen. However, at HD conditions, despite results also revealed that *N. californicus* was excluded by *E. stipulatus* in both treatments, the presence of pollen might have extended the coexistence of the two species through the experiment. Therefore, the effect exerted by alternative food on the avocado mite community dynamics depended on abiotic conditions.

In the absence of pollen, exclusion of *N. californicus* by *E. stipulatus* at both abiotic conditions could be explained by the initial IG-prey/IG-predator ratio and the initial stage structure of populations, which determine patterns of coexistence and exclusion in intraguild predator-prey systems (Montserrat et al. 2008b). At the beginning of my experiments predator populations had no stage structure and prey availability was different for the two predators. *Neoseiulus californicus* have the ability of penetrating nests (Montserrat et al. 2008a) and likely females foraged on *O. perseae* individuals inside. This could have conferred *N. californicus* an initial advantage over *E. stipulatus*, which are unable to go inside nests (Montserrat et al. 2008a; González-Fernández et al. 2009). Initial higher food intake by *N. californicus* might have result in production of offspring, increasing the density of *N. californicus* stages that are vulnerable to predation by *E. stipulatus* females. Building on this hypothetical initial scenario, the combination of a reduced availability of herbivore females wandering outside nests caused by predation of *N. californicus* inside nests, and a higher availability of vulnerable IG-prey, during the initial phase of the interaction, could have resulted in high predation pressure of *E. stipulatus* on *N. californicus*, preventing *N. californicus* populations from growing, and increasing the likelihood of exclusion of *N. californicus* by *E. stipulatus* through the experiment because of lack of turnover in *N. californicus* populations.

The presence of pollen for *E. stipulatus* should have reduced the levels of predation on *N. californicus*. However, results suggested that this did not occur. At M conditions, all the adults collected from plants with or without pollen in the final destructive sampling were *E. stipulatus*, suggesting that this species excluded *N. californicus* from the community, independently of the presence or the absence of pollen. At HD conditions, phytoseiid adults that were collected from plants in which pollen was added revealed that both predator species coexisted in one of the plants. In the rest of plants, with or without pollen, all the adults were *E. stipulatus*. Nevertheless, in both abiotic conditions total exclusion of *N. californicus* cannot be totally ascertained because juveniles and eggs that were collected at the end of the experiment could not be identified. Even so, from the results I can conclude that the addition of pollen did not alleviate predator-prey interactions between the two species at both abiotic conditions, as expected. Competitive effects can be discarded, as *N. californicus* is better at exploiting spider mite prey than *E. stipulatus*.

The low effect of pollen on the phytoseiid populations may have been caused by the set up. Pollen was provided in a plastic vial glued on the top of a single leaf of the whole plant. This likely made it more difficult for *E. stipulatus* to find the patch with pollen. This could have led to local spatial segregation of *E. stipulatus*, with some individuals foraging preferentially on pollen near the pollen patch, and other individuals preying preferentially on immatures of *N. californicu*s on leaves far from the pollen patch. Therefore, *N. californicus* immatures might again have suffered high rates of predation by *E. stipulatus*. However, at HD conditions, high temperatures and low relative humidities probably reduced the effectiveness of *E. stipulatus* as predator and, consequently, a segregated population of this species could have exerted less predation pressure on *N. californicus* immatures during the initial phase of the interaction. Therefore, this could explain delayed exclusion of *N. californicus* by *E. stipulatus* or, alternatively, the coexistence of both predator species through the experiment, being the probable cause of a better control of pest population at these abiotic conditions.

On the other hand, results showed that, at M conditions, the final number of predators per leaf was higher in the treatment with pollen. This indicated that once *N. californicus* was excluded from the system, the availability of pollen for *E. stipulatus* probably promoted a numerical response of this predator species. On the contrary, at HD conditions, results revealed that the final predator abundance per leaf was similar in both treatments, showing that pollen did not favour the growth of *E. stipulatus* populations. Although at the individual level the presence of pollen at extreme abiotic conditions contributed to increase the rate of egg laying of this species, extended exposure to adverse abiotic conditions was detrimental for *E. stipulatus* populations, as it has been described that eggs of this species are highly vulnerable to desiccation (Ferragut et al. 1987; Guzmán 2014).

Summarising, the beneficial effects of the supply of pollen to the system on *O. perseae* population control were only partially detected. One of the aims of such supply was to alleviate predator-prey interactions occurring between the two predator species through supplemental feeding for *E. stipulatus*. Conversely, at both abiotic conditions, results revealed that *E. stipulatus* probably excluded *N. californicus*. There are two explanations for the failed results: (1) pollen was provided in a concentrated form. Instead, pollen should have been uniformly added on all leaves, favouring the expected separation of the avocado mite community in two trophic chains (i.e. *E. stipulatus* feeding on pollen and *N. californicus* preying on the pest) throughout the plant; and (2) predator populations had no stage structure at the beginning of the experiment. Likely, eradication of *N. californicus* by *E. stipulatus* might have been avoided if the experiment had started with predator populations well-stage structured. The other aim was to promote apparent competition between the alternative food and the pest mediated by an increase in the population size of *E. stipulatus*. Although at M conditions the final abundance of *E. stipulatus* was higher in the treatment with pollen, this increase did not translate into a better pest control at the end of the experiment. On the other hand, at HD conditions, the combination of high temperatures with low air humidity probably affected negatively the growth of *E. stipulatus* populations. Therefore, this result evidenced that abiotic conditions were central to predator population dynamics. Periods of high temperatures combined with severe drought that are predicted by models of climate change in Mediterranean and temperate regions will lead to loss of predatory mite populations, which will provide herbivores, less sensitive to warming than higher trophic levels (Voigt et al. 2003; Schweiger et al. 2008), the opportunity of escaping predator control.

These results allow predicting that climate change represents a threat to the effective maintenance of agricultural communities under biocontrol management. Under climate warming, the success of biocontrol agents will depend on their capacity to respond adaptively to heat stress (Hoffmann and Sgrò 2011). However, it is needed to take into account that stressful temperatures will exert a selection pressure simultaneously on all the interacting populations within the whole agricultural community, spurring evolutionary responses that will affect population dynamics and, ultimately, community dynamics (Fussmann et al. 2007). Therefore, evolutionary adaptation of each species will be determined by the interplay between ecological and abiotic factors. Accordingly, the approach of natural enemy´s adaptation responses to oncoming warming should be considered at the community level (Pelletier et al. 2009). Hence, the development of strategies that allow facing effects derived from climate change for each specific agricultural system needs to integrate knowledge on community ecology and evolutionary biology (Moya-Laraño et al. 2012; Moya-Laraño et al. 2014).

5. CONCLUSIONS

5. CONCLUSIONS

When each predator species is alone with its prey

1) The effect of increasing warming and dryness on key life-history parameters (e.g. predation rates on the prey, oviposition rates, food-to-egg conversion efficiency and survival) differs between predatory mite species. Negative effects are stronger for *E. stipulatus* than for *N. californicus*.

2) *Euseius stipulatus* shows a clear preference for foraging on pollen over the prey when the two food types are available. Rates of oviposition increase when this species forages on pollen and egg-laying at harsh abiotic conditions only occurs in the presence of this alternative food. On the contrary, the addition of pollen does not influence predation rates on *O. perseae*, or oviposition rates, in *N. californicus*.

When both predator species are together and share a prey

3) Trophic interactions in the avocado mite community when a community module with intraguild predation is simulated are strongly affected by changes in the abiotic conditions, resulting in community configurations that would often be negative for biological pest control (BPC).

4) The addition of pollen to the system contributes considerably to reduce the trophic interaction strength between predator species, and re-shapes the community into two trophic chains at the three abiotic conditions, one with *E. stipulatus* feeding exclusively on pollen, and another with *N. californicus* preying exclusively on *O. perseae*. This community configuration could improve pest control at the three abiotic conditions because weakening negative predator-prey interactions between *E. stipulatus* and *N. californicus*, would likely strengthen predator-prey interactions between *N. californicus* and the pest. Also, pollen might promote a numerical response

in *E. stipulatus* under favourable abiotic conditions that could induce apparent competition between *O. perseae* and pollen.

5) Events of predation of *E. stipulatus* eggs by juveniles of *N. californicus* were observed in the presence of pollen, but not in its absence. There is a tendency towards higher predation of *N. californicus* juveniles on *E. stipulatus* eggs located in leaf domatia when eggs are laid by females that have been previously fed on pollen. This could be an adaptive behaviour meant to scare predators away from patches occupied by IG-prey.

6) *Neoseiulus californicus* juveniles can penetrate inside *O. perseae* nests through the entrances built by the persea mite. The accessibility to the eggs of *O. perseae* inside the nests enhances the survival of juveniles when abiotic conditions are adverse, likely because feeding on the eggs provides water and shields juveniles from desiccation.

7) At the population level, the beneficial effects for BPC of adding pollen to the system are only partially detected. On the one hand, the expected reduction of negative trophic interactions occurring between predator species is not observed, probably due to methodological errors. Instead, the most common pattern is that *E. stipulatus* excludes *N. californicus* from avocado plants, at the two tested abiotic conditions, and independently of the presence or absence of pollen. On the other hand, at M conditions the presence of pollen favours an increase of *E. stipulatus* populations, but such increase does not translate into a better pest control. Therefore, adding alternative food does not induce apparent competition between pollen and prey, probably because of the strong preference of *E. stipulatus* for pollen over the prey. The general negative effect on the growth of *E. stipulatus* populations observed at HD conditions evidences that abiotic conditions can be key drivers of population dynamics.

8) Results from this thesis allow anticipating that in the near future climate will be a threat for the success of BPC, because warming will likely cause changes in the trophic structure and dynamics of agricultural communities that often contribute to pest control disruption.

6. RESUMEN EN ESPAÑOL

6. RESUMEN EXTENDIDO EN ESPAÑOL

Según el Quinto Informe de Evaluación (IE5) del Grupo Intergubernamental de Expertos sobre el Cambio Climático (IPCC) (IPCC 2014), el calentamiento del sistema climático es inequívoco y la influencia de la actividad humana es innegable. En el último siglo la temperatura global de la superficie de la Tierra ha aumentado en 0.85ºC, han mermado las reservas de agua en forma de nieve y de hielo, y ha subido el nivel del mar. La emisión antropogénica de los gases de efecto invernadero (GEI) ha contribuido a incrementar las concentraciones de dióxido de carbono, metano, and óxido nitroso desde la era pre-industrial, y es muy probable (entre 99 – 100 %) que sean la principal causa del calentamiento observado desde la mitad del siglo pasado hasta la actualidad. El IE5 también ha revelado que se han producido numerosos cambios en muchos de los fenómenos meteorológicos y climáticos extremos desde 1950, y que algunos de estos cambios están asociados a actividades humanas pasadas y presentes. Como consecuencia, se ha producido un aumento en la intensidad, duración y número de períodos de temperaturas extremas en gran parte de Europa, Asia y Australia, y cada vez hay más regiones donde han aumentado el número de episodios de precipitación y sequía extrema. La emisión continua de GEI conducirá a un mayor calentamiento y a cambios más duraderos en todos los componentes del sistema climático. De acuerdo con el IE5, la temperatura global de la superficie de la Tierra seguirá aumentando a lo largo del siglo XXI en todos los escenarios de emisiones evaluados, y es muy probable que las olas de calor ocurran con mayor frecuencia y duren más, y que los episodios de precipitación y sequía extremas sean más intensos y frecuentes en muchas regiones.

Los sistemas naturales están siendo fuertemente perturbados por el cambio climático, y ya han sido documentados cambios en la fenología, distribución, composición y diversidad de especies en los ecosistemas acuáticos y terrestres (Walther et al. 2002; Parmesan 2006; Warren et al. 2011; Bellard et al. 2012). Las respuestas de los individuos a cambios en los factores abióticos puede afectar a la forma en la cual interaccionan con otros individuos, tanto de la misma como de otras especies, viéndose modificada la composición y la estructura de comunidades ecológicas (Dunson and Travis 1991; Gilman et al. 2010; Woodward et al. 2010). De hecho, existen evidencias de que el calentamiento global está alterando la fuerza y/o dirección de prácticamente todos los tipos de interacciones bióticas, entre las que se incluyen competencia, mutualismo, depredación, parasitismo, etc. (Tylianakis et al. 2008). Por tanto, uno de los principales desafíos a los que se enfrentan los ecólogos es tratar de predecir la forma en las comunidades responderán a un clima que está cambiando, lo cual resulta de especial interés en comunidades que proporcionan relevantes servicios ecológicos, tales como el control biológico de plagas.

En la agricultura basada en prácticas respetuosas con el medio ambiente como el control biológico, cambios en las condiciones abióticas podrían afectar al buen funcionamiento de la comunidad agrícola, ya que la mayoría de las plagas y los enemigos naturales que las controlan son artrópodos y, como organismos ectotermos, muchos parámetros relacionados con su eficiencia biológica (p.ej. supervivencia, reproducción, longevidad) así como la forma en la que interaccionan entre sí (p.ej. tasa de depredación, habilidad competitiva), están fuertemente determinados por la temperatura ambiental (Beveridge et al. 2010; Gilman et al. 2010). Es por ello que la investigación en las ciencias agrarias debe ir orientada hacia la identificación y cuantificación del efecto del calentamiento global en las interacciones entre las especies, determinando el modo en el que se ve afectada la estructura trófica y la dinámica de cada comunidad agrícola (Bascompte and Stouffer 2009).

En base a los antecedentes expuestos, el **objetivo principal de esta tesis** fue generar conocimiento sobre los efectos de cambios en las condiciones abióticas en las interacciones entre las especies que forman comunidades agrícolas bajo control biológico, y como estos efectos determinan la dinámica y la estructura de estas comunidades. Para cumplir este objetivo se estudió una comunidad de ácaros presente en el agro-ecosistema del aguacate (*Persea Americana* Mill., Lauraceae) en el sureste de España. El bajo número de especies que integran esta comunidad la convierten en un modelo de estudio idóneo para evaluar los efectos del cambio climático en las interacciones entre las plagas y sus enemigos naturales. En áreas costeras, donde las condiciones climáticas son suaves, esta comunidad de ácaros está compuesta por una especie plaga *Oligonychus perseae* (Tuttle, Baker & Abbatiello) (Acari: Tetranychidae), denominada comúnmente como 'ácaro cristalino', y dos especies de ácaros fitoseidos que son enemigos naturales de la plaga: el depredador especialista de tetraníchidos *Neoseiulus californicus* (McGregor) y el omnívoro *Euseius stipulatus* (Athias-Henriot) que puede reproducirse cuando se alimenta de polen. La especie plaga construye nidos densos de seda en el envés de las hojas, principalmente a lo largo del nervio central y en los nervios laterales (Aponte and McMurtry 1997a). Dentro de los nidos los adultos se alimentan y se reproducen, y los juveniles se desarrollan. La alimentación de este herbívoro causa daño a la hoja, el cual es detectado por la aparición de manchas necróticas que pueden llegar a ocupar hasta el 90% de la superficie foliar, afectando a la eficiencia fotosintética de la planta (Aponte and McMurtry 1997a). Los nidos tienen una forma semicircular y presentan una o más aberturas que funcionan como entradas o salidas para el ácaro cristalino. Los nidos actúan protegiendo a esta plaga frente a las condiciones abióticas adversas y frente al ataque de algunas especies de enemigos naturales (Mori et al. 1999; Montserrat et al. 2008a). En el caso de las dos especies de ácaros fitoseidos que se encuentran en asociación con *O. perseae* en los cultivos de aguacate del sureste de España, se ha demostrado que las hembras de *N. californicus* son capaces de entrar en los nidos rasgando la tela con sus primer par de patas y atacar a los individuos que se encuentran en su interior (Montserrat et al. 2008a). En cambio, las hembras de *E. stipulatus* no puede penetrar en el interior de los nidos, y sólo atacan a los individuos que se encuentran deambulado en el exterior. En cuanto a los juveniles de ambas especies, aún no se ha explorado si éstos son capaces o no de entrar en los nidos.

La capacidad para predecir la influencia que tendrá el cambio climático sobre comunidades agrícolas depende de la identificación de aquellas interacciones entre especies que son más susceptibles a variaciones en las condiciones abióticas, y que son clave para el funcionamiento y mantenimiento de la comunidad. Para estudiar estas interacciones resulta muy útil el uso de módulos comunitarios (Holt 1997), en los que comunidades enteras se simplifican en módulos de dos a seis especies que interaccionan a través de depredación, competencia o competencia aparente, lo que facilita un mejor entendimiento del funcionamiento de comunidades complejas. Por ello, para evaluar el efecto de cambios en las condiciones abióticas en la fuerza y la dirección de las interacciones que ocurren en la comunidad de ácaros que habita en el agro-ecosistema del aguacate se eligieron cuatro módulos comunitarios que son de interés para el control biológico de plagas:

(i) *Cadena Trófica* - Aunque en las comunidades agrícolas las especies están conectadas formando redes tróficas complejas (Gallopin 1972; Polis and Strong 1996), el estudio de cadenas tróficas simples proporciona un punto de partida para entender los cambios que se producen al añadir más especies y/o interacciones a la comunidad.

(ii) *Competencia aparente* - Este módulo comunitario está compuesto por dos presas que no compiten entre ellas pero que comparten un mismo depredador. Esta interacción resulta de especial interés para el control biológico ya que la presencia de una presa alternativa que es inocua para el cultivo puede conducir a una reducción de la especie plaga a través de un aumento en la densidad del depredador que comparten (Holt 1977). De esta manera, aunque la depredación *per capita* de la especie plaga sea menor, un aumento numérico de la población de depredadores conduce a que depredación total de la plaga sea mayor que en ausencia de la presa alternativa.

(iv) *Depredación intragremial* (IGP) - En el módulo de IGP se combinan competencia y depredación, donde dos especies interaccionan compitiendo por un recurso (presa compartida), y a su vez una de ellas (IG-depredador) consume también a la otra (IG-presa) (Polis et al. 1989).

(v) *Depredación intragremial-Competencia aparente* - En sistemas agrícolas donde se dan interacciones IGP entre los enemigos naturales de la plaga, la presencia de un alimento alternativo (p.ej. polen) para el IG-depredador puede mejorar el control biológico a través de una reducción de la presión de depredación del IG-depredador sobre la IG-presa, y por la aparición de competencia aparente entre el alimento alternativo y la plaga.

Con el fin de lograr el objetivo principal de esta tesis, el trabajo se centró, por un lado, en la evaluación a nivel de individuo del efecto de cambios en los factores abióticos (i.e. temperatura y humedad relativa) en la fuerza y la dirección de las interacciones entre las especies que componen esta comunidad de ácaros haciendo uso de los módulos comunitarios anteriormente expuestos. Dichas interacciones fueron evaluadas en disco de hojas de aguacate a tres combinaciones diferentes de temperatura y humedad relativa que fueron denominadas como "óptimas" (O, en adelante), "cálidas-secas" (CS, en adelante) y "muy cálidas-secas" (MCS, en adelante), comprendidas entre unas condiciones óptimas de temperatura y humedad relativa, y unas condiciones más extremas, predichas por modelos de cambio climático. Por otro lado, se examinó como los efectos de los factores abióticos en las interacciones entre las especies observados a nivel de individuo fueron traducidos a nivel poblacional mediante el estudio de las dinámicas poblacionales de la plaga y los depredadores en plantones de aguacate a condiciones O y CS, determinando a su vez la estructura y la dinámica de la comunidad.

(i) A nivel de individuo

Módulos comunitarios: Cadena trófica y Competencia aparente

El estudio comenzó evaluando cómo las condiciones abióticas influyeron en las interacciones depredador–presa entre las dos especies de ácaros depredadores, *E. stipulatus* y *N. californicus*, y su presa herbívora *O. perseae* en la presencia y en la ausencia de un alimento alternativo (i.e. polen), a través de los módulos comunitarios de cadena trófica y competencia aparente.

En el <u>módulo comunitario de cadena trófica</u>, los resultados expusieron que el efecto de cambios en las condiciones abióticas sobre las interacciones entre los depredadores y la presa fue diferente dependiendo de la especie de ácaro depredador. Mientras que la fuerza de la interacción entre las hembras de *N. californicus* y las hembras de *O. perseae* fue similar a las tres condiciones abióticas, las hembras de *E. stipulatus* sólo depredaron a las hembras de ácaro cristalino a condiciones O. Dado que ambas especies se alimentaron de *O. perseae* a condiciones O, la comparación de sus tasas de depredación mostró que *N. californicus* fue capaz de depredar más hembras por día que *E. stipulatus*. Sin embargo, no hubo diferencias entre las tasas de ovoposición de ambas especies. Por tanto, el ratio entre la ovoposición diaria y la depredación diaria fue mayor para *E. stipulatus* que para *N. californicus*. La estimación de este ratio puede informar sobre la eficiencia de los depredadores convirtiendo su presa en huevos, ya que los ácaros fitoseidos destinan una importante fracción del alimento ingerido a la reproducción (Sabelis and Janssen 1994). Así, este ratio reveló que las hembras de *E. stipulatus* exhibieron una mayor eficiencia que aquellas de *N. californicus* convirtiendo las hembras de *O. perseae* que depredaron en huevos. Tales diferencias entre especies podrían ser explicadas si cada especie depredadora ingirió diferentes cantidades de alimento de la presa. Algunos autores han encontrado diferencias en el ratio de ovoposición/depredación en función del nivel de disponibilidad de presa: algunas especies de ácaros fitoseidos convierten la presa ingerida en huevos más eficientemente a bajos niveles de disponibilidad de presa que a altos (Friese and Gilstrap 1982; Cuellar et al. 2001; Saber 2013). Esto es causado por un efecto de estimulación-interferencia (Sandness and McMurtry 1970), es decir, a densidades de presa altas, un mayor contacto con la presa puede tener un efecto estimulatorio en los depredadores dando lugar a un incremento en la tasa de depredación. Consecuentemente, el tiempo empleado alimentándose de cada individuo disminuye y, así, también disminuye la cantidad de alimento ingerido. De esta manera, los depredadores pueden matar a un mayor número de individuos antes de alcanzar la saciedad. La metodología del experimento realizado llevó a la conclusión que la disponibilidad de presa fue mayor para *N. californicus* que para *E. stipulatus*, ya que esta última no es capaz de penetrar los nidos y depredar a los huevos y las hembras de *O. perseae* que se encuentran en su interior. Así, un efecto de estimulación-interferencia podría haber sucedido en *N. californicus*. De hecho, ha sido documentado que a bajas densidades de presa *N. californicus* es más eficiente convirtiendo la presa en huevos que a altas densidades de presa (Saber 2013). Este comportamiento puede ser considerando beneficioso para el control biológico, ya que aumenta la tasa de depredación cuando los niveles de presa son altos (Ferragut et al. 1992).

Los resultados mostraron que *E. stipulatus* no depredó a hembras de *O. perseae* a condiciones CS y MCS. Sin embargo, se sabe que cuando las condiciones son de alta temperatura y baja humedad relativa el ácaro cristalino puede aumentar la producción de tela para incrementar la humedad en el interior de los nidos y proteger los estadios más vulnerables, como los huevos, frente a la desecación (Hazan et al. 1974, 1975). Por tanto, las hembras de *O. perseae* podrían haber estado tejiendo nuevas capas de tela. Si esto es verdad, no explicaría porque *E. stipulatus* no incrementó sus tasas de depredación sobre las hembras de *O. perseae* a medida que aumentó la temperatura, simplemente porque la tasa de encuentro entre los depredadores y la presa sería mayor que a condiciones O, con un menor número de hembras de *O. perseae* fuera de los nidos. Una posible explicación es que unas condiciones abióticas desfavorables podrían haber afectado al estado de esta especie disminuyendo su actividad y movimiento. Esto ya ha sido demostrado para otras especies de ácaros fitoseidos (Shipp et al. 1996; Skirvin and Fenlon 2013). No obstante, se deberían realizar otros estudios en los que se examinase cómo unas condiciones abióticas desfavorables afectan al comportamiento de esta especie.

A condiciones CS y MCS, las tasas de depredación de *N. californicus* sobre hembras de *O. perseae* no fueron significativamente diferentes a la obtenida a condiciones O. En cambio, esta especie consumió un menor número de huevos de *O. perseae* en el interior de los nidos a medida que las condiciones abióticas fueron más adversas. Estos resultados sugirieron que las hembras de *N. californicus* pasaron menos tiempo en el interior de los nidos probablemente debido a que las hembras de *O. perseae* permanecieron más tiempo fuera de los nidos a condiciones extremas. Al contrario que *E. stipulatus*, un aumento de la temperatura podría haber promovido un incremento del movimiento en *N. californicus*, tal y como muestran algunos trabajos (Auger et al. 1999). De hecho, el ratio entre la ovoposición diaria y la depredación diaria para este depredador disminuyó a condiciones CS y, además, la tasa de ovoposición fue nula a condiciones MCS poniendo de manifiesto que un aumento del movimiento pudo haber dado lugar a la disminución de la cantidad de recursos que fueron destinados a la formación de huevos en pro del mantenimiento somático. Posiblemente debido a esto las hembras de *N. californicus* no sobrevivieron en la ausencia de alimento bajo condiciones abióticas extremas, a diferencia de las hembras de *E. stipulatus*, cuya supervivencia fue mayor al 70% incluso sin consumir ningún alimento. Por tanto, las diferentes estrategias adoptadas por cada especie de ácaro depredador frente a las altas temperaturas, en un caso reduciendo su movimiento y en otro caso aumentándolo, podrían explicar las diferencias en la supervivencia que fueron observadas entre especies.

En el módulo comunitario de competencia aparente los resultados revelaron que la presencia de un alimento alternativo (polen) para los depredadores influyó en la fuerza de la interacción entre *E. stipulatus* y *O. perseae*, y tal influencia fue a su vez afectada por las condiciones abióticas. En cambio, la disponibilidad de polen no tuvo efectos en la interacción entre *N. californicus* y el ácaro cristalino a ninguna de las condiciones abióticas.

Como fue observado en el módulo comunitario de cadena trófica, *E. stipulatus* depredó a hembras de *O. perseae* en la ausencia de polen a condiciones O. Sin embargo, este ácaro depredador claramente prefirió alimentarse de polen cuando este recurso estuvo disponible a estas condiciones. Este resultado era esperable ya que el polen es considerado un alimento óptimo para las especies del género *Euseius*, las cuales alcanzan su mayor potencial reproductivo cuando consumen este tipo de recurso (Ferragut et al. 1987; McMurtry and Croft 1997; Bouras and

Papadoulis 2005; González-Fernández et al. 2009). En efecto, las tasas de ovoposición de *E. stipulatus* fueron siempre más altas cuando esta especie dispuso de polen. Además, este ácaro depredador sólo ovopositó bajo condiciones extremas cuando el polen estuvo presente. Sin embargo, dado que la tasa de eclosión de huevos de *E. stipulatus* es muy baja cuando las condiciones son secas (Ferragut et al. 1987; Guzmán 2014), es muy probable que tras largos períodos a condiciones de altas temperaturas y humedad relativa baja se produjera un descenso de las poblaciones de esta especie, incluso en presencia de polen como alimento alternativo.

En cuanto a *N. californicus*, los resultados sugirieron que cuando las hembras dispusieron de polen y *O. perseae*, sólo depredaron al herbívoro a las tres condiciones abióticas. Además, ni las tasas de depredación ni las tasas de ovoposición difirieron entre los tratamientos con polen y sin polen.

Por tanto, la adición de polen como un alimento alternativo no tendría efectos en las poblaciones de *N. californicus*. Sin embargo, la presencia de polen podría promover una respuesta numérica en *E. stipulatus* y favorecer el control de la plaga a través de la inducción de competencia aparente entre el herbívoro y el polen. Pero esto sólo sería probable a condiciones suaves, ya que las condiciones adversas perjudicarían el crecimiento de las poblaciones de este depredador.

Módulos comunitarios: Depredación intragremial y Depredación intragremial-Competencia aparente

Tras evaluar el efecto de las condiciones abióticas y la presencia de un alimento alternativo en las interacciones entre *E. stipulatus* y *N. californicus*, y la especie plaga *O. perseae*, a continuación se determinó el efecto de estos dos factores sobre las interacciones intragremiales entre los dos ácaros depredadores con *O. perseae* como especie presa compartida. Estas interacciones fueron analizadas usando los módulos comunitarios de depredación intragremial y depredación intragremial-competencia aparente.

Las interacciones intragremiales entre los dos ácaros depredadores fueron evaluadas con hembras de *E. stipulatus* y *N. californicus* actuando como IG-depredador, y juveniles de *N. californicus* y *E. stipulatus* actuando como IG-presa, respectivamente.

En el <u>módulo comunitario de depredación intragremial,</u> los resultados pusieron de manifiesto el importante papel que jugaron las condiciones abióticas determinando la estructura trófica de esta comunidad de ácaros.

A condiciones O, cuando hembras de *E. stipulatus* actuaron como IG-depredador y los juveniles de *N. californicus* como IG-presa, los resultados revelaron que las hembras de *E. stipulatus* sólo depredaron a los juveniles de *N. californicus*, mientras que estos últimos consumieron a la presa compartida, *O. perseae*. De este modo, la estructura de la comunidad se asemejó más a una cadena trófica que a una comunidad con depredación intragremial, con las hembras de *E. stipulatus* depredando a juveniles de *N. californicus* y con juveniles de *N. californicus* depredando a *O. perseae*. Cabe destacar que la presencia de huevos de *O. perseae* depredados dentro de los nidos reveló que los juveniles de *N. californicus* fueron capaces de penetrar en estos y alimentarse dentro. Dado que los nidos estaban intactos, los juveniles utilizaron las entradas construidas por *O. perseae* para pasar al interior de los nidos (observación personal).

Cuando las interacciones fueron evaluadas con hembras de *N. californicus* actuando como el IG-depredador y con juveniles de *E. stipulatus* actuando como la IG-presa, los resultados mostraron que *N. californicus* no depredó ni a *O. perseae* ni a la IG-presa, siendo los juveniles de *E. stipulatus* los únicos que depredaron a la presa compartida. Por tanto, la estructura de la comunidad también se pareció a una cadena trófica, sólo con juveniles de *E. stipulatus* consumiendo a las hembras de ácaro cristalino y con las hembras de *N. californicus* no interaccionando en absoluto. Es muy probable que los juveniles de *E. stipulatus* indujeran cambios en el comportamiento de las hembras de *N. californicus*, causando su inactividad. Cabe la posibilidad que las hembras de *N. californicus* percibieran a los juveniles como depredadores potenciales, lo que habría desencadenado un comportamiento anti-depredador. De hecho, en otro trabajo se observó que los juveniles de *E. stipulatus* pueden ejercer este tipo de efectos no letales sobre las hembras de *N. californicus* (Abad-Moyano et al. 2010b).

Con respecto al control de la plaga, estos resultados llevaron a concluir que las configuraciones tróficas obtenidas a condiciones O siempre favorecerían la herbivoría a través de efectos indirectos de *E. stipulatus* sobre *O. perseae*. Por un lado, la depredación de los juveniles de *N. californicus* por parte de las hembras de *E. stipulatus*, mermarían las poblaciones de *N. californicus* conduciendo a un incremento en la abundancia de la plaga. Por otro lado, el efecto que los juveniles de *E. stipulatus* pueden ejercer sobre el comportamiento de las hembras de *N. californicus* reducirían la actividad depredadora de esta especie sobre el ácaro cristalino, conduciendo de nuevo a un aumento de las poblaciones de la plaga.

A condiciones CS y MCS, cuando las hembras de *E. stipulatus* actuaron como IG-depredador y los juveniles de *N. californicus* como IG-presa, los resultados sugirieron que bajo condiciones abióticas desfavorables la estructura de la comunidad dejó de asemejarse a una cadena trófica y cambió a una comunidad dominada por competencia por explotación entre las dos especies de depredadores. Es muy probable que este cambio en la configuración de la comunidad respecto a condiciones O, donde las hembras de *E. stipulatus* pasaron de depredar sólo a la IG-presa a depredar sólo la presa compartida, fuera la consecuencia de un cambio en la disponibilidad de ambas presas en función de las condiciones abióticas. La disponibilidad de los juveniles de *N. californicus* podría haber disminuido a condiciones CS y MCS debido a dos causas: (i) a una tendencia de los juveniles de *N. californicus* a escapar de las arenas experimentales bajo estas condiciones; (ii) a la habilidad de los juveniles de *N. californicus* a penetrar en los nidos de *O. perseae*, los cuales podrían haber entrado para resguardarse de las condiciones abióticas adversas. La presencia de juveniles de *N. californicus* en el interior de los nidos podría haber provocado que las hembras de *O. perseae* que se encontraban en el exterior se abstuvieran de penetrar en ellos, prolongando de esta forma el riesgo de ser depredadas por las hembras de *E. stipulatus*.

Cuando las hembras de *N. californicus* fueron el IG-depredador y los juveniles de *E. stipulatus* la IG-presa, los resultados indicaron que sólo las hembras de *N. californicus* se alimentaron de la presa. Además, no se observó depredación del IG-depredador a los juveniles de *E. stipulatus*. Así, la configuración de la comunidad fue de nuevo una cadena trófica, pero esta vez sólo con las hembras de *N. californicus* depredando a *O. perseae*, y con los juveniles de *E. stipulatus* no interaccionando con ninguna de las otras dos especies. Este cambio en la estructura de la comunidad con respecto a condiciones O condujo a hipotetizar que unas condiciones abióticas adversas afectaron fuertemente a los juveniles de *E. stipulatus*. Por tanto, éstos habrían dejado de inducir un comportamiento anti-depredador en las hembras de *N. californicus*, las cuales reanudaron su actividad depredadora.

Cabe destacar que cuando esta comunidad de ácaros simuló un módulo comunitario con IGP, todos los resultados obtenidos sugirieron que las dos especies de depredadores podrían no estar involucradas en IGP, ya que ninguna de ellas depredó a la vez tanto a la IG-presa como a la presa compartida cuando los dos tipos de presa estuvieron disponibles.

En el módulo comunitario de depredación intragremial-competencia aparente, la presencia de un alimento alternativo (i.e. polen) redujo las interacciones entre las dos especies de ácaros depredadores, dando lugar a configuraciones tróficas que favorecerían el control biológico de la plaga.

A las tres condiciones abióticas estudiadas, en ambas combinaciones de IG-depredador y IG-presa (i.e. *E. stipulatus* y *N. californicus* actuando como IG-depredador o como IG-presa), la presencia de polen dividió esta comunidad de ácaros en dos cadenas tróficas: una con *E. stipulatus* (hembras o juveniles) alimentándose de polen y la otra con *N. californicus* (hembras o juveniles) depredando a *O. perseae*. Como fue mencionado anteriormente, *E. stipulatus* es una especie que consume preferencialmente polen cuando éste está presente (McMurtry and Croft 1997). Así, cuando este recurso estuvo disponible, las hembras de *E. stipulatus* redujeron sus ataques sobre los juveniles de *N. californicus*, y los juveniles de *E. stipulatus* minimizaron sus intentos de ataque o interferencia sobre las hembras *N. californicus*, dejando de influir en la actividad depredadora de esta especie, lo que fortalecería la interacción entre *N. californicus* y *O. perseae*. Además, la presencia de polen contribuyó a aumentar las tasas de ovoposición de las hembras de *E. stipulatus* a las tres condiciones abióticas, y contrarrestó los efectos negativos que las condiciones abióticas adversas ejercieron sobre los juveniles de *E. stipulatus*.

(ii) A nivel poblacional

A nivel de individuo la presencia de polen en el sistema dividió la comunidad de ácaros en dos cadenas tróficas independientemente de las condiciones abióticas, una con *E. stipulatus* alimentándose de polen y la otra con *N. californicus* depredando a *O. perseae*. Por tanto, en base a estos resultados, a nivel poblacional la adición de polen como alimento alternativo para *E. stipulatus* mejoraría el control de las poblaciones de *O. perseae* debido a que (i) debilitaría las interacciones negativas entre *E. stipulatus* y *N. californicus* a condiciones óptimas y extremas, y (ii) promovería una respuesta numérica de las poblaciones de *E. stipulatus* que podría inducir la aparición de competencia aparente entre el polen y *O. perseae*, principalmente a condiciones óptimas, lo que ejercería un impacto extra sobre las poblaciones del ácaro cristalino.

Por un lado, contrario a lo que se esperaba, la adición de polen al sistema no redujo las interacciones entre ambas especies depredadoras, ya que los resultados revelaron que probablemente *E. stipulatus* excluyó a *N. californicus* a ambas condiciones abióticas, independientemente de la presencia o ausencia de polen. En ausencia de polen este resultado podría ser explicado por el ratio inicial de IG-presa/IG-depredador y la estructura de edades de las poblaciones de depredadores, lo cual determina los patrones de coexistencia y exclusión en los sistemas con depredación intragremial (Montserrat et al. 2008b). Al comienzo del experimento las poblaciones de ambos depredadores no tenían una estructura de edades ya que sólo estaban formadas por hembras de ambas especies, y además la disponibilidad de la presa compartida fue diferente para ambas especies de depredadores. La habilidad las hembras de *N. californicus* de penetrar en los nidos del ácaro cristalino y alimentarse en su interior podría haberle conferido a esta especie una ventaja inicial sobre las hembras de *E. stipulatus*, las cuales son incapaces de entrar en los nidos. Por tanto, una mayor ingesta de alimentos por parte de *N. californicus* podría haber sido traducida en una mayor descendencia, incrementando la densidad de los estadios que fueron vulnerables de ser depredados por las hembras de *E. stipulatus*. Este escenario inicial, en el que la combinación de una baja disponibilidad de *O. perseae* fuera de los nidos debido a la depredación de *N. californicus* en el interior de los nidos, y una alta disponibilidad de estadios inmaduros de *N. californicus* podría haber dado lugar a una fuerte presión de depredación de *E. stipulatus* sobre *N. californicus*. Esto habría impedido el crecimiento de las poblaciones de *N. californicus*, incrementando la probabilidad de que *E. stipulatus* excluyera a esta especie a través del experimento debido a la falta de renovación de las poblaciones de *N. californicus*. Considerando este escenario inicial, la presencia de polen debería haber reducido los niveles de depredación de *E. stipulatus* sobre *N. californicus*. Sin embargo, los resultados sugirieron que esto no sucedió. Probablemente la adición del polen como un único foco a la planta pudo ser la causa de los resultados obtenidos. Esto podría haber conducido a una segregación de las poblaciones de *E. stipulatus*, con individuos alimentándose preferencialmente de polen cerca del foco de polen y con individuos depredando preferencialmente a inmaduros de *N. californicus* lejos del foco de polen. De esta forma, incluso cuando se añadió polen al sistema, *N. californicus* podría haber estado sometido a altas tasas de depredación. No obstante, a condiciones CS, las altas temperaturas y la baja humedad relativa posiblemente redujeron la efectividad de *E. stipulatus* como depredador, y en consecuencia, una población segregada de *E. stipulatus* podría haber ejercido menos presión de depredación sobre *N. californicus* durante las fase inicial del experimento. Por tanto, esto pudo haber retrasado la exclusión de *N. californicus* por *E. stipulatus*, siendo la causa de un mejor control de las poblaciones de *O. perseae* que fue observado sólo a estas condiciones en la presencia de polen.

Por otro lado, la adición de polen promovió un aumento de las poblaciones *E. stipulatus* a condiciones O. Este resultado indicó que una vez esta especie excluyó a *N. californicus* del sistema, *E. stipulatus* pudo alimentarse preferencialmente de polen lo que promovió una respuesta numérica de esta especie pero que no fue traducida en una mayor reducción de las poblaciones de *O. perseae* en comparación al tratamiento sin polen. En cambio, a condiciones CS, los resultados revelaron que la abundancia final de depredadores fue similar en los tratamientos con polen y sin polen, indicando que el polen no favoreció el aumento de las poblaciones de *E. stipulatus*. Aunque a nivel de individuo la presencia de polen contribuyó a aumentar las tasas de ovoposición de esta especie, una exposición prolongada a unas condiciones abióticas adversas fueron perjudiciales para las poblaciones de *E. stipulatus*, ya que sus huevos son altamente vulnerables a la desecación. Estos resultados pusieron de manifiesto que las condiciones abióticas fueron fundamentales en la dinámica de las poblaciones de depredadores. Así, cambios en los factores abióticos que son predichos por los modelos de cambio climático podrían conducir una pérdida de las poblaciones de ácaros depredadores, lo cual proporcionaría a los herbívoros, que son menos sensibles al calentamiento que los niveles tróficos superiores (Voigt et al. 2003; Schweiger et al. 2008), la oportunidad de escapar del control biológico.

Por tanto, los resultados obtenidos en esta tesis muestran que las respuestas de cada especie a cambios en las condiciones abióticas modifican la forma en la que interaccionan entre sí, afectando a la estructura trófica y la dinámica de la comunidad. Bajo el escenario de cambio climático actual, esto implica que para lograr un manejo eficiente de enemigos naturales en comunidades agrícolas es fundamental tener en cuenta la influencia que los factores abióticos ejercen sobre las interacciones que ocurren entre las especies que componen cada comunidad.

7. REFERENCES

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APPENDICES

Appendix 1

Protocol for extraction of pollen from flowers of *Carpobrotus edulis*

1. Pink flowers of *Carprobrotus edulis* are collected three times a week during spring months (March and April).

2. Sepals and petals are removed from flowers, and stamens are taken with a clamp (Figure A1.1).

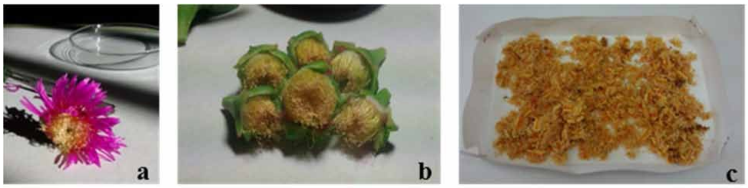


Figure A1.1. a. Flower of *C. edulis* without sepals and a part of petals removed; b. Flowers only with stamens; c. Stamens removed.

3. Stamens are dried in a stove at 37 ºC for 48h.

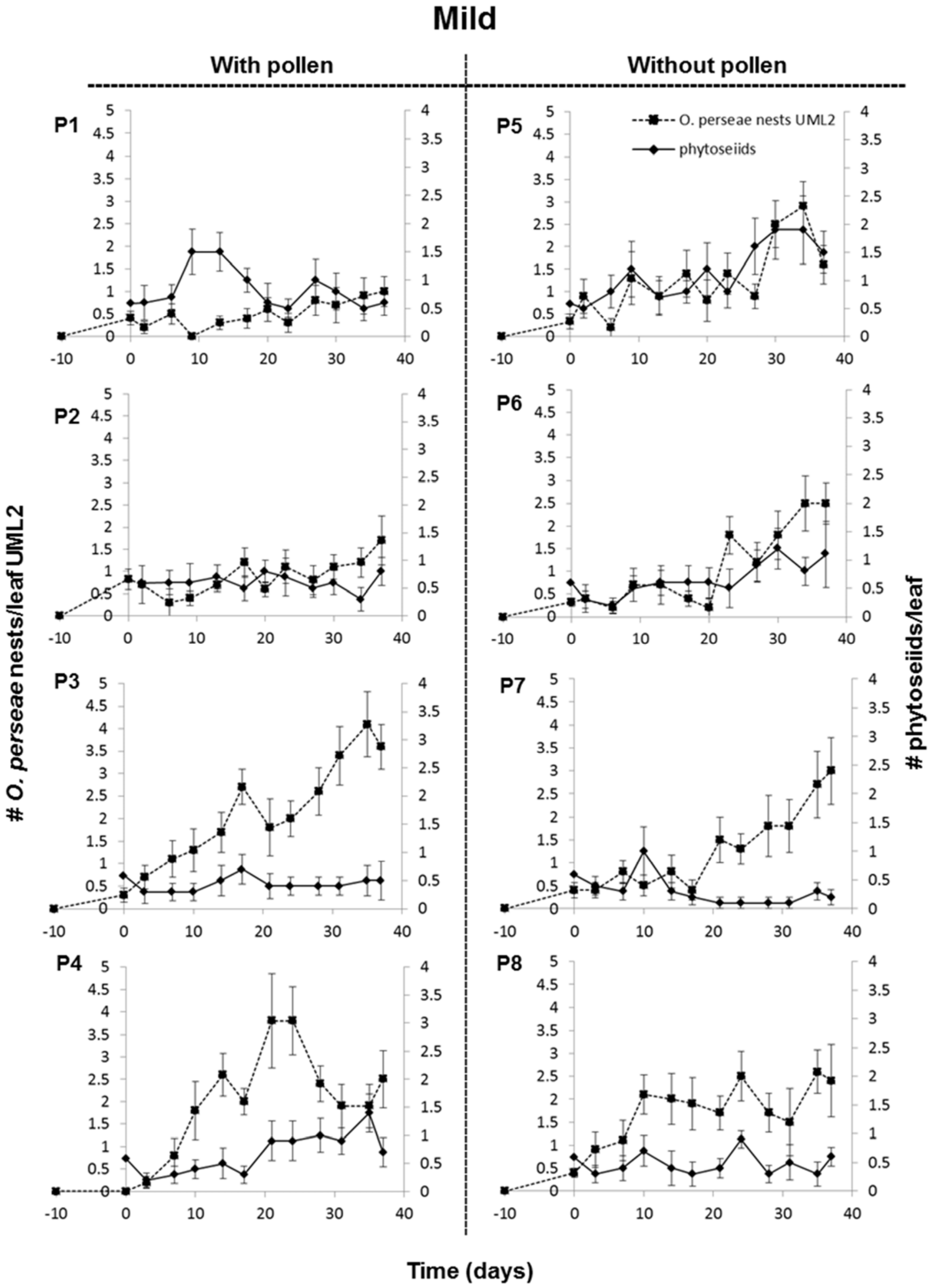
Figure A1.2. First sieving (and b); Second sieving (c); Pollen extraction (d); Pollen storage (e)

4. Stamens are sieved through two different pore diameters using a brush: the first sieving (0.25 mm) is to remove stamen remains (Figure A1.2; a and b) and the second sieving (0.075 mm) is to extract the pollen (Figure A1.2; c and d).

5. Pollen is stored in plastic containers at 4ºC (Figure A1.2e).

Appendix 2

Mite predator/prey population dynamics



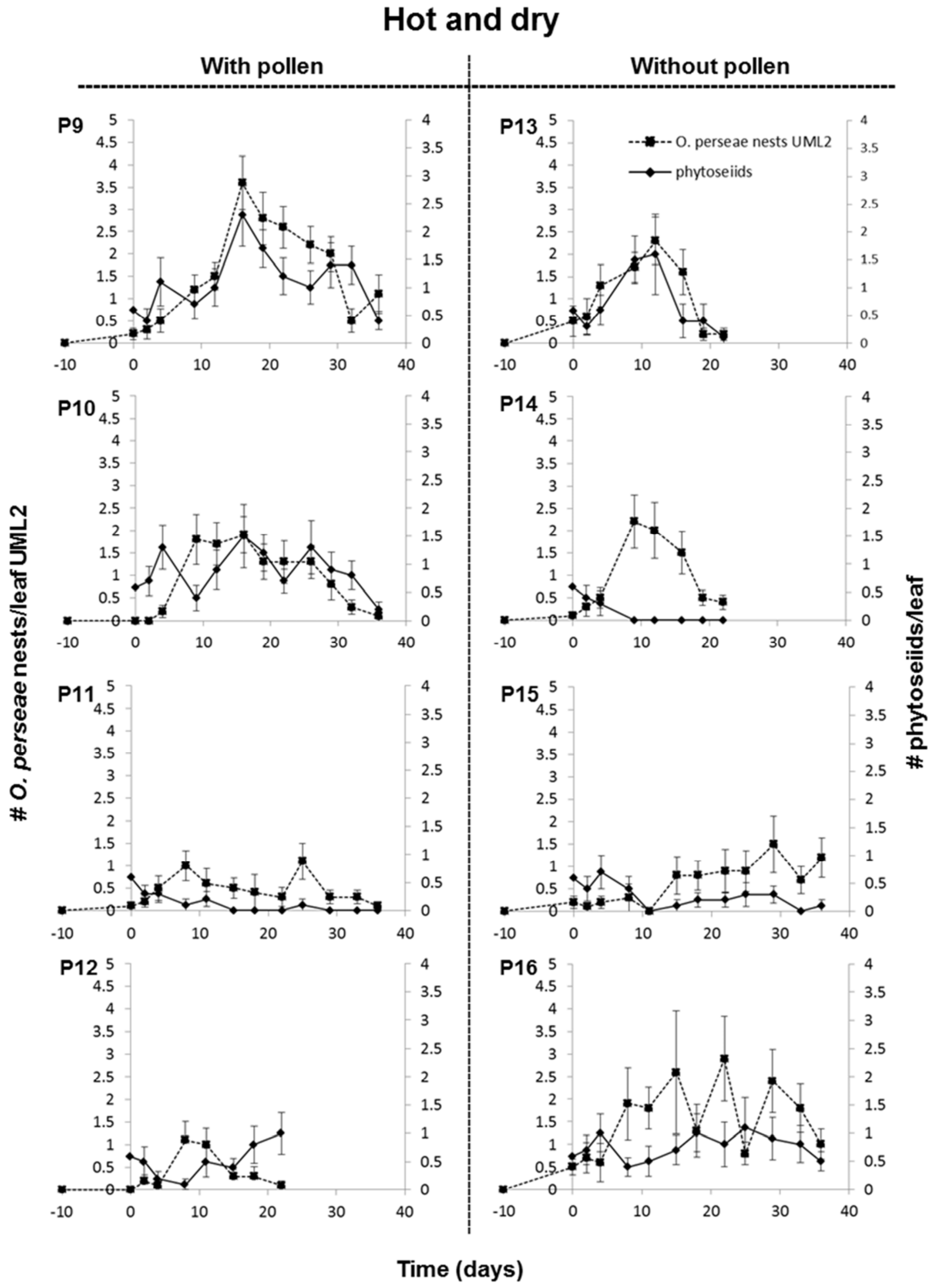


Figure A2. Population dynamics of *O. perseae* and predatory mites (*E. stipulatus* and *N. californicus*) for each plant (16 plants) depending on the two abiotic conditions ("mild" and "hot and dry"), and treatment (with pollen and without pollen). Each point corresponds to the average (± SE) of number of occupied nests in the UML2, and the total number of mobile stages (i.e. adults and juveniles) of phytoseiid mites per leaf for each recount date. Note that the number of predatory mites on day 0 was set to 0.59 in all replicates, i.e., initial number of predators (20 females) divided by average number of leaves per plant (34 leaves).

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