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Towards the integrated management of the Texas citrus mite *Eutetranychus banksi* (Acari: Tetranychidae) in Spain

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Towards the integrated management of the Texas citrus mite *Eutetranychus banksi* (Acari:Tetranychidae) in Spain

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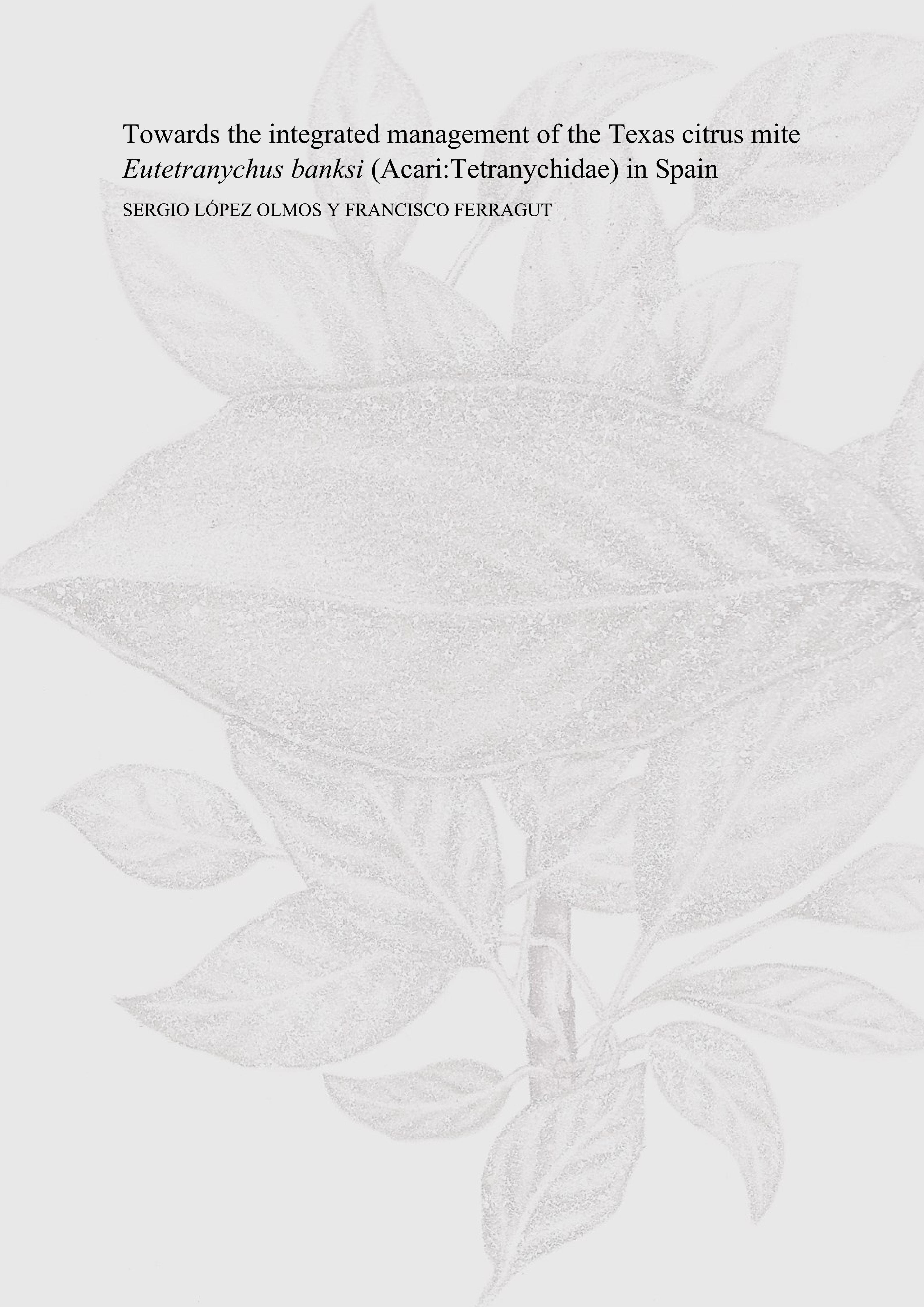

A Caterina, por todo

A mis padres, por su apoyo incondicional

“Everything is connected to everything else”

First law of Ecology

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Abstract

Agricultural ecosystems are usually poor in species richness and subject to constant disturbances through agricultural practices, making them more prone to the establishment of exotic species. The Texas citrus mite, *Eutetranychus banksi* McGregor, is native to the Americas and widely distributed across this continent. In 2013 it was detected in the south of the province of Valencia, affecting the main citrus-growing area in Spain. The mite produces severe damage, reducing photosynthesis, causing defoliation, and producing a lack in fruit pigmentation, which could affect its economic value.

Firstly, this work evaluated the ecological impact produced by the invasive species on the resident spider mites *Panonychus citri* (McGregor) and *Eutetranychus orientalis* (Klein). Since its arrival in 2013, *E. banksi* has become the most frequent and abundant spider mite on citrus, competitively displacing the other species and reducing their presence and geographic range, which is influenced by its colonisation history. Secondly, this work studies the within-tree distribution and seasonal trends of the pest and associated phytoseiid mites, which may contribute to its control. The Texas citrus mite was feeding mainly on the adaxial (upper) side of leaves in the periphery of the tree canopy showing a single population peak in late summer-early autumn, while phytoseiids preferred the abaxial (lower) sides inside the canopy showing two peaks, a main spring peak and a second, less abundant, in autumn. *Euseius stipulatus* (Athias-Henriot) was the most frequent and abundant phytoseiid, and when *E. banksi* increased, it moved to the adaxial sides on outer leaves and fruits to feed on its prey and changed its colouring from white to reddish-brown, evidencing its contribution to biological pest control. However, it was not capable of maintaining *E. banksi* populations under tolerable densities due to the unfavourable predator/prey ratios in summer and early autumn.

Thirdly, this work aimed to describe the pest population structure and define a sampling plan. There were differences in the age structure on fruits and leaves, as well as between leaves from different flushes. Furthermore, over time, there were fluctuations in its composition correlated with variations in sex-ratio. No aggregation differences among plant strata were found, but there were significant differences between immature and adult stages, the females being the less aggregated. The high correlation of the total population with the motile forms and females allowed the use of both as a reference stage in the sampling plan, establishing a presence/absence sampling of 100 leaves for females or 400 leaves for motile forms. Finally, a recently described phytoseiid mite, *Neoseiulus madeirensis* Papadoulis & Kapaxidi, was found to be associated with *E. banksi*, suggesting that it could be a promising candidate for pest suppression. Fourthly, this work aimed to evaluate the potential of this predator to control pest populations. *Neoseiulus madeirensis* exhibited a short developmental time, high survival and reproductive rates feeding on *E. banksi*. The predator was fed mainly on immature stages, with eggs being the preferred stage, showing a type II functional response for all the prey stages tested, that stabilises at high prey densities with high egg laying. Survival and reproduction values were the best obtained so far for any phytoseiid previously tested against *E. banksi*, as well as comparable to values reported for the most common phytoseiids used in the biological control of spider mites. All this, together with its high predation rate and associated egg deposition, makes it a suitable candidate for the development of a biological control program based on augmentative releases or importation aiming for the establishment of permanent populations on citrus.

Resumen

Los ecosistemas agrícolas suelen ser pobres en diversidad de especies y están sometidos a constantes perturbaciones generadas por las prácticas agrícolas, lo que los hace más propensos al establecimiento de especies exóticas. El ácaro de Texas, *Eutetranychus banksi* McGregor, es nativo de América y está ampliamente distribuido por el cultivo de cítricos de este continente. En 2013, esta especie se detectó en el sur de la provincia de Valencia, afectando a la principal zona citrícola de España. El ácaro produce graves daños reduciendo la fotosíntesis, causando defoliación y decoloración de los frutos, lo que podría afectar a su valor económico.

En primer lugar, este trabajo evaluó el impacto ecológico producido por la especie invasora sobre las especies de ácaros tetraníquidos residentes en los cítricos valencianos *Panonychus citri* (McGregor) y *Eutetranychus orientalis* (Klein). Desde su llegada en 2013, *E. banksi* se ha convertido en el tetraníquido más frecuente y abundante en cítricos desplazando competitivamente a las otras especies, reduciendo su presencia y distribución geográfica, a su vez influenciada por su historia de colonización. En segundo lugar, este trabajo estudió la distribución dentro del árbol y las tendencias estacionales de la plaga y de los ácaros fitoseidos asociados, que pueden contribuir a su control. El ácaro de Texas se alimentó principalmente en la cara adaxial (haz) de las hojas en la periferia de la copa del árbol, mostrando un único pico de población a finales de verano-principios de otoño, mientras que los fitoseidos prefirieron la cara abaxial (envés) de las hojas del interior de la copa, mostrando dos picos, uno principal en primavera y otro menos abundante en otoño. *Euseius stipulatus* (Athias-Henriot) fue el fitoseido más frecuente y abundante, y cuando *E. banksi* aumentó se desplazó a las caras adaxiales de las hojas de la parte exterior de la copa y los frutos para alimentarse de su presa, cambiando su coloración de blanco a marrón rojizo evidenciando su contribución al control biológico de la plaga. Sin embargo, no fue capaz de mantener las poblaciones de *E. banksi* bajo densidades tolerables debido a la desfavorable relación depredador/presa que existe en verano y principios de otoño.

En tercer lugar, este trabajo pretendió describir la estructura de población del ácaro de Texas y definir un plan de muestreo. Existieron diferencias en la estructura de edad en frutos y hojas, así como entre hojas de diferentes brotes. Además, a lo largo del tiempo se observaron fluctuaciones en su composición correlacionadas con variaciones en la proporción de sexos. No se observaron diferencias de agregación entre estratos vegetales, pero sí entre estadios inmaduros y adultos, siendo las hembras las menos agregadas. La alta correlación de la población total con las formas móviles y las hembras permitió utilizar ambas como estadio de referencia para el desarrollo del plan de muestreo, estableciendo un muestreo de presencia/ausencia de 100 hojas para las hembras o 400 hojas para las formas móviles. Finalmente, un ácaro fitoseido recientemente descrito, *Neoseiulus madeirensis* Papadoulis & Kapaxidi, se encontró asociado a *E. banksi*, sugiriendo que podría ser un candidato prometedor para su control biológico. En cuarto lugar, este trabajo pretendió evaluar el potencial de este depredador para controlar las poblaciones de la plaga. *Neoseiulus madeirensis* mostró un rápido desarrollo y altas tasas de supervivencia y reproducción alimentándose de *E. banksi.* El depredador se alimentó principalmente de estadios inmaduros, siendo los huevos el estadio preferido, y mostrando una respuesta funcional de tipo II para todos los estadios de presa ensayados, que se estabilizó a altas densidades de presa con una elevada puesta de huevos. Los valores de supervivencia y reproducción han sido los mejores obtenidos hasta el momento para cualquier fitoseido ensayado previamente contra *E. banksi,* así como comparables a los valores que presentan los fitoseidos más comunes utilizados en el control biológico de arañas rojas. Todo ello, unido a su elevada tasa de depredación y a la puesta de huevos asociada, lo convierte en un candidato idóneo para el desarrollo de un programa de control biológico basado en sueltas aumentativas o en la importación con vistas al establecimiento de poblaciones permanentes en cítricos.

Resum

Els ecosistemes agrícoles solen ser pobres en diversitat d'espècies i estan sotmesos a constants pertorbacions generades per les pràctiques agrícoles, cosa que els fa més propensos a l'establiment d'espècies exòtiques. L´àcar de Texas, *Eutetranychus banksi* McGregor, és nadiu d´Amèrica i està àmpliament distribuït pel cultiu de cítrics d´aquest continent. El 2013, esta espècie es va detectar al sud de la província de València, afectant la principal zona citrícola d'Espanya. L'àcar produeix greus danys reduint la fotosíntesi, causant defoliació i decoloració dels fruits, cosa que podria afectar el seu valor econòmic.

En primer lloc, aquest treball va avaluar l'impacte ecològic produït per l'espècie invasora sobre les espècies d'àcars tetraníquids residents als cítrics valencians *Panonychus citri* (McGregor) i *Eutetranychus orientalis* (Klein). Des de la seva arribada el 2013, *E. banksi* s'ha convertit en el tetraníquid més freqüent i abundant en cítrics desplaçant competitivament les altres espècies, reduint la seva presència i distribució geogràfica, alhora influenciat per la seva història de colonització. En segon lloc, aquest treball va estudiar la distribució dins de l'arbre i les tendències estacionals de la plaga i dels àcars fitoseids associats, que poden contribuir al seu control. L'àcar de Texas es va alimentar principalment en la cara adaxial (fes) de les fulles a la perifèria de la copa de l'arbre, mostrant un únic pic de població a finals d'estiu-principis de tardor, mentre que els fitoseids van preferir la cara abaxial (revers) de les fulles de l'interior de la copa, mostrant dos pics, un de principal a la primavera i un altre menys abundant a la tardor. *Euseius stipulatus* (Athias-Henriot) va ser el fitoseid més freqüent i abundant, i quan *E. banksi* va augmentar es va desplaçar a les cares adaxials de les fulles de la part exterior de la copa i els fruits per alimentar-se de la presa, canviant la seva coloració de blanc a marró vermellós evidenciant la seva contribució al control biològic de la plaga. Tot i això, no va ser capaç de mantenir les poblacions d'*E. banksi* sota densitats tolerables a causa de la desfavorable relació depredador/presa que hi ha a l'estiu i principis de tardor.

En tercer lloc, aquest treball va voler descriure l'estructura de població de l'àcar de Texas i definir un pla de mostreig. Hi hagué diferències en l'estructura d'edat de fruits i fulles, així com entre fulles de diferents brots. A més, al llarg del temps es van observar fluctuacions en la composició correlacionades amb variacions en la proporció de sexes. No es van observar diferències d'agregació entre estrats vegetals, però sí entre estadis

immadurs i adults, sent les femelles les menys agregades. L'alta correlació de la població total amb les formes mòbils i les femelles va permetre utilitzar totes dues com a estadi de referència per al desenvolupament del pla de mostreig, establint un mostreig de presència/absència de 100 fulls per a les femelles o 400 fulls per a les formes mòbils. Finalment, una espècie fitoseid recentment descrit, *Neoseiulus madeirensis* Papadoulis & Kapaxidi, es va trobar associat a *E. banksi*, suggerint que podria ser un candidat prometedor per al seu control biològic. En quart lloc, aquest treball va voler avaluar el potencial d'aquest depredador per controlar les poblacions de la plaga. *Neoseiulus madeirensis* va mostrar un desenvolupament ràpid i altes taxes de supervivència i reproducció alimentant-se d'*E. banksi*. El depredador es va alimentar principalment d'estadis immadurs, sent els ous l'estadi preferit, i mostrant una resposta funcional de tipus II per a tots els estadis de presa assajats, que es va estabilitzar a altes densitats de presa amb una posta d'ous elevada. Els valors de supervivència i reproducció han estat els millors obtinguts fins ara per a qualsevol espècie de fitoseid assajat prèviament contra *E. banksi*, així com comparables als valors que presenten els fitoseids més comuns utilitzats en el control biològic d'aranyes roges. Tot plegat, unit a la seva elevada taxa de depredació i a la posta d'ous associada, el converteix en un candidat idoni per al desenvolupament d'un programa de control biològic basat en soltes augmentatives o en la importació amb vista a l'establiment de poblacions permanents en cítrics.

CHAPTER 1

Introduction

1.1 Geographical distribution and invasion history

The Texas citrus red mite (TCM), *Eutetranychus banksi* McGregor (Acari: Tetranychidae), was first described from castor bean, *Ricinus communis* L., and velvet bean, *Mucuna deeringiana* (Bort) Merrill, leaves in Orlando, Florida (McGregor 1914), but it was not collected until 1935 on citrus from Rio Grande Valley in Texas (Dean 1952, 1959b, 1980). Considered native to the Americas, TCM was cited in North, Central and South America (Jeppson et al. 1975), and is currently widely distributed across the continent, from southern United States to northern Argentina, and reported from most countries in Latin America (Migeon and Dorkeld 2022). This species is considered a major spider mite pest on citrus (Gerson 2003), being an economic Texas citrus problem since the late 1920's and has been the most important spider mite pest in the warm and humid areas of Florida since 1960 (Childers et al. 1991). Furthermore, it is one of the most polyphagous spider mite species (Gerson 2003) reported on 110 hosts belonging to 34 plant families, mostly Fabaceae (22 species) and Rutaceae (9 species), infecting other crops and many ornamental plants (Migeon and Dorkeld 2022).

Far from its native region, TCM was found on citrus in Egypt (Abdel-Shaheed et al. 1973), and more recently in Portugal (Carvalho et al. 1999), Spain (García et al. 2003) and Iran (Beyzavi et al. 2013). Since its arrival to the Iberian Peninsula, severe damage to citrus has been recorded in the Algarve region of southern Portugal, making it a key pest (Gonçalves et al. 2002). In 2001, Spanish citrus orchards in Ayamonte and Isla Cristina (Huelva province) were invaded by *E. banksi*. Although we have no information about the dissemination pathway followed by the mites, it has been suggested that they were carried in fruit containers that are frequently exchanged by Portuguese and Spanish farmers along the border area (García et al. 2003). In 2013, *E. banksi* was suddenly detected in La Safor (Valencia province) more than 800 Km away from Ayamonte, and the following year in other regions of the provinces of Valencia and Alicante, affecting the main citrus-growing area of the eastern Mediterranean in Spain. Because no geographical spread of this species throughout the citrus-growing regions of southern Spain was detected, it seems that the Valencian populations came directly from Huelva via contaminated plant material (Ferragut 2016).

Before the arrival of TCM in Valencian citrus, three spider mite species had already occurred in the crop. The two-spotted spider mite *Tetranychus urticae* Koch, the citrus red mite *Panonychus citri* (McGregor), and the Oriental red mite *Eutetranychus orientalis* (Klein). Only *T. urticae* is thought to be native to the Mediterranean basin while the others had invaded the region in the past. The Asian species *P. citri* was the first invasive spider mite to infest the Spanish citrus in 1981 (García-Marí and del Rivero 1981), spreading rapidly throughout the Valencian region and becoming a key pest (García-Marí et al. 1983). *Eutetranychus orientalis* is a common species in many countries in Asia and North Africa (Migeon and Dorkeld 2022), and, in 2001, arrived to the citrus-growing area of southern Spain, being detected for the first time in the province of Málaga (García et al. 2003). Since its arrival, *E. orientalis* spread rapidly from this initial focus westward and eastward, reaching Murcia and Alicante province in the south of the Valencian region within a few years (Ledesma et al. 2011; Vela et al. 2013).

1.2 Distinctive morphological and biological features

Species in the genus *Eutetranychus* have distinctive characteristics and can be easily separated from other spider mites belonging to other genera found on citrus in the Mediterranean region, such as *T. urticae* and *P. citri*. When resting, both females and males have their first two pairs of legs oriented straight forward and the third and fourth pairs backward (Ferragut et al. 2013b). Adult females are broadly oval, flattened, with variable color, from brownish green with dark brown to greenish spots dorsally and stripes near the lateral margins. The legs are pale with the basal segments orange to brown. Males are more slender, with a triangular posterior body shape and legs longer than those in females; they are very active on leaves (Dean 1952). Newly hatched larvae are light yellow with pale legs, while nymphs, except for size, appear much like the adult females in form and colour. Eggs are flat and disk-like with a fine rolled rim or edge, transparent when first laid, but, before hatching, reddish to brownish (Muma et al. 1953) (Figure 1.1a, b). In contrast to *T. urticae*, *Eutetranychus* do not produce silk and usually feed on the upper side of the leaf, like *P. citri*, producing similar feeding symptoms. However, morphologically they are distinct: *Eutetranychus* lack the dorsal strong tubercles which characterize the dorsal not-flattened surface in *P. citri*. Furthermore, eggs of *P. citri* are almost red and spherical, somewhat flattened and with a straight and tapering filament emerging from the top, and the adults, and all immature stages, are reddish or purple (Ferragut et al. 2013b).

Figure 1.1 *Eutetranychus banksi,* a. Deutonymph, male and exuvia next to the midrib. b. Protonymph, larva, female and eggs close to midrib. In the images it is possible to distinguish the feeding damage caused by *E. banksi* on the leaf ‘s surface.

The two *Eutetranychus* species present in the Iberian Peninsula are very similar morphologically and it is not possible to distinguish them with the naked eye, or by using a hand lens in the field or a stereoscopic microscope in the laboratory. Intraspecific variability in some characteristics, such as the dorsal setae, has been reported in both species, and morphological variability, depending on the host plant, has been demonstrated in *E. banksi* (Mattos and Feres 2009). Despite this, reliable features for species identification include the pattern formed by the setae *e1* and *f1* on the dorsal surface and the number of ventral setae on the coxa of leg II (Figure 1.2). In *E. banksi*, setae *f1* are broadly separated by a distance greater than setae *e1*, forming the four bases a trapezoid pattern (Figure 1.3a). In *E. orientalis*, the distance between the insertions of the *f1* pair of setae is short, and similar to the distance between the bases of the *e1* pair of setae, forming a square pattern (Figure 1.3c). In addition, coxa II has two ventral setae in *E. banksi* (Figure 1.3b), but only one in *E. orientalis* (Figure 1.3d). These differences can be observed in female specimens, making it unnecessary for males to be present, processed and slide mounted (Ferragut et al. 2013b).

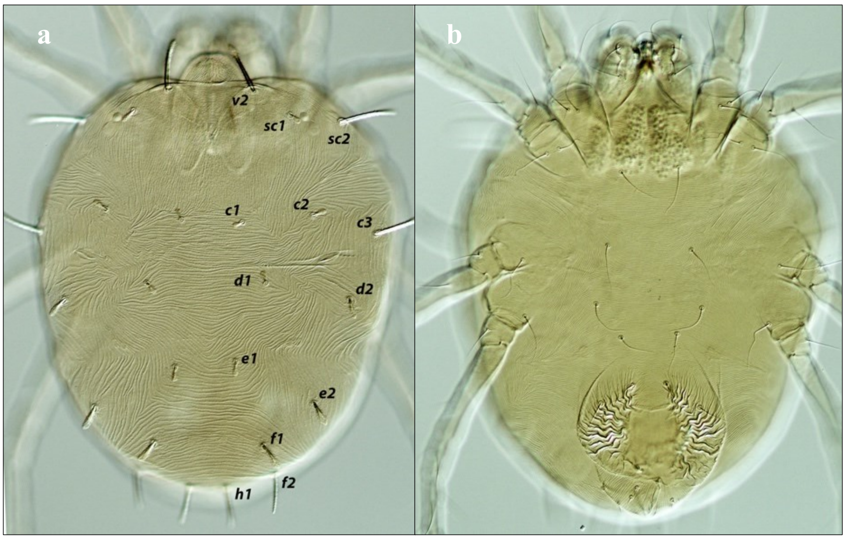


Figure 1.2 Morphological features to separate *Eutetranychus* species in Mediterranean citrus crops. Dorsal (a) and ventral (b) surface of *Eutetranychus banksi* showing the naming of dorsal setae.

1.3 Biology of *Eutetranychus banksi*

Eutetranychus banksi mainly feed on leaves and is observed on fruits only at high infestation levels (Childers 1992), when the mites move from the leaves to the fruit (Rogers and Stansly 2017). Furthermore, *E. banksi* has a non-random distribution in the tree canopy. TCM prefers to feed at the periphery of the canopy and on the adaxial (upper) side of leaves, as well as on the sunniest sides of the canopy (Dean 1952; Muma et al. 1953), so low pest densities only occur in the northern quadrants of the canopy (Dean, 1959a). At the beginning of leaf infestation, females prefer to lay their eggs along the midrib (Figure 1.1b). After hatching all developmental stages remain in the same position, and when the population grows, they move towards the leaf margins (Rogers and Stansly

2017), and to the abaxial (lower) side of leaves (Dean 1959b). A similar spatial preference has been reported for *E. orientalis* by Bodenheimer (1951), and Vela et al. (2017) in southern Spain. *Panonychus citri* also showed the same preferences as the two *Eutetranychus* species mentioned above (Ebeling 1959; García-Marí et al. 1983).

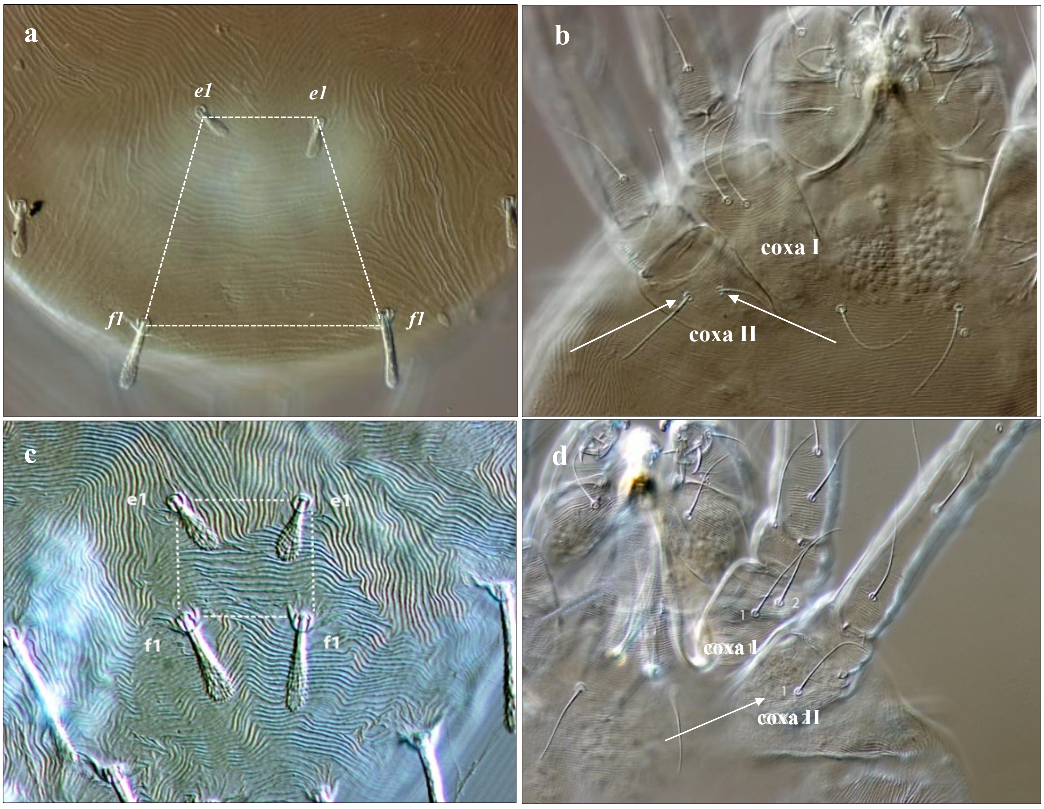


Figure 1.3 Morphological features to separate *Eutetranychus* species in Mediterranean citrus crops. *Eutetranychus banksi* show (a) a trapezoid pattern between the third (*e1*) and fourth (*f1*) dorsocentral hysterosomal setae, and (b) two ventral setae in the coxa II. *Eutetranychus orientalis* show (c) a square pattern between the same dorsocentral histerosomal setae, and (d) one ventral setae in coxa I.

Varietal structural features have been associated with less or more spider mite proliferation (van de Vrie et al. 1972). Dean (1980) found TCM preferences among sweet orange varieties in Texas citrus, while Monzó et al. (2016) point to the existence of a preference for early Navel group sweet orange varieties, and the same preference was recorded for *P. citri* in the Valencian region (García-Marí and del Rivero 1981). In California, with dry summers typical under Mediterranean climate conditions, *E. banksi* shows unimodal population dynamics with a population peak at the end of summer-beginning of autumn. On the contrary, Florida has two peaks; infestations may occur at any time of the year but the maximum in abundance occurs in late spring and early summer (Anonymous 2022). After that, populations decline sharply during the summer due to heavy rainfall and high humidity and show a second peak of lower abundance in late autumn and winter (Muma et al. 1953; Childers 1992; Rogers and Stansly 2017). In Texas it is also possible to find *E. banksi* all year round with the same population trends. Low relative humidity and rainfall, and temperatures above 21.2 °C favour the development of the main population peak, and the decline in summer is related to excessive heat. Subsequently, heavy rains in early autumn also contribute to reducing mite abundance, and cold winter temperatures keep populations low or even undetectable. High rainfall during spring contributes to population decline (Dean 1952, 1959b, 1980). In Mexico, the Texas citrus mite could also be collected all year round and showed the same bimodal dynamics where low winter temperatures, as well as spring and autumn rainfall, contributed to reduced mite density (Landeros et al. 2004). Childers (1992), based on field observations, reported that a competitive interaction took place between TCM and *P. citri* in Florida citrus, where TCM has become the dominant species in the orchards since 1955, displacing *P. citri*. The seasonal trends of *P. citri* and *E. banksi* in Florida overlap, which, in addition to the coinciding spatial feeding preferences, may have played an important role in the competitive displacement of *P. citri* (Rogers and Stansly 2017).

1.4 Feeding damage, sampling, and thresholds

Eutetranychus banksi on citrus trees feeds on leaves and fruits forming dense colonies (Figure 1.4). They consume the contents of mesophyll plant cells producing stippling damage, which may reduce photosynthesis and promote premature leaf drop at high population levels with dry winds, resulting in a decrease in plant vigour and yield. On fruits, mites feed mainly on the sunny side, and may intensify the discolouration caused by sunlight producing aesthetic damage based on a lack of pigmentation and silvery appearance, which may reduce their economic value (García et al. 2003; Monzó et al. 2016) (Figure 1.5). Damage is most severe on the upper side of the leaves located in the periphery of the tree canopy, as well as in the quadrants most exposed to the sun, where *E. banksi* populations are highest (Dean 1959a). In addition, damage is also higher on trees located along roadsides (García et al. 2003), and populations growing earlier in trees along a dusty road before a general increase was observed in the rest of the grove (Dean 1959b).

TCM is currently the prevalent species of spider mite throughout the citrus growing areas in Florida, and a set of sampling and treatment threshold guidelines, developed empirically for *E. banksi*, are provided to farmers (Rogers and Stansly 2017). These guidelines recommend binomial sampling of 96–32 leaves on 24–8 trees to estimate densities of 5–15 motile forms of *P. citri* and *E. banksi* per leaf (Table 1.1), respectively, since an average of 5 motile forms per leaf corresponds to 70–80% infestation, representing a reasonable intervention threshold (Rogers and Stansly 2017). However, this sampling plan and intervention thresholds are empirical and are not based on studies that relate pest densities to plant damage or economic losses in yield (Monzó et al. 2016).

Figure 1.4 Dense colonies of *E. banksi* on clementine citrus. a, b. fruit colony and c. leaf colony where all stages of development are feeding. In addition, stippling damage can be observed, as well as the characteristic residues of accumulated moult visible at high populations.

1.5 Biological Control

Chemical control is commonly used in Valencian citrus to maintain mite populations at low levels. TCM has been shown to be sensitive to numerous acaricides, being easy to keep under control with this strategy (Monzó et al. 2016). However, under the perspective of Integrated Pest Management (IPM), cultural practices, biological and biotechnological control should be combined with chemicals to produce healthy crops and minimize the use of pesticides, reducing the risks to human health and the environment.

Figure 1.5 Damaged fruit and leaves by *E. banksi.* a. Unripe sweet orange tree fruit, the left fruit shows its normal colouring while the right one is damaged. b. Ripe orange fruit, the upper fruit row shows damage on the sunny side, while the lower row shows normal colouring. c. Clementine citrus leaves discoloured and with the *"silvery"* appearance observed after severe *E. banksi* infestation.

Different organisms have been cited as natural enemies of *E. banksi*. The fungus *Neozygites floridana* (Weiser & Muma) and *Hirsutella thompsonii* Fisher, insects including the coccinellid *Stethorus atomus* Casey and *Stethorus siphonulus* Kapur, and predatory mites such as the ascid *Lasioseius scapulatus* Kennet and the stigmeid *Agistemus fleschneri* Summers (Vacante 2010).

Table 1.1 Intervention thresholds and sample sizes for *E. banksi* infestations in Florida citrus.

If the control threshold is:	**Sample size** (Sample trees should be uniformly scattered across a 10-acre* block. Do not sample adjacent trees)
5 mites/leaf	Examine 4 leaves/tree from 6 trees/area from 4 areas/10 acres = 96 leaves on 24 trees/10 acres
8 mites/leaf	Examine 4 leaves/tree from 6 trees/area from 3 areas/10 acres = 72 leaves on 18 trees/10 acres
10 mites/leaf	Examine 4 leaves/tree from 5 trees/area from 2 areas/10 acres = 40 leaves on 10 trees/10 acres
15 mites/leaf	Examine 4 leaves/tree from 4 trees/area from 2 areas/10 acres = 32 leaves on 8 trees/10 acres

Adapted from Rogers and Stansly (2017)
* 1 acre = 40468.6 m^2 = 0.040 Km^2

Predatory mites of the family Phytoseiidae are the most effective and widely used natural enemies for biological control of mite pests on citrus and other crops worldwide. (McMurtry 1983; Gerson 2003). In Florida citrus *E. banksi* has two annual population peaks, in late spring-early summer and late autumn-early winter. However, infestations may occur at any time of the year (Childers 1992), but populations are maintained at low levels due to the biological control provided by its rich complex of associated phytoseiid mites (Villanueva and Childers 2005; Rogers and Stansly 2017). Therefore, three species of phytoseiids, common in Florida citrus, have been tested in laboratory experiments to evaluate their potential to control *E. banksi* infestations. *Euseius mesembrinus* (Dean) was the most abundant in Florida citrus in summer (Villanueva and Childers 2005), and a common species in Texas citrus associated with *E. banksi* (Dean 1959b). In the life-history experiments on this species, the development was not assessed, but the mortality and fecundity of newly mated females were studied at 26 °C and 75% RH. Total fecundity (eggs/female) was estimated at 13.25 with an oviposition rate (eggs/female/day) of 1.558. The net reproductive rate (R_0) was 6.36, generation time (T) was 9.68, $r_m = 0.191$ and $\lambda = 1.21$. Adult females of *E. mesembrinus* who were not able to feed on *E. banksi* eggs, reached 50% mortality at 13.25 days, and showed low offspring survival (4.5%) with a low sex-ratio of 0.54 (Abou-Setta and Childers 1989). *Galendromus helveolus* (Chant) was the next species subjected to life-history experiments to study its effectiveness as a predator of *E. banksi* (Caceres and Childers 1991). *Galendromus helveolus* was observed feeding on the immature stages of *E. banksi*, 70-80% of the offspring reached adulthood after 8 days, and the oviposition rate (eggs/female/day) for the first 12 days of the oviposition period on newly mated females was 1.96 ± 0.94. The last species assessed for *E. banksi* was *Iphiseiodes quadripilis* (Banks). This species has been described as a generalist facultative predator that can feed on different families of phytophagous mites (Muma 1971). *Iphiseioides quadripilis* completed its development satisfactorily feeding on *E. banksi* in 8.5 ± 0.2 days, with an egg to adult survival of 48%, and offspring sex ratio of 0.58 (Villanueva and Childers 2007).

The surveys that have compiled the phytoseiid fauna of Spanish citrus, agree that *Euseius stipulatus* (Athias-Henriot) is the most abundant phytoseiid species on citrus, representing more than 90% of the total number of phytoseiids collected, as well as the most frequent, found in about 90% of the samplings carried out per year (Ferragut et al. 1988; Abad-Moyano et al. 2009a; Ledesma et al. 2011). In Spain, *E. stipulatus* is capable of performing a good biological control on *P. citri* (Ferragut et al. 1988), and it has been hypothesized that predators that attack *P. citri* may also be effective against *Eutetranychus* spp. (McMurtry 1985). However, *E. stipulatus* was unable to control other spider mites on citrus such as *T. urticae* due to their inability to reproduce feeding on sweet orange leaves (Ferragut et al. 1987), and not even completing its development on clementine leaves (Abad-Moyano et al. 2009b). The phytoseiid mite *Neoseiulus madeirensis* Papadoulis & Kapaxidi was described from females collected in the Botanical Garden of Madeira Island on *Lantana sp.* (Verbenaceae) and *Dombeya wallichii* (Sterculiaceae) (Papadoulis and Kapaxidi 2011). Before the publication of the original description, specimens of this mite had already been collected in Alicante and Valencia, Spain, on *Pinus halepensis* (Pinaceae) and *Brugmansia candida* (Solanaceae) infested with the spider mites *Tetranychus turkestani* (Ugarov & Nikolskii), *T. ludeni* Zacher and *T. urticae* Koch, respectively (Ferragut, unpublished data). Later, it was found in Huelva province on citrus orchards associated with *E. banksi*, since then it has been frequently recorded in the Spanish Mediterranean coast on different host plants and always in association with spider mites. Nothing is known about its biology since it has never been evaluated or used in biological control programs, but its evident relationship with spider mites, including *E. banksi*, suggests that it can be a promising candidate for pest suppression, and it seems worthwhile to investigate the possibility of using it as a biocontrol agent against *E. banksi*.

1.6 Objectives

In order to understand the magnitude of the invasion and its ecological impact, provide farmers with guidelines for early detection and to estimate pest population densities in their orchards, as well as to investigate the potential of natural enemies found in the crop to suppress *E. banksi* populations, the following objectives were proposed for this doctoral thesis:

i. To study the geographical distribution of *E. banksi* in Valencian citrus, as well as to evaluate its ecological impact on local communities of phytophagous mites.

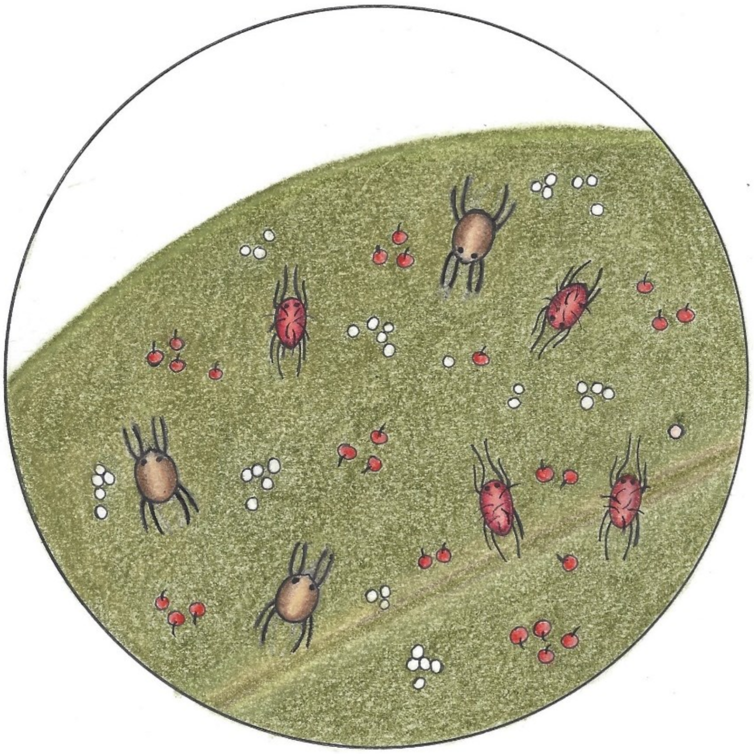
ii. To determine the within-tree distribution and seasonal dynamics of *E. banksi* and its associated predatory phytoseiids, and its implications for the biological control of the pest.

iii. To study the developmental stage structure, as well as their spatial distribution pattern and establish a sampling plan for *E. banksi* on citrus.

iv. To evaluate the biological control capabilities of *N. madeirensis*, a phytoseiid species recently found in association with *E. banksi* in Spanish citrus crops.

CHAPTER 2

The newcomer takes it all: the invader Texas citrus mite, *Eutetranychus banksi* (Acari: Tetranychidae), displaces the resident relatives on citrus agrosystems



The newcomer takes it all: the invader Texas citrus mite, *Eutetranychus banksi* (Acari: Tetranychidae), displaces the resident relatives on citrus agrosystems

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Doctoral thesis adapted version.

Abstract: Many studies have emphasized the importance of interspecific competition in shaping natural ecosystem communities. In contrast, few investigations have explored the role of competition in agricultural environments after the arrival of an invasive pest species. This work evaluated the ecological impact produced by the invasive Texas citrus mite, *Eutetranychus banksi* (McGregor), on the resident spider mites *Panonychus citri* (McGregor) and *Eutetranychus orientalis* (Klein) on the main citrus crop area in Eastern Spain. Since its arrival in 2013, *E. banksi* has become the most frequent and abundant spider mite on citrus, apparently leading to a reduction in the presence and geographic range of the other related species. Competitive relationships were detected between *E. banksi–E. orientalis* and *E. banksi–P. citri* pairs using co-occurrence analysis. Furthermore, the generalized linear model analysis showed that the probability of finding *E. orientalis* or *P. citri* decreases with increasing *E. banksi* density and vice versa. Principal component analysis and permutational multivariate analysis of variance found competition between these two pairs, and also between the *E. orientalis–P. citri* pair. Redundancy and variation partitioning analysis revealed how the geographic distribution of the three spider mites is not caused by the environmental conditions, but it is strongly influenced by their colonization history and competitive relationships, since the areas with the highest density of the three species are related to their place of first detection, and do not coincide geographically. Finally, the mechanisms that may be involved in the competitive displacement and the possible future scenarios are discussed.

2.1 Introduction

Agricultural ecosystems are usually poor in species richness due to the loss of natural biodiversity, showing simple trophic networks (Duru et al. 2015). Furthermore, these environments are also subjected to constant disturbances through agricultural practices, making them more prone to the establishment of exotic species (Horvitz et al. 1998). Moreover, the use of fertilizers, phytohormones and biostimulants increases food production, but, at the same time, enhances the level of available resources in the ecosystem, while a low level of available resources may prevent invasions by alien species (Tilman 1997, 1999; Tilman et al. 2002).

Phytophagous mites have a high potential to become invasive species, because their small size makes them difficult to detect under routine inspections (Navia et al. 2010). Among them, spider mites, belonging to the family Tetranychidae, cause significant damage in many crops worldwide, being the most economically important phytophagous mites (Jeppson et al. 1975). On citrus crops these mites feed on the cells of the epidermis and parenchyma of leaves and fruits, reducing the photosynthetic capacity of leaves, as well as affecting the coloration of the fruits, thus producing cosmetic damage that greatly reduces their commercial value (Vacante 2010). Four spider mite species currently occur in the Spanish citrus crop area, the two-spotted spider mite *Tetranychus urticae* Koch, the citrus red mite *Panonychus citri* (McGregor), the Oriental red mite *Eutetranychus orientalis* (Klein), and the Texas citrus mite *Eutetranychus banksi* (McGregor). Only *T. urticae* is thought to be native to the Mediterranean basin, while the others invaded the region in the past, the most recent being *E. banksi* which arrived in 2013 to the Valencia region, the most important Spanish citrus crop area (Ferragut 2016). The behaviour and spatial distribution of these species on the leaves is different. While *T. urticae* lives on the underside of leaves forming dense colonies, the remaining species prefer to live at lower densities on the upperside, resulting much more likely in competition between *Panonychus* and both *Eutetranychus*, as they share the same habitat and compete for space, shelter, and food (Ferragut et al. 2013b)

The Asian species *P. citri* was the first invasive spider mite to infest the Spanish citrus in 1981 (García-Marí and del Rivero 1981), spreading rapidly through the Valencian region and becoming a key pest (García-Marí et al. 1983). It was not until 2001 when *E. banksi* and *E. orientalis* reached the citrus area in southern Spain (García et al.

2003). Although in both cases the pathway is unknown, *E. orientalis* is a common species in many countries in Asia and North Africa, while *E. banksi* is native to the Americas, from southern United States to northern Argentina. Since its arrival in Spain, *E. orientalis* spread rapidly westward and eastward, reaching the Valencian region in a few years, while the relative *E. banksi* remained, for about a decade, in a small geographical area. However, in 2013 *E. banksi* was suddenly detected in citrus orchards in the Valencian southern region, where it most likely arrived through the trade of fruit or plant material (Ferragut 2016). The mite spread rapidly in the region, reaching high populations, producing severe damage to leaves and fruits, and forcing farmers to apply acaricides to maintain populations at low levels.

Since the arrival of the Texas citrus mite (TCM) to the Valencian region, farmers and technicians have realized that the spread of the new species coincided with a reduction in the frequency and abundance of *P. citri*. There is evidence that competition between closely related spider mite species, sharing the same habitat, may limit their geographic range (Fujimoto et al. 1996; Takafuji et al. 1997). Childers (1992), based on field observations, reported that competition took place between TCM and *P. citri* in Florida citrus, where TCM has become the dominant species in the orchards since 1955, displacing *P. citri*.

Competition is a powerful force that regulates the species abundance within communities, but despite their economic importance very few studies have been carried out on the competition between mite species (Reitz and Trumble 2002). This study aims to evaluate the competitive relationships among the three citrus pests, *P. citri*, *E. orientalis*, and *E. banksi* in the Valencian region, assessing the impact of the recent arrival of TCM in spider mite communities. For this purpose, we address the following objectives: (i) study species co-occurrence using three different approaches: contingency tables, null model, and probabilistic model analysis; (ii) define the current spatial distribution of the three mite species using kernel density estimation (KDE); (iii) determine the competitive relationships among species using generalized linear models (GLMs), principal component analysis (PCA) and permutational multivariate analysis of variance (PERMANOVA); and finally (iv) analyse the relative importance of environmental and spatial factors structuring the community using redundancy (RDA) and variation partitioning analysis (VPA).

2.2 Materials and Methods

Sampling sites

A total of 275 unsprayed citrus orchards were sampled once in a year throughout the three provinces (Castellón, Valencia and Alicante) of the Valencian region from September to December, over a 4-year period (2017-2020). This sampling period was chosen since previous studies have shown that the three mite species have coinciding population peaks in these months, with little differences between regions due to climatic differences (Ferragut et al. 1988; Ledesma et al. 2011; Monzó et al. 2016). During the first 2 years, 153 orchards with symptomatic leaves and 95 orchards without symptoms were sampled. In the last 2 years TCM moved northwards, entering the province of Castellón; therefore, during that period 27 additional orchards were sampled in that province (Figure 2.1). We used a chi-square goodness of fit test (Gardener 2012) to assess whether the number of samples per province (sampling effort) was significantly different from the expected value according to the citrus area per province.

Abad-Moyano et al (2009a) surveyed 147 citrus orchards infested with tetranychid mites once a year during 4 years of sampling across the Valencian citrus area. In order to detect the presence/absence of natural enemies, which are at best 10 times less abundant than the pest, as well as to conduct a quantitative study, the sample size was 50 leaves from the outer part of the canopy per orchard. Therefore, and considering the small size of plots, 20 symptomatic leaves were considered sufficient in our study for detecting the presence/absence of pest mites. Leaves were placed in plastic bags and transported to the laboratory inside a portable cooling box. In the laboratory, the occurrence of *P. citri* and *Eutetranychus* spp. was confirmed under a stereomicroscope. *Eutetranychus banksi* and *E. orientalis* cannot be differentiated under stereoscopic microscope, and, according to Ferragut et al. (2013b), females must be processed and slide mounted to separate them using a compound microscope. Reliable characters to separate females of both species include the pattern formed by the setae *e1* and *f1* on the dorsal surface and the number of ventral setae on the coxa of leg II, and is not necessary to inspect the males to separate the two species with certainty (Ferragut et al. 2013b). *Eutetranychus* density on citrus usually varies between 0–10 females/leaf. Thus, a reasonable number of 20 females per sample were randomly collected from the leaves and identified to species level in order to obtain presence/absence data for both species.

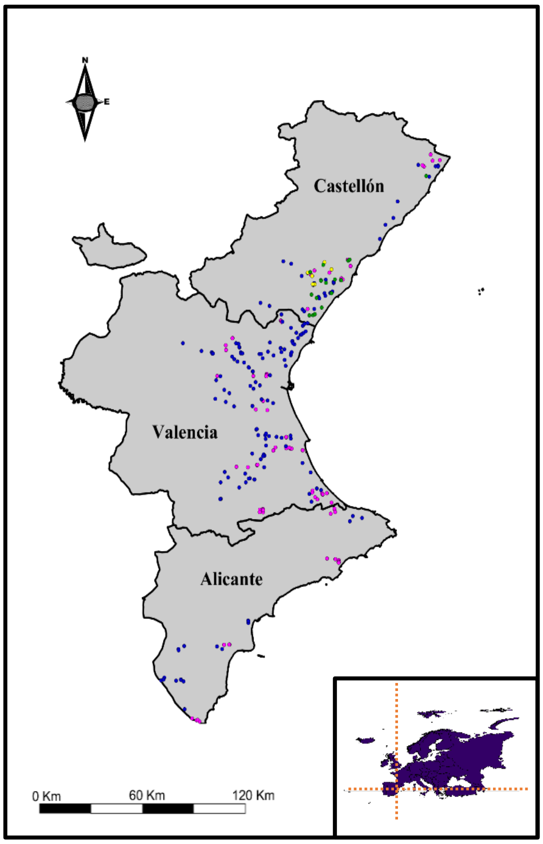


Figure 2.1 Location of sampling sites in the Valencian region (Spain). (Legend: *Pink* corresponds to sites sampled during 2017, *Blue* is sampled sites in 2018, *Yellow* 2019, and *Green* 2020. Map was obtained using *Q-sig*.

Occurrence analysis

Three types of analysis were used to assess co-occurrence patterns in the mite community to check the competitive interactions. First, we mapped the occurrence (i.e., absence/presence) of the three mite species (*P. citri, E. banksi* and *E. orientalis*) in all 275 orchards, and then we carried out a (i) null model analysis (Gotelli 2000) by computing two binary (presence/absence) indices for the observed community: checkerboards score (C-score), and variance ratio (V-ratio). C-score quantifies the average amount of co-occurrence among all unique pairs of species in the assemblage (Stone and Roberts 1990), and the V-ratio is calculated as the ratio of the variance in total species number in samples (columns) to the sum of the variances of the individual species (rows). For a presence-absence matrix, this is the ratio of the variance in species richness to the sum of the variance in species occurrence (Schluter 1984). Once the indices were obtained for the data-matrix, the values were compared with a distribution of index values obtained from 9999 randomly generated null assembled communities using a randomised algorithm with the R-package *EcosimR* (Gotelli et al. 2015). To generate the null communities, we used two sequential-swap algorithms with the best statistical properties in terms of Type I and II errors: SIM 2 and SIM 10. SIM 2 algorithm randomizes the occurrence of each species among the sites, assuming the sites are equiprobable. It corresponds to a simple model of community assembly in which species colonize sites independently of one another (Gotelli 2000). SIM 10 algorithm randomizes a binary species-data matrix by reshuffling all of its elements equiprobably (Gotelli et al. 2010). Statistical significance of the observed matrix was calculated as the frequency of simulated matrices that had indices which were equal to or more extreme than the observed index, being $\alpha = 0.05$ the frequency significance level in two-tailed tests. In this way, for the C-Score index competitively structured communities should have an index significantly larger than expected by chance, located in the upper tail of the randomly simulated communities index distribution. On the other hand, guild communities will have an index smaller than expected by chance and located in the lower tail. However, for the V-ratio index competitive communities should have a significantly smaller index, and guild communities a larger one, than expected by chance. Finally, the standardized effect size (SES) was calculated following the same methodology as in meta-analysis. This metric was calculated as: observed index – mean (simulated indices)/standard deviation (simulated indices). It scales the results in units of standard deviations, which allows for meaningful comparisons among different studies (Gotelli and McCabe 2002).

However, species do not occur randomly in nature; they all respond to environmental variation, and algorithms for randomizing species presence–absence data have different statistical properties, computational complexities, and biological realism. Therefore, we calculated species frequency and overlap using the R-package *eulerr* (Larsson 2021), and used two more methods to assess the co-occurrence patterns. In the first one, we used (ii) contingency tables and a Chi-square test (Gardener 2012) to assess the competitive interactions between mite species by testing whether the observed frequencies were statistically different from those expected under the null hypothesis of independence. The second one was made using the R-package *coocur* (Griffith et al. 2016), computing a (iii) probabilistic model of species co-occurrence, which uses basic probability theory to measure co-occurrence as the number of sampling sites where two species co-occur in the community data matrix. Through this method we employed combinatorics to determine the probability that the observed frequency of co-occurrence is significantly large and greater than expected (positive association, i.e. mutualism), significantly small and less than expected (negative association, i.e. competition), or not significantly different and approximately equal to expected (random association). Within any species matrix, there will be a mixture of competitive, mutualistic, and random species pairs, and in the above null model analysis, all of which contribute to the observed index value. In contrast to this type of analysis, a probabilistic model analyses the co-occurrence between pairs of species instead of the entire community, which is strictly analytical and requires no randomization being a more parsimonious approach (Veech 2012).

Species distribution and GLM competition models

Geographical position at all 275 orchards was registered in datum WGS84 and reprojected to Cartesian coordinates in ETRS89/UTM zone 30N with the R-packages *sp* (Pebesma and Bivand 2005; Bivand et al. 2013) and *maptools* (Bivand and Lewin-Koh 2021). Using these sampled locations, kernel density estimation (KDE) based on a Gaussian function was computed to assess the spatial distribution of *P. citri*, *E. orientalis* and *E. banksi* (Baddeley et al. 2015). We estimated the optimal bandwidth using likelihood cross-validation and a Gaussian function to assess the probability density of the three species per square kilometre, in raster grid cells i.e., raster maps with 3284 x 1890 pixel array and 0.1 square km area per pixel. Once the raster maps were obtained, we extracted the density values for each of the sampled locations by interpolation using the *spatstat* R-package (Baddeley et al. 2015), and using the density values for each mite species, in each sampled orchard, we obtained the data species matrix for the competition models and multivariate analysis. We performed a Mann-Whitney *U-test* (Mann and Whitney 1947) to compare the mean density value of each mite species with the presence/absence of the remaining species. Finally, we used binomial (logit-link) generalized linear models (GLMs) (R Core Team 2019) to assess the influence of density on the occurrence, analysing the competitive relationships between the three mite species across the 275 citrus orchards sampled (Zuur et al. 2009).

Environmental and spatial variables

Temperature, relative humidity, and rainfall have been shown to be important factors affecting the development, survival, and reproduction of spider mites, while wind has been described as the main mode of long-distance dispersal (van de Vrie et al. 1972). To examine the influence of these environmental variables on the distribution and competitive relationships between species, a total of sixteen parameters related to temperature, relative humidity, wind speed, and precipitation were considered (Table 2.1).

Table 2.1 Meaning of the seventeen variables related to temperature, relative humidity, wind speed, precipitation, and crop density.

Variable group	Code	Meaning
Temperature	Tmitmit	Average monthly mean temperatures
	Tmitmax	Average temperature of monthly maximums
	Tmitmin	Average temperature of monthly minimums
	Tminmit	Minimum temperature of monthly average temperatures
	Tminmax	Minimum temperature of monthly maximums
	Tminmin	Minimum temperature of monthly minimums
	Tmaxmit	Maximum temperature of monthly averages
	Tmaxmax	Maximum temperature of monthly maximums
	Tmaxmin	Maximum temperature of monthly minimums
Relative humidity	Hmitmit	Average relative humidity of monthly averages
	Hmaxmax	Maximum relative humidity of monthly maximums
	Hminmin	Minimum relative humidity of monthly minimums
Wind speed	Vmit	Average wind speed
	Vmax	Maximum wind speed
Precipitation	Pctot	Total annual precipitation
	Pcmax	Maximum annual precipitation
Crop density	Citridens	Citrus crop density

The data were obtained from 262 weather stations from the Valencian Meteorology Association (AVAMET) scattered around the Valencian region. The span of years for which data were recorded varies across stations, and, for this reason a common time series was used (2013-2020). The stations record environmental data daily, and the annual average in each station for each variable was calculated (Figure 1a in Supplementary Material 1). The geographical position of the 262 climatic stations in datum WGS84 was reprojected to cartesian coordinates in ETRS89/UTM zone 30N as on the species distribution maps. The location and value for the climatic variables in each station were used to perform a spatial smoothing by kernel weighting (KSW), using the Gaussian function with least-squares cross-validation selected bandwidth. We computed the KSW to calculate values for output cells in a raster grid, as in the previous point (Figure 2 in

Supplementary Material 1). Finally, values for each sampled location were extracted from climate raster maps by interpolation using the *spatstat* R-package (Baddeley et al. 2015).

In addition to climatic variables, other factors such as the citrus crop density can provide information about species competition. We used QGIS to calculate the centroid in datum WGS84 for each municipality (polygon) of a polygons layer (QGIS DT 2009) (Figure 1b in Supplementary Material 1), and they were later reprojected in the same way as the climatic stations. The dataset of citrus crop area by municipality was obtained from (CADRECTE 2019), and the value for each municipality was associated with its centroid. The centroid geographical locations and its citrus crop area values were used to perform kernel density weighted estimation (KDWE), to assess the probability density of this variable per square kilometre, using the Gaussian function with the crop area value to weigh the points, and the Scott's rule of thumb to determine the smoothing bandwidth (Baddeley et al. 2015). The KDWE allowed us to obtain a raster map with the same number of cells and area per pixel as the climate maps (Figure 2 in Supplementary Material 1), and then values for each sampled location were extracted from the raster map according to the same approach. In this way, it was possible to obtain a dataset containing the values of 16 climatic variables and the variable related to the crop area for each of the sampled citrus orchards, building the environmental data matrix for the multivariate analysis. In addition, considering only the localities where the species were present, three more environmental data matrices corresponding to each of the mite species were obtained.

We calculated a set of spatial variables as Moran eigenvector maps (MEMs) from the cartesian coordinates of the 275 sampled orchards with at least one mite species, as well as three datasets, one for each mite species, considering only the localities where each was present (Dray et al. 2006, 2012). For this purpose, four connection networks were built following a distance based spatial weighting matrix (SWM) using the minimum connection distance as a threshold to define the connected site pairs (Borcard et al. 2011), and the best choice for the weighting functions was done according to the adjusted *p-value* (Bauman et al. 2018). These analyses were performed with the R-package *adespatial* (Dray et al. 2021).

Multivariate analysis

To determine the relationship between the three mite species in all 275 sampled locations, the species density data matrix was transformed with the Wisconsin double standardization. This procedure was conducted to normalize sites to the percentage abundance of species and implies first standardize species by their maximum value (column maximum), and then sites by their total value (row total) (Legendre and Gallagher 2001). After that, each locality was assigned to a mite species according to its presence. When more than one species was present in the same locality it was assigned to the species with the highest density value, building 3 groups of localities: *E. banksi*, *E. orientalis* and *Panonychus*. Sampled locations with no presence of any of the three mite species were not considered. To test if the grouping was statistically significant, we used non-parametric permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001), considering Chi-square dissimilarity as a measure of distance. The statistical significance was assessed via comparison with 9999 randomized datasets. In addition, an analysis of multivariate homogeneity of group dispersions (PERMDISP2) (Anderson 2006) was done to test differential multivariate dispersion (variance) between groups that can affect the results of PERMANOVA. Finally, pairwise PERMANOVA with Bonferroni *p-value* correction was also carried out to analyse the differences between groups two by two (Martinez-Arbizu 2017). Later, species distribution was analysed with principal component analysis (PCA) showing the significant grouping obtained by the PERMANOVA analysis.

The relative role of the environment and space on the density distribution of the mite species was determined with redundancy analysis (RDA) and variation partitioning analysis (VPA) (Borcard et al. 1992; Peres-Neto et al. 2006), corrected by Moran spectral randomisation (Wagner and Dray 2015). RDA allows us to determine the percentage of variance in a community of species across a set of sites that is due to the variance of a group of variables across those sites. To consider two or more groups of variables, e.g., spatial and environmental variables, the VPA allows us to define what percentage of the variance in the community is due to each of the groups (Borcard et al. 1992). This procedure was applied across the 275 sampled locations with at least one mite species for the whole community, and for each mite species only with locations where the species was present. Multicollinearity among environmental predictors was controlled by the variance inflation factor (VIFs) using VIF < 10 as a preselected threshold (Zuur et al.

2010). Furthermore, we used a forward selection (FS) procedure with two stopping criteria to select environmental and spatial variables in each case (Blanchet et al. 2008) (1) the variance explained by each variable selected had to be significant ($P < 0.05$), and (2) the procedure stopped when the adjusted R^2 accumulated by the variables selected exceeded adjusted R^2 of all the explanatory variables considered. We performed PCA, RDA, PERMANOVA, PERMDISP2 and VIF with the *vegan* R-package (Oksanen et al. 2020) .The R-package *adespatial* (Dray et al. 2021) was used to perform FS, VPA and Moran spectral randomisation correction.

2.3 Results

Sampling effort

Considering the data for 2017 and 2018, the province with the highest sampling effort was Valencia with 160 (64.52%) orchards sampled, followed by Castellón with 47 (18.95%), and Alicante with 41 (16.53%). There were no significant differences between the number of samples per province and the expected value according to the citrus area per province in Valencia (58.23%), Castellón (21.46%) and Alicante (20.32%) ($\chi^2 = 4.15$, $df = 2$, $P = 0.12$). During 2019 and 2020 the sampling effort was increased only in the province of Castellón with 27 orchards, with the aim of updating the geographical distribution of the three mite species (Figure 2.1).

Occurrence Analysis

We detected at least one species in 87.6% (n=241) of the sampled orchards (n=275). Most occupied orchards harboured only one species (70.95%), whereas all three species were detected in only 6.22% of occupied orchards (Figure 2.2a). Of the three species, we detected TCM in the largest proportion of orchards (67.22% of occupied orchards), followed by *P. citri* (39.83%), and *E. orientalis* (28.21%) (Figure 2.2b). Overall, we found that TCM showed the least overlap in orchard occupancy (38.27%), meanwhile *E. orientalis* and *P.citri* showed 55.88% and 57.29% respectively (Figure 2.2c).

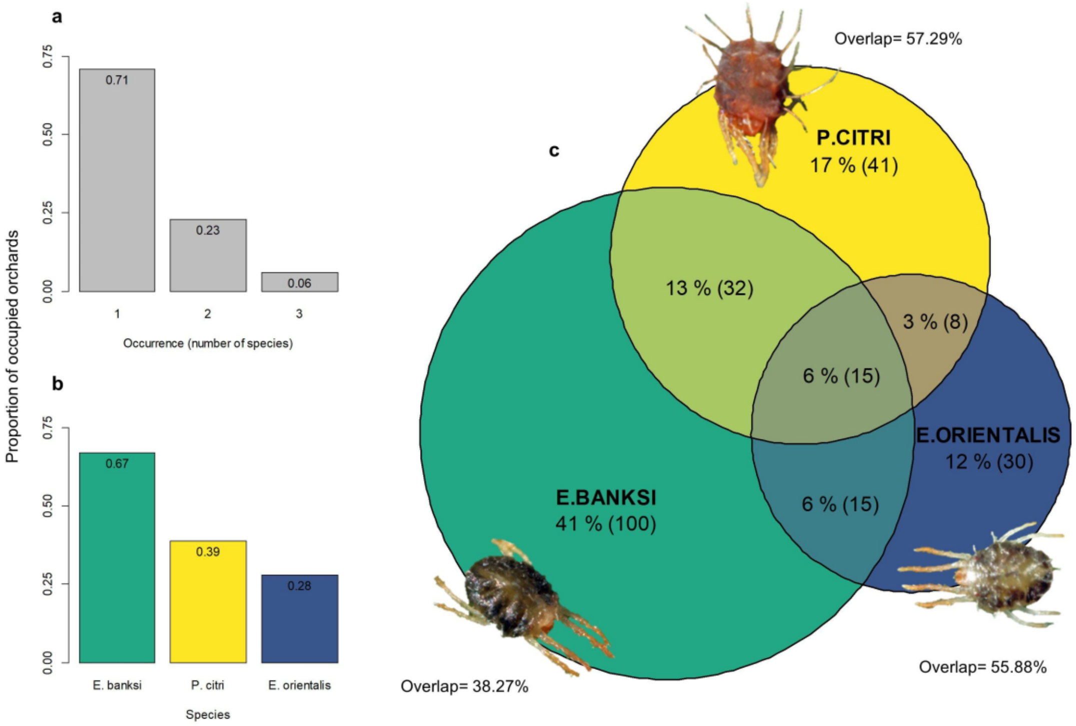


Figure 2.2 Patterns of species occurrence across the sampled orchards. Species occur more frequently singly than together with other species. a. Proportion of occupied orchards by occurrence (i.e., number of species detected). b. Proportion of occupied orchards by species: *E. banksi*, *E. orientalis*, and *P. citri.* c. Area-proportional Euler diagram illustrating the number of orchards occupied by each species, and by multiple species (indicated in areas of overlap).

Patterns of co-occurrence in the mite community were analysed using three different approaches. First, for the null model analysis, the C-score index results, under the sim2 and sim10 algorithms, showed a non-random pattern of species association. Using both algorithms, the C-score observed value (4645.3; $P < 0.05$) was significantly higher than the simulated index expected by chance, indicating a negative covariance between mite species, and providing evidence of a competitively structured community. Secondly, the V-ratio index for the two algorithms also showed a non-random pattern of species association. V-ratio observed values (0.7; $P < 0.05$) were significantly smaller than those expected by chance, showing negative covariance between species pairs, being evidence again of a competitively structured community. Finally, for the two algorithms, the C-score SES was larger than 2 and smaller than -2 for the V-ratio, providing evidence of non-random community patterns (Table 2.2).

Table 2.2 Results for the null model co-occurrence analysis. The analysis was based on the C-score and V-ratio index for sim 2 and sim 10 algorithms.

Index	*Algorithm*	*nº Rep*	*Obs Index*	*Mean of Sim Index*	*Lower 95%*	*Upper 95%*	*Simulated > Observed*	*Simulated < Observed*	*Simulated = Observed*	*p - value*	*Standardized Effect Size*	*Interpretation*
C-score	sim2	9999	4645.3	3602.2	3048.0	4170.4	0	9999	0	< 0.05	3.59	Competition
C-score	sim10	9999	4645.3	2297.7	734.6	3396.0	0	9999	0	< 0.05	3.21	Competition
V-ratio	sim2	9999	0.7	1.0	0.8	1.1	9994	3	2	< 0.05	-3.22	Competition
V-ratio	sim10	9999	0.7	1.2	1.1	1.4	9999	0	0	< 0.05	-6.37	Competition

Abbreviations: (*Index*)= Index used; (*Algorithm*)= Algorithm used; (*nº Rep*) = number of random matrices obtained; (*Obs index*) = Index value for the observed data matrix; (*Mean of Sim index*) = Mean simulated index from 9999 simulated matrices; (*Lower 95%*) = Value of the index below which the lowest 5% of the values in the set of simulated data matrices are found; (*Upper 95%*) = Value of the index above which 5% of the largest values of the set of simulated data matrices are found; (*Simulated > Observed*) = Number of simulated matrices with an index value greater than the observed matrix value; (*Simulated < Observed*) = Number of simulated matrices with an index value lower than the observed matrix value; (*Simulated = Observed*) = Number of simulated matrices with the same index value as the observed matrix value. (*Standardized effect size*) = Indicates the intensity of the relationship. *Note*: *Lower 95%* and *Upper 95%* intervals are not based on a normal or other parametric distribution. Instead, they are always estimated directly from the set of index values obtained from the simulated random matrices, so they can be considered cut-off points above (*Upper 95%*) or below (*Lower 95%*) of which 5% of the least frequent values under the null hypothesis are found and constitute a proper 95% confidence interval for the null distribution.

Contingency table analysis indicated that TCM was detected in 162 (59%) of the 275 orchards sampled and was absent in 113 (41%). There was competition between *E. banksi* and *E. orientalis,* since *E. orientalis* was only present in 30 (19%) of the orchards where TCM occurred, while it was absent in the remaining 132 plots (81%). In the orchards where TCM was absent, *E. orientalis* increased its presence (38; 34%) and decreased its absence (75; 66%) (χ^2= 7.37, *df* = 1, *P* = 0.0066) (Figure 2.3a). Furthermore, a competitive relationship between *E. banksi* and *P. citri* was also detected (Figure 2.3b). In the same way as in the previous case, the presence of *P. citri* increased in the orchards where TCM was absent (49; 43%) and decreased with its presence (47; 29%). In parallel, its absence also gained strength with the presence (115; 71%) of TCM and declined with the absence (64; 57%) (χ^2= 5.42, *df* = 1, *P* = 0.019). On the other hand, no statistically significant evidence of a competitive interaction between *E. orientalis* and *P. citri* could be found (Figure 2.3c) (χ^2=0.0048, *df* = 1, *P* = 0.9443).

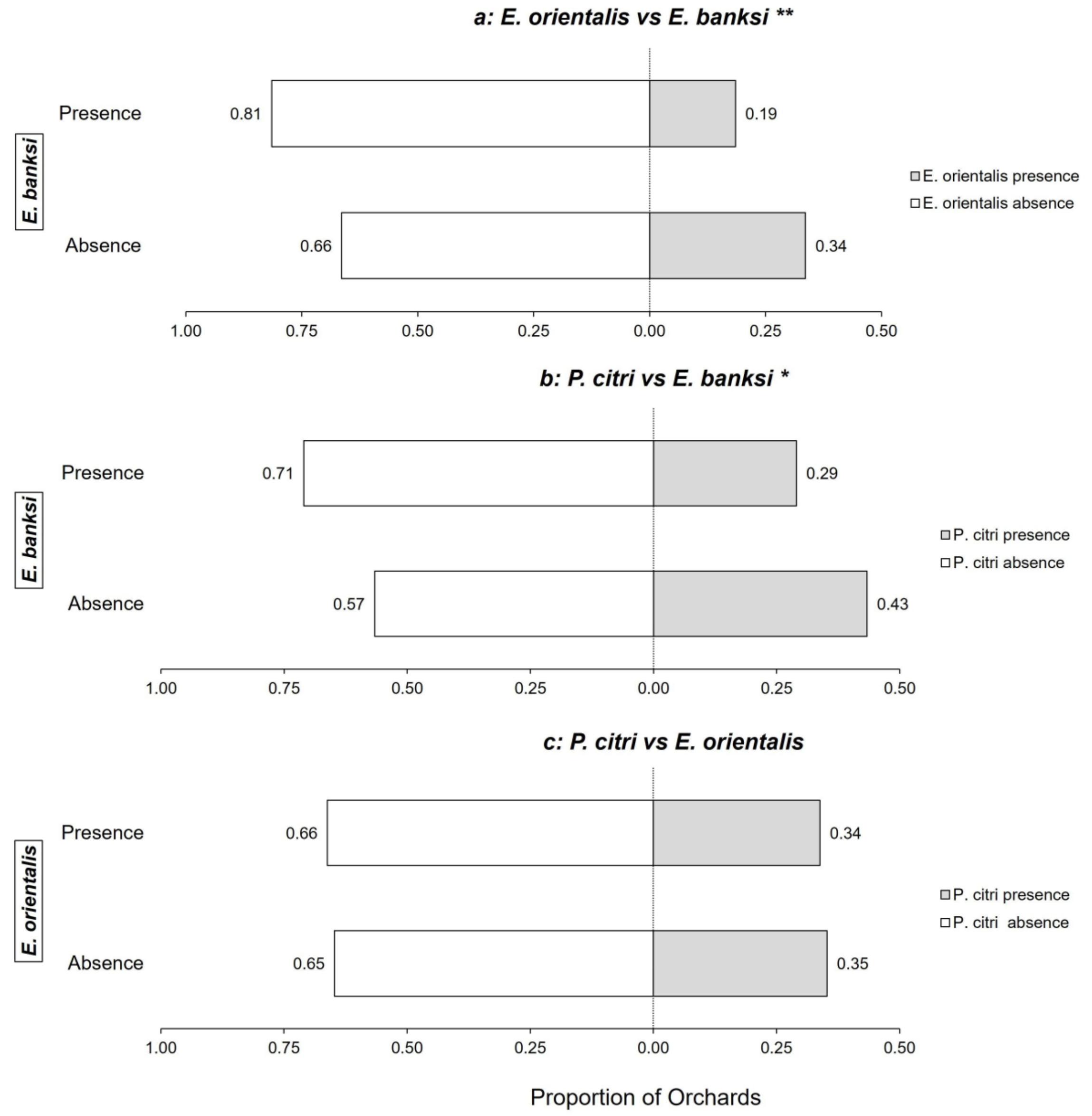


Figure 2.3 Proportion of orchards with occurrence of each species (framed), when another one occurs (grey legend) or is absent (white legend). a. *E. orientalis vs E. banksi*, b. *P. citri* vs *E. banksi,* c. *P. citri vs E. orientalis*. For each pair, significant differences are denoted with asterisks. Chi square contingency test: **P* < 0.05; ***P* < 0.01.

Probabilistic model of species co-occurrence also allowed us to analyse the co-occurrence between pairs of species in the mite community. *Eutetranychus banksi* and *E. orientalis* significantly coexisted (30) in fewer orchards than expected by chance (40.1) showing a strong competitive relationship (*p-lt*= 0.0034). In the same way, TCM had a negative relationship with *P. citri* as both also appear significantly together in fewer orchards (47) than expected (56.6) (*p-lt*= 0.0101). However, between *P. citri* and *E. orientalis* it was not possible to detect either a negative (*p-lt*= 0.4751) or a positive (*p-gt*= 0.6391) relationship. The effect size of the competition for *E. banksi* with *E. orientalis* and *P. citri* was similar (Table 2.3).

Table 2.3 Results for the probabilistic model of co-occurrence analysis.

	Obs-cooccur	***Exp-cooccur***	***p-lt***	***p-gt***	***Effect size***	***Interpretation***
E. banksi vs *E. orientalis*	30	40.1	0.0034**	0.9985	-0.036	Competition
E. banksi vs *P. citri*	47	56.6	0.0101*	0.9950	-0.034	Competition
E. orientalis vs *P. citri*	23	23.7	0.4751	0.6391	-0.002	Random

Signif. Codes: $^*p < 0.05$ $^{**}p < 0.01$

Abbreviations: (*Obs-cooccur*) = Observed number of orchards in which the species overlap; (*Exp-cooccur*) = Expected number of orchards in which the species should coincide under random distribution; (*p-lt*) = Probability that two mite species would co-occur less frequent than the observed number of co-occurrences. It can be interpreted as p-value indicating significance level for negative co-occurrence patterns with significance threshold at $P < 0.05$; (*p-gt*) = Probability that two mite species would co-occur more frequent than the observed number of co-occurrences. It can be interpreted as p-value indicating significance level for positive co-occurrence patterns with significance threshold at $P < 0.05$. (*Effect size*) = Indicates the intensity of the relationship, negative ($x < 0$), positive ($x > 0$) or random ($x = 0$).

Species distribution and GLM competition models

The current geographic distribution of the three mite species is shown in Figure 2.4. TCM showed the highest mean density values with 0.0068 occurrences/km^2 distributed in three high-density areas with about 0.05 occurrences/km^2. The most important of these areas is located in the south of the Valencia province. Furthermore, two other smaller areas can also be observed, the first between the provinces of Valencia and Castellón, and the second one between the provinces of Valencia and Alicante (Figure 2.4, left). *Panonychus citri* presented 0.0041 occurrences/km^2 of mean density with two high-density areas with around 0.05 occurrences/km^2. The most important of them coincides with the northern nucleus of TCM between the provinces of Castellón and Valencia, and the second smaller one is in the north of the province of Castellón (Figure 2.4, middle). Finally, *E. orientalis* with 0.0028 occurrences/km^2 showed the lowest mean density value in two low density areas (0.026 occurrences/km^2). The first one located in the north of the province of Valencia, matching with *P.citri* and *E. banksi* areas, and the other one in the south of the Alicante province (Figure 2.4, right) (Figure 2.1).

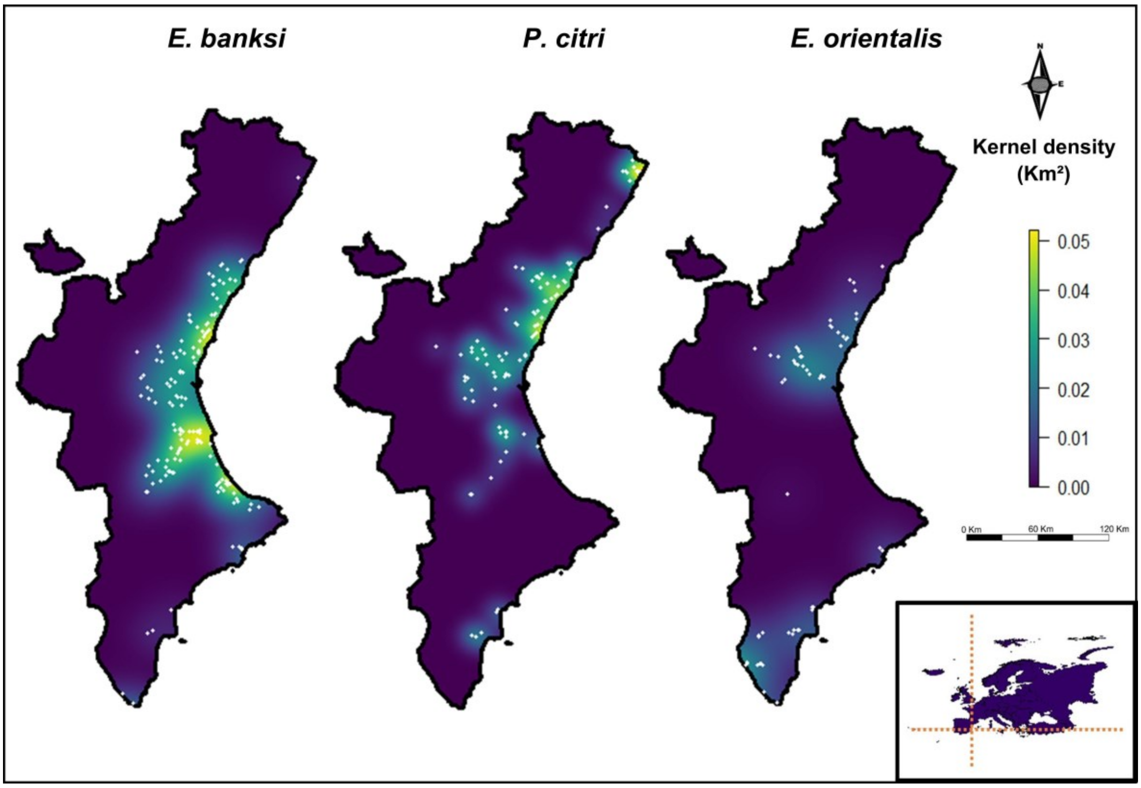


Figure 2.4 Kernel density distribution of the three mite species in the Valencian region (Spain). Kernel density estimation (KDE) was computed with the optimal bandwidth using likelihood cross-validation, and a Gaussian function to assess the probability density of the three species per square kilometre in raster maps with 3284 x 1890 pixel array and 0.1 km^2 area per pixel. The yellow areas represent high mite densities while dark violet colour indicates low mite densities. The white dots indicate the citrus orchards sampled with the occurrence of each species. Maps were performed using the R-package *spatstat.*

The mean TCM density in orchards without *E. orientalis* (0.028 ± 9.59e-04) was significantly higher than in orchards with *E. orientalis* (0.016 ± 1.55e-03) (*W* = 10470, *P* = 1.631e-09) (Figure 2.5a, left). To a lesser extent, *P. citri* also showed the highest TCM density values in orchards with its absence (0.026 ± 1.08e-03), while orchards with its presence showed low values (0.022 ± 1.45e-03) (*W* = 10113, *P* = 0.01558) (Figure 2.5a, right). The mean density values of *E. orientalis* was significantly higher in orchards not infested with TCM (0.010 ± 6.95e-04) and lower in infested ones (0.006 ± 5.56e-04) (*W* = 11845, *P* = 3.355e-05) (Figure 2.5b, left). There was no significant difference for the *E. orientalis* density values between orchards with or without *P. citri* (*W* = 8040, *P* = 0.3804) (Figure 2.5b, right). Finally, *P. citri* mean density value was significantly lower in the presence (0.016 ± 1.01e-03) of TCM than in its absence (0.022 ± 1.43e-03) (*W* =

11262, *P* = 0.001159) (Figure 2.5c, left), and it was not significantly affected by the presence of *E. orientalis* (*W* = 7294, *P* = 0.6534) (Figure 2.5c, right).

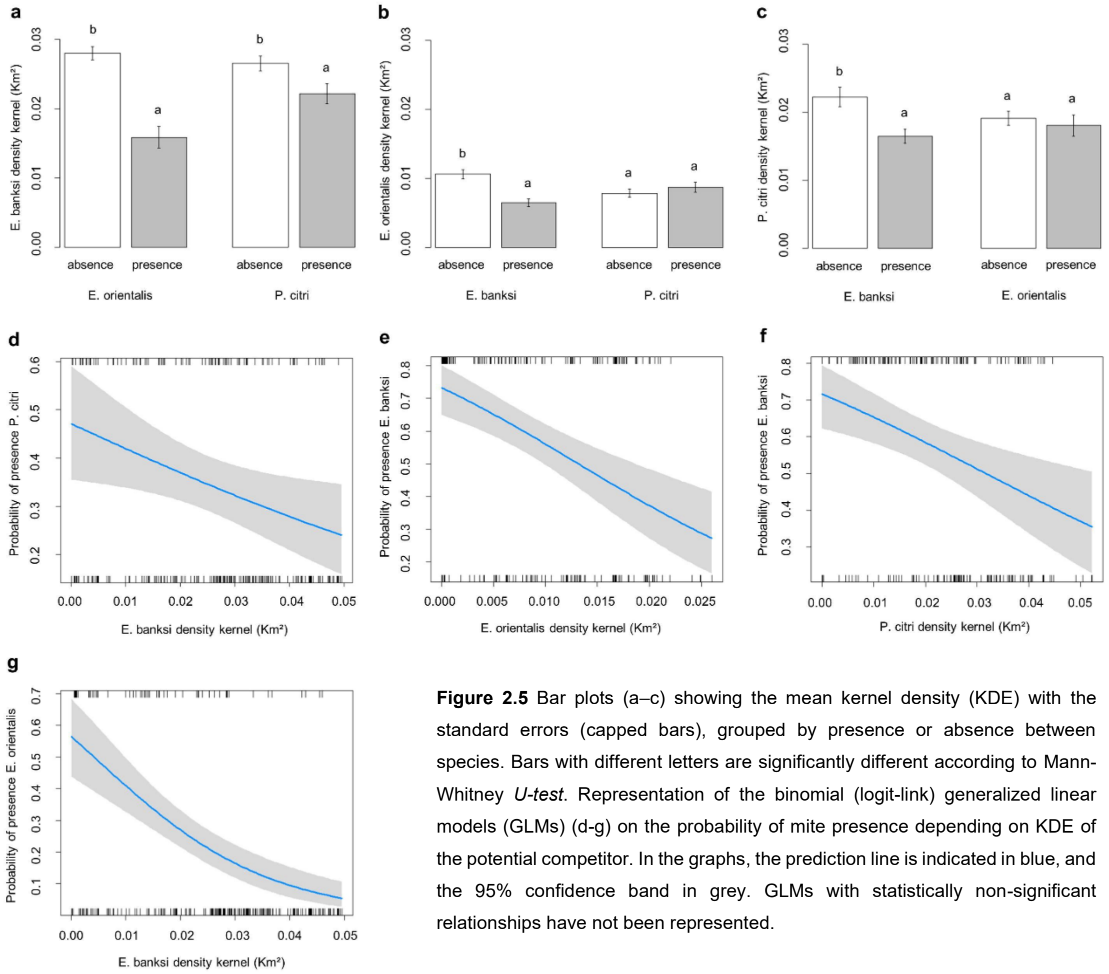


Figure 2.5 Bar plots (a–c) showing the mean kernel density (KDE) with the standard errors (capped bars), grouped by presence or absence between species. Bars with different letters are significantly different according to Mann-Whitney *U-test*. Representation of the binomial (logit-link) generalized linear models (GLMs) (d-g) on the probability of mite presence depending on KDE of the potential competitor. In the graphs, the prediction line is indicated in blue, and the 95% confidence band in grey. GLMs with statistically non-significant relationships have not been represented.

A summary for the GLM analysis is provided in Table 2.4. The first model for TCM shows a significantly intense decline in the presence of *E. orientalis* with increasing *E. banksi* density (Figure 2.5g) (Table 2.4a). To a lesser extent, the presence of *P. citri* was also negatively affected by the density of *E. banksi* (Figure 2.5d) (Table 2.4b). Complementarily, in *E. orientalis* and *P. citri* models, the probability of finding TCM was strongly affected by the density of *E. orientalis* (Figure 2.5e), and in a smaller proportion by *P. citri* (Figure 2.5f) (Table 2.4c). There was no statistically significant evidence about the influence of *E. orientalis* density on the presence of *P. citri* and vice versa.

Table 2.4 Results of the binomial (logit-link) generalized linear models (GLMs) on the probability of presence depending on the density kernel (KDE) for the three mite species.

	Coefficients	*Std Error*	*Resid. Dev*	*Pr (>Chi)*	*Pseudo R^2*	*AIC*
a. *Eutetranychus orientalis* GLMs						
E. orientalis occurrence vs *E. banksi* density						
(Intercept)	0.26	0.26	307.63	-	-	-
Kernel *E. banksi*	-63.01	11.16	270.33	1.01e-09 ***	0.1212	274.33
E. orientalis occurrence vs *P. citri* density						
Intercept	-1.01	0.23	307.63	-	-	-
Kernel *P. citri*	-5.30	9.88	307.34	0.5911	0.0009	311.34
b. *Panonychus citri* GLMs						
P. citri occurrence vs *E. banksi* density						
(Intercept)	-0.11	0.25	355.79	-	-	-
Kernel *E. banksi*	-20.87	8.84	350.12	0.0173*	0.0159	354.12
P. citri occurrence vs *E. orientalis* density						
(Intercept)	-0.75	0.19	355.79	-	-	-
Kernel *E. orientalis*	15.43	16.87	354.95	0.3607	0.0024	358.95
c. *Eutetranychus banksi* GLMs						
E. banksi occurrence vs *P. citri* density						
(Intercept)	0.92	0.22	372.45	-	-	-
Kernel *P. citri*	-29.14	8.91	361.38	8.73e-04 ***	0.0297	365.38
E. banksi occurrence vs *E. orientalis* density						
(Intercept)	1.01	0.20	372.45	-	-	-
Kernel *E. orientalis*	-76.41	17.31	351.76	5.39e-06 ***	0.0556	355.76

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Abbreviations: GLM Coefficients, standard error (*Std error*), residual deviance (*Resid. Dev*), significance *(Pr(>Chi))*, pseudo R^2, and AIC value.

Multivariate analysis

PCA shows a clear difference between the orchards colonised by each of the mite species. The first axis explains 65.72% of the total variance, while the second axis explains 34.28% (Figure 2.6a). PERMANOVA test provided statistical support for the visual interpretation of the ordination results, and evidence that samples within groups are more similar than samples from different groups. Differences were highly significant, due only in a small proportion to multivariate dispersion (Table 2.5a, b). Furthermore, PERMANOVA models including only pairs of mite species also indicated that there were highly significant differences between groups (Table 2.5c).

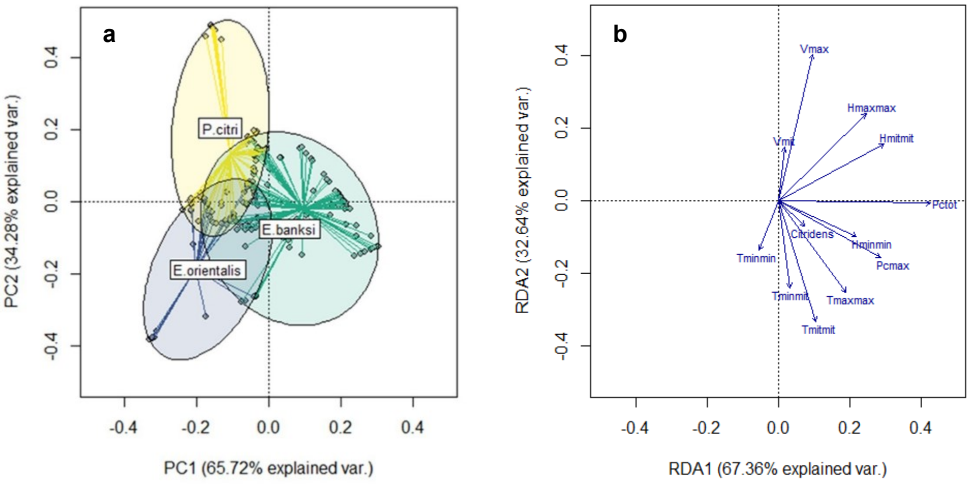


Figure 2.6 a. Principal component analysis (PCA) showing all the sampled orchards grouped according to the mite occurrence and kernel density estimation (KDE) values. b. Ordination plot of the two axes of RDA constrained by the selected environmental variables (variable codes are in table 2.1). Graphs were performed using the R-package *vegan.*

Table 2.5 (a) Permutational multivariate analysis of variance (PERMANOVA) and (b) multivariate dispersion analysis (BETADISPER) of kernel density (KDE) data, in relation to the different groups of orchards grouped by the mite occurrence and KDE values. (c) PERMANOVA pairwise comparison between groups of orchards.

	df	*SS*	*MS*	*Pseudo F*	*R²*	*N. perm*	*p-value*
a. PERMANOVA							
Groups	2	62.65	31.32	96.60	0.45	9999	1e-04 ***
Residuals	238	77.18	0.32		0.55		
Total	240	139.82			1.00		
b. BETADISPERS							
Groups	2	0.34	0.17	2.66		9999	0.07
Residuals	238	15.45	0.06				
c. Pairwise PERMANOVA							
P. citri vs *E. banksi*	1	27.32	27.32	81.22	0.28	9999	3e-04 **
P. citri vs *E. orientalis*	1	15.55	15.55	53.86	0.36	9999	3e-04 **
E. banksi vs *E. orientalis*	1	37.81	37.81	109.38	0.39	9999	3e-04 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Across all the sampled locations with at least 1 mite species (241), the initial 17 environmental variables were reduced to 12 using VIF<10, and FS for environmental RDA did not exclude any variable (Table 1 in Supplementary Material 2). However, this procedure selected 38 broad-scale MEMs with positive eigenvalues out of the initial 241

(Table 2 in Supplementary Material 2) (Figure 1,2,3 in Supplementary Material 3). For TCM sites (162), environmental variables were reduced to 11 using VIF < 10, and FS chose only 9 of them (Table 3 in Supplementary Material 2). On the other hand, FS selected 38 broad-scale MEMs with positive eigenvalues out of the initial 162 (Table 4 in Supplementary Material 2) (Figure 4,5,6 in Supplementary Material 3). *Eutetranychus orientalis* showed 10 environmental variables using VIF < 10, and FS chose 7 of them (Table 5 in Supplementary Material 2). Moreover, only 7 MEMs with positive eigenvalues out of the initial 68 were included by the FS (Table 6 in Supplementary Material 2) (Figure 7,8,9 in Supplementary Material 3). *Panonychus citri* showed 11 environmental variables after VIF < 10 reduction, and FS chose 9 of them (Table 7 in Supplementary Material 2). This procedure chose 22 MEMs with positive eigenvalues out of the initial 96 (Table 8 in Supplementary Material 2) (Figure 10,11, 12 in Supplementary Material 3).

The RDA constrained by the selected environmental variables across all the sampled locations (Figure 2.6b), indicated that TCM is associated with high temperatures as well as not being negatively affected by rainfall or high relative humidity. On the other hand, *P. citri* is related with low temperatures, high relative humidity, and areas with high wind speed, and *E. orientalis* is found in areas with high temperatures, slow wind velocity and low relative humidity. TCM was present in areas with the highest density of citrus crops. A summary of the environmental variables for the sites where each mite species was found is shown in Table 2.6. The results for the RDAs constrained by the selected environmental and spatial variables, and VPA are summarised in Table 2.7. Selected environmental variables explain 75.4% of the total observed variance across all sampled locations. However, despite this, most of the variance explained by the environment is shared with space (74.9%). If we look at the variance explained purely by the environment (0.5%), it is much smaller than the variance explained purely by space (22.2%). The shared variance is also high for *E. banksi*, *E. orientalis* and *P. citri* sites. Furthermore, the spatial fraction was also more important than the environmental fraction in all three scenarios. However, *E. orientalis* with 64.6% showed the highest purely spatial fraction followed by *E. banksi* (46.3%) and finally *P. citri* (23.8%). On the other hand, *P. citri* with 1% had the best purely environmental fraction followed by *E. banksi* (0.9%) and *E. orientalis* (0.1%) (Table 2.7).

Table 2.6 Environmental variables between sampling sites for the three mite species. Variables are given as mean (mean plus standard deviation -SD-, minimum, and maximum values recorded. Variables are grouped by temperature, relative humidity (HUM), wind speed (WIN) and precipitation (PRE). The meaning for the codes of the environmental variables is available in Table 2.1.

	Variables	Banksi sites (162)			Orientalis sites (68)			Panonychus sites (96)		
		Mean ± SD	Min.	Max.	Mean ± SD	Min.	Max.	Mean ± SD	Min.	Max.
	Citrus crop density (Km2)	17.8 ± 5.3	2.4	25.8	15.9 ± 7.0	2.2	27.8	16.0 ± 5.5	2.2	25.0
TEMPERATURE	Tmitmit (°C)	18.2 ± 0.6	16.9	19.4	18.3 ± 0.8	16.9	19.7	17.7 ± 0.7	15.6	19.4
	Tmitmax (°C)	29.5 ± 0.9	27.8	30.8	29.3 ± 0.8	27.8	30.9	28.8 ± 0.8	27.8	30.7
	Tmitmin (°C)	6.4 ± 0.8	4.1	8.4	6.7 ± 1.1	2.9	8.6	6.1 ± 1.1	3.0	8.6
	Tminmit (°C)	13.3 ± 0.8	11.0	14.9	13.4 ± 1.1	11.0	14.9	12.9 ± 0.9	10.4	15.0
	Tminmax (°C)	24.0 ± 0.5	23.0	25.3	24.3 ± 0.9	21.5	25.5	23.6 ± 0.6	21.5	25.4
	Tminmin (°C)	1.1 ± 1.0	0.0	3.8	1.4 ± 1.0	0.0	3.8	1.0 ± 0.8	0.0	2.4
	Tmaxmit (°C)	23.4 ± 1.0	21.9	25.0	23.4 ± 0.9	21.9	24.9	22.9 ± 1.0	21.3	24.9
	Tmaxmax (°C)	37.8 ± 2.4	33.4	41.6	37.4 ± 2.0	33.9	40.6	36.5 ± 2.1	33.3	41.3
	Tmaxmin (°C)	10.5 ± 0.9	7.7	13.1	10.6 ± 1.1	6.9	13.3	10.1 ± 1.2	6.9	13.2
HUM	Hmitmit (%)	67.2 ± 0.9	64.8	70.5	66.5 ± 0.9	64.6	68.6	67.0 ± 0.8	64.6	68.9
	Hmaxmax (%)	91.7 ± 1.2	88.2	94.4	90.7 ± 1.2	88.1	94.4	91.7 ± 1.5	88.1	94.4
	Hminmin (%)	35.6 ± 2.0	30.5	39.7	34.4 ± 1.9	30.5	37.4	34.5 ± 2.1	30.8	39.3
WIN	Vmit (km/h)	5.3 ± 0.6	3.9	6.4	5.1 ± 0.9	3.9	7.2	5.4 ± 0.7	3.9	7.2
	Vmax (km/h)	80.9 ± 3.7	72.1	97.6	77.8 ± 5.0	69.0	97.6	83.2 ± 7.4	75.9	104.0
PRE	Pctot (mm)	585.2 ± 141.8	312.8	875.3	455.7 ± 75.6	312.8	617.2	513.1 ± 96.3	312.8	828.1
	Pcmax (mm)	106.7 ± 33.8	51.6	190.5	88.0 ± 25.5	51.6	151.8	87.7 ± 26.8	51.6	176.4

Table 2.7 Results from redundance (RDAs) and variation partitioning analysis (VPA) corrected by Moran spectral randomisation.

	All sampled sites		*E. banksi sites*		*E. orientalis sites*		*P. citri sites*	
	Adjusted R^2	*p-value*	*Adjusted R^2*	*p-value*	*Adjusted R^2*	*p-value*	*Adjusted R^2*	*p-value*
a. Partition table								
E [a + b]	0.754	0.001**	0.509	0.001**	0.292	0.001**	0.721	0.001**
S [b + c]	0.970	0.001**	0.962	0.001**	0.936	0.001**	0.949	0.001**
E + S [a + b + c]	0.975	0.001**	0.971	0.001**	0.937	0.001**	0.958	0.001**
b. Individual fractions								
Pure E [a]	0.005	0.001**	0.009	0.001**	0.001	0.001**	0.010	0.001**
Pure S [c]	0.222	0.001**	0.463	0.001**	0.646	0.001**	0.238	0.001**
Shared [b]	0.749	-	0.500	-	0.291	-	0.711	-
Residuals [d]	0.025	-	0.029	-	0.063	-	0.042	-

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Adjusted R^2= Amount of variance explained by the explanatory variables; *P- value*= Statistical significance from an ANOVA like permutation test; *E*= Environmental explanatory variables; *S*= Spatial explanatory variables (MEMs); *Shared*= Variance fraction shared by environment and space; *Residuals*: Residual variance (no explained).

2.4 Discussion

Agricultural ecosystems have become excellent laboratories for the study of invasion ecology. The increased international mobility of people and goods during recent decades has caused the accidental introduction of invasive pests in agriculture to rise, leading to economic losses for farmers and food producers (Pimentel et al. 2000). Furthermore, the ecological impact produced by the invaders may change the resident community, altering the delicate web of relationships among species (Parker et al. 1999). Phytophagous mites have high potential as an invasive species because of their small size (from 0.2 to 0.5 mm length), short generation time, high reproductive potential, and their ability to be spread by the wind. Despite this, the study of the ecological consequences after invasion and the competitive relationships between spider mite species, the most damaging group to cultivated plants, has only been carried out on a few cases (Reitz and Trumble 2002). Recently, Ferragut et al. (2013a) studied the mite species composition on cultivated and non-cultivated plants in vegetable agrosystems before and after the invasion by the tomato spider mite *Tetranychus evansi* Baker and Pritchard, demonstrating that the invasive mite had reduced the presence and abundance of other *Tetranychus* species. However, a different approach was applied in that study and no spatial analysis was carried out to assess interspecific competition. The present work is the first study to conduct a spatial analysis to examine interspecific competition among spider mite species.

Occurrence analysis

We have studied the impact of TCM after its arrival. Our results have confirmed the rapid spread of this species since 2013, becoming the most frequent and abundant spider mite on citrus and leading to a significant reduction in the presence of their relatives *P. citri* and *E. orientalis*. These conclusions are supported by the patterns of co-occurrence, which provide evidence for a community structured by competition. The highest percentage of sampled orchards were occupied with only one species, while those occupied by all three species were extremely rare. Likewise, the probabilistic model and Chi-square contingency test detected competitive relationships between the pairs *E. banksi* – *E. orientalis* and *E. banksi* – *P. citri*, while there was no statistical evidence about the existence of competition between *E. orientalis* and *P. citri*.

Species distribution and GLM competition models

The current geographic distribution of the three spider mites do not coincide geographically, and GLM analysis shows how the probability of finding *E. orientalis* or *P. citri* decreases with increasing TCM density and vice versa, evidence of current competitive relationships. Unfortunately, there is no information about the geographic distribution of *E. orientalis* prior to the invasion of TCM. On the other hand, *P. citri* spread rapidly to the north and south of the entire Valencian territory soon after the first detection in April 1981, causing higher incidence in the Valencia province (García-Marí et al. 1983). A survey carried out by Abad-Moyano et al. (2009a) on citrus crops, before the arrival of TCM, clearly showed *P. citri* widely distributed throughout the citrus-growing area of the provinces of Castellón and Valencia. According to our results, the arrival of TCM to the province of Valencia has decreased the presence of *P. citri,* which now has its core distribution further north, in the south of the province of Castellón.

Multivariate Analysis

PCA and PERMANOVA indicated that there were segregated geographical distribution patterns between all pairs of mite species. Segregated patterns can be explained by competitive relationships between species. Nevertheless, they also may be caused by abiotic factors such as environmental heterogeneity across a set of sites (Kraft et al. 2015), acting as filters that hinder the establishment of the species (Pearson et al. 2018). Despite the economic importance of spider mites, there are no studies on the influence of environmental factors on the structure of their communities, and climatic factors such as temperature, relative humidity (RH), and precipitation have been considered the most influential in the development of their populations (van de Vrie et al. 1972; Vacante 2010). Furthermore, wind velocity or crop density may also be underlying non-random distribution patterns, since spider mites are considered passive dispersers, and wind dispersal has been proposed as one means of long-distance dissemination, especially when spider mite colonies are overcrowded, or the host plant is no longer suitable (Boyle 1956; van de Vrie et al. 1972). VPA allows partitioning of the variance of species abundance community data into variance that can be explained by environmental factors or by spatial variables. In practice, in a VPA the spatial component represents the community variance that cannot be explained by the environmental factors considered. In our study we have selected two sets of variables, a first set of environmental variables related to the temperature, relative humidity, precipitation, wind speed and crop density;

and a second set of spatial variables that tries to integrate the spatial variance of the set of sites. RDA constrained by the environmental variables across all the sampled sites suggests that the spider mite communities were significantly affected by these environmental factors, explaining 75.4 % of the community variance. However, when we separate the variance due to the environment, as well as the shared variance using VPA, the purely environmental contribution to the variance of mite communities (0.5%) is much lower than the spatial one (22.2%) (non-environmental). Furthermore, we found a large proportion of the explained variance shared by the environmental and spatial variables (74.9%). This situation is common, since environmental factors often vary through space, especially on a broad scale. In this scenario, we should be careful when assigning cause-effect relationships between species and space or with environmental conditions (Borcard et al. 1992). Furthermore, purely spatial fraction also could be explained by unmeasured environmental variables (Borcard and Legendre 1994). Despite these two observations, our findings indicate the existence of spatial patterns of geographical distribution of these species which do not seem to be governed mainly by the environmental variables selected. These results agree with Ferragut et al. (2013b) who point out that the climatic conditions under which citrus crops are grown throughout the Valencian region, are suitable for the establishment of *E. banksi*, *E. orientalis* and *P. citri* populations all year-round.

In addition to the environmental conditions, historical factors and interspecific competition can act spatially structuring the community in VPA (Borcard and Legendre 1994). In our work we have demonstrated the existence of competition among the three spider mite species using different approaches, and most of the classical publications point to competitive interactions as the main important factor in species segregation (Diamond 1975; Connor and Simberloff 1979) determining the community assembly (Kunstler et al. 2012). Furthermore, processes such as colonization/extinction history may also be underlying non-random distribution patterns (Gilpin and Diamond 1982). Current geographic distribution for the three mite species is not coincident and evidence their competitive relationships, but also their density cores, at least for TCM and *E. orientalis,* recently introduced, are related to the place of their first detection, the south of the province of Valencia for TCM, and the south of the province of Alicante for *E. orientalis*. For all these reasons, we believe that competitive interactions, and the colonization history, are the most important factors conditioning the spider mite community structure across all the sampled sites, contributing to the spatial component in the VPA. In our VPA study for each species, we found that (as in the analysis for all the sampled localities), the pure spatial component is much greater than the pure environmental component. If we focus on the analysis of the purely environmental fraction, *P. citri* followed by TCM have the highest values, while *E. orientalis* has a very low value. However, if we pay attention to the purely spatial component, *E. orientalis* has the highest value, followed by *E. banksi* and finally *P. citri*. We believe that these results, can be explained by historical colonisation factors and competitive interactions between species. According to the historical colonisation, *P. citri* has been present in the region for about 40 years; therefore, there is currently no spatial evidence of its colonisation process (historical factors) in its spatial distribution, and its pure environmental component is large, reflecting its adaptive process. Despite this, its current competition with TCM is reflected in its pure spatial component. *Eutetranychus banksi*, although recently introduced, has spread rapidly throughout the citrus area displacing the other two mite species and, accordingly, its spatial distribution is less affected by competition and historical colonisation processes (spatial component) compared to *E. orientalis*. However, its rapid adaptation process is reflected in its broad environmental component. Finally, *E. orientalis* has a very small pure environmental component, as it has a very large pure spatial component due to intense competition with TCM and the historical colonisation processes.

Competitive mechanisms

In our opinion, there are different non-exclusive hypotheses that can explain the impact of TCM on the whole community. One of the best-known hypotheses to explain the spread of invasive species is the *Enemy Release Hypothesis.* Following this approach, invasive species are less impacted by natural enemies (e.g., predators, parasites, and pathogens) than native species, because, in the new geographic location, invasive species lack the enemies that keep their growth under control in their native environment (Jeffries and Lawton 1984). There is little information available on field efficacy of predators on TCM in its native geographical area. The predatory mites of the family Phytoseiidae are the most important biological control agents used against spider mites, and essential elements in pest management programs (McMurtry 1982). In Florida, the phytoseiids *Euseius mesembrinus* (Dean) (Abou-Setta and Childers 1989), *Iphiseiodes quadripilis* (Banks) (Villanueva and Childers 2007) and *Galendromus helveolus* (Chant) (Caceres and Childers 1991) are common species on citrus and were tested in the laboratory with

TCM as a suitable food source, being a suitable prey. Furthermore, Landeros et al. (2004) observed a synchronization of the population peaks of TCM with their predator *E. mesembrinus*, as well as a correlation in the abundance of both species on Mexican citrus crops. Nevertheless, these natural enemies were not introduced with TCM in the Spanish invaded areas. *Euseius stipulatus* (Athias-Henriot) is the predominant native phytoseiid on citrus in Spain and is a useful biocontrol agent of *P. citri* (Ferragut et al. 1987, 1988). According to McMurtry (1985), predators that attack *P. citri* may also be effective against *Eutetranychus spp*. because those spider mites have a similar colonization pattern on the leaves and produce small amounts of webbing. This predator was studied under laboratory conditions to test its predatory potential on *E. banksi*. The predator was able to complete its life cycle when feeding on TCM, but mortality was high, the reproductive parameters very low and almost no eggs were produced, indicating that this prey is not a suitable food source (López-Olmos and Ferragut, in preparation). According to the results obtained in laboratory tests, we believe that *E. stipulatus* in the presence of *P. citri* and *E. banksi* as a food source, will prefer the former. Therefore, *E. stipulatus*, by selecting *P. citri* over *E. banksi*, would be contributing to tip the balance in favour of TCM in the interspecific competition between both phytophagous. Despite the spread of TCM, *E. stipulatus* populations do not seem to become reduced, since this phytoseiid is highly generalist and can complete its development feeding on other mite species, small insects, and even pollen or fungal spores (Ferragut et al. 1987; McMurtry and Croft 1997).

In addition to the ability of TCM to escape the attack by native predators, the biology of this species can turn it into a superior competitor of *P. citri* at high temperatures. The developmental time of TCM at 25 °C is 13.1 days (Childers et al. 1991), while at the same temperature *P. citri* has a shorter development time of 10.9 days (Tanaka and Inoue 1970). On the contrary, at higher temperatures the development time of TCM is considerably reduced, being of 11.6 days at 28 °C and 61% RH. Greater differences are reflected in the fecundity values. The highest fecundity for TCM was reported at 28 °C with 37.08 eggs/female, and 8.84 being the maximum fecundity rate (eggs/female/day) at 30 °C. This behaviour is the opposite of that of *P. citri,* which produces 40.4 eggs/female and 6 eggs/female/day at 24 °C (Yasuda 1982). The temperature range of 28-30 °C seems to be optimal for TCM, while the range of 24-25 °C is optimal for *P. citri*. In the Valencian region in late spring-early summer, with average daily temperatures between 24-26 ºC, low populations of *P. citri* can be detected which are controlled by *E. stipulatus*. During July-September, mean daily temperatures reach values of 27-30 °C, providing conditions more favourable for the development of TCM. Low populations of this mite are found in July, increasing rapidly to produce population peaks at the end of September and beginning of October (López-Olmos and Ferragut, accepted). According to Ferragut et al. (1988), *P. citri* has a population peak in late summer-early fall. However, this peak is currently not observed in the field (López-Olmos and Ferragut, in preparation). We believe that the demise of the population peak of *P. citri* is related to the lack of effective natural enemies against TCM, as well as the indirect competition for resource exploitation between both species. TCM seems not to reject feeding on leaves slightly damaged by previous consumption of low *P. citri* populations, while high TCM populations completely cover the habitable space on the leaves and leave them without nutrients available for *P. citri*. The *Resource Depletion* hypothesis (space and nutrient) has been cited as the leading mechanism of interspecific competition between the spider mites *Tetranychus urticae* Koch and *Eotetranychus carpini borealis* (Ewing) on red raspberry (Bounfour and Tanigoshi 2001), while the ability of *Tetranychus telarius* (L.) to feed on leaves previously consumed by *Panonychus ulmi* (Koch) gives them an advantage in a competitive situation on peach and apple crops (Foott 1963).

In addition to these two hypotheses, some studies have shown how indirect competition can exist between two herbivores through changes in the quality of the shared plant, representing interactions between competitors mediated by the host plant (Karban and Myers 1989; Tallamy and Raupp 1991). Different studies have demonstrated the ability of several spider mites to *Induce Plant Defences* that negatively affect other phytophagous competitors. Early season occurrence of *Eotetranychus willamettei* (McGregor) on grapevines in California, resulted in a population reduction of *Tetranychus pacificus* (McGregor) through the induction of a systemic response (Hougen-Eitzman and Karban 1995). On tomato plants, *Tetranychus evansi* and *T. urticae* are often competitive species. Both can inhibit host plant defences, which facilitates their own development, although they also favour the growth of other species (Sarmento et al. 2011). Therefore, the induction of plant defences and their tolerance by the inducing species determine the outcome of their competition with less tolerant species. Although there are no studies on the ability of TCM to manipulate citrus defences in its favour to affect potential competitors, we cannot rule out that this mechanism might play a role in the competitive displacement carried out by *E. banksi.*

Our results show, for the first time, the existence of competition between two invasive mite species of the genus *Eutetranychus* originating from different parts of the world. In this case, we cannot consider the enemy release hypothesis as a mechanism that regulates the competition because *E. orientalis*, like *E. banksi*, lacks effective predators in the Spanish citrus area. However, our results point to the existence of a competitive relationship between them that is even more intense than that recorded between *E. banksi* and *P. citri*. Both species are phylogenetically closer, so their ecological niche may be more similar, and therefore the indirect competition through resource exploitation between both species would be more intense. Previous laboratory studies demonstrated that *E. orientalis* performs better at high temperatures around 30 ºC, in the same way as TCM; however, its biological parameters, especially those related to fecundity, are lower than those of TCM in the same environmental conditions. *Eutetranychus orientalis* showed a fecundity of 14.56 eggs/female at 25 ºC and 16.33 eggs/female at 30 ºC (Imani and Shishehbor 2009), while TCM can produce twice as many eggs as *E. orientalis* (29.96 eggs/female at 25 ºC and 32.08 eggs/female at 30 ºC) (Childers et al. 1991). *Fecundity differences* between both species would be able to lead the competitive displacement of *E. orientalis* from the citrus area (Reitz and Trumble 2002).

As both *Eutetranychus* are closely related species, *Reproductive Interference* is another hypothesis that may explain the intense competition. Reproductive interference is an interspecific interaction during the process of mate acquisition caused by incomplete species recognition resulting in the lack of courtship and mating discrimination, which adversely affects the fitness of at least one of the species involved (Gröning and Hochkirch 2008). Since the native ranges of both *Eutetranychus* do not overlap, they likely do not have recognition mechanisms that act as a barrier to avoid interspecific mating. Geographic displacement driven by this mechanism has been demonstrated in spider mites on apple trees in Japan, where *Panonychus mori* Yokoyama predominates in the north and *P. citri* in the south. Interspecific mating occurs without fertilization and, because of their reproduction mode by arrhenotoky, females involved in interspecific copulation do not produce females in their offspring, resulting in a decrease in their biological fitness (Fujimoto et al. 1996). However, the degree of reproductive interference is greater in *P. mori* than in *P. citri* and, consequently, *P. citri* geographically has displaced *P. mori*, in the southern area (Takafuji et al. 1997). Following this hypothesis, the intense competition detected by us between *E. banksi* and *E. orientalis* could suggest the existence of reproductive interference between them. It is possible that males of *E. orientalis* do not discriminate between conspecific and heterospecific females as much as males of TCM, or alternatively, females of TCM discriminate against interspecific mating more than females of *E. orientalis*. In either scenario, *E. orientalis* females may be involved in interspecific mating more frequently than TCM females, being more affected by reproductive interference. Interspecific copulations could be totally ineffective, but not affect the successive fertilization with males of the same species (Ozawa and Takafuji 1987). However, in the worst case, the eggs produced are non-viable, no females are produced in the offspring, or hybrid and infertile females are generated, affecting in all cases subsequent intraspecific mating (Boudreaux 1963; Smith 1975).

Niche partitioning and future scenarios

Interspecific competition may cause competitors to evolve to reduce the impact of that competition (Sakai et al. 2001). We can consider future scenarios at plant leaf level and at plant species level. One way to reduce the impact of competition and make possible the coexistence between competitive species should involve a niche partitioning process (Albrecht and Gotelli 2001). In a broad scale spatial niche partitioning, *P. citri* could be displaced to more temperate citrus-growing areas, where it can compete better, specializing in those temperate ranges, while TCM would occupy the warmer zones. However, this option seems less plausible in the case of *E. orientalis*, as both *Eutetranychus* prefer hot temperatures. On the other hand, on a fine scale, the movement of inferior competitors *E. orientalis* and *P. citri* to the lower face of the citrus leaves avoiding the presence of TCM, who prefers to live on the upper side, could allow the coexistence of these two species on the same leaves with their superior competitor. However, this hypothesis seems unlikely, at least in clementine citrus and lemons, since the lower side is already occupied by the two-spotted spider mite *T. urticae*, which produces large amounts of silk covering the leaf surface and probably rendering this habitat unfavourable (Morimoto et al. 2006).

A food niche partitioning event was observed after the invasion of *T. evansi* in the Valencian region, as the native spider mite species *T. urticae* and *T. turkestani* Koch moved to occupy non-crop plants more frequently, while *T. evansi* established mainly on crop plants (Ferragut et al. 2013a). Thus, another option should be a change in the host plant range by the inferior competitors in the invaded area. The three mite species are highly polyphagous, reported on more than one hundred plant species worldwide (Migeon and Dorkeld 2022) and the observed interactions may be expected on other hosts. We have demonstrated the existence of competition on citrus plants, but there is no information on the population behaviour of these mites on other plants.

Although food and space are the major mechanisms for niche partitioning, time can also provide a scenario for the niche partitioning on an annual temporal scale (Loureau 1989). This scenario is not very plausible for both *Eutetranychus* species that show optimal biological parameters related to high temperatures. However, *P. citri* could present a spring population dynamic in warm temperatures, while TCM populations could develop during the hot summer. Nevertheless, this scenario is improbable, since *E. stipulatus*, the main predator of *P. citri*, has its population peak in spring (Ferragut et al. 1988). Competition among *E. banksi* and *P. citri* in Florida citrus has been reported before by Childers (1992), where TCM has become the dominant species in the orchards since 1955, displacing the *P. citri*. Therefore, the establishment of *E. banksi* and the resulting competitive displacement and extinction of *P. citri* from entire or most of the citrus growing areas in the Valencian region seems the most likely hypothesis. On the other hand, this is the first time that *E. orientalis* and *E. banksi* have coexisted on citrus crops worldwide, but the phylogenetic proximity, and the consequent niche similarity and intense competition between both *Eutetranychus* species, makes their coexistence even less plausible. Whatever the final outcome, under a total competitive displacement scenario, TCM will be more exposed to intraspecific competition, a powerful force in the case of agricultural pests which, by definition, produce intense population outbreaks in short periods of time.

2.5 Acknowledgments

Authors like to thank the owners and technicians of the orchards for allowing us to use their plantations and J. Gavara-Vidal (UPV) for his help in sampling. This research was supported by a predoctoral grant (ACIF to S. López-Olmos from Generalitat Valenciana) and the Pest Control Collaboration Project S7296000 between the Polytechnic University of Valencia (UPV) and the Generalitat Valenciana. We thank Phil Barker for English revision, and AVAMET for providing the climate data.

2.6 Supplementary Materials

Supplementary Material 1: Climate Variables

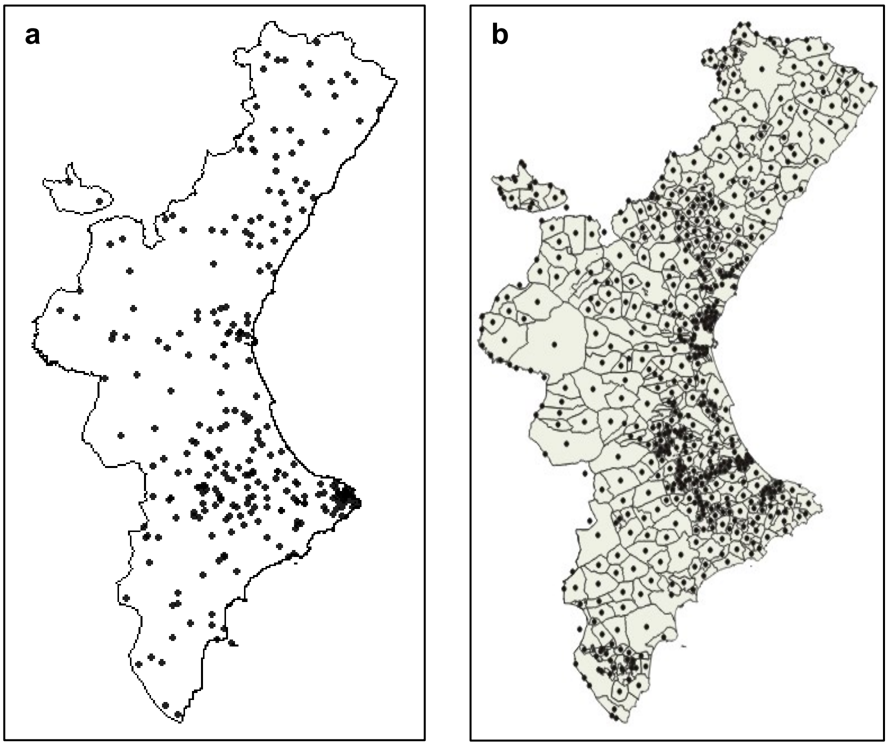
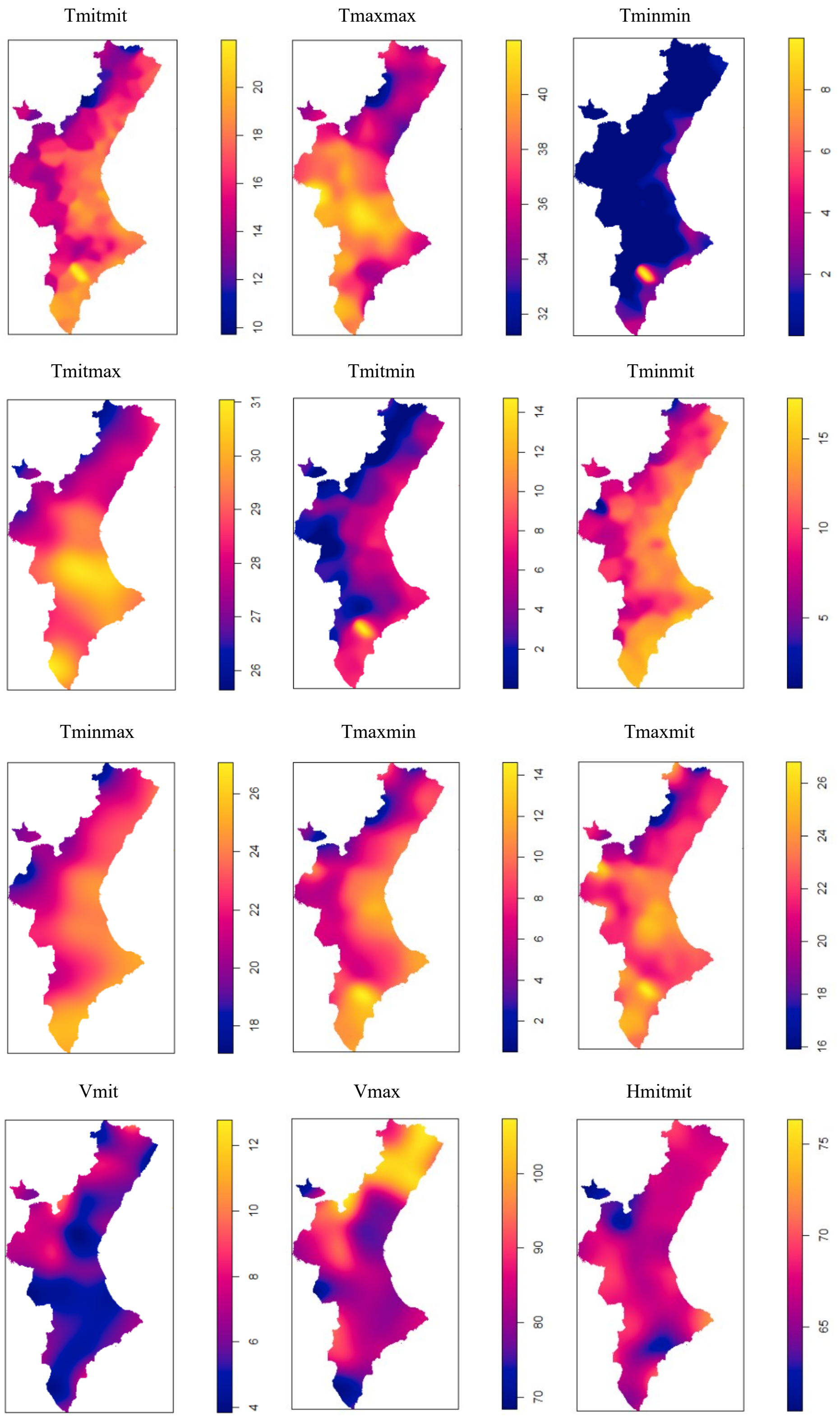


Figure 1 a. Geographical location of 262 weather stations from the Valencian Meteorology Association (AVAMET) scattered around the Valencian region. The span of years for which data were recorded varies across stations, by this reason a common time series was used (2013-2020). The stations record environmental data daily, and annual average in each station for each variable was calculated. The map was obtained using the R-package *spatstat*. b. Geographical location of the centroids (points) for the municipalities (polygons) in the Valencian region. The map was obtained using *Q-sig*.


Tmitmit
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Tmitmax
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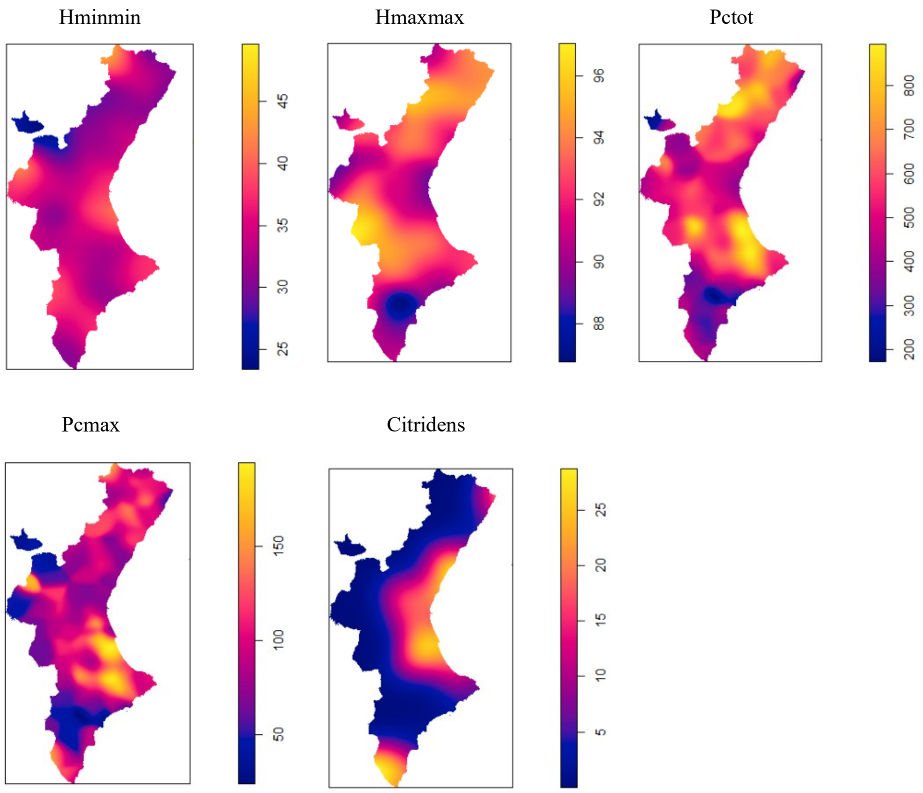


Figure 2 Raster maps with 3284 x 1890-pixel array and 0.1 square km area per pixel of the 16 climate variables, and the variable related with the crop density (Citridens). Climate variable maps were obtained through kernel smooth weighting (KSW) for the climate station values, using the Gaussian function with least-squares cross-validation selected bandwidth. The variable related with crop density was obtained through kernel density weighted estimation (KDWE) for the municipality citrus crop area values, to assess the probability density of this variable per square kilometre, using the Gaussian function with the crop area value to weight the municipality centroids, and the Scott's rule of thumb to determine the smoothing bandwidth. Temperature maps in degrees Celsius, relative humidity in %, and rainfall in mm. Crop density is measured as citrus municipalities/Km^2 weighted by the cultivated citrus area in each municipality. The meaning for the codes of the environmental variables is available in Table 2.1. Maps were performed using the R-package *spatstat.*

Supplementary Material 2: Forward selection procedure for climate and spatial variables

A. Across all the sampled orchards: Only sites with at least 1 mite species have been considered (241)

Climate variables: Variable inflation factor (VIF<10), and forward selection procedure chose 12 climate variables.

Table 1 Forward selection procedure for climate variables across all the sampled orchards computed by the R-package *adespatial.*

Variables	R^2	R^2 cum	Adjusted R^2 cum	F	*p*-value
Pctot	0.425	0.425	0.423	176.587	0.001
Vmax	0.210	0.634	0.631	136.461	0.001
Hminmin	0.044	0.679	0.674	32.455	0.001
Hmaxmax	0.074	0.753	0.749	71.071	0.001
Tminmit	0.036	0.789	0.785	40.218	0.001
Tmaxmax	0.027	0.816	0.811	34.016	0.001
Tminmin	0.022	0.838	0.833	31.443	0.001
Pcmax	0.007	0.845	0.839	10.647	0.001
Citridens	0.012	0.857	0.852	20.001	0.001
Vmit	0.004	0.861	0.855	7.070	0.001
Hmitmit	0.004	0.865	0.858	5.960	0.004
Tmitmit	0.004	0.869	0.862	7.178	0.001

Forward selection procedure included all variables selected by VIF<10

Spatial Variables: The forward selection procedure chose 38 broad-scale spatial predictors (MEMs).

Table 2 Forward selection procedure for environmental variables (MEMs) across all the sampled orchards computed by the R-

Variables	R^2	R^2 cum	Adjusted R^2 cum	*p* value
MEM1	0.257	0.257	0.253	0.010
MEM3	0.196	0.452	0.448	0.010
MEM15	0.093	0.545	0.539	0.010
MEM5	0.064	0.609	0.602	0.010
MEM4	0.064	0.673	0.666	0.010
MEM12	0.043	0.716	0.709	0.010
MEM7	0.042	0.758	0.751	0.010
MEM6	0.027	0.785	0.778	0.010
MEM10	0.024	0.809	0.801	0.010
MEM11	0.022	0.830	0.823	0.010
MEM8	0.018	0.849	0.841	0.010
MEM9	0.014	0.863	0.856	0.010
MEM2	0.014	0.877	0.870	0.010
MEM25	0.012	0.889	0.882	0.010
MEM36	0.012	0.901	0.895	0.010
MEM29	0.006	0.908	0.901	0.010
MEM46	0.006	0.914	0.907	0.010
MEM13	0.005	0.919	0.913	0.010
MEM17	0.005	0.924	0.917	0.010
MEM32	0.005	0.929	0.922	0.010
MEM30	0.005	0.933	0.927	0.010
MEM34	0.004	0.938	0.931	0.010
MEM27	0.004	0.942	0.936	0.010
MEM35	0.004	0.946	0.940	0.010
MEM14	0.004	0.950	0.944	0.010
MEM58	0.003	0.953	0.947	0.010
MEM39	0.003	0.956	0.950	0.010
MEM61	0.002	0.958	0.952	0.010
MEM28	0.002	0.960	0.955	0.010
MEM20	0.002	0.962	0.957	0.010
MEM31	0.002	0.964	0.959	0.010
MEM47	0.002	0.966	0.961	0.010
MEM43	0.002	0.968	0.963	0.010
MEM64	0.002	0.969	0.964	0.010
MEM60	0.002	0.971	0.966	0.010
MEM48	0.001	0.972	0.968	0.010
MEM50	0.001	0.974	0.969	0.010
MEM19	0.001	0.975	0.970	0.010

Procedure stopped (R^2 more criteria): variable 39 explains only 0.000875 of the variance.

B. *Eutetranychus banksi* sites (162)

Climate variables: Variable inflation factor (VIF<10), and forward selection procedure chose 9 climate variables.

Table 3 Forward selection procedure for climate variables to *E. banksi* sites computed by the R-package *adespatial.*

Variables	R^2	R^2 cum	Adjusted R^2 cum	F	*p*-value
Pcmax	0.373	0.373	0.369	95.170	0.001
Tmitmit	0.147	0.520	0.514	48.739	0.001
Tminmax	0.129	0.649	0.642	57.860	0.001
Vmax	0.029	0.678	0.670	14.353	0.001
Citridens	0.028	0.707	0.697	15.124	0.001
Vmit	0.019	0.726	0.715	10.733	0.002
Hmitmit	0.013	0.739	0.727	7.724	0.004
Tmaxmax	0.007	0.746	0.733	4.387	0.028
Tminmit	0.010	0.756	0.741	6.144	0.006

Procedure stopped (alpha criteria): *p*-value for variable 10 is 0.135000 (> 0.050000).

Spatial Variables: The forward selection procedure chose 38 broad-scale spatial predictors (MEMs).

Table 4 Forward selection procedure for spatial variables (MEMs) to *E. banksi* sites computed by the R-package *adespatial.*

Variables	R^2	R^2 cum	AdjustedR^2 cum	*p*-value
MEM1	0.520211	0.520211	0.517212	0.01
MEM8	0.101773	0.621984	0.617229	0.01
MEM2	0.049761	0.671745	0.665512	0.01
MEM4	0.034223	0.705968	0.698476	0.01
MEM29	0.025168	0.731136	0.722519	0.01
MEM10	0.020533	0.751669	0.742056	0.01
MEM13	0.019224	0.770893	0.760479	0.01
MEM14	0.017212	0.788105	0.777026	0.01
MEM3	0.016539	0.804644	0.793077	0.01
MEM24	0.015747	0.820391	0.808497	0.01
MEM9	0.014341	0.834732	0.822613	0.01
MEM6	0.013841	0.848574	0.836378	0.01
MEM41	0.011396	0.859969	0.847669	0.01
MEM17	0.01078	0.870749	0.858439	0.01
MEM28	0.009174	0.879922	0.867586	0.01
MEM11	0.007855	0.887777	0.875394	0.01
MEM7	0.007191	0.894968	0.882568	0.01
MEM25	0.005663	0.900631	0.888123	0.01
MEM22	0.00557	0.906201	0.893651	0.01
MEM34	0.005328	0.911529	0.89898	0.01
MEM40	0.005031	0.916561	0.904045	0.01
MEM43	0.005014	0.921574	0.909162	0.01
MEM18	0.004733	0.926308	0.914026	0.01
MEM5	0.004404	0.930712	0.918574	0.01
MEM19	0.004297	0.935008	0.923062	0.01
MEM23	0.004281	0.93929	0.927597	0.01
MEM42	0.00421	0.943499	0.932115	0.01
MEM12	0.003998	0.947497	0.936444	0.01
MEM27	0.003388	0.950885	0.940095	0.01
MEM30	0.003328	0.954214	0.943728	0.01
MEM36	0.003175	0.957389	0.947228	0.01
MEM15	0.003159	0.960548	0.950761	0.02
MEM16	0.002658	0.963206	0.953719	0.01
MEM21	0.002255	0.965461	0.956214	0.01
MEM35	0.001908	0.967369	0.958305	0.01
MEM39	0.001403	0.968772	0.959778	0.03
MEM31	0.001377	0.970148	0.961241	0.04
MEM26	0.001052	0.9712	0.962303	0.04

Procedure stopped (R^2 more criteria): variable 39 explains only 0.000980 of the variance.

C. *Eutetranychus orientalis* sites (68)

Climate variables: Variable inflation factor (VIF<10), and forward selection procedure chose 7 climate variables.

Table 5 Forward selection procedure for climate variables to *E. orientalis* sites computed by the R-package *adespatial.*

Variables	R^2	R^2 cum	Adjusted R^2 cum	F	*p*-value
Tmaxmax	0.474	0.474	0.466	59.497	0.001
Tminmin	0.131	0.605	0.593	21.530	0.001
Pctot	0.063	0.668	0.652	12.124	0.001
Pcmax	0.053	0.721	0.704	12.056	0.001
Vmax	0.033	0.754	0.734	8.244	0.003
Hminmin	0.017	0.770	0.748	4.402	0.024
Tmaxmin	0.035	0.805	0.782	10.671	0.003

Procedure stopped (adjusted R^2 threshold criteria) adjusted R^2 cum = 0.788 with 8 variables (> 0.786)

Spatial Variables: The forward selection procedure chose 7 broad-scale spatial predictors (MEMs).

Table 6 Forward selection procedure for spatial variables (MEMs) to *E. banksi* sites computed by the R-package *adespatial.*

Variables	R^2	R^2 cum	Adjusted R^2 cum	*p*-value
MEM1	0.377141	0.377141	0.367704	0.01
MEM2	0.188353	0.565494	0.552124	0.01
MEM4	0.136591	0.702085	0.68812	0.01
MEM3	0.107388	0.809473	0.797376	0.01
MEM5	0.051994	0.861467	0.850295	0.01
MEM6	0.042224	0.903692	0.894219	0.01
MEM7	0.039343	0.943035	0.936389	0.01

Forward selection procedure included all spatial variables (MEMs)

D. *Panonychus citri* sites (96)

Climate variables: Variable inflation factor (VIF<10), and forward selection procedure chose 9 climate variables.

Table 7 Forward selection procedure for climate variables to *P. citri* sites computed by the R-package *adespatial.*

Variables	R^2	R^2 cum	Adjusted R^2 cum	F	*p*-value
Vmax	0.436	0.436	0.430	72.723	0.001
Pcmax	0.225	0.661	0.654	61.827	0.001
Citridens	0.106	0.767	0.760	41.960	0.001
Hmitmit	0.027	0.794	0.785	11.930	0.001
Pctot	0.020	0.814	0.804	9.581	0.001
Tminmit	0.009	0.823	0.811	4.636	0.016
Vmit	0.006	0.830	0.816	3.212	0.048
Tminmax	0.009	0.838	0.823	4.713	0.017
Tmaxmax	0.009	0.847	0.831	4.965	0.012

Procedure stopped (adjR2thresh criteria) adjR^2 cum = 0.833710 with 10 variables (> 0.832797).

Spatial Variables: The forward selection procedure chose 22 broad-scale spatial predictors (MEMs).

Table 8 Forward selection procedure for spatial variables (MEMs) to *P. citri* sites computed by the R-package *adespatial.*

Variables	R^2	R^2 cum	Adjusted R^2 cum	*p*-value
MEM3	0.389876	0.389876	0.383385	0.01
MEM5	0.126268	0.516143	0.505738	0.01
MEM4	0.104481	0.620625	0.608254	0.01
MEM10	0.044296	0.664921	0.650192	0.01
MEM2	0.043699	0.708619	0.692432	0.01
MEM6	0.041524	0.750143	0.733299	0.01
MEM9	0.041269	0.791412	0.77482	0.01
MEM11	0.039002	0.830414	0.81482	0.01
MEM1	0.029514	0.859928	0.845269	0.01
MEM23	0.017665	0.877593	0.863192	0.01
MEM25	0.017381	0.894974	0.881221	0.01
MEM21	0.011908	0.906882	0.89342	0.01
MEM17	0.008004	0.914887	0.901393	0.01
MEM12	0.007703	0.92259	0.90921	0.01
MEM15	0.007468	0.930058	0.916944	0.01
MEM26	0.00613	0.936188	0.923264	0.02
MEM13	0.005602	0.94179	0.929103	0.01
MEM18	0.00539	0.94718	0.934832	0.01
MEM27	0.004588	0.951767	0.939709	0.01
MEM22	0.004506	0.956273	0.944612	0.01
MEM24	0.002393	0.958666	0.946936	0.01
MEM7	0.001846	0.960512	0.948611	0.04

Procedure stopped (alpha criteria): *p-value* for variable 22 is 0.070000 (> 0.050000).

Supplementary Material 3: Summary of the MEMs computed

A. Across all the sampled orchards: Only sites with at least 1 mite species have been considered (241)

Figure 1 Map across all the sampled orchards with the orchard's locations (white points), and connection network following distance based spatial weighting matrix (SWM) using the minimum connection distance as a threshold. Map performed using R-package *adespatial*

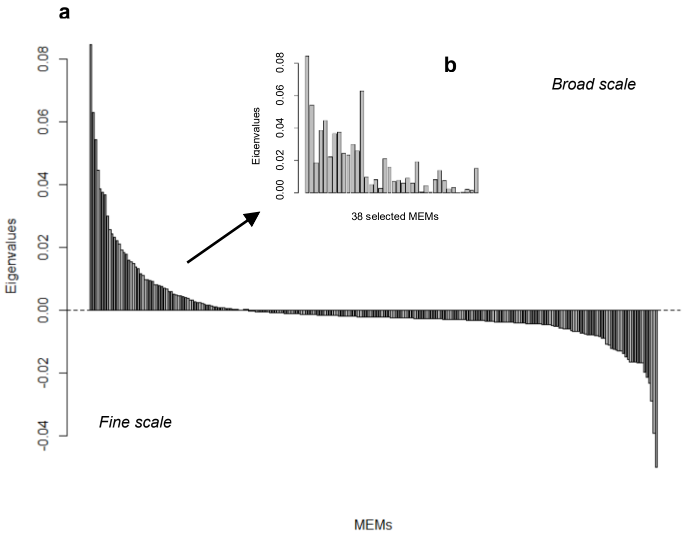


Figure 2 (a) Eigenvalues associated with each MEM (eigenvectors) across settings. Positive values correspond to broad scale spatial structures, whereas negative values represent fine scale space. (b) Eigenvalues associated with the 38 selected MEMs (p value < 0.05).

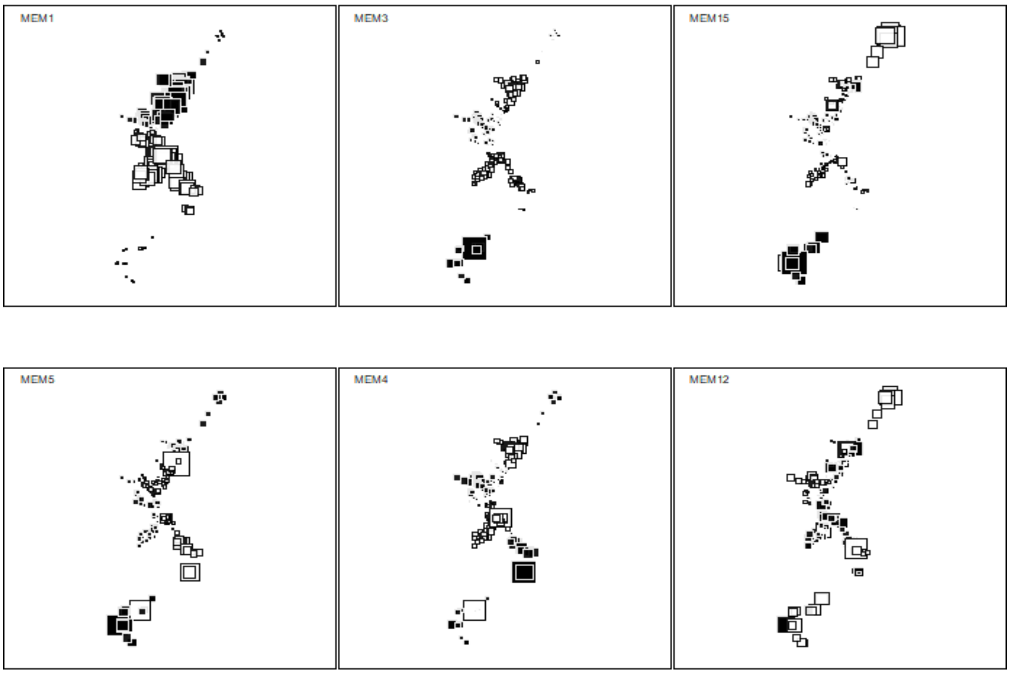


Figure 3 Maps across all the sampled orchards showing the values of each MEM in each site. Black squares indicate positive values and white squares negative values. The size of the rectangles depends on the absolute value. We only plotted 6 MEMs of the total selected by the forward procedure.

B. *Eutetranychus banksi* sites (162)

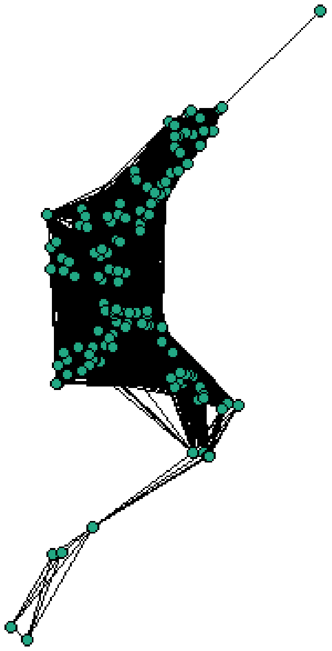


Figure 4 Map for *E. banksi* sites with the orchard's locations (green points), and connection network following distance based spatial weighting matrix (SWM) using the minimum connection distance as a threshold. Map performed using R-package *adespatial.*

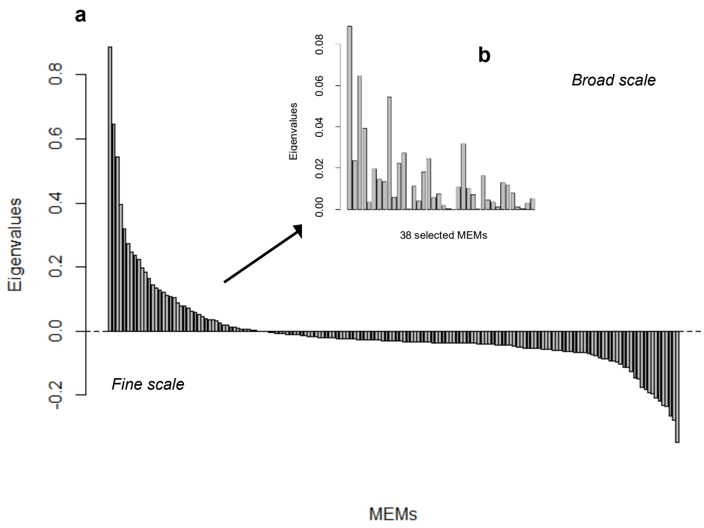


Figure 5 (a) Eigenvalues associated with each MEM (eigenvectors) for *E. banksi* sites. Positive values correspond to broad scale spatial structures, whereas negative values represent fine scale space. (b) Eigenvalues associated with the 38 selected MEMs (*p* value < 0.05).

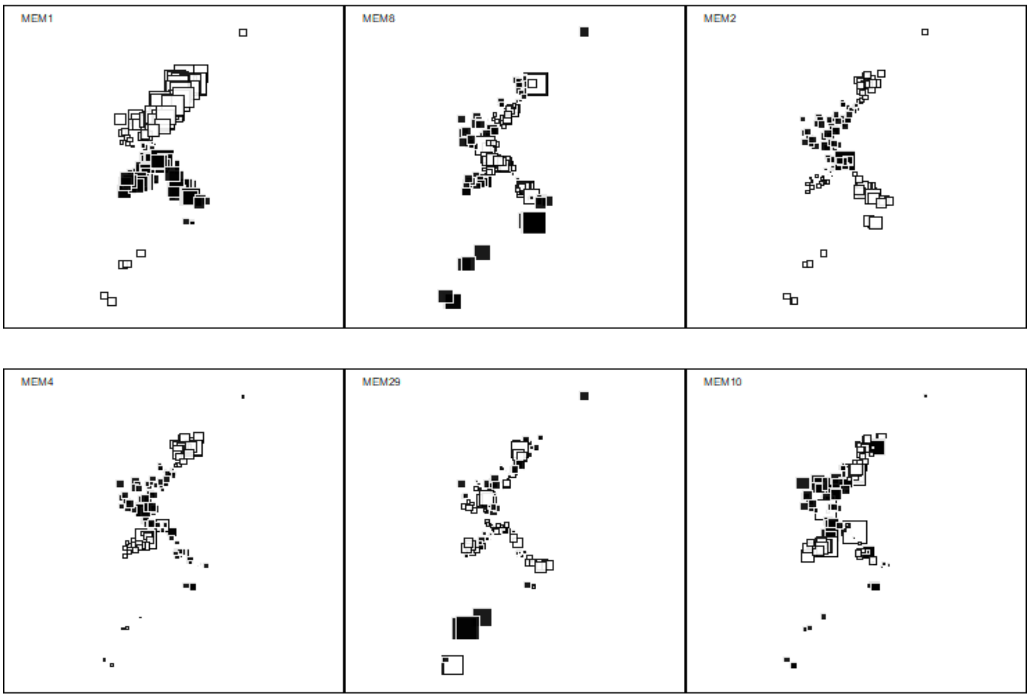


Figure 6 Maps for the *E. banksi* sites showing the values of each MEM in each site. Black squares indicate positive values and white squares negative values. The size of the rectangles depends on the absolute value. We only plotted the first 6 MEMs selected by the forward procedure.

C. *Eutetranychus orientalis* sites (68)

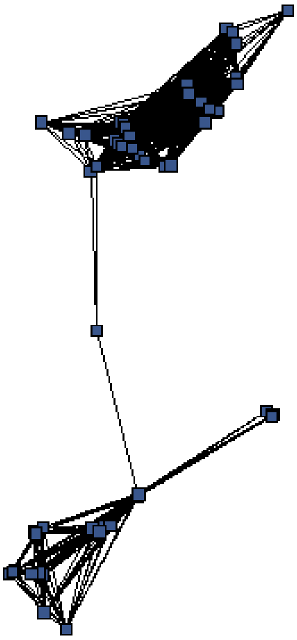


Figure 7 Map for *E. orientalis* sites with the orchard's locations (blue squares), and connection network following distance based spatial weighting matrix (SWM) using the minimum connection distance as a threshold. Map performed using R-package *adespatial*.

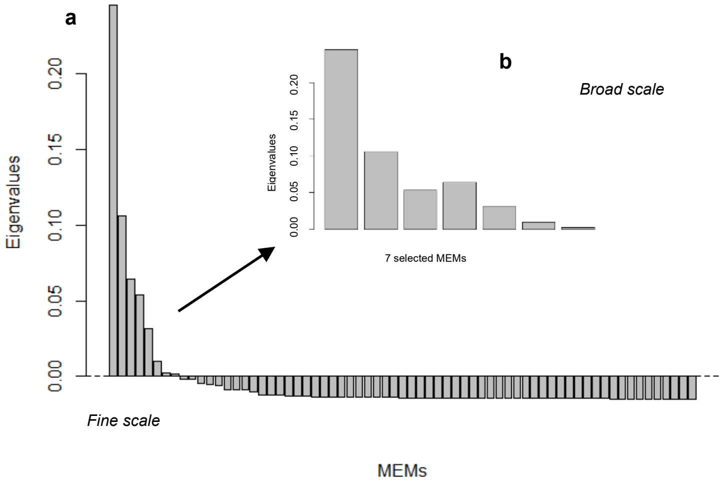


Figure 8 (a) Eigenvalues associated with each MEM (eigenvectors) for *E. orientalis* sites. Positive values correspond to broad scale spatial structures, whereas negative values represent fine scale space. (b) Eigenvalues associated with the 7 selected MEMs (p value < 0.05).

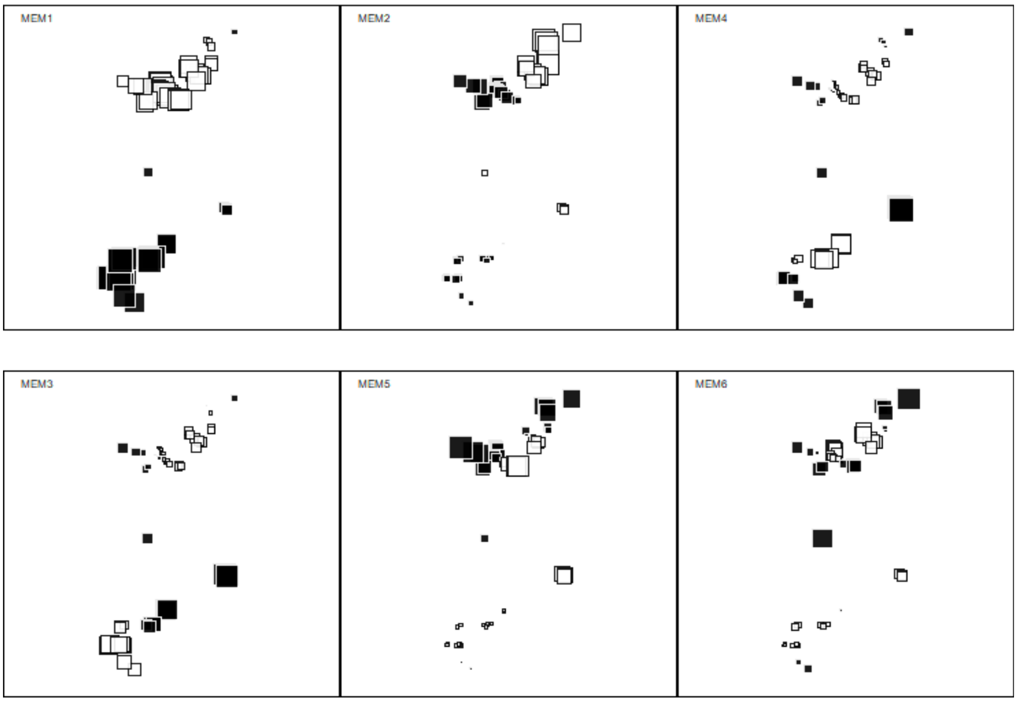
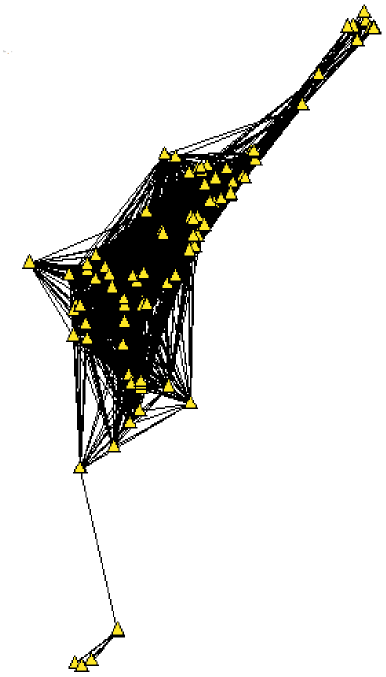


Figure 9 Maps for the *E. orientalis* sites showing the values of each MEM in each site. Black squares indicate positive values and white squares negative values. The size of the rectangles depends on the absolute value. We only plotted the first 6 MEMs selected by the forward procedure.

C. *Panonychus citri* sites (96)



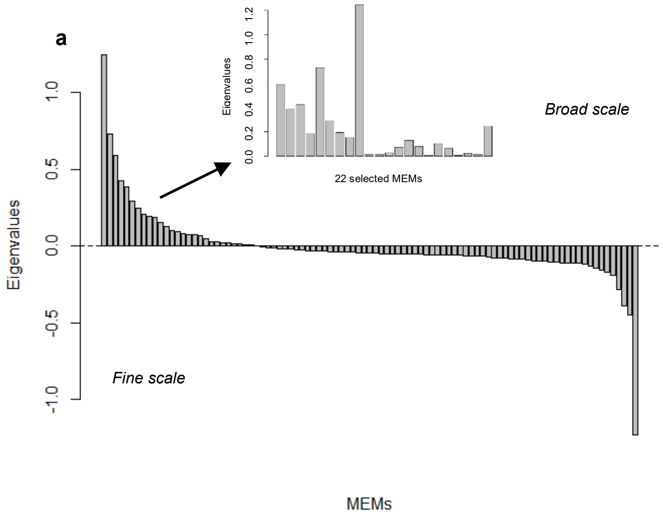


Figure 11 (a) Eigenvalues associated with each MEM (eigenvectors) for *P. citri* sites. Positive values correspond to broad scale spatial structures, whereas negative values represent fine scale space. (b) Eigenvalues associated with the 22 selected MEMs (*p* value < 0.05).

Figure 10 Map for *P. citri* sites with the orchard's locations (yellow triangles), and connection network following distance based spatial weighting matrix (SWM) using the minimum connection distance as a threshold. Map performed using R-package *adespatial.*

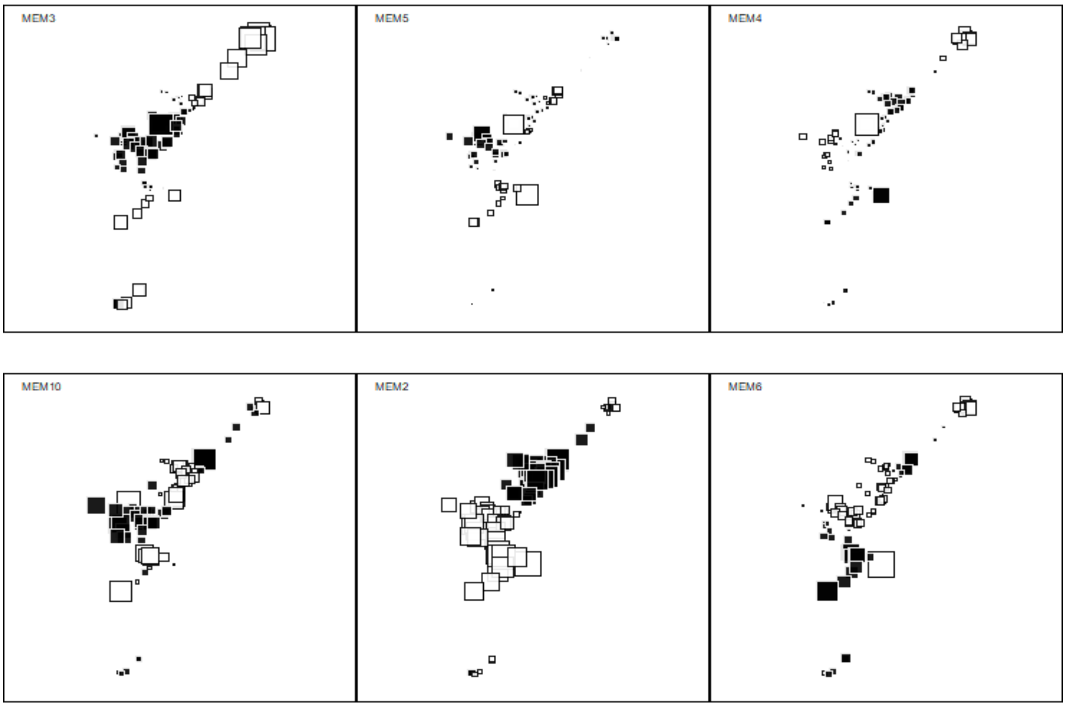


Figure 12 Maps for the *P. citri* sites showing the values of each MEM in each site. Black squares indicate positive values and white squares negative values. The size of the rectangles depends on the absolute value. We only plotted the first 6 MEMs selected by the forward procedure.

CHAPTER 3

Within-tree distribution and seasonal dynamics of *Eutetranychus banksi* (Acari: Tetranychidae) and *Euseius stipulatus* (Acari: Phytoseiidae) on citrus: Implications for the biological control of the pest

Within-tree distribution and seasonal dynamics of *Eutetranychus banksi* (Acari: Tetranychidae) and *Euseius stipulatus* (Acari: Phytoseiidae) on citrus: Implications for the biological control of the pest

López-Olmos S and Ferragut F (2023). Within-tree distribution and seasonal dynamics of *Eutetranychus banksi* (Acari: Tetranychidae) and *Euseius stipulatus* (Acari: Phytoseiidae) on citrus: Implications for the biological control of the pest. Acarologia. Accepted minor revisions. Doctoral thesis adapted version.

Abstract: The Texas citrus mite *Eutetranychus banksi* McGregor (Acari: Tetranychidae), is a citrus pest widely distributed throughout the Americas. In 2013 it was detected in the south of the province of Valencia, affecting the main citrus-growing area in Spain. The mite produces severe damage reducing photosynthesis, causing defoliation, and producing a lack in fruit pigmentation, which may affect its economic value. A better understanding of the population biology and natural enemies associated with the pest is necessary prior to establishing an Integrated Pest Management programme. Phytoseiid mites have been widely cited as the main natural enemies of spider mites around the world. Therefore, we aimed to study the within-tree distribution of *E. banksi* and phytoseiid mites, describe their seasonal trends, and, finally, identify the phytoseiid species associated with *E. banksi,* as well as evaluate the biological control implications. The Texas citrus mite was mainly found on the adaxial (upper) side of leaves from the current year's shoots located at the periphery of the tree canopy, whereas phytoseiids prefer the abaxial (lower) side of leaves inside the tree canopy. The spider mite preferred to feed on leaves rather than fruits, whereas phytoseiids use both substrates equally. *Eutetranychus banksi* showed a seasonal trend on leaves with a single population peak in late summer-early autumn, while phytoseiids showed two peaks, a main spring peak and a second less abundant in autumn. The seasonal dynamics of *E. banksi* and phytoseiids on fruits occurred in parallel to those recorded on leaves. *Euseius stipulatus* was the most frequent and abundant phytoseiid species, apparently displacing other predatory mite species. In autumn, when *E. banksi* reaches high densities, phytoseiids moved from the leaf abaxial sides inside the tree canopy to the adaxial sides in periphery and to the fruits, to feed on *E. banksi.* In addition, a change in phytoseiid colouration from white to reddish-brown was observed. Both, the proportion of leaves occupied by red phytoseiids and the number of coloured phytoseiid increased significantly in response to the prey population growth, which was indirect evidence of their contribution to the biological pest control.

3.1 Introduction

The Texas citrus red mite, *Eutetranychus banksi* McGregor (Acari: Tetranychidae), is an important citrus pest first recorded on citrus in 1935 from Rio Grande Valley in Texas (Dean 1952, 1959b, 1980), and now widely distributed throughout the Americas, being cited from the southern United States to northern Argentina and reported from most countries in Latin America (Migeon and Dorkeld 2022). Far from its native region, the Texas citrus mite was found in Egypt (Abdel-Shaheed et al. 1973), and more recently in Portugal (Carvalho et al. 1999), Spain (García et al. 2003) and Iran (Beyzavi et al. 2013). Since its arrival in the Iberian Peninsula, severe damage to citrus has been recorded in the Algarve region of southern Portugal, making it a key pest (Gonçalves et al. 2002). In 2001, Spanish citrus orchards in Ayamonte and Isla Cristina (Huelva province) were invaded by *E. banksi*. Although we have no information about the dissemination pathway followed by the mites, it has been suggested that they were carried in fruit containers that are frequently exchanged by Portuguese and Spanish farmers in the border area (García et al. 2003). In 2013 *E. banksi* was suddenly detected in La Safor (Valencia province) more than 800 km away from Ayamonte, and the following year in other regions of the provinces of Valencia and Alicante, affecting the main citrus-growing area of the eastern Mediterranean in Spain. Because no geographical spread of this species throughout the citrus-growing regions of southern Spain was detected, it seems that the Valencian populations came directly from Huelva via contaminated plant material (Ferragut 2016). On citrus trees, *Eutetranychus banksi* feeds by consuming the contents of mesophyll plant cells, reducing the leaf photosynthetic capacity and promoting defoliation, which results in a decrease in yield. In addition, an aesthetic damage to the fruits is produced, resulting in a lack in pigmentation, which may reduce their economic value (García et al. 2003; Monzó et al. 2016). Furthermore, it is one of the most polyphagous spider mite species reported on 110 hosts belonging to 34 plant families, mostly Fabaceae (22 species) and Rutaceae (9 species), infecting other crops and many ornamental plants (Migeon and Dorkeld 2022).

Chemical control is commonly used to maintain mite populations at low levels. The Texas citrus mite has been shown to be sensitive to numerous acaricides, being easy to keep under control with this strategy (Monzó et al. 2016). However, under the perspective of Integrated Pest Management (IPM), cultural practices, biological and biotechnological control should be combined with chemicals to produce healthy crops and minimize the use of pesticides, reducing the risks to human health and the environment. Before developing an IPM programme against *E. banksi*, it is necessary to have a better understanding about the population biology of the pest, as well as its natural enemies. Phytoseiid mites have been widely cited as the main natural enemies of spider mites on citrus crops around the world (Gerson 2003; Vacante 2010). Therefore, we aimed to: (i) study the within-tree distribution of *E. banksi* and phytoseiid mites, (ii) describe the seasonal trends of *E. banksi* and phytoseiids, and finally, (iii) identify the phytoseiid species associated with *E. banksi*, as well as analyse the behaviour of the predatory mites in response to the increase in pest density.

3.2 Materials and Methods

Sampled orchards

The study was conducted across four citrus orchards in 2018 and six in 2019; only two of them were monitored during the two years. All the orchards were commercial citrus plantations situated in an extensive citrus monoculture region in the Valencian Community (Spain). The orchards were selected due to the presence of evident symptoms of damage produced by *E. banksi* on the leaves of the previous year. Furthermore, the selection was carried out aiming to include different groups of citrus varieties and different environmental conditions. In the first year, two sampling areas were selected according to their climatic differences, Picassent in the centre of Valencia province and Pego in the north of Alicante province. The second year, in addition to these two areas, a third one was incorporated in the locality of Riola, in the south of the Valencia province (Table 3.1). The orchards were planted with sweet orange, *Citrus sinensis* (L.) Osbeck [including the varieties Valencia late (one orchard), Salustiana (one orchard), and Navelina (three orchards)], clementine mandarin, *Citrus reticulata* Blanco [including the variety Oronules (one orchard)], and the hybrids Ortanique [*C. sinensis* x *C. reticulata* (one orchard)], and Clemenvilla [*Citrus clementina* Hort x (*Citrus paradisi* Macf. x *Citrus tangerina* Hort), (one orchard)]. All the orchards ranged from 0.2 to 2 ha in land area and had normally developed 11 to 15-year-old trees in full production drip irrigated. The selected orchards were under several pest management strategies which included (i) zero residue, based on the use of low-impact chemicals for pest management that allows fruit without chemical residues, as well as chemical nutrition for the crop, (ii) conventional, based on the use of chemical pesticides that leave residues on fruit, as well as chemical nutrition, (iii) organic, based on the use of organic pesticides and nutrition. Despite this, orchards had not been sprayed with pesticides for at least 6 months before sampling (Table 3.1).

Table 3.1 Sampled sites, years, varieties and pest management practices.

Sampling year	Locality	Citrus variety	Variety Group	Management
2018-2019	Picassent	Oronules	Clementine	Conventional
2018	Picassent	Ortanique	Hybrid	Organic
2018-2019	Pego	Navelina	Navel	Zero residue
2018	Pego	Valencia late	White	Organic
2019	Picassent	Salustiana	White	Conventional
2019	Pego	Navelina	Navel	Zero residue
2019	Pego	Clemenvilla	Hybrid	Conventional
2019	Riola	Navelina	Navel	Organic

Sampling procedure

In each orchard, approximately 10% of the trees were marked and randomly sampled regularly. The marked trees were not sprayed with pesticides during the sampling period. Sampling was conducted weekly, during the period of peak pest abundance (July-November), and fortnightly during the rest of the year. On each sampling date, 25 inner and 25 outer leaves from each citrus sprouting (spring, summer, and autumn), belonging to the previous and the current year, were randomly collected around the marked trees canopy in each sampled orchard. In addition, and depending on their availability, 25 fruits per orchard were randomly collected from the outer part of the marked trees canopy. All the leaves of the same position and sprouting were combined in a plastic bag and transported to the laboratory inside a portable cooler. Fruits from the same orchard were transported in plastic containers. Within the next 24 h, leaves and fruits were examined under the stereomicroscope. All the development stages of *E. banksi* and phytoseiid mites were counted on leaves and fruits. Furthermore, in the case of leaves, their position on the adaxial (upper) or abaxial (lower) side of the leaf was recorded. The motile forms of phytoseiids from each sampling and orchard were extracted from the leaves and fruits using a fine brush and placed inside plastic tubes containing 70 % ethanol. They were subsequently mounted on microscope slides, using Heinze-PVA after clearing with Nesbitt's medium and, identified at the species level. In total, 49,350 leaves and 5,175 fruits were counted from 69 samplings carried out during 2018 and 2019. Regarding phytoseiids, 5,338 individuals were determined at species level.

Data analysis

In the sampling data all the development stages were pooled together and averaged per sampling (date) and sample unit (leaf or fruit), using this value for graphics and statistical analysis. Samplings with 0 values were eliminated from the analysis. The average number of mites per cm^2 was used to compare fruits and leaves, due to the differences in surface area between these two structures. The within-tree distribution analysis was carried out at four levels (canopy, leaf, leaf age, and fruit) by comparing the mean number of mites in different positions at each level. For this purpose, data were tested for normal distribution using the Shapiro-Wilk test and for homogeny of variances using Levene's test. Data were log-transformed to fulfil the homogeneity of variances requirements of the Wilcoxon *rank sum* test (Wilcoxon 1945), used to evaluate the difference between the means in the different positions.

Data from seasonal monitoring of *E. banksi* and phytoseiids were represented graphically to show the seasonal abundance trends on leaves and fruits. Daily air temperature and relative humidity data were obtained from three meteorological stations from the Valencia Association of Meteorology (AVAMET), each one situated in each three sampled localities (Picassent, Pego and Riola). The maximum, mean, and minimum daily air temperature, as well as the daily relative humidity were represented in the seasonal abundance graphs. The absolute and relative abundance, and the frequency of occurrence for all the phytoseiid species were calculated.

Finally, to evaluate the biological control possibilities by the phytoseiid mites, we aimed to detect changes in the spatial distribution of these predatory mites at three spatial levels: canopy, leaf, and fruit. If phytoseiids are actively preying on *E. banksi* individuals, they should appear associated with them at different spatial levels. In addition, we expected a change in the coloration of the phytoseiids (from white to red), indicative of predation on their presumptive prey *E. banksi*, since no other potential prey with reddish-brown coloration was detected on the leaves at the same time. To explore this change in behaviour, we plotted the proportion of phytoseiids at each position for each of the three levels, as well as the proportion of phytoseiids with different colour along the seasonal dynamics of *E. banksi*. To statistically test whether the predatory mites were modifying their behaviour, because of the increase in prey density, three approaches were carried out at the three levels above mentioned. Firstly, (i) Binomial (logit-link) generalized linear models (GLMs) were performed to evaluate the effect of the mean number of *E. banksi* on occupancy of phytoseiids. To evaluate the change in mite coloration, the relationship between the mean number of *E. banksi* on the proportion of phytoseiids with different coloration was tested using the same methodology. To deal with the overdispersion in the data analysis, the standard errors of the estimated coefficients in the model were corrected by using a quasi-binomial error distribution (Zuur et al. 2009). Secondly, (ii) Linear models (LM) were used to evaluate the influence of the mean number of *E. banksi* on the mean number of phytoseiids. To test the coloration hypothesis, the relationship between the mean number of *E. banksi* and red phytoseiids found was tested using the same approach. The data were log-log transformed to fulfil the assumptions of normality tested with Shapiro-Wilk’s test, and homogeneity of the residual variance tested with Breusch-Pagan’s test (Breusch and Pagan 1979). Finally, (iii) Contingency tables and Chi-square test (Gardener 2012) were used to assess the association between *E. banksi* and predatory phytoseiids (García Marí et al. 1991). This methodology was used to study the effect of the presence of *E. banksi* on the occupancy and the proportion of phytoseiids at the three spatial levels of study. The colour change of phytoseiids was also evaluated by this methodology. We performed all statistical analysis by means of an R version 3.6.1 (R Core Team 2019). Wilcoxon’s *rank sum* test was performed using the package *coin* (Hothorn et al. 2006), and Breusch-Pagan’s test was performed using the package *car* (Fox and Weisberg 2019). The graphs of the GLM and LM models were plotted with the package *visreg* (Breheny and Burchett 2017).

3.3 Results

Within-tree distribution analysis

The mean number of *E. banksi* per leaf (mean ± standard error, SE) on the periphery (outside) of the canopy (12.08 ± 2.38) was significantly higher than inside (2.95 ± 0.62) (Z = -2.41, P = 0.015) (Figure 3.1a). At the leaf level, *E. banksi* was more abundant on the adaxial side of the leaf (8.93 ± 1.77) than on the abaxial side (1.96 ± 0.40) (Z = -3.50, P = 0.0003) (Figure 3.1b). Leaf age was also a factor influencing the abundance of the species, since *E. banksi* density on leaves of the current year "New leaves" (17.07 ± 3.23), was higher compared to the leaves of the previous year "Old leaves" (10.88 ± 2.19) (Z = 3.92, P = 3.766e-05) (Figure 3.1c). Finally, statistically significant differences were observed between leaves and fruits, as we found a higher mean number of mites per cm^2 on the leaves (0.23 ± 0.04) than on the fruits (0.10 ± 0.02) (Z = 2.06, P = 0.040) (Figure 3.1d).

Phytoseiids showed a different pattern of spatial distribution. When we focus on the canopy, the mean number of phytoseiids per leaf inside the canopy (0.78 ± 0.10) was significantly higher than outside (0.088 ± 0.012) (Z = 6.96, P < 2.2e-16) (Figure 3.1e). Regarding leaves, phytoseiid density was higher on the abaxial (0.45 ± 0.07) side compared to the adaxial side (0.04 ± 0.005) (Z = 7.004, P < 2.2e-16) (Figure 3.1f). Leaf age also affected the abundance of phytoseiids, since the population density on leaves from the previous year (0.16 ± 0.02) was significantly higher than those on the current year (0.04 ± 0.006) (Z = -5.48, P = 2.666e-10) (Figure 3.1g). When we compared leaves and fruits, we did not observe significant differences in the mean number of phytoseiids per cm^2 in both substrates (Z = -1.52, P = 0.132) (Figure 3.1h).

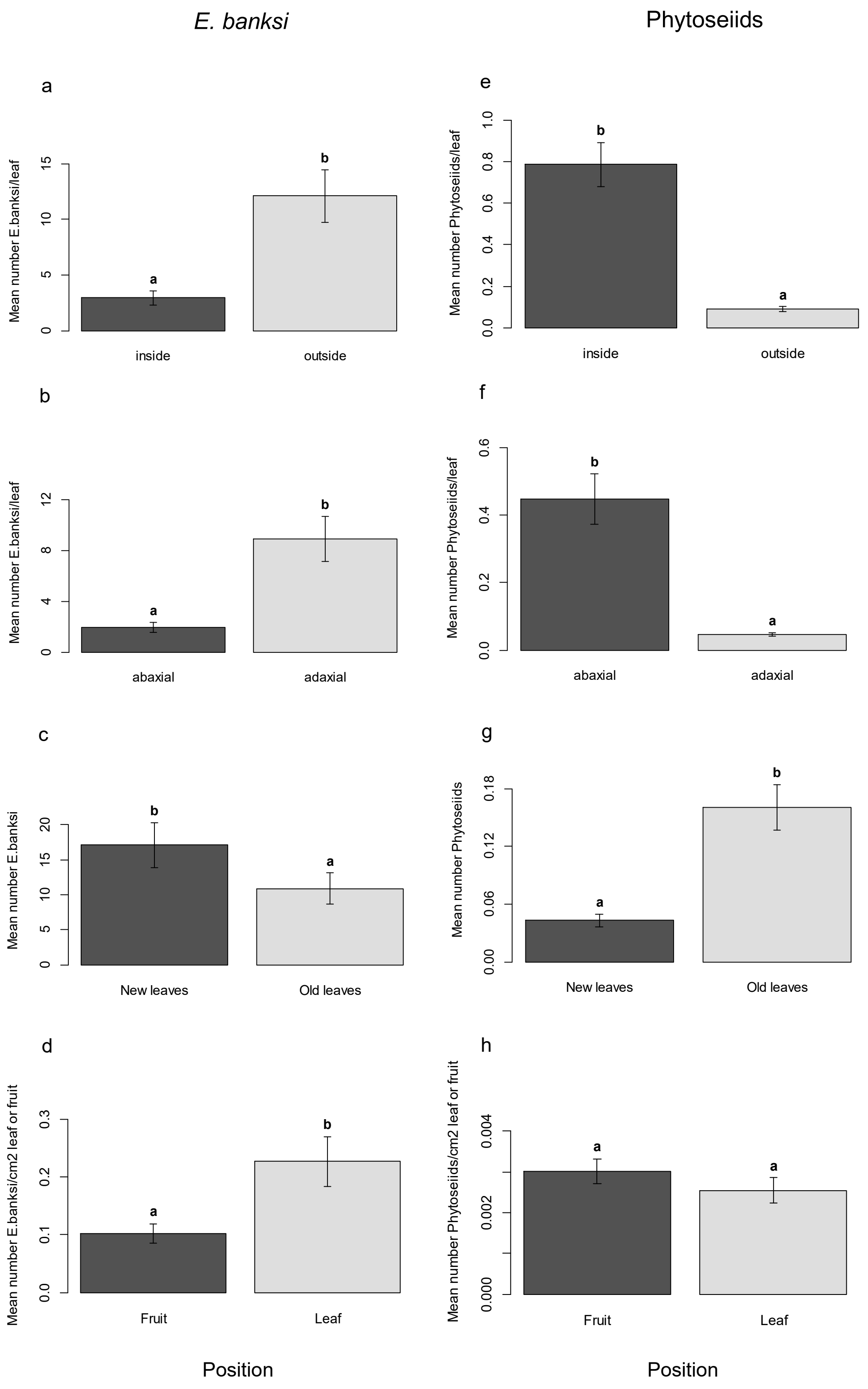


Figure 3.1 Mean number of *E. banksi* (a–d) and phytoseiid mites (e–h) per leaf or per cm2 of leaves and fruits. Bars with different letters are significantly different (Wilcoxon *rank sum* test).

Seasonal trends of E. banksi and phytoseiid mites

The development of *E. banksi* showed a similar trend over the two-year period of study. The density of this mite on the citrus leaves started to increase rapidly in late summer, during the month of August, showing unimodal dynamics, with one single annual peak in late summer-early autumn. The peak population density on leaves reached an average of 25.69 ± 2.17 and 58.76 ± 3.27 mites per leaf in 2018 and 2019, respectively. Afterwards, the population decreased and was extremely low and even undetectable during winter. The pest density on fruits followed the same seasonal trend as on the leaves. The peak population density on fruits reached an average of 28.76 ± 5.90 and 52.16 ± 6.31 mites per fruit in 2018 and 2019, respectively (Figures 3.2 and 3.3).

Phytoseiids also showed a similar seasonal trend during the two years of the study. They presented bimodal dynamics with a spring peak of greater importance during May–June, and a second autumn peak of lesser importance in October–November. Furthermore, in 2018, a peak prior to the main one occurred in April. The main spring peak population density reached an average of 1.38 ± 0.17 and 1.17 ± 0.11 mites per leaf in 2018 and 2019, respectively. In autumn, the maximum density was 0.25 ± 0.06 and 0.34 ± 0.03 mites per leaf. Subsequently, the phytoseiids increased in 2018 and decreased in 2019 resulting in values that remain around 0.25 phytoseiids per leaf during winter. On fruits, the phytoseiids presented low values during the summer, and their abundance increased during autumn reaching average values of around 0.5 or even 1 mite per fruit in 2018 and 2019, respectively (Figures 3.2 and 3.3).

Phytoseiid species on the trees

The most abundant and frequent species was *Euseius stipulatus* (Athias-Henriot) with 4,844 (90.75% of the total phytoseiids) individuals retrieved in 88% of the samplings. *Typhlodromus phialatus* Athias-Henriot was the following species in abundance with 290 (5.43%) individuals and 37.82% in frequency. A third species was *Neoseiulus californicus* (McGregor), which showed an abundance of 182 (3.41%), and occurred in 8.36% of the samplings. Finally, the remaining species *Paraseiulus talbii* (Athias Henriot), *Neoseiulus barkeri* Hughes and *Neoseiulella litoralis* (Swirski & Amitai) were scarcely present on the leaves (Table 3.2).

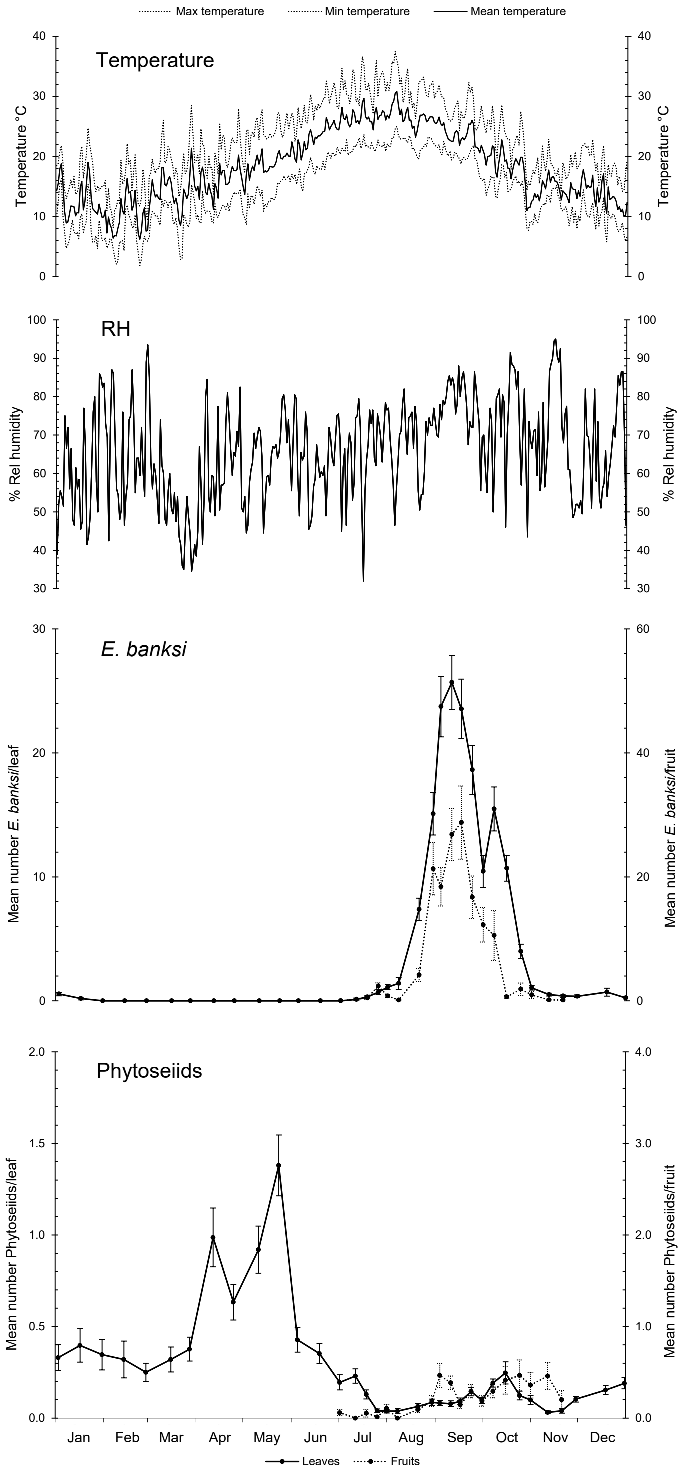


Figure 3.2 Seasonal trends of *E. banksi* and phytoseiid mites on leaves (solid line, first y-axis) and fruits (broken line, second y-axis) in four citrus orchards in 2018. Mean number of mites collected per sampling unit (all the stages were pooled together). Note that first and second y-axis scales are different. Mean (solid line), maximum and minimum daily temperatures in °C (broken lines) and mean daily relative humidity (RH) were represented. Capped bars represent ± standard error (SE).

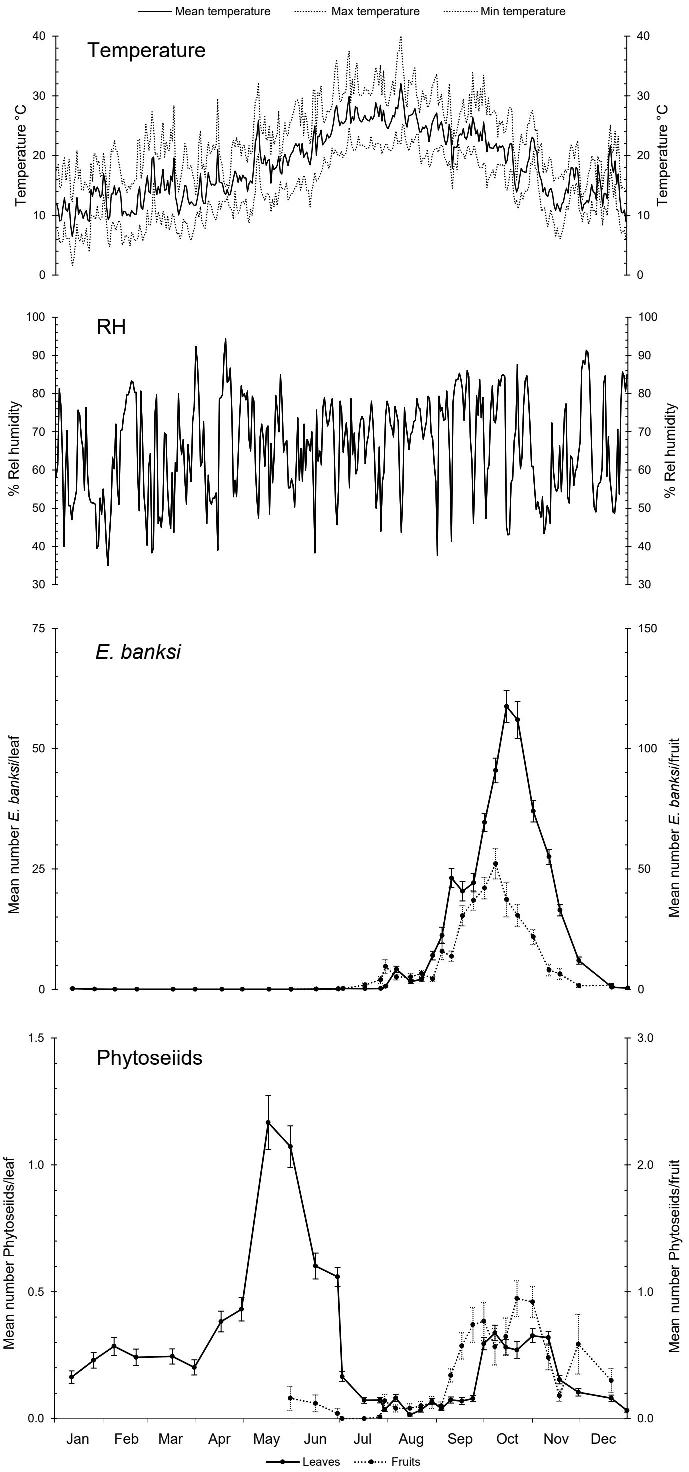


Figure 3.3 Seasonal trends of *E. banksi* and phytoseiid mites on leaves (solid line, first y-axis) and fruits (broken line, second y-axis) in six citrus orchards in 2019. Mean number of mites collected per sampling unit (all the stages were pooled together). The first and second y-axis scales are different. Mean (solid line), maximum and minimum daily temperatures in °C (broken lines) and mean daily relative humidity (RH) were represented. Capped bars represent ± standard error (SE).

Table 3.2 Abundance, relative abundance, and frequency of phytoseiid mites. Abundance represents the number of individuals of each species collected; Relative abundance is the percentage of individuals of each species with respect to the total number of phytoseiids; Frequency is the percentage of samples in which a certain phytoseiid species occurred with respect to the total number of samples collected.

Phytoseiid specie	Abundance	Relative abundance (%)	Frequency (%)
Euseius stipulatus (Athias- Henriot)	4,844	90.75	88.00
Typhlodromus phialatus Athias-Henriot	290	5.43	37.82
Neoseiulus californicus (McGregor)	182	3.41	8.36
Paraseiulus talbii (Athias Henriot)	17	0.32	2.91
Neoseiulus barkeri Hughes	2	0.04	0.73
Neoseiulella litoralis (Swirski & Amitai)	2	0.04	0.36
Neoseiulus sp.	1	0.02	0.36

Euseius stipulatus was the most abundant phytoseiid species during winter and spring of 2018 and 2019. However, in the month of July of both years, we found a decline in its abundance. Following this decline, in both years there was an increase in the abundance of *T. phialatus* and *N. californicus* in early autumn. This effect was more pronounced in 2019. In August of that year, *T. phialatus* with 29 individuals collected (80.56%, relative abundance), was more abundant than *E. stipulatus* (7 individuals; 19.44% relative abundance). In September, both *T. phialatus* (22; 41.51%) and *N. californicus* (22; 41.51%) were more abundant and frequent than *E. stipulatus* (9; 16.98%). In October, *E. stipulatus* (121; 40.33%) recovered, while *N. californicus* (108; 36.00%) increased and outcompeted *T. phialatus* (71; 23.67%). In November, *E. stipulatus* did not increase in absolute abundance but continued its increase in relative abundance (123; 77.36%), compared to *T. phialatus* (6; 3.77%) and *N. californicus* (30; 18.87%). With the arrival of winter, in December, *Euseius stipulatus* (145; 91.77%) recovered its complete dominance over *T. phialatus* (5; 3.16%) and *N. californicus* (7; 4.43%) (Figure 3.4).

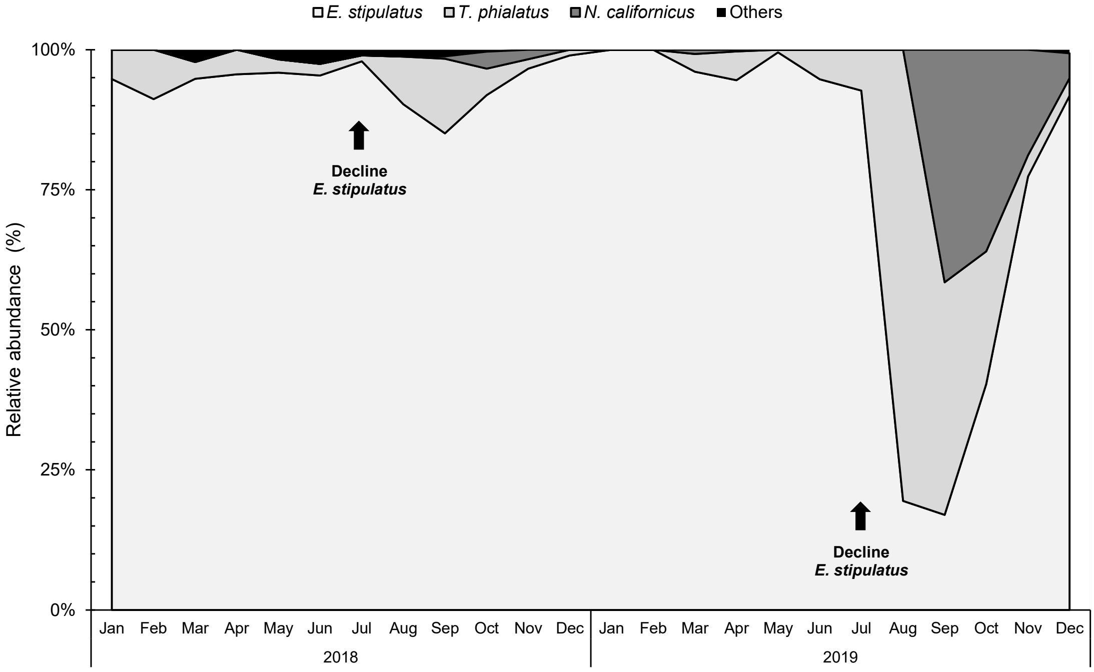


Figure 3.4 Seasonal relative abundance of motile forms of phytoseiid species in four (2018) and six (2019) citrus orchards. Percentage of each species per sampling is represented. The summer decline of *E. stipulatus* is represented by arrows in the graph.

Implications for the biological control of E. banksi

Using the data obtained under field conditions, we plotted the relationship of the seasonal dynamics of *E. banksi* against the proportion of phytoseiids at four spatial levels throughout 2018 and 2019 (Figure 3.5). As graphically we observed that *E. banksi* density was influencing the spatial distribution of phytoseiids we performed a statistical analysis of the data. Regarding the canopy, we observed a significative increase in the proportion of leaves occupied by phytoseiids on the periphery of the canopy when the *E. banksi* population increased (Table 3.3a, Figure 3.6a). Furthermore, we also detected a significative increase in the number of phytoseiids outside the canopy when *E. banksi* increased in abundance (Table 3.4a, Figure 3.6e). When focusing on leaves outside the tree canopy, the proportion of adaxial leaf surfaces occupied by phytoseiids increased with *E. banksi* population (Table 3.3b, Figure 3.6b). In parallel, the number of phytoseiids on the adaxial side of leaves also increased (Table 3.4b, Figure 3.6f). The colour of the phytoseiids was also significantly affected by the presence of *E. banksi*. The proportion of leaves occupied by red phytoseiids and the number of coloured phytoseiid increased significantly in response to *E. banksi* populations (Tables 3.3c and 3.4c, Figures 3.6c and 3.6g). Finally, we also found a significant influence of *E. banksi* density in fruits on the proportion of occupied fruits by phytoseiids (Table 3.3d, Figure 3.6d), as well as the number of them per fruit (Table 3.4d, Figure 3.6h).

The Contingency tables and Chi-square tests confirmed the results obtained from the GLM and LM models. In relation to occupancy, there was a significant increase in leaf occupancy by phytoseiids on the periphery of the tree canopy when *E. banksi* was present on leaves (*X-squared* = 381.96, *df* = 1, *P* < 2.2e-16) (Figure 3.7a). In parallel, an increase in the occupancy of the adaxial side of the leaves was observed (*X-squared* = 375.76, *df* =1, *P* < 2.2e-16) (Figure 3.7b), as well as an increase in the proportion of phytoseiids with reddish coloration (*X-squared* = 750.15, *df*= 1, *P* < 2.2e-16) (Figure 3.7c). Finally, we observed a significant rise in the occupation of fruits by phytoseiids when *E. banksi* was present on them (*X-squared* = 122.77, *df*= 1 *P* < 2.2e-16) (Figure 3.7d). When we examined the different levels of study, from the point of view of the number of phytoseiids, we observed that the proportion of these predators outside the tree canopy increased in the presence of *E. banksi* (*X-squared* = 16.79, *df*= 1, *P* = 4.164e-05) (Figure 3.8a). In addition, the proportion of phytoseiids found on the adaxial side of the leaves (*X-squared* = 12.48, *df*= 1, *P* = 0.000411) (Figure 3.8b), as well as the reddish coloured phytoseiids (*X-squared* = 74.43, *df*= 1, *P* < 2.2e-16) (Figure 3.8c) also increased significantly in the presence of *E. banksi*. Finally, the presence of *E. banksi* on fruits significantly increased the proportion of phytoseiids per fruit (*X-squared* = 35.19, *df*= 1, *P* = 2.985e-09) (Figure 3.8d).

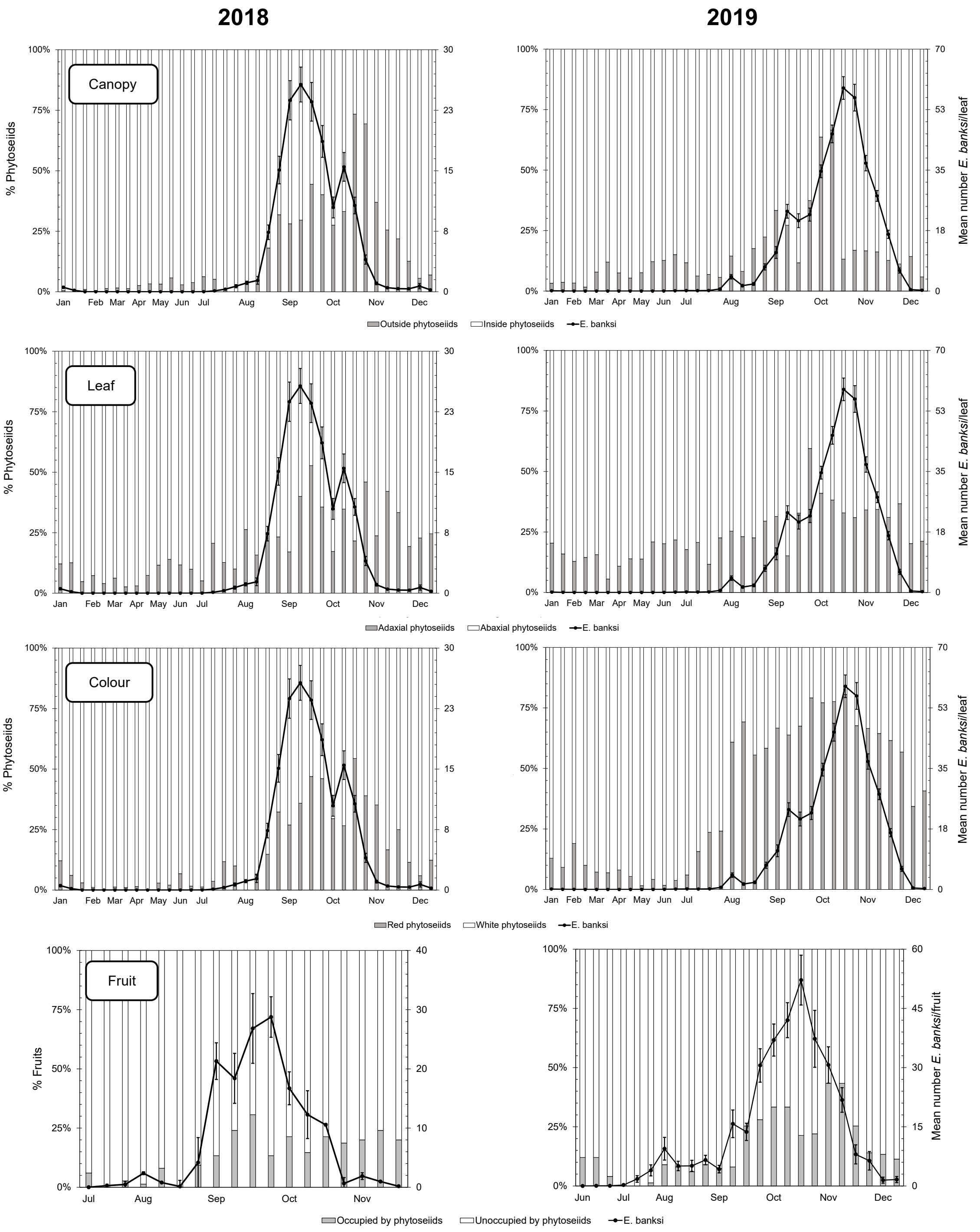


Figure 3.5 Variation in the spatial distribution and body coloration of phytoseiids in relation to the abundance of *E. banksi* in four (2018) and six (2019) citrus orchards. Percentage of phytoseiids in each position in the tree canopy (outside/inside), leaf (adaxial/abaxial), fruits (occupied/unoccupied by phytoseiids), and different body coloration (white/red) (primary y-axis), compared with the mean number of *E. banksi* per fruit or leaf (secondary, y-axis). Capped bars represent ± standard error (SE).

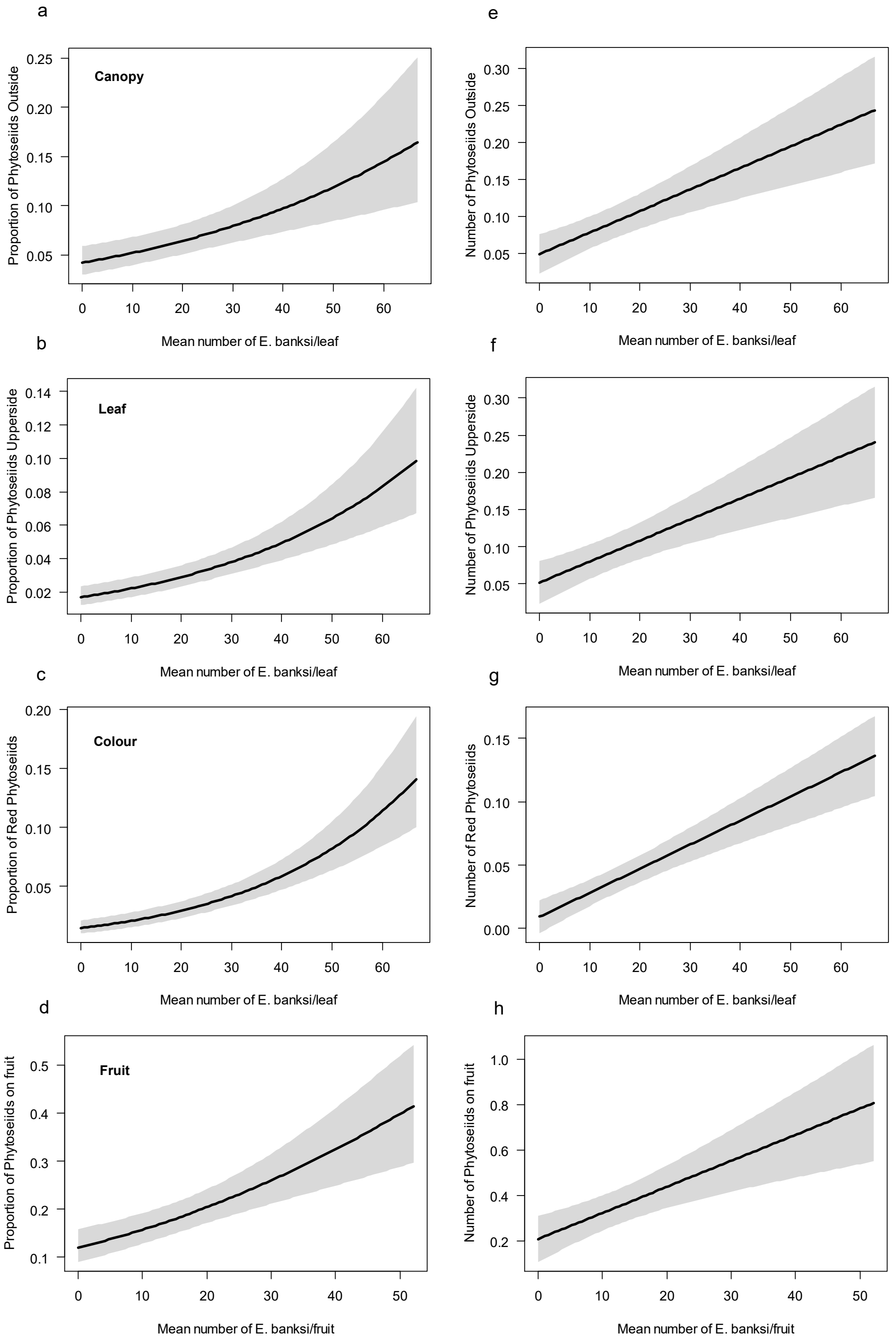


Figure 3.6 (a–d) Representation of the binomial (logit-link) generalized linear models (GLMs) showing the relationship between the proportion of leaves occupied by phytoseiids outside the canopy (canopy), on the adaxial side of the leaves (leaf), the occupation on leaves by red-coloured phytoseiids (colour), and the proportion of fruits occupied by phytoseiids (fruit) (y-axis), compared with the mean number of *E. banksi* per fruit or leaf (x-axis). In the graphs the prediction lines were plotted in black and the 95% confidence bands in grey. (e–h) Representation of the linear models (LM) showing the relationship between the mean number of phytoseiids per sample unit on the outside canopy, on the adaxial leaf side, red-coloured, and collected on fruits (y-axis), compared with the mean number *E. banksi* per leaf or fruit (x-axis). Prediction lines and confidence bands were plotted in the same way as on the GLMs.

Table 3.3 Results of the binomial (logit-link) generalized linear models (GLMs) on the occupancy by phytoseiids outside the canopy, on the adaxial side of the leaves, as well as the occupation on leaves by red-colour phytoseiids, and the occupancy by phytoseiids on fruits, depending on the mean number of *E. banksi* per sample unit.

	Coefficients	*Std Error*	*Resid. Dev*	*Pr (>Chi)*	*Pseudo R^2*
Biological Control GLMs					
a. Canopy (Outside)					
(Intercept)	-3.131	0.182	1417.3	-	-
Mean number of *E. banksi*	0.023	0.006	1004.5	0.0001063 ***	0.291
b. Leaf (Adaxial side)					
Intercept	-4.069	0.170	723.4	-	-
Mean number of *E. banksi*	0.028	0.005	400.8	1.348e-08 ***	0.446
c. Colour (Red phytoseiids)					
Intercept	-4.220	0.193	998.0	-	-
Mean number of *E. banksi*	0.036	0.005	408.3	1.720e-13 ***	0.591
d. Fruit					
Intercept	-1.994	0.166	444.4	-	-
Mean number of *E. banksi*	0.032	0.007	294.0	8.477e-06 ***	0.338

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 3.4 Results of the linear models (LM) on the mean number of phytoseiids per sample unit outside the canopy, on the adaxial leaf side, red-coloured, and collected on fruits, compared with the mean number of *E. banksi* per sample unit.

	Coefficients	*Std Error*	*Pr (>F)*	*Adjusted R^2*
Biological Control LMs				
a. Canopy (Outside)				
(Intercept)	-1.384	0.068	-	-
Mean number of *E. banksi*	0.198	0.062	0.00249 **	0.158
b. Leaf (Adaxial side)				
Intercept	-1.356	0.069	-	-
Mean number of *E. banksi*	0.190	0.064	0.00449 **	0.147
c. Colour (Red phytoseiids)				
Intercept	-1.934	0.063	-	-
Mean number of *E. banksi*	0.391	0.057	2.90e-08 ***	0.530
d. Fruit				
Intercept	-1.384	0.068	-	-
Mean number of *E. banksi*	0.198	0.062	0.00249 **	0.158

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

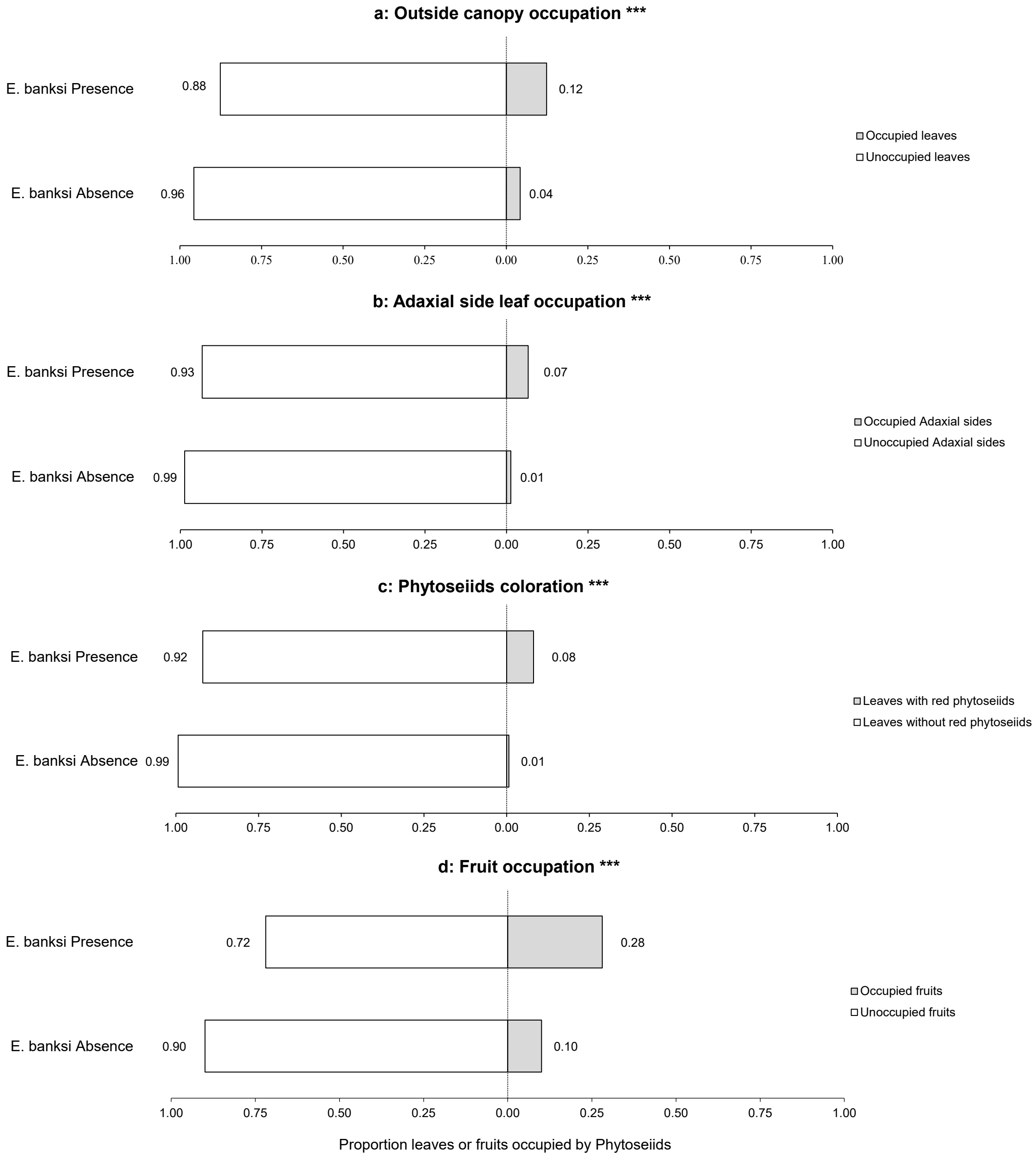


Figure 3.7 Proportion of leaves occupied (grey legend) or unoccupied (white legend) by phytoseiids outside the canopy (a), on adaxial side of leaves (b), red coloured phytoseiids on leaves (c), and phytoseiid on fruits (d), when *E. banksi* occurred or was absent. Significant differences are denoted with asterisks. Chi square contingency test: ***$P < 0.001$.

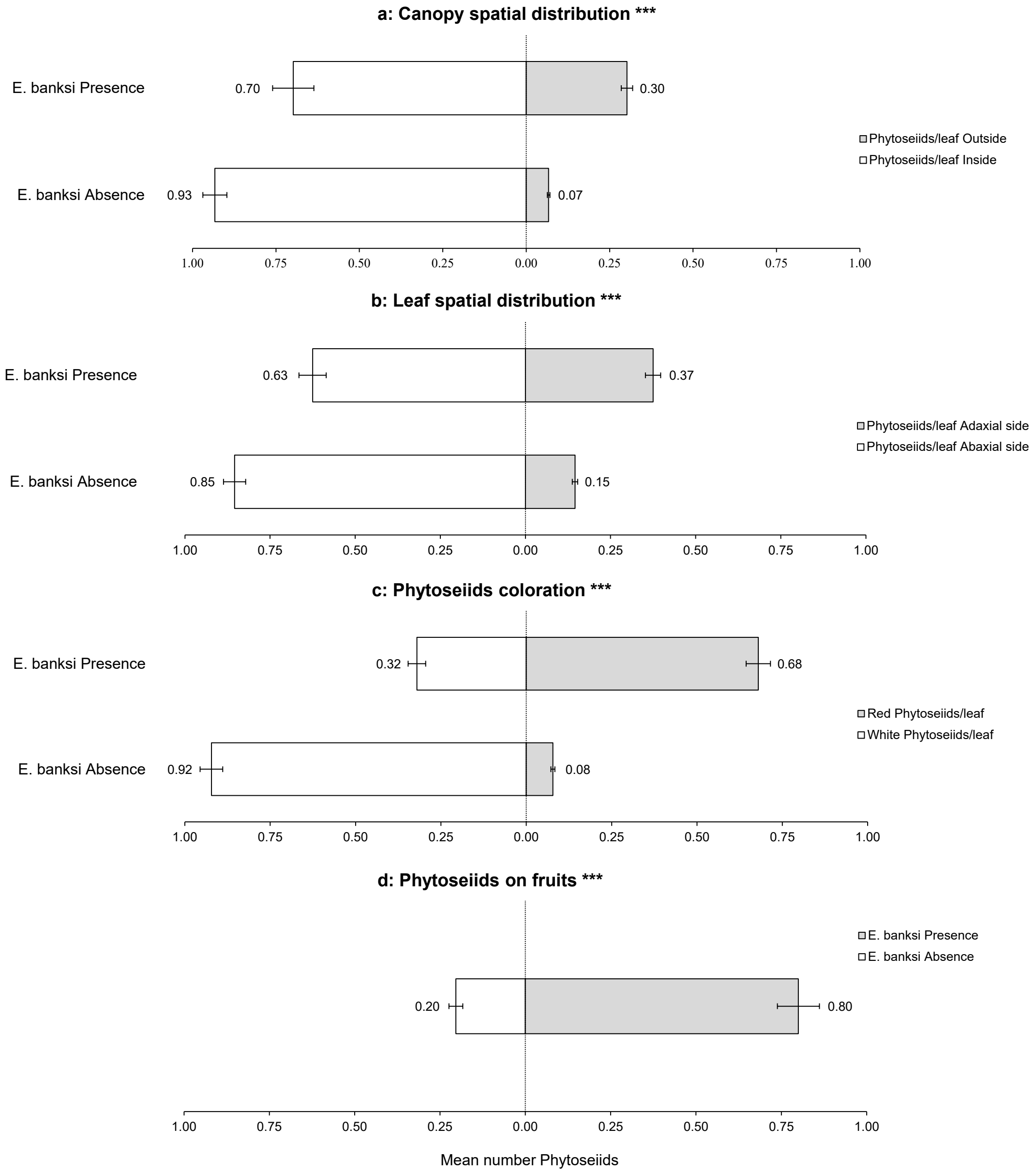


Figure 3.8 Phytoseiid proportion per leaf outside or inside the canopy (a), on the adaxial or abaxial side of the leaves (b), white or red coloured (c), and collected on fruits (d), when *E. banksi* occurred or was absent. Capped bars represent ± standard error (SE). Significant differences are denoted with asterisks. Chi square contingency test: ***$P < 0.001$.

3.4 Discussion

Whitin-tree distribution analysis

This is the first study that compares the population of *E. banksi* and phytoseiid mites on fruits and leaves in citrus groves. The Texas citrus mite showed a clear preference for leaves over fruits. On citrus crops from the western Mediterranean, a single flowering period occurs during April-May, after which fruit development begins (Agustí 2003). When *E. banksi* populations start to increase in the canopy in late summer-early autumn, the fruits are an available substrate for harbouring mites. Despite this, the population per unit area (cm^2) on leaves was much higher than that on fruits, where the mites are more difficult to detect in field observations and being visible only when their density on leaves is very high. This suggests that leaves are a better food substrate for its development, since the mites prefer to live on them rather than move to the fruits, despite being more densely clustered. Our assumptions agree with Childers (1992) and Rogers and Stansly (2017) who point out that this species feeds mainly on citrus leaves and is observed on fruits only at high levels of infestation (McCoy 1985), when the mites move from the leaves to the fruit (Rogers and Stansly 2017). The preference for leaves over fruit as a food substrate has been described for other spider mites in the eastern Mediterranean, such as *Eutetranychus orientalis* (Klein)*,* which causes more damage to leaves than to fruit (Bodenheimer 1951), and *Panonychus citri* (McGregor)*,* which showed in laboratory experiments that lemon leaves are more suitable than fruits for its development (Chiavegato 1988). There is little information about the presence and abundance of phytoseiids on citrus fruits. Usually, it is considered that the phytoseiids in the tree canopy live mainly on leaves, so the population of these is considered representative of the tree canopy (Muma 1964). Summer populations of phytoseiids on Spanish citrus trees are usually very low because of the high temperatures and low humidity, recovering partially in autumn (Ferragut et al. 1988). In our results, the average number of phytoseiids per fruit in autumn was higher than on leaves; however, if we take into account the surface area (cm^2), we can see that both substrates were used equally. We believe that this fact is related to the presence of *E. banksi* on the fruit as will be discussed later.

When we focus on the leaves, our results indicate that *E. banksi* and phytoseiids are not randomly distributed in the tree canopy. Microclimatic variations that occur at different sites in the canopy and leaves play an important role in the spatial distribution of phytophagous and predatory mites (Ferro et al. 1979). The Texas citrus mite clearly prefers to feed at the periphery of the canopy and on the adaxial (upper) side of leaves, where radiation and temperatures are higher, and relative humidity is lower. These same preferences have already been described in Texas citrus by Dean (1952) and Florida citrus by Muma et al. (1953), who point out that most of the mites were found on the adaxial side of the leaves, as well as on the sunniest sides of the canopy, so low pest densities only occur in the northern quadrants of the canopy (Dean 1959a). At the beginning of leaf infestation, females prefer to lay their eggs along the midrib. After hatching all developmental stages remain in the same position, and when the population grows, they move towards the leaf margins (Rogers and Stansly 2017), and to the abaxial (lower) side of leaves (Dean 1959b). Recently, Landeros et al. (2004), in Mexican citrus, also recorded a higher density of mites on the outside of the canopy and on the adaxial side of the leaves. A similar spatial preference has been reported for *E. orientalis* by Bodenheimer (1951), and Vela et al. (2017) in southern Spain. *Panonychus citri* also showed the same preferences as the two *Eutetranychus* species mentioned above (Ebeling 1959; García-Marí et al. 1983). In the orchards sampled, phytoseiids preferred the abaxial side of leaves located inside the canopy. These spatial preferences were already reported by García-Marí et al. (1983) in Spanish citrus, while recently important differences between the abundance of phytoseiids outside and inside of the canopy were found in sweet oranges in Florida (Villanueva and Childers 2005), and grapefruit in Mexico (Landeros et al. 2004). As phytoseiids are severely affected by high temperatures and low relative humidity (Ferragut et al. 1987) they shelter on the underside of the leaves inside the canopy, being more abundant in plantations and citrus varieties with dense imbricate canopies (McMurtry et al. 1970; Ferragut et al. 1988).

Finally, when we studied the mite distribution outside the canopy, we detected the preference of *E. banksi* for leaves coming from current year's shoots as opposed to leaves from the previous year. We consider that different factors could be playing a role in this preference. Leaves of the current year are always located in the outermost part of the canopy, where more favorable microclimate conditions for the establishment of the spider mites occur (Ferro et al. 1979). Normally, orchards with high infestations show the presence of this mite every year with greater or lesser intensity, so previous year’s leaves are often damaged by mite feeding, and the level of injury influences the mite density, reducing egg laying (Henderson and Holloway 1942). On the other hand, changes associated with aging may be conditioning leaf mite preferences. On citrus, leaves remain on trees for 1–2 years during which there are constant changes in their structure and nutritional composition (Scott et al. 1948). A higher population of *Panonychus ulmi* (Koch) was observed on leaves with a thicker palisade mesophyll, as well as a higher nitrogen content (Post 1962). The number of eggs laid by *P. citri* on young and middle-aged citrus leaves is greater than on old leaves (Henderson and Holloway 1942). Furthermore, a higher speed of development and survival of immature stages has also been recorded (Garcia-Marí et al. 1992). Finally, newly developed leaves on the outermost part of the canopy accumulate greater dust deposition. The Texas citrus mite does not produce a web, and the development of non-producing web spider mites is favoured by the presence of dust or inert debris on the leaf surface (Holloway et al. 1942). In agreement with this, Dean (1959b), in Texas grapefruit crops, observed *E. banksi* populations growing earlier in trees along a dusty road before a general increase was observed in the rest of the grove. Contrary to *E. banksi,* phytoseiids prefer the leaves of the previous year instead of the current year leaves. Phytoseiids spend most of their lifetime inside the canopy; however, they are moving continuously while foraging for prey or other foods (Sabelis 1985). The foraging behaviour may depend not only upon prey availability, but also on abiotic factors such as relative humidity, temperature, and light intensity, being aggregated on the old leaves during day and moving to the periphery of the canopy during the night searching for spider mites and showing a random aggregation pattern on leaves (García-Marí et al. 1985; Villanueva and Childers 2005). Thus, when they must move outside the canopy to forage, they prefer leaves of the previous year, located at mid-level in the canopy, and less exposed to unfavourable climatic conditions.

Seasonal trends of E. banksi and phytoseiid mites

Our results indicate that different citrus varieties are susceptible to the development of *E. banksi* populations. Varietal structural features have been associated with less or more spider mite proliferation (van de Vrie et al. 1972). Different studies have reported a preference of *E. orientalis* for several citrus species or varieties, in which it has a higher biotic potential (Rasmy 1977; Aljboori and AlDahwi 2020; Yalçin et al. 2022), being able to develop abundant populations (Bodenheimer 1951; Halawa et al. 2020). Dean (1980) found Texas citrus mite preferences among sweet orange varieties in Texas citrus, while Monzó et al. (2016) point to the existence of a preference for early Navel group sweet orange varieties, and the same preference was recorded for *P. citri* in the Valencian region (García-Marí and del Rivero 1981). In our study, clementine and Navel group early varieties showed abundant populations (data not shown); however, we do not consider this to be evidence of preference, as many other factors such as nutrition or the environmental conditions of each locality may play an important role in the development of spider mite populations (van de Vrie et al. 1972).

The Texas citrus mite was detected on fully developed leaves of the current year's spring and summer shoots in mid-summer during July-August, and the main population peak was registered in late summer-early autumn, during September-October. Once autumn leaves are available, they move to occupy them, and this new, better quality, food substrate could have an impact on increasing autumn populations and even in developing secondary autumn population peaks, as we observed in 2018. Laboratory studies on citrus leaves has shown that *P. citri* lays significantly more eggs on leaves from autumn than spring flushes (Beitia-Crespo and Garrido-Vivas 1991). This migration from old to new leaves is consistent with behaviour observed in other spider mite species (Gotoh 1987), and probably related to changes in hardness, dryness, wax deposition, and nutrients that occur in leaves with age, making it difficult for spider mites to feed (van de Vrie et al. 1972).

The Texas citrus mite was undetectable during the winter of 2018-2019, however, small populations composed by all developmental stages were observed in January 2018. In January, February, and March the average of mean temperatures registered were 12.67, 10.01 and 14.26 °C in 2018, and 11.41, 12.20 and 14.21 °C in 2019, respectively. These temperatures are below 15 °C, which is the lowest temperature reported for *E. banksi* in laboratory experiments on Marsh grapefruit leaves (Childers et al. 1991). After the population peak in autumn, falling temperatures cause a progressive decline in populations, with development practically stopped in the winter months and early spring. In this way, and depending on the population density reached in the autumn peak, during winter the mites are very scarce, even being undetectable. In April, the average temperatures recover, with values of 16.02 and 15.18 °C in 2018 and 2019 respectively. At these temperatures, the development of *E. banksi* probably restarts, producing a progressive population growth that, in orchards with high density levels the previous autumn or with some physiological disorders, could lead to population peaks at the end of spring and beginning of summer (unpublished data). Winter populations were also reported for *P. citri* in Spanish citrus (Ferragut et al. 1988), because climatic conditions, with warm summers and mild winters, permit the maintenance of spider mite populations all year round. In other locations with warmer temperatures, such as Texas, Dean (1959b) observed *E. banksi* populations throughout the year, but in years with extremely cold winter temperatures he was unable to find any mites on the leaves (Dean 1952).

In July, August, and September the population peak coincided with the highest monthly average temperatures, which reached values of 26.60 and 26.72 °C in July, 26.55 and 26.23 °C in August, and 23.55 and 23.54 °C in September, in 2018 and 2019, respectively. During these summer months, the maximum daily temperature was under 28 °C only 14 days in 2018 and 2019. Furthermore, the daily maximum temperature was above 31°C 36 days in July–August 2018 and 30 days in the same period in 2019, while that temperature was only surpassed one day in September 2018, and three days in September 2019. Our observations agree with the life history laboratory work carried out by Childers et al. (1991) on grapefruit leaves, which indicates that the optimum temperature range for *E. banksi* is 28–30 °C, as well as those of Badii et al. (2003) on sweet orange leaves, that found 28–31 °C as the optimal temperatures. Both authors conclude that temperatures above 30 or 32.5 °C had a negative effect on the development rate, survival, and fecundity of the mite. In our orchards, these temperatures are only reached during some days in July and August, which could contribute to the slowing down of population growth during these months, while September presents the optimal temperature conditions that cause the typical population peak.

In addition to temperature, relative humidity (RH) and precipitation are climatic factors that could affect the development of spider mite populations (van de Vrie et al. 1972). Badii et al. (2003) indicate that the optimum relative humidity for *E. banksi* development is 61%, and a relative humidity of 74% would lead to a delay in the developmental time of males and females. In Valencia, the lowest average relative humidity was recorded during the summer months with values between 63-70% in 2018 and 2019. These values could have contributed to a rapid growth of the populations. The autumn of both years was different, while 2018 was wetter with average RH of 77.71, 72.06 and 70.83% for September, October, and November respectively, 2019 was drier, with values of 73.32, 69.33 and 56.96% for these months. In addition, the autumn of 2018 was very rainy with a cumulative rainfall of 204.3, 320.9 and 312.10 mm for September, October and November, respectively (values obtained from all the weather stations), while 2019 showed 409.3, 180.1, and 27.9 mm for the same months (data not shown). High RH and rainfall in September 2018 may have reduced the mite abundance and accelerated its decline, and the lower values during late autumn in 2019 could have contributed to a slower decline of the abundance in these months. In California, with dry summers typical under Mediterranean climate conditions, *E. banksi* shows unimodal population dynamics with a population peak at the end of summer-beginning of autumn as we have described in our latitudes. On the contrary, Florida has two peaks; infestations may occur at any time of the year but the maximum in abundance occurs in late spring and early summer (Anonymous 2022). After that, populations decline sharply during the summer due to heavy rainfall and high humidity and show a second peak of lower abundance in late autumn and winter (Muma et al. 1953; Childers 1992; Rogers and Stansly 2017). In Texas it is also possible to find *E. banksi* all year round with the same population trends. Low relative humidity and rainfall, and temperatures above 21.2 °C favour the development of the main population peak, and the decline in summer is related to excessive heat. Subsequently, heavy rains in early autumn also contribute to reducing mite abundance, and cold winter temperatures keep populations low or even undetectable. High rainfall during spring contributes to population decline (Dean 1952, 1959b, 1980). In Mexico, the Texas citrus mite could also be collected all year round and showed the same bimodal dynamics where low winter temperatures as well as spring and autumn rainfall contributed to reduced mite density (Landeros et al. 2004). The population peak shown by *E. banksi* in our Mediterranean conditions coincides in time with the population outbreaks of *E. orientalis* (Ledesma et al. 2011; Vela et al. 2017) and *P. citri* (Ferragut et al. 1988). This contributes to the existence of interspecific competition in which *E. banksi* behaves as a superior competitor displacing the other two species geographically (López-Olmos and Ferragut, published).

Euseius stipulatus was the most abundant phytoseiid, representing 90.75% of the total number of phytoseiids collected, as well as the most frequent, found in 88% of the samplings carried out. For this reason, although the total number of collected phytoseiids is represented in our graphs, the remaining discussion will be focused on *E. stipulatus*. This species presented a seasonal dynamic with two population peaks (spring and autumn), the first and most abundant being in May–June. *Euseius* species were classified as a primary pollen feeder and generalist predators whose density can be correlated with the availability of wind-borne pollen settling on the foliage (McMurtry and Croft 1997;

McMurtry et al. 2013), so the spring peak could be related to the availability of this resource, not only from the crop, but also from surrounding plants or even ground cover plants which is transported by the wind (Warburg et al. 2018). Moreover, the spring mild temperature regime under a Mediterranean climate can stimulate the reproduction of this species (Warburg et al. 2019), since its highest survival and reproduction can be reached at 18 °C (Ferragut et al. 1987). A strong population decline occurs during summer, reaching minimum densities in July and August, when maximum daily temperatures exceed 30 °C and RH drops below 40%. However, decreases in relative humidity by themselves can cause population declines of phytoseiids as happened in the spring of 2018. Our results agree with those obtained by Ferragut et al. (1988) in Spanish citrus, where mean temperatures above 30–35 °C and relative humidity below 40% produced drastic population reductions. The summer collapse of *E. stipulatus* in Mediterranean citrus has also been described by other authors; in Italy, mite abundance collapses after four hours with temperatures above 40 °C (Ragusa 1986), and in Tunisia, temperatures above 30–35 °C reduced populations (Sahraoui et al. 2014), while citrus seedlings in semi-field trials in France showed a reduction in mite numbers after 40 hours below 30% relative humidity (Warburg et al. 2019). Furthermore, laboratory trials show that at a constant temperature of 32 °C, fertilised females are unable to lay eggs and die within 5–15 days, while relative humidity below 50% impedes egg hatching (Ferragut et al. 1987). In September, when daily maximum temperatures remain below 30 °C and RH above 40%, populations recover leading to an autumn peak of lower abundance. This second peak has been related to the availability of tetranychids as a food source, contributing to their reduction in populations (Ferragut et al. 1988; Vela et al. 2017); however, we have seen that with a shortage of or even in absence of pest populations, this second peak also occurs, so it could be related to the presence of pollen from plant species that flower during this season or to the availability of other food resources. Populations were maintained at low values throughout the winter. During autumn and even in winter this polyphagous species can feed on other prey sources. We have observed *E. stipulatus* females feeding on Psocoptera larvae that are relatively abundant on the underside of leaves inside the canopy, where the phytoseiids remain (personal observation), and on whitefly eggs and honeydew, as well as mealybug larvae. The latest foods were tested in laboratory trials demonstrating that they allow survival but not reproduction of the mite (Ferragut et al. 1987). Furthermore, these food sources are often available simultaneously on citrus trees enhancing its biotic potential. Aphid or whitefly honeydew supplementation, together with different spider mite species, have been successful in reducing development time and increasing oviposition in phytoseiids (Zhimo and McMurtry 1990). A recent study has shown how *E. stipulatus*, like other *Euseius* species, is a zoophytophagous predator able to feed on plant fluids from the citrus leaves, which undoubtedly contributes to the maintenance of overwintering populations (Cruz-Miralles et al. 2021).

This is the first time that a detailed study of the seasonal dynamics of *E. banksi* and phytoseiids on fruits has been carried out. We have observed that both mites increase simultaneously on fruits and leaves. Fruits are available from the beginning of summer, so when *E. banksi* starts growing, fruits and leaves are available as food substrate; on the contrary, phytoseiids can only inhabit leaves in the spring peak and is its minor autumn peak that occupies both substrates at the same time. As we mentioned above, fruits are used as substrate by *E. banksi* with values comparable to those of leaves per unit sample but not per area, so they are difficult to detect on fruit. In contrast, the average number of phytoseiids per fruit is higher than leaves, but the surface area of both substrates are used equally. The Texas citrus mite remains on the external side of the fruit, which receives the direct solar light (García et al. 2003), while phytoseiids use the region of the primitive calyx of the flower (Gurra et al. 1997) or the navel of sweet orange varieties for shelter, laying their eggs in their refuges or even on the fruit surface. In the same way as *E. banksi*, phytoseiids are difficult to detect in field fruit observations, so economic thresholds against mite pests use leaf sampling plans for estimating the phytophagous and predatory mite densities (Garcia-Marí et al. 1994).

Seasonal variation of phytoseiid species on the trees

In addition to *E. stipulatus*, other phytoseiid species such as *T. phialatus* and *N. californicus* were relatively abundant, while the remaining species can be considered scarce. These data agree with those published in previous studies on phytoseiid fauna on citrus in Spain (Abad-Moyano et al. 2009a; Ferragut et al. 1988; Ledesma et al. 2011). We found that *T. phialatus* and *N. californicus* are present throughout the year with a population peak in autumn and that they are more abundant in periods with low densities of *E. stipulatus*. The seasonal trends of *T. phialatus* and *N. californicus* can be explained by biotic and abiotic factors. Among the abiotic factors, high temperature and low RH also have been shown to have a negative effect (Ferragut et al. 1987; Gotoh et al. 2004; Walzer et al. 2007), leading to a decline during summer under Mediterranean climate conditions (Ragusa 1986). However, during winter and spring, temperatures and RH are suitable for the development of these species, and their populations are very low. Among the biotic factors, intraguild interactions such as intraguild predation may play an important role, as *E. stipulatus* show high populations during these months, and has been defined as a strong intraguild predator on other phytoseiids on citrus (Abad-Moyano et al. 2010). The summer decline of phytoseiids sets the counter to 0 for all species, and with the start of autumn they begin to increase in number, competing for space, food, and shelter. In this competition, the greater capacity of *E. stipulatus* to act as a highly competitive and intraguild predator progressively reduces the population of the remaining phytoseiid species during autumn, becoming the predominant species again in winter. In this fight, *E. stipulatus* seems to displace *T. phialatus* more easily than *N. californicus*. The ability of *N. californicus* to delay the impact of *E. stipulatus* may be related to the biology of that species. We have found individuals of *N. californicus* only on clementine citrus orchards where *Tetranychus urticae* Koch is a key pest. This phytoseiid is a Type II lifestyle selective predator of spider mites associated with dense web producing species such as *T. urticae* (McMurtry et al. 2013), and shows adaptations for living inside heavy webbing spider mite colonies, walking on the web, and cutting strands of webbing with the chelicerae (Shimoda et al. 2009). On the other hand, polyphagous phytoseiids, like *E. stipulatus,* do not do well on mite prey of this type, often getting stuck in their webbing. The inability of *E. stipulatus* to enter inside the *T. urticae* web, where *N. californicus* lives, most likely reduces intraguild predation and delays the displacement of *N. californicus* to autumn.

Implications for the biological control of E. banksi

We have found behavioural changes in phytoseiids when *E. banksi* increases, which is indicative of the existence of a predator-prey relationship. Phytoseiids move to the adaxial side of the leaves on the periphery of the canopy, where *E. banksi* density is higher, to feed on it. In addition, the increased population of *E. banksi* on the surface of the fruit also stimulates the movement of the predators, which in the absence of its prey do not usually inhabit the fruit. Within-plant daily migrations seem to be a common behaviour in phytoseiids. To avoid adverse climatic conditions during the day, such as high temperatures, low relative humidity, rain, or high ultraviolet radiation, phytoseiids remain sheltered and move during the night to forage (Onzo et al. 2003, 2010). Daily migrations on citrus phytoseiids have been described for *E. stipulatus* on sweet oranges

(García-Marí et al. 1985). According to these authors, during sunlight hours, when temperature and humidity conditions are more adverse, the phytoseiids are mainly found sheltering on the underside of the leaves inside the canopy, with an aggregate distribution. At dusk, when the sun disappears, the humidity and temperature conditions are more favourable and encourages the phytoseiids to move to the upper side of the leaves on the periphery of the canopy where the populations of spider mites are abundant, showing a random distribution pattern typical of a predator searching for prey. In addition, seasonal changes in the predatory behaviour of *E. stipulatus* have been observed in laboratory studies, with increased attacks on prey during the summer months and reduced attacks during the winter. However, no daily differences were observed in this species, while *Phytoseiulus persimilis* (Athias-Henriot) was shown to be a diurnal predator regardless of the season, with maximum activity at midday in winter and in the evening in summer, and *N. californicus* was shown to be a nocturnal predator in summer and a diurnal predator in winter (Pérez-Sayas et al. 2018). Our surveys were carried out all year-round during sunlight hours, and therefore we cannot corroborate these hypotheses. However, despite the adverse weather conditions for phytoseiids during the day-light hours, we were able to detect a change in their presence and abundance inside/outside of the canopy, as well as on the upper/under side of the leaves in response to the presence and growth of *E. banksi,* which supports their contribution to the biological control of this spider mite.

Moreover, we have tested statistically how the presence of a spider mite in fruits is able to stimulate the movement of its predatory phytoseiid towards the fruit. The presence of *E. banksi* in the fruit significantly encourages the movement of phytoseiids to this substrate, which they do not occupy in its absence, since with the arrival of winter and the decrease in *E. banksi* abundance, although the population of phytoseiids in leaves remains relatively high, there is a decrease in abundance and occupancy on fruits. Finally, we have statistically demonstrated that the change in the colour of phytoseiids provides evidence of the use of *E. banksi* as prey and an indirect estimation of the biological control. However, we must be careful when relating the color of phytoseiids to prey consumption, as we do not know, in detail, how long the coloring remains, and this could lead to over- or underestimates of prey consumption.

There is little information available on field efficacy of phytoseiid predators on *E. banksi* in its native geographical area. In Texas citrus, the most widely distributed predatory mite, capable of reducing populations of *E. banksi,* was identified as *Euseius* *finlandicus* (Oudemans) (Dean 1952), while *E. mesembrinus* (Dean) increased during May and was abundant in summer and autumn (Dean 1959b). In Mexico, *E. mesembrinus,* and its presumed prey *E. banksi*, showed spatio-temporal synchronisation, as well as a high correlation, allowing to maintain *E. banksi* populations under control most of the year (Landeros et al. 2004). In Florida, *E. banksi* is the prevalent mite pest species on citrus, where the rich phytoseiid complex keeps its populations under control most of the year. *Euseius mesembrinu*s, *Iphiseiodes quadripilis* (Banks) and *Galendromus helveolus* (Chant) are common species in Florida (Villanueva and Childers 2005) tested successfully under laboratory conditions with *E. banksi* as a food resource (Abou-Setta and Childers 1989; Caceres and Childers 1991; Villanueva and Childers 2007). In Spain, *E. stipulatus* is capable of performing a good biological control on *P. citri* (Ferragut et al. 1988); however, it is unable to reproduce preying *T. urticae* on sweet orange leaves (Ferragut et al. 1987), and even not complete its development on clementine leaves (Abad-Moyano et al. 2009b). It has been hypothesized that predators that attack *P. citri* may also be effective against *Eutetranychus* spp. because all these species, have similar colonization patterns on the leaves and produce small amounts of webbing (McMurtry 1985). The behavioural change observed in phytoseiids clearly indicates that they are playing a role in the biological control of *E. banksi.* However, we should wonder whether this biological control is effective or not. *Eutetranychus banksi* increased exponentially from mid-summer, growing in parallel with phytoseiid populations during late summer-early autumn. Despite this synchrony between predator and prey, the differences in their abundance values were very high, since *E. banksi* had a density 60–100 times higher than its predator. The presence of high levels of the pest encourages phytoseiids to move in to feed on this abundant food source. However, this prey is not a suitable food source for *E. stipulatus*, delaying its development, and strongly reducing its survival rate and fecundity in laboratory studies (López-Olmos and Ferragut, in preparation). Eventually, our field observations confirm that *E. stipulatus* is not able to perform an efficient biological control on *E. banksi* and the impact of predation is not enough to keep populations at tolerable economic levels for farmers.

3.5 Acknowledgements

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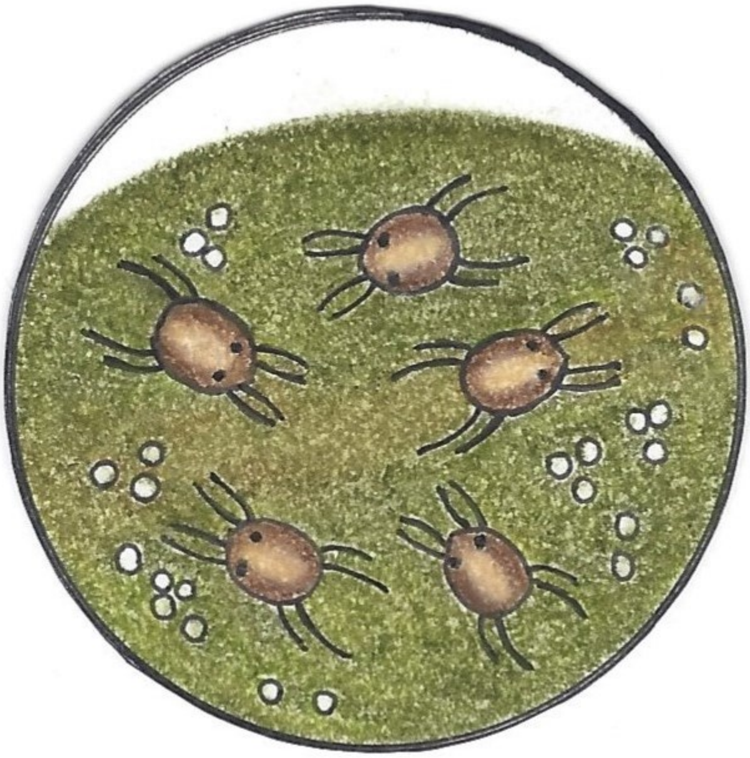
CHAPTER 4

Population structure, spatial distribution, and sampling Plan for the Texas citrus mite, *Eutetranychus banksi* (Acari: Tetranychidae) on citrus



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Abstract: *Eutetranychus banksi* (McGregor) (Acari: Tetranychidae) is an invasive spider mite native to the Americas. In 2013 it invaded the main citrus-growing area in Spain producing significant damage and requiring chemical treatments. This article examines its population structure, spatial distribution and presents a sampling plan to develop an integrated pest management (IPM) strategy. Four citrus orchards were periodically surveyed during 2018 in eastern Spain, sampling both sides of leaves from different flushes on the outer and inner part of the tree canopy. The fruits were also monitored. Our results showed that there were differences in the population structure on fruits and leaves, as well as between leaves from different flushes. Furthermore, over time, there were fluctuations in its composition correlated with variations in sex-ratio. There were no differences in the aggregation pattern at the different plant strata sampled; however, immature stages showed a higher aggregation than adults, with females being the sex with the lowest aggregation. There was a highly significant linear relationship between *E. banksi* motile forms and adult females with the total population, thus both were used as reference stages to develop sampling plans for the species. This study recommends a binomial sampling of 100 leaves for female monitoring, sampling 2 leaves per tree on 25 trees per transect regularly spaced along two diagonal transects, the first oriented north-east to south-west and the second north-west to south-east. To be more accurate, it is possible surveying the presence/absence of motile forms. In this case, 4 leaves per tree on 50 trees per transect should be monitored.

4.1 Introduction

The introduction of invasive pests in agriculture is an increasing problem, leading to economic losses for farmers and food producers (Pimentel et al. 2000). Phytophagous mites have high potential as an invasive species because their small size (from 0.2 to 0.5 mm in length) makes them difficult to detect and they can be easily distributed in world trade (Navia et al. 2010). Among them, spider mites, belonging to the family Tetranychidae, cause significant damage in many crops worldwide, being the most economically important phytophagous mites on citrus (Gerson 2003; Jeppson et al. 1975; Vacante 2010). The Texas citrus mite, *Eutetranychus banksi* (McGregor) (Acari: Tetranychidae), is native to the Americas, reported as a major pest on citrus from southern United States to northern Argentina (Gerson 2003; Migeon and Dorkeld 2022). Far from its native region, this species was found in 1999 in the Algarve region of southern Portugal (Carvalho et al. 1999), and in 2001 it reached the citrus producing area in southern Spain (García et al. 2003) and remained, for about a decade, in a small geographical area. In 2013, *E. banksi* was suddenly detected in citrus orchards from the Valencian region, more than 800 km away from the location of its first detection, where it most likely arrived through the trade of fruit or plant material (Ferragut 2016). The mite spread rapidly in the region affecting the main citrus-growing area in Spain. *Eutetranychus banksi* is one of the most polyphagous spider mites, reported on 110 hosts belonging to 34 plant families, mostly Fabaceae (22 species) and Rutaceae (9 species), infecting other crops and many ornamental plants (Migeon and Dorkeld 2022). On citrus, the mite feeds mainly on leaves consuming the contents of mesophyll plant cells, reducing the leaf photosynthetic capacity and promoting defoliation, which results in a decrease in yield. Moreover, it also feeds on fruits producing aesthetic damage, resulting in a lack in pigmentation, which may reduce their economic value (García et al. 2003; Monzó et al. 2016).

Different chemicals are commonly used to maintain mite population at low levels on citrus. The Texas citrus mite has been shown to be sensitive to various acaricides, being easy to keep under control with this strategy (Monzó et al. 2016). However, under the perspective of Integrated Pest Management (IPM) programmes, a better understanding of the biology and ecology of the target pest is required, as well as efficient sampling techniques and management action thresholds (Binns and Nyrop 1992). In addition to *E. banksi*, three other spider mite species currently occur in the Spanish citrus crop area, the two-spotted spider mite *Tetranychus urticae* Koch, the citrus red mite *Panonychus citri* (McGregor), and the Oriental red mite *Eutetranychus orientalis* (Klein). *Tetranychus urticae* is native to the Mediterranean basin and has been described as a cosmopolitan species of minor pest importance on citrus (Gerson 2003); however, it is the main pest of clementine mandarins, *Citrus reticulata* Blanco, on the eastern coast of Spain (Aucejo-Romero et al. 2004). On the other hand, *P. citri* is an Asian species considered a major important cosmopolitan pest species (Gerson 2003), which has become a key pest of citrus fruit since its arrival on the eastern citrus growing area of Spain (García-Marí et al. 1983). Sampling plans have been developed for both mite species (Garcia-Marí et al. 1992, 1994; Jones and Parrella 1984a, 1984b; Martínez-Ferrer et al. 2006); however, none have been developed for any *Eutetranychus* species, and, only in Florida, a set of sampling and treatment threshold guidelines developed empirically for *E. banksi* are provided to farmers (Rogers and Stansly 2017). Since its arrival to the Valencian region, *E. banksi* has spread rapidly through citrus-growing areas, becoming the most frequent spider mite, geographically displacing other species with which it competes. (Lopez-Olmos and Ferragut, published). For all these reasons, our research aims to (i) study the population age structure on leaves and fruits and its variation over time, (ii) calculate the aggregation patterns for different developmental stages and plant strata, (iii) and finally, define a sampling methodology for *E. banksi* on citrus as a first step towards the development of an IPM programme for this species.

4.2 Materials and Methods

Sampling groves

The study was conducted in four citrus groves in 2018. All of them were commercial citrus plantations situated in an extensive citrus monoculture region in the Valencian Community (Spain). Groves were selected due to the presence of evident symptoms of damage produced by *E. banksi* on the leaves from the previous year. Furthermore, the selection was carried out aiming to include different groups of citrus varieties and different environmental conditions across the geographical area of distribution of the species, to obtain robust data for the development of the sampling plan (Naranjo and Hutchinson 1997). Two sampling areas were selected according to their climatic conditions, Picassent in the centre of Valencia province with a cooler and drier climate and Pego in the north of Alicante province characterized by a warm and humid climate. The orchards were of sweet orange, *Citrus sinensis* (L.) Osbeck [including the varieties Valencia-late (one grove), and Navelina (one grove)], clementine mandarin, *Citrus reticulata* Blanco [including the variety Oronules (one grove)], and the hybrid Ortanique [*C. sinensis* x *C. reticulata* (one grove)]. All the orchards ranged from 0.2 to 2 ha in land area and had normally developed 11- to 15-year-old trees in full production drip irrigated. The trees had not been sprayed with pesticides for at least 6 months before sampling.

Sampling procedure

In each grove, approximately 10% of the trees were marked and randomly sampled regularly. The marked trees were not sprayed with pesticides during the sampling period. Sampling was conducted weekly during the period of peak pest abundance (July-November), and fortnightly during the rest of the year. With the aim of detecting differences in aggregation at various positions and leaf types in the trees, at each sampling date: 25 inner leaves, 50 outers from the previous year, and 25 outers belonging to the current year from each citrus sprouting (spring, summer, and autumn), were randomly collected. In addition, and depending on their availability, 25 fruits per orchard were randomly collected from the outer part of the canopy. All the leaves of the same position and sprouting were combined in a plastic bag and transported to the laboratory inside a portable cooler. Fruits from the same orchard were transported in plastic containers. Within the next 24 h, leaves and fruits were examined under the stereomicroscope. All the development stages of *E. banksi* were counted on the leaves and fruits. Larvae, nymphs, and immature quiescent stages were counted together, and adults were separated by sex. In this way, mites were grouped into four categories: Eggs, Nymphs, Females and Males. Furthermore, in the case of leaves, their position on the adaxial (upper) or abaxial (lower) side of the leaf was recorded. The total number of sampling units counted was 15,196 leaves and 1,800 fruits, making a total of 213 samples: 136 integrated by leaves and 77 by fruits. For the population structure studies each leaf type was considered independently, while for the analysis of spatial distribution and sampling plan, leaves developed in the previous year were considered together as *"Old leaves"*, and leaves developed in the current year were grouped as *"New leaves"*. Fruit and leaf samples with no mites were not considered. On leaf-side analyses, all leaf types were pooled together.

Population structure and sex ratio

The study of the population age structure (relative proportion of individuals in different age classes) and sex-ratio were carried out on leaves and fruits (sample units), as well as on each leaf type. For this purpose, the proportion of each developmental stage, and the sex-ratio value (females/ [males + females]) were calculated based on the total number of mites collected on each substrate. In the case of leaves, upper (adaxial) and lower (abaxial) sides were analysed independently. All the statistical analyses in this study were carried out in R-environment (R Core Team 2019). Chi-square test was used to assess differences in the proportion of developmental stages (Gardener 2012). Population structure and sex-ratio were also analysed through time on fruits and leaves, by plotting fortnightly the percentage of each stage of development and sex-ratio per sampling unit over time. Linear models (LMs) were used to perform ANOVA analyses to assess statistical differences between developmental stages and sex-ratio on fruits and leaves (Zuur et al. 2009). Normality assumption was tested with Geary's test for normality (Geary 1935) using the R-package *normtest* (Gavrilov and Pusev 2014), while the homogeneity of variances was assessed with the Breusch-Pagan test (Breusch and Pagan 1979) computed with the R-package *car* (Fox and Weisberg 2019). Finally, LMs were also used to compute linear regressions to test the similarity of trends between the age structure and the sex-ratio or mite density on leaves and fruits. Normality and homogeneity of variances assumption was tested in the same way as above, and goodness-of-fit of the models were evaluated using r^2 values.

Dispersion pattern

The spatial aggregation was analysed using Taylor's power law that relates the mean density to variance by equation: $s^2 = am^b$ (Taylor 1961). After *log$_e$-log$_e$* transformation ($\ln s^2 = \ln a + b \ln m$), linear models (LM) (Zuur et al. 2009) were used to estimate Taylor's parameters a and b, as well as to compute a t- test for a slope b ($d.f. = n$-2, $\alpha = 0.05$) assessing significant differences from unit. The goodness-of-fit of the LMs, normality and homogeneity of variances assumption was tested in the same way as above. When the variance of the residuals was mis-specified, a *sandwich* estimator for the variances of the coefficients was used to correct the standard errors used in the Wald test, obtaining consistent standard errors even though the variance was not constant (Zeileis 2004). In Taylor equation s^2 is the variance; m is the sample mean; meanwhile a

and *b* are the Taylor coefficients. The first parameter *a* is a scaling factor related to sample size, and *b* is the Taylor's index of aggregation which is an intrinsic property of the species. According to the value of *b*, populations are classified as regular ($b < 1$), random ($b = 1$), or aggregated ($b > 1$) (Taylor 1984). When no mites were found in a sample, it was not considered for the analysis. LMs were used to perform an analysis of covariance (ANCOVA) to determine whether the aggregation pattern (slope *b*) differ between developmental stages, plant strata, seasons, and groves (Cayuela and de la Cruz 2022). Furthermore, a 95% confidence interval based on the *t*-distribution was also used to test for significant differences based on interval overlap.

Determination of a representative developmental stage

We used motile forms or only females as a representative stage of the total population, since the eggs are difficult to detect in field observations, and using females simplify the sampling since they are easy to detect, and their lower abundance makes counting less time consuming (Jones and Parrella 1984a). This approach must be preceded by verification that the chosen developmental stage is representative of the total population (Gonzalez-Zamora et al. 1993). To test this hypothesis, an LM analysis was first used to evaluate the linear relationship between the mean number of mites in the total population and the mean number of motile stages per leaf and sample. Subsequently, the same methodology was used to study the relationship between total population and females (Jones and Parrella 1984b). A $\log_e$+1 transformation was applied to the total population (y-axis) and the presumed reference stage (x-axis) to stabilise the variance. No intercept was fitted in the models (regression line was forced through the origin), because if the total population sample is 0, then the number of females and motile stages will also be 0. Normality and homogeneity of variance assumptions were tested as above.

Enumerative and binomial sampling

Taylor's power law coefficients were further used to determine the minimum sample size necessary for estimating population density for both enumerative and binomial sampling.

Enumerative sampling. Minimum required sample size was obtained using Green's formula, which establishes sampling precision based on the standard error ($s/\sqrt{n}$) as a prefixed proportion (*D*) of the known sample mean (*m*). However, the mean and variance are not fixed, and change over time. Therefore, the variance is expressed as a function of the mean using the relationship between the mean and variance: $s^2 = am^b$ proposed by Taylor (1961), where a and b are Taylor's indices (Green 1970).

$$n = a\, m^{(b-2)}/D^2$$

The value of D = 0.25 is often applied for extensive studies of pest insect populations. Many of these species show a 10-fold or even 100-fold population change within a single season and estimating population density with a standard error of about 25% of the mean, is sufficiently accurate to allow detection of a doubling or halving of the population (Southwood and Henderson 2000).

Binomial sampling. It can be applied if the relationship between the mean (m) number of individuals and the proportion (p) of occupied sampling units are correlated. Two models were used to assess this relationship. Based on the negative binomial distribution (NBD) Wilson & Room's model relates m and p according to Taylor's indices a and b (Wilson and Room 1983)

$$p = 1 - \exp\left[-m\, ln(a\, m^{(b-1)})/(a\, m^{(b-1)} - 1)\right]$$

In cases where our data do not fit well with the negative binomial distribution, it is possible to establish this relationship using the empirical model independently proposed by Kono and Sugino (1958), Gerrard and Chiang (1970) working on insects, and Nachman (1984) on mites

$$p = 1 - exp\,(-a'\, m^{b'})$$

where a' and b' are constants and can be obtained from the regression

$$\ln(m) = a' + b' \ln\left[-\ln\ (1 - p)\right]$$

Linear models (LMs) were used to test if there was a good fit between the predicted infestation levels (p) of the two models and those observed by us in the field. For this purpose, we regressed the observed proportion of occupied sample units on the values of p predicted by the two models. The goodness-of-fit of the LMs, normality and homogeneity of variances assumption was tested in the same way as above.

Finally, the number of experimental units (n) required in the presence-absence sampling was calculated using the formula proposed by Kuno (1986).

$$n = D^{-2}\,(1 - p_0)\,p_0^{-(2/k)-1}\,[k\,(p_0^{-1/k} - 1)]^{-2}$$

where p_0 is the proportion of non-infested sample units per sample (1-*p*), D is the pre-fixed sampling precision, and k is a parameter characteristic of the negative binomial distribution calculated from the mean (m) and the Taylor's indices using the equation proposed by Wilson and Room (1983)

$$k = m^2/(am^b - m)$$

In the same way as for enumerative sampling, we have obtained the number of experimental units that have to be sampled as a function of population size considering an error of *D* = 0.25 (Southwood and Henderson 2000).

Sampling plan validation

To test the reliability of the developed enumerative and binomial sampling plans, we used the Resampling Software for Validation Sampling Plans (RVSP) (Naranjo and Hutchinson 1997). The simulation selected samples randomly, with replacement, from each independent data set until the stop line was exceeded. The prefixed precision level was *D* = 0.25, and the parameters *a* and *b* from Taylor's power law, were used in the simulation for the optimum sample size. Mean actual density, sample size, and precision were obtained based on 500 sampling bouts for each dataset. Following the protocol developed by Naranjo and Hutchison (1997), six motile mite and female independent extra datasets, that were not used to develop the sampling plans, were employed for their validation. Motile mite datasets covered a range of 0.42–71.18 mites per leaf and those of females between 0.12–3.04 females per leaf. Datasets consisted of leaves collected from the periphery of the tree canopy, each one obtained from different citrus varieties, and sampled in different months during 2019 following the methods described above.

4.3 Results

Population age structure and sex ratio

Table 4.1 summarises the proportion of the different developmental stages and sex ratio on sample units and leaf type. Immatures were much more abundant than adults. Eggs were the most abundant stage ranging between 40.65 – 66.82%, followed by nymphs with 25.84 – 46.83%. Adults were far less abundant with 5.10 – 8.17% for the females, while males with 1.28–5.21% were the least abundant (*X-squared* = 121809; *df* = 3; *P* < 2.2e-16). Population size was highest on the upper side of the leaves (75,601), compared to the lower side (12,382) and fruits (12,695). We observed statistically significant differences in age structure of the sampling units (*X-squared* = 1517.8; *df* = 6; *P* < 2.2e-16). The egg-to-nymph ratio on fruits was similar (0.99), while on leaves was favourable to eggs, especially on the lower side (2.46) (*X-squared* = 1183; *df* = 2; *P* < 2.2e-16). The percentage of adults was higher on the lower side of leaves (12.23%) and fruits (13,11%) than on the upper side of leaves (8.94%) (*X-squared* = 5386.1, *df* = 2, *P* < 2.2e-16). Finally, leaves showed higher sex-ratio values than fruits (0.60), with the upper side showing the highest value (0.70). The total number of mites collected was higher in spring (28,486) and summer (22,745) *"New leaves"*, and lower on leaves from the previous year, *"Old leaves"* (12,539), and *"New leaves"* from autumn sprouting (10,585). Differences in the proportion of developmental stages were also observed in the different leaf types (*X-squared* = 1898, *df* = 9, *P* < 2.2e-16). The egg-to-nymph ratio on *"Old leaves"* (0.86) was the most closely matched, while on *"New leaves"* eggs predominated, moderately in spring (1.31) and summer (1.58) sprouting, and distinctly in autumn (2.58) (*X-squared* = 1502.3, *df* = 3, *P* < 2.2e-16). The percentage of adults was high on *"Old leaves"* (12.52%), mid in spring (7.50%), summer (9.38%), and low in autumn (7.35%) *"New leaves"* (*X-squared* = 748.92, *df* = 3, *P* < 2.2e-16). Finally, autumn *"New leaves"* showed the highest sex-ratio (0.83), which decreased with leaf age in summer (0.72), spring (0.68), and previous year leaves (0.64).

Table 4.1 Total individuals collected of different developmental stages in each sampling unit (fruit, upper and lower leaf side) and leaf type. In brackets, the proportion of each stage of development in each sample unit and leaf type. The last row summarises the sex-ratio (females/ [males + females]). Significant differences are denoted with asterisks. Chi square test: ***P < 0.001.

Stage	Sample units ***			Leaf type ***			
	Fruit (%)	Upper (%)	Lower (%)	Last year (%)	Spring (%)	Summer (%)	Autumn (%)
Egg	5,507 (43.38)	37,982 (50.24)	7,725 (62.39)	5,096 (40.65)	14,977 (52.58)	12,638 (55.56)	7,073 (66.82)
Nymph	5,522 (43.49)	30,855 (40.81)	3,142 (25.37)	5,873 (46.83)	11,373 (39.92)	7,973 (35.05)	2,735 (25.84)
Female	1,004 (7.90)	4,702 (6.22)	1,012 (8.17)	1,000 (7.89)	1,453 (5.10)	1,543 (6.78)	642 (6.07)
Male	662 (5.21)	2,062 (2.73)	503 (4.06)	570 (4.54)	683 (2.40)	591 (2.60)	135 (1.28)
Total	12,695	75,601	12,382	12,539	28,486	22,745	10,585
Sex-ratio	0.60	0.70	0.67	0.64	0.68	0.72	0.83

Seasonal analysis of the age structure on leaves and fruits showed a fluctuation of eggs over time with values between 29.37 ± 5.03 and 68.28 ± 6.61%, while nymphs ranged between 21.34 ± 6.67 and 55.33 ± 13.73%, and adults from 21.94 ± 4.64% and 31.13 ± 7.04%. Adult stages showed higher relative percentages at the beginning and end of the period; moreover, they were more represented on fruits (11.25 ± 1.32 and 15.29 ± 2.31%) than on leaves (6.74 ± 0.62 and 10.92 ± 3.41%) ($F = 9.7418$, $df = 1$, $P = 0.007$). Furthermore, the synchrony between the two substrates was notable, with three peaks of egg production at 68.28 ± 6.61, 50.35 ± 5.32, 44.90 ± 32.05% on leaves and at 53.12 ± 6.48, 41.88 ± 10.10, 66.67 ± 19.72% on fruits, occurring simultaneously with no statistical differences ($F = 0.004$; $df = 1$; $P = 0.95$) in July-August, September-October, and November (Figure 4.1a). Mean female percentage (72.47 ± 2.21%) was higher than male (27.52 ± 2.21%) on both substrates ($F = 224.63$; $df = 1$; $P < 2e\text{-}16$). The interaction between the two factors, sex and substrate, revealed that females on leaves (76.13 ± 2.92%), were significantly higher than on fruits (68.80 ± 3.07%), so respectively, male percentage was superior on fruits (31.19 ± 3.07%) and inferior on leaves (23.86 ± 2.92%) ($F = 5.98$; $df = 1$; $P = 0.018$). Despite these differences in the relative proportions, seasonal monitoring of the sex-ratio on leaves and fruits showed that there are constant fluctuations on the values, appearing to be related with the age structure and population density. We observed three synchronic female peaks in the sex-ratio at 82.97 ± 6.84, 78.04 ± 7.39, 90.32 ± 24.39% on leaves, and at 76.47 ± 33.43, 71.42 ± 25.25, 87.50 ± 33.85% on fruits, occurring in July-August, September-October, and November. Female peaks in the sex-ratio were associated with the egg peaks found in the age structure (Figure 4.1a, b), since a statistically significant positive linear relationship was found between the egg-ratio and the sex-ratio on leaves ($F = 590.06$; $df = 1$; $P = 5.102e\text{-}08$) ($r^2 = 0.99$) and fruits ($F = 294.63$; $df = 1$; $P = 9.512e\text{-}09$) ($r^2 = 0.97$). In addition, we can observe that the proportion of females is lower at high population densities, being the lowest at the peak of the population in fruits and leaves (Figure 4.1b), since a statistically significant negative linear relationship was found between two variables on leaves ($F = 16.59$; $df = 1$; $P = 0.006$) ($r^2 = 0.69$), and fruits ($F = 8.02$; $df = 1$; $P = 0.036$) ($r^2 = 0.54$).

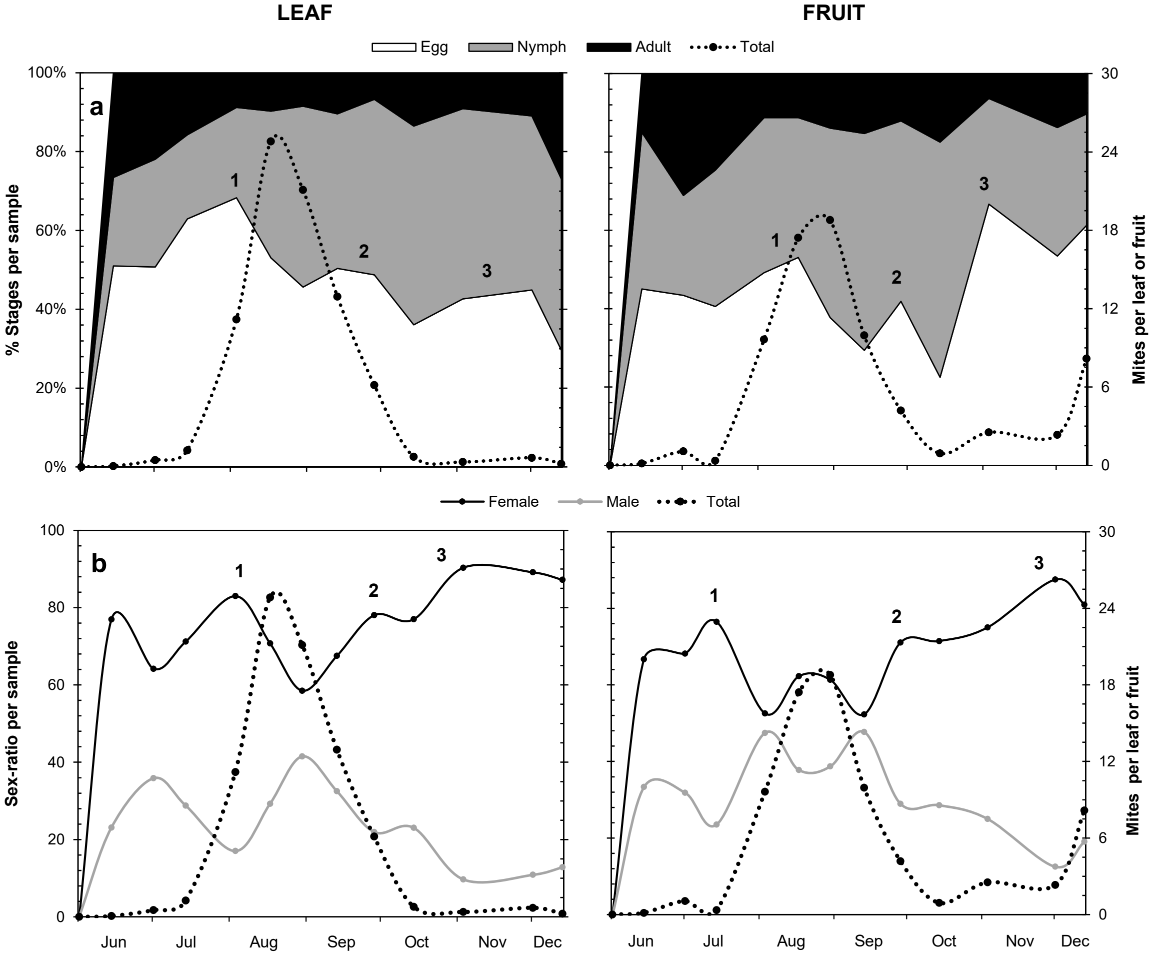

Figure 4.1 a. Seasonal relative abundance of developmental stages and population trends of *E. banksi* on citrus leaves and fruits. Percentage of each developmental stage per sample unit (fruit or leaf) and date are plotted in colored areas, and the number of mites per sample unit by a dotted line. b. Seasonal sex ratio and population trends of *E. banksi* on leaves and fruits. Sex ratio per sampling unit (fruit or leaf) and date are represented by solid lines, while the number of mites per sample unit by a dotted line. The peaks of eggs (a) and females (b) have been highlighted with ordinal numbers.

Dispersion pattern

Taylor's power law was used to study the spatial aggregation patterns of *E. banksi* on different citrus plant strata. Significant regressions with high determination coefficients (r^2) were obtained ranging from 0.91-0.99 (Table 4.2) when the logarithms of the sample mean and sample variance were plotted. The values of the slope (b) were significantly > 1 (t- test for a slope: P < 0.01; Table 4.2) in all cases, indicating an aggregated population distribution. ANCOVA analysis revealed no significant differences in the aggregation parameter *b* for the total mite population between groves ($F = 0.16$; $df = 3$; $P = 0.92$), despite their differences in abundance ($F = 2.90$; $df = 3$; $P = 0.034$). If we compare the 95% confidence intervals, there was a high overlap between the four groves indicating also non-significant differences [Navelina (1.51-1.60, 95% CI), Valencia-late (1.47-1.61, 95% CI), Ortanique (1.48-1.59, 95% CI) and Oronules (1.49-1.59, 95% CI) ($P > 0.05$)]. Regarding seasons, no significant differences in the aggregation parameter was found using ANCOVA analysis ($F = 0.71$; $df = 2$; $P = 0.490$), and this was confirmed through the calculation of 95% confidence intervals [Winter (1.43-1.81, 95% CI), Summer (1.51-1.57, 95% CI), and Autumn (1.53-1.60, 95% CI)].

ANCOVA analysis found no significant differences in aggregation of any of the mite developmental stages between plant strata ($F = 2.03$; $df = 5$; $P = 0.072$), in parallel with confidence interval overlapping ($P > 0.05$) (95% CI; Table 4.2). High significance was found in aggregation among the developmental stages ($F = 92.25$; $df = 3$; $P < 2e\text{-}16$). The ANCOVA two-by-two comparison of the different stages showed no significant differences in the aggregation pattern between immatures ($F = 0.22$; $df = 1$; $P = 0.63$), small differences between sexes ($F = 5.10$; $df = 1$; $P = 0.024$), and high differences between any of the immature and adult stages, egg/females ($F = 149.23$; $df = 1$; $P < 2.2e\text{-}16$), egg/males ($F = 83.93$; $df = 1$; $P < 2.2e\text{-}16$), nymph/females ($F = 172.27$; $df = 1$; $P < 2.2e\text{-}16$), and nymph/males ($F = 97.72$; $df = 1$; $P < 2.2e\text{-}16$). Small differences in aggregation between sexes could not be detected through the calculation of confidence intervals ($P > 0.05$) (95% CI; Table 4.2), but differences between adults and immatures were clearly detected ($P < 0.05$) (95% CI; Table 4.2), so differences between sexes should be considered with prudence. Thus, the aggregation spatial pattern between stages of development from higher to lower aggregation was Eggs ranging from 1.53–1.58, 95% CI = Nymphs from 1.43–1.57, 95% CI > Males from 1.11–1.35, 95% CI ≥ Females from 1.20–1.31, 95% CI.

Finally, if we pool all the developmental stages together ANCOVA detect no aggregation differences of the total population with any of the immature egg ($F = 0.045$; $df = 1$; $P = 0.83$) or nymph stages ($F = 0.090$; $df = 1$; $P = 0.76$), similarly to the comparison of confidence intervals ($P > 0.05$) (95% CI; Table 4.2). However, high differences were detected with both female ($F = 173.08$; $df = 1$; $P < 2e\text{-}16$) and male sexes ($F = 93.97$; $df = 1$; $P < 2e\text{-}16$) ($P < 0.05$) (95% CI; Table 4.2).

Table 4.2 Taylor's power law parameters for the different developmental stages of *E. banksi* on different plant strata. Parameter values *a* and *b* used for the sampling plans are highlighted in bold.

Life stage	Plant strata		Samples *n*	Taylor Power law					Test for slope($b \neq 0$)		CI (*b*)	
				a	*b*	SE(*b*)	r^2	*P*	*t*	*P*	*Inf*	*Sup*
Egg	Canopy	Outside	119	9.89	1.54	0.03	0.97	<2e-16 ***	58.23	<2e-16 ***	1.49	1.59
		Inside	36	6.09	1.55	0.06	0.95	<2e-16 ***	26.53	<2e-16 ***	1.43	1.67
	Leaf	New leaves	58	10.96	1.53	0.04	0.96	<2e-16 ***	37.29	<2e-16 ***	1.46	1.59
		Old leaves	61	9.03	1.53	0.04	0.96	<2e-16 ***	40.32	<2e-16 ***	1.45	1.60
	Leaf side	Upper	154	8.79	1.56	0.02	0.96	<2e-16 ***	63.27	<2e-16 ***	1.51	1.61
		Lower	66	11.85	1.58	0.04	0.97	<2e-16 ***	44.79	<2e-16 ***	1.51	1.65
	Fruit		52	6.34	1.53	0.05	0.94	<2e-16 ***	28.21	<2e-16 ***	1.42	1.64
	Total		427	8.80	1.55	0.02	0.96	<2e-16 ***	101.83	<2e-16 ***	1.52	1.58
Nymph	Canopy	Outside	119	9.56	1.53	0.02	0.97	<2e-16 ***	65.92	<2e-16 ***	1.48	1.57
		Inside	30	3.97	1.43	0.08	0.92	<2e-16 ***	18.77	<2e-16 ***	1.28	1.59
	Leaf	New leaves	59	10.36	1.46	0.04	0.96	<2e-16 ***	36.98	<2e-16 ***	1.38	1.54
		Old leaves	60	9.27	1.56	0.03	0.98	<2e-16 ***	55.00	<2e-16 ***	1.50	1.62
	Leaf side	Upper	146	8.48	1.57	0.02	0.97	<2e-16 ***	66.52	<2e-16 ***	1.52	1.62
		Lower	59	9.33	1.57	0.04	0.96	<2e-16 ***	37.95	<2e-16 ***	1.49	1.65
	Fruit		50	5.03	1.57	0.04	0.96	<2e-16 ***	35.04	<2e-16 ***	1.48	1.66
	Total		404	8.02	1.56	0.01	0.97	<2e-16 ***	106.22	<2e-16 ***	1.53	1.59
Female	**Canopy**	**Outside**	**116**	**3.04**	**1.27**	**0.03**	**0.96**	**<2e-16 *** **	**50.02**	**<2e-16 *** **	**1.22**	**1.33**
		Inside	24	1.89	1.20	0.09	0.91	<2e-13 ***	13.81	2.6e-12 ***	1.02	1.38
	Leaf	New leaves	60	2.96	1.24	0.04	0.95	<2e-16 ***	31.95	<2e-16 ***	1.16	1.32
		Old leaves	56	3.15	1.31	0.03	0.96	<2e-16 ***	38.01	<2e-16 ***	1.24	1.38
	Leaf side	Upper	138	2.95	1.28	0.02	0.95	<2e-16 ***	52.43	<2e-16 ***	1.24	1.33
		Lower	67	3.00	1.29	0.04	0.95	<2e-16 ***	34.93	<2e-16 ***	1.21	1.36
	Fruit		44	2.08	1.23	0.06	0.90	<2e-16 ***	22.17	<2e-16 ***	1.12	1.35
	Total		389	2.80	1.27	0.01	0.95	<2e-16 ***	99.40	<2e-16 ***	1.25	1.30
Male	Canopy	Outside	89	3.71	1.34	0.03	0.97	<2e-16 ***	53.63	<2e-16 ***	1.29	1.39
		Inside	22	1.43	1.11	0.04	0.99	<2e-16 ***	30.54	<2e-16 ***	1.04	1.19
	Leaf	New leaves	52	3.73	1.34	0.04	0.96	<2e-16 ***	33.55	<2e-16 ***	1.26	1.42
		Old leaves	37	3.66	1.35	0.03	0.98	<2e-16 ***	45.20	<2e-16 ***	1.29	1.41
	Leaf side	Upper	100	3.64	1.35	0.02	0.97	<2e-16 ***	60.98	<2e-16 ***	1.31	1.39
		Lower	37	3.14	1.29	0.04	0.96	<2e-16 ***	29.20	<2e-16 ***	1.20	1.38
	Fruit		43	2.27	1.26	0.04	0.95	<2e-16 ***	28.50	<2e-16 ***	1.17	1.35
	Total		291	3.24	1.32	0.01	0.96	<2e-16 ***	92.16	<2e-16 ***	1.29	1.35
Motile Stages	**Canopy**	**Outside**	**129**	**8.35**	**1.53**	**0.02**	**0.97**	**<2e-16 *** **	**63.35**	**<2e-16 *** **	**1.48**	**1.57**
		Inside	39	3.34	1.36	0.05	0.93	<2e-16 ***	26.15	<2e-16 ***	1.26	1.47
	Leaf	New leaves	66	8.98	1.48	0.04	0.96	<2e-16 ***	41.04	<2e-16 ***	1.41	1.55
		Old leaves	63	7.98	1.56	0.03	0.97	<2e-16 ***	48.08	<2e-16 ***	1.49	1.62
	Leaf side	Upper	171	8.28	1.57	0.03	0.96	<2e-16 ***	62.20	<2e-16 ***	1.52	1.62
		Lower	84	6.79	1.52	0.04	0.96	<2e-16 ***	42.19	<2e-16 ***	1.45	1.59
	Fruit		50	4.67	1.54	0.05	0.96	<2e-16 ***	33.96	<2e-16 ***	1.45	1.64
	Total		475	7.26	1.55	0.01	0.96	<2e-16 ***	145.29	<2e-16 ***	1.53	1.57
All Stages	Canopy	Outside	132	10.27	1.53	0.02	0.97	<2e-16 ***	70.32	<2e-16 ***	1.49	1.58
		Inside	43	5.72	1.55	0.05	0.96	<2e-16 ***	32.04	<2e-16 ***	1.46	1.65
	Leaf	New leaves	66	11.32	1.51	0.03	0.97	<2e-16 ***	45.20	<2e-16 ***	1.45	1.58
		Old leaves	66	9.49	1.54	0.03	0.98	<2e-16 ***	53.74	<2e-16 ***	1.48	1.60
	Leaf side	Upper	171	8.70	1.56	0.02	0.97	<2e-16 ***	73.27	<2e-16 ***	1.52	1.61
		Lower	93	9.89	1.57	0.03	0.96	<2e-16 ***	50.20	<2e-16 ***	1.51	1.63
	Fruit		53	6.06	1.54	0.05	0.95	<2e-16 ***	31.73	<2e-16 ***	1.44	1.64
	Total		490	8.49	1.55	0.01	0.97	<2e-16 ***	126.38	<2e-16 ***	1.53	1.57

For motile forms, ANCOVA analysis and confidence intervals also showed no differences with any immature, egg ($F = 0.0061$; $df = 1$; $P = 0.94$) or nymph stages ($F = 0.16$; $df = 1$; $P = 0.69$), and high differences with both female ($F = 147.48$; $df = 1$; $P <$ 2e-16) and male sexes ($F = 79.33$; $df = 1$; $P <$ 2e-16) ($P > 0.05$) (95% CI; Table 4.2). No significant statistical differences were found between the aggregation patterns of the total and motile population (F = 0.0177; df = 1; P = 0.89); in fact b coefficient and its CI were the same for the two populations considering all substrates (P > 0.05) (95% CI; Table 4.2).

Determination of representative developmental stages

To determine a representative developmental stage for sampling plan, we selected leaves at the periphery of the canopy as a plant stratum, because they harbour more than 98% of the total leaf mite population over the year and fruit populations are lower and difficult to detect. We pooled together the leaves from the previous year's shoots (*"Old-leaves"*) and those of the current year (*"New-leaves"*), as no differences in the aggregation pattern were found between them, and in practice they are difficult to differentiate, especially in some clementine varieties that show a not well-defined staggered sprouting (Agustí 2003).

Linear models revealed highly significant linear relationship between motile forms and total mite population on leaves at the periphery of the canopy by the equation $y = 1.29x$ (F = 1871.3; df = 1; P < 2.2e-16) (r^2 = 0.98) (Figure 4.2a). Furthermore, highly significant linear relationship was also found between female and total mite populations by the equation $y = 3.78x$ (F = 1660.9; df = 1; P < 2.2e-16) (r^2 = 0.98) (Figure 4.2b). This means that it is feasible to count only motile stages or females to assess the total mite population, although it is always more accurate to sample all the developmental stages. Adult female and the motile mite population showed significantly different aggregation patterns (Table 4.2), so in the following sections we will use a and b Taylor parameters to develop separate sampling plans for the female population and the motile *E. banksi* population.

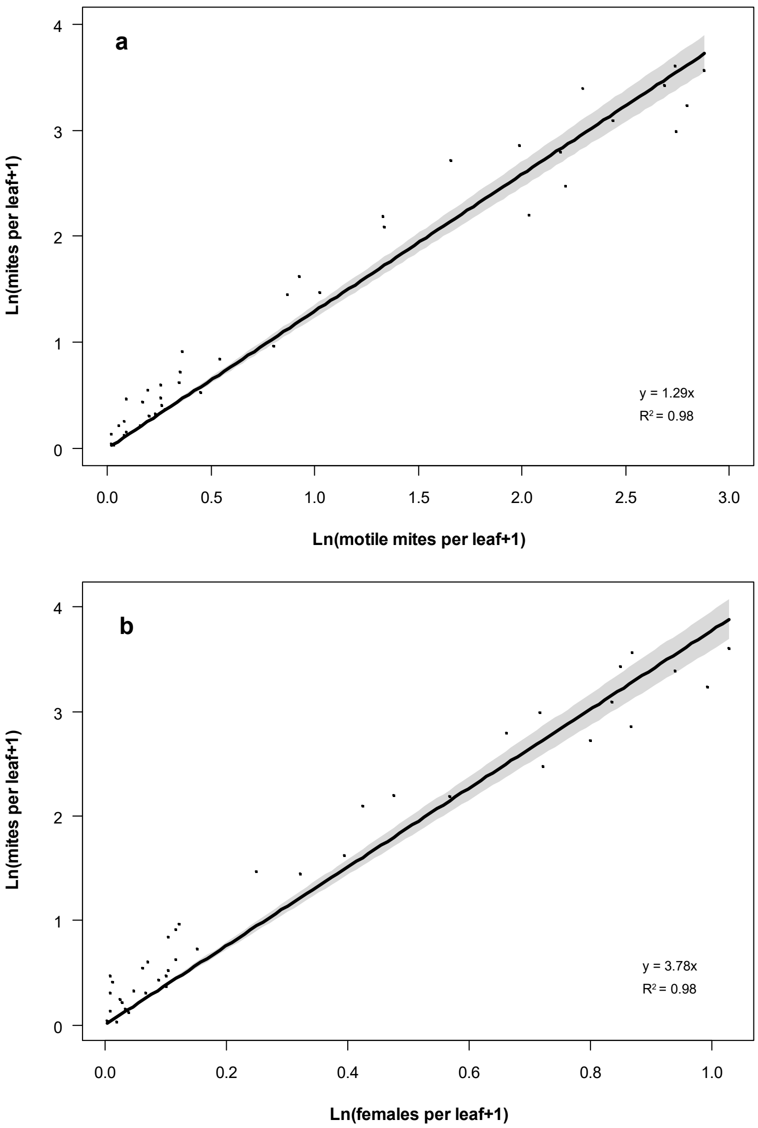

Figure 4.2 Representation of the linear models (LMs) showing the relationship between the mean motile developmental stages (a) or mean females (b) (x-axis) and the mean total mites (y-axis) of *E. banksi* per leaf in the periphery of the canopy. In the graphs, the prediction line is represented in black and the 95% confidence bands in grey. The goodness of fit of the model was assessed by computing the r^2 value.

Figure 4.3 shows the differences in spatial aggregation pattern of motile forms pooled together and adult females on leaves from the periphery of the canopy.

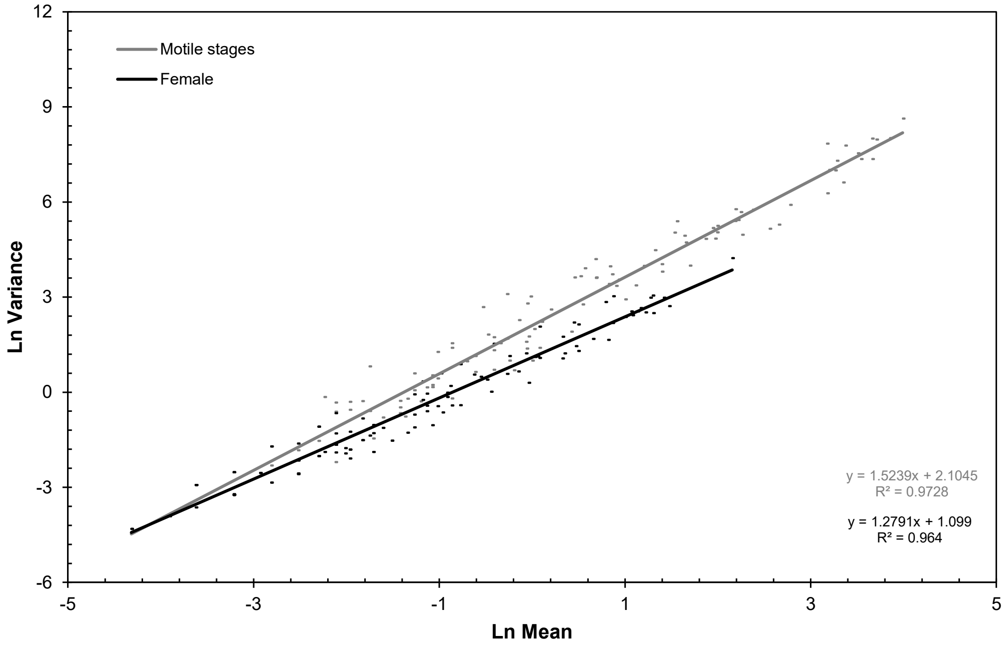

Figure 4.3 Representation of the linear models (LM) showing the relationship between mean density (x-axis) and variance (y-axis) of motile stages of *E. banksi* pooled together (gray) and adult females (black) on citrus leaves in the periphery of the tree canopy. The goodness of fit of the models was assessed by computing the r^2 value.

Enumerative and binomial sampling

To develop a practical and efficient sampling plan for IPM programs we selected as plant strata *"Old"* and *"New leaves"* from the periphery of the canopy pooled together for the same reasons explained in the previous section. Taylor parameters *a* and *b* used for female and the motile population sampling plan are highlighted in Table 4.2.

Enumerative sampling. The number of leaves from the periphery of the citrus tree canopy per sample (sample size) required to estimate *E. banksi* populations was calculated using Green's sampling plan at $D = 0.25$ precession level. The sample size was calculated separately for females and the motile developmental stages pooled together as they had shown significantly different aggregation patterns (Figure 4.4). According to our sampling plan, for population densities from 5 to 25 motile mites per leaf, 60–30 leaves should be monitored in enumerative sampling. If it is decided to use females as the reference developmental stage for monitoring, at values from 1 to 5 females per leaf, only 50–20 leaves should be examined.

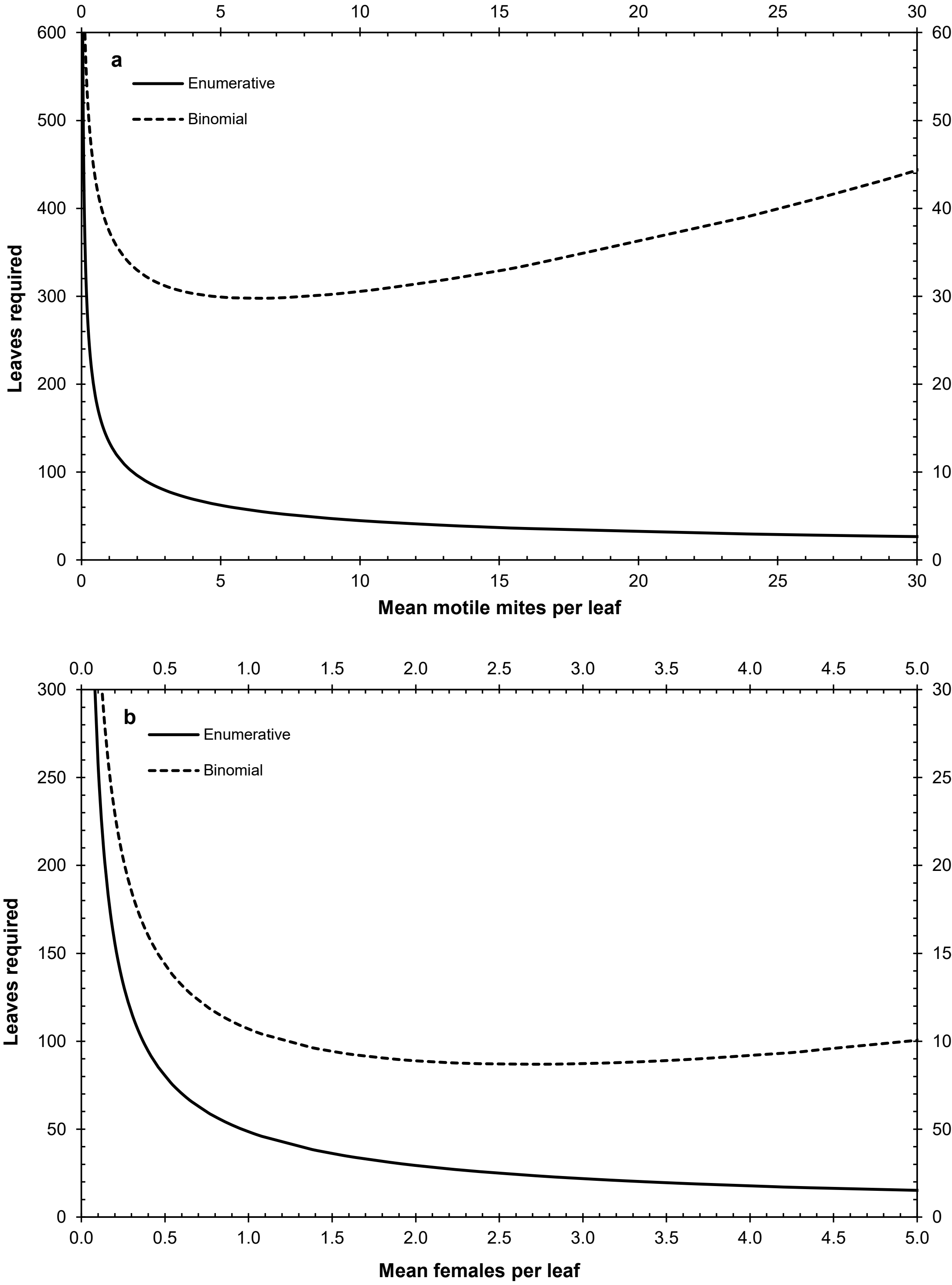

Figure 4.4 Enumerative and binomial sampling for *Eutetranychus banksi* motile mites (a) and females (b) on citrus. Number of leaves from the periphery of the canopy required (y-axis) based on the mean motile mites or mean females per leaf (x-axis). Green's method was used for enumerative (solid line) and Kuno's method for binomial sampling (dashed line), to achieve a precision level of *D* = 0.25.

Binomial sampling. The determination of the relationship between the reference developmental stage density per leaf and proportion of infested leaves (incidence-density relation) can reduce the time of sampling, as it allows the estimating of density without counting all individuals present on each leaf. The relation incidence-density for *E. banksi* was calculated on citrus following two different models, based on the NBD model of Wilson & Room and the empirical model of Kono & Sugino. The motile mite levels on leaves observed in the field adequately fitted the estimated indices (*p*) using Wilson & Room's model ($F = 3360.6$; $df = 1$; $P < 2.2e\text{-}16$) ($r^2 = 0.97$), while they did not fit well with the Kono & Sugino's model predicted values ($F = 214.27$; $df = 1$; $P < 2.2e\text{-}16$) ($r^2 = 0.65$). The adult female's percentage infestation levels also showed a strong fit with two models, Wilson & Room's ($F = 5275.5$; $df = 1$; $P < 2.2e\text{-}16$) ($r^2 = 0.98$) and Kono & Sugino's ($F = 4644.7$; $df = 1$; $P < 2.2e\text{-}16$) ($r^2 = 0.98$). These results indicate that the Wilson and Room's (1983) NBD approach, rather than the Kono and Sugino's (1958) empirical approach, could be good for describing the incidence of both the motile forms and females. Following this approach, values between 5–25 *E. banksi* motile forms per leaf correspond to 55–90% of occupied leaves, while for the females, values of 1–5 correspond to 40–90% (Figure 4.5).

The number of leaves from the periphery of the canopy per sample (sample size) required to estimate *E. banksi* populations by presence/absence, sampling for motile developmental stages, as well as only females, was calculated using the Kuno's (1986) model at $D = 0.25$ precision level (Figure 4.4). For binomial sampling of motile forms, densities between 5 to 25 mites per leaf would require 300–400 leaves, and 110–90 leaves for monitoring female densities between 1 to 5 individuals per leaf.

Sampling plan validation

The validation of the sampling plan with a desired precision of 0.25 resulted in a sample size ranging from 206 to 19 leaves for motile forms, and from 234 to 22 leaves for females (Table 4.3). Actual mean precision levels varied from 0.18–0.46 and 0.15–0.32 for motile mites and females, respectively, averaged in both cases the desired precision of 0.25.

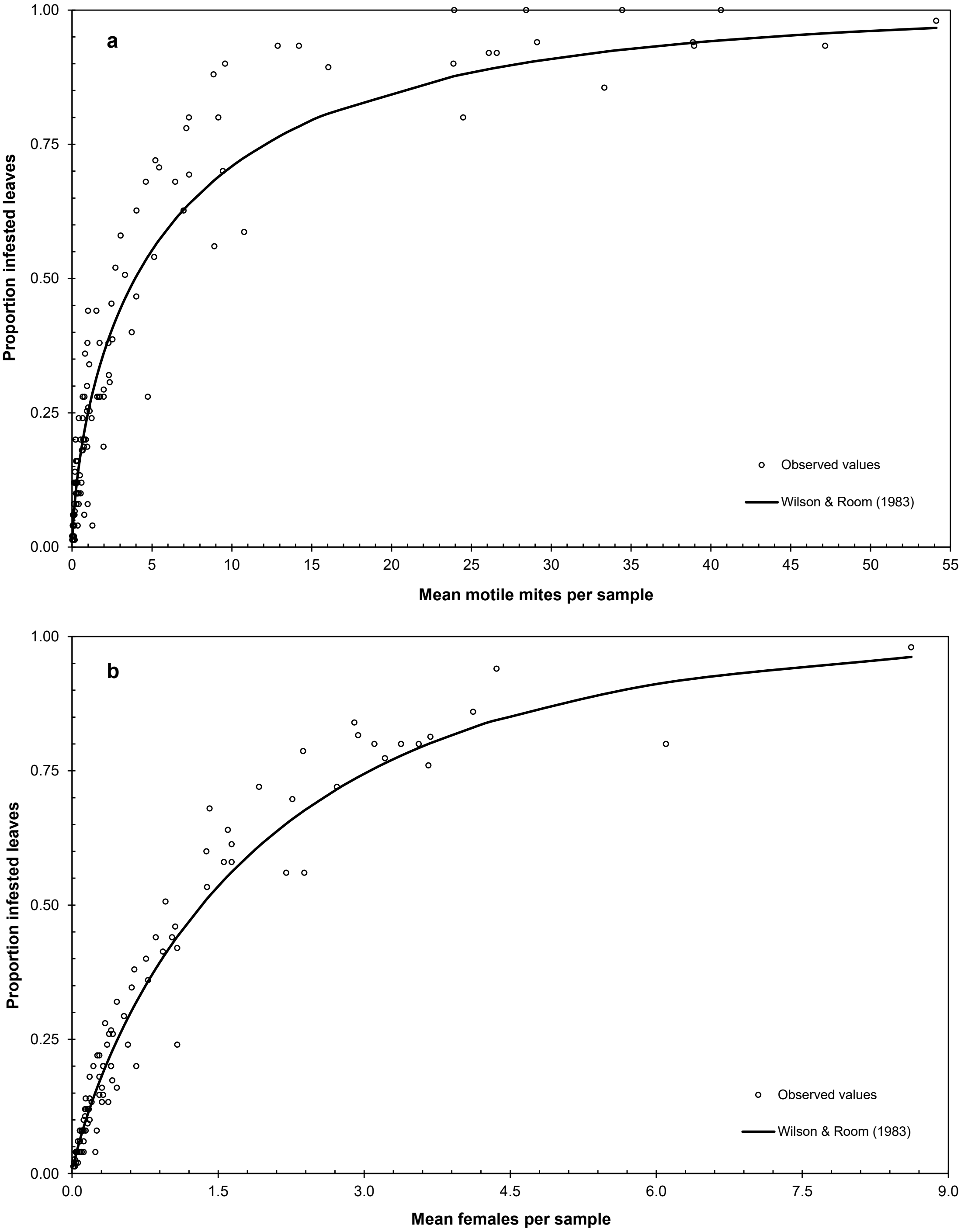

Figure 4.5 Relationship between the proportion of leaves on the periphery of the canopy infested with *E. banksi* motile forms (a) and females (b) (y-axis) and the mean number of motile mites or females per sample unit (x-axis). Lines represent predicted values from the NBD Wilson & Room approach, while points represent the observed values in the field.

Table 4.3 Resampling simulation results used to validate Green's fixed precision sequential sampling plan for *E. banksi* motile developmental stages (a) and females (b) on citrus with desired precision levels adjusted to 0.25 with replacement (Motile stages' Taylor *a* = 8.35 and *b* = 1.53; Female's Taylor *a* = 3.04 and *b* =1.27)

Datasets	Observed mean density	Avg. Statistics for 500 sequential sampling simulations						
		Density mean	Precision (*D*)			Average sample no.		
			Mean	Max.	Min.	Mean	Max.	Min.
a. Motile mite								
1	71.18	73.25	0.21	0.33	0.10	19	25	14
2	52.34	53.28	0.18	0.27	0.10	21	27	16
3	15.92	16.51	0.20	0.31	0.11	37	47	27
4	6.22	6.30	0.18	0.22	0.14	58	81	46
5	3.74	4.08	0.46	0.67	0.32	77	165	41
6	0.42	0.43	0.27	0.33	0.20	206	294	147
Overall	24.97	25.64	0.25	0.35	0.16	69.67	106.50	48.50
b. Female								
1	3.04	3.11	0.15	0.21	0.09	22	31	16
2	2.8	2.90	0.24	0.34	0.15	24	37	13
3	1.2	1.27	0.25	0.36	0.16	44	72	24
4	0.86	0.91	0.32	0.42	0.22	58	100	24
5	0.16	0.17	0.29	0.35	0.23	192	321	107
6	0.12	0.13	0.26	0.28	0.22	234	398	110
Overall	1.36	1.41	0.25	0.33	0.18	95.67	159.83	49.00

4.4 Discussion

Population structure and sex-ratio

This is the first time that a detailed study of the age structure of a spider mite on leaves, through different sprouting events, and fruits has been carried out on citrus. The population structure of *E. banksi* was characterised by a higher abundance of juvenile stages showing the same pattern already described for other spider mites feeding on different crops (Carey 1982). Differences in the proportion of developmental stages, as well as sex-ratio, were observed in the different feeding substrates, and these differences could be related to the nutritional quality of the food substrate. The proportion of eggs and nymphs was similar in fruits, while leaves had a higher proportion of eggs, especially on the lower side. Furthermore, the upper side of leaves showed the highest sex ratio, followed by the lower side and finally the fruits. It is possible that leaves have a more suitable nutritional content for development than fruits, while the upper side of the leaf favors mite growth (Ferro et al. 1979). On the lower side, the proportion of eggs was much higher than that of nymphs, and the proportion of adults higher than on the upper side. Females prefer to lay their eggs on leaves along the midrib of the upper surface, and when the population grows, they move towards the leaf margins and to the lower side of leaves to explore new oviposition sites (Dean 1959b). This movement leads to an increase in the proportion of eggs and adults on the underside, as the larvae, once hatched, quickly move to the upper side.

When we analyze the age structure throughout the different leaf types, there is a progressive increase in the proportion of eggs and sex-ratio, which is negatively correlated with leaf age. Leaves from the previous year harbor a low density of mites and the lowest egg proportion and sex ratio. On citrus, leaves remain on trees for 1-2 years (Scott et al. 1948), so previous year's leaves are often damaged by mite feeding, and the level of injury influences the mite density, reducing egg laying and sex-ratio (Henderson and Holloway 1942). The current year's leaves integrate the greatest part of the population (Lopez-Olmos and Ferragut, accepted), and differences in the composition of developmental stages, in addition to the reasons already noted, may be due to two factors. First, laboratory studies with *T. urticae* showed that when the population density increases there is a sex-ratio bias towards males (Wrensch and Young 1978), so the spring and summer shoots with a higher *E. banksi* population density could have experienced this bias. Second, the sex-ratio study of *T. urticae* in corn showed that although all mobile stages move upward to colonize newly expanded leaves, it is the females that do so to a greater extent (Krainacker and Carey 1991). The same pattern of dispersal could be observed for *Panonychus ulmi* (Koch) that overwinters on Dwarf Bamboo as diapause eggs on leaves and with the arrival of spring, and sprouting of new leaves, the adults move from the previous year's leaves to the new ones (Gotoh 1987). We have observed the same dispersal pattern in *E. banksi* on citrus during autumn. In our latitudes, citrus shows three more or less defined flushing periods, spring, summer and autumn (Agustí 2003). Mild winters, with daily average temperatures above 4 °C, the threshold temperature of development obtained for *E. banksi* in laboratory conditions (Badii et al. 2003; Childers et al. 1991), allow mite survival on leaves without entering diapause and constituting small populations often undetectable, as has been observed for other spider mites such as *Panonychus citri* that also affect citrus (Ferragut et al. 1988). Usually, the first individuals are detected in the field during summer on the spring and summer leaves of the current year, coming from leaves of the previous year. The population grows rapidly in a few weeks occupying the leaves of the current year, those of the previous year and fruits. The vegetative development of the trees stops during summer due to the high temperatures, and restarts with the beginning of autumn, giving rise to a final flushing (Agustí 2003). When these autumn leaves are fully developed, there is a displacement, mainly of females, to occupy this new resource available to establish a new population. However, the drop in winter temperatures prevents these leaves from harboring as high a number of mites as those in spring and summer leaves (Figure 4.6).

Figure 4.6 Autumn leaf with females coming from the spring and summer leaves. In the picture more than 20 females can be seen at the first stages of leaf colonization, while the number of females per leaf ranges from 1 to 10 in already established populations.

Spider mites have short development times between 6 to 10 days and several overlapping and almost indistinguishable generations may be produced each year, the number of which is correlated with temperature and influenced by the nutritional status of the plant (van de Vrie et al. 1972). *Eutetranychus banksi* shows a development time ranging from 16–9.6 days from egg to adult at temperatures between 20–32 °C (Badii et al. 2003; Childers et al. 1991), so we decided to plot the observed age structure on a fortnightly schedule. We observed three generations occurring simultaneously on leaves and fruits. Carey (1982), after an exhaustive theoretical study using the Leslie matrix method on different spider mite species, concluded that when mortality and fecundity in a population are kept constant at each age, the population over generations converges to a stable age distribution (SAD), which in the case of tetranychid mites was fixed at approximately 65% eggs, 25% immatures and 10% adults. *Eutetranychus banksi* showed a fluctuation of proportions over time and does not seem to reach this SAD on leaves; however, on fruits it seems to finally reach these values. Developmental time and fecundity are highly influenced by temperature and have been shown to be the demographic determinants with the greatest capacity to alter the structure of developmental stages (Carey 1984). Under field conditions, temperature is constantly changing, altering development time and fecundity, which impedes convergence towards SAD. Moreover, regarding leaves, the citrus generates new leaves three times per year, which represent a new food substrate susceptible to alter demographic parameters.

Sex-ratio is another parameter that fluctuates constantly over time in *E. banksi*, although it is always biased towards females with 0.7–0.75 female proportion. Sex-ratio fluctuations over time are common in tetranychids (Boudreaux 1963), but female biased values around 0.75 occurs frequently (Helle and Pijnacker 1985). We observed that the proportion of eggs in the population was highly correlated with the proportion of females in the adult proportion, which is consistent with the observations of other authors in other spider mites (Krainacker and Carey 1991; Yasuda 1982). As in the case of leaf types, the sex ratio was a highly density-dependent factor, since the lowest proportion of females, both in leaves and fruits, coincided with the peak of population density. Spider mites reproduce by arrhenotokous parthenogenesis, with females controlling the sex-ratio of their offspring (Young et al. 1986). In presence of a deteriorated food resource, such as old-damaged leaves, females increase the proportion of female daughters in their offspring that will disperse to colonize a new flush, which contribute to the low sex-ratio observed in older flushes and the high sex-ratio observed in the new ones. Furthermore, males produced remain in old flushes and fertilize females, increasing their success in colonization, since fertilized females are more efficient in colonizing new environments than unfertilized females (Wrensch and Young 1975).

Dispersion pattern

Eutetranychus banksi showed strongly clumped distribution on leaves and fruits with significant differences between developmental stages. Eggs showed *b* values ranging from 1.53–1.58, followed by nymphs with 1.43–1.57, males 1.11–1.35, and finally females with values between 1.20–1.29. Immature stages were significantly more aggregated than adults, there were no differences between eggs and nymphs, while small differences were detected between sexes, with females being the sex with the lowest aggregation. Differences in aggregation patterns between immature and adult stages are common in spider mites (García Marí et al. 1991; García-Marí and Costa-Comelles 1992; Mico et al. 1992), because it is the adult stages, especially the females, that disperse to colonize new food sources, such as a new flush, and establish new populations (Gotoh 1987; Krainacker and Carey 1991). The comparative study of spatial aggregation in different plant strata and developmental stages has been carried out for other spider mites on citrus. *Tetranychus urticae* females had a significantly higher aggregation on leaves on the outer part of the canopy (1.43 ± 0.03) than on the inner (1.37 ± 0.02), as well as a lower aggregation on fruits (1.18 ± 0.03) than on leaves (Martinez-Ferrer et al. 2006). Unfortunately, there is no information published about aggregation values of immature stages for this species on citrus. On the other hand, *Panonychus citri* did not show significant differences in aggregation between the different plant strata in Spanish orange trees, showing *b* values from1.51–1.61 for eggs, 1.34–1.49 for nymphs, 1.04–1.15 for adult females, and 1.26–1.39 for motile forms (Mico et al. 1992). Studies on Californian orange and lemon trees found higher aggregation values for *P. citri*, which may be due to differences between citrus varieties, climatic factors, and cultural practices such as pruning (Jones and Parrella 1984a; Zalom et al. 1985).

Eutetranychus banksi females showed a lower aggregation than *T. urticae* but higher values than *P. citri* on leaves, with 1.27 ± 0.03 on the outer side, 1.20 ± 0.09 on the inner side of the canopy. However, the aggregation on fruits (1.23 ± 0.06) was comparable to that of *T. urticae*. There is no published information about the aggregation pattern of *P. citri* on citrus fruits. *Eutetranychus banksi* showed a similar egg aggregation to *P. citri,* but the aggregation of nymphs and motile forms (1.36–1.57) was higher. *Tetranychus urticae* on citrus lives all year round in dense colonies on the lower side of leaves where it builds a web on which all stages of development reside, and it is not until August-September when it colonizes the fruit infecting its stylar area (Ansaloni et al. 2008). This delay in reaching the fruit probably contributes to the reduction of its aggregation on this substrate (Martinez-Ferrer et al. 2006). The feeding behavior of *E. banksi* on fruits is different, because from the beginning of its population increase on leaves, we could observe individuals feeding on the sunny side of the fruits (Lopez-Olmos and Ferragut, accepted). *Panonychus citri*, unlike *T. urticae*, can feed on both sides of the leaf, preferring the upper side over the lower side, as well as *E. banksi.* Both species also prefer the outer part of the tree canopy to live in, as well as the leaves rather than the fruit to feed on (García et al. 2003). Accordingly to this shared behavior, both species showed no differences in aggregation patterns between plant strata; however, the motile forms of *E. banksi* are more aggregated on the leaves than those of *P. citri,* which are organized into dense colonies that completely occupy the leaf's upper surface in the outer part of the citrus canopy (Figure 4.7).

Figure 4.7 A dense *E. banksi* colony where all stages of development can be observed. There is a large accumulation of molts and hatched eggs, as well as discoloration on the leaf surface because of feeding.

Despite the importance of the *Eutetranychus* species as pests on citrus, as well as other crops and ornamental plants, there is very little information about their spatial distribution on plants. Landeros et al. (2004) in Mexican orange trees obtained highly variable values of Taylor's parameter *b* ranging from 0.65–2.39 for the total *E. banksi* population over a year. We have not studied the spatial distribution in each sampling; however, we did not find differences in the aggregation pattern over time between seasons. Jones (1990) compiled aggregation values for different mite species of the genera *Tetranychus* and *Panonychus* on different cultivated plant species. In this review, he also included the unpublished value of b=1.54 for the mobile forms of *E. banksi* on cassava leaves. This value is very similar to that obtained by us for the motile forms and the total population on citrus leaves. Finally, Yarahmadi and Rajabpour (2013) obtained the value of b=1.45 for the total population of *E. orientalis* on leaves of the ornamental legume species *Albizia lebbeck* (L.), a value lower than the values obtained by us for the total population.

Sampling plans

The existence of a significant linear relationship between motile forms and adult females with the total *E. banksi* population makes it possible to use either as representative stages for the development of a sampling methodology. Binomial sampling involves presence-absence monitoring of the species, which is usually faster than enumerative sampling, despite the fact that it implies counting a larger number of leaves. The use of females as a representative stage in mite monitoring reduces effort making monitoring feasible without losing excessive accuracy. Therefore, we recommend monitoring the presence/absence of females on 100 leaves for binomial sampling or, to be more accurate, we can survey mite motile forms on 400 leaves. In our latitudes *E. banksi* tends to increase in mid-summer to reach its peak in late summer-early autumn. However, on rare occasions there are orchards that, due to nutritional, physiological problems, or other causes, can maintain relatively high populations during winter and show population peaks in late spring-early summer (Lopez-Olmos and Ferragut, data not published). Therefore, we advise farmers to starting weekly monitoring for *E. banksi* at the beginning of summer.

There is evidence that *E. banksi* populations on citrus are spatially structured at the orchard level, since the mites aggregate on the sunniest sides of the tree canopy, so in our latitudes high pest densities occur in the south-eastern quadrants of the canopy, as previously reported in Texas grapefruit orchards (Dean 1959a). Furthermore, on trees along dusty roads, *E. banksi* rose before a general increase was observed in the rest of the orchard (Dean 1959b), because the development of non-producing web spider mites, as *E. banksi*, is favored by the presence of dust or inert debris on the leaf surface (Holloway et al. 1942). When pest mites exhibit aggregation among trees as well as among areas within an orchard, the use of transects has been an effective tool for estimating population density accurately (Hall et al. 1994). Therefore, following this methodology we recommend sampling along 2 diagonal transects, a first transect between the north-east and south-west corners and a second transect between the north-west and south-east corners. In each of the transects 25 trees regularly spaced should be sampled to cover the whole diagonal length of the orchard. Two leaves of the outer part of the canopy per tree, one from each side of the tree facing the line, should be sampled. In the case of orchards with non-linearly planted trees, the four canopy orientations should be alternated among trees to collect the leaves. For each leaf, the presence/absence of females on both sides of the leaf should be monitored. In case of checking motile forms, 4 leaves per tree in 50 trees per transect should be monitored. If orchards have a small number of trees that impedes transect sampling, simply divide the number of leaves to be monitored (100 or 400) by the total number of trees in the orchard, and the leaves to be collected from each tree in each of its four quadrants (NE, NW, SE and SW).

Comparing our sampling plans to those obtained for other spider mites affecting citrus, around 450 leaves from the outer part of the canopy are recommended in the enumerative sampling of *T. urticae* to estimate densities of 0.1 females per leaf, and 240 leaves in the binomial sampling to estimate densities of 0.3 females per leaf. However, if only symptomatic leaves are sampled, 250 leaves are needed for low female densities, while at high densities it is sufficient to collect 100 leaves (Martinez-Ferrer et al. 2006). In *P. citri*, it is recommended to randomly sample 50 leaves fully expanded from the last two flushes in the enumerative sampling to estimate densities around 1 motile form per leaf (Garcia-Marí et al. 1994). The binomial sampling for motile forms requires 100 leaves from the last flush collected from 25 random selected trees (4 leaves per tree), as a good relationship between the number of motile forms per leaf and the percentage of occupied leaves has been described, showing that an average of 1-2 motile forms per leaf corresponds to 50% infestation (Garcia-Marí et al. 1992). García-Marí and Ferragut (2019), as well as the IPM citrus guidelines in Spain (GIPCITRI 2022), recommend using the same sampling plan for *E. banksi* and *E. orientalis* as described for *P. citri.* To estimate population densities of 1 motile form per leaf of *E. banksi* in an enumerative sampling*,* would be require 135 leaves, almost 3 times more leaves than those needed to estimate that density in *P. citri*. Regarding binomial sampling, in *E. banksi* 4 motile forms per leaf correspond to 50% infestation. Because of its more spatially aggregated pattern, *E. banksi* needs 4 times the population size of *P. citri* to infect the same proportion of leaves, so it is not surprising that to estimate the same density as *P. citri* (1 motile form per leaf), we need nearly 4 times as many leaves (400) in a binomial sampling. On the other hand, in our sampling plan for *E. banksi* we have not made distinctions between flushes, as they are sometimes difficult to identify in the field. Furthermore, the migratory process of the mites, especially females, can generate alterations in the age structure in the last flushes that lead us to overestimate population density, in a sampling plan based on females, or underestimate it when we used the motile forms. We have not found significant differences in the aggregation pattern on leaves in the outer part of the canopy from different flushes, so we think it is better to randomly gather leaves from the periphery of the canopy and dilute the effects generated by the mite mobility.

There are no accurate sampling methods for *Eutetranychus* species in the literature. In Florida, binomial sampling of 96–32 leaves on 24–8 trees is recommended to estimate densities of 5–15 motile forms of *P. citri* and *E. banksi* per leaf, respectively, since an average of 5 motile forms per leaf corresponds to 70–80% infestation (Rogers and Stansly 2017). According to our findings, *E. banksi* should need to infect 70–80% of leaves to reach values of 10–15 motile forms per leaf. Furthermore, it is thought that spider mites belonging to the same genus share a similar spatial aggregation pattern (Jones 1990); therefore, the sampling plans we have developed and validated for *E. banksi* on citrus could be useful as well for *E. orientalis*.

4.5 Acknowledgements

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CHAPTER 5

Life-history, prey-stage preference, functional and numerical response of the predatory mite *Neoseiulus madeirensis* (Acari: Phytoseiidae) to *Eutetranychus banksi* (Acari: Tetranychidae) on citrus

Life-history, prey-stage preference, functional and numerical response of the predatory mite *Neoseiulus madeirensis* (Acari: Phytoseiidae) to *Eutetranychus banksi* (Acari: Tetranychidae) on citrus

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Doctoral thesis adapted version.

Abstract: The Texas citrus mite, *Eutetranychus banksi* McGregor (Acari: Tetranychidae) is an American mite pest that has recently invaded the Spanish Mediterranean citrus crop. The current management of this new pest is carried out using chemical control, since natural enemies found on citrus are not effective for its control. It is necessary to implement a biological control program as a strategy to control *E. banksi* populations and reduce the risks to human health and the environment caused by chemicals. For this purpose, studies have been carried out to evaluate the biological control possibilities using the predatory mite *Neoseiulus madeirensis* Papadoulis & Kapaxidi (Acari: Phytoseiidae) through life-history, prey-stage preference, functional and numerical response experiments. *Neoseiulus madeirensis,* feeding on *E. banksi,* exhibits a short developmental time and high survival and reproductive rates, suggesting high capacity for population increase. The predator shows a clear preference for the immature stages of *E. banksi*, with eggs being slightly preferred when they are offered together with other immature prey-stages. *Neoseiulus madeirensis* displayed type II functional response, and the highest attack rate and shortest handling time was recorded for nymphs and larvae, respectively. The numerical response fitted the hyperbolic model, showing the higher maximum daily oviposition for the nymphs, and the lower prey density needed to display half of the egg-laid for the eggs. Results of this study suggest that *N. madeirensis* is a suitable candidate for the development of a biological control program to manage *E. banksi* infestations especially at low population densities.

5.1 Introduction

The Texas citrus mite, *Eutetranychus banksi* McGregor (Acari: Tetranychidae) is one of the main citrus spider mite pests in the Americas (Gerson 2003), where it is considered native. Far from its region, in 1999 *E. banksi* invaded the Iberian Peninsula producing severe damage to citrus in the Algarve region of southern Portugal (Gonçalves et al. 2002), and in 2001 it spread and colonized citrus orchards in south-western Spain in Ayamonte and Isla Cristina (Huelva province) (García et al. 2003). In 2013 it invaded La Safor (Valencia province) more than 800 km away from Ayamonte, and the following year other regions of the provinces of Valencia and Alicante, affecting the main citrus-growing area of the eastern Mediterranean in Spain (Ferragut 2016). The adults and immature of *E. banksi* feed mainly on leaves producing stippling damage, which may reduce photosynthesis and promote premature leaf drop at high population levels, resulting in a decrease in plant vigour and yield. Furthermore, aesthetic damage to the fruits is produced, resulting in a lack in pigmentation and silvery, which may reduce their economic value (García et al. 2003; Monzó et al. 2016).

Three overlapping generations per year were recorded for *E. banksi* in Spanish citrus orchards with a single population peak in late-summer early-autumn (Lopez-Olmos and Ferragut, submitted), when population densities were greater than the empirical economic threshold proposed by Rogers and Stansly (2017). Currently, pest management in Spain depends on chemical control, since *E. banksi* has been shown to be sensitive to numerous acaricides, being easy to keep under control with this strategy (Monzó et al. 2016). However, under the perspective of Integrated Pest Management (IPM), biological control should be combined with chemicals to produce healthy crops and minimize the use of pesticides, reducing the risks to human health and the environment. Predatory mites of the family Phytoseiidae have been widely used for biological control of pest mites on citrus and other crops worldwide (Gerson 2003; McMurtry 1983). Surveys that have compiled the phytoseiid fauna of Spanish citrus agree that *Euseius stipulatus* (Athias-Henriot) is the most abundant phytoseiid species on citrus, representing more than 90% of the total number of phytoseiids collected, as well as the most frequent, found in about 90% of the samplings carried out per year (Abad-Moyano et al. 2009a; Ferragut et al. 1988; Ledesma et al. 2011; López-Olmos and Ferragut, submitted). However, this predatory mite was recorded in orchards at densities too low for effective suppression of

E. banksi, possibly because this prey is not a suitable food for the development and reproduction of the predator (López-Olmos and Ferragut, in preparation).

The phytoseiid mite *Neoseiulus madeirensis* Papadoulis & Kapaxidi was described from females collected in the Botanical Garden of Madeira Island on *Lantana sp.* (Verbenaceae) and *Dombeya wallichii* (Sterculiaceae) (Papadoulis and Kapaxidi 2011). Before the publication of the original description, specimens of this mite had already been collected in Alicante and Valencia, Spain, on *Pinus halepensis* (Pinaceae) and *Brugmansia candida* (Solanaceae) infested with the spider mites *Tetranychus turkestani* (Ugarov & Nikolskii), *T. ludeni* Zacher and *T. urticae* Koch, respectively (Ferragut, unpublished data). Later, it was found in Huelva province on citrus orchards associated with *E. banksi*, since then it has been frequently recorded in the Spanish Mediterranean coast on different host plants and always in association with spider mites. Nothing is known about its biology, and it has never been evaluated or used in biological control programs, but its evident relationship with spider mites, including *E. banksi*, suggests that it can be a promising candidate for pest suppression, and it seems worthwhile to investigate the possibility of using it as a biocontrol agent against *E. banksi*.

The aim of this work was to explore the efficacy of *N. madeirensis* for the biological control of *E. banksi*, as part of an IPM programme. For this purpose, (i) a life-history experiment was carried out to determine whether *N. madeirensis* can develop and reproduce when feeding solely on *E. banksi.* After that, (ii) prey stage preference experiments were performed to determine *E. banksi* prey stages preferred by *N. madeirensis* for feeding, and finally (iii) functional response experiments were conducted on suitable *E. banksi* prey stages to determine the potential of *N. madeirensis* to reduce pest populations.

5.2 Material and Methods

Stock mite colonies

Laboratory mite cultures of *E. banksi* and *N. madeirensis* were established from field collections and maintained in separate climatic rooms at 25 ± 1 °C, 60-70% RH under a 16:8 hours L:D. *Eutetranychus banksi* was collected from a clementine mandarin citrus orchard, *Citrus reticulata* Blanco, of the variety Oronules located in Picassent (39.325936S, -0.438279W) in January 2018, and taken to the Acarology laboratory at the

Mediterranean Agroforestry Institute (IAM) of the Polytechnic University of Valencia (UPV). *Eutetranychus banksi* colony was maintained in the laboratory on castor bean leaves (*Ricinus communis* L.), as this spider mite can easily develop high populations by feeding on this species. The leaves were kept turgid for about a week in a plastic container with water (Figure 5.1a). To maintain the culture, once a week all the leaves were cut into 5-10 cm fragments, and those with lower mite populations were used for culture maintenance infecting new leaves. The leaf fragments with the highest mite populations were used to infect detached sour orange, *Citrus* × *aurantium* L., leaves, prepared as follows. Several young, fully expanded citrus leaves were placed upperside up on a wet cotton wool layer in a plastic tray (29.5 x 19 cm) surrounded by cotton wool raised margins. The wet cotton wool prevented mite escape and maintain leaf freshness for one week. The cotton wool was kept wet by adding water when necessary (Figure 5.1b). In this way, *E. banksi* remained for one week feeding on citrus leaves before being used as a food source for *N. madeirensis* in the different experiments.

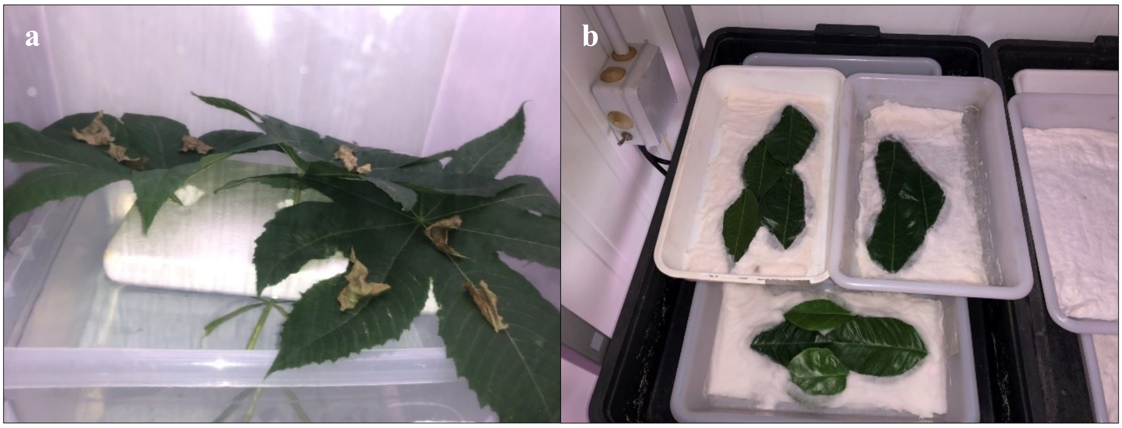

Figure 5.1 a. *E. banksi* colony maintained on turgid castor been leaves for about a week in a plastic container with water. b. *E. banksi* rearing units on detached citrus leaves kept fresh using wet cotton wool.

The predatory mite *N. madeirensis* was collected from castor plants (*R. communis*) in the city of Valencia. On these plants, this mite was found feeding on *Tetranychus urticae* Koch, so this prey was used for the maintenance of the predator culture in the laboratory. Therefore, it was necessary to establish a previous culture of this spider mite in the laboratory to feed the predator. The culture of *T. urticae* was carried out on bean plants (*Phaseolus vulgaris* L.) that were replaced weekly. The phytoseiid mite colony was maintained in similar trays as described for *E. banksi*, but using young, detached bean leaves as a substrate placed with their underside up on the cotton wool layer (Figure 5.2a, b). The leaves were infested with *T. urticae* three days before *N. madeirensis* females were transferred to them. These rearing units were also renewed weekly. To prevent the escape of phytoseiids, in addition to periodically wetting the cotton wool, a *Tanglefoot*® barrier was applied at the edge of each tray.

Figure 5.2 *Neoseiulus madeirensis* rearing units on detached bean leaves previously infested with *T. urticae* and kept fresh using wet cotton wool. a. Dense colony of *N. madeirensis* where all stages of development are present. b. Gravid female of *N. madeirensis* walking on the web of *T. urticae*.

Life-history experiment

Life-history experiment was carried out in a climate-controlled incubator at 25 ± 1 °C, 60-70% RH under a 16:8 hours L:D photoperiod. To obtain synchronized eggs for the experiment, about 100 *N. madeirensis* adult females from the stock colony were confined in rearing units with enough *T. urticae* as a prey to oviposit. Eggs were collected 12 hours later with a fine brush and introduced individually in the experimental arenas to follow the life cycle. These experimental arenas consisted of 3 x 3 cm square cells, prepared as follows. One sour orange leaf was placed inside a Petri dish (14 cm in diameter) with the underside up on cotton wool moistened with water, and to perform the cell, cotton strips also moistened with water were used to prevent the escape of individuals. Inside the cell, a piece of leaf was provided as a shelter. Petri dishes were provided with a perforated lid covered with a fine mesh (4.5 x 4.5 cm), in order to ventilate the interior and avoid humidity condensation (Figure 5.3a, b).

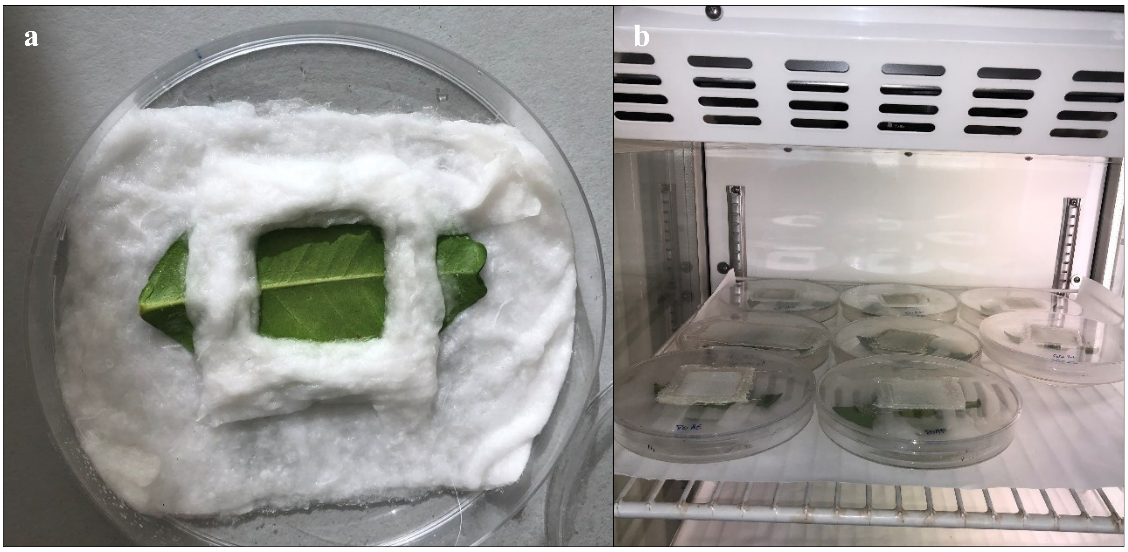

Figure 5.3 a. Experimental arena consist of 3 x 3 cm square cells on orange leaves, placed inside a Petri dish with the underside facing upwards and on moistened cotton wool and strips of cotton wool used to prevent individuals from escaping. b. Petri dishes inside the climate-controlled incubator and closed with a perforated lid covered with a fine mesh.

A mixture of all the developmental stages of *E. banksi* was added everyday to the experimental arenas to provide abundant prey for *N. madeirensis*. During the juvenile development, experimental units were checked every 8 h to determine the duration and the survivorship for each stage, and the presence of exuvia was used as evidence of moulting. Recently emerged *N. madeirensis* adult females were transferred to new experimental units. A male taken from the stock colony was then added to each unit containing one female. Survival, and the number of deposited eggs, were recorded daily until all females had died. Citrus leaves were replaced fortnightly and phytoseiid mites were transferred to the new arenas with a fine brush. The eggs laid were placed together daily in a rearing unit with enough *T. urticae* as prey and reared to adulthood to determine the secondary sex-ratio (female percentage of the studied female cohort offspring). The experiment was done in two rounds of 100 individually reared mites in each one.

A life table was constructed from the observed survival (l_x) and fecundity (m_x) rates. The net reproductive rate (R_o), mean generation time (T), doubling time (D_t), finite rate of increase (λ) and the intrinsic rate of increase (r_m) were calculated following Birch (1948). The r_m was estimated using Lotka-Euler equation:

$$\sum_{0}^{\infty} e^{-rx}\, l_x\, m_x = 1$$

where r is the intrinsic rate of natural increase (r_m), x is the female age in days, l_x is the fraction of females surviving to age x, and m_x is the expected number of daughters produced per female alive at age x, obtained by multiplying the number of eggs by the age-specific sex-ratio, which is defined as the proportion of females in the progeny. Calculation of r_m values was performed by iteration using the non-linear least squares regression procedure based on the Gauss-Newton algorithm with the R-package *petitr* (Pierre 2018), and the uncertainty estimation was conducted using jackknife technique following Meyer et al. (1986). Wilcoxon *rank sum* test were used to assess the differences on developmental time between males and females, the requirements for homogeneity of variances were tested with Levene's test computed with the R-package *car* (Fox and Weisberg 2019).

Prey stage preference experiment

Prey stage preference experiments were conducted to determine which *E. banksi* stage is preferred as a prey for *N. madeirensis*. In the experiments *N. madeirensis* females in their peak oviposition period (9-10 days old) were used. To obtain females of this age, synchronised egg cohorts were obtained in the same way as explained above. For the predator to experience lifelong contact with *E. banksi*, the synchronised eggs were then transferred to the citrus rearing units described above with all *E. banksi* stages, until they reached 9-10 days of age, and starved 24 h prior to participate in the experiment in order to motivate them. To avoid escape, a *Tanglefoot*® barrier was applied at the edge of each tray. Prey preference experiments were conducted at the same place and environmental conditions used for the life-table experiment, and the experimental arenas, were performed in the same way. Prey preferences of *N. madeirensis* among *E. banksi* developmental stages were determined by choice and no-choice tests. Single *N. madeirensis* females were transferred into arenas with specific densities of *E. banksi* stages and the numbers of each stage killed during a period of 24 h was recorded by subtracting the number of individuals remaining from the total number provided.

Two no-choice tests were carried out. In the first one, 30 *E. banksi* individuals of each developmental stage and sex (egg, larvae, nymph, male and female) for a total of 150 prey items, were offered individually to *N. madeirensis* females. In the second one, 30 *E. banksi* individuals of each immature developmental stage (egg, larvae, and nymph) for a total of 90 prey items were offered. Prey individuals were transferred from *E. banksi* rearing units using a fine brush. Twenty *N. madeirensis* females were used as replicates in each experiment. Significant differences in the consumption of the different types of prey were assessed using poisson (log-link) generalized linear models (GLMs). In addition, binomial (logit-link) GLMs were used to assess the proportion of each developmental stage consumed relative to the total. Means were separated through multiple comparisons using Tukey's HSD method (R Core Team 2019).

Under choice conditions we tested 3 ratios: 1:1, 1:2 and 2:1, for the 3 immature developmental stage combinations: larvae:eggs (L:E), nymphs:larvae (N:L), nymphs:eggs (N:E). The 1:1 ratio was performed to evaluate the prey preference, meanwhile 1:2 and 2:1 ratios were conducted to evaluate the prey-stage switching. A total of 60 mite prey per arena were tested for the 1:1 ratio, and 90 for the 1:2 and 2:1 ratio. For each ratio, ten *N. madeirensis* females were used as replicates. Prey stage preferences were quantified with the index β (Manly et al. 1972), as described by Blackwood et al. (2001):

$$\beta = \left[\frac{\ln{(N'/N'_c)}}{\ln{(N/N_c)}} + 1\right]^{-1}$$

where N and N' are the numbers of each prey-stage provided and N_c and N'_c are the numbers of each prey-stage killed. The index assigns preference values from 0–1, where 0.5 represents no preference. The β value was calculated for each replicate and averaged to determine the mean β value for each ratio and prey stage combinations. Prey-stage preference was also assessed in terms of the significant differences in prey consumption proportion (prey consumed/total prey offered) between stages in the 1:1 ratios for each combination of developmental stages (L:E, N:E and N:L) using Student's *t*-test (R Core Team 2019). The normality and homogeneity of variances requirements were tested with Shapiro-Wilk and Levene's test respectively. Prey-stage switching was evaluated on the basis of the differences in the β-index between the 1:2, 1:1 and 2:1 ratios for each of the prey stage combinations. Significant differences were assessed using linear models (LM) for ANOVA analysis (R Core Team 2019). The assumptions of normality and homogeneity of variances were tested in the same way as above.

Functional and numerical response experiment

Ten *E. banksi* densities 5, 10, 15, 20, 30, 40, 60, 80, 100 and 150 prey, per arena of each immature developmental stage (egg, larvae, or nymph), were offered to *N. madeirensis* 9-10 days aged starved females, obtained as described above, and on the same arenas performed in previous experiments. A single *N. madeirensis* female was transferred into an arena with a fixed *E. banksi* egg, larvae or nymph density, and the numbers of prey killed during a period of 24 h was recorded in the same way as explained above. Furthermore, the number of eggs laid by the female phytoseiid was recorded.

The experiment was carried out with prey replacement, whereby for 6 consecutive days the number of prey consumed in each of the arenas were replenished daily. For each density and prey stage, four *N. madeirensis* females were used as replicates, so that a total of 24 prey consumption and oviposition data in 24 h were obtained for each density and prey stage.

The type of functional response of *N. madeirensis,* preying on *E. banksi* eggs, larvae and nymph, was determined using binomial (logit-link) generalized linear models (GLMs) (R Core Team 2019) of prey consumed as a function of prey density offered as described by Juliano (2001):

$$\frac{N_e}{N_0} = \frac{\exp\,(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + \exp\,(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}$$

where N_e is the number of prey consumed, N_0 is the initial prey density, and P_0, P_1, P_2 and P_3 are the maximum likelihood estimates of the intercept, linear, quadratic and cubic coefficients respectively. The sign of P_1 and P_2 are used to determine the type of functional response. Type I responses are described by an intercept ($P_0 > 0$) and constant positive slope ($P_1 > 0$). When the linear coefficient is significantly negative ($P_1 < 0$), the predator displays a type II functional response, which indicates that the proportion of prey consumed declines monotonically with the initial prey density offered. When the linear coefficient is positive ($P_1 > 0$) and the quadratic coefficient is negative ($P_2 < 0$), the predator displays a type III functional response. Maximal models were fitted with linear, quadratic, and cubic terms and simplified in stepwise forward manner by the AIC using the R-package *MASS* (Venables and Ripley 2002). The binomial regression analysis indicated that our data fit a type II functional response for all stages of *E. banksi*, therefore, further analysis were restricted to type II response. Since the experiment was carried out with prey replacement, the appropriate model to estimate handling times (T_h) and the attack rates (*a*) for a type II functional response, is the Holling's disc equation (Holling 1959a, 1959b), since this model provides a good fit, as characterized by high R^2 values, in experiments with sampling and replacement (Fenlon and Faddy 2006).

$$N_e = \frac{aTN_0}{1 + aN_0T_h}$$

where N_e is the number of killed prey, N_0 is the density of prey offered, *T* is the prey exposure time to the predator (in this case, it was 1 day), *a* is the attack rate (proportion of prey captured by the predator per unit of searching time) and T_h is the handling time (time that the predator spends identifying, pursuing, killing, consuming and digesting a prey).

In order to lead with the overdispersion, the R-package *nlme* (Pinheiro et al. 2021) was used to perform a generalized non-linear least squares regression (GNLS) based on the Gauss-Newton algorithm to estimate the parameters a and T_h of the Holling's disc equation (Ritz and Streibig 2008), and the R^2 value was computed as a measure of fit between the data obtained in the experiment and the Holling's model. After a and T_h were determinate for the original data, the differences among a values, as well as T_h values among the different prey stages were tested for significance by estimating the variance using the *bootstrap* technique (Meyer et al. 1986). The R-package *nlraa* (Miguez 2022) was used to compute the GNLSs bootstrapping, obtaining 999 resamples, and the mean values of bootstrap pseudo-values for a and T_h of each prey stage were subjected to Kruskall-Wallis test. Subsequently, post-hoc pairwise Wilcoxon *rank sum* test comparations with Holm's *p-value* correction was performed to separate the means. Data were square root or log transformation to fulfil the variance requirements tested through Breusch-Pagan test using the R-package *car* (Fox and Weisberg 2019).

The numerical response represents the change in the daily oviposition rate as a function of prey density, the relationship between the two parameters was described using the hyperbolic model:

$$y = \frac{nD}{m + D}$$

where y is the daily oviposition rate by *N. madeirensis* at the various prey densities (D), n is the maximum daily oviposition (i.e. plateau), and m is the prey density necessary for the predatory mite to oviposit half the maximum response. To estimate the parameters n and m of the hyperbolic model equation, non-linear least squares regression procedure (NLS), based on the Gauss-Newton algorithm, was used (Ritz and Streibig 2008), and the R^2 value was calculated as a measure of the fit of the fitted model. The R-package *nlstools* (Baty et al. 2015) was used to compute the NLSs bootstrapping obtaining 999 resamples, and the mean values of bootstrap pseudo-values for n and m of each prey stage were subjected to the same statistical analysis as above for GNLSs.

Finally, significant differences in *N. madeirensis* mean prey consumption, percentage of predation (N_e/N_0), eggs laid, and contribution of feeding to reproduction (eggs laid/prey killed) on different prey stages of *E. banksi,* were assessed using poisson (log-link) GLMs and binomial (logit-link) GLMs, followed by Tukey's HSD to separate the means using the R-package *multcomp* (Hothorn et al. 2008), or Kruskall-Wallis test followed by pairwise Wilcoxon *rank sum* test with Holm's *p-value* correction to separate the means. In this case, fourth root transformation was conducted to fulfil the variance requirements tested through Breusch-Pagan test using the R-package *car* (Fox and Weisberg 2019).

5.3 Results

Life table experiment

Neoseiulus madeirensis successfully completed its development feeding on *E. banksi* (Table 5.1). The egg stage was the longest, meanwhile the larval stage was the shortest. Survival decreased throughout development from 100-95%. The duration of the protonymphal and deutonymphal stages were similar. No statistically significant differences in egg hatching between sexes were found (*W*=2310.5; *P*= 0.43); however, the larval stage was longer in males (*W*= 1869.5; *P*= 0.016) while nymphal stages, protonymph (*W*= 3196.5; *P*= 0.003) and deutonymph (*W*= 2992; *P*= 0.032) were longer in females. Therefore, the egg-adult development time was significantly longer in females than in males (*W*= 2952.5; *P*= 0.040).

Table 5.1 Development time in hours (mean ± SE), and survival of immature stages of *N. madeirensis* when fed on *E. banksi*.

	n	Egg	Larva	Protonymph	Deutonymph	Egg-Adult
Female	122	47.26 ± 0.60 a	14.31 ± 0.48 a	31.80 ± 0.63 a	33.20 ± 0.87 a	126.57 ± 1.09 a
Male	47	48.26 ± 0.87 a	16.91 ± 0.85 b	28.21 ± 0.92 b	30.38 ± 1.04 b	123.77 ± 1.44 b
Survival (%)	-	99.5	100	97.5	98	95

Numbers within the same column followed by different letters are significantly different (Wilcoxon *rank sum* test $P < 0.05$)

The age-specific survival *lx* (percentage of surviving females at age x) and age-specific fecundity rate *mx* (number of female eggs laid per female per day) for *N. madeirensis* feeding on *E. banksi* is shown in the Figure 5.4. Survival of females begins to decline progressively at an early age, so the average longevity of the females was around 15 days (Table 5.2a). The form of the time-specific fecundity curve was triangular or trapezoid. Oviposition rate increases rapidly from the beginning of the reproductive period to its peak (2.10–2.13 eggs/day) between the first five to eight days of the oviposition phase; thereafter it drops gradually with fluctuations until the end of the reproductive stage. Females of *N. madeirensis* required around 2 days to initiate egg-laying, after reaching maturity and mating, and the oviposition period was about 12 days during which they laid a total of around 30 eggs on average at an approximate rate of 2.5 eggs per day. Offspring sex-ratio was female biased on average (Table 5.2a). However, if we examine the female age-specific offspring sex-ratio, it ranged between 70.26-86.91%, being 41.60% and 50% at the beginning and end of the oviposition period respectively (Figure 5.4). In relation to demographic parameters, the value of the intrinsic rate of increase (r_m) and finite rate on increase (λ) was 0.273 days^{-1} and 1.31 females/female/day respectively. The average time needed to complete one generation (T) was estimated at 13.44 days, while 2.54 days was calculated to be capable of doubling its population size (D_t). Finally, female offspring production was estimated by calculating the net reproductive rate (R_0) in 21.87 females/female (Table 5.2b).

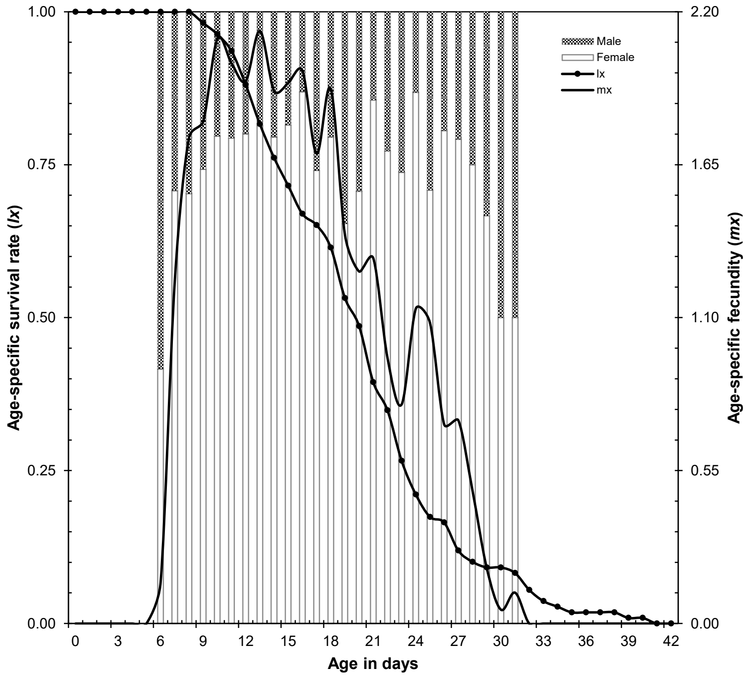

Figure 5.4 Age-specific survival rate (lx) (y-first axis) and age-specific fecundity rate (mx) (y-secondary axis) curves of *N. madeirensis* fed on *E. banksi.* The age-specific offspring sex-ratio is represented in bars (y-first axis).

Table 5.2 Mean ± SE duration of female adult phases, longevity, fecundity, and demography parameters of *N. madeirensis* feeding on *E. banksi.*

a. Female longevity and fecundity	
N	122
Pre-oviposition (days)	1.95 ± 0.08
Oviposition (days)	11.74 ± 0.49
Post-Oviposition (days)	1.40 ± 0.30
Longevity (days)	15.10 ± 0.62
Total fecundity (eggs/female)	28.47 ± 1.27
Oviposition rate (eggs/female/day)	2.44 ± 0.04
Offspring sex-ratio (%)	75.03
b. Demographic parameters	
Net reproductive rate (R_o)	21.87
Mean generation time (T)	13.44
Doubling time (D_t)	2.54
Finite rate of increase (Λ)	1.31
Intrinsic rate of increase (r_m)	0.273

R_0 (female eggs/female), T (days), D_t (days), Λ (females/day), r_m (days^{-1}).

Prey stage preference experiments

The mean prey consumption of *N. madeirensis* significantly differed across the type of prey offered in the first (χ^2 = 491.35; *df* = 4; *P* < 2.2e-16) and second (χ^2 = 7.66; *df* = 2; *P* = 0.022) non-choice experiments. The highest number of prey consumed was observed for eggs, followed by larvae, nymphs, males, and females (Table 5.3a). Significant differences were observed in the relative percentages of each type of prey consumed in the first experiment (χ^2 = 599.79; *df* = 4; *P* < 2.2e-16), where around 40% of the prey ingested were eggs, 35% larvae, 18% nymphs, 10% males and 2% females. These percentages remained significantly (χ^2 = 13.72; *df* = 2; *P* = 0.001) stable in the second experiment, where the consumption of nymphs increased to compensate for the absence of males and females (Table 5.3b).

The choice experiments at 1:1 ratio indicated that *N. madeirensis* showed a slight preference for *E. banksi* eggs when eggs and larvae were offered simultaneously (mean ± SE; β = 0.36 ± 0.035), in the same way as when eggs and nymphs were offered simultaneously (β = 0.41 ± 0.043). When larvae and nymphs were offered simultaneously, a slight preference for larvae versus nymphs was observed (β = 0.44 ± 0.037) (Figure 5.5). Furthermore, the analysis of prey consumption showed that egg consumption was significantly higher when eggs were offered simultaneously and at the same ratio (1:1) with larvae (*t* = -5.07, *df* = 18, *P* = 7.916e-05) and nymphs (*t* = -2.94, *df* = 18, *P* = 0.0087). Similarly, larval consumption was significantly higher when larvae were offered with nymphs at the same ratio (*t* = -3.13, *df* = 18, *P* = 0.0058). The preferences when offered different prey combinations (L:H, N:H and N:L) at ratios 1:1, 1:2 and 2:1 were compared to determine prey-switching behaviour, indicated by significant change in the β index value. No significant prey-switching was recorded for *N. madeirensis* at L:H (*F* = 1.11; *df* = 2; *P* = 0.34), N:H (*F* = 2.23; *df* = 2; *P* = 0.13) and N:L (*F* = 1.40; *df* = 2; *P* = 0.26) prey-stage combinations (Figure 5.5). Therefore, there was no increase in preference for the non-preferred stage in response to the increased density of the preferred stage, to compensate for the relationship between the two prey-stages offered.

Table 5.3 Mean ± SE number of prey consumed and relative consumption of *N. madeirensis* when offered 30 prey items of different developmental stages for 24 h.

Prey stage	Number of prey consumed	% Relative Consumption*
a. First experiment		
Eggs	17.70 ± 0.84 a	39.58 ± 1.76 a
Larvae	14.60 ± 0.97 b	33.08 ± 2.40 b
Nymphs	7.85 ± 1.15 c	16.50 ± 2.20 c
Males	4.25 ± 0.58 d	9.11 ± 1.15 d
Females	0.80 ± 0.32 e	1.73 ± 0.70 e
b. Second experiment		
Eggs	17.17 ± 0.85 a	39.82 ± 2.33 a
Larvae	14.29 ± 1.40 ab	31.88 ± 2.51 ab
Nymphs	12.82 ± 1.14 b	28.29 ± 1.84 b

* Percentage of prey consumed of one stage respect of the total prey consumed. Means within the same column followed by the same letter are not significantly different (Tukey's HSD, *P* < 0.05).

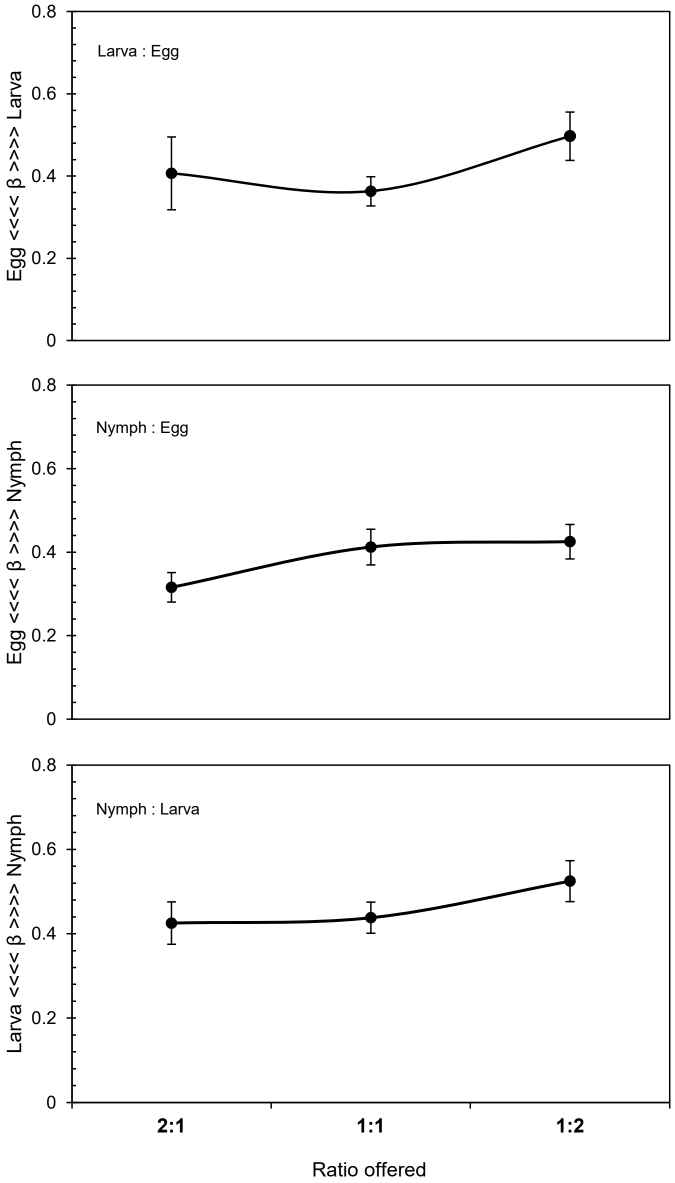

Figure 5.5 Prey-stage preferences according to mean *β* ± SE value when provided varying prey ratios (1:1, 1:2 or 2:1) for the three different immature prey combinations (Larva:Nymph, Egg:Nymph or Egg:Larva).

Functional and numerical response experiment

The number of *E. banksi* eggs killed (mean ± SE) increased from 3.75 ± 0.23 (75.00 ± 4.45; predation: $[N_e/N_0]$ x 100), at a prey density of 5 per arena, to a maximum of 36.21 ± 1.85 (45.27 ± 2.26% predation) at a prey density of 80 per arena. Larvae consumption increased from 4.29 ± 0.23 (85.83 ± 4.48% predation), at a prey density of 5 per arena, to a maximum of 87.00 ± 1.56 (58.00 ± 1.04% predation) at a prey density of 150. Finally, nymphal consumption increased from 4.70 ± 0.13 (94.17 ± 2.49% predation), at a prey density of 5 per arena, to a maximum of 47.43 ± 1.68 (31.62 ± 1.10% predation) at a prey density of 150 per arena (Figure 5.6).

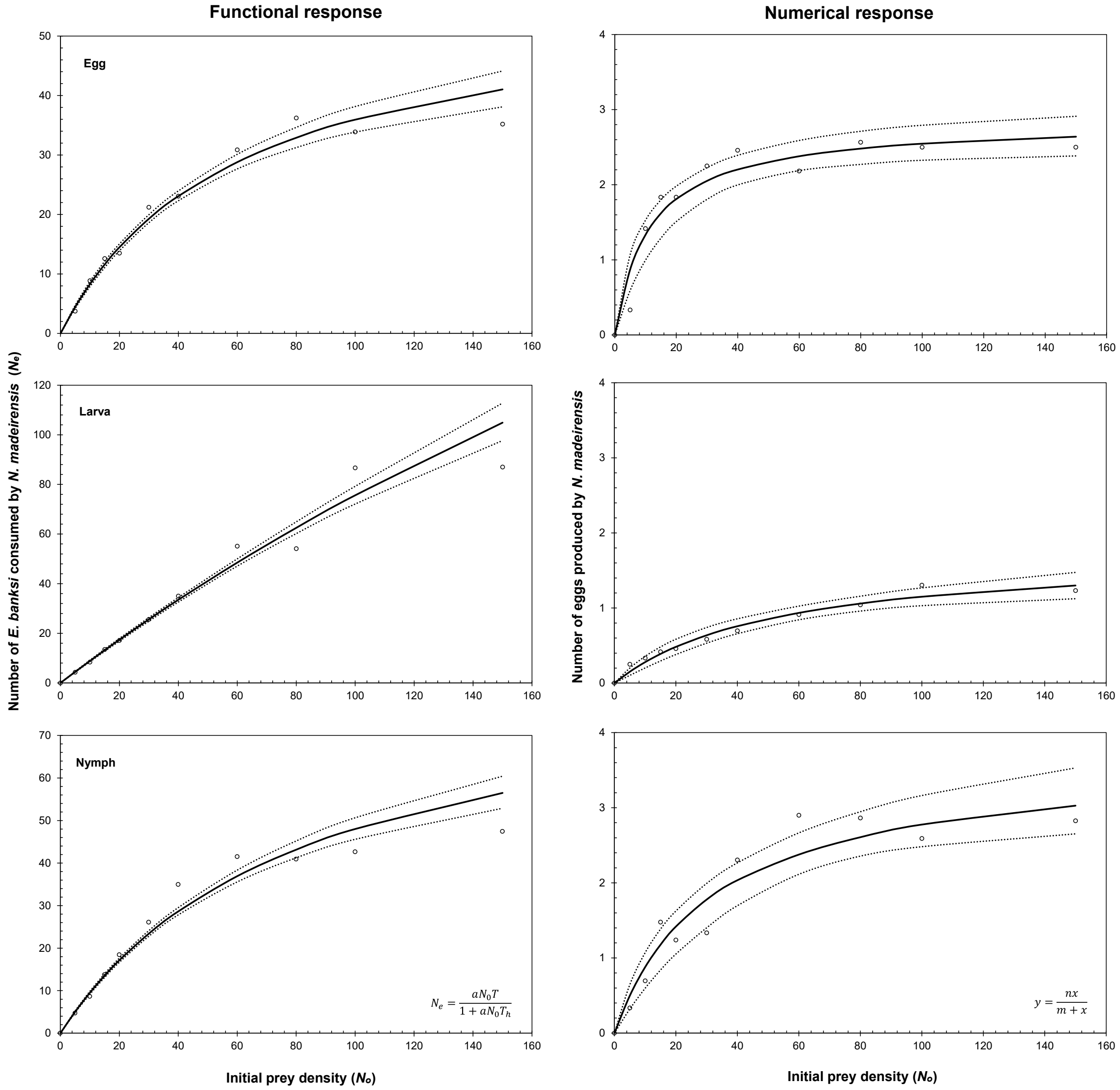

Figure 5.6 Functional and numerical responses of adult female *N. madeirensis* to different developmental stages of *E. banksi*. In functional response graphs, points represent the observed mean prey consumed at each initial prey density, and solid lines were predicted by the Holling's disc equation. In numerical response graphs, points represent the observed mean eggs laid at each initial prey density, and solid lines were predicted by the hyperbolic model. Dashed lines represent the 95% confidence intervals.

In all the three prey stages the proportion of prey killed (N_e/N_0) decreased as a function of prey density offered, and the linear parameter estimated from the binomial (logit-link) regression was significantly negative ($P1 < 0$), suggesting that the predator displayed a type II (convex) functional response on all life stages (Table 5.4). Holling's model fits well to describe the consumption of *N. madeirensis* females on different stages of *E. banksi* prey (Figure 5.6; Table 5.5).

Table 5.4 Maximum likelihood estimates from binomial (logit-link) GLMs of the proportion of prey consumed as a function of initial *E. banksi* prey density by adult female *N. madeirensis*.

Prey stage	Parameters	Estimates	SE	χ^2	*P*-value
Egg	Intercept (P_0)	1.83965	0.13660	13.468	< 0.0001
	Linear (P_1)	-0.04347	0.00696	40.376	< 0.0001
	Quadratic (P_2)	0.00026	0.00009	7.754	0.0054
	Cubic (P_3)	-7.64e-07	3.83e-07	3.985	0.0459
Larva	Intercept (P_0)	2.31165	0.17124	13.499	< 0.0001
	Linear (P_1)	-0.02551	0.00864	8.942	0.0027
	Quadratic (P_2)	0.00031	0.00011	7.314	0.0068
	Cubic (P_3)	-1.57e-06	4.63e-07	11.753	0.0006
Nymph	Intercept (P_0)	2.82978	0.19498	14.513	< 0.0001
	Linear (P_1)	-0.02396	0.00899	7.358	0.0067
	Quadratic (P_2)	-0.00024	0.00011	4.223	0.0399
	Cubic (P_3)	1.64e-06	4.50e-07	12.923	0.0003

The number of eggs laid per day per predator female, feeding on *E.banksi* eggs (mean ± SE), increased from 0.33 ± 0.11, at a prey density of 5 per arena, to a maximum of 2.56 ± 0.23 at a prey density of 80 per arena. Predator oviposition feeding on *E. banksi* larvae ranged from 0.25 ± 0.13, at 5 larvae per arena, to a maximum of 1.30 ± 0.13 at 100 larvae per arena. Finally, oviposition feeding on nymphs ranged from 0.33 ± 0.11, at 5 nymphs per arena, until 2.90 ± 0.20 at 60 nymphs per arena. (Figure 5.6). Estimates of numerical response parameters of *N. madeirensis* females for the three prey stages of *E. banksi* showed that the nymph is the prey-stage with the maximum daily oviposition value (*a*), followed by egg and finally larva (χ^2 =2588.9; *df* = 2; $P < 2.2e\text{-}16$), while the prey density to elicit half the maximum response (*b*), was the highest for larvae, followed by nymphs and finally eggs (χ^2 =2485.1; *df* = 2; $P < 2.2e\text{-}16$) (Table 5.5).

Table 5.5 Estimates of functional response parameters (a) by generalized non-linear least squares regression (GNLS) of Holling 's Type II model, and numerical response parameters (b) by non-linear least squares regression (NLS) of the Hyperbolic model.

a. Functional response

Prey stage	Attack rate (*a*) value ± SE	Asymptotic 95% CI		Handling time (T_h) value ± SE	Asymptotic 95% CI		R^2
		Lower	Upper		Lower	Upper	
Egg	0.9729 ± 0.0356 a	0.9030	1.0428	0.0175 ± 0.0010 a	0.0155	0.0195	0.75
Larva	0.9040 ± 0.0198 b	0.8650	0.9430	0.0021 ± 0.0004 b	0.0012	0.0030	0.95
Nymph	1.0687 ± 0.0261 c	1.0174	1.1200	0.0114 ± 0.0006 c	0.0101	0.0128	0.84

b. Numerical response

Prey stage	Maximum daily oviposition (*n*) value ± SE	Asymptotic 95% CI		Prey needed for a half daily oviposition (*m*) value ± SE	Asymptotic 95% CI		R^2
		Lower	Upper		Lower	Upper	
Egg	2.8511 ± 0.1935 a	2.4657	3.3174	11.3379 ± 3.0813 a	6.1496	19.6374	0.88
Larva	1.7561 ± 0.1616 b	1.4394	2.2358	52.7621 ± 10.9169 b	32.6863	87.4170	0.96
Nymph	3.7116 ± 0.4432 c	2.9070	4.9798	32.2634 ± 10.3482 c	15.9188	65.6431	0.90

Mean having no letters in common are significantly different ($P < 0.05$ Kruskall-Wallis test, pairwise Wilcoxon *rank sum* test with Holm's p-adjust method)

(a) Attack rate coefficient *a* (in units of the proportion of prey captured by each predator per unit of searching time) and handling time T_h (in units of the proportion of 24 h exposure period).

(b) Maximum daily oviposition *n* (maximum number of eggs per day) and the prey density necessary (*m*) for the predatory mite to oviposit half the maximum daily oviposition.

The mean daily prey consumption by female *N. madeirensis* was significantly different for each of the developmental stages of *E. banksi* ($\chi^2 = 873.76$; $df = 2$; $P < 2.2e\text{-}16$), with larvae being the most consumed stage on average, followed by nymphs and finally eggs. Furthermore, the rate of predation (Ne/N_0) was also different ($\chi^2 = 2885.8$; $df = 2$; $P < 2.2e\text{-}16$), following the same trend. The mean daily oviposition rate (eggs laid/day) of *N. madeirensis* females was the same when feeding on *E. banksi* eggs or nymphs, while it was lower when feeding on larvae ($\chi^2 = 167.8$; $df = 2$; $P < 2.2e\text{-}16$). Finally, the contribution of food to reproduction (egg laid/prey killed) was highest for eggs, followed by nymphs and finally larvae ($\chi^2 = 215.9$; $df = 2$; $P < 2.2e\text{-}16$) (Table 5.6).

Table 5.6 *Neoseiulus madeirensis* female summary of prey consumption and egg-laying on the different prey stages of *E. banksi*

Prey stage	**Mean ± SE prey killed/day**^{a}	**Mean ± SE Ne/N_0 %**^{b}	**Mean ± SE eggs laid/day**^{c}	**Mean ± SE eggs laid/prey killed**^{d}
Egg	21.34 ± 0.85 a	60.89 ± 1.68 a	1.965 ± 0.074 a	0.107 ± 0.005 a
Larva	36.07 ± 1.92 b	83.36 ± 1.20 b	0.694 ± 0.053 b	0.026 ± 0.004 b
Nymph	27.70 ± 1.10 c	73.46 ± 1.75 c	1.834 ± 0.082 a	0.071 ± 0.004 c

Mean having no letters in common are significantly different (a) ($P < 0.05$ poisson GLM [log-link], Tukey's HSD, (b) ($P < 0.05$ binomial GLM [logit-link], Tukey's HSD, (c) ($P < 0.05$ poisson GLM [log-link], Tukey's HSD, and (d) ($P < 0.05$ Kruskall-Wallis test, pairwise Wilcoxon *rank sum* test with Holm's p-adjust method).

5.4 Discussion

Life-history experiment

This is the first study about the life-history and feeding behaviour of the phytoseiid *N. madeirensis*, a recently described species with a possible Macaronesian origin, whose existence had gone unnoticed from science until a few years ago (Papadoulis and Kapaxidi 2011). The development time of this species was short in comparison with other phytoseiid species (Gotoh et al. 2004), and significantly longer in females 5.27 ± 0.04 days, than males 5.15 ± 0.06 days. During its development larvae moved slowly and rested most of the time; furthermore, no feeding was observed in that stage, and there was no change in body color from white to dark brown, as was the case for other developmental stages that fed during the experiment. Non-feeding of larvae is not an unusual behaviour, as it has been described for other phytoseiid species. The ecological explanation for this is not clear; however, McMurtry et al. (1970) suggested that it may be advantageous if the larva did not have to find food, assuming that six-legged larva had lower searching ability than the four-legged protonymph. In addition, Chitteden and Saito (2001) concluded that non-feeding behaviour of larvae may be an adaptation to avoid sibling cannibalism. Larvae, protonymphs and deutonymphs remained hidden in the piece of leaf shelter provided, and nymphs moved outside searching for prey. Adults also remained hidden, leaving the shelter to feed and oviposition occurred mainly in the leaf shelter throughout their life. The oviposition period started (0.42) and ended (0.5) with low values of offspring sex-ratio, while most of the time it remained at high values (0.70-0.87). Phytoseiids oviposit only after insemination, and usually require repeated insemination for continued oviposition. Cytological studies present evidence that sex may be determined by a haploid-diploid mechanism (arrhenotoky), and ovigenesis requires some stimulus associated with mating or presence of sperm (McMurtry et al. 1970). In the life-history experiment, when adults were paired, copulation occurred in a few minutes, and within 48 hours the female began to grow in size, which secondarily indicates the beginning of egg production, with the first egg deposited most likely to be a male. The same fact has been observed in other phytoseiid species (Putman 1962) and could be an evolutionary selected life-history trait, since it confers to the female the possibility of dispersing to other plants after the first copulation, carrying her future reproductive partner. This assumption would agree with the dispersal behaviour observed in *N. madeirensis* females, and not in males, after their first copulation, which, in the first 48 h after mating, tried to escape from the arenas, being recovered alive in the wet cotton barrier in the first 24 h and returned to the arena. Once the females began the oviposition period, both male and female adults remained in the arena with few escape attempts. The low offspring sex-ratio recorded by *N. madeirensis* females at the end of the oviposition period could be related to their senescence. Further studies on these features are needed to confirm these assumptions.

The Texas citrus mite is the prevalent spider mite on Florida citrus (Childers et al. 1991). In this region, it has two annual population peaks in late spring-early summer and late autumn-early winter, and although infestations may occur at any time of the year (Childers 1992), populations are maintained at low levels due to the biological control provided by its rich complex of associated phytoseiid mites (Rogers and Stansly 2017; Villanueva and Childers 2005). Therefore, three species of phytoseiids common in Florida citrus have been tested with *E. banksi* as prey. *Euseius mesembrinus* (Dean) was the most abundant in summer (Villanueva and Childers 2005), and a common species in Texas citrus associated with *E. banksi* (Dean 1959a). Its development was not assessed, but the mortality and fecundity of newly mated females were studied at 26 °C and 75% RH. Total fecundity (eggs/female) was estimated at 13.25 with an oviposition rate (eggs/female/day) of 1.558. The net reproductive rate (R_0) was 6.36, generation time (T) was 9.68, $r_m = 0.191$ and $\Lambda = 1.21$. The demographic parameter values obtained for *E. mesembrinus* feeding on *E. banksi* were lower than those obtained in our study for *N. madeirensis*. Furthermore, adult females of *E. mesembrinus* were not able to feed on *E. banksi* eggs, and newly hatched offspring larvae attempted to escape from arenas resulting in low immature survival (4.5%), with a low sex-ratio of 0.54. In our study, *N. madeirensis* females were able to feed on *E. banksi* eggs, and although we did not assess the emergence of offspring, 75.21% of the eggs reached adulthood with a mean sex-ratio value of 0.75. In addition, *N. madeirensis* females took 14.70 days to reach 50% mortality, while *E. mesembrinus* females had a lower survival rate, reaching this value at 13.25 days (Abou-Setta and Childers 1989). In another study, *Galendromus helveolus* (Chant) was observed feeding on the immature stages of *E. banksi* and 70-80% of the offspring reached adulthood after 8 days (Caceres and Childers 1991). This is a very long development time compared to *N. madeirensis*, whose egg-adult development averaged 5.21 ± 0.05 days (average both sexes). Unfortunately, in that study only the oviposition rate for the first 12 days was obtained (1.96 ± 0.94 eggs/female/day). In comparison, *N. madeirensis* showed an oviposition rate of 2.21 ± 0.18 in the same period evaluated on *G. helveolus* females. The last species assessed for *E. banksi* was *Iphiseiodes quadripilis* (Banks), described as a generalist facultative predator that can feed on several families of phytophagous mites (Muma 1971). This predatory mite completed its development satisfactorily feeding on *E. banksi* in 8.5 ± 0.2 days, with an egg to adult survival of 48%, and offspring sex ratio of 0.58 (Villanueva and Childers 2007). This phytoseiid also has a long development compared to *N. madeirensis*, and the developmental survival and offspring sex-ratio were very low compared to the 95% and 0.75 values respectively obtained for *N. madeirensis*.

Generally, demographic and life-history parameters from different studies are difficult to compare, as small differences may be due to strains, experimental methodology, the size and type of arenas, supply of feed, relative humidity, photoperiod and even the methodology of computing the parameters. Despite this, the wide differences are often reliable. Gotoh et al. (2004) compiled demographic parameter values for many phytoseiid species, while Gotoh et al. (2006) studied the response of *Neoseiulus californicus* (McGregor) to different spider mites, and Escudero and Ferragut (2005) conducted life-history experiments on *N. californicus* and *Phytoseiulus persimilis* (Athias-Henriot) using four *Tetranychus* species as a food source. Comparing the life-history traits of *N. madeirensis* feeding on *E. banksi* with those obtained for this two phytoseiids widely used in biological control against spider mites, its developmental time was comparable to both species. Summarizing the survival and reproductive behavior of *N. californicus* and *P. persimilis* when feeding on spider mites, we can see that *P. persimilis* females survived around 30 days while *N. californicus* females until day 40. *Neoseiulus californicus* has a long oviposition period, about 20 days at a lower oviposition rate of 2-3 eggs/female/day, while *P. persimilis* has a shorter one with around 15 days with a higher rate of 3-4 eggs/female/day. As a result, the number of eggs/female is comparable in both species (R_0), the generation time (T) of *N. californicus* is longer, while the intrinsic rate of increase (r_m) in *P. persimilis* is higher. Life span of *N. madeirensis* was similar to that of *N. californicus* and had an oviposition period (11.74 ± 0.49 days) comparable to *P. persimilis*, but with an oviposition rate (2.44 ± 0.04 days) comparable to *N. californicus*, which implies a lower reproductive behavior than both. However, the intrinsic rate of increase (r_m) of *N. madeirensis* was 0.273 which is comparable with the values between 0.227–0.283 obtained for *N. californicus* and inferior to 0.317–0.428 obtained for *P. persimilis*. The r_m was considered the best demographic parameter useful for predicting the potential population growth under given environmental conditions (Andrewartha and Birch 1954).

Prey stage preference experiments

Non-choice experiments showed a clear preference of *N. madeirensis* for preying on immature stages, which decreases towards the adult stages (egg > larvae > nymph > adult). Moreover, predation is greater on males than on females, so that the latter was negligible (<2%). Choice tests among immature stages confirm these preferences, as $\beta < 0.5$ in the Egg:Larvae, Larvae:Nymph and Egg:Nymph ratios. This preference scheme is common and has been described for females of different phytoseiid species (Badii et al. 2004; Reichert et al. 2016). However, if we consider the immature stages of the predator, preferences may change (Devasia and Neravathu 2019). We believe that these preferences can be explained by a trade-off between the energy obtained vs invested by the predator to obtain a prey. According to optimal foraging theory, natural selection should favour foraging behaviours that maximize energetic gains while minimizing the costs involved in searching, handling, and processing food items (Begon et al. 1996). The consumption rate of *N. madeirensis* is inversely related to the life stage, thus size, of *E. banksi.* Larvae, being smaller than nymphs, would be easier to kill, but their small size probably gives them a low nutritional value compared to nymphs or adults, which are more difficult to prey on because of their greater ability to evade the predator due to their size and mobility (Reitz et al. 2006). Therefore, the predator needs to consume a larger amount of prey with low biomass to obtain the same energy it gets from a few larger ones. Eggs are an immobile and defenceless stage and would be a priori the most easily handling stage of *E. banksi*, which would give them preference over the motile stages. However, eggs have a different composition, and their nutritional value probably does not follow the same size rules as in the case of the motile stages. Apparently, the oviposition rate of *Amblyseius largoensis* Muma fed on an egg diet of *Raoiella indica* Hirst is higher than the one obtained from a mixture of stages, suggesting that an egg diet may result in a greater fecundity than a mixed prey one (Carrillo et al. 2010, 2012). Furthermore, according to Badii et al. (2003) and Childers et al. (1991) egg stage is the longest immature stage of *E. banksi* (4.85–13.70 days, depending on temperature), therefore, eggs are available for predators for more time than other prey immature stages.

No significant prey-switching was recorded for the 3 prey-stage ratios tested (Egg:Larva, Egg:Nymph, and Larva:Nymph), so the predator did not compensate the increase in density of the preferred stage with an increase in consumption of the non-preferred stage. The non-significant prey-switching results obtained by us are similar to those obtained for *Neoseiulus fallacis* (Garman) females at ratios of 1:2, 1:1 and 2:1 for eggs:larvae of *T. urticae* as prey (Blackwood et al. 2001). *Neoseiulus madeirensis* is taxonomically close to *N. fallacis*, suggesting that the feeding behaviour of phytoseiids may be partly governed by the evolutionary history of the species. McMurtry and Croft (1997) defined four life-style types in phytoseiids based on diet and related biological and morphological traits, and Blackwood et al. (2001) quantified phytoseiid preferences for preying on specific stages (egg or larva) of *T. urticae* showing that prey-stage preferences correlate well with the life-styles of phytoseiids. Finally, Blackwood et al. (2004), working with *T. urticae* as a prey, conclude that prey-stage preference on phytoseiids performs well as a single-trait indicator of life-style type. In general, species that preferred larvae to eggs ($\beta > 0.5$) were classified as type IV, species with no preference ($\beta = 0.5$) were classified as type III, species with moderate egg preference ($\beta < 0.5$) type II and species with strong egg preference ($\beta << 0.5$) type I. *Neoseiulus madeirensis* showed moderate preference for *E. banksi* eggs to larvae ($\beta \approx 0.4$), and these preferences were maintained when *T. urticae* was used as prey (López-Olmos and Ferragut, unpublished data). Therefore, this species appears to be a type II phytoseiid, usually associated with *Tetranychus* species that produce a dense web-nest and perform worse feeding on little webbing spider mites (McMurtry and Croft 1997). *Neoseiulus madeirensis* showed a high reproductive potential feeding on *E. banksi,* however, it has been frequently found associated with other *Tetranychus* species which produce a dense web like *T. turkestani*, *T. ludeni* or *T. urticae*, showing with the latter even higher demographic parameters than those shown with *E. banksi* as prey (López-Olmos and Ferragut, unpublished data). *Neoseiulus idaeus* Denmark and Muma, a species taxonomically close to *N. madeirensis*, belongs to type II life-styles and has a great reproductive potential for *T. urticae,* but it also performs well feeding on the cassava green mite, *Mononychellus tanajoa* (Bondar), which produces little webbing (Mesa et al. 1990). *Panonychus ulmi* Koch, another spider mite that produces little webbing, was a less favourable food than *T. urticae* in terms of development and oviposition for *N. fallacis* (Genini et al. 1991), another phytoseiid species belonging to type II life-styles and closely related to *N. madeirensis*.

Functional and numerical response experiment

The negative values estimated for the linear parameters, as well as the inverse density-dependent relationship between the proportion of prey consumed and the initial prey density offered on all life stages of the prey, are compatible with a type II functional response. The type II functional response is the most common in phytoseiids (Sabelis 1985), being the functional response shown by *N. californicus* and *P. persimilis* which have amply demonstrated their efficacy in the biological control of spider mites on different crops (Xiao and Fadamiro 2010). Although type II responses are the most common in phytoseiids, it is important to note that this behaviour may change to type III depending on the plant species (Skirvin and Fenlon 2001), the size of foraging areas (Takafuji and Deguchi 1980) or the predator age (Fathipour et al. 2018) among others. Predation and oviposition of *N. madeirensis* increased as a function of an increase in the population density of all stages of the Texas citrus mite. Females consumed higher proportions of prey at lower densities, suggesting high search ability. The proportion of prey killed decreased at high population densities probably because of satiation (Sandness and McMurtry 1970). Some predators at high prey densities reduce their consumption leading to dome-shaped response curves, as at high densities a "swarming effect" is produced which causes some predators to experience a conflict of stimuli that blocks feeding responses or causes disturbances in terms of repeated erratic movements (Holling 1961; Mori and Chant 1966). The feeding behaviour of *N. madeirensis* does not seems to be negatively influenced at the high prey densities tested, since above a certain density, consumption seems to be constant and does not decrease generating a dome-shaped curve.

Mean daily consumption values were significantly different for each of the *E. banksi* prey-stages tested, stabilising at values of 30–40 for eggs, 40–50 for nymphs and 80–100 for larvae. The number of eggs laid per day increased as a function of the number of prey consumed for all *E. banksi* prey-stages showing a good fit with the hyperbolic model. Egg-laying with *E. banksi* nymphs and eggs stabilised between 2–3 eggs/female/day, while with larvae this occurred at values of 1 egg/female/day. No differences were found between the mean number of eggs laid when feeding solely on *E. banksi* eggs or nymphs, but for larvae egg production it was significantly lower.

The attack rate and handling time are parameters used to determine the magnitude of the functional responses. The handling time is a good indicator of the consumption rate and predator efficacy since it determines the cumulative time spent on identifying, pursuing, killing, consuming, and digesting a prey. Meanwhile the attack rate represents the proportion of prey captured by the predator per unit of searching time (Holling 1959a, 1959b). The magnitude of handling time followed the order eggs > nymphs > larvae, while the attack rate followed nymphs > eggs > larvae. The attack rate is higher in nymphs than in eggs, since nymphs are mobile, which favours the encounter between predator and prey (Sabelis 1985). However, the attack rate is higher in eggs than in larvae, which is evidence of the low nutritional content of the latter (Begon et al. 1996). It is common that with the increase in prey size comes an increase in handling time, as larger prey is stronger, more mobile and defend more effectively against predators (Reitz et al. 2006). This would explain the increase in handling time from larvae to nymphs of *E. banksi*. However, the results obtained for eggs are surprising, as it is usual for predatory phytoseiids to have the shortest handling times when feeding on the egg stage of their prey. Eggs are immobile and defenceless, therefore, they usually are easier to handle and subdue, in specially for the type I phytoseiids specialist on spider mite eggs (Sabelis 1990). *Neoseiulus madeirensis* normally required a single attack to consume the larvae, while several attacks were frequently required to kill the nymphs. During the experiments, it was common to observe *N. madeirensis* feeding on a nymph and stop feeding to initiate cleaning behaviours and then continue feeding on the same prey. All these probably increased handling time on nymphs. In the case of eggs, we observed a different behaviour, as the females seemed to have difficulty feeding on them, circling around the eggs and needing several attempts to consume their contents. In general, it has always been thought that the chorion of spider mite eggs is easier to penetrate than the cuticle of motile immatures, which is one of the explanations offered for the preference for eggs over the motile forms of predatory phytoseiids feeding on *Tetranychus* (Sabelis 1985). However, some studies point out that other genera of spider mites have a thick egg-shell, which could be difficult to penetrate (Rahman et al. 2013). Furthermore, we have frequently found this predatory mite associated with *Tetranychus* species, which produce spherical eggs often laid suspended in the webbing they produce for protection. Nevertheless, *E. banksi* does not produce webbing and its eggs are disk-shaped and attached to the leaf surface.

The nutritional benefit that phytoseiids obtain from each of the prey-stage supplied can be estimated by comparing the fecundity of females when feeding solely on each prey-stage (Carrillo and Peña 2012). Fitting the hyperbolic model to the numerical response allows us to estimate the parameters of the maximum daily oviposition rate and the prey density required to obtain half the maximum response. Both parameters allow us to compare the numerical response as a function of the different prey-stages consumed. The maximum daily oviposition followed the following pattern: nymphs > eggs > larvae, while the amount of prey required for the predator to display half of its egg production was: larvae > nymphs > eggs. Furthermore, the contribution of each prey to the total egg production followed the same pattern. These results indicate that eggs are the most nutritious prey-stage for *N. madeirensis*, which explains the preference observed in prey-stage preference experiments. However, maximum egg laying is achieved by using the nymphs as a food source. This apparent contradiction can be explained through the existence of a trade-off in the predatory behaviour (Begon et al. 1996). Eggs are highly nutritious, however, a lot of energy is invested in their consumption, while nymphs, despite being less nutritious are easier to consume, so the energy balance in a solely stage diet could be higher. The larvae are poor in nutrients and too much energy is invested in attacking many prey, which would limit the egg laying. Different studies have pointed out the wide nutritional content of spider mite eggs to phytoseiid mites, achieving egg laying sizes even higher than those obtained by consuming a mixture of prey-stages (Blackwood et al. 2001; Carrillo and Peña 2012). *Galendromus occidentalis* (Nesbitt) feeding on a single prey-stage of *Tetranychus pacificus* McGregor, killed twice as many larvae as eggs but achieve similar fecundities in each case (Croft and McMurtry 1972), so the nutritional benefits of prey eggs to larvae was estimated around 3:2 in terms of predator egg production (McMurtry and Croft 1987).

This study has been conducted to obtain information on the ability of *N. madeirensis* to control *E. banksi*. Life-history experiments revealed that *N. madeirensis* exhibits shorter developmental times, and higher survival and reproductive rates when fed solely on *E. banksi* compared to other *E. banksi* phytoseiid predators currently tested. Furthermore, its demographic parameters were comparable to those shown by the most common phytoseiids commercially used for spider mite control. Prey-stage preference experiments showed a clear preference for the immature stages of *E. banksi*, with eggs being slightly the most preferred stage. Preference for eggs, from a pest management point of view, is a desirable attribute in a natural enemy, as it kills its prey before it causes damage to its host plant. Functional and numerical response experiments suggest that *N. madeirensis* could play a role in reducing *E. banksi* populations; however, its high consumption rates at low prey densities suggest that the predator is more effective at low pest populations. Therefore, in an augmentative release program, it should be released early in the infestation. For all these reasons, we believe that *N. madeirensis* is a suitable candidate for the development of a biological control program to manage *E. banksi* infestations. However, the results obtained must be considered with caution, as they have been obtained under laboratory conditions, in a climatic chamber and on detached leaves. Under natural conditions, when predators live on the whole plant, they can disperse to other plants and interact with other prey, food sources, as well as with other predators, which can lead to a reduction in their efficiency. Future studies on prey preference and possible intra-guild predation with other phytoseiids that have high densities in Spanish Mediterranean citrus may provide answers to resolve these questions. Finally, field studies based on releases of known quantities of phytoseiids to control known pest densities are needed to determine the efficiency of this predator in citrus orchards.

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CHAPTER 6

General Discussion

The international mobility of people and goods usually cause unintentional transportation and introduction of invasive pests in agriculture leading to economic losses for farmers (Pimentel et al. 2000). Phytophagous mites are small and difficult to detect, having a high potential to become an invasive species (Navia et al. 2010). Among them, spider mites belonging to the family Tetranychidae are the most damaging group to cultivated plants (Jeppson et al. 1975). In addition to economic losses, invasive species provide an excellent opportunity to study the ecological consequences after the invasive process and the changes occurring in the resident community as a consequence of the establishment and population growth of alien species. Assessment of the economic impact has been performed in the case of some invasive spider mites, but their effects on native or already naturalized biota in agricultural ecosystems have only been carried out on a few cases (Reitz and Trumble 2002; Ferragut et al. 2013a).

The second chapter of this thesis studied the impact of Texas citrus mite in the local communities of spider mites on citrus after its arrival to the Valencian region. TCM has spread since 2013 quickly becoming the most frequent and abundant spider mite on citrus and competitively displacing the relatives *P. citri* and *E. orientalis*. There is no accurate information about the geographic distribution of *E. orientalis* prior to the invasion by TCM in 2013. However, García-Marí et al. (1983) and Abad-Moyano et al. (2009a) reported that *P. citri* was widely distributed throughout the Valencian region, and a recession in its distribution area is currently occurring probably due to the invasion of *E. banksi*. The results of the present work have demonstrated that the invasive TCM is widespread in the region, moving north and reaching the northern citrus-growing regions. The findings also suggested that spider mite communities on Valencian citrus showed spatial patterns of geographical distribution which do not seem to be only driven by climatic conditions. Historical factors as well as interspecific competition can act spatially structuring communities (Borcard and Legendre 1994). Current geographic distribution for the three mite species is not coincident and provides evidence of their competitive relationships, as well as their density cores and dispersal from the establishment sites. In fact, the current geographical range of both, TCM and *E. orientalis,* is related to the place of their first detection, the south of the province of Valencia for TCM, and the south of the province of Alicante for *E. orientalis*. *Panonychus citri* is the least spatially and the highest environmentally structured species, since it has been present in the Valencian region over 40 years (García-Marí and del Rivero 1981), so there is currently no spatial evidence of its colonisation, but its geographic range is affected by competition with *Eutetranychus* species. *Eutetranychus banksi*, recently introduced, has spread rapidly displacing the other two mite species and, accordingly, its spatial distribution is less affected by competition and colonization history compared to *E. orientalis*, that has a very large spatial component due to intense competition with TCM and the recently historical colonisation processes.

There are different non-exclusive hypotheses that may explain the ecological impact of TCM on the whole community. Following the *"Enemy Release Hypothesis" E. banksi* could be favoured in its competition with *P. citri*, as it escapes attack by *E. stipulatus*, which control *P. citri* populations for most of the year (Ferragut et al. 1988). Seasonal dynamics of *Eutetranychus* species are coincident with that of *P. citri* (Ferragut et al. 1988; Ledesma et al 2011; Monzó et al 2016), and these mites perform better at higher temperatures (Childers et al. 1991; Imani and Shishehbor 2009), being better competitors than *P. citri* for space and nutrients during summer, and able to suppress its population peak in late summer-early autumn, which would explain its displacement by competition according to the "*Resource Depletion Hypothesis"*. Likewise, indirect competition can exist between two herbivores through changes in the quality of the shared plant (Karban and Myers 1989; Tallamy and Raupp 1991). Some spider mites can induce plant defences of their host plant to their advantage and to the detriment of a competing species (Hougen-Eitzman and Karban 1995), so this mechanism could play a role in the competitive displacement carried out by *E. banksi.* In our study, intense competition was detected between the two *Eutetranychus* species. Although both species perform well at high temperatures, *E. banksi* produce twice as many eggs than *E. orientalis* (Childers et al. 1991; Imani and Shishehbor 2009), and fecundity differences could be another factor driving the competitive displacement of *E. orientalis*. Furthermore, they are a taxonomically related species, so reproductive interference could contribute to the competition between them. As their native ranges do not overlap, they likely do not have recognition mechanisms that act as a barrier to avoid interspecific mating which are ineffective producing no viable eggs, no females in the offspring, or hybrid and infertile females (Boudreaux 1963; Smith 1975).

Niche partitioning processes make possible the coexistence between competitive species (Albrecht and Gotelli 2001), and spatial, food and time niche partitioning could promote relaxing competitive relations between competitors. Competition between *E.*

banksi and *P. citri* in Florida citrus has been reported before by Childers (1992), where TCM has become the dominant species in the orchards since 1955, displacing *P. citri*. Therefore, with the progressively spread of *E. banksi*, the competitive displacement and decline of *P. citri,* from the entire or most of the citrus growing areas in the Valencian region, seems a reasonable hypothesis. On the other hand, the phylogenetic proximity, and the consequent niche similarity and intense competition between both *Eutetranychus*, makes coexistence even less plausible.

Following the introduction of a new invasive pest into an agricultural area, strategies for its management need to be developed. Under the perspective of Integrated Pest Management (IPM), cultural practices, biological and biotechnological control should be combined with chemicals to produce healthy crops and minimize the use of pesticides, reducing the risks to human health and the environment. Before developing an IPM programme against *E. banksi*, it is necessary to better understand the population biology of the pest, as well as its associated natural enemies in the invaded area. Phytoseiid mites are considered as the main natural enemies of spider mites on citrus crops around the world (Gerson 2003; Vacante 2010).

For these reasons, the third chapter of this thesis studies the spatial and temporal dynamics of *E. banksi* and the main phytoseiid predator, *E. stipulatus*. TCM showed a clear preference for leaves over fruits in agreement with observations made in Florida citrus (Childers 1992; Rogers and Stansly 2017), while both substrates are used equally by phytoseiid mites. When we focus on leaves, TCM preferred to feed on the sunniest sides at the periphery of the canopy, and on the adaxial (upper) side of leaves close to midrib, as was reported by Dean (1952, 1959a). Furthermore, it also prefers leaves coming from current year's shoots as opposed to leaves from the previous year, probably due to the microclimatic conditions, nutrients, previous damage, or the presence of dust to refuge. On the contrary, phytoseiids preferred the underside of leaves located inside the tree canopy, where they spend most of their lifetime, as was previously observed by Ferragut et al. (1988) on Valencian citrus, Villanueva and Childers (2005) on sweet oranges in Florida, and Landeros et al. (2004) on grapefruit in Mexico. However, they must move outside to feed on spider mites preferring leaves of the previous year, located at mid-level in the canopy, and less exposed to unfavourable climatic conditions. This finding is related with the pest population growth as we will explain in detail later.

Texas citrus mite was detected on fully developed leaves of the current year's spring and summer shoots in mid-summer during July-August, showing a single population peak in late summer-early autumn (September–October). Once autumn leaves are available, they move to occupy them, and this migration from old to new leaves is consistent with the behaviour observed in other spider mite species (Gotoh 1987), and probably related to changes in hardness, dryness, wax deposition, and nutrients that occur in aged leaves, making it difficult for spider mites to feed. In April, average temperatures increase and the growth of *E. banksi* probably restarts, being usually detectable in the field in late July–early August leading the single population peak coinciding with the highest monthly average temperatures, which are in the optimum range between 28–31 °C for the species (Childers et al. 1991; Badii et al. 2003). High RH and precipitation in autumn may contribute to reduced TCM populations, as were reported by different authors (Dean 1952, 1959b, 1980; Muma et al. 1953; Childers 1992; Rogers and Stansly 2017), and low winter temperatures cause usually undetectable mite densities, although small populations composed of all developmental stages could be observed in some localities.

Euseius stipulatus was the most abundant and frequent phytoseiid species, showing a seasonal dynamic with two population peaks (spring and autumn). The first one in May–June was the most abundant and could be related to the availability of pollen from the crop and non-crop plants (McMurtry and Croft 1997; McMurtry et al. 2013; Warburg et al. 2018), together with the spring mild temperature regime under a Mediterranean climate (Warburg et al. 2019; Ferragut et al. 1987). In summer, strong population decline occurs related to high temperature and low RH (Ferragut et al. 1987, 1988; Ragusa 1986; Sahraoui et al. 2014; Warburg et al. 2019), and in September densities recover leading to an autumn peak of lower abundance and maintained at low values throughout the winter, when the mite feeds on other food sources (Ferragut et al. 1987; Zhimo and McMurtry, 1990). On fruits, phytoseiids display the autumn population peak using the navel of sweet oranges or the calix region for shelter and lay eggs, as was described in other crops (Gurra et al. 1997). In addition to *E. stipulatus*, other phytoseiid species, such as *T. phialatus* and *N. californicus,* were relatively abundant, in agreement with Ferragut et al. (1988), Abad-Moyano et al. (2009a); and Ledesma et al. (2011). Both predators are also affected by unfavourable summer conditions (Ragusa 1986; Ferragut et al. 1987; Gotoh et al. 2004; Walzer et al. 2007), and are more abundant in periods with low densities of *E. stipulatus* showing their respective population peaks in autumn. *Euseius stipulatus* has been described as a strong intraguild predator on other phytoseiids on citrus (Abad-Moyano et al. 2010), probably maintaining other predatory mites at low densities during spring and winter.

Phytoseiids exhibit different behaviour and changes in colour from white to reddish-brown when the *E. banksi* population increases on leaves and fruits, demonstrating the existence of predator-prey relationships. Phytoseiids move to the upper side of leaves on the periphery of the canopy and also increase the movement to the fruit to feed on *E. banksi,* since, in the absence of its prey, do not usually inhabit the upper side of peripheral leaves and fruit. Within-plant daily migrations seem to be a common behaviour in phytoseiids, which remain sheltered during the day inside the tree canopy to avoid unfavourable climatic conditions and migrate at dusk to feed on spider mites outside the tree canopy during the night, coming back inside at dawn (García-Marí et al. 1985; Onzo et al. 2003, 2010). Surveys were carried out all year-round during sunlight hours, and therefore we cannot corroborate this hypothesis. However, despite the adverse weather conditions for phytoseiids during the day-light hours, changes in behaviour and body colour indicate that they respond readily to the increase of the pest and feed on it, which supports the idea that they contribute to the biological control. Nevertheless, the results also indicated that this contribution is not sufficient for two main reasons: (1) the population peaks of both, predator and prey are not coincident and thus, the prey consumption does not have a significant impact on the pest, and (2) the predator-prey ratio in summer and early autumn always favours the prey, which is 60–100 times more abundant than its predator, making biological control impossible. Furthermore, *E. banksi* is not a suitable food source for *E. stipulatus*, delaying its development, and strongly reducing its survival rate and fecundity in laboratory studies (López-Olmos and Ferragut, in preparation). For all these reasons, *E. stipulatus* is not a suitable candidate for the biological control of *E. banksi*, despite its predominance as a predatory mite on citrus.

Chemicals are commonly used to maintain mite population at low levels on citrus, and TCM has been shown to be sensitive to various acaricides, being easy to keep under control with this strategy (Monzó et al. 2016). However, a better knowledge of pest biology and the development of a sampling plan are necessary for successful IPM. Sampling plans have been developed for *P. citri* and *T. urticae* on citrus (Jones et al. 1984a, 1984b; Garcia-Marí et al. 1992, 1994; Martínez-Ferrer et al. 2006); however, none have been developed for any *Eutetranychus* species, and only in Florida a set of sampling and treatment threshold guidelines developed empirically for *E. banksi* are provided to farmers (Rogers and Stansly 2017).

Therefore, the study of the population age structure of *E. banksi*, as well as the development of a sampling plan have been carried out in the fourth chapter of this thesis. The age structure was characterised by a higher abundance of juvenile stages as was described for other spider mites feeding on different crops (Carey 1982). Results showed differences in the relative proportion of developmental stages, as well as in the sex-ratio observed in the different plant feeding substrates. A progressive increase in the proportion of eggs and sex-ratio has been found negatively correlated with leaf age. Leaves from the previous year harbor a low mite density and the lowest egg proportion and sex ratio. Previous year's leaves are often damaged by mite feeding, and the level of injury influences the mite reducing egg laying and sex-ratio (Henderson and Holloway 1942). Furthermore, females are the main mobile stage that moves to colonize newly expanded leaves (Gotoh 1987; Krainacker and Carey 1991), producing sex-ratio bias towards females on newly expanded leaves in autumn, and the corresponding bias towards males in the spring and summer leaves. Three overlapping generations occurring simultaneously on leaves and fruits have been recognized. Sex-ratio fluctuates constantly over time although it is always biased towards females with 0.7–0.75 female proportion, as was described for other tetranychid species (Boudreaux 1963; Helle and Pijnacker 1985). Furthermore, the proportion of eggs in the population was highly correlated with the proportion of females in the adult proportion, which is consistent with the observations of other authors in other spider mites (Krainacker and Carey 1991; Yasuda 1982). As in the case of leaf types, the sex-ratio was a highly density-dependent factor over time, since the lowest proportion of females, both in leaves and fruits, coincided with the population peak. Spider mites reproduce by arrhenotokous parthenogenesis, with females controlling the sex-ratio of their offspring (Young et al. 1986). In presence of a deteriorated food resource, such as old-damaged leaves, females increase the proportion of female daughters in their offspring that will disperse to colonize a new flush. However, males remain on old leaves fertilizing the females, increasing their success in colonization, since fertilized females are more efficient in colonizing new environments than unfertilized females (Wrensch and Young 1975).

Eutetranychus banksi showed strongly clumped distribution on leaves and fruits with significant differences between developmental stages. Immatures were significantly more aggregated than adults, no differences were found between eggs and nymphs, while small differences were detected between sexes, with females being less aggregated. Similar differences between developmental stages were obtained for *P. citri* (Jones and Parrella 1984a; Zalom et al. 1985; Mico et al. 1992), and both species also have the same within-tree distribution. According to this shared behavior, both spider mites showed no differences in aggregation patterns between plant strata, although the motile forms of *E. banksi* are more aggregated on the leaves than those of *P. citri,* being organized into dense colonies that completely occupy the leaf upper surface in the outer part of the canopy and the sunny sides on fruits. On the other hand, *T. urticae* on citrus lives all year round in dense colonies on the lower side of leaves, and is not until August–September when it colonizes the fruit (Ansaloni et al. 2008), with a lower aggregation pattern (Martinez-Ferrer et al. 2006). For this reason, TCM females showed a lower aggregation than *T. urticae* on leaves, but comparable on fruits.

A significant linear relationship between motile forms and adult females with the total *E. banksi* population makes it possible to use either as representative stages for the development of a sampling methodology. The use of females as a representative stage, and presence-absence monitoring (binomial sampling), reduces effort making sampling feasible without losing excessive accuracy. Monitoring the presence/absence of females on 100 leaves for binomial sampling or, to be more accurate, motile forms on 400 leaves since the beginning of summer, is sufficient to early detect and estimate *E. banksi* populations. Furthermore, there is evidence that *E. banksi* populations on citrus are spatially structured at orchards spatial level (Holloway et al. 1942; Dean 1959a), and in this situation the use of transects has been an effective tool for estimating population density accurately (Hall et al. 1994). Observation of 2 leaves per tree from 25 trees per transect along 2 diagonal transects across the orchard, a first transect between the north-east and south-west corners, and a second transect between the north-west and south-east corners is recommended according to the obtained results. This sampling plan involves monitoring for presence-absence of females on a similar number of symptomatic leaves as suggested for monitoring of *T. urticae* females at high densities (Martinez-Ferrer et al. 2006). However, since *E. banksi* has a more aggregated spatial distribution than *P. citri* (Garcia-Marí et al. 1992), the sampling effort to estimate the population with the same pre-fixed error should be higher. No accurate sampling methods for the *Eutetranychus* species have been found in the literature. In Florida, motile forms binomial sampling of

96–32 leaves on 24–8 trees is recommended to estimate densities of 5–15 motile forms of *P. citri* and *E. banksi* per leaf, respectively, since an average of 5 motile forms per leaf corresponds to 70–80% infestation (Rogers and Stansly 2017). However, the binomial sampling plan for *E. banksi* described in this thesis requires more leaves since this species should need to infect 70–80% of leaves to reach values of 10–15 motile forms per leaf.

In addition to chemical control, biological control is an environmentally respectful pest management strategy that prevents the development of chemically resistant pest populations, so under the IPM perspective both strategies should be combined. As *E. stipulatus* is not able to keep the pest under control in conservation techniques, it would be necessary to select other natural enemies as biocontrol agents. For this purpose, we have selected the phytoseiid mite *Neoseiulus madeirensis* Papadoulis & Kapaxidi, a predator recently discovered and associated to *E. banksi* on citrus in Huelva and Valencia and to *T. urticae* on ornamental plants in different localities of the Mediterranean coast. Nothing is known about its biology, and it has never been evaluated or used in biological control programs, but its evident relationship with *E. banksi*, suggests that it can be a promising candidate for pest suppression.

Therefore, the fifth chapter of this thesis aims to assess the ability of *N. madeirensis* to control *E. banksi* populations on citrus leaves. *Neoseiulus madeirensis* showed high immature survival when fed on *E. banksi*, as well as a short developmental time, being longer in females than males. Furthermore, female survival and reproduction was much higher than that shown by other phytoseiids previously tested for biological control of *E. banksi* (Abou-Setta and Childers 1989; Caceres and Childers 1991; Villanueva and Childers 2007), making it the most promising phytoseiid predator tested so far. In addition, *N. madeirensis* displayed life-history traits comparable to the two phytoseiids widely used in biological control against spider mites: *Neoseiulus californicus* and *Phytoseiulus persimilis*, which evidences its capacity to suppress spider mite populations (Gotoh et al. 2004, 2006; Escudero and Ferragut 2005).

Females of *N. madeirensis* showed a clear preference for preying on immature stages as common in other phytoseiid species (Badii et al. 2004; Reichert et al. 2016). No significant prey-switching was recorded for this predator, so it did not compensate for the increase in density of the preferred stage with an increase in consumption of the non-preferred stage. This behaviour was recorded before for other phytoseiid species taxonomically close (Blackwood et al. 2001), suggesting that feeding behaviour may be partly governed by the evolutionary history of the species. Due to the moderate preference for *E. banksi* eggs to larvae, this predator could be classified as type II life-styles phytoseiid (Blackwood et al. 2004), usually associated with *Tetranychus* species that produce a dense web-nest and perform worse feeding on little webbing spider mites (McMurtry and Croft 1997). *Neoseiulus madeirensis* showed a high reproductive potential feeding on the little-webbing spider mite *E. banksi;* however, it has been frequently found associated with *Tetranychus* species, which produce a dense web, showing even higher demographic parameters (López-Olmos and Ferragut, data not published). Other type II phytoseiid related species perform well with little-webbing spider mites, and also feed on the dense webbing *T. urticae* (Mesa et al. 1990; Genini et al. 1991), evidencing once again food preferences related to the evolutionary history.

Neoseiulus madeirensis displayed a type II functional response, the most common on phytoseiids (Sabelis 1985) and the same type displayed for *N. californicus* and *P. persimilis* (Xiao and Fadamiro 2010). Predation and oviposition increased as a function of an increase in population density of all stages of the prey. The proportion of prey killed decreased at high population densities probably because of satiation (Sandness and McMurtry 1970). Mean daily consumption values were significantly different for each of the *E. banksi* prey-stages tested, stabilising at values of 30–40 for eggs, 40–50 for nymphs and 80–100 for larvae. Egg-laying with *E. banksi* nymphs and eggs do not significantly differ and was stabilised between 2–3 eggs/female/day, while with larvae this occurred at significantly lower values around 1 egg/female/day. The magnitude of handling time followed the order: eggs > nymphs > larvae, while the attack rate followed nymphs > eggs > larvae. Handling time usually increases with the prey size, being the lowest in the eggs since they are immobile and defenceless (Reitz et al. 2006; Sabelis 1990); however, sometimes eggs have a thick shell that is difficult to penetrate (Rahman et al. 2013). Maximum female daily oviposition followed the following pattern: nymphs > eggs > larvae, while the amount of prey required for the predator to display half of its egg production was: larvae > nymphs > eggs. Eggs are the most nutritious prey-stage, which explains the preference observed in the experiments. However, maximum egg laying is achieved by using the nymphs as a food source. This apparent contradiction can be explained through the existence of a trade-off in the predatory behaviour (Begon et al. 1996). Eggs are highly nutritious, but a lot of energy is invested in their handling-time and consumption, while nymphs, despite being less nutritious are easier to consume, so the energy balance in a solely stage diet could be higher. In agreement with the results, different studies have pointed out the wide nutritional content of spider mite eggs to phytoseiid mites (Blackwood et al. 2001; Carrillo and Peña 2012; Croft and McMurtry 1972; McMurtry and Croft 1987)

According to the experimental results, *N. madeirensis* seems to be a suitable candidate for the development of a biological control program to manage *E. banksi* through augmentative releases or importation aiming for the establishment of permanent populations on citrus to provide long-term control. However, the results obtained must be considered with caution, as they were obtained in laboratory conditions. Future laboratory studies on prey preference and intra-guild predation with other phytoseiids, that have high densities on Spanish citrus, may provide answers to resolve these questions. Finally, field studies based on releases of known quantities of phytoseiids to control known pest densities are needed to determine the efficiency of this predator on citrus orchards.

CHAPTER 7

Conclusions

The newcomer takes it all: the invader Texas citrus mite, *Eutetranychus banksi* (Acari: Tetranychidae), displaces the resident relatives on citrus agrosystems

i. Since its arrival to the Valencian region in 2013, *E. banksi* has become the most frequent and abundant spider mite species on citrus leading to a significant reduction in the presence of their relatives *P. citri* and *E. orientalis*.

ii. Co-occurrence analysis provide evidence for a community structured by competition between the specie pairs *E. banksi–E. orientalis* and *E. banksi–P. citri*.

iii. *E. banksi* lead to a reduction in the geographic range of the other related species, since generalized linear model (GLM) analysis showed that the probability of finding *E. orientalis* or *P. citri* decreases with increasing *E. banksi* geographic density and vice versa.

iv. Principal component analysis (PCA) and permutational multivariate analyses of variance (PERMANOVA) found competition between the two pairs above mentioned, and also between the *E. orientalis–P. citri* pair.

v. Redundancy (RDA) and variation partitioning (VPA) analyses revealed that the geographic distribution of the three spider mites is not caused by environmental conditions, but it is strongly influenced by their colonization history and competitive relationships.

vi. Several factors could be playing an important role in the competitive displacement. Among them, the lack of effective natural enemies of *E. banksi* in the invaded area, as well as its better performance at high temperatures could be causing the displacement of *P. citri*, while the lower reproductive interference and higher fecundity of *E. banksi* could be reducing *E. orientalis*. The coexistence of the three mite species on Valencian citrus seems unlikely.

Within-tree distribution and seasonal dynamics of *Eutetranychus banksi* (Acari: Tetranychidae) and *Euseius stipulatus* (Acari: Phytoseiidae) on citrus: Implications for the biological control of the pest

i. Texas citrus mite feeds mainly on the adaxial (upper) side of leaves from the current year's shoots located at the periphery of the tree canopy, whereas phytoseiids prefer the abaxial (lower) side of leaves inside the tree canopy. Furthermore, TCM preferred to feed on leaves rather than fruits, whereas phytoseiids use both substrates equally.

ii. *Eutetranychus banksi* showed a seasonal trend on leaves with a single population peak in late summer-early autumn, while phytoseiids showed two peaks, a main spring peak and a second less abundant in autumn. Moreover, the seasonal dynamics of *E. banksi* and phytoseiids on fruits occurred in parallel to those recorded on leaves.

iii. *Euseius stipulatu*s was the most frequent and abundant phytoseiid species, apparently displacing other predatory mites on Valencian citrus acting as a strong intra-guild predator.

iv. In autumn, when *E. banksi* reaches high densities, phytoseiids moved from the leaf abaxial sides inside the tree canopy to the adaxial sides in periphery and to the fruits, to feed on *E. banksi*. In addition, a change in phytoseiid colouration from white to reddish-brown was observed, revealing that the predator actually feed on the pest.

v. Chi-square, linear models (LM) and GLM analyses showed that both, the proportion of leaves occupied by red phytoseiids and the number of coloured phytoseiid, increased significantly in response to the prey population growth, which was indirect evidence of their contribution to the biological control.

Population structure, spatial distribution, and sampling plan for the Texas citrus mite, *Eutetranychus banksi* (Acari: Tetranychidae) on citrus

i. *Eutetranychus banksi* showed differences in the population structure and sex-ratio on fruits and leaves, as well as between leaves from different flushes, since a progressive increase in the proportion of eggs and sex-ratio has been found negatively correlated with leaf age.

ii. Three overlapping *E. banksi* generations occur simultaneously on leaves and fruits with sex-ratio fluctuating over time although it is always biased towards females with 0.7–0.75 female proportion.

iii. The sex-ratio was correlated with the population structure since female and egg peaks coincide, but was a highly density-dependent factor, since the lowest proportion of females coincided with the peak of population density.

iv. There were no differences in the aggregation pattern at the different plant strata sampled; however, immature stages showed a higher aggregation than adults, females being the sex with the lowest aggregation index.

v. There was a highly significant linear relationship between *E. banksi* motile forms and adult females with the total population, thus both were used as reference stages to develop sampling plans.

vi. We recommend a binomial sampling of 100 leaves for female monitoring, sampling 2 leaves per tree on 25 trees per transect regularly spaced along two diagonal transects, the first oriented north-east to south-west and the second north-west to south-east. To be more accurate, it is possible to examine the presence/absence of motile forms. In this case, 4 leaves per tree in 50 trees per transect should be monitored.

Life-history, prey-stage preference, functional and numerical response of the predatory mite *Neoseiulus madeirensis* (Acari: Phytoseiidae) to *Eutetranychus banksi* (Acari: Tetranychidae) on citrus

i. *Neoseiulus madeirensis* showed high immature survival when fed on TCM, as well as short developmental time, longer in females than males. Furthermore, female survival and reproductive rates suggest a high capacity for population increase, making it the most promising phytoseiid predator of *E. banksi* tested so far.

ii. The predator shows clear preference for the immature stages of the prey, with eggs being slightly preferred when are offered together with other immature prey-stages.

iii. *Neoseiulus madeirensis* displayed a type II functional response, with increased predation as a function of an increase in the population density of all stages of the TCM. The highest attack rate and shortest handling time was recorded for nymphs and larvae, respectively.

iv. The predator number of eggs laid per day increased as a function of the number of prey consumed for all prey-stages fitting the hyperbolic model. The maximum daily oviposition was achieved with the nymph's prey, and the lower prey density needed to display half of the egg-laid with the eggs prey.

v. Mean daily consumption values were significantly different for each of the *E. banksi* prey-stages tested, stabilising at values of 30–40 for eggs, 40–50 for nymphs and 80–100 for larvae. Mean egg-laying feeding on *E. banksi* nymphs and eggs do not significantly differ and was stabilised between 2–3 eggs/female/day. While using larvae as a prey, values were significantly lower and stabilizes at around 1 egg/female/day.

vi. *N. madeirensis* seems to be a suitable candidate for the development of a biological control program to manage *E. banksi* infestations on citrus. However, future laboratory studies on prey preference and intra-guild predation, as well as field release studies to control known pest densities are needed to determine accurately the efficiency of this predator.

CHAPTER 8

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