Efectos de las condiciones abióticas sobre las interacciones entre tres depredadores de *Tetranychus urticae* (Acari: Tetranychidae) con distintos hábitos alimenticios

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Resum

El canvi climàtic pot afectar de forma significativa a les comunitats vives, directament i indirectament. Per una part, pot afectar la fisiologia, la fenologia i la distribució de qualsevol espècie viva independentment de la seua posició en la cadena tròfica. D'altra banda, es poden produir canvis addicionals en la composició i diversitat de comunitats en tots els nivells tròfics. En conseqüència, el canvi climàtic pot afectar dramàticament els sistemes agrícoles i la seva productivitat. Especialment l'augment de les temperatures pot alterar les diferents comunitats d'artròpodes a través dels seus efectes sobre les interaccions de les espècies. *Tetranychus urticae* és un dels àcars més perjudicials que afecten els cítrics a Espanya, clementins en la seva majoria. Els seus principals enemics naturals en aquest agro-ecosistema pertanyen a la família Phytoseiidae entre els que destaquen *Euseius stipulatus, Neoseiulus californicus* i *Phytoseiulus persimilis. Tetranychus urticae* és un herbívor afavorit per les altes temperatures i la baixa humitat relativa. Per aquesta raó, el canvi climàtic podria incrementar la incidència de *T. urticae*. No obstant això, no se sap com aquest fenomen afectaria el sistema tritròfic clementina - *T. urticae* - fitoseids. L'objectiu principal d'aquesta tesi ha estat avaluar com les condicions abiòtiques (temperatura i humitat relativa) afecten les tres espècies de fitoseids citats anteriorment, en diferents mòduls comunitaris sota condicions de camp i de laboratori. Els nostres resultats han demostrat que les densitats poblacionals de *T. urticae* augmenten més de l'esperat en condicions abiòtiques típiques del estiu, que hem pres com aproximació del canvi climàtic atribuint aquest resultat a un efecte a través de la planta hoste, potser dels metabòlits secundaris. Quan es van estudiar els efectes d'aquestes condicions sobre els enemics naturals de *T. urticae, E. stipulatus* va aparèixer com la espècie més susceptible, que no pot controlar les poblacions de *T. urticae* a l'estiu, encara que va poder regular aquesta presa en primavera. Aquesta espècie és la més freqüent en els camps de cítrics valencians, però podria desaparèixer del sistema. Els nostres resultats de laboratori apunten que l'addició d'aliment alternatiu (pol·len) al sistema podria considerar-se com una forma de mitigar aquests efectes negatius, millorant la supervivència d'aquest àcar pal·linòfag , i reduint la competència específica amb els altres dos fitoseids (*N. californicus* i *P. persimilis*). Aquestes dues espècies estudiades individualment, especialment la raça de *P. persimilis*

seleccionada a la nostra regió, podrien controlar *T. urticae* tant a la primavera com a l'estiu. Tanmateix, quan es va alliberar *N. californicus* amb qualsevol de les altres dues espècies, la regulació no va ser tan bona, i aquest resultat es va atribuir a un augment de la competència, que difícilment podria ser mitigat per l'addició de pol·len al sistema. Amb tot, la combinació *P. persimilis* i *E. stipulatus* es va adaptar bé a les condicions extremes, a més a més, sota aquestes circumstàncies *P. persimilis* podria estar utilitzant *E. stipulatus* com a font alternativa d'aliment i no viceversa. Resumint, hem demostrat que: a) El canvi climàtic pot posar en perill el control biològic de *T. urticae* en clementins degut a l'efecte positiu que es pot produir sobre *T. urticae*, i els efectes negatius que es produeixen en els enemics naturals, directament o a través de les seues interaccions tròfiques. b) no tots els fitoseids estudiats suporten de la mateixa forma de condicions de canvi climàtic; c) encara que el subministrament de pol·len pot mitigar parcialment aquests efectes negatius, els diferents estils de vida dels tres fitoseids estudiats en aquest document i la diferent competència interespecífica entre ells, no permeten que aquest aliment alternatiu es convertisca en la clau per mantenir un bon control biològic en condicions de Canvi climàtic.

Abstract

Climate change can significantly shape living communities directly and indirectly. On the one hand, it can affect the physiology, phenology and distribution of any living species independently of its position in the trophic chain. On the other hand, it can produce further changes in composition and diversity of communities at all trophic levels. Consequently, climate change can dramatically affect agricultural systems and their productivity. Especially rising temperatures may alter the food web configuration of arthropod communities through its effects on species interactions. *Tetranychus urticae* is one of the most damaging mites affecting citrus orchards in Spain, mostly clementines. Its main natural enemies in this agro-ecosystem belong to the Phytoseiidae family, namely *Euseius stipulatus*, *Neoseiulus californicus* and *Phytoseiulus persimilis*. *Tetranychus urticae* is a herbivore favored by high temperatures and low relative humidity. For this reason, Climate Change could trigger outbreaks of *T. urticae*. However, it is not known how this phenomenon would affect the tri-trophic system clementine - *T. urticae* - phytoseiids. The main objective of this thesis has been to explore how abiotic conditions (temperature and relative humidity) affect the performance of the three phytoseiid species mentioned above on different community modules in field and laboratory conditions. Our results have shown that *T. urticae* population densities increase more that expected at abiotic conditions typical of the summer, which we have taken as proxy for Climate Change, and we have attributed this result to a the host-mediated effect maybe through secondary metabolites. When considering the effects of these conditions on *T. urticae* natural enemies, *E. stipulatus* appeared as the most susceptible species, resulting in no control of *T. urticae* in summer although it could regulated this prey in spring. This phytoseiid species is prevalent in Valencian citrus orchards but may disappear from the system. Our laboratory results point at the provision of alternative food (pollen) as a way to mitigate these effects, allowing a better survival of this pallinophagous mite and reducing interspecific competition with the other two phytoseiids (*N. californicus* and *P. persimilis*). These two species alone, especially the strain of *P. persimilis* selected in our region, could control *T. urticae* both in spring and in summer. However, when *N. californicus* was released with any of the other two species, regulation was not as good, and this result was attributed at increased competition, which could be hardly mitigated by the addition of pollen to the system. However, the combinations

including *P. persimilis* and *E. stipulatus* did well at extreme conditions although we suspect that under these circumstances *P. persimilis* may be actually exploiting *E. stipulatus* as an alternative food source and not vice-versa. To sum up, we have demonstrated that (a) Climate Change can actually jeopardize biological control of *T. urticae* in clementines because of the combined positive effect on *T. urticae*, partly through its host plant, and the negative effects on its key phytoseiid natural enemies, either directly or through their interactions; (b) not all phytoseiids studied suffer the same form Climate Change conditions; (c) although pollen supply may partly mitigate these negative effects, the different life-styles of the three phytoseiids studied herein and the different strength of interspecific competition among them, may not allow this alternative food item to become the key to maintain a good biological control at Climate Change conditions.

Resumen

El cambio climático puede afectar de forma significativa a las comunidades vivas, directa e indirectamente. Por un lado, puede afectar la fisiología, la fenología y la distribución de cualquier especie viva independientemente de su posición en la cadena trófica. Por otro lado, puede producir cambios adicionales en la composición y diversidad de comunidades en todos los niveles tróficos. En consecuencia, el cambio climático puede afectar dramáticamente a los sistemas agrícolas y su productividad. Especialmente el aumento de las temperaturas puede alterar las distintas comunidades de artrópodos a través de sus efectos sobre las interacciones de las especies. *Tetranychus urticae* es uno de los ácaros más perjudiciales que afectan a los huertos de cítricos en España, en su mayoría clementinos. Sus principales enemigos naturales en este agro-ecosistema pertenecen a la familia Phytoseiidae entre los que destacan *Euseius stipulatus*, *Neoseiulus californicus* y *Phytoseiulus persimilis*. *Tetranychus urticae* es un herbívoro favorecido por altas temperaturas y baja humedad relativa. Por esta razón, el cambio climático podría desencadenar brotes de *T. urticae*. Sin embargo, no se sabe cómo este fenómeno afectaría el sistema tritrófico clementina - *T. urticae* - fitoseidos. El objetivo principal de esta tesis ha sido evaluar cómo las condiciones abióticas (temperatura y humedad relativa) afectan el rendimiento de las tres especies de fitoseidos mencionadas anteriormente, en diferentes módulos comunitarios bajo condiciones de campo y de laboratorio. Nuestros resultados han demostrado que las densidades poblacionales de *T. urticae* aumentan más de lo esperado en condiciones abióticas típicas del verano, que hemos tomado como aproximación del cambio climático atribuyendo este resultado a un efecto influido por la planta huésped, tal vez a través de metabolitos secundarios. Cuando se estudiaron los efectos de estas condiciones sobre los enemigos naturales de *T. urticae*, *E. stipulatus* apareció como la especie más susceptible, sin controlar las poblaciones de *T. urticae* en verano, aunque pudo regular esta presa en primavera. Esta especie es la más frecuente en los huertos de cítricos valencianos, pero puede desaparecer del sistema. Nuestros resultados de laboratorio apuntan a que la adición de alimento alternativo (polen) al sistema podría considerarse como una forma de mitigar estos efectos negativos, permitiendo una mejor supervivencia de este ácaro palinófago, y reduciendo la competencia interespecífica con los otros dos fitoseidos (*N.*

californicus y *P. persimilis*). Estas dos especies asolas, especialmente la raza de *P. persimilis* seleccionada en nuestra región, podría controlar *T. urticae* tanto en primavera como en verano. Sin embargo, cuando se liberó *N. californicus* con cualquiera de las otras dos especies, la regulación no fue tan buena, y este resultado se atribuyó a un incremento de la competencia, que difícilmente podría ser mitigada por la adición de polen al sistema. Sin embargo, la combinación *P. persimilis* y *E. stipulatus* se adaptó bien en condiciones extremas, aunque sospechamos que bajo estas circunstancias *P. persimilis* puede estar utilizando a *E. stipulatus* como una fuente alternativa de alimento y no viceversa. Resumiendo, hemos demostrado que: a) El cambio climático puede poner en peligro el control biológico de *T. urticae* en clementinos debido al efecto positivo que puede producir sobre *T. urticae*, y los efectos negativos que produce en sus enemigos naturales clave, directamente o a través de sus interacciones. b) no todos los fitoseidos estudiados sufren la misma forma de condiciones de cambio climático; c) aunque el suministro de polen puede mitigar parcialmente estos efectos negativos, los diferentes estilos de vida de los tres fitoseidos estudiados en este documento y la diferente competencia interespecífica ejercida entre ellos, no permiten que este alimento alternativo se convierta en la clave para mantener un buen control biológico en condiciones de cambio climático.

1

Introducción

1.1 Importancia económica de los cítricos

España es el sexto productor y el mayor exportador de cítricos frescos en el mundo, con una producción alrededor de siete millones de toneladas en la campaña 2013/2014 (FAO, 2015). La producción de cítricos en España principalmente agrupa los siguientes grupos de cítricos, naranjas (52 %), mandarinas (31 %), limones (16 %) y pomelos (1 %), la mayoría de la ellos destinado para consumo fresco (75 %). Además, un 57 % total de la producción se exporta a otros países (FAO, 2015). Prácticamente la totalidad de esta producción se concentra en tres comunidades autónomas: Andalucía, Comunidad Valenciana y Murcia. Concretamente, la Comunidad Valenciana ocupa alrededor del 55 % de la superficie total destinada a la producción citrícola de toda España. Las naranjas y las mandarinas ocupan un mayor porcentaje de superficie que los limones (44, 50 y 6 %, respectivamente). Lo mismo ocurre en cuanto al porcentaje de producción de estos cítricos, con un 49, 43 y 8 %, respectivamente (Generalitat Valenciana, 2017). En los últimos 10 años, en la Comunidad Valenciana se ha producido un incremento de más de un 250 % en la superficie citrícola gestionada de acuerdo a los reglamentos de la Producción Integrada, pasando de 8.673 ha en 2005 a 23.372 ha en 2015, alrededor del 15% de la superficie citrícola total (MAPAMA 2017). El valor de los cítricos españoles asciende aproximadamente a unos 2.200 millones de euros anuales, lo que representa más del 6% de la producción agraria del país (MAPAMA 2017).

1.2 Gestión Integrada de Plagas en cítricos

Desde 2014, con la entrada en vigor de la Directiva 2009/128/CE del Parlamento Europeo y del Consejo de 21 de octubre de 2009 los principios generales de la Gestión Integrada de Plagas (GIP) son obligatorios para todos los productores europeos. El objetivo principal de la GIP es mantener las densidades de los fitófagos plaga por debajo de sus umbrales económicos de daños (UED) (Tena el al., 2011). La GIP consiste en la aplicación racional de una combinación de métodos biológicos, biotecnológicos, químicos y culturales, de modo que la utilización de

productos fitosanitarios se limite al mínimo necesario. El muestreo periódico con fines de realizar el correcto seguimiento de los distintos fitófagos que afectan a este cultivo es necesario para la aplicación de la GIP. Los métodos para realizar estos seguimientos pueden ser directos (muestreos de campo), o indirectos, calculado las integrales térmicas (grados-día). La web del Instituto Valenciano de Investigaciones Agrícolas (IVIA) sobre la "Gestión Integrada de Plagas y Enfermedades en Cítricos" (www.gipcitricos.ivia.es) proporciona información sobre estos métodos, así como los de control.

En los cítricos españoles se han citado más de 90 fitófagos potencialmente plaga, pero de estos sólo unos 15 alcanzan la categoría de especie plaga En función del grado de control que ejercen sus enemigos naturales (excelente, satisfactorio o insuficiente), estos se agrupan en: fitófagos secundarios, plagas ocasionales y plagas clave (Urbaneja and Jacas 2008). La mayor parte de plagas potenciales en cítricos se encuentran bajo un excelente control biológico, por parte de sus enemigos naturales, tanto exóticos como autóctonos (Jacas et al., 2010). Sin embargo, hay algunos fitófagos cuyo control biológico no llega a ser satisfactorio y sus densidades se encuentran normalmente por encima de sus umbrales económicos de daño (UED), y constituyen las plagas clave de este cultivo (Urbaneja & Jacas, 2008). En este grupo encontramos al piojo rojo de California, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), a los pulgones *Aphis spiraecola* Patch y *A. gossypii* Glover (Hemiptera: Aphididae), a la mosca mediterránea de la fruta *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) y a la araña roja *Tetranychus urticae* Koch (Prostigmata: Tetranychidae), plaga clave de los clementinos en la que se centra esta tesis doctoral.

1.3 *Tetranychus urticae*

La araña roja (Figura 1.1) es una especie polífaga y cosmopolita, que puede desarrollarse sobre más de 1000 huéspedes distintos de los que más de 150 tienen importancia económica (Attia et al. 2013; Helle and Sabelis 1985; Abad-Moyano et al. 2008). En cítricos afecta principalmente a clementinos y limoneros (Garcia-Marí et al., 1991; Abad-Moyano et al., 2008) y se considera el tetraníquido más perjudicial a nivel mundial (Helle and Sabelis 1985).

1.3.1 Biología

Tetranychus urticae es una especie que sigue una estrategia de desarrollo tipo "*r*" (en referencia al modelo logístico de crecimiento poblacional), con un gran potencial biótico, tasa de desarrollo rápido, ciclo de vida corto, y rápida dispersión (Speight et al. 2008). *Tetranychus urticae* desarrolla sus colonias en el envés de las hojas de los cítricos (Figura 1.2). La telaraña que produce, puede retener la humedad de la transpiración de la planta, creando un microclima favorable que les permite sobrevivir en climas secos (Garcia-Marí et al., 1991; Aucejo y Jacas, 2005). Además, esa telaraña las protege de algunos de sus depredadores, que no pueden desplazarse en su interior, e incluso de algunos tratamientos acaricidas.

Figura 1.1. Hembra adulta y huevo de *T. urticae*

Figura 1.2. Colonia de *T. urticae* en el envés de una hoja de clementino

El ciclo de vida consta de cuatro estados de desarrollo: huevo, larva, 2 estados ninfales (protoninfa y deutonifa) y adulto. En condiciones óptimas, aproximadamente 30ºC, completa su ciclo en 9-10 días (Garcia-Marí et al., 1991; Aucejo y Jacas, 2005). Este ácaro favorecido por temperaturas elevadas y bajas humedades relativas, pero que se mantiene activo en clementino durante todo el año, puede llegar alcanzar elevadas densidades que ocasionan graves daños económicos (Martínez-Ferrer et al. 2006; Pascual-Ruiz et al. 2014).

1.3.2 Daños

La alimentación de *T. urticae* en clementino ocasiona manchas cloróticas y un abombamiento característico en el haz de las hojas (Figura 1.3) y manchas en los frutos que deprecian el valor comercial del fruto (Figura 1.4), ya que la producción de clementinas se destina principalmente al consumo en fresco (Pascual-Ruiz et al. 2014). Si la infestación coincide con altas temperaturas y/o estrés hídrico, puede provocar además, graves defoliaciones (Aucejo y Jacas 2005). Por esta razón, verano es la época critica en la gestión de esta especie en clementino, ya que incluso bajas poblaciones en hojas pueden suponer graves daños en frutos (Ansaloni et al., 2008).

Figura 1.3. Manchas cloróticas

Figura 1.4. Daño en fruto

1.3.3 Control químico

El control químico sigue siendo el método más utilizado para controlar *T. urticae* en cítricos, especialmente en mandarino clementino. Los tratamientos químicos deben hacerse siempre que la densidad de *T. urticae* sobrepase el UED (Pascual-Ruiz et al. 2014), y no de manera preventiva. Es muy importante, debido a su alta fecundidad y corto ciclo de vida, la alternancia entre sustancias activas con distintos modos de acción para evitar el desarrollo de resistencias en pocas generaciones (IRAC 2017). Diversos autores han estudiado diferentes mutaciones y mecanismos de resistencia para *T. urticae* en distintos cultivos (Lee et al. 2003; García-Marí 2005; Van Leeuwen et al. 2010, 2015; Grbić et al. 2011; Khajehali et al. 2011; Attia et al. 2013;

Santamaría et al. 2015; Brown et al. 2017; Ilias et al. 2017). El control químico es una práctica eficaz a corto plazo, lo que a menudo lleva a los productores a utilitzar sustancias que pueden causar serios problemas de eliminación de la fauna útil de *T. urticae* (Jacas and Garcia-Marí 2001; Kim and Yoo 2002; Castagnoli et al. 2005; Duso et al. 2008; Urbaneja et al. 2008; Sá Argolo et al. 2013, 2014). La aplicación correcta y eficaz de un acaricida para el control de *T. urticae* en cítricos exige un conocimiento completo de la biología de esta plaga. En España, actualmente, las sustancias activas registradas y recomendadas para el control de *T. urticae* en cítricos están disponibles en la página web de los órganos competentes del Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente (MAPAMA, 2017) (Tabla 1.1).

Modo acción	**Sustancia activa**	**Plazo seguridad**	**Efectos secundarios (OILB)**		
			Es	**Nc**	**Pp**
Activador canal cloro	Abamectina	10	3	3	3
Inhibidores del crecimiento	Clofentezin	21	1 - 2	1	1
	Hexitiazox	14	1	1	1
	Etoxazol	14	2	-	2
Inhibidores transporte electrones	Tebufenpirad	7	-	1 - 2	4
	Fenpiroximato	14	3	3	3
Inhibidores síntesis de lípidos	Spirodiclofen	14	1 - 2	2	2 - 3
Físico asfixia	Aceite parafinico 78 %	0	1	2	2

Table 1.1. Materias activas actualmente registradas y recomendadas contra *T. urticae*. Modo de acción, Materia activa, Plazo de seguridad (días) y efectos secundarios en *E. stipulatus* (Es), *N. claifornicus* (Nc) y *P. persimilis* (Pp) clasificada según la OILB (*International Organisation for Biological and Integrated Control*): - (no hay datos); 1 (Inocuo); 2 (Ligeramente toxico), 3 (Moderadamente toxico) y 4 (Tóxico).

En la actualidad, el control químico se está realizando de manera más sostenible, dando prioridad a la conservación de los enemigos naturales, mediante la utilización de plaguicidas con menor impacto sobre ellos. El uso de plaguicidas selectivos combinados junto a la conservación y/o sueltas de ácaros depredadores, pueden ser métodos importantes para el éxito de la GIP en cultivos de cítricos (Urbaneja and Jacas 2008).

1.4 Enemigos naturales

En los cítricos españoles se ha identificado un variado complejo de enemigos naturales de *T. urticae*, pertenecientes a distintos grupos taxonómicos, cuya abundancia en el cultivo y eficacia en el control de esta plaga varía en función de la época del año, del cultivo y de la gestión a que esté sometida. Entre ellos, destacan los ácaros depredadores de la familia de los Phytoseiidae (Mesostigmata) (Abad- Moyano et al., 2009a; Aguilar-Fenollosa et al., 2011b; Aguilar-Fenollosa et al., 2012).

1.4.1 Phytoseiidae

Las especies más comunes en cítricos en el este de la Península Ibérica, que se consideran clave para la regulación natural de las poblaciones de *T. urticae* en mandarino clementino (Abad-Moyano et al. 2009a; Aguilar-Fenollosa et al. 2011a) son las tres siguientes:

Euseius stipulatus (Athias-Henriot) (Figura 1.5) es un depredador omnívoro (McMurtry y Croft, 2013), que se alimenta de diversos tipos de polen, ácaros fitófagos, pequeños insectos y melaza (Abad-Moyano et al. 2008; Pérez-Sayas et al. 2015). Es el fitoseido más abundante en los cítricos españoles, tanto en los árboles como en la flora adventicia asociada a los mismos (Garcia-Marí et al., 1991; Abad-Moyano et al., 2009a; Aguilar-Fenollosa et al., 2011b). Además, se le considera una especie palinófaga ya que es capaz de sobrevivir alimentándose únicamente de polen e incluso mantenerse a densidades relativamente altas en el cultivo cuando las presas son escasas (Pina et al. 2012). Se sospecha que su omnivoría, combinada con una superioridad como depredador intregremial, puede llevar al desplazamiento, de otros fitoseidos más eficaces, cuando hay disponibilidad de polen de forma continuada durante toda la campaña, lo que llevaría a aun control deficiente de *T. urticae* (Aguilar-Fenollosa et al. 2011a). Por ello, se recomiendan prácticas que no fomenten en exceso su abundancia en el cultivo (p.e., el uso de una cubierta vegetal gramínea cuyo polen no es el óptimo para *E. stipulatus*).

Neoseiulus californicus (McGregor) (Figura 1.6), es un omnívoro especializado en ácaros tetraníquidos (McMurtry y Croft, 2013), que está ampliamente distribuido tanto en los cítricos como en la cubierta vegetal asociada a los mismos (Abad-Moyano et al., 2009a; Aguilar-Fenollosa et al., 2011b). Este fitoseido es considerado como un buen depredador de *T. urticae* (Escudero y Ferragut, 2005; Abad-Moyano et al., 2010b). Además, es capaz de alimentarse también de polen y de otros microartrópodos, por lo que puede permanecer en el cultivo cuando *T. urticae* escasea (Escudero y Ferragut, 1996).

Finalmente *Phytoseiulus persimilis* (Figura 1.7) es un depredador especialista en *Tetranychus* y se le considera como el depredador más eficaz de *T. urticae*. Se le considera la especie clave para el control natural de *T. urticae* en mandarino clementino (Aguilar-Fenollosa et al. 2011b; Pérez-Sayas et al. 2015). Presenta un rápido desarrollo, elevada fecundidad, elevada capacidad de consumo de presas y gran movilidad (Abad-Moyano et al., 2009a). Sin embargo, presenta la desventaja que cuando la presa es escasa, puede desaparecer del agroecosistema. En estudios anteriores, Aguilar-Fenollosa et al. (2011a) vieron que la adecuada gestión de la cubierta vegetal podía asegurar la presencia continuada de esta especie en cultivo, probablemente a través de la provisión de alimento alternativo para sus competidores (Gómez-Martínez et al. 2017) y de una raza de *T. urticae* especializada en la cubierta como presa, que se traducía en una mejor regulación de las poblaciones de *T. urticae* en el árbol (Aguilar-Fenollosa et al. 2012).

Figura 1.5. *Euseius stipulatus* depredando un huevo de *Neoseiulus californicus*

Figura 1.6 Hembra de *Neoseiulus californicus* recién alimentada de *T. urticae* con su puesta.

Figura 1.7 Hembra, macho, inmaduros y puesta del depredador *Phytoseiulus persimilis*

Euseius stipulatus ha sido descrito como un depredador intragremial más fuerte que *N. californicus* y *P. persimilis* (Abad-Moyano et al., 2010a), por lo que esta especie podría desplazarlos del agroecosistema y aumentando aún más a su abundancia. Esto podría explicar el deficiente control de *T. urticae* en los huertos comerciales de cítricos (Aguilar-Fenollosa et al. 2011c). Sin embargo, *N. californicus* presenta una alta capacidad de crecimiento, además de una elevada tolerancia a las altas temperaturas y a la baja humedad, por lo que se le considera un buen candidato en el control de *T. urticae* en muchos cultivos (Ragusa et al. 2009). El especialista *P. persimilis* es poco abundante en los cítricos españoles, lo que se ha atribuido a su baja tolerancia a las altas temperaturas y, a que suele ser desplazado por otros fitoseidos mejor adaptados a las condiciones climáticas locales, como *E. stipulatus* y *N. californicus* (Abad-Moyano at al., 2009a; McMurtry y Croft, 2013).

1.4.2 Efecto del polen como alimento alternativo

Existe una gran cantidad de autores que han estudiado el efecto del polen como alimento alternativo sobre diferentes especies de ácaros depredadores de la familia de los Phytoseiidae en distintos cultivos, como el aguacate (González-Fernández et al. 2009; Maoz et al. 2011), la viña (Pozzebon et al. 2009), cítricos (Ferragut et al. 1987; Abad-Moyano et al. 2010b), hortícolas (Nomikou et al. 2010; Pappas et al. 2013; Kumar et al. 2015; Nguyen et al. 2015) e incluso en palmeras (Carrillo et al. 2010). El polen puede ser suministrado al sistema artificialmente (Maoz et al. 2011; Put et al. 2012; Montserrat et al. 2013a), de modo natural en especies presentes en la cubierta vegetal (Coli et al. 1994; Raworth et al. 1994; Auger et al. 1999; Aguilar-Fenollosa et al. 2011a) u ofrecido por plantas intercaladas entre cultivos (Smith and Papacek 1991; González-Fernández et al. 2009; Maoz et al. 2011). Además, algunos ácaros depredadores pueden mejorar sus parámetros biológicos en función de la calidad del polen consumido (Bouras and Papadoulis 2005; Pina et al. 2012; Delisle et al. 2015; Leman and Messelink 2015). La utilización de polen como alimento alternativo, tiene como objetivo, mantener las poblaciones de depredadores en períodos de escasez o ausencia de la plaga (Nomikou et al. 2002; González-Fernández et al. 2009; Pozzebon et al. 2009; Maoz et al. 2011) e incrementar el número de los depredadores y el control biológico a través de la competencia aparente que se genera entre la plaga y el alimento alternativo (Montserrat et al. 2013a). Recientemente, se ha demostrado que la adición de polen al sistema puede disminuir los ataques entre *E. stipulatus* y *E. scutalis* (Guzmán 2014).

1.5 Calentamiento global

Actualmente los expertos del “Intergovernmental Panel on Climate Change” (IPCC) han expresado su preocupación y han reiterado la gran responsabilidad del Hombre en las consecuencias que llevan al cambio climático (IPCC, 2014). En este último informe del Panel se destaca la predicción de un incremento fenómenos meteorológicos extremos, como olas de calor y de frío, sequías, inundaciones y las supertormentas. Además, estos expertos insisten en un aumento de la frecuencia, duración e intensidad de estos fenómenos en un futuro que ya está aquí. Muchos

autores se han hecho eco de este último informe, en forma de revisiones (Beck and Mahony 2017; O'Neill et al. 2017) diseñadas para categorizar y representar los crecientes riesgos del cambio climático. En general, las predicciones proyectadas para un futuro próximo se basan en un aumento de la temperatura global entre 1,1 y 6,4 ° C y una disminución de las precipitaciones alrededor de un 25 % (IPCC, 2014; Figura 1.8)

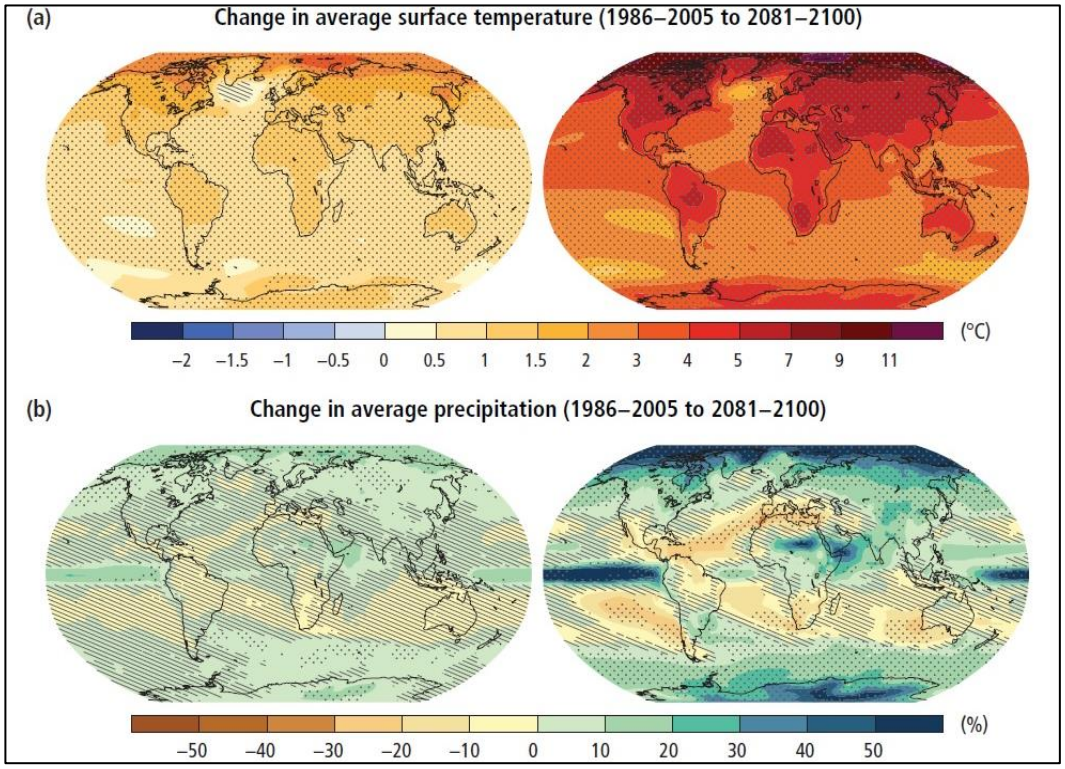


Figura 1.8 Cambio de las temperaturas (a) y de la precipitación (b) medias en la superficie terrestre proyectadas para finales del siglo XXI (Fuente: IPCC, 2014).

En el caso de la Cuenca Mediterránea concretamente, se ha pronosticado un incremento entre 1,5 y 2 ºC en las temperaturas de invierno y verano respectivamente y un decrecimiento alrededor del 5 % en las precipitaciones (Giorgi and Lionello, 2008; Gualdi et al., 2012; Navarra y Tubiana, 2013).

A raíz del evidente cambio climático y sus alarmantes predicciones, en 2015 se celebró la Conferencia de París sobre el Clima, donde 195 países firmaron el primer acuerdo vinculante mundial sobre el clima. La UE (unión europea) se ha fijado objetivos para reducir progresivamente

las emisiones de gases de efecto invernadero de aquí a 2050. Este paquete de medidas contiene legislación vinculante que garantizará el cumplimiento de los objetivos climáticos y de energía asumidos por la UE para 2020. Los objetivos fundamentales pretenden en reducir un 20 % estas emisiones, e incrementar un 20 % las energías renovables y la eficiencia energética (European Commission, 2017).

1.6 Efectos del calentamiento global en los sistemas agrícolas

El principio del siglo XXI, marcó el inicio de las investigaciones en las que se estudiaba el efecto del cambio climático en relación a distintos ámbitos de la ecología y sistemas naturales (Rott and Ponsonby 2000; Walther et al. 2002; Karl and Trenberth 2003; Parmesan and Yohe 2003; Voigt et al. 2003). Muchos de estos estudios sobre la influencia del calentamiento global en los cambios ecológicos en la fenología y distribución de plantas y animales (comunidades marinas, de agua dulce y terrestres) están recogidos en una revisión realizada por Parmesan, 2006. También, en 2008, Tylianakis y colaboradores realizaron una revisión de 688 publicaciones donde demostraron los efectos causados por el cambio climático en la intensidad y/o dirección de las interacciones bióticas, como la competencia (planta-planta, animal-animal), la depredación, el parasitismo, el mutualismo (planta-polinizador) y otras relaciones intra e interespecíficas.

El cambio climático puede perturbar la fenología, distribución, diversidad y composición de diferentes especies de insectos (Pelini et al. 2009) y afectar dramáticamente a los sistemas agrícolas y su productividad (Aguilar-Fenollosa and Jacas 2014). En los ecosistemas naturales, las interacciones entre las plantas, los insectos herbívoros y sus enemigos naturales son el resultado de largos procesos coevolutivos específicos de entornos particulares y condiciones climáticas relativamente estables (Van Der Putten et al. 2004). En las comunidades agrícolas, muchas especies plaga y sus enemigos naturales son artrópodos y los efectos del calentamiento global podrían tener importantes implicaciones en el control biológico de plagas. Por lo tanto, se espera que los cambios en las condiciones ambientales locales o regionales, especialmente los incrementos de temperatura

y períodos de calor y sequía extremos, puedan afectar profundamente al control de plagas (Araújo and Luoto, 2007; Hegland et al., 2009).

Los macroclimas están cambiando más rápido de lo que se predijo anteriormente (van Oldenborgh et al. 2008). Por tanto, un problema clave para la comunidad científica es predecir las consecuencias del calentamiento global a las pequeñas escalas en las que viven la mayoría de los organismos (Potter et al. 2013). Existe una elevada diversidad de estudios que predicen los efectos que originan las condiciones de cambio climático sobre: la biodiversidad de distintas especies (Garcia et al. 2014), cadenas tróficas (Sentis et al. 2015), enemigos naturales (Thomson et al. 2010), modelos de adaptación de especies (Valladares et al. 2014), relaciones presa-depredador en el control biológico de conservación (Schmitz and Barton 2014) y una elevada cantidad de estudios de gran interés sobre futuras predicciones del calentamiento global. Además, varios autores están estudiando el efecto del alimento alternativo sobre la abundancia de los depredadores y el control de plagas en condiciones extremas (González-Fernández et al., 2009; Guzmán et al., 2016b; Montserrat et al., 2013a, 2013b).

Como anteriormente se ha descrito, en nuestro sistema la araña roja es un herbívoro favorecido por las altas temperaturas y la baja humedad relativa. Por esta razón, el calentamiento global podría desencadenar explosiones de *T. urticae* en nuestro sistema. Sin embargo, no se sabe cómo este fenómeno podría afectar al conjunto de la comunidad de ácaros depredadores de *T. urticae* en clementino. Este desconocimiento llevó a plantearse esta tesis doctoral, cuyos objetivos se describen a continuación.

1.7 Objetivos

Las poblaciones de *T. urticae* en cítricos podrían verse afectadas positivamente por el calentamiento global. Además, los mecanismos de control biológico pueden verse influidos negativamente por ese mismo calentamiento, lo que podría llevar a un aumento de la incidencia de esta especie plaga en un futuro cada vez más cercano. Por esta razón, es necesario el estudio y evaluación del efecto de la temperatura y la humedad relativa en la dinámica y la estructura de la comunidad constituida por la araña roja, *Tetranychus urticae* y sus tres principales depredadores (*Euseius stipulatus*, *Neoseiulus californicus* y *Phytoseiulus persimilis*) en los cítricos valencianos. La consecución de los siguientes objetivos, proporcionarán información sobre la adaptación de este sistema al cambio climático, a la vez que apoyo científico para el desarrollo de métodos de control biológico en cítricos:

1. Evaluar en condiciones de campo el control ejercido sobre *T. urticae* por distintas combinaciones de los fitoseidos más abundantes en los cítricos valencianos (*E. stipulatus*, *N. californicus* y *P. persimilis*), tanto en primavera (aproximación de condiciones óptimas) como en verano (aproximación a condiciones de cambio climático).

2. Evaluar en condiciones de laboratorio, como afectan distintas combinaciones de temperatura y humedad relativa a los distintos parámetros biológicos de *T. urticae* y a sus tres principales depredadores en clementino, incluyendo distintos módulos comunitarios: cadena trófica y competencia.

3. Estudiar en condiciones de laboratorio, si la adición de polen al sistema podría mitigar los efectos de las altas temeperaturas y la baja humedad relativa sobre la regulación natural de *T. urticae* en clementino.

2

Common Material and Methods

2.1 Plant material

2.1.1 Clementine plants

Two-year-old clementine plants (*C. clementina* cv. Clementina de Nules) were used as experimental units in the field experiment but also as a source of leaves for some of our laboratory assays. Between 1 $^{1}/_{2}$ and 2 months before the beginning of each assay, around 100 plants were defoliated and kept in a greenhouse at Universitat Jaume I (UJI) set at 22 ± 2ºC, 55 ± 10% relative humidity and natural photoperiod. These plants were grown on vermiculite and peat (1:3; vol:vol) in 320 ml pots and were fertilized using a modified Hoagland's solution (Bañuls et al. 1997) and received no pesticide treatments.

2.1.2 Other plant material

Leaves of beans (*Phaseolus vulgaris* L.) were obtained from the plants kept in a greenhouse at UJI. Lemon, fruits (*Citrus lemon* Burm. f.) were obtained from a pesticide-free orchard. Furthermore, we used for some of our laboratory assays *Carpobrotus edulis* (L.) pollen obtained from IVIA (Montcada, Valencia, Spain), where this plant is used as an ornamental. Pollen of *C. edulis* was obtained from male flowers that were dried in a stove at 37ºC for 48 h, and then sieved (350 µm), before being frozen until use.

2.2 Mite stock colonies

Four different mite species were used in our studies: the two-spotted spider mite *Tetranychus urticae*, and the Phytoseiidae *Euseius stipulatus*, *Neoseiulus californicus*, and *Phytoseiulus persimilis*. Their colonies were maintained in separate climatic chambers set at 25 ± 1ºC, 65 ± 5 % relative humidity and a 16-hour light photoperiod.

Spider mites were originally collected in a Clementina de Nules orchard in the municipality of Les Alqueries (UTM: 39°59'15.1"N 0°03'02.0"W) in 2010. This colony has been maintained ever since using standard procedures on detached leaves of clementine mandarins (Aguilar-Fenollosa et al. 2012) and, when necessary, on organic lemon fruits (Abad-Moyano et al. 2010a).

For practical reasons, when this mite was used to feed the Phytoseiidae stock colonies, bean leaflets were used. Leaflets were periodically infested by exposure to the lemon colonies.

When needed, individuals of *N. californicus* were obtained from Koppert Biological Systems (SPICAL®) to initiate a laboratory colony. Contrarily, *P. persimilis* and *E. stipulatus* were originally collected in 2012 in two clementine orchards in Les Alqueries (same location as *T. urticae*) and Montcada (UTM: 39° 32' 42.906" N 0° 23' 45.699" W), respectively. Phytoseiid stock colonies were maintained on detached leaf arenas using standard procedures (Pina et al. 2012). These arenas consisted of single bean leaflets placed upside down on moistened filter paper placed on top of a water saturated foam cube (3–4 cm thick) in an open plastic box half-filled with water. These colonies were maintained on detached bean leaves infested with *T. urticae* and supplied with *C. edulis* pollen.

2.3 Laboratory experiments

2.3.1 Arenas

Arenas consisted of a 5 cm in diameter petri dish, with a 3 cm in diameter hole in the cover. The base of the dish was filled with bacteriological agar (2.5 w/v) to maintain the turgor of the leaves used during the assays. When agar was cold and solid enough, a fully expanded healthy clementine leaf was placed upside down on top of the agar. The cover was subsequently put in place in such a way that the leaf substrate formed a 3 cm in diameter exposed area. To prevent individual mites from the experimental arena, a permanent glue (Tree Tanglefoot®; Grand Rapids, MI, USA) was applied along the rim of the arena. Finally, the dishes were lateratelly sealed with Parafilm® (Pechiney Plastic Packaging, Menasha, WI, USA).

2.3.2 Phytoseiid performance: fate, predation and oviposition

A fully expanded healthy clementine leaf was introduced onto a *T. urticae*-infested lemon stock colony. 24 h later, the infested leaf was moved into a phytoseiid colony and left there for an additional 24-hour period. At that moment, leaves were inspected under binocular microscope to remove all motile stages. A separate colony was started with each single leaf and they constituted

the phytoseiid cohorts used in our assays. As this method did not work for *E. stipulatus*, the eggs of this species were obtained by exposing a few cotton threads to an existing colony. 24 hour later, all motile forms on these threads were removed and the remaining eggs used to start a new cohort. Phytoseiids were reared up to the adult stage following the same procedure as for the stock colonies.

In our assays, we used gravid adult females at their peak oviposition rate (12 to 14 days from egg hatching) (Janssen and Sabelis 1992; Aucejo-Romero et al. 2004). To ensure the same level of satiety in all females tested, these were randomly selected from a cohort and individualized in plastic arenas (same as for the stock colonies but with no plant material) placed on top of sponges in water-containing trays where they starved for 24 hours. The edges of these arenas were covered with tissue paper in contact with the sponge and the water, served as both a barrier and a water source for mites.

Once the experimental arenas had received the corresponding phytoseiid specimen, they were introduced into a desiccator (where the desired relative humidity was achieved by using different salt concentrations) in an environmental chamber set at the target temperature. Because *T. urticae* eggs used in the arenas could start hatching in 48 h, the assay had to be stopped at this time. Arenas were checked 24 and 48 h after the onset of the assay (i.e., 48 and 72 hours after the end of the starvation period for adult phytoseiid females) under a binocular microscope and several parameters were scored: fate (i.e., live and dead specimens and escapees), oviposition (number of eggs laid) and predation (number of *T. urticae* eggs eaten).

3

Effect of climatic conditions on the biological control of *Tetranychus urticae* in clementine mandarins by *Euseius stipulatus*, *Neoseiulus californicus* and *Phytoseiulus persimilis* at field conditions

Hypothesis

Climate change may disrupt existing natural regulation of *Tetranychus urticae* by its main predators in Valencian citrus orchards: *Euseius stipulatus*, *Neoseiulus californicus* and *Phytoseiulus persimilis*. Field summer conditions have been used as a proxy of Climate Change conditions and compared to spring (used as proxy of optimal conditions) to assess the effects of Climate Change on the natural regulation of *T. urticae* by its main phytoseiid predators, either alone or in combinations of two when released simultaneously (competing adults) or sequentially (to assess any priority effect).

3.1 Material and methods

3.1.1 Experimental procedure

Two experiments were carried out in spring (1 to 31 May) and summer (5 to 28 July) 2015 as proxy of optimal and climate change conditions, respectively. A third assay, planned for the autumn of that year, had to be cancelled because of the repeated heavy rains falling that year at the place were the experiment had been set up. The assays took place in a plot located in Les Alqueries in a commercial clementine orchard. It consisted of two lines of 12 trees each (N-S orientation) which had been replaced by 24 mesh cages (3 x 3 x 3 m) with a zipped door (1.5 m high) on one of their sides. 13 of these cages were randomly selected and used for different treatments (see below). Each cage received five potted clementine plants (= five replicates) (see the general M&M section) and were provided with a drip irrigation system. Three Datalogger (model TESTO 175-H), Madrid, Spain) were placed in three different cages to measure temperature and relative humidity values within the cage.

At the beginning of the assay, each plant was artificially infested with 10 *T. urticae* adult females obtained from the laboratory rearing mentioned in the general M&M section. Every mite was deposited onto a different leaf with the aid of a fine brush. One week later, and in coincidence with the appearance of the first typical symptoms of *T. urticae* attack in clementines (chlorotic spots

on leaves), *T. urticae* density was individually estimated per plant (see below). Subsequently, the phytoseiids *E. stipulatus, N. californicus* and *P. persi*milis were released on different plants depending on the treatment. One cage constituted the control treatment and received no phytoseiids.

The other 12 treatments consisted of either the release of only one species of phytoseiid (trophic chain, three cages, one for each predatory species), the simultaneous release of two species (competition, three cages, one for each phytoseiid combination), or the sequential release in a 1-week interval of two species (priority effects, six cages, one for each phytoseiid combination). The release rate for all phytoseiid treatments was 10 females in total. These corresponded to 10 individuals of the same species for the trophic chain treatments, five mites of each species for the competition treatments, and again five mites of each species released one week apart for the priority effects treatments. Experiments were discontinued as soon as plants in the control treatment collapsed and this event happened nine and seven weeks after initial *T. urticae* infestation, in spring and summer, respectively.

Tetranychus urticae density was estimated by counting (a) the number of symptomatic leaves per plant (those showing the typical chlorotic spots) and then (b) the number of females on up to eight of these symptomatic leaves randomly selected without removing them from the plant. By multiplying both figures, we obtained an estimation of *T. urticae* population per plant. Additionally, as a measure of damage, we calculated the cumulative mite days (CMD) per plant as:

$$\sum \Delta_t \frac{(X_1 + X_2)}{2}$$

Where $\sum$ is summation over all sampling dates from the sampling date; Δ_t is the interval between two successive sampling dates (seven days); and X_1 and X_2 are *T. urticae* densities on those consecutive dates.

At the end of the assay, in order to assess phytoseiid numbers on each plant, around 100 leaves were randomly collected from the trees. These samples were transported to the laboratory in a plastic bag in a cooler for microarthropod extraction using Berlese funnels. Extracted arthropods were preserved in 70 % ethanol. Mites were later separated using a binocular microscope.

Subsequently, they were digested in lactic acid (65%) and mounted for microscope observation in Hoyer's medium (Gutiérrez, 1985). Identification to species level using a phase contrast microscope was based on the number, length and position of setae on dorsal and ventral plates, their morphology and that of the spermatheca for females (Ferragut et al. 2010). Immature forms of *N. californicus* and *E. stipulatus* in treatments where these two species were released together were distributed between species based on the proportion of adult females found. In the case of *P. pers*imilis, because of the orange color of motiles, all forms, both adult and immature, were identified at species level.

3.1.2 Statistical analyses

Tetranychus urticae densities were analyzed using General Linear Mixed Models (GLMM), with "season" (spring and summer) and "treatment" (control, trophic chain, competition and priority effects) as fixed factors and "time" (expressed either as days or as degree-days, DD, since initial *T. urticae* infestation) as a random factor. To calculate DD, a lower development threshold of 10.45ºC (Bounfour and Tanigoshi, 2001) was used. As our main goal was to identify seasonal differences (spring versus summer as proxy of present conditions versus Climate change), we started the analysis of *T. urticae* densities by considering all factor combinations including the "season" factor ("season" and "season*treatment"). Once the signification of the "season" factor was clear, we continued the study of *T. urticae* density for each season separately. Because our main goal was to identify species-specific differences, we grouped the different treatments as follows: 1) One model for the trophic chain including the three different phytoseiid species and the control, 2) One model for competition including the three 2-species combinations possible and the control, and 3) Three different models for the priority effects, one for each first introduced phytoseiid species including the two 2-species combinations possible and the control.

Akaike information criteria (AIC) (Akaike 1974) was used to select the best model. Pairwise comparisons of the fixed factors levels (i.e., the sampling time points) were made using the Bonferroni post-hoc test ($P < 0.05$) upon a significant treatment effect. Finally, we compared the cumulative mite days (CMD) and the variation of this parameter relative to control for every

treatment at the end of the assay using a General Linear Model (GLM), with a Poisson distribution of the error. When necessary, we used Bonferroni post-hoc test ($P < 0.05$) for mean separation. Additionally, differences between seasonal values of "CMD" and their variation (difference between spring and summer conditions) were analyzed using Student t-test ($P < 0.05$).

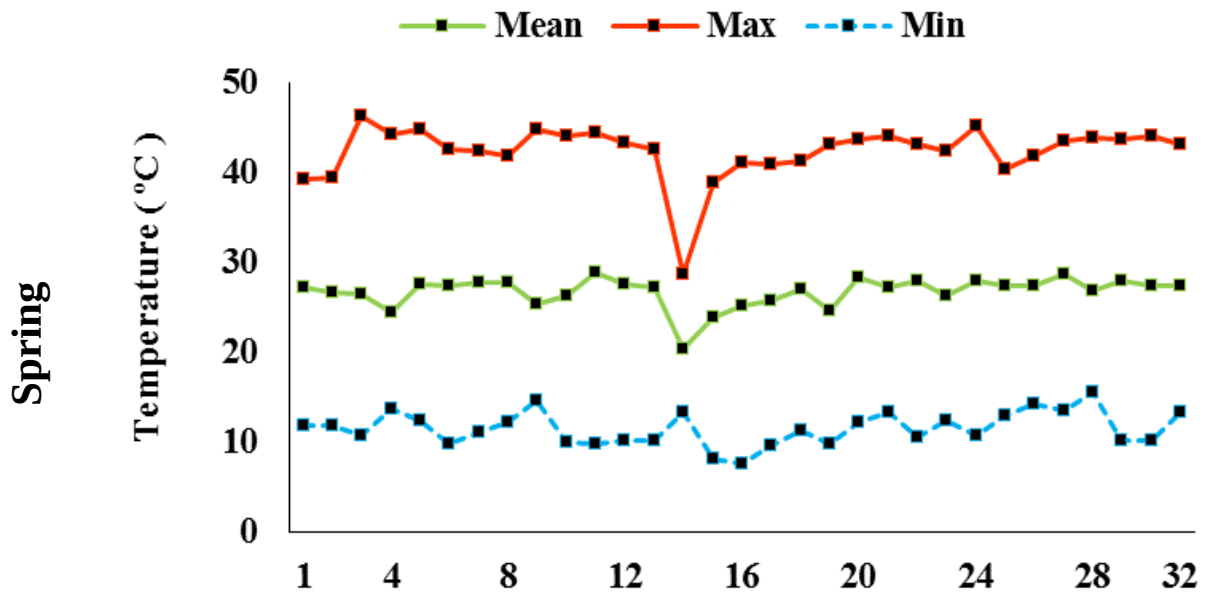
3.2 Results

Mean daily temperature and relative humidity values for the spring and summer experiments are shown in Figure 3.1. Mean temperatures were 26.2 ± 0.4 and 31.9 ± 0.2 ºC during the spring and summer periods, respectively. Likewise, mean relative humidity values were 64.7 ± 1.2 and 45.2 ± 2.0 %, respectively.

When we looked at the effect of "season" on the density of *T. urticae*, significant differences could be observed for all the treatments included in this experiment (Table 3.1). Therefore, we further continued our analyses separately for each season. Same as before, significant differences were observed for all treatments (Table 3.2) and the same occurred when we compared cumulative mite day values (CMD) (Table 3.3). When we considered the dynamics of *T. urticae* in the control treatments in spring and summer and referred these values to DD as the time measure, we observed that, for the same DD value, *T. urticae* densities were much higher during the summer than during the spring (Figures 3.2 to 3.6). Indeed, at the end of the spring assay, when almost to 500 DD had been accumulated, *T. urticae* density reached 500 mites per plant, whereas this value was reached at around 400 DD in the summer. Likewise, CMD values in the control were much higher at the end of the summer assay (2511.6 ± 61.6 versus 1693.2 ± 31.7 CMD for summer and spring, respectively). These results point at an effect of season independent of temperature, which could be related to photoperiod but also to a plant-mediated effect, which could make plants growing at summer conditions a better substrate for *T. urticae* than spring. The results of the identification of the phytoseiid species found at the end of the two assays are shown in Table 3.3. As expected, we never found any alien phytoseiid species in our treatments and figures were in general higher in summer than in spring. We recovered a minimum of 3 and a maximum of 13 individuals in the trophic chain treatments, which corresponded to *E. stipulatus* in spring and *P.*

persimilis in summer, respectively. In the case of competition, no specimens of *N. californicus* were recovered in spring irrespective of the combination considered, and the same happened to both this species and *E. stipulatus* when competing with each other in summer. When looking at the results of the sequential introduction of phytoseiids, we could see that in spring *E. stipulatus* could be recovered in half of the treatments and *N. californicus* in one fourth of them only. As a consequence, when looking at the grand totals recovered each season, *P. permimilis* resulted the most abundant species, with figures much higher than those reached by the other two phytoseiids. Therefore, it seems that priority effects do not play an important role in this system. However, *P. persimilis* appears as a better competitor both in spring and in summer, as its densities were higher in all cases where it was released, regardless of being introduced first, second or simultaneously in the system.

a)


Mean
Max
Min
Spring
Temperature (°C)
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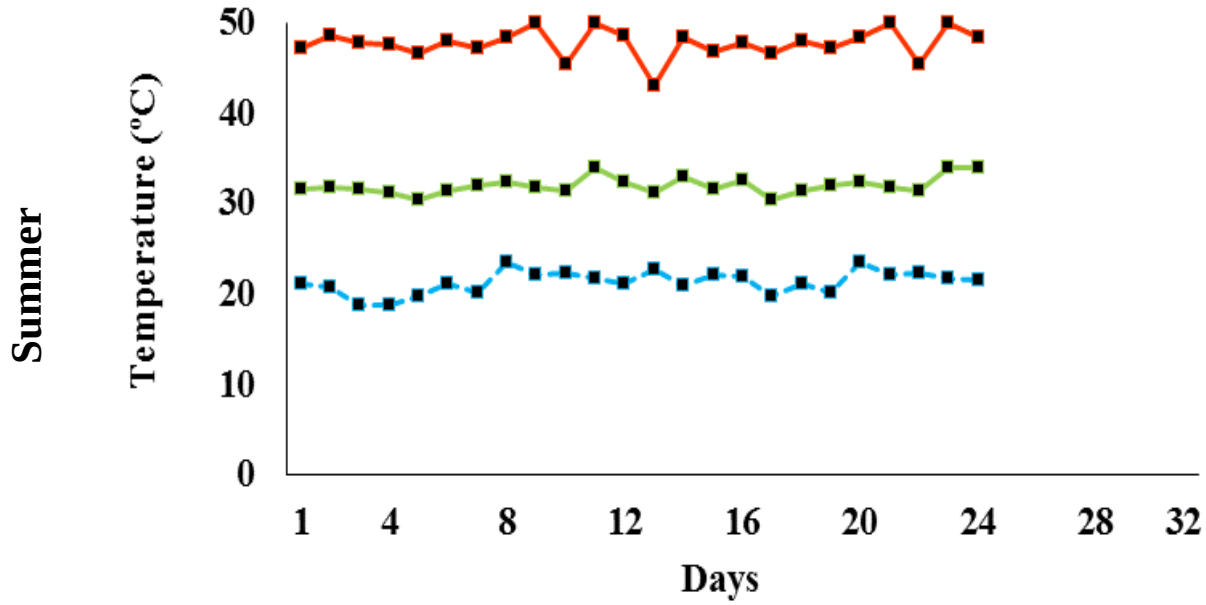
30
20
10
0
1
4
8
12
16
20
24
28
32


Summer
Temperature (°C)
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40
30
20
10
0
1
4
8
12
16
20
24
28
32
Days

b)

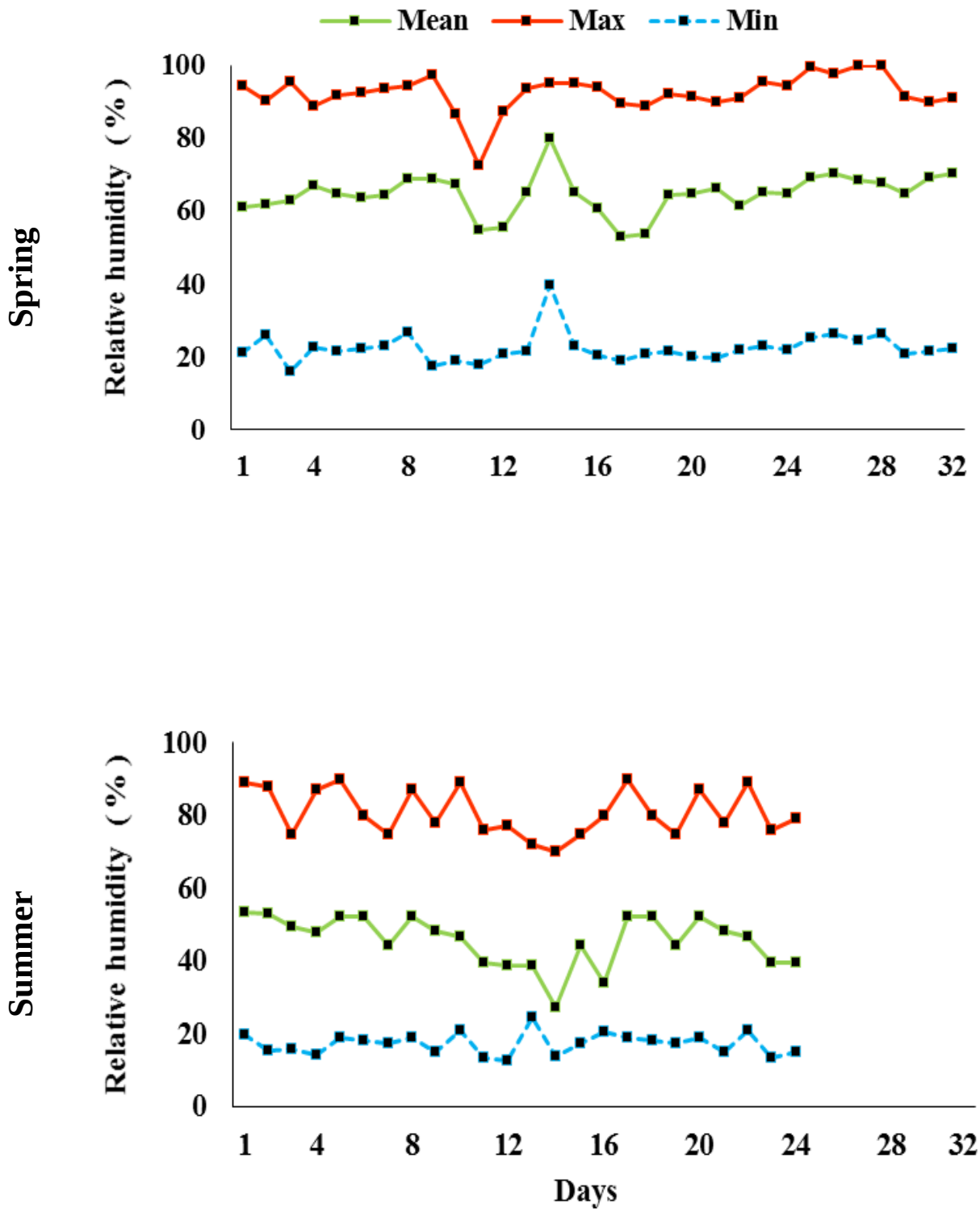


Figure 3.1. Temperature (a) and relative humidity (b) per day (max., min. and mean of data recorded every half hour) for spring and summer.

Treatment	Factor	*T. urticae* density
Trophic chain	Season	1.035; 1; 0.310
	Season * Combination	11.89; 7; < 0.001
Competition	Season	3.26; 1; 0.072
	Season * Combination	14.73; 7; < 0.001
Es-sequentially	Season	0.75; 1; 0.968
	Season * Combination	38.57; 5; < 0.001
Nc-sequentially	Season	0.41; 1; 0.531
	Season * Combination	22.95; 5; <0.001
Pp-sequentially	Season	0.71; 1; 0.429
	Season * Combination	18.01; 5; <0.001

Table 3.1. Statistics (F; *df*; P-value) of the GLMM models adjusted for each season (spring and summer) and the interaction between combinations (phytoseiid mite releases) for each treatment on *T. urticae* density per plant.

Treatment	Factor	*T. urticae* density	
		Spring	Summer
Trophic chain	Combination	21.82; 3; < 0.001	37.27; 3; < 0.001
	Combination* DD	3.50; 24; 0.038	6.59; 16; < 0.001
Competition	Combination	41.98; 3; < 0.001	397.19; 3; < 0.001
	Combination* DD	3.12; 24; < 0.001	76.29; 16; < 0.001
Es-sequentially	Combination	46.13; 2; < 0.001	141.05; 2; < 0.001
	Combination* DD	5.50; 18; 0.004	18.55; 12; < 0.001
Nc-sequentially	Combination	25.50; 2; < 0.001	67.81; 2; < 0.001
	Combination* DD	4.06; 18; < 0.001	16.75; 12; < 0.001
Pp-sequentially	Combination	86.94; 2; < 0.001	15.77; 2; < 0.001
	Combination* DD	8.89; 18; < 0.001	3.29; 12; < 0.001

Table 3.2. Statistics (F; *df*; P-value) of the GLMM models adjusted for combination (within each treatment) and the interaction between the degree days (DD) in each trophic module considering the factors treatment and combination *degree days within each season on *T. urticae* density per plant.

3.2.1 Trophic chain: effects of the release of a single phytoseiid species

The densities of *T. urticae* for the different treatments (control and each of the three phytoseiid species) in spring significantly differed starting at 232.9 DD after infestation (Figure 3.2a). From that date until the end of the assay, *T. urticae* densities were lower for the three phytoseiid species, with no clear differences between them until the end of the assay, when the density of *T. urticae* for the *E. stipulatus* and the *P. persimilis* treatments were significantly lower than that achieved when releasing *N. californicus* (Fig. 3.2a). At that time, the number of phytoseiids recovered was similar in the three treatments, from 5 to 6 (Table 3.3). In summer, *T. urticae* density was not different in control and *E. stipulatus* treatments (Figure 3.2b). Therefore, the efficacy of *E. stipulatus* in that experiment was nil (Table 3.4). For the other two phytoseiid treatments, significant differences were first observed at around the same degree-days as in spring, 227.38 DD after infestation. From this date until the end of the assay, we observed the lowest density of *T. urticae* in the *P. persimilis* treatment. Therefore, under summer conditions, *P. persimilis* was faster and had the highest efficacy at reducing *T. urticae* densities, *E. stipulatus* was not effective at all, although this species did not disappear from the system (Table 3.3), and *N. californicus* showed an intermediate result (Figure 3.2b). These results are in agreement with the number of phytoseiid specimens recovered at the end of the summer, which was higher than in spring for *P. persimilis* and *N. californicus* (Table 3.3). Consequently, when we compared CMD values at the end of the season, we found no differences between phytoseiids during the spring season, with the three species resulting in similar reductions (Table 3.4). However, when we compared the same treatments during the summer, significant differences between species were detected. The efficacy of *E. stipulatus* was nil. *T. urticae* CMD values achieved in the *N. californicus* treatment were not different between seasons but, as *T. urticae* populations were higher in the summer, the efficacy of this phytoseiid was also higher in summer. Finally, in the case of *P. persimilis*, there were differences between CMD values, which were lower in summer and, as a consequence, the efficacy of this mite almost doubled when comparing spring and summer CMD reductions, resulting in *P. persimilis* as the most efficient predator at regulating *T. urticae* populations, especially in summer.

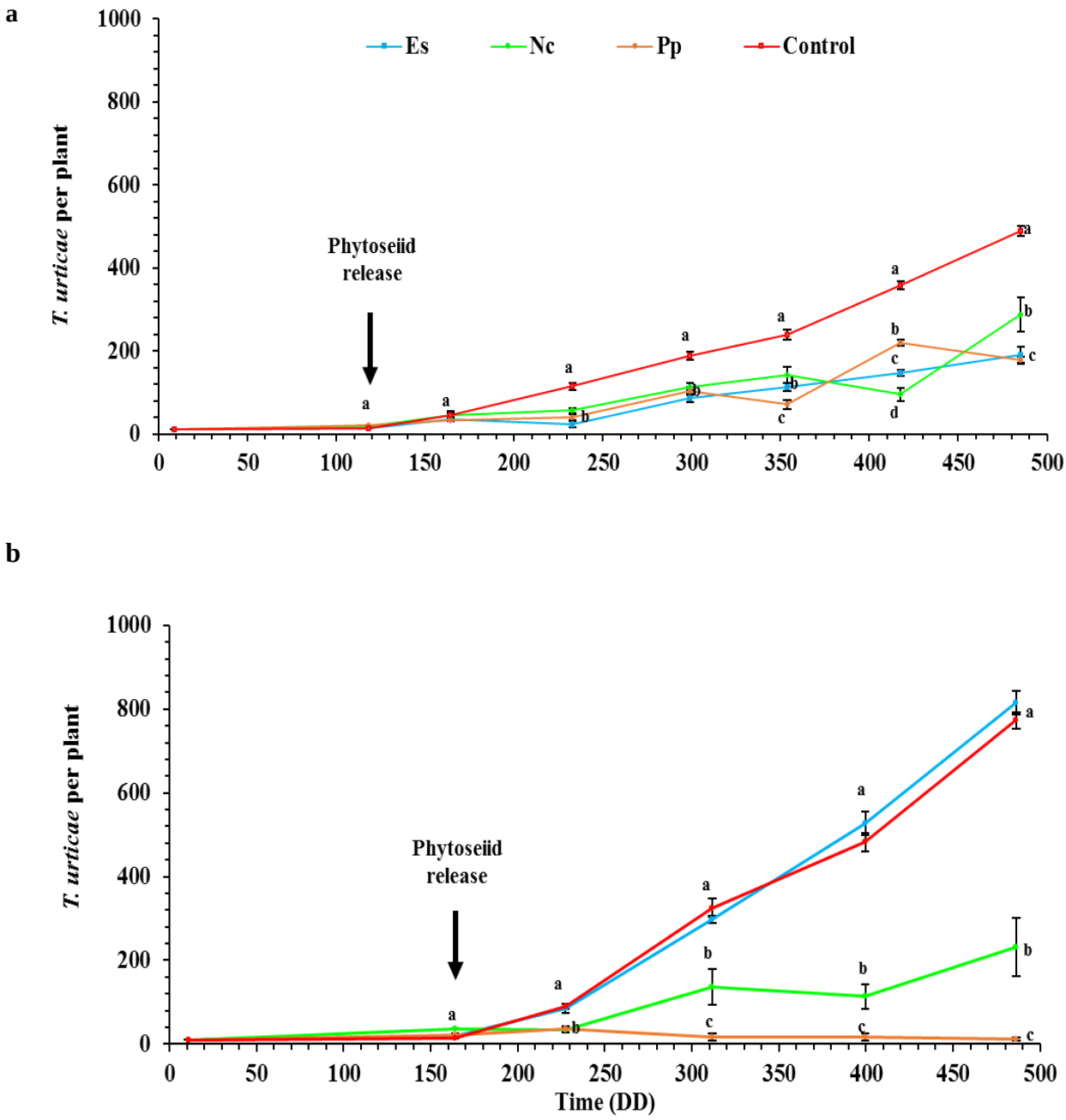


Figure 3.2. Density of *T. urticae* adult females on clementine plants when different phytoseiid species (Es = *E. stipulatus*, Nc = *N. californicus*, and Pp = *P. persimilis*; Control = no phytoseiid released) were released at a rate of 10 adult females per plant two weeks after the introduction of 10 adult females of *T. urticae* per plant during spring (a) and summer (b). A lower development threshold of 10.45ºC (Bounfour and Tanigoshi 2001) was set for the calculation of the degree days (DD). For each time point, values with different letters mean significant differences between species (Bonferroni; $p < 0.005$).

3.2.2 Competition: effects of simultaneous release of two phytoseiid species

Same as observed in the trophic chain in spring, *T. urticae* densities in control and the phytoseiid release treatments started showing significant differences at 232.9 DD after infestation and these differences remained until the end of the assay (Figure 3.3a). Additionally, during the fourth sampling date (299.0 DD after infestation), we observed significant differences between phytoseiid combinations, with the highest density of *T. urticae* in the *N. californicus-P. persimilis* combination, which was not significantly different from the other combination including *N. californicus* (*E. stipulatus-N. californicus*). Therefore, the *P. persimilis-E. stipulatus* combination resulted the most efficient (Table 3.4). In summer, same as before, we observed the first differences between treatments occurring at the second sampling date (227.4 DD after infestation). However, for most of the assay only the *P. persimilis-E. stipulatus* combination effectively reduced *T. urticae* density, with the other two combinations resulting in even higher densities than control until the end of the assay (486.3 DD after infestation), when both combinations eventually reduced *T. urticae* density to a similar value higher than that obtained for *P. persimilis-E. stipulatus* combination (Figure 3.3b). As a consequence of these results, when we looked at CMD values (Table 3.4), we observed when comparing spring and summer values a significant reduction in the efficiency of all combinations except for *P. persimilis-E. stipulatus*, which showed consistent results during both seasons. These results point at a negative effect of competition on *T. urticae* regulation in combinations including *N. californicus*. Interestingly though, as the densities of *P. persimilis* within the same season were similar at the end of the assay irrespective of the competing pair (*N. californicus* or *E. stipulatus*) (Table 3.3), reasons for the low efficacy found for the combination of this mite with *N. californicus* could be time dependent. However, as the same trend in *T. urticae* density was observed for the *N. californicus-E. stipulatus* combination in summer, when no phytoseiids could be recovered at the end of the assay, the reasons for these results remain open.

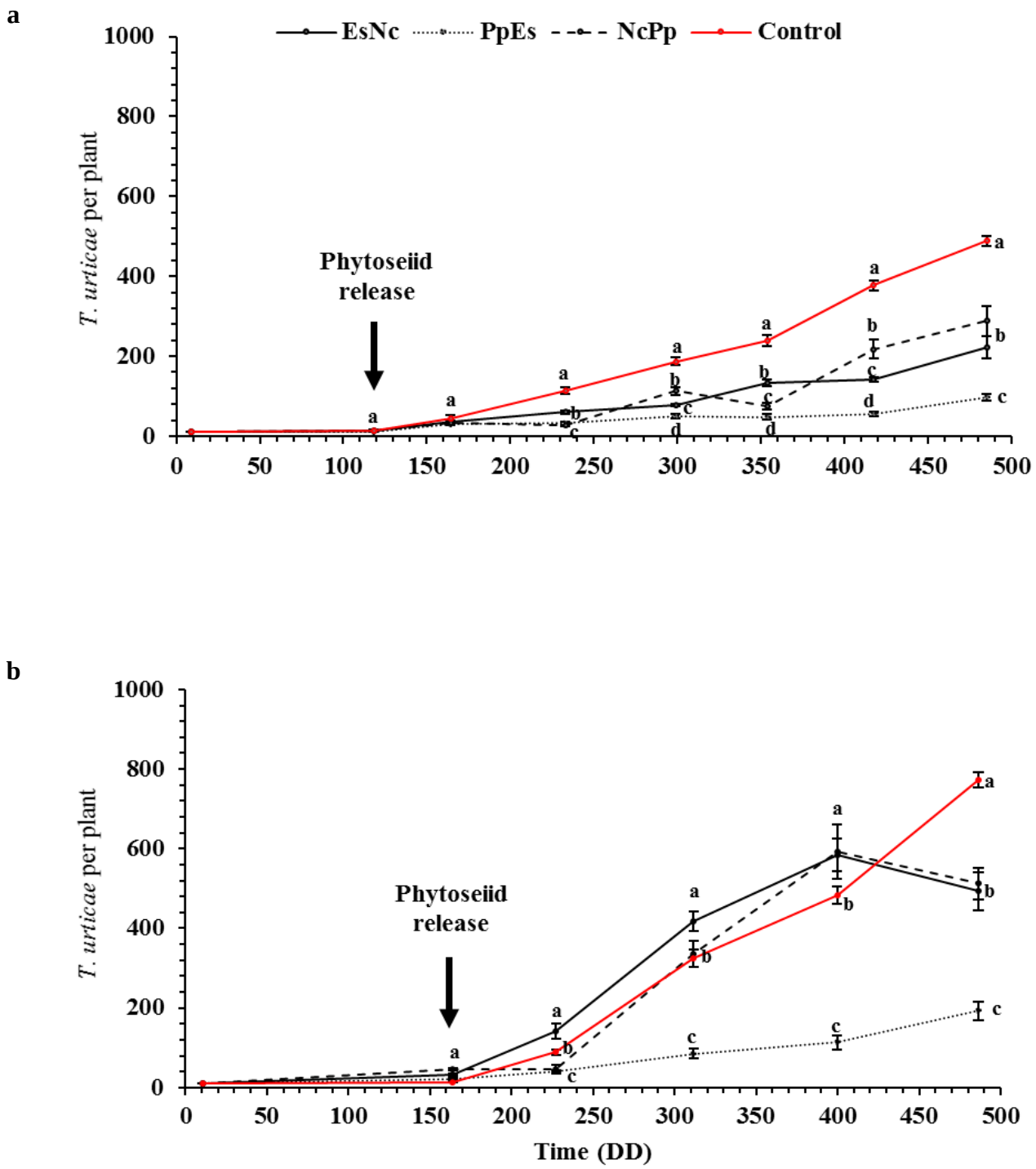


Figure 3.3. Density of *T. urticae* adult females on clementine plants when different combinations of phytoseiid (EsNc = *E. stipulatus* + *N. californicus*; PpEs = *P. persimilis* + *E. stipulatus*; NcPp = *N. californicus* + *P. persimilis*; Control = no phytoseiid released) were simultaneously released in pairs at a rate of 5 adult females of each mite species per plant two weeks after the introduction of 10 adult females of *T. urticae* per plant during spring (a) and summer (b). A lower development threshold of 10.45 ºC (Bounfour and Tanigoshi, 2001) was set for the calculation of the degree days (DD). For each time point, values with different letters mean significant differences between species (Bonferroni; $p < 0.005$).

3.2.3 Priority effects: sequential release of two phytoseiid species

When comparing the effect of the sequential release of *E. stipulatus* and *N. californicus* in spring, we observed that starting again at 232.9 DD there were differences with the control (Figure 3.4a). From that date until 353.8 DD, efficacies were similar for all treatments including any of these species, although for the sequential release treatments the number of adult phytoseiid mites initially released was half that of the other treatments. From that moment until the end of the assay, the sequential release of *N. californicus* first and then *E. stipulatus* showed the lowest efficacy (Table 3.4). Remarkably, the opposite sequence (*E. stipulatus* first and then *N. californicus*) resulted in a reduction similar to that achieved by *E. stipulatus* alone, although the numbers of these two phytoseiids recovered at the end of the assay were extremely low irrespective of the sequence (Table 3.3). In summer, we found again significant differences for the sequential release of these two species. Same as in spring, the release of *E. stipulatus* first and then *N. californicus* resulted in the highest efficacy from all combinations where any of these two species was considered (Figure 3.4b; Table 3.4). Remarkably, in this case, the opposite release sequence resulted in the same *T. urticae* densities as control and *E. stipulatus* alone, although the final numbers of phytoseiids recovered at the end of the assay for these two phytoseiids were not that different, especially for *E. stipulatus* (Table 3.3).

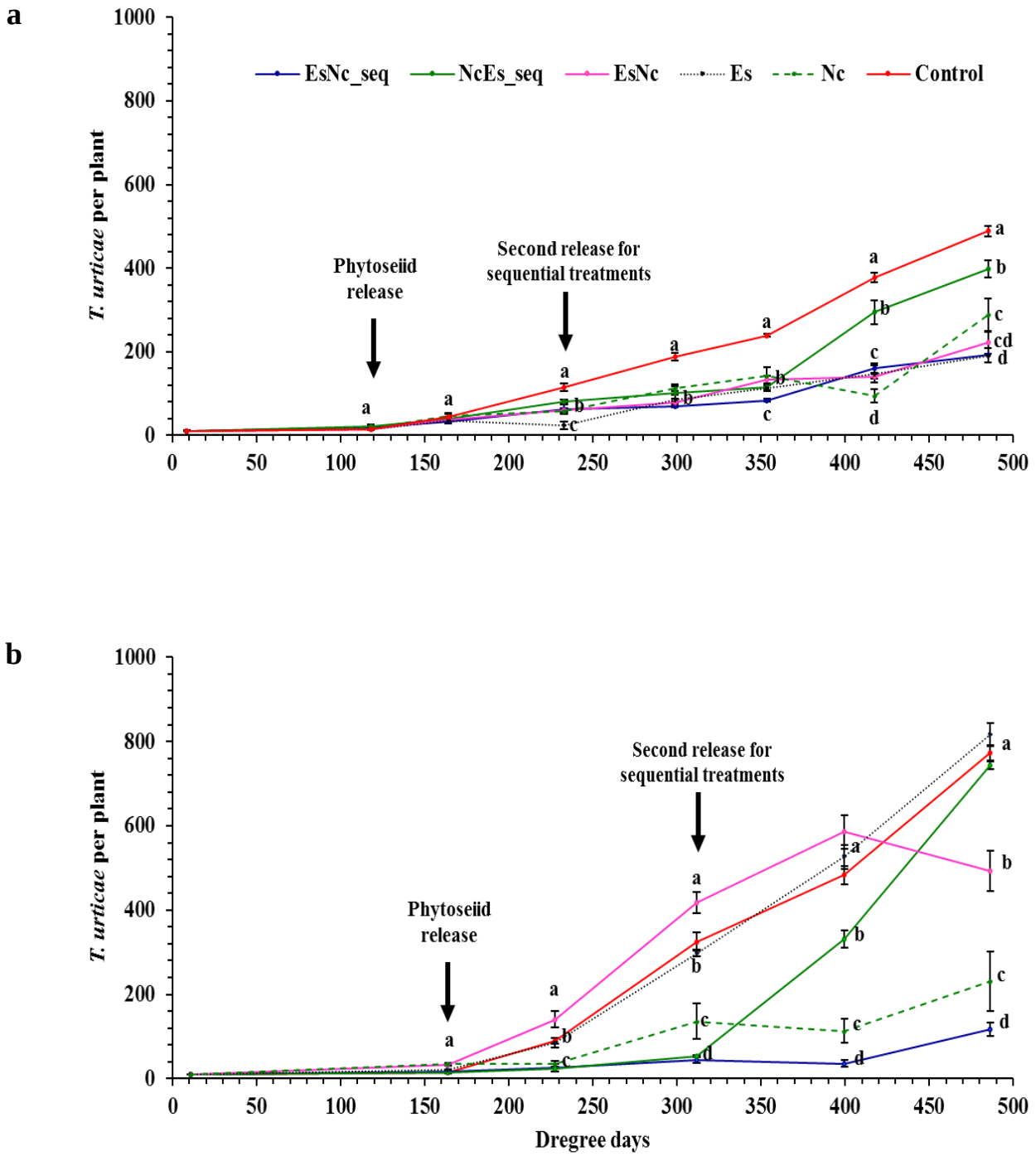
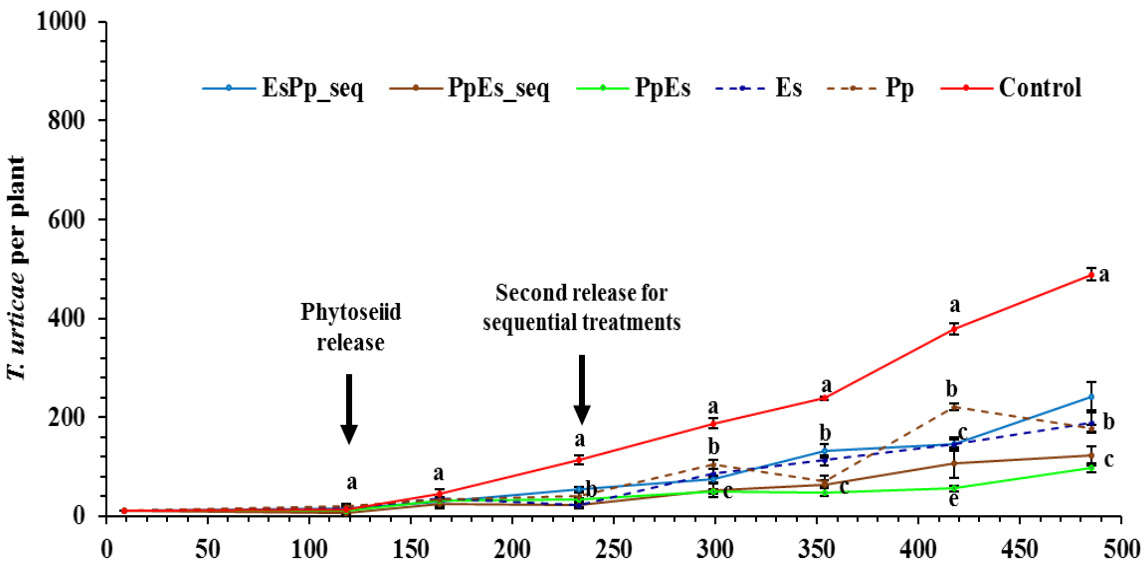


Figure 3.4. Density of *T. urticae* adult females on clementine plants when different species of phytoseiid were sequentially released at a rate of 5 adult females of each mite species per plant (EsNc_seq = 1º *E. stipulatus* + 2º *N. californicus*; NcEs_seq = 1º *N. californicus* + 2º *E. stipulatus*), simultaneously released in pairs at a rate of 5 adult females of each mite species per plant (EsNc = *E. stipulatus* + *N. californicus)* and Control (no phytoseiid released) during the spring (a) and summer (b). Arrows represent the introduction of the second phytoseiid specie (day 8). A lower development threshold of 10 ºC (Bounfour and Tanigoshi, 2001) was set for the calculation of the degree days (DD). For each time point, values with different letters mean significant differences between species (Bonferroni; $p < 0.005$).

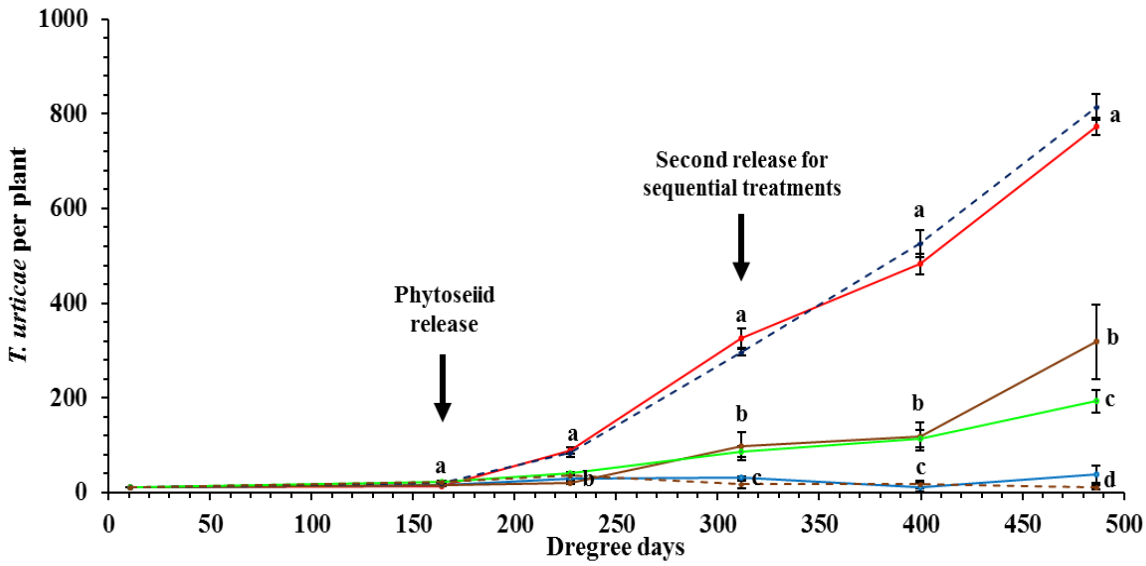
When we compared the releases of *E. stipulatus* and *P. persimilis* in spring, differences between treatments were not as huge as in the previous case. Releasing *P. persimilis* before *E. stipulatus* resulted in a better regulation than the opposite, and that result was similar to that obtained when releasing both species simultaneously (competition) and better than releasing them separately (Figure 3.5a; Table 3.4). Interestingly though, the number of *P. persimilis* recovered at the end of the assay, when released after *E. stipulatus,* was much higher than the opposite (table 3.3). In summer, the situation changed: starting at 227.4 DD after initial infestation, the two sequences including *E. stipulatus* and *P. persimilis* diverged. On the one hand, releasing *E. stipulatus* first resulted better and almost as good as releasing *P. persimilis* alone. On the other, the opposite sequence was a slightly worse than releasing them simultaneously (Figure 3.5b; table 3.3). It is important to recall here that the efficacy of *E. stipulatus* alone in summer had no effect on the density of *T. urticae* (Figure 3.5b; Table 3.4).

Figure 3.5. Density of *T. urticae* adult females on clementine plants when different species of phytoseiid were sequentially released at a rate of 5 adult females of each mite species per plant (EsPp_seq = 1º *E. stipulatus* + 2º *P. persimilis*; PpEs_seq= 1º *P. persimilis*+ 2º *E. stipulatus*), simultaneously released in pairs at a rate of 5 adult females of each mite species per plant (EsPp = *E. stipulatus* + *P. persimilis)* and Control (no phytoseiid released) during the spring (a) and summer (b). Arrows represent the introduction of the second phytoseiid specie (day 8). A lower development threshold of 10 ºC (Bounfour and Tanigoshi, 2001) was set for the calculation of the degree days (DD). For each time point, values with different letters mean significant differences between species (Bonferroni; $p < 0.005$).

a



b



The last couple under scrutiny, *N. californicus* and *P. persimilis* showed almost no differences in spring (Figure 3.6a) although no *N. californicus* specimens could be found at the end of the assay in any of the treatments where it was jointly released with *P. persimilis* (Table 3.3). This situation changed in summer and specimens of all species were recovered although *P. persimilis* was much more abundantly found (Table 3.3). Nevertheless, these higher phytoseiid counts did not result in a better regulation when simultaneously released (Table 3.4). During that season, sequential release of these two species resulted as efficient as releasing *P. persimilis* alone but less than releasing *N. californicus* only (Figure 3.6b; table 3.4).

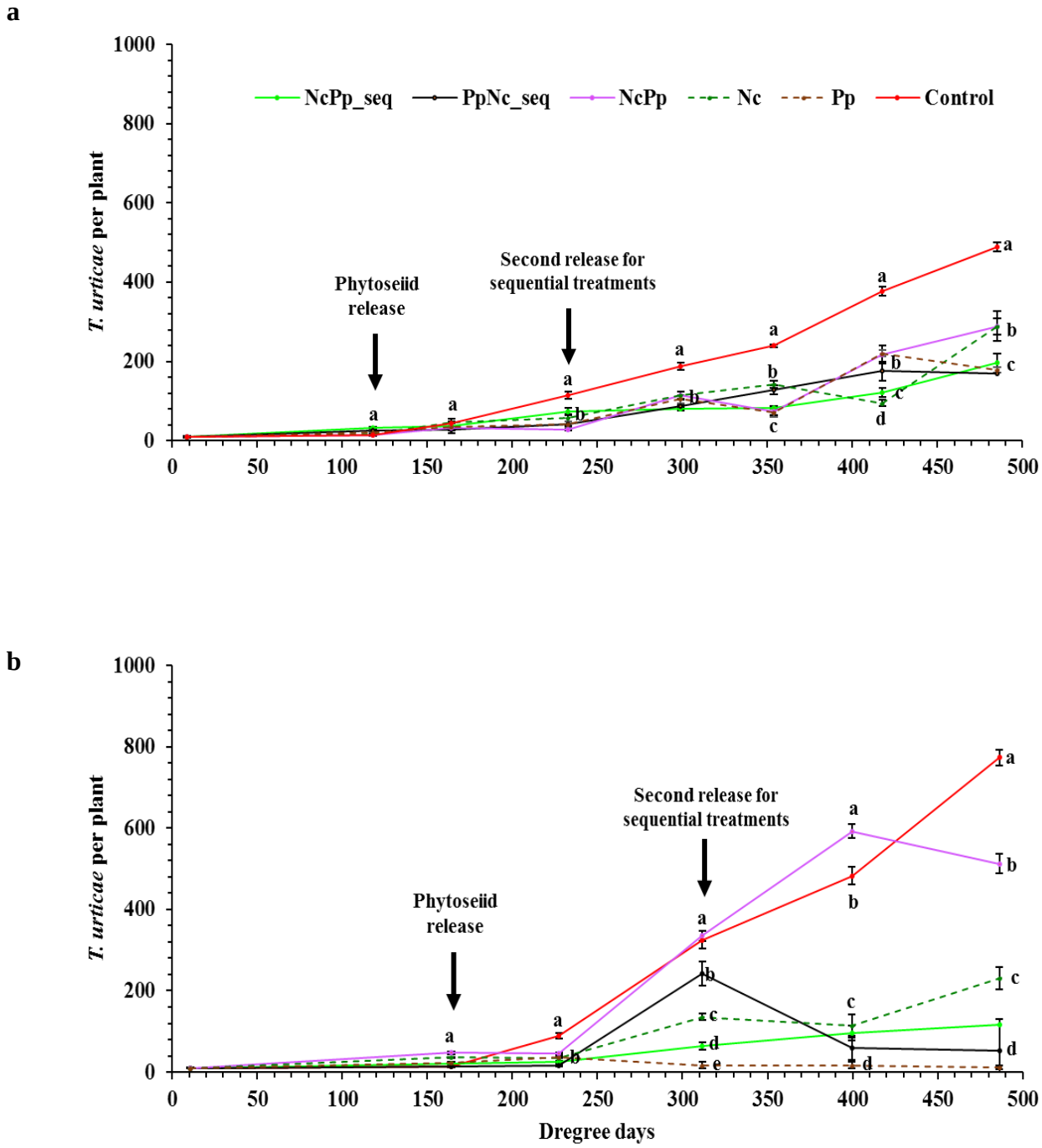


Figure 3.6. Density of *T. urticae* adult females on clementine plants when different species of phytoseiid were sequentially released at a rate of 5 adult females of each mite species per plant (NcPp_seq = 1º *E. stipulatus* + 2º *N. californicus*; PpNc_seq = 1º *P. persimilis*+ 2º *N. californicus*), simultaneously released in pairs at a rate of 5 adult females of each mite species per plant (NcPp = *N. californicus* + *P. persimilis)* and Control (no phytoseiid released) during the spring (a) and summer (b). Arrows represent the introduction of the second phytoseiid specie (day 8). A lower development threshold of 10 ºC (Bounfour and Tanigoshi, 2001) was set for the calculation of the degree days (DD). For each time point, values with different letters mean significant differences between species (Bonferroni; $p < 0.005$).

Treatment / Combination		Spring (26.4 ± 0.4 ºC; 63.7 ± 2.1 % RH)			Summer (30.4 ± 0.2 ºC; 45.7 ± 1.7 % RH)		
		E.stipulatus	*P. persimilis*	*N. californicus*	*E.stipulatus*	*P. persimilis*	*N. californicus*
Trophic chain	*E. stipulatus* (Es)	1 (6)	-	-	1 (3)	-	-
	P. persimilis (Pp)	-	1 (5)	-	-	5 (13)	-
	N. californicus (Nc)	-	-	1 (5)	-	-	2 (8)
Competition	Es + Nc	1 (1)	-	0 (0)	0 (0)	-	0 (0)
	Pp + Es	1 (1)	5 (6)	-	2 (3)	11 (14)	-
	Nc + Pp	-	3 (3)	0 (0)	-	10 (14)	2 (7)
Priority effects	Es + Nc	0 (0)	-	0 (0)	3 (7)	-	5 (11)
	Nc + Es	0 (1)	-	0 (1)	5 (5)	-	1 (1)
	Pp + Es	0 (1)	1 (1)	-	1 (1)	2 (2)	-
	Es + Pp	0 (0)	8 (12)	-	0 (2)	5 (7)	-
	Nc + Pp	-	1 (1)	0 (0)	-	4 (4)	3 (3)
	Pp +Nc	-	2 (2)	0 (0)	-	10 (0)	2 (3)
Grand Total		3 (10)	21 (30)	1 (6)	12 (21)	47 (54)	15 (33)

Table 3.3. Number of adult females of each phytoseiid species recovered at the end of each assay in the spring and summer treatments for each release combination: single species (trophic chain); two species simultaneously (competition) and two species released one week apart from each other (priority effects). The figure in brackets corresponds to the total number of specimens recovered (females and remaining immature motiles) which, in the case of the combinations including both *E. stipulatus* (Es) and *N. californicus* (Nc), were distributed between species based on the proportion of females found. In the case of *P. persimilis* (Pp), because of the orange color of motiles, all forms, both adult and immature, were identified at species level.

Treatment / Combination		Spring (26.4 ± 0.4 ºC; 63.7 ± 2.1 % RH)		Summer (30.4 ± 0.2 ºC; 45.7 ± 1.7 % RH)		*t*-test spring-summer	
		CMD	Decrease	CMD	Decrease	CMD	Decrease
Control		1693.2 ± 31.7 a	-	2511.6 ± 61.6 a	-	P < 0.001	-
Trophic chain	*E. stipulatus (Es)*	671.8 ± 41.5 d	60.7 ± 6.1 b	2684.5 ± 44.6 a	0 d	P < 0.001	P < 0.001
	P. persimilis (Pp)	797.3 ± 13.3 d	52.8 ± 1.5 bc	57.2 ± 4.8 f	97.6 ± 1.2 a	P = 0.235	P < 0.001
	N. californicus (Nc)	764.4 ± 89.2 d	51.6 ± 9.3 bc	688.6 ± 98.6 c	70.5 ± 13.4 b	P = 0.235	P < 0.001
Competition	Es + Nc	727.4 ± 64.8 d	60.6 ± 8.0 b	2156.3 ± 67.6 b	20.10 ± 10.0 c	P < 0.001	P < 0.001
	Pp + Es	307.8 ± 23.9 e	75.4 ± 3.3 a	612.2 ± 79.3 c	75.9 ± 9.5 b	P = 0.024	P = 0.962
	Nc + Pp	1012.7 ± 61.8 c	43.6 ± 8.8 c	2208.1 ± 105.3 b	16.3 ± 4.5 c	P < 0.001	P < 0.001
Priority effects	Es + Nc	704.9 ± 50.9 d	56.3 ± 8.6 bc	304.4 ± 71.9 d	86.9 ± 2.6 b	P < 0.001	P < 0.001
	Nc + Es	1382.5 ± 79.1 b	16.3 ± 7.4 d	2149.5 ± 52.7 b	10.8 ± 7.6 c	P < 0.001	P = 0.457
	Pp + Es	456.3 ± 22.1 e	77.1 ± 3.8 a	874.2 ± 84.6 c	68.5 ± 5.8 b	P = 0.015	P = 0.903
	Es + Pp	773.9 ± 59.3 d	53.8 ± 5.4 bc	100.7 ± 25.3 e	96.1 ± 7.7 a	P < 0.001	P < 0.001
	Nc + Pp	634.7 ± 60.8 d	62.3 ± 7.5 ab	424.3 ± 51.5 c	83.4 ± 14.8 b	P = 0.027	P = 0.084
	Pp +Nc	649.6 ± 61.3 d	66.0 ± 7.6 ab	226.1 ± 34.0 d	90.1 ± 3.8 a	P = 0.003	P = 0.019
GLM (Wald χ^2;df; *P*)		98.76; 12; < 0.001	68.56; 11; < 0.001	52.68; 12; < 0.001	124.93; 11; < 0.001	-	-

Table 3.4. Cumulative Mite Day (CMD; mean ± standard error) per plant and percentage of *T. urticae* density reduction compared with control treatment at two different seasons (spring and summer) observed for control (no phytoseiid released), single release of either *E. stipulatus* (Es), *N. californicus* (Nc) or *P. persimilis* (Pp), simultaneous release of the same species in pairs (Es+Nc, Pp+Es, Nc+Pp) and sequential release of the same pairs one week apart. Within each column, values followed by the same letter are not significantly different (Bonferroni post hoc test at P < 0.05).

3.3 Discussion

The objective of this study was to check whether Climate Change could disrupt existing natural regulation of *Tetranychus urticae* by its main predators in Valencian citrus orchards. Our results provide evidence that this may be indeed the case. On the one hand, we have proved that the regulation of *T. urticae* provided by its predators *E. stipulatus*, *N. californicus*, and *P. persimilis* depends on the season (Table 3.1) and in our assays, we took season as proxy for present versus Climate Change conditions. Actually, mean temperature was around 5 ºC higher and relative humidity around 20 % lower during our summer assay and this is close to predicted future Climate Change conditions for the Mediterranean basin (Lionello 2008; Navarra & Tubiana 2013; Gualdi et al., 2012). On the other hand, this regulation is species-specific and is affected by interspecific competition but apparently not by priority effects (Tables 3.2 and 3.3).

In general, we observed a better natural regulation of *T. urticae* populations in spring than in summer. Although the average decreases in *T. urticae* populations observed in spring and summer were similar (55.9 ± 4.6 versus 59.7 ± 5.1 %, respectively) (Table 3.4), differences between treatments were higher in summer (from 0 to 97.6 % for *E. stipulatus* and *P. persimilis*, respectively) than in spring (from 16.3 to 77.1 % for the sequential release of *N. californicus* plus *E. stipulatus* and *P. persimilis* plus *E. stipulatus*, respectively). The three species under scrutiny provided the same level of control when released singly in spring. However, in summer, *E. stipulatus* provided no control whereas the other two species were even more effective than in spring (Table 3.4). In line with our observations, Grafton-Cardwell et al. (1997) recognized *E. stipulatus* to significantly reduce *T. urticae* populations in 2-year-old orange trees in a semifield experiment in spring. Conversely, at summer conditions (hot temperature and low relative humidity values) either *N. californicus* (Palevsky et al., 2008; Walzer et al., 2007) or *P. persimilis* (Schausberger and Walzer 2001) alone have been considered as better suited for immediate suppression of local spider mite populations in greenhouse crops. As *E. stipulatus* is the most abundant phytoseiid species in Valencian clementine orchards (Garcia-Marí et al., 1991; Abad-Moyano et al., 2009a; Aguilar-Fenollosa et al., 2011b), one might consequently expect *T. urticae* problems to worsen with Climate Change. However, as under these conditions *P. persimilis* appears as

the most tolerant species (Table 3.3) and this species is also considered as the most efficient predator of *T. urticae* in clementines (Pérez-Sayas et al., 2015), the situation could be reverted. However, as *N. californicus* also thrived under these conditions (Table 3.3), competition between *E. stipulatus* and the other two phytoseiids could decrease with Climate Change but increase for *P. persimilis* and *N. californicus*. When looking at the results of the simultaneous release of pairs of these three species, the combination *P. persimilis* plus *E. stipulatus* was in fact the only one that did not lose efficacy in summer compared to spring (Table 3.4). Intriguingly, the numbers of *P. persimilis* at the end of the assay when released with either *E. stipulatus* or *N. californicus* were similar (Table 3.3). Because the initial populations of *P. persimilis* and *E. stipulatus* used in our assays were collected in the vicinity of the site where our assays took place, whereas the origin of *N. californicus* remains ignored as it was purchased from Koppert Biological Systems, we could hypothesize that the former two local strains were adapted to coexist and endure the climatic fluctuations occurring during our assays. Yet, *N. californicus* was not. In fact, *N. californicus* was again implicated in the only combination of sequentially released phytoseiids where efficacy was below 20 % in both seasons (table 3.4), and this was the case of *N. californicus* being first released and followed by *E. stipulatus*.

As, in general, *P. persimilis* alone or in combination with the other predators, produced the highest efficacies both in spring and in summer, our assays reinforce the idea that this is the key species to maintain in the system to ensure a good natural regulation of *T. urticae* populations in clementines (Aguilar-Fenollosa et al., 2011b). Interestingly, some authors (Skirvin and Fenlon 2003; Walzer et al. 2007), consider this *T. urticae* specialist a suitable predator until 25 ºC but not at higher temperatures. Once again, the fact of using a local strain of *P. persimilis* presumably used to prevailing climatic conditions in clementine orchards may explain our results and highlights the importance of locally adapted natural enemies to cope with Climate Change (Aguilar-Fenollosa and Jacas 2014). To further challenge this hypothesis, it would be important to repeat our assays with a local strain of *N. californicus* occurring in clementines.

One striking result of our experiments is that in almost half of the combinations including *N. californicus* (6 out of 14) and one third of those including *E. stipulatus* (3 out of 14), we could not

recover any motile of these species at the end of the assay whereas this situation was never encountered for *P. persimilis* (Table 3.3). Although we assume that our samplings are representative of the species abundance in our trees, we cannot exclude some circadian rhythms occurring in our system, which may have hindered the estimation of the real abundance of these species. Indeed, Pérez-Sayas et al. (2017) demonstrated that the three phytoseiids included in our study exhibit diel and seasonal predatory patterns when feeding on *Tetranychus urticae* which may explain why *N. californicus*, which is usually present in clementine orchards at densities above those of *P. persimilis* (Aguilar-Fenollosa et al., 2011b), was barely found during samplings performed around midday in summer (Pérez-Sayas et al., 2015). Moreover, additional semifield trials where *N. californicus* was released demonstrated that this species is prone to seek refuge in citrus branches and trunks (Pina et al., 2013). Therefore, the results of Table 3.3 should be taken with caution and, if possible, further assays digging on the same issue should consider the sampling of not only leaves but also branches to discard any of the effects commented above.

To sum up, our data are indicative that Climate Change may actually disrupt existing natural regulation of *T. urticae* in clementines, same as other authors have described for similar systems (Montserrat et al. 2013a, b; Guzmán et al. 2016b). In order to examine all the possible interactions between the three phytoseiids above and *T. urticae*, further laboratory research under controlled conditions could help explaining the results obtained and better predicting future outcomes. In the following chapters of this thesis, we will explore different community modules (trophic chain, and competition) and whether the provision of pollen as alternative diet could mitigate these detrimental effects of Climate Change.

4

Effect of abiotic conditions on three predators of *Tetranychus urticae*: *Euseius stipulatus*, *Neoseiulus californicus* and *Phytoseiulus persimilis* at laboratory conditions

Hypothesis

The extreme temperatures and relative humidity values typical of Climate Change will differentially affect the biological parameters of *Tetranychus urticae* and its predators *Euseius stipulatus*, *Neoseiulus californicus* and *Phytoseiulus persimilis*. Because species with the highest specializations in terms of lifestyle or habitat are typically most at risk of Climate Change (Aguilar-Fenollosa & Jacas, 2014), a positive or neutral effect on this polyphagous and cosmopolitan herbivore is expected but negative on its specialized phytoseiid predators, maximal for the *Tetranychus* sp.-specialist *P. persimilis*, minimal for the pallinophagous omnivore *E. stipulatus*, and intermediate for *N. californicus*.

4.1 Material and methods

4.1.1 Environmental conditions

Different temperature (10 to 40ºC in 5ºC steps) and relative humidity values, RH (30, 50 and 70 %) were combined in our assays. Constant relative humidity values were obtained by using different salt solutions (Winston and Bates, 1960) in desiccators kept inside environmental chambers (Sanyo Electric Co., Ltd., Japan) set at a photoperiod of 16:8 h L:D and the different target temperatures.

4.1.2 Phytoseiid performance: fate and oviposition

The fate of the three most abundant predators of *T. urticae* in Valencian clementine orchards (Abad-Moyano et al., 2009a; Aguilar-Fenollosa et al., 2011b): *E. stipulatus*, *N. californicus* and *P. persimilis*, was studied following the procedure explained in Chapter 2 (Common Material and Methods).

4.1.3 *T. urticae* performance: fate and oviposition

A few days prior to the start of the test, several hundred ovipositing females were randomly taken from the rearing units and transferred to a new unit (consisting of a detached bean leaf) for 24 h. Subsequently females were removed. These units were held separately in a climatic chamber (25ºC, 65 % RH) and constituted the cohorts used in our assays. When attaining the adult stage, immediately after

the quiescent teliochrysalis stage, less than 24 h old presumably-mated females were selected and individually moved into a clean experimental arena. Fate and oviposition were assessed.

4.1.4 Statistical analyses

To study the effects of the different factors included in our assays on fate, predation (only for phytoseiids) and oviposition, we used general linear models (GLM) and separately analyzed *T. urticae* and phytoseiids. In the case of fate, we used a GLM with a multinomial distribution of the error. Likewise, for predation and oviposition we used a GLM with a Poisson distribution of the error. In all cases, the factors "species", time period "time" (i.e., evaluation 24 and 48 hours after the onset of the assay), temperature "T" and relative humidity "RH" were used as fixed effects. As one of our main goals was to identify phytoseiid species-specific differences, in the case of predators, we started our analyses by considering all combinations including the "species" factor. Once the signification of the "species" factor was clear, we similarly continued the analyses of fate, predation and oviposition by studying the effect of the "time" factor for each species. Eventually, for these parameters, we separately analyzed for each species and time period the effects of, "T", "RH" and their interaction. When necessary, we used Bonferroni post-hoc test for mean separation. In the case of *T. urticae*, fate and oviposition rates were analyzed using the same models as those already selected for phytoseiids.

4.2 Results

4.2.1 Effects of temperature and relative humidity on phytoseiids

We observed significant differences between phytoseiid species for all parameters considered (fate, predation and oviposition) (Table 4.1). Consequently, we further analyzed the effect of time when measurements were done (24 and 48 hours after the onset) for each species separately. This factor, either alone or through the interactions with other factors, was significant for all parameters of the three phytoseiid species, except for oviposition in *E. stipulatus* (Table 4.2a, b and c) and this result should be probably attributed to the extremely low fecundity of this species in our assays. To decide the time period better suited for our purposes, we looked at survival. The fact that number of individuals evaluated 48 hours after the onset of the assay was much lower than that evaluated during the first 24 hour period (i. e 22, 32 and 55 % of survival during the second time period, for *E. stipulatus, P.*

persimilis and *N. californicus*, respectively), made us decide to further focus our analyses on the results obtained during the first 24 hours only. Consequently, we finally analyzed the effect of temperature "T", relative humidity "RH" and their interactions for each mite species separately 24-hour after the onset of the assay (i.e., 48 hours after the start of the starvation period).

Table 4.1. Signification (Wald X^2; *df*; P-value) of the different GLM fit to four different parameters (fate, predation, oviposition, and egg hatching) using the factors phytoseiid species (sp), temperature (T), relative humidity (RH), and evaluation time (Time) and their interactions as explanatory variables.

Factors	Fate	Predation	Oviposition
Model	259.14; 62; <0.001	7089.74; 102; <0.001	2891.71; 102; <0.001
sp	28.76; 2; <0.001	1666.78; 2; <0.001	115.06; 2; <0.001
T * sp	53.58; 18; ; <0.001	3954.17; 18; <0.001	2019.30; 18; <0.001
RH * sp	18.92; 8; <0.001	964.66; 8; <0.001	151.63; 8; <0.001
Time * sp	452.21; 5; <0.001	1250.804; 5; <0.001	127.17; 5; <0.001
T * RH * sp	153.95; 54; <0.001	7026.59; 54; <0.001	2269.18; 54; <0.001
T * Time * sp	1421.12; 37; <0.001	6475.11; 37; <0.001	2407.44; 37; <0.001
RH * Time * sp	756.21¸17; <0.001	1872.85; 17; <0.001	339.78; 17; <0.001
T * RH * Time * sp	7453.41; 102; <0.001	8066.09; 102; <0.001	2646.95; 102; <0.001

Table 4.2. Signification (F; *df*; P-value) of the different GLM fit to three different parameters (fate, predation, and oviposition) for (a) *E. stipulatus*, (b) *N. californicus*, (c) *P. persimilis* and (d) *T. urticae* using the factors temperature (T), relative humidity (RH), and evaluation time (Time) and their interactions as explanatory variables.

a)

Factors	Fate	Predation	Oviposition
Model	12.33; 29; < 0.001	589.36; 29; < 0.001	70.04; 29; < 0.001
Time	96.3; 1; 0.001	1.70; 1; 0.192	1.64; 1; 0.200
T * Time	145.39; 9; < 0.001	322.21; 9; < 0.001	2.52; 9; 0.773
RH * Time	7.08; 5; 0.021	4.08; 5; 0.538	1.65; 5; 0.648
T * RH * Time	963.32; 29; < 0.001	732.822; 29; < 0.001	0.137; 1; 0.711

b)

Factors	Fate	Predation	Oviposition
Model	4563.21; 35; < 0.001	1866.64; 35; < 0.001	436.09; 35; < 0.001
Time	7894.45; 1; < 0.001	140.68; 1; < 0.001	32.56; 1; < 0.001
T * Time	963.32; 11; < 0.001	1821.56; 11; < 0.001	258.87; 11; < 0.001
RH * Time	442.11; 5; < 0.001	524.24; 5; < 0.001	70.35; 5; < 0.001
T * RH * Time	3112.01; 35; < 0.001	2234.45; 35; < 0.001	61.31; 35; < 0.001

c)

Factors	Fate	Predation	Oviposition
Model	321.12; 35; < 0.001	2261.39; 35; < 0.001	407.55; 35; < 0.001
Time	5.44; 1; 0.003	1.08; 1; 0.299	2.01; 1; 0.354
T * Time	1025.31; 11; < 0.001	2074.52; 11; < 0.001	321.61; 11; < 0.001
RH * Time	45.11; 5; < 0.001	25.18; 5; < 0.001	3.24; 5; 0.519
T * RH * Time	56.32; 35; < 0.001	58.95; 35; < 0.001	33.51; 35; 0.014

d)

Factors	Fate	Oviposition
Model	12.21; < 0.001	789.41; < 0.001
Time	23.54; 1; < 0.001	145.32; 1; < 0.001
T * Time	78.89; 11; < 0.001	14.25; 11; < 0.001
RH * Time	54.36; 5; < 0.001	13.01; 5; < 0.001
T * RH * Time	685.12; 41; < 0.001	789.12; 41; < 0.001

4.2.2 Fate of Phytoseiids

The GLM model best fitting fate 24 hours after the onset of the assay for the three phytoseiids predators *E. stipulatus*, *N. californicus* and *P. persimilis* included "T" and "RH" (Table 4.3).

Factors	*E. stipulatus*	*N. californicus*	*P. persimilis*
Model	85.58; 20; < 0.001	59.19; < 0.001	66.88; < 0.001
T	24.07; 6; 0.001	10.79; 6; 0.095	9.82; 6; 0.132
RH	6.68: 2; 0.035	3.31: 2; 0.191	6.37: 2; 0.041
T*RH	52.77; 20; < 0.001	42.60; 20; < 0.001	50.92; 20; < 0.001

Table 4.3. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to fate (multinomial). Estimated during the first 24-hour period after the onset of the assay for *E. stipulatus, N. californicus* and *P. persimilis* considering the factors temperature (T) and relative humidity (RH) as well as their interaction.

For *E. stipulatus* (Figure 4.1), both temperature and relative humidity and their interaction were significant (Table 4.3). This species could not survive temperatures beyond 30 ºC. Interestingly, around half of the individuals at these conditions tried to escape and this may be taken as indicative that in the real world these individuals would have been able to survive in refuges. Below this threshold, survival usually increased with relative humidity. However, the percentage of escapees was around 50 % in all combinations of temperature and relative humidity considered except 25 ºC and either 50 or 75 % RH.

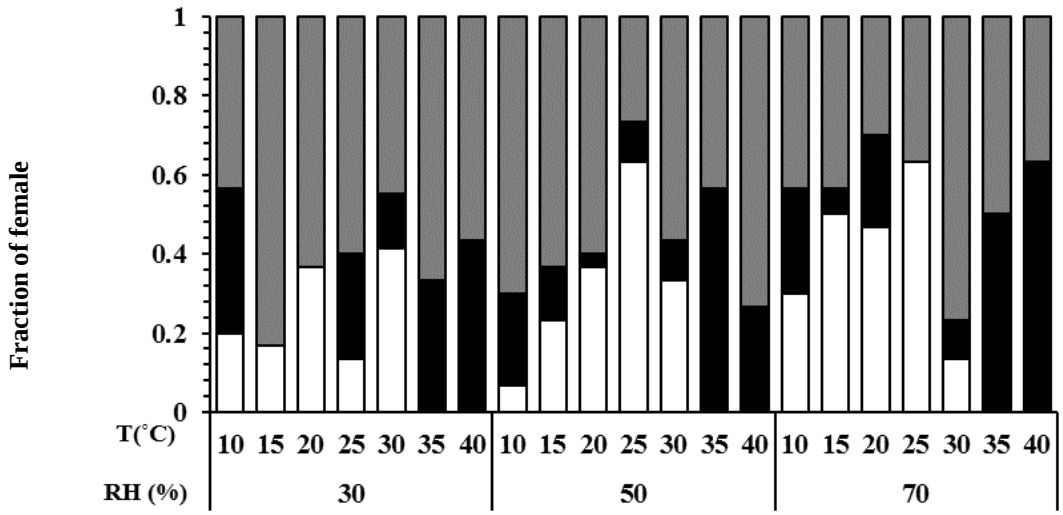


Figure 4.1. Fraction of females (fate) either stuck in the glue (escapees, grey bars), dead (black bars) and alive (white bars) during the first 24-hour period after the onset of the assay for *E. stipulatus.*

For *N. californicus* only the interaction between temperature and relative humidity was significant (Table 4.3). This species could not survive 40ºC at any of the three relative humidity values considered. Same as *E. stipulatus*, around half of the individuals tried to escape from these arenas. However, for the remaining conditions, survival was similar and relatively high (Figure 4.2). At 25 ºC and 70 % relative humidity survival was 100 %. The fact that this species was purchased from a commercial producer may explain this tolerance to a wide range of temperature and relative humidity conditions.

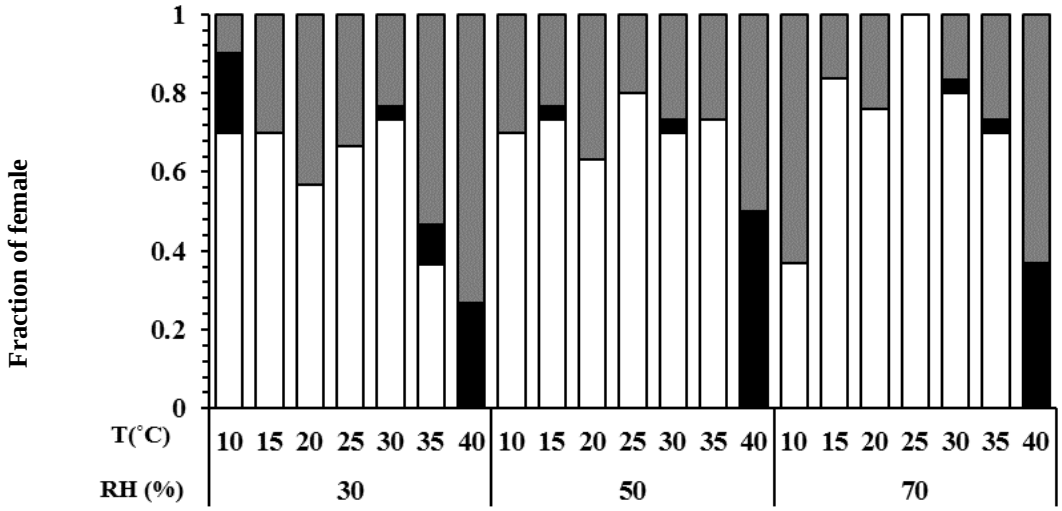


Figure 4.2. Fraction of females (fate) either stuck in the glue (grey bars), dead (black bars) and alive (white bars) during the first 24-hour periods after the onset of the assay, for *Neoseiulus californicus*.

For *P. persimilis*, both relative humidity and temperature (through the interaction these two factors) significantly affected fate (Table 4.3). Same as with *N. californicus*, this species did not survive 40ºC, and in line with the previous species, almost half of these individuals tried to escape and seek refuge, which may be taken as evidence of likely survival in true field conditions. For the remaining conditions, maximal survival was observed in the range 15-20ºC. Contrary to the other two species, in this case survival at 35ºC was maximal at the lowest relative humidity tested.

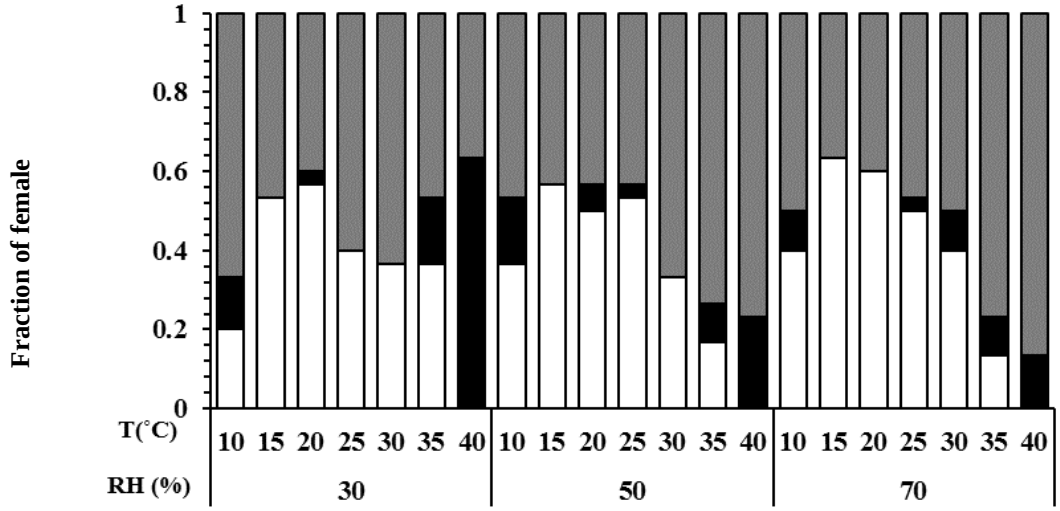


Figure 4.3. Fraction of females (fate) either stuck in the glue (grey bars), dead (black bars) and alive (white bars) during the first 24-hour periods after the onset of the assay, for *Phytoseiulus persimilis*.

4.2.3 Predation

Contrary to what we observed for fate, predation was significantly affected by temperature, relative humidity and their interaction for the three phytoseiid species considered (Table 4.4).

Factors	*E. stipulatus*	*N. californicus*	*P. persimilis*
Model	380.17; 14; < 0.001	1368.63; 17; < 0.001	1227.38; 17; < 0.001
T	252.181; 4; < 0.001	1125.27; 5; < 0.001	1283.33; 5; < 0.001
RH	32.88; 2; < 0.001	6.85; 2; 0.033	16.74; 2; < 0.001
T*RH	495.75;14 ; < 0.001	1378.35;17 ; < 0.001	1620.61; 17; < 0.001

Table 4.4. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to predation estimated during the first 24-hour period after the onset of the assay for *E. stipulatus, N. californicus* and *P. persimilis* considering the factors temperature (T) and relative humidity (RH) as well as their interaction.

In the case of *E. stipulatus*, lowest predation rates were observed at 10ºC irrespective of relative humidity (Figure 4.4). Above this temperature and up to 25ºC, predation increased and maximum rates were usually associated with 70 % relative humidity. A maximum of 15.6 ± 1.9 eggs per female was observed at 15 and 25 ºC in combination with this relative humidity. Beyond 30ºC, as survival was nil (Figure 4.1) no predation was observed.

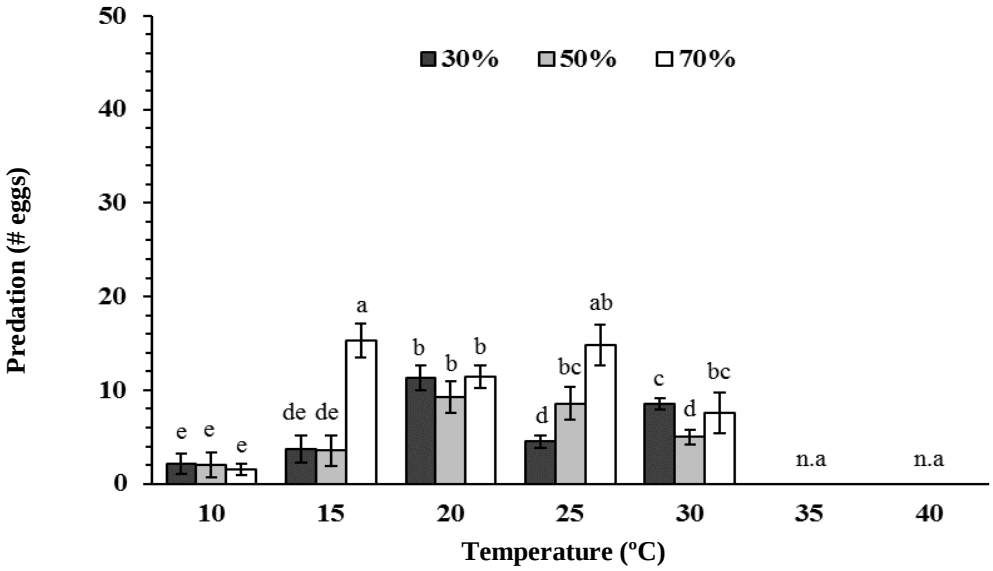


Figure 4.4. Predation (number of *T. urticae* eggs eaten per female during the first 24-hour period after the onset of the assay) for *E. stipulatus* when exposed to temperatures in the range 10 to 40 °C in combination with 30 (black bars), 50 (grey bars) and 70 % (white bars) relative humidity values.

Predation rates for *N. californicus* (Figure 4.5) were minimal at 10ºC irrespective of relative humidity. Above this temperature, they increased up 30ºC, then decreased at 35ºC and were nil at 40 as a consequence of no survival at this temperature in our arenas. Interestingly, from 15 to 20ºC and again at 35ºC, predation was maximal at 50 % relative humidity, whereas at 25 and 30ºC, the highest predation rates were associated with the highest relative humidity tested, with a mean of 21.6 ± 1.2 *T. urticae* eggs eaten per female.

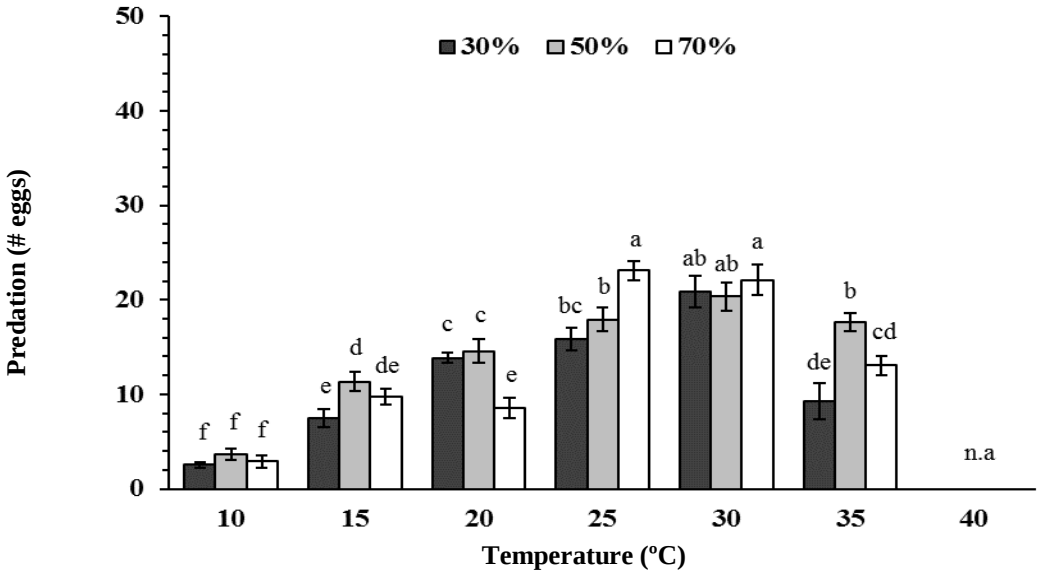


Figure 4.5. Predation (number of *T. urticae* eggs eaten per female during the first 24-hour period after the onset of the assay) for *N. californicus* when exposed to temperatures in the range 10 to 40 °C in combination with 30 (black bars), 50 (grey bars) and 70 % (white bars) relative humidity values.

Phytoseiulus persimilis was the most voracious mite at any of the temperature-relative humidity combinations tested and presented a trend closely matching what we observed for *N. californcius* (Figure 4.6). In this case, maximum predation rates were reached at 30ºC independently of relative humidity and at 25ºC with 70% relative humidity with a mean of 39.3 ± 2.5 eggs per female. The number of eggs eaten per female decreased dramatically to 16.3 ± 2.5 eggs at 35 ºC but these values were still higher than those observed at 10 ºC.

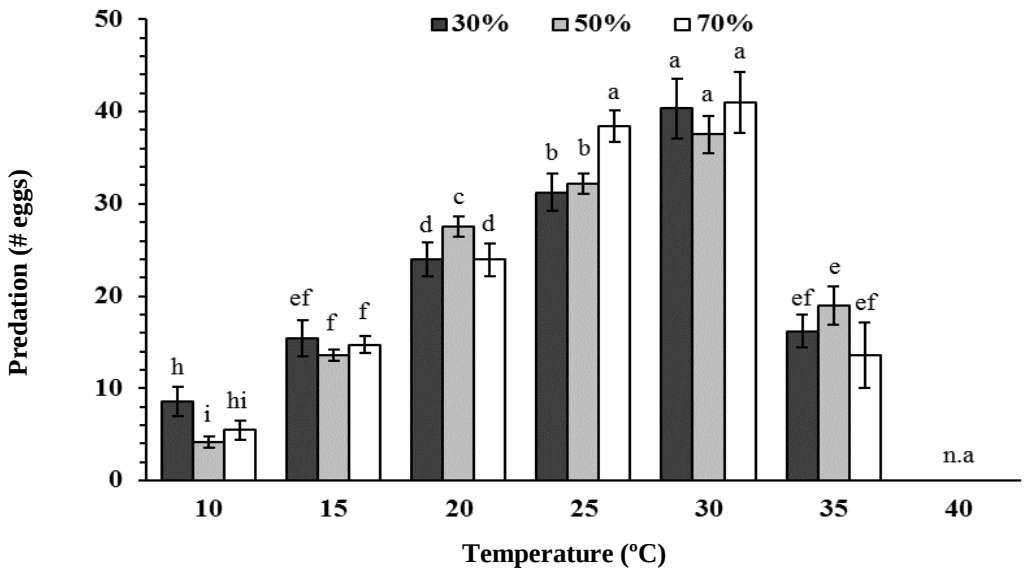


Figure 4.6. Predation (number of *T. urticae* eggs eaten per female during the first 24-hour period after the onset of the assay) for *P. persimilis* when exposed to temperatures in the range 10 to 40 °C in combination with 30 (black bars), 50 (grey bars) and 70 % (white bars) relative humidity values.

4.2.4 Oviposition of phytoseiids

Oviposition during the first 24 hr of the assay was affected both by "T" and "RH" in *N. californicus,* by temperature and the interaction of this factor with relative humidity for *P. persimilis,* and it was independent of these factors for *E. stipulatus* (Table 4.5). This lack of effect should be probably attributed to the low oviposition rates observed for this phytoseiid at all combinations tested (0 to 0.2 eggs per female and day; Figure 4.7).

Factors	*E. stipulatus*	*N. californicus*	*P. persimilis*
Model	28.29; 14; 0.042	376.62; 17; < 0.001	233.28; 17; < 0.001
T	0.123; 4; 0.989	354.15; 5; < 0.001	195.52; 5; < 0.001
RH	0.180; 2; 0.914	34.67; 2; < 0.001	2.71; 2; 0.259
T*RH	0.201;14 ; 0.654	397.01; 15; < 0.001	265.55; 17; < 0.001

Table 4.5. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to oviposition (Poisson). Estimated during the first 24-hour period after the onset of the assay for *E. stipulatus, N. californicus* and *P. persimilis* considering the factors temperature (T) and relative humidity (RH) as well as their interaction.

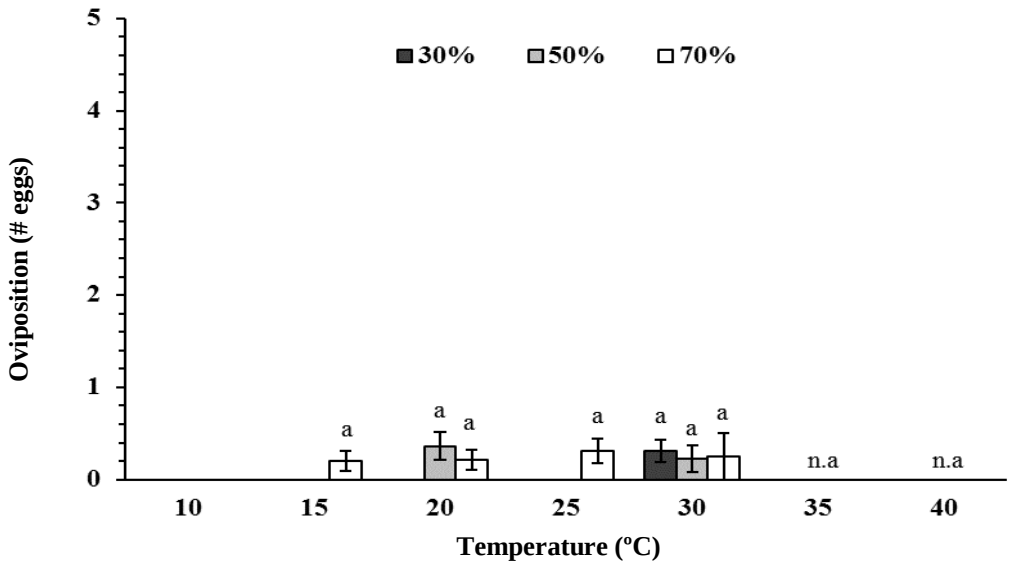


Figure 4.7. Oviposition (number of eggs laid per female) during the first 24-hour period after the onset of the assay) for *E. stipulatus* when exposed to temperatures in the range 10 to 40 °C in combination with 30 (black bars), 50 (grey bars) and 70 % (white bars) relative humidity values.

Oviposition rate of *N. californicus* (Figure 4.8) increased from about 0.1 to 2.5 eggs per female between 15 and 30 °C with the absolute maximum number of eggs laid per female at 30 ºC and 50 % relative humidity (3.2 ± 0.1 eggs). Beyond 15 and 30ºC, oviposition was minimal and at 10ºC only a few eggs could be collected in the arenas kept at 70% relative humidity. Intriguingly, oviposition at 20ºC and 70% relative humidity was as low as the reported minimum values.

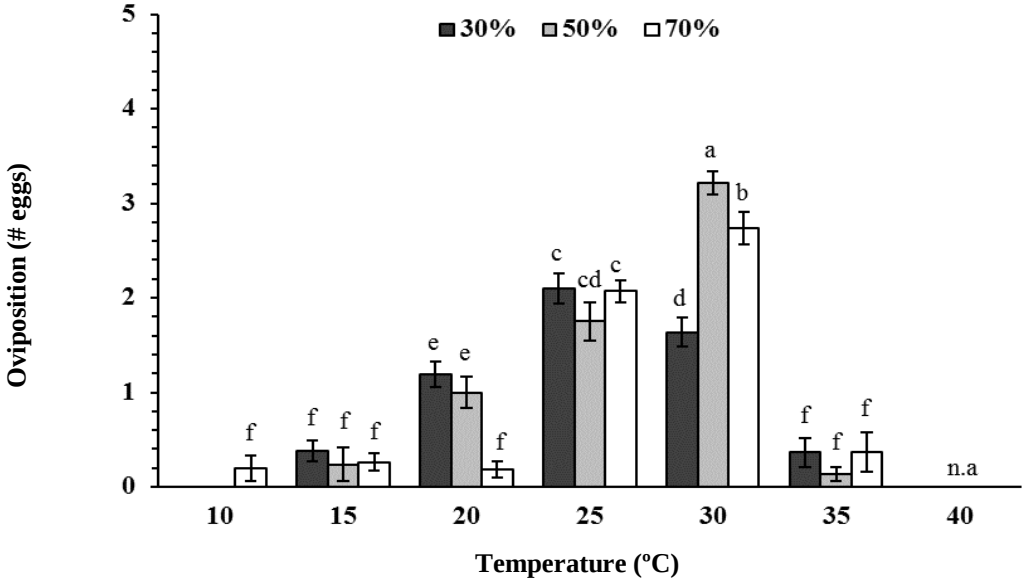


Figure 4.8. Oviposition (number of eggs laid per female) during the first 24-hour period after the onset of the assay) for *N. californicus* when exposed to temperatures in the range 10 to 40 °C in combination with 30 (black bars), 50 (grey bars) and 70 % (white bars) relative humidity values.

A similar trend was observed for the response of *P. persimilis* to temperature (Figure 4.9). In this case, oviposition increased from about 0.66 to around 4.01 eggs per female between 10º and 30 °C. However, in this case, the effect of relative humidity changed direction depending on the temperature and maximum oviposition rates at 25 and 30ºC were associated with highest and intermediate relative humidity values (around 4.2 ± 0.2 eggs per female).

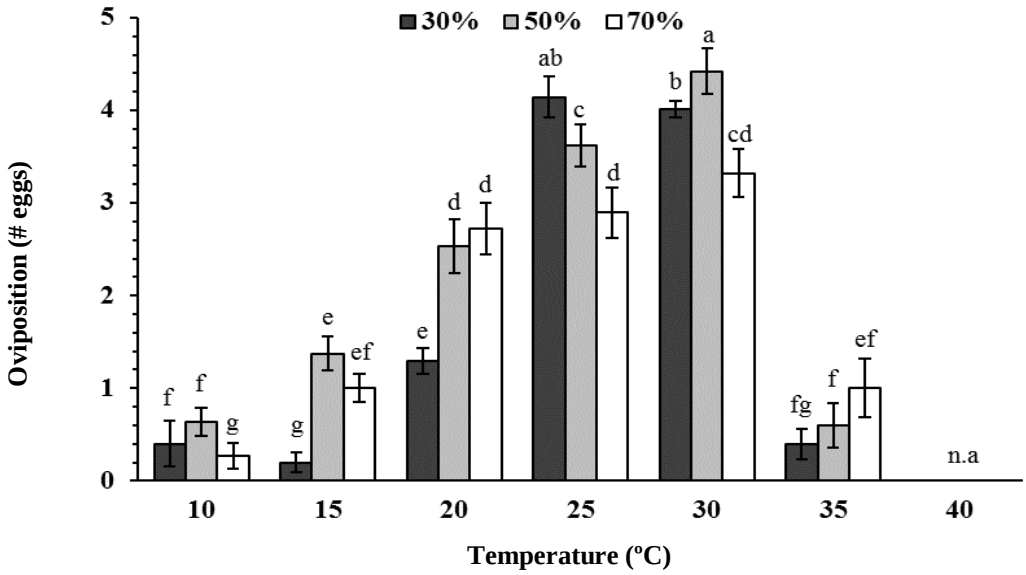


Figure 4.9. Oviposition (number of eggs laid per female) during the first 24-hour period after the onset of the assay) for *P. persimilis* when exposed to temperatures in the range 10 to 40 °C in combination with 30 (black bars), 50 (grey bars) and 70 % (white bars) relative humidity values.

4.2.5 Fate and oviposition of *T. urticae*

The GLM best fitting *T. urticae* fate included "T" and "RH" (Table 4.6). Absolute highest survival was observed at 25 ºC and 30 % relative humidity (100 % survival) (Figure 4.10). However, survival was always above 60 %, even at the extreme temperatures tested (10 and 40ºC). In the case of oviposition, minimum values were observed at 10 and 40ºC (0.3 ± 0.1 and 0.4 ± 0.1 eggs, respectively). Between these temperatures, oviposition increased with no clear trends for relative humidity. Indeed, at 35ºC maximum oviposition rates were attained independently of temperature (mean of 7.4 ± 0.9 eggs per female).

Factors	Fate	Oviposition
Model	52.04; 20; <0.001	936.97; 20; < 0.001
T	24.05; 6; 0.001	760.50; 6; < 0.001
RH	1.29: 2; 0.525	8.17; 2; 0.017
T*RH	23.03; 20; 0.027	841.18; 20; < 0.001

Table 4.6. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to fate and oviposition estimated during the first 24-hour period after the onset of the assay considering the factors temperature (T) and relative humidity (RH) as well as their interaction.

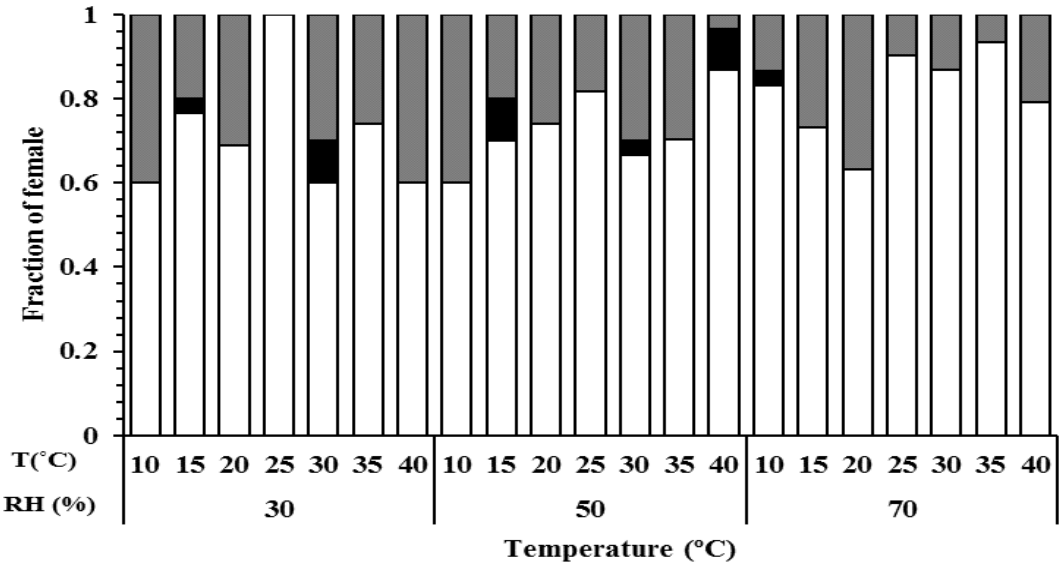


Figure 4.10. Fraction of *T. urticae* females (fate) either stuck in the glue (grey bars), dead (black bars) or alive (white bars) during the first 24-hour periods after the onset of the assay.

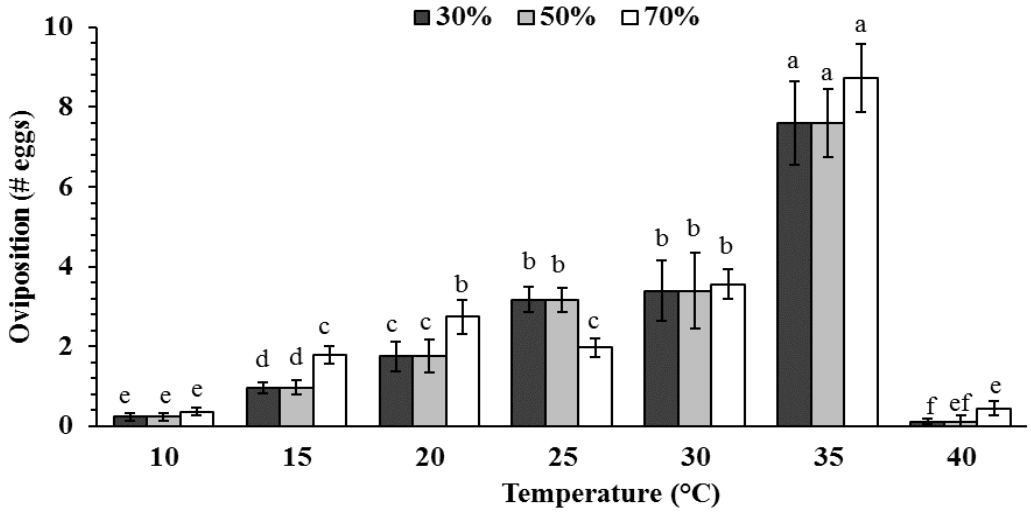


Figure 4.11. Oviposition (number of eggs laid per female) during the first 24-hour period after the onset of the assay) for *T. urticae* when exposed to temperatures in the range 10 to 40 °C in combination with 30 (black bars), 50 (grey bars) and 70 % (white bars) relative humidity values.

4.3 Discussion

We hypothesized that Climate Change would differentially impact first and second trophic levels of the mite community established around *T. urticae* in clementines. We have actually demonstrated that the different parameters studied (fate, oviposition and predation) depend on both temperature and relative humidity for the four species considered. However, the magnitude of the impact is species dependent and temperatures above 25ºC, which can be taken as proxy of Climate Change conditions, are more detrimental to phytoseiids than to *T. urticae*, which presents its maximum values of survival and oviposition at temperatures about 5ºC higher than best adapted phytoseiids, independently of relative humidity. However, probably as a consequence of the experimental setup used, the highest impact among phytoseiids has been observed for the omnivore *E. stipulatus*, and this is not what we initially assumed.

Mean survival of *T. urticae* in our experimental conditions was always above 60 % and it even reached 100 % at 25ºC and 30% relative humidity (Figure 4.10). This means that the strain we worked with, which had been originally collected in a clementine orchard of La Plana Region, was quite tolerant to hot and dry conditions. Other authors had also reported high survival rates for *T. urticae*, at either constant or fluctuating temperatures, similar to our results (Gotoh et al., 2010; Vangansbeke et al., 2013). Likewise, the maximal oviposition rate (8.7 eggs) obtained at 35ºC independently of relative humidity, falls into the range of what other authors had previously reported (7.1 to 9.1 eggs) (Rao et al., 1996; Bounfour and Tanigoshi, 2001; Vangansbeke et al., 2013). These values contrast with what was observed for the phytoseiids. Only *N. californicus* presented a survival comparable to *T. urticae* at high temperatures (Figure 4.2). For the other two phytoseiids, survival barely exceeded 60 % in the best case, which was always at temperatures below 25ºC (Figures 4.1 and 4.3). Although, *N. californicus* and, especially, *P. persimilis* could increase their predation rates on *T. urticae* eggs up to 30ºC (Figures 4.5 and 4.6), and probably effectively regulate the herbivore within this temperature range, at higher temperatures this positive relationship would probably collapse. The combined effect of lower predation and extremely lower oviposition rates for the two phytoseiids plus the lower survival rate for *P. persimilis*, would probably release *T. urticae* from effective natural regulation. However, this was not

the case when we studied the same trophic chain modules at field conditions (Figure 3.2, Chapter 3). Indeed, *E. stipulatus* lost effectiveness during the summer experiment, and this is in agreement with what we have found in this laboratory assay. However, both *N. californicus* and *P. persimilis* could keep *T. urticae* under control within the fluctuating temperature and relative humidity regimes registered during our field assays (Figure 3.1, Chapter 3).

Some authors (Ferragut et al. 1987) do not consider *E. stipulatus*, which is the most abundant phytoseiid in Spanish clementine orchards (Abad-Moyano et al. 2009b), as a suitable biological control agent for *T. urticae*. However, both field (Pérez-Sayas et al., 2016) and field (Grafton-Cardwell et al., 1997) assays point at the important role of this phytoseiid in the regulation of *T. urticae* populations in clementines. This predator is known to be poorly adapted to prey on *T. urticae* because it cannot invade the web produced by this spider mite (Ferragut et al. 1992; Abad-Moyano et al. 2009b). However, we observed a non-negligible predation rate at low temperatures and high relative humidity regimes (15.6 eggs at 15-20 ºC and 70 % RH), which may explain the field regulation observed in spring (Fig. 3.2, Chapter 3). As this is an omnivore phytoseiid, considered as a specialized pollen feeder (Ferragut et al. 1987; González-Fernández et al. 2009; Pina et al. 2012; Guzmán et al. 2016a), the provision of pollen as alternative food could compensate these negative results observed both in the field during summer and in the laboratory at almost all conditions tested (oviposition was almost nil). Therefore, it would be interesting to check the effect of pollen on this species and this will be the focus of the next Chapter of this thesis.

Neoseiulus californicus has been traditionally associated with higher tolerance to heat and drought (Escudero and Ferragut 2005; Abad-Moyano et al. 2009b; Walzer et al., 2007; Ahn et al. 2010). However, this has not always been the case (Croft et al. 1998; Castagnoli and Simoni 1999; Castagnoli et al. 2001; Ghazy et al. 2014; Nguyen et al. 2015) and this may be partly attributed to the use of different strains which may differ in their tolerance to these harsh conditions. In our field assay, the commercial strain used performed well both in spring and in summer (Figure 3.2). As this species was also the most tolerant to Climate Change conditions in our laboratory assays, its good performance in the field was not a surprise and it indeed performed better than what could be forecasted based on these

laboratory results. This enhanced performance could be the result of two factors acting in a synergistic way. On the one hand, in the laboratory we worked at constant temperature regimes, which do not allow for the mite to recover, most likely at night, from maximum temperatures attained during the day at field conditions. It is known that fluctuating temperatures have usually a lower impact on arthropod physiology and behavior than a constant temperature equivalent to their mean (Nguyen and Amano 2010; Vangansbeke et al. 2013; Gotoh et al. 2014). On the other hand, we saw that the number of escapees in our assays was larger than that of dead specimens (Figure 4.2). Our arena did not allow phytoseiids to leave the arena and seek refuge. This behavior, which may impact predator fitness in terms of lost foraging and reproduction opportunities when looking for shelter (Gillespie et al., 2012), may though increase its survival at field conditions. These two factors could well apply also to the third predator considered in this study, *P. persimilis*, as the number of escapees for this mite was even larger than observed for *N. californicus* (Figure 4.3). Indeed, *P. persimilis* spends relatively more time searching and moving around the leaf than other predatory mite species (Sabelis and Dicke 1985; Gontijo et al., 2012). However, Skirvin and Fenlon (2003) showed that the mobility of *P. persimilis* is reduced at temperatures above 25 °C. Nonetheless, we observed the highest number of escapees at 30 and 35 ºC. Therefore, if the local *P. persimilis* strain used in our assays, which generally performed better than other strains of this mite at higher temperatures and lower relative humidity regimes (Skirvin and Fenlon, 2003; Escudero and Ferragut 2005; Abad-Moyano et al. 2009b; Vangansbeke et al. 2015a, b), could actually find refuge and escape extreme temperatures at field conditions, it would result in a satisfactory regulation of *T. urticae* populations, as it was observed in our field assays.

To sum up, the laboratory results reported here support part of what we actually observed under field conditions. On the one hand the extremely fine tuning of *T. urticae* response to hot and dry conditions. On the other, the extremely poor adaptation of *E. stipulatus* and the intermediate effect on *P. persimilis* and *N. californicus*, which combined with some behavioral adaptations which could not be taken into account in our laboratory assays, may explain field results where *T. urticae* was still naturally regulated at conditions matching Climate Change.

5

Can pollen supply mitigate the deleterious effects of climate change on the biological control of *Tetranychus urticae*? Effects on the predators *Euseius stipulatus*, *Neoseiulus californicus* and *Phytoseiulus persimilis* at laboratory conditions

Hypothesis

It is well documented that some phytoseiid predators, including the omnivorous *Euseius stipulatus* and the Tetranychidae-specialist *Neoseiulus californicus*, are able to persist in the crop when spider mite prey is scarce, feeding on other food sources such as pollen (Castagnoli and Simoni 2003; (González-Fernández et al. 2009; Pozzebon et al. 2009; Maoz et al. 2011; Mcmurtry et al. 2013). In a previous field study (Chapter 3), we observed that summer environmental conditions, which were taken as proxy of Climate Change, negatively affected the regulation of *Tetranychus urticae* by *E. stipulatus*. Further laboratory studies (Chapter 4) can be taken as evidence of the poor performance of *E. stipulatus* at hot and dry conditions when *T. urticae* eggs were the only food source available. Therefore, we hypothesize that pollen supply could mitigate these negative effects on this pallinophagous mite with no or a slightly positive impact on either *N. californicus* or the *Tetranychus* sp.-specialist *Phytoseiulus persimilis*.

5.1 Material and methods

5.1.1 Environmental conditions

The combination of three temperature (T) and three relative humidity values (RH) (15, 25 and 30 ºC and 30, 50 and 70 %, respectively) were chosen. Constant relative humidity values were obtained by using different salt solutions (Winston and Bates 1960) in desiccators kept inside environmental chamber (Sanyo Electric Co., Ltd., Japan) set at the target temperature and a photoperiod of 16:8 h L:D.

5.1.2 Phytoseiid performance

The fate, predation, and oviposition of the three most abundant predators of *T. urticae* in Valencian clementine orchards (Abad-Moyano et al., 2009a; Aguilar-Fenollosa et al., 2011b): *E. stipulatus*, *N. californicus* and *P. persimilis*, was studied following the procedure explained in Chapter 2 (Common Material and Methods). In this case, three different diets were considered. These consisted of (1) less than 48 h old *T. urticae* eggs, (2) pollen of *Carpobrotus edulis*, which is considered as good quality pollen for phytoseiids in general (Pina et al., 2012), and (3) a combination of both. Following th standard procedures explained in Chapter 2, to obtain spider mite eggs to feed the corresponding

arenas, 48 h prior to the introduction of the phytoseiid into the experimental setup, these arenas received 15 *T. urticae* females each, which fed, laid eggs and produced web. Subsequently, we removed all mobile forms of *T. urticae* and only ≤ 48 h old eggs were left. For those arenas with pollen supply, we added *C. edulis* pollen *ad libitum* in a single point in the center of the arena. Arenas were checked 24 hours after the onset of the assay (i.e., 48 hours after the end of the starvation period for adult phytoseiid females) under a binocular microscope and several parameters were scored: fate (i.e., live and dead specimens and escapees), oviposition (number of eggs laid), predation (number of *T. urticae* eggs eaten).

5.1.3 Statistical analyses

To study the effects of the different factors included in our assays on the above mentioned parameters, we used general linear models (GLM) assuming different probability distributions and different link functions according to the characteristics of the data. In the case of fate, which had three different possible outputs, we used a GLM with a multinomial distribution of the error and a generalized logit link function. Likewise, for egg hatching we used a binomial distribution of the error and a logit link function. Finally, for oviposition and predation we used a GLM with a Poisson distribution of the error and a logistic link function. For fate, oviposition and predation, the factors “species”, “diet”, temperature (“T”) and relative humidity (“RH”) were used as fixed effects. In the case of egg hatching, we used a similar approach but only the factors “species”, “T” and “RH” were included in the model. As our main goal was to identify species-specific patterns of response to climate change and whether diet could modulate this response, we started our analyses by considering all combinations including the “species” factor. Once the signification of the “species” factor was tested, we similarly continued the analyses of fate, predation and oviposition in a sequential manner studying the effect of “diet”. Eventually, we separately analyzed for each species and diet, the effects of “T” and “RH” during the first 24 h after the introduction of the phytoseiid into the arena. When necessary, we used Bonferroni post-hoc test for mean separation.

5.2 Results

There were significant differences ($P < 0.001$) between the three phytoseiid species included in this study for the four parameters considered (fate, predation and oviposition) (Table 5.1). As a consequence, we further analyzed the influence of the diet for each species separately. We found that this factor either alone or through the interactions with relative humidity and temperature was always significant (Table 5.2) and this result led us to eventually analyze the influence of "T", "RH" and their interaction for each mite species and diet individually (Table 5.3, 5.4 and 5.5 for fate, predation and oviposition, respectively).

Table 5.1. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to fate (multinomial), predation, oviposition and egg hatching (Poisson) considering the factors phytoseiid species "sp" and their interactions with temperature "T", relative humidity "RH" and diet "Diet" as explanatory variables.

Factors	Fate	Predation	Oviposition
Model	280.57; 161; <0.001	754.69; 107; <0.001	637.31; 161; <0.001
sp	17.12; 2; <0.001	637.61; 2; <0.001	381.39; 2; <0.001
T * sp	1.51; 8; 0.959	485.29; 8; <0.001	241.70; 8; <0.001
RH * sp	1.45; 8; 0.963	28.94; 8; <0.001	13.98; 8; <0.001
Diet * sp	10.58; 8; 0.122	60.96; 5; <0.001	356.25; 8; <0.001
T * RH * sp	3.46; 26; 0.991	63.85; 26; <0.001	9.23; 26; 0.056
T * Diet * sp	2.73; 26; 0.997	67.20; 17; <0.001	101.53; 26; <0.001
RH * Diet * sp	2.04; 26; 0.999	12.63; 17; 0.049	5.79; 26; 0.215
T * RH * Diet * sp	5.73; 80; 0.914	79.87; 53; <0.001	19.21; 80; 0.014

a)

Factors	Fate	Predation	Oviposition
Model	73.56; 26; <0.001	195.13; 17; <0.001	39.53; 26; 0.043
Diet	75.31; 2; < 0.001	4.23; 1; 0.002	9.63; 2; <0.001
T * Diet	16.3; 8; 0.003	66.37; 5; <0.001	63.47; 8; <0.001
RH * Diet	6.41; 8; 0.932	89.73; 5; <0.001	56.59; 8; <0.001
T * RH * Diet	85.99; 26; 0.292	228.29; 17; <0.001	85.99; 26; <0.001

b)

Factors	Fate	Predation	Oviposition
Model	36.89; 23; <0.001	491.54; 17; <0.001	230.58; 23; <0.001
Diet	54.27; 2; < 0.001	0.106; 1; 0.745	6.54; 2; <0.001
T * Diet	15.59; 8; 0.004	427.98; 5; <0.001	309.61; 8; <0.001
RH * Diet	15.91; 8; 0.003	54.23; 5; <0.001	141.82; 8; <0.001
T * RH * Diet	17.36; 23; 0.027	593.01; 17; <0.001	346.02; 23; <0.001

c)

Factors	Fate	Predation	Oviposition
Model	94.06; 26; <0.001	94.06; 17; <0.001	216.27;26; <0.001
Diet	19.54; 2; < 0.001	39.68; 1; <0.001	31.91; 2; <0.001
T * Diet	25.67; 8; < 0.001	380.684; 5; <0.001	201.20; 8; <0.001
RH * Diet	3.06; 8; 0.548	57.19; 5; <0.001	149.78; 8; <0.001
T * RH * Diet	21.54; 26; 0.006	415.41; 17; <0.001	258.02; 26; <0.001

Table 5.2. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to fate (multinomial), predation and oviposition (Poisson) for (a) *E. stipulatus*, (b) *N. californicus*, and (c) *P. persimilis* considering the factor diet "Diet and their interactions with temperature "T", relative humidity "RH"," as explanatory variables.

5.2.1 Fate

For the omnivorous *E. stipulatus*, contrary to what would be expected for a generalist predator, survival was highest when it had access to the pollen only diet (means of 77.1 versus 49.3 and 33.8 % for pollen only, mixed and *T. urticae* eggs only diets, respectively). The presence of pollen (either alone or with *T. urticae* eggs) made fate independent of relative humidity (Table 5.3). This result should be probably attributed to the zero death rates observed at 25 ºC in the pollen only diet, as well as at 15 and 25 ºC in the mixed diet. Contrarily, in the *T. urticae* eggs only diet, the effect of temperature and relative

humidity did not follow any clear trend and, although 30 % relative humidity provided the lowest survival rates at 15º and 25ºC, it provided the highest at 30ºC. Remarkably, the percentage of escapees was similar in the two diets supplemented with *T. urticae* eggs (up to 52 %) and higher than when pollen only was available. The number of dead individuals was highest when only *T. urticae* eggs were offered as a food source (around 11 %).

Diet	Factors	*E. stipulatus*	*N. californicus*	*P. persimilis*
***T. urticae* eggs + pollen**	Model	16.49; 8; 0.036	9.80; 8; 0.279	27.92; 8; < 0.001
	T	5.25; 2; 0.038	3.08; 2; 0.214	14.06; 2; 0.001
	RH	1.58; 2; 0.455	1.59; 2; 0.451	6.27; 2; 0.043
	T*RH	9.82; 8; 0.056	5.83; 8; 0.212	4.20; 8; 0.379
***T. urticae* eggs**	Model	11.59; 8; 0.170	4.77; 8; 0.782	15.77; 8; 0.046
	T	3.11; 2; 0.211	2.75; 2; 0.252	4.39; 2; 0.011
	RH	1.45; 2; 0.049	1.03; 2; 0.598	1.78; 2; 0.411
	T*RH	8.04; 8; 0.034	1.36; 8; 0.852	9.17; 8; 0.057
Pollen	Model	25.68; 8; 0.001	10.99; 6; 0.012	37.19; 8; < 0.001
	T	14.61; 2; 0.001	0.14; 2; 0.011	17.87; 2; < 0.001
	RH	2.49; 2; 0.287	5.05; 2; 0.080	2.48; 2; 0.289
	T*RH	6.37; 4; 0.173	5.92; 6; 0.041	8.64; 8; 0.071

Table 5.3. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to fate (multinomial), for *E. stipulatus*, *N. californicus* and *P. persimilis* in each diet separately (*T. urticae* eggs + pollen, *T. urticae* eggs and pollen) considering the factors temperature "T", relative humidity "RH" and their interactions as explanatory variables.

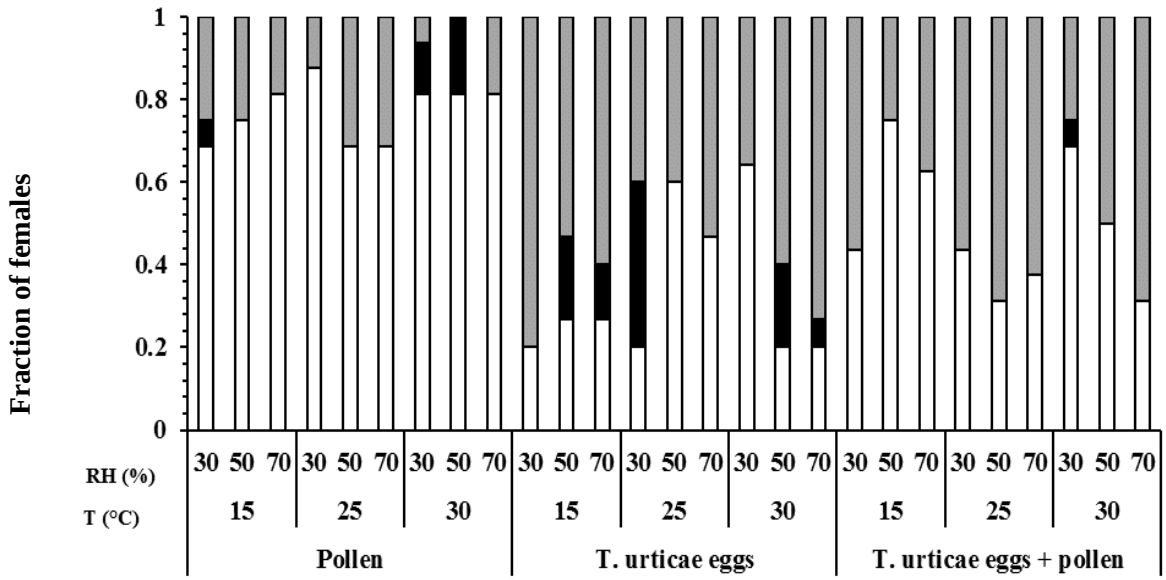


Figure 5.1. Fate of *Euseius stipulatus* (stuck in the glue (grey bars), dead (black bars) and alive (white bars)) when offered three different diets (Pollen, *T. urticae* eggs and *T. urticae* eggs + pollen) considering three temperature (15, 25 and 30 ºC) combined with three relative humidity (30, 50 and 70 % RH).

In the case of the tetranychid-specialist predator *N. californicus*, survival was higher when the mite had access to *T. urticae* eggs, either alone (86.5 %) or combined with pollen (85.9 %), and decreased when pollen was the only food source available (31.6 %) (Figure 5.2). However, when we analyzed the fate for each diet, we observed that the GLM model provided a good fit ($P < 0.05$) only in the case of the pollen only diet (Table 5.3). This failure was attributed to the fact that all individuals survived (no dead or stuck-in-the-glue specimens) at the combinations 25 and 30ºC with 50 % relative humidity for the *T. urticae* eggs and mixed diets, respectively. For the pollen only diet, fate was highest at 25 ºC, much lower at 15 ºC and became nil at 30 ºC. For the other two diets, survival never dropped below 60.0 %.

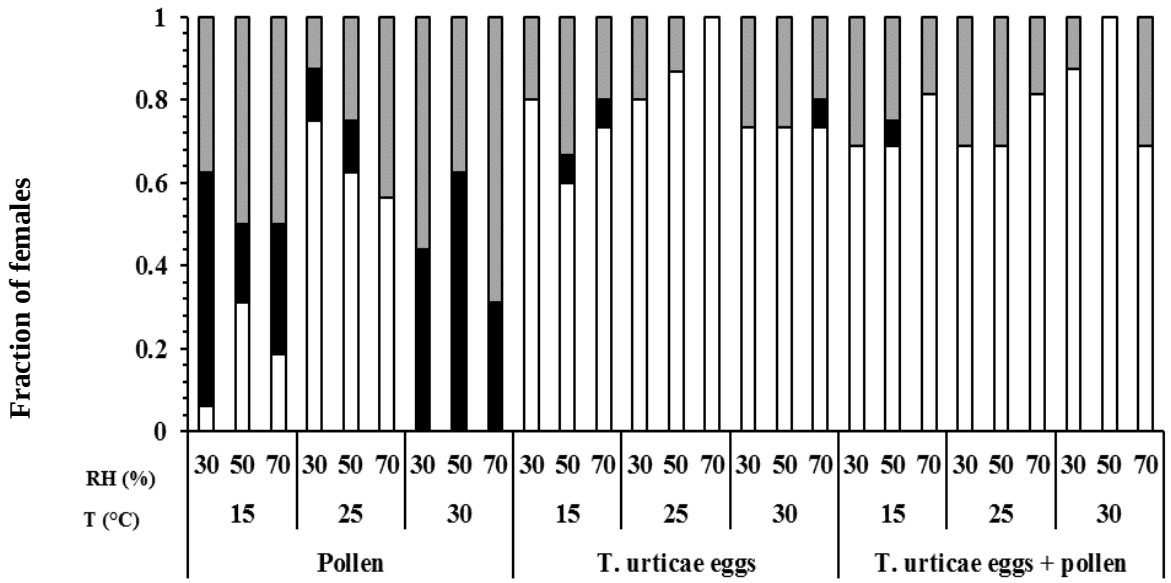


Figure 5.2. Fate of *Neoseiulus californicus* (stuck in the glue (grey bars), dead (black bars) and alive (white bars)) when offered three different diets (Pollen, *T. urticae* eggs and *T. urticae* eggs + pollen) considering three temperature (15, 25 and 30 ºC) combined with three relative humidity (30, 50 and 70 % RH).

For the *Tetranychus* sp.-specialist predator *P. persimilis*, fate (Figure 5.3) was significantly affected by diet (Table 5.3). Highest survival occurred when this phytoseiid had access to *T. urticae* eggs only (47.7%), closely followed by the mixed diet (44.4%). Lowest survival was observed when this phytoseiid had access to pollen only (21.5%). Interestingly, the mixed diet was the only one resulting in no dead specimens. However, same as the other two phytoseiids, moderate to high escapee rates were observed even in this case. Temperature was the single factor affecting fate for all diets. Relative humidity was significant for the mixed diet only (Table 5.3). This mixed diet resulted in absolute highest survival at 25 ºC and 70 % relative humidity (93. 8 %) and no dead individuals in any of the different combinations of temperature and relative humidity tested. When we offered pollen and *T. urticae* eggs separately, survival was highest at 15 ºC (31.2 and 63 %, respectively).

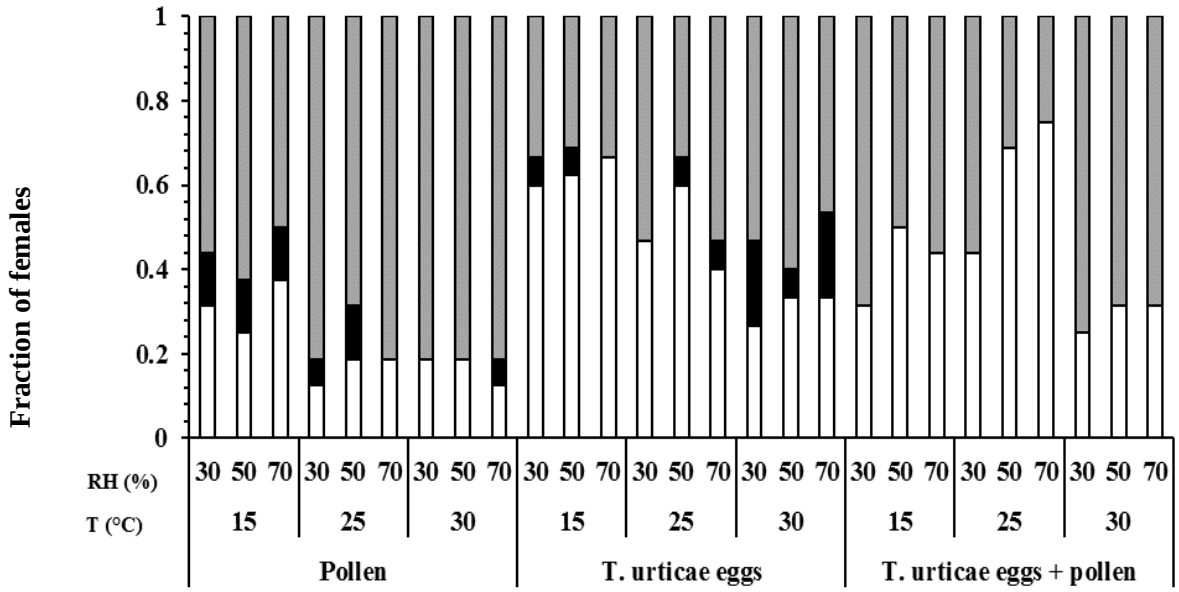


Figure 5.3. Fate of *Phytoseiulus persimilis* (stuck in the glue (grey bars), dead (black bars) and alive (white bars)) when offered three different diets (Pollen, *T. urticae* eggs and *T. urticae* eggs + pollen) considering three temperature (15, 25 and 30 ºC) combined with three relative humidity (30, 50 and 70 % RH).

5.2.2 Predation

Predation of *E. stipulatus* was affected by temperature and relative humidity in both diets (Table 5.4). Again, contrary to what would be expected for a generalist predator, highest predation rates were observed when *E. stipulatus* was offered a mixture of pollen and *T. urticae* eggs (average of 11.4 ± 1.4 eggs eaten versus 7.9 ± 1.6 for the *T. urticae* eggs only diet) (Figure 5.4). However, we observed similar predation rates at 15 and 25 ºC and 70 % relative humidity when *T. urticae* eggs constituted the only food source available.

Diet	Factors	*E. stipulatus*	*N. californicus*	*P. persimilis*
***T. urticae* eggs + pollen**	Model	67.09; 8; < 0.001	274.11; 8; < 0.001	66.07; 8; < 0.001
	T	3.46; 2; 0.178	189.87; 2; < 0.001	29.48; 2; < 0.001
	RH	14.51; 2; 0.001	37.21; 2; < 0.001	2.11; 2; 0.349
	T*RH	38.95; 8; < 0.001	46.23; 8; < 0.001	24.49; 8; < 0.001
***T. urticae* eggs**	Model	99.01; 8; < 0.001	223.83; 8; < 0.001	292.20; 8; 0.001
	T	5.67; 2; 0.059	151.15; 2; < 0.001	269.03; 2; < 0.001
	RH	20.59; 2; < 0.001	16.95; 2; < 0.001	1.78; 2; 0.682
	T*RH	66.10; 8; < 0.001	22.87; 8; < 0.001	4.13; 8; 0.038

Table 5.4. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to predation (Poisson), for *E. stipulatus*, *N. californicus* and *P. persimilis* in each diet separately (*T. urticae* eggs + pollen, *T. urticae* eggs and pollen) considering the factors temperature "T", relative humidity "RH" and their interactions as explanatory variables.

a)

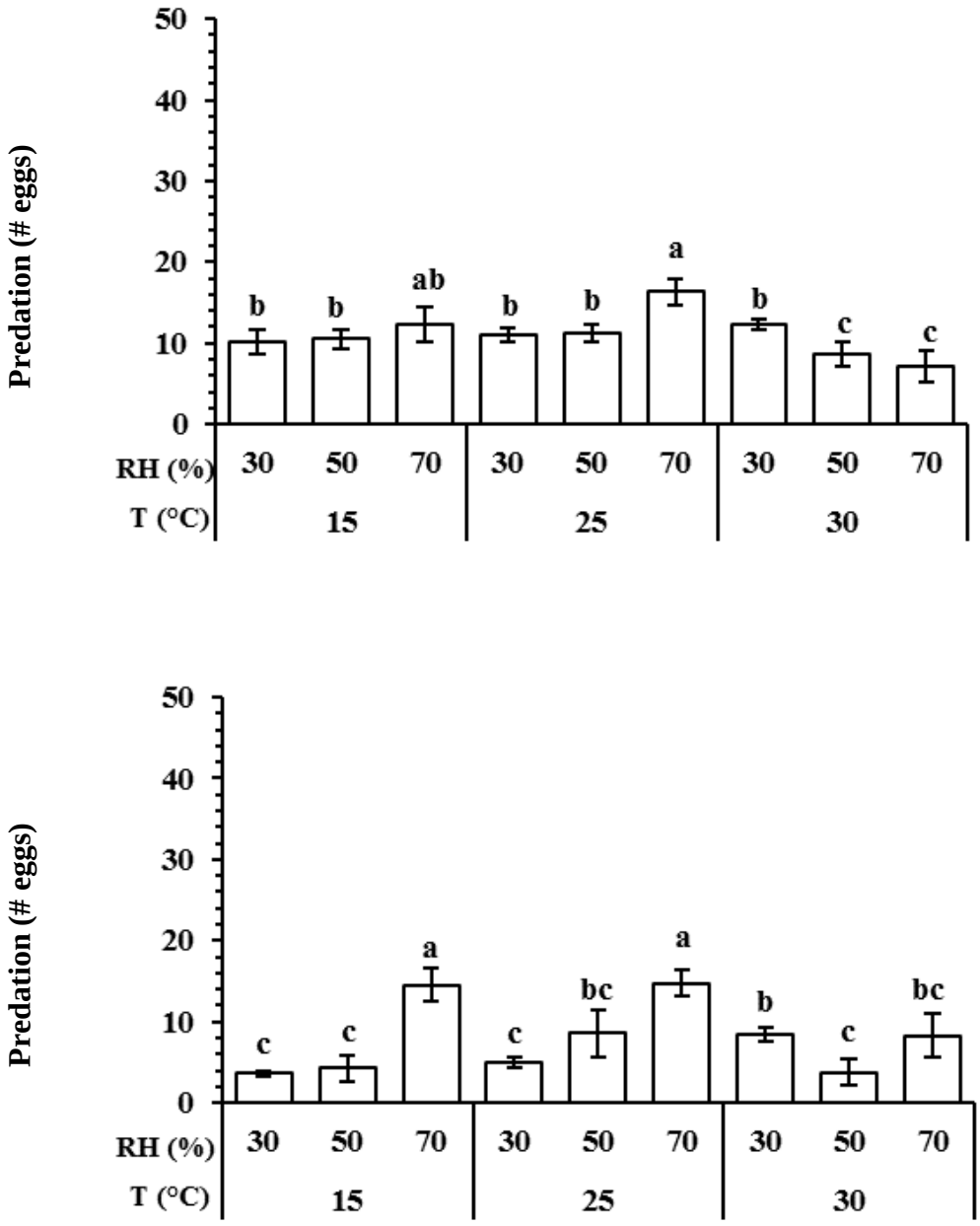


Figure 5.4. Predation of *E. stipulatus* when offered two different diets, (a) *T. urticae* eggs + pollen and (b) *T. urticae* eggs at 15º, 25º and 30° C combined with 30, 50 and 70 % of RH. For each figure, bars with the same letter are not statistically different (Bonferroni P<0.05).

Predation rate of *Neoseiulus californicus* also was significantly affected by temperature and relative humidity for the two diets considered (Table 5.4). The mixed diet provided a higher and more homogeneous response (Figure 5.5). In general, highest predation rates were observed at 30 ºC irrespective of relative humidity and, within each temperature, at 70 % relative humidity.

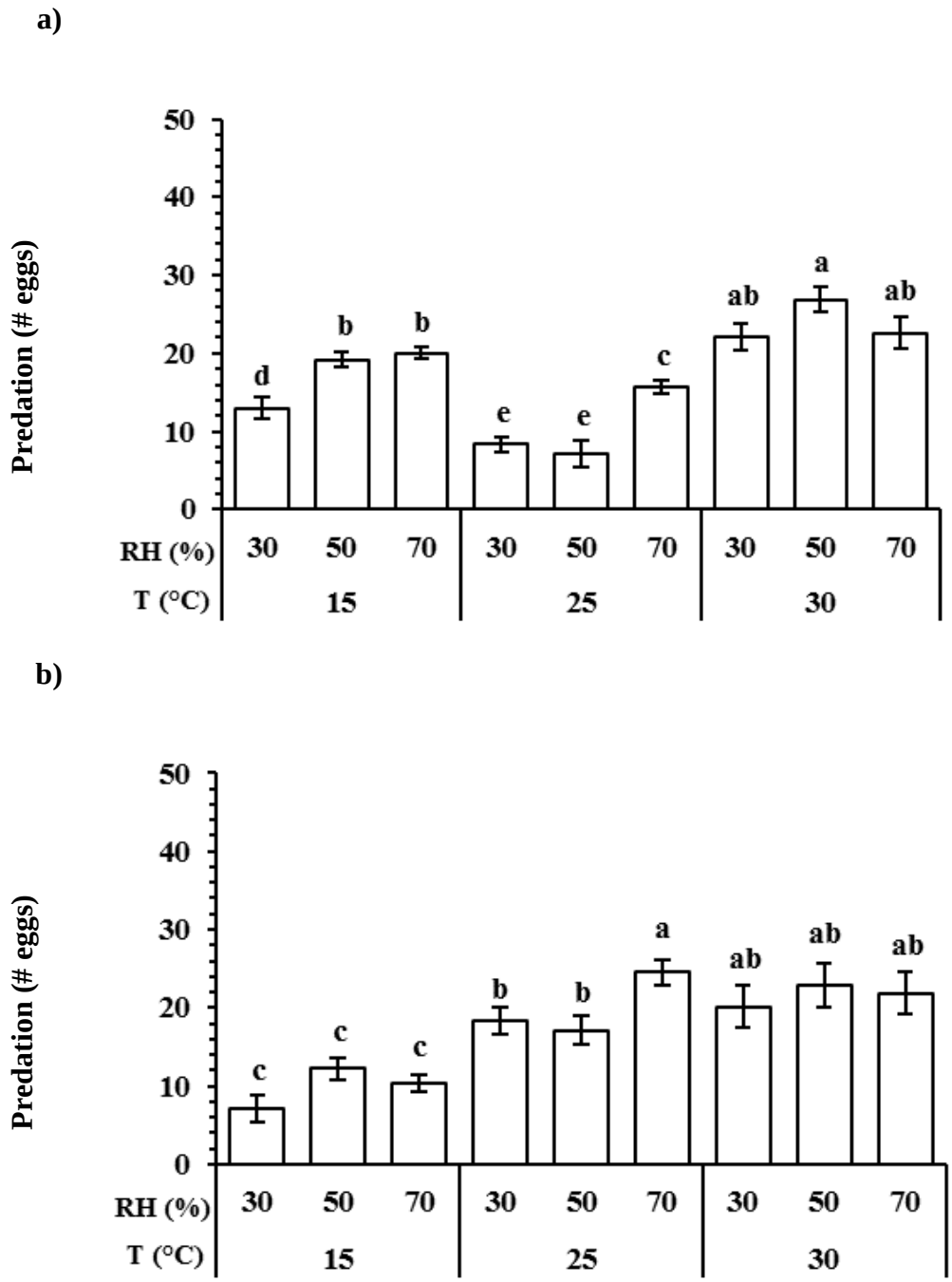


Figure 5.5. Predation of *N. californicus* when offered two different diets (a) *T. urticae* eggs + pollen and (b) *T. urticae* eggs at 15º, 25º and 30° C combined with 30, 50 and 70 % of RH. For each figure, bars with the same letter are not statistically different (Bonferroni P<0.05).

The number of eggs preyed by *P. persimilis* (Figure 5.6) was affected by temperature and relative humidity and their interaction in both diets (Table 5.4). Pollen provision affected predation rates because the number of eggs preyed decreased when offered a mixed diet (20.6 ± 1.6) compared with *T. urticae* eggs only (28.5 ± 2.1). For both diets, predation significantly decreased at 15 ºC independently of relative humidity and there were no differences between 25 and 30ºC.

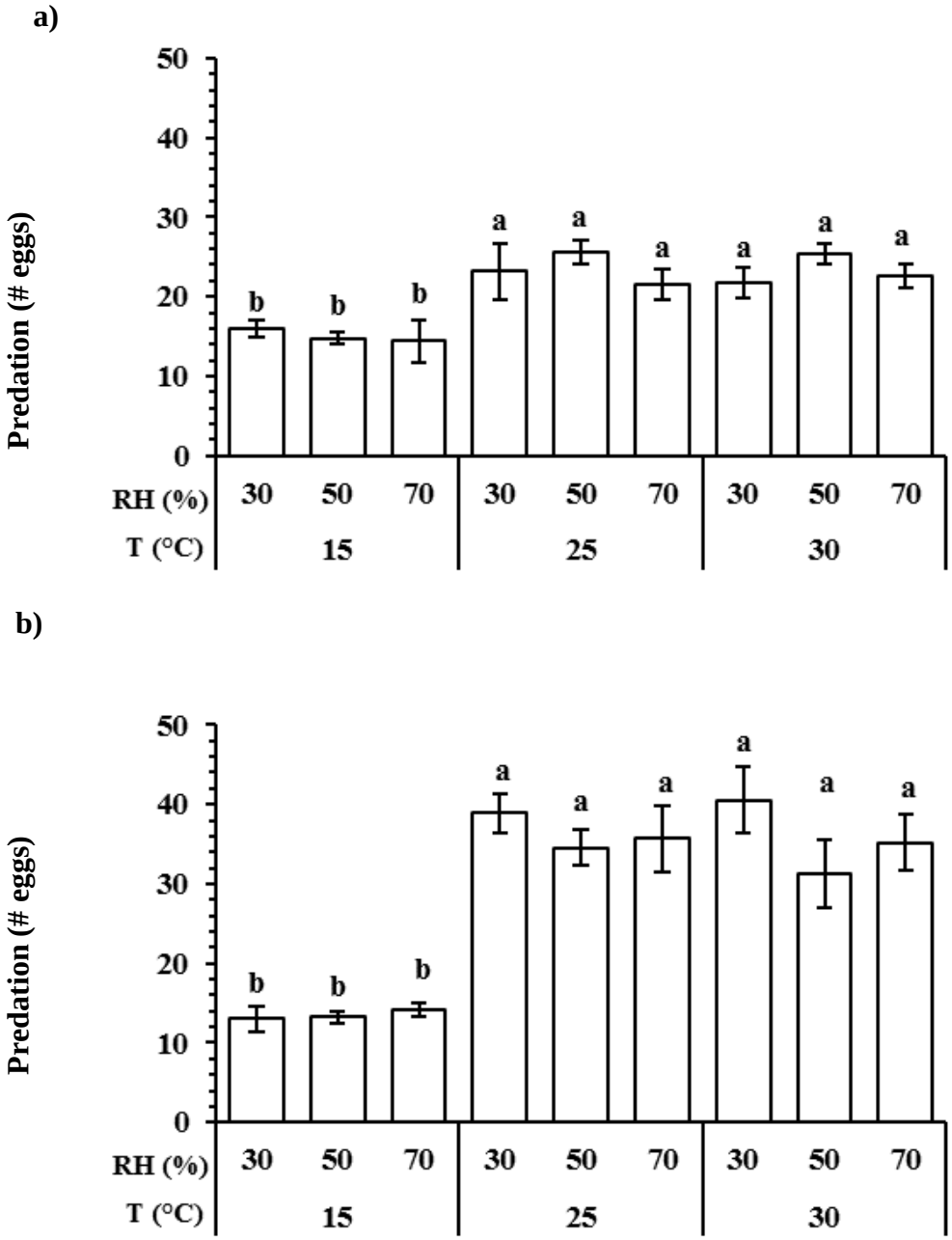


Figure 5.6. Predation of *Phytoseiulus persimilis* when offered two different diets (a) *T. urticae* eggs + pollen and (b) *T. urticae* eggs at 15º, 25º and 30° C combined with 30, 50 and 70 % of RH. For each figure, bars with the same letter are not statistically different (Bonferroni P<0.05).

5.2.3 Oviposition

The GLM model used to analyze oviposition was not significant in the case of *E. stipulatus* (Table 5.5) and, same observed in Chapter 4, this result should be probably attributed to the extremely low fecundity of this species in our assays, which was always below one egg per female and day (Figure 5.7).

Diet	Factors	*E. stipulatus*	*N. californicus*	*P. persimilis*
***T. urticae* eggs + pollen**	Model	9.99; 8; 0.266	99.26; 8; < 0.001	89.31; 8; < 0.001
	T	2.82; 2; 0.244	50.42; 2; < 0.001	210.71; 2; < 0.001
	RH	2.78; 2; 0.249	6.85; 2; 0.033	1.96; 2; 0.376
	T*RH	4.23; 8; 0.375	103.54; 8; < 0.001	199.20; 7; < 0.001
***T. urticae* eggs**	Model	8.73; 8; 0.365	71.59; 8; < 0.001	70.66; 8; < 0.001
	T	3.64; 2; 0.162	84.66; 2; < 0.001	62.37; 2; < 0.001
	RH	0.27; 2; 0.873	1.52; 2; 0.772	2.91; 2; 0.232
	T*RH	13.42; 4; 0.009	93.94; 8; < 0.001	94.95; 8; < 0.001
Pollen	Model	12.29; 8; 0.139	12.39; 5; 0.030	25.499; 8; 0.001
	T	28.50; 2; < 0.001	7.00; 1; 0.008	17.67; 2; <0.001
	RH	24.64; 2; < 0.001	3.50; 2; 0.061	15.69; 2; <0.001
	T*RH	39.01; 7; < 0.001	16.01; 2; 0.003	11.08; 2; 0.011

Table 5.5. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to oviposition (Poisson), for *E. stipulatus*, *N. californicus* and *P. persimilis* in each diet separately (*T. urticae* eggs + pollen, *T. urticae* eggs and pollen) considering the factors temperature "T", relative humidity "RH" and their interactions as explanatory variables.

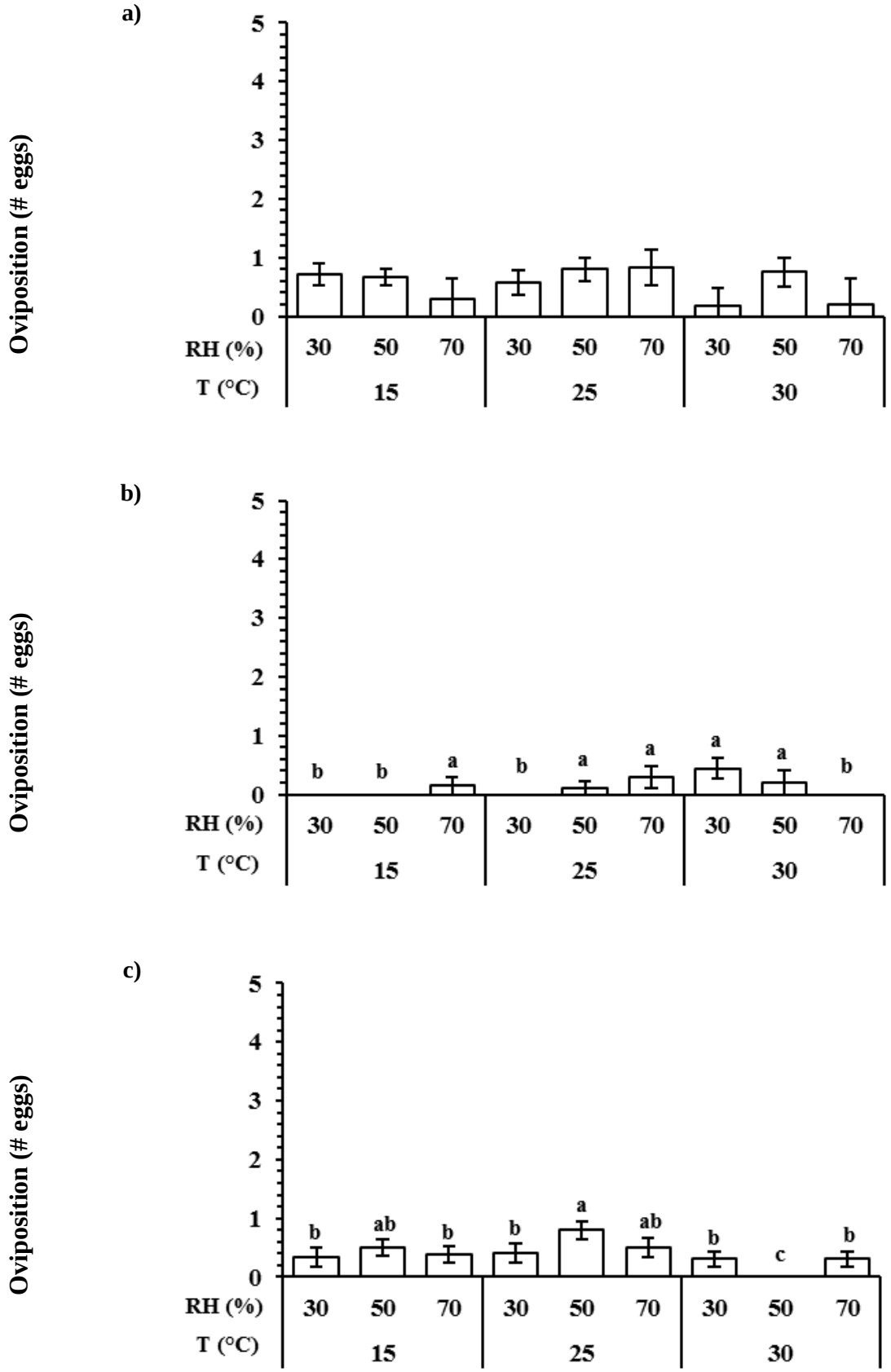
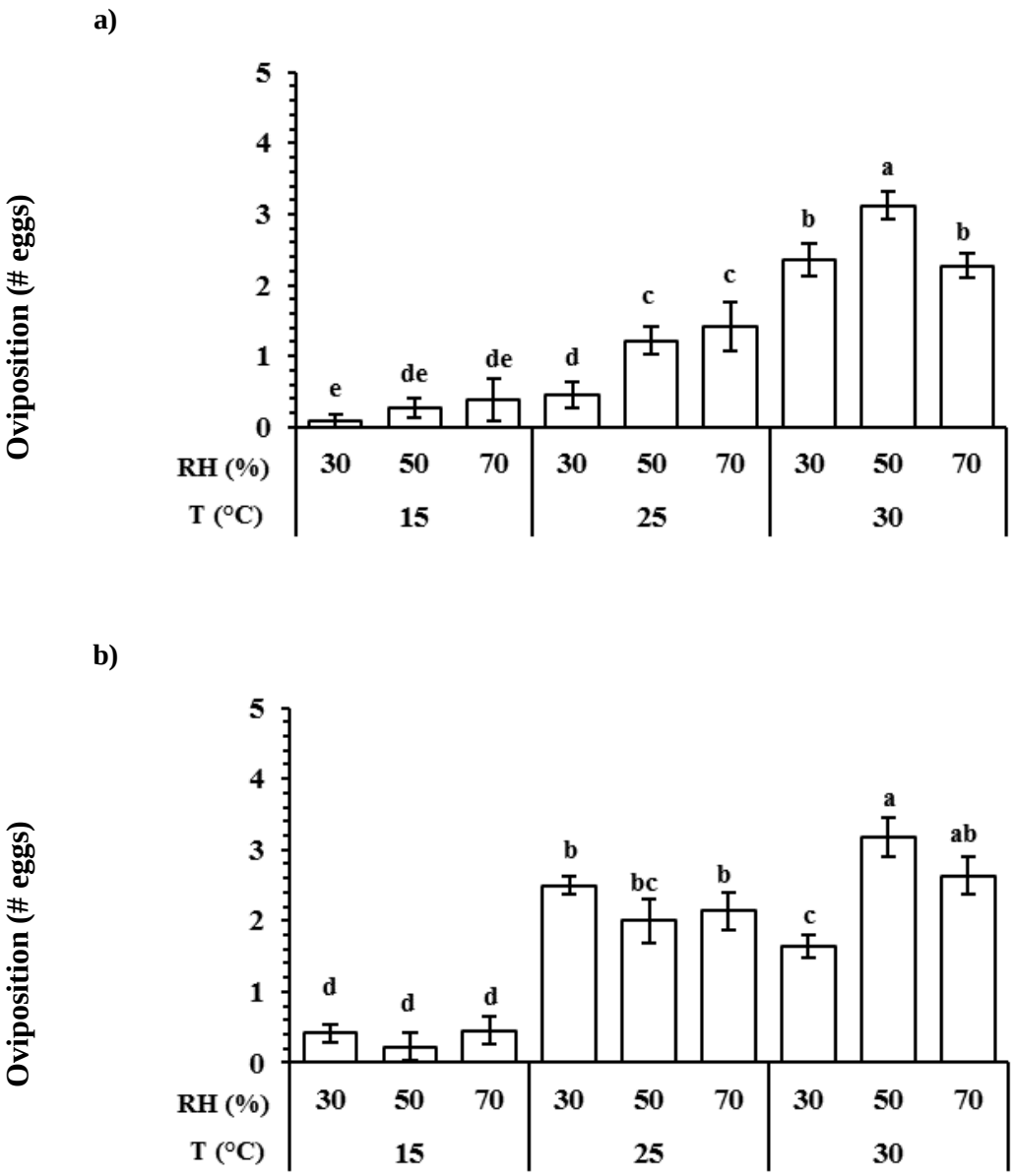
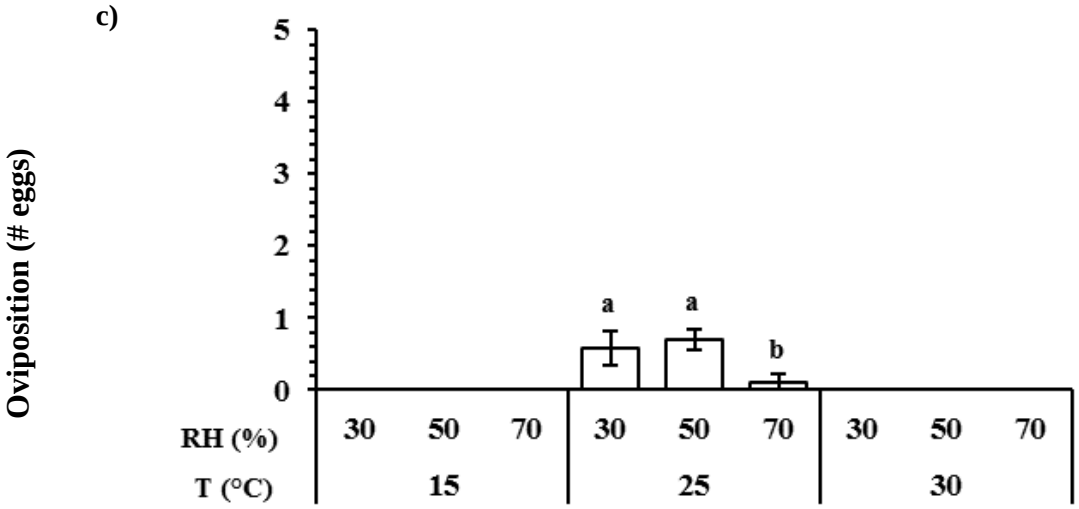


Figure 5.7. Oviposition of *E. stipulatus* when offered three different diets (a) *T. urticae* eggs + pollen, (b) *T. urticae* eggs and (c) pollen at 15º, 25º and 30° C combined with 30, 50 and 70 % of RH. For each figure, bars with the same letter are not statistically different (Bonferroni P<0.05).

For *N. californicus*, oviposition was affected by temperature and relative humidity (Table 5.5) and it was lowest when the phytoseiid had access to pollen only (it was actually nil at 15º and 30ºC irrespective of relative humidity) (Figure 5.8). Interestingly, this species was especially fecund at 30ºC with much lower values at 15 ºC and intermediate at 25 ºC.

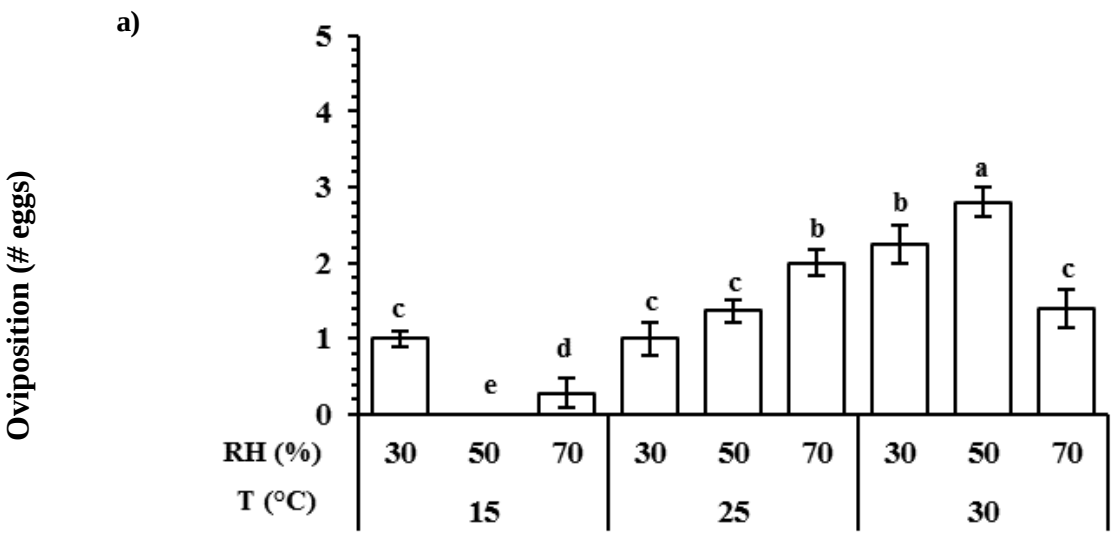
Figure 5.8. Oviposition of *N. californicus* when offered three different diets (a) *T. urticae* eggs + pollen, (b) *T. urticae* eggs and (c) pollen at 15º, 25º and 30° C combined with 30, 50 and 70 % of RH. For each figure, bars with the same letter are not statistically different (Bonferroni P<0.05).



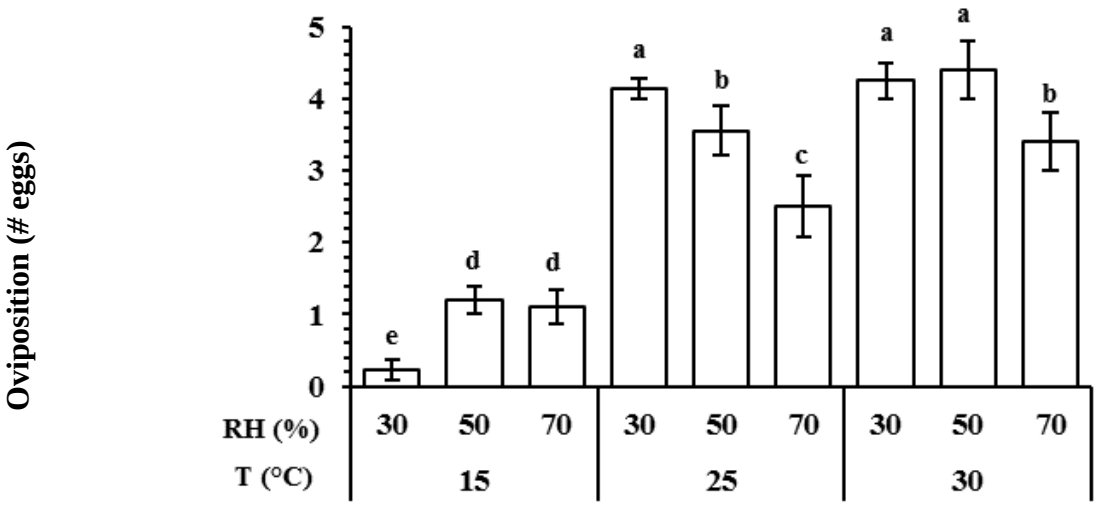


Same as before, in the case of *P. persimilis*, temperature and relative humidity significantly affected oviposition (Table 5.5), which became almost nil when this mite had access to pollen only (Figure 5.9). From the other two food sources, the *T. urtice* eggs only diet presented higher values (mean 2.8 ± 0.3 eggs) than the mixed diet (1.3 ± 0.2). For these food sources, oviposition was lowest at 15ºC and increased along with temperature with maximum values usually associated to intermediate relative humidity values rather than to 70%.

Figure 5.9. Oviposition of *P. persimilis* when offered three different diets (a) *T. urticae* eggs + pollen, (b) *T. urticae* eggs and (c) pollen at 15º, 25º and 30° C combined with 30, 50 and 70 % of RH. For each figure, bars with the same letter are not statistically different (Bonferroni P<0.05).



b)



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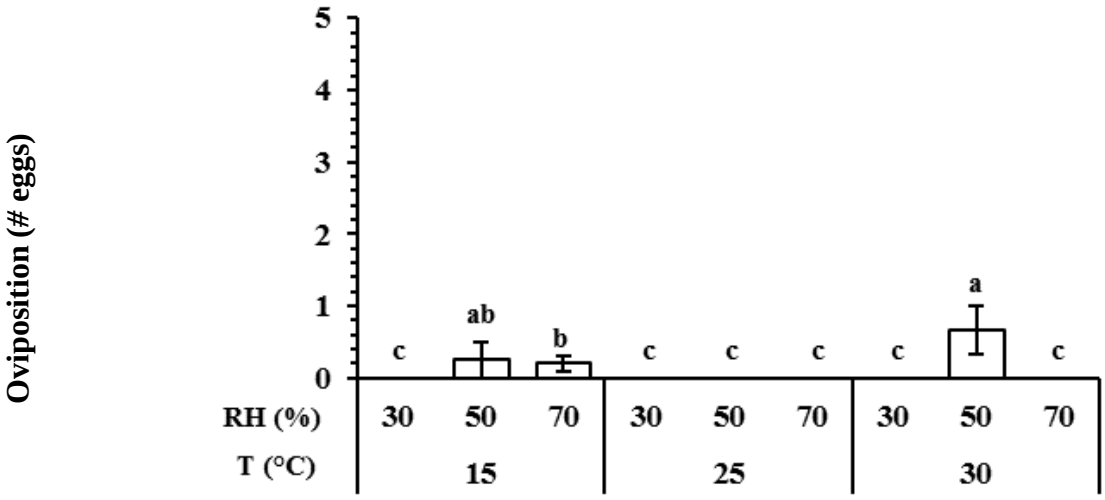


5.3 Discussion

Our initial hypothesis assumed that pollen supply could at least partly mitigate the negative effects of Climate Change on the regulation of *T. urticae* by pallinophagous *E. stipulatus*. However, we also assumed that this supply would have no or a minor positive impact on the Tetranychidae-specialist *N. californicus* and the *Tetranychus* sp.-specialist *P. persimilis*. Results basically demonstrate that pollen supply may enhance *E. stipulatus* survival, predation and oviposition rates. However, even the access to this preferred food source kept fecundity below one egg per female and day. Such a low value may not be enough to maintain *T. urticae* under regulation at hot and dry conditions typical of Climate

Change, where this spider mite may reproduce much faster, up to eight eggs per female and day (Figure 4.11, Chapter 4). For the other two phytoseiids, access to pollen in combination with *T. urticae* eggs decreased the number of specimens found dead in the arenas but also reduced predation and oviposition relative to the *T. urticae*-only diet.

Several authors have reported the positive effects of pollen supply on *E. stipulatus* performance (Ferragut et al. 1987; Bouras and Papadoulis 2005; Pina et al. 2012; Montserrat et al. 2013a). Although other mixed diets including honeydew and microarthropods can also enhance it (Zhimo and McMurtry 1990; Ferragut et al. 1992), pollen is considered the most suitable alternative diet for this mite and this may explain why in our assays, best results (i.e., highest survival, predation and oviposition rates) corresponded to the pollen only diet. Whether this better performance could compensate the negative effects of hot temperatures and low relative humidity observed on this mite in our previous assays (Chapters 3 and 4) and elsewhere (Ferragut et al., 1987; El Taj and Jung, 2012; Ghazy et al., 2012a; Guzmán et al., 2016) though, remains quite doubtful because of the high oviposition rate observed for *T. urticae* at 30ºC in our previous studies (Chapter 4). Indeed, Montserrat et al. (2013a) found that although pollen supply in avocadoes could boost the populations of omnivorous predatory mites occurring in that system (including *E. stipulatus*), this increase did not translate into a better control of the populations of another tetranychid pest mite, the persea mite, *Oligonychus perseae* Turtle, Baker and Abbatiello. The intrinsic rate of increase of *O. perseae* at 30ºC has been reported to be 0.144 day^{-1} (Aponte and McMurtry 1997), which is about half what is reported for *T. urticae* (0.219 to 0.336 day^{-1}; Sabelis, 1985, 1991) and this difference may be taken as indicative that pollen supply would most likely fail to prevent the negative effects of Climate Change on the regulation of *T. urticae* provided by *E. stipulatus* in citrus.

Pollen can also allow *N. californicus* to survive and even reproduce (Castagnoli and Simoni, 1999; Ragusa et al., 2009). Importantly, pollen supply in our assays reduced the rate of dead specimens registered in the experimental arenas, both for this mite and for *P. persimilis*. This reduction would increase the chances of these two species to either survive or to effectively escape from these harsh conditions, which could improve their impact on *T. urticae*. However, no further positive effects on

oviposition and predation were observed for any of these mites when having access to a mixed diet of *T. urticae* eggs and pollen. Indeed, in general higher oviposition rates were obtained in the *T. urticae* eggs-only diet than in the mixed pollen and *T. urticae*-eggs diet for both *N. californicus* (1.7 ± 0.2 versus 1.3 ± 0.2 eggs, respectively) and *P. persimilis* (2.8 ± 0.3 versus 1.3 ± 0.2 eggs, respectively). Likewise, predation rates were higher for *P. persimilis* when no pollen was offered (28.5 ± 2.7 versus 20.6 ± 1.8 eggs) and they did not change for *N. californicus* (17.2 ± 1.7 eggs), and this should be probably attributed at the positive effect of pollen on predation at 15ºC but negative at 25ºC (Figure 5.5).

To sum up, the effects reported here point at a better survival of the three phytoseiid species when having access to a mixed diet of pollen and *T. urticae* eggs. However, as no further positive effects on oviposition and predation were observed in any case, the question is whether this enhanced survival would justify the use of pollen in citrus to counteract the deleterious effects of Climate Change on the natural regulation of *T. urticae*. As pollen could also affect interspecific relationships between these species, in the next Chapter we will investigate whether pollen supply may modulate the effect of interspecific competition.

6

Can pollen supply mitigate the deleterious effects of climate change on the biological control of *Tetranychus urticae*? Effects of pollen on the competition between the predators *Euseius stipulatus*, *Neoseiulus californicus* and *Phytoseiulus persimilis* at laboratory conditions

Hypothesis

The extreme temperatures and relative humidity values typical of Climate Change may affect competition between *Euseius stipulatus*, *Neoseiulus californicus* and *Phytoseiulus persimilis* and this may have consequences on the natural regulation of *T. urticae* in citrus, where these phytoseiids are the spider mite key natural enemies. Furthermore, competition in co-occurrence with alternative food and prey may promote the abundance of pallinophagous predators (i.e., *E. stipulatus*) but demote pest control through apparent competition, as observed in avocado by Montserrat et al. (2013a). As in our case, only one of the predators at play is pallinophagous, our hypothesis assumes that pollen supply may boost its populations, even at Climate Change conditions, resulting in increased competition between phytoseiids, which together with apparent competition between pollen and *T. urticae* may lead to a disruption of existing biological control.

6.1 Material and Methods

6.1.1 Environmental conditions

Temperature and relative humidity values for the experiments were chosen as proxy for the present average conditions in our region (Mediterranean basin) during the summer season (25 ± 1 ˚C and 70 ± 5 % RH) (AEMET 2017) called "optimal conditions" (OC). These conditions were compared with the future climate change predictions, with an increase of temperature around 5 ºC (30 ºC) and 25 % of rainfall decrease (50% RH) (IPCC, 2014), which we called "climate change conditions" (CCC). The stable relative humidity values were obtained in desiccators (L × W × H: 250 × 320 × 355 mm) by using different salt solutions (Winston and Bates, 1960). Experiments were carried out at laboratory conditions in a climate chamber (Sanyo Electric Co., Ltd., Japan) with 16:8 h L:D.

6.1.2 Experimental procedure

The fate, predation, and oviposition of the three most abundant predators of *T. urticae* in Valencian clementine orchards (Abad-Moyano et al., 2009a; Aguilar-Fenollosa et al., 2011b): *E. stipulatus*, *N. californicus* and *P. persimilis*, was studied following the procedure explained in Chapter

2 (Common Material and Methods). In this case, though we additionally studied the different two-species (one specimen of each) and used four different diet treatments: control (unfed), *Carpobrotus edullis* pollen (P), *T. urticae* eggs (T) and *T. urticae* eggs plus *C. edullis* pollen (TP). When necessary, pollen was supplied ad libitum to each arena immediately before the introduction of the phytoseiid. In all cases, we checked predation (number of *T. urticae* eggs eaten), oviposition (number of eggs deposited) and fate (i.e., live, dead and escapees) as in previous chapters both for the single species arenas and those where two specimens from different species were competing. As all *P. persimilis* stages have a distinctive red-orange color, eggs deposited by this species could be easily distinguished from those deposited by the other two. However, for the arenas where *E. stipulatus* and *N. californicus* were competing, eggs were isolated on leaf discs provided with *T. urticae eggs* and pollen, and allowed to develop to adults at OC. Once they reached adulthood, they were identified to the species level under the microscope following the procedure explained in Chapter 3 (Experimental procedure). A total of 15 replicates (five replicates per 3 repetitions during the time) were performed for each treatment (phytoseiid combination*diet*abiotic condition).

6.1.3 Statistical analyses

To study the effects of the different factors included in our assays on the above mentioned parameters, we used general linear models (GLM) assuming different probability distributions and different link functions according to the characteristics of the data. In the case of fate, which had three different possible outputs, we used a GLM with a multinomial distribution of the error and a generalized logit link function. For oviposition and predation, we used a GLM with a Poisson distribution of the error and a logistic link function. In all three cases, the factors “combination”, “diet” and “abiotic conditions” were used as fixed effects. As our main goal was to identify for each phytoseiid species separately, the competition-specific patterns of response to climate change and whether diet could modulate this response, we started our analyses by considering all combinations including the “competition” factor. Once the signification of the “combination” factor was tested, we similarly continued the analyses of fate, predation and oviposition in a sequential manner by studying the effect

of “diet”. Eventually, we analyzed for each phytoseiid combination and diet, the effects of “abiotic conditions”. When necessary, we used Bonferroni post-hoc test for mean separation. Because in the case of predation when evaluating the combinations including two species, it was not possible to separate which eggs had been consumed by which species, we could not perform the analyses as for the other two parameters (fate and oviposition). Therefore, in this case, we had to analyze this parameter independently for the arenas where one single predator species had been evaluated and those where two species competed. To better characterize the effects of competition on predation, we compared the number of eggs actually eaten by the two species together with the expected predation without competition, which corresponded to the addition of mean predation observed for each species when evaluated singly (predation species 1 + predation species 2). In this case, Chi square (x^2) tests was used to compare predation obtained and predation expected. In all cases, SPSS 23.0 was used for statistical analysis.

6.2 Results

6.2.1 Fate

There were significant differences ($P < 0.05$) between combinations for each species included in this study for fate (Table 6.1). However, for *P. persimilis* only the interaction diet*climate*combination was barely significant.

Factors	*E. stipulatus*	*N. californicus*	*P. persimilis*
Model	64.71; 23; < 0.001	215.38; 23; < 0.001	127.72; 23; < 0.001
Combination	9.64; 2; 0.008	115.42; 2; < 0.001	0.42; 2; 0.896
Diet * Combination	23.37; 11; 0.005	13.47; 11; 0.143	6.79; 11; 0.659
Climate * Combination	5.32; 5; 0.150	32.53; 5; < 0.001	2.33; 5; 0.506
Diet * climate * Combination	26.4; 23; 0.002	37.37; 23; < 0.001	17.62; 23; 0.040

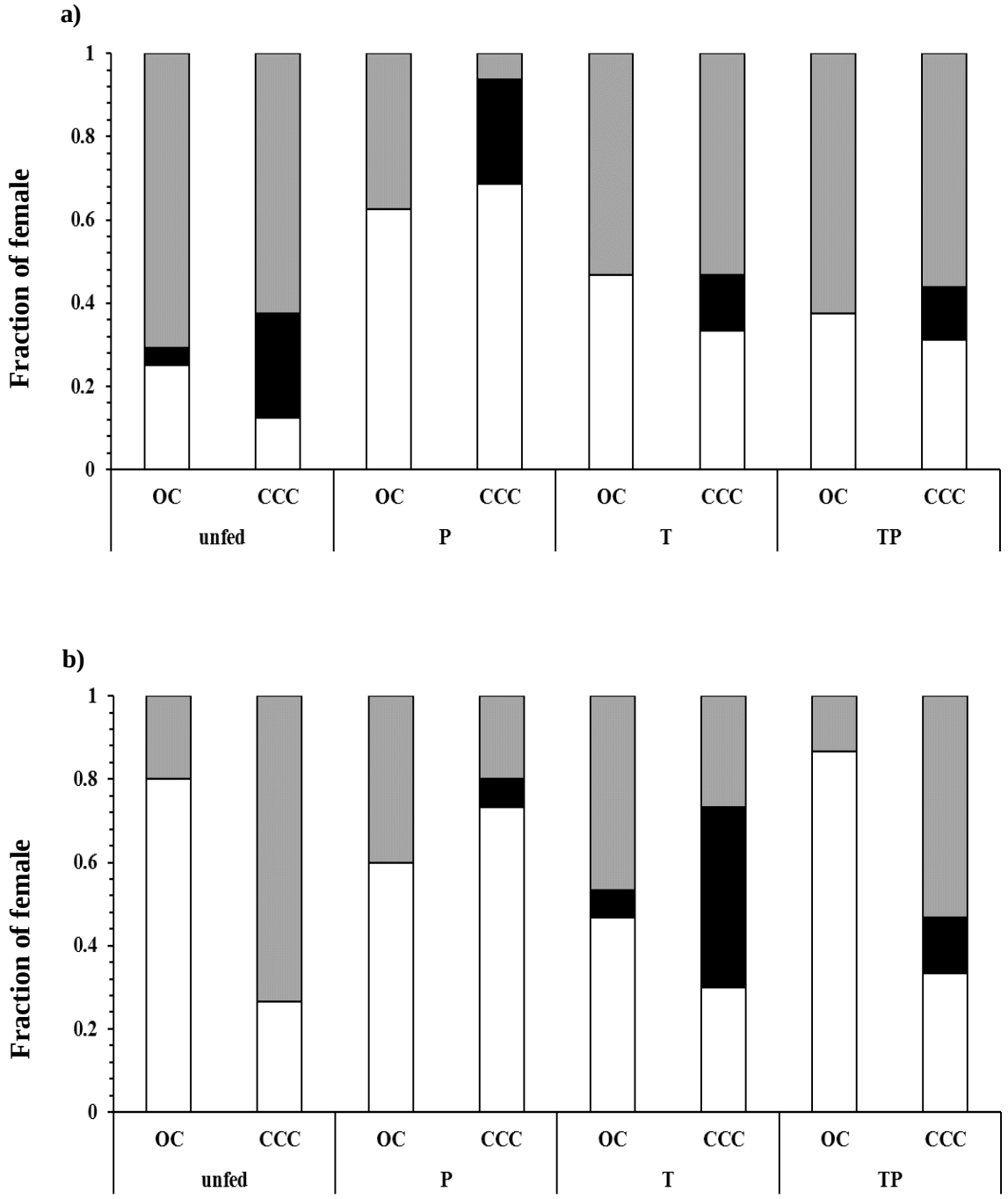
Table 6.1. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to fate (multinomial) for *E. stipulatus*, *N. californicus*, and *P. persimilis* considering the factor combination and their interactions with diet and climate as explanatory variables for each phytoseiid species.

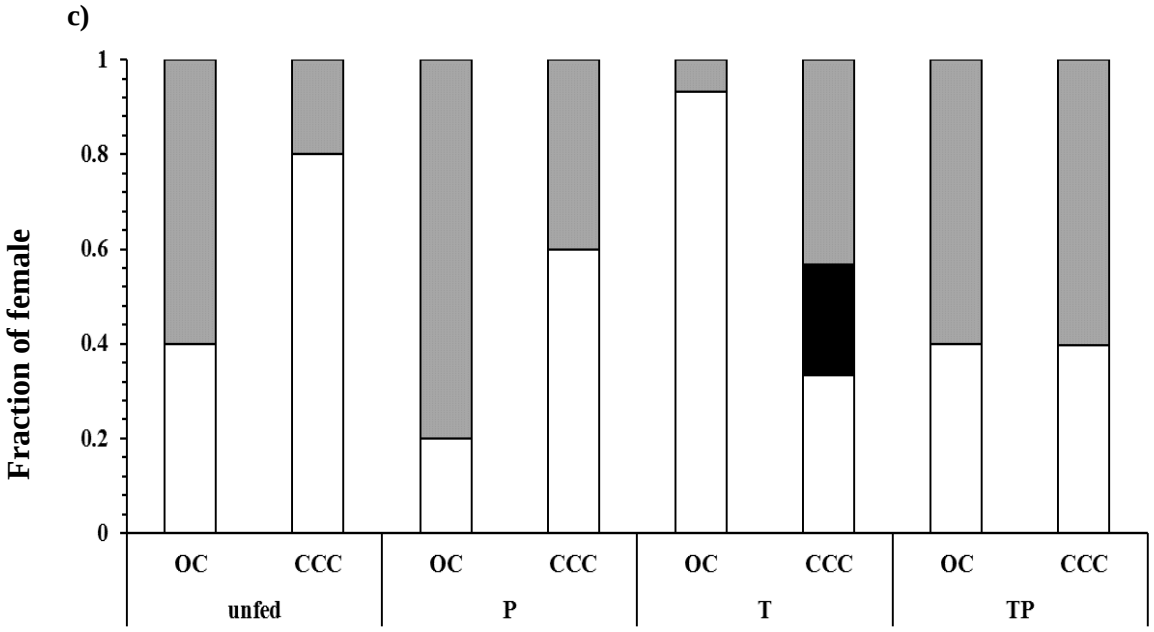
For *E. stipulatus* diet and climate but not their interaction affected fate when it was alone (Table 6.2). Same as observed in previous assays (Chapter 4) survival was highest when pollen was the only food source available and lowest when no food was available. In general, mortality was associated to Climate Change conditions (Figure 6.1). The addition of a competitor in the system changed the situation and fate now depended on the interaction of diet and climate (Table 6.2). For both competitors, survival of *E. stipulatus* dramatically increased when no additional food was added. No dead specimens were found in these arenas (Figure 6.1). Interestingly, mortality only increased when the diet consisted of *T. urticae* eggs only, especially at Climate Change conditions. The mixed diet increased survival relative to what was observed when *E. stipulatus* was alone but still some mortality occurred when competing with *N. californcius* at Climate Change conditions. Therefore, pollen increased survival of *E. stipulatus* when competing with the other two phytoseiids but could not completely avoid some mortality when exposed to *N. californicus* at harshest conditions.

Factors	**Combination**		
	***E. stipulatus* alone**	**+ *N. californicus***	**+ *P. persimilis***
Model	18.34; 7; 0.011	19.51; 7; 0.007	17.59; 7; 0.014
Diet	11.58; 3; 0.009	4.20; 3; 0.240	5.64; 3; 0.130
Climate	4.20; 1; 0.040	0.09; 1; 0.753	0.14; 1; 0.907
Diet * Climate	1.68; 7; 0.640	14.59; 7; 0.002	11.05; 7; 0.011

Table 6.2. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to fate (multinomial) for *E. stipulatus* either alone, in competition with *N. californicus* and *P. persimilis* considering the factors diet, climate, and their interactions as explanatory variables.

Figure 6.1. Fate of *E. stipulatus*. Escapees stuck in the glue (grey bars), dead (black bars) and alive (white bars) specimens when the phytoseiid was challenged either (a) alone, competing with (b) *N. californicus*, and with (c) *P. persimilis* (c). Phytoseiids were offered four different diets (unfed, P = pollen, T = *T. urticae* eggs, TP = *T. urticae* eggs + pollen) at optimal (OC; 25 ºC – 70 %RH) and climate change (CCC; 30 ºC – 50 %RH) conditions.



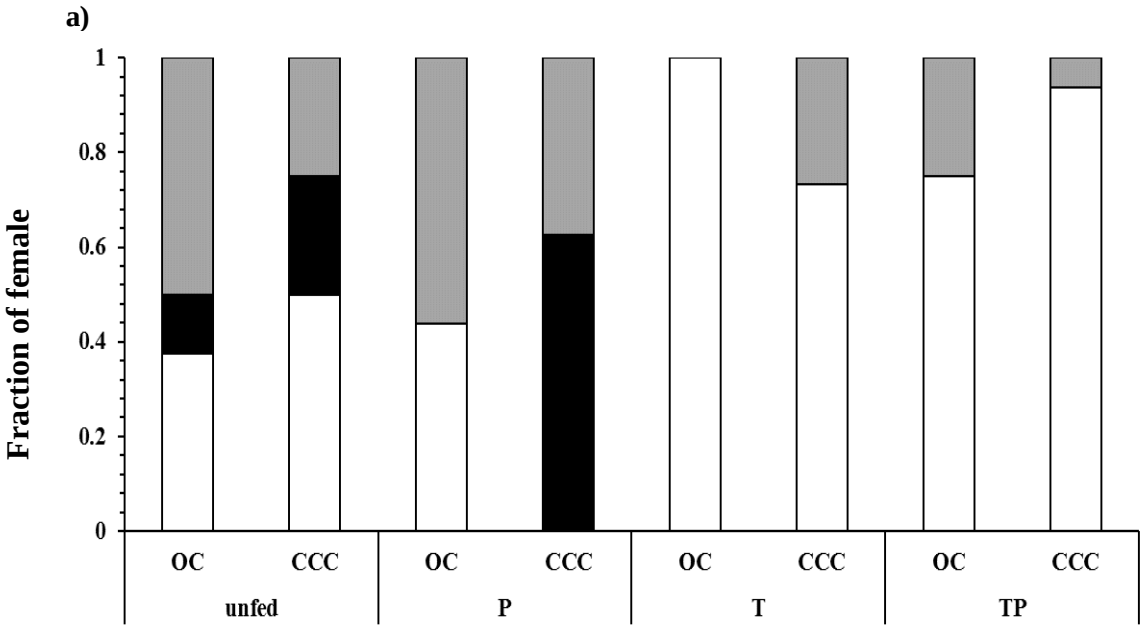


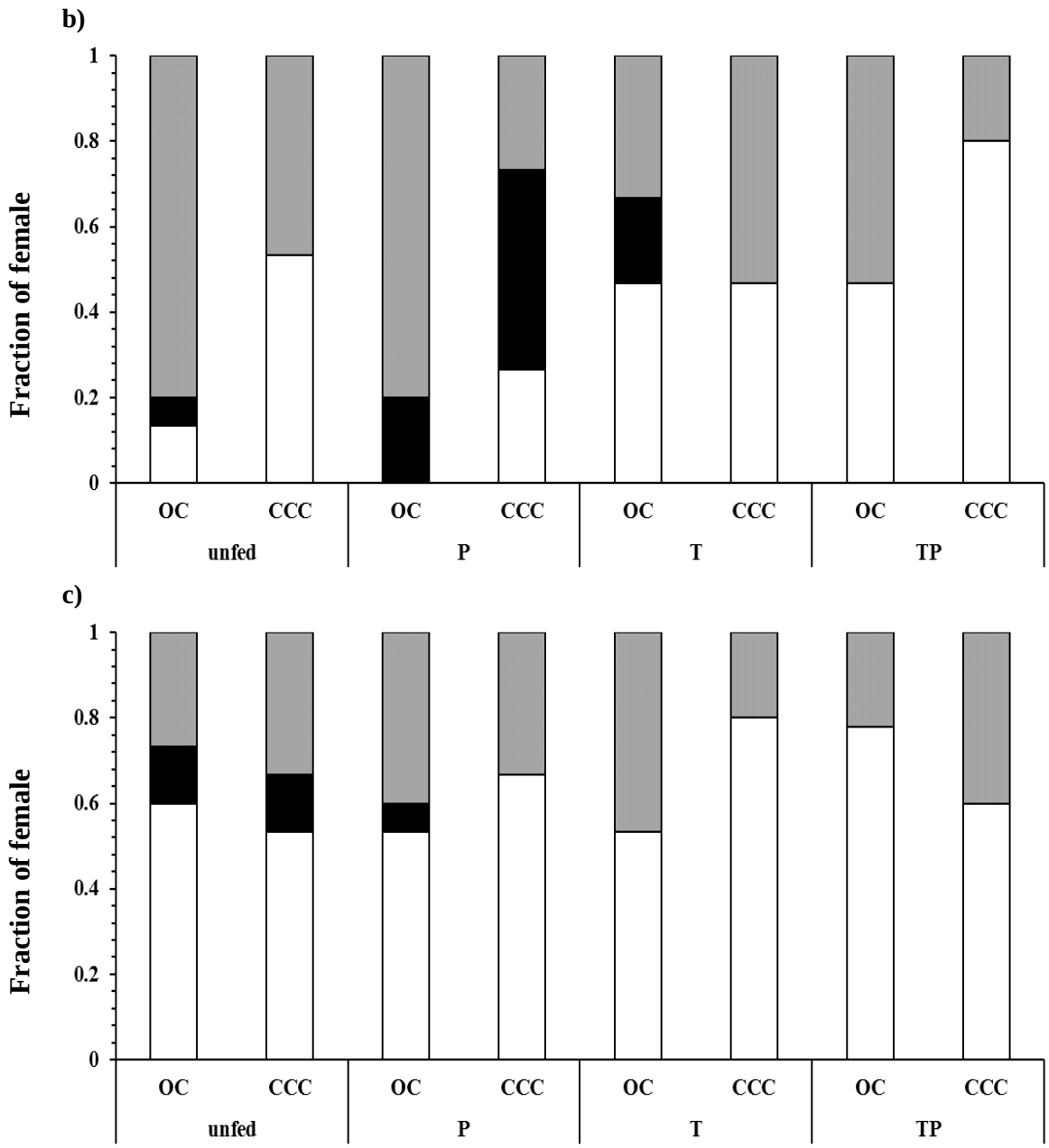
For *N. californicus* climate and the diet*climate interaction were significant when it was singly challenged (Table 6.3). Survival was high when the mite had access to *T. urticae* eggs, either alone or in the mixed diet (no mortality) and it reached 100 % when eggs were the only food source at optimal conditions. Mortality was especially high when this predator was offered pollen only at Climate Change conditions (Figure 6.2). These results changed when *N. californcius* was exposed to competition. When the other phytoseiid was *E. stipulatus*, the factor diet was significant only through its interaction with climate (Table 6.3). In general, survival rates decreased in all cases at the expenses of an increased rate of escapees rather than to increased mortality (Figure 6.2). When *P. persimilis* was the competitor, only climate was significant (Table 6.3). In general, escapee rates increased relative to the situation where *N. californicus* was alone and dead specimens were associated to diets lacking *T. urticae* eggs (Figure 6.2). Therefore, this species seemed to suffer increased deleterious effects from competition from *E. stipulatus* than from *P. persimilis* and pollen provision partly compensated them (no mortality when competing with *E. stipulatus* at optimal conditions).

Table 6.3. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to fate (multinomial) for *N. californicus* either alone, in competition with *E. stipulatus* and *P. persimilis* considering the factors diet, climate, and their interactions as explanatory variables.

Factors	Combination		
	***N. californicus* alone**	**+ *E. stipulatus***	**+ *P. persimilis***
Model	21.91; 6; 0.003	35.06; 7; <0.001	28.08; 7; <0.001
Diet	0.74; 3; 0.865	3.95;: 3; 0.266	3.98; 3; 0.264
Climate	8.94; 1; 0.003	15.99; 1; <0.001	13.92; 1; <0.001
Diet * Climate	13.48; 6; 0.004	17.08; 6; 0.001	5.88; 3; 0.118

Figure 6.2. Fate of *N. californicus*. Escapees stuck in the glue (grey bars), dead (black bars) and alive (white bars) specimens when the phytoseiid was challenged either (a) alone , competing with (b) *E. stipulatus* and with (c) *P. persimilis.* Phytoseiids were offered four different diets (unfed, P = pollen, T = *T. urticae* eggs, TP = *T. urticae* eggs + pollen) at optimal (OC; 25 ºC – 70 %RH) and climate change (CCC; 30 ºC – 50 %RH) conditions.





In the case of *P. persimilis* alone, diet and climate significantly affected fate only through their interaction (Table 6.4). Survival was nil when no food was available and pollen just allowed some survival below 30 %, especially at optimal conditions (Figure 6.3). The availability of *T. urticae* eggs made the difference and no mortality was observed when this food item was accessible, either alone or with pollen. Nevertheless, the rate of escapees was around 50% even in these cases. The addition of *E. stipulatus* to the system made fate dependent on both diet and climate. In all cases survival increased and mortality became nil. The most remarkable change was survival at "unfed" conditions, which may

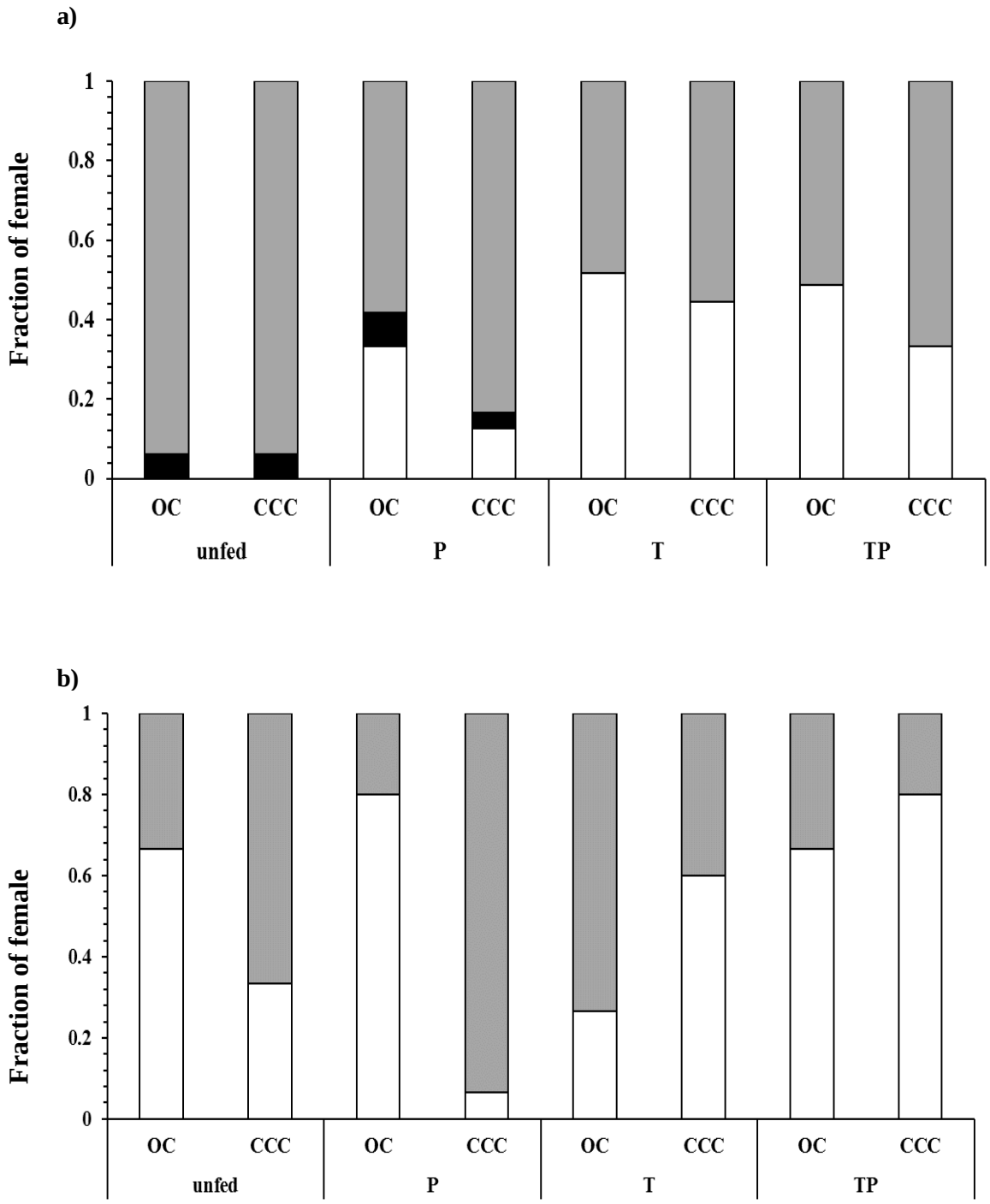
be taken as evidence that *P. persimilis* probably fed on *E. stipulatus* eggs because no *E. stipulatus* adult was found dead under these conditions (Figure 6.1). Importantly, at Climate Change conditions, survival of *P. persimilis* also increased, and same as before, the lack of dead *E. stipulatus* specimens found in the mixed diet (Figure 6.2) may indicate that its eggs were exploited by *P. persimilis* but no predation on adults occurred. However, higher survival at Climate Change conditions when only *T. urticae* eggs were available coincided with increased *E. stipulatus* mortality, which may indicate that at these conditions, *P. persimilis* actually killed some of them. The addition of *N. californicus* affected *P. persimilis* fate in a different way. In this case, fate became independent of diet and climate (Table 6.4). Survival of *P. persimilis* also increased at "unfed" conditions and also in this case this coincided with the observation of some dead specimens of *N. californicus* (Figure 6.2). For the remaining conditions, some mortality occurred, which may indicate that *N. californicus* actually killed some *P. persimilis* specimens, especially when pollen was not available and at Climate Change conditions (Figure 6.3). At these conditions, though, no dead *N. californicus* adult was found (Figure 6.2).

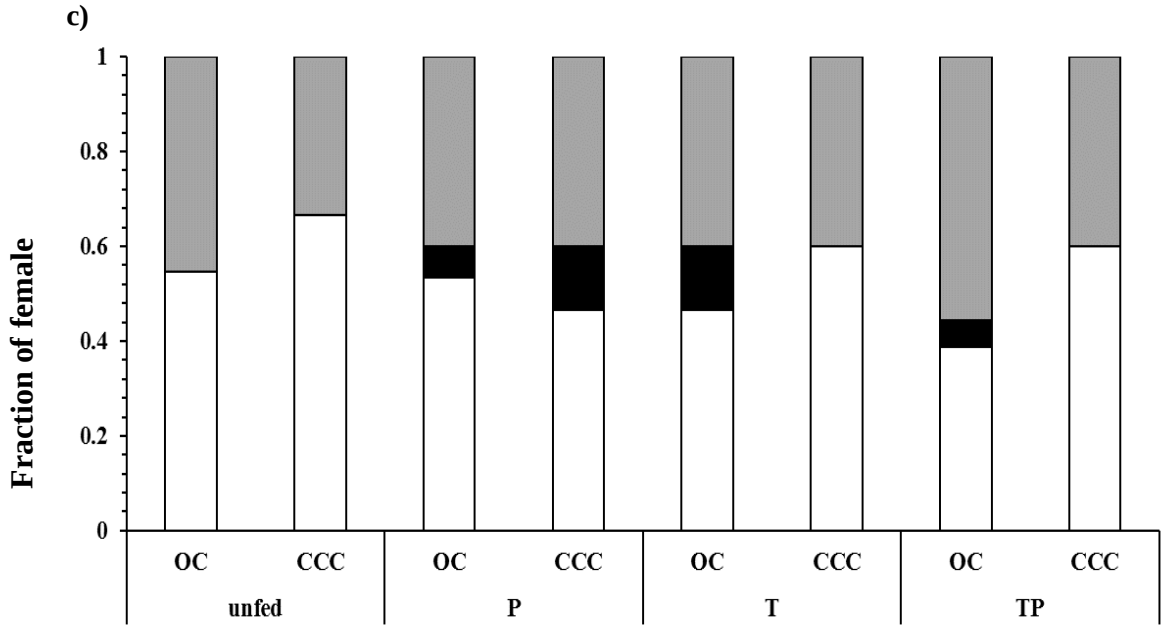
To sum up, we have seen that, in general, *E. stipulatus* is the species most at risk from competition from the other two predators and that *N. californicus* is the most successful competitor, with *P. persimilis* occupying an intermediate position. The provision of pollen in a mixed diet may help *E. stipulatus* to better survive when competing with *P. persimilis* but this is not the case when the competitor is *N. californicus* (Figure 6.1).

Factors	**Combination**		
	***P. persimilis* alone**	**+ *E. stipulatus***	**+ *N. californicus***
Model	59.24; 5; <0.001	39.48; 7; < 0.001	7.08; 7; 0.020
Diet	4.46; 3; 0.217	65.4; 3; <0.001	2.28; 3; 0516
Climate	0.123; 1; 0.998	24.1; 1; <0.001	2.49; 1; 0.115
Diet * Climate	14.67; 5; 0.002	38.87; 7; <0.001	2.39; 3; 0.494

Table 6.4. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to fate (multinomial) for *P. persimilis* either alone, in competition with *E. stipulatus* and *N. californicus* considering the factors diet, climate, and their interactions as explanatory variables.

Figure 6.3. Fate of *P. persimilis*. Escapees stuck in the glue (grey bars), dead (black bars) and alive (white bars) specimens when the phytoseiid was challenged either (a) alone, competing with (b) *E. stipulatus* and with (c) *N. californicus*. Phytoseiids were offered four different diets (unfed, P = pollen, T = *T. urticae* eggs, TP = *T. urticae* eggs + pollen) at optimal (OC; 25 ºC – 70 %RH) and climate change (CCC; 30 ºC – 50 %RH) conditions.





6.2.2 Predation

6.2.2.1 Predators individually challenged

Same as observed in the chapters 4 and 5, there were significant differences ($P < 0.001$) between the three phytoseiid species included in this study for predation. The omnivorous *E. stipulatus* was the less voracious predator, followed by *N. californicus* and *P. persimilis* (9.1, 22.1 and 27.7 *T. urticae* eggs per female, respectively) (Table 6.5).

Factors	Predation
Model	443.04; 11; < 0.001
Diet	1.24; 1; 0.551
Climate	2.65; 1; 0.421
species	197.58; 2; < 0.001
Diet*Climate	9.66; 3; 0.009
Diet*species	251.56; 5; < 0.001
Climate*species	275.40; 5; < 0.001
Diet*Climate*species	541.67; 11; < 0.001

Table 6.5. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to predation (Poisson) for predators individually challenged considering the factors diet, climate, species and their interactions as explanatory variables.

In general, same as reported in chapter 5 of this thesis, diet, climate and their interactions affected the predation rate of *E. stipulatus, N. californicus* and *P. persimilis* (Table 6.6). In the case of *E. stipulatus*, maximum and minimum predation rates were observed at optimal conditions with the mixed diet (16.3 eggs) and at Climate Change conditions when offered *T. urticae* eggs only (3.7 eggs), respectively (Table 6.7). For *N. californicus*, highest predation rates were observed at Climate Change conditions independently of the diet (26.5 eggs) and minimum at optimal conditions with the mixed diet (15.2 eggs) (Table 6.7). In the case of *P. persimilis* the highest predation rate was observed for the *T. urticae* eggs only diet at optimal conditions (35.7 eggs, respectively) and the smallest one at optimal conditions as well with the mixed diet (Table 6.6). All these results are in agreement with what was reported in Chapter 5 (Figures 5.4 to 5.6).

Factors	**Phytoseiids**		
	E. stipulatus	***N. californicus***	***P. persimilis***
Diet	23.96; 1; <0.001	35.42; 1; 0.128	18.26; 1; 0.021
Climate	17.44; 1; 0.014	62.34; 1; 0.004	18.37; 1; 0.034
Diet*Climate	13.78; 7; <0.001	29.65; 7; <0.001	15.42; 7; 0.013

Table 6.6. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to predation (Poisson) for *E. stipulatus, N. californicus* and *P. persimilis* considering the factors Diet, Climate and their interactions as explanatory variables

Phytoseiid sp.	Diet	Climate	Predation
E. stipulatus	T	OC	7.9 ± 2.1 b
		CCC	3.7 ± 1.0 c
	TP	OC	16.3 ± 1.9 a
		CCC	8.6 ± 1.5 b
N. californicus	T	OC	19.9 ± 0.9 b
		CCC	26.1 ± 2.6 a
	TP	OC	15.2 ± 0.6 c
		CCC	26.9 ± 0.8 a
P. persimilis	T	OC	35.7 ± 4.1 a
		CCC	28.3 ± 3.8 ab
	TP	OC	21.5 ± 1.4 c
		CCC	25.4 ± 2.7 bc

Table 6.7. Predation of *E. stipulatus, N. californicus* and *P. persimilis* when the phytoseiids were challenged either alone. Phytoseiids were offered two different diets (unfed, P = pollen, T = *T. urticae* eggs, TP = *T. urticae* eggs + pollen) at optimal (OC; 25 ºC – 70 %RH) and climate change (CCC; 30 ºC – 50 %RH) conditions. For each phytoseiid specie within each column of o predation values followed by the same letter are not significantly different (Bonferroni post hoc test at P < 0.05).

6.2.2.2 Predators Challenged at competing conditions

Predation rates of three different combinations of phytoseiids included in this thesis were significantly affected either directly or through their interactions by all factors considered (Table 6.8). However, when we analyzed each combination separately, it was clear that none of these factors were significant for the combination *E. stipulatus-P. persimilis* (Table 6.9). Predation was the same independently of diet and climatic conditions. Furthermore, when we compared these values with those expected (i.e., the addition of what we had observed for each species separately) (Table 6.10), we could see that competition only affected predation at optimal conditions when no pollen was available with a reduction of 20 %. As this reduction disappeared when providing pollen (mixed diet), we could conclude that in this case, pollen successfully reduced the detrimental effects of competition at optimal conditions (Table 6.10). For the other two combinations either diet or climate and their interaction significantly affected predation (Table 6.9). For the competing pair *E. stipulatus-N. californicus,* higher

predation rates were observed at optimal conditions with the *T. urticae* eggs only-diet, or at Climate Change conditions with the mixed diet (mean of 22.1 eggs). For this pair, observed predation was always lower than expected (Table 6.10) with reductions around 40 %. Interestingly, pollen addition (i.e., mixed diet) could partly compensate this effect at Climate Change conditions, but increased the reduction at optimal conditions (apparent competition). A similar situation was observed for the last pair (*N. californcius-P. persimilis*). However, in this case, the reduction in predation (around 44 %) was partially compensated by the addition of pollen at optimal conditions but not at climate change conditions (Table 6.10). To sum up, we have seen that competition may impact predation almost exclusively in pairs including *N. californicus*. When the second species is *E. stipulatus*, the addition of pollen can partly compensate this reduction at Climate Change conditions. However, when the other species is *P. persimilis*, pollen addition may in fact increase this negative impact on predation.

Factors	**Predation**
Model	299.19; 11; < 0.001
Diet	3.37; 1; 0.066
Climate	1.47; 1; 0.4 93
Combination	7.25; 2; < 0.001
Diet*Climate	9.66; 3; 0.064
Diet* Combination	71.78; 5; < 0.001
Climate* Combination	80.13; 5; < 0.001
Diet*Climate* Combination.	153.41; 11; < 0.001

Table 6.8. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to predation (Poisson) for predators challenged at competing conditions considering the factors diet, climate, combination and their interactions as explanatory variables.

Factors	**Phytoseiids combinations**		
	E. stipulatus - *N. californicus*	*N. californicus* - *P. persimilis*	*E.stipulatus* - *P. persimilis*
Diet	5.31; 1; 0.123	14.35; 1; 0.003	2.32; 1; 0.712
Climate	14.1; 1; 0.078	8.96; 1; 0.059	1.32; 1; 0.451
Diet*Climate	15.36; 1; <0.036	14.21; 1; 0.002	12.12; 1; 0.078

Table 6.9. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to predation (Poisson) for three combinations of competition *E. stipulatus-N. californicus, N. californicus-P. persimilis* and *E.stipulatus-P. persimilis* considering the factors Diet, Climate and their interactions.

Phytoseiid combination	Diet	Climate	Predation			
			Observed	Expected	(X^2)	Reduction (%)
E. stipulatus* - *N. californicus	**T**	**OC**	21.2 ± 2.8 a	27.7	P < 0.001	23.5
		CCC	15.8 ± 2.7 b	29.8	P < 0.001	47.0
	TP	**OC**	14.6 ± 1.4 b	31.5	P < 0.001	53.7
		CCC	22.8 ± 3.8 a	35.5	P < 0.001	35.8
N. californicus* - *P. persimilis	**T**	**OC**	26.5 ± 3.2 b	55.5	P < 0.001	52.3
		CCC	34.7 ± 2.5 a	54.4	P < 0.001	36.2
	TP	**OC**	25.9 ± 1.8 b	36.7	P < 0.001	29.4
		CCC	22.6 ± 3.6 b	52.27	P < 0.001	56.8
E. stipulatus* - *P. persimilis	**T**	**OC**	34.9 ± 1.7 a	43.6	P < 0.001	20.0
		CCC	32.8 ± 2.5 a	32.1	P = 0.076	0
	TP	**OC**	31.2 ± 1.8 a	37.8	P = 0.003	0
		CCC	31.9 ± 1.1 a	34.1	P = 0.032	0

Table 6.10. Predation observed and expected when phytoseiid co-occurring in competition against each other phytoseiid sp. (*E. stipulatus-N. californicus, N. californicus-P. persimilis, E.stipulatus-P. persimilis*). Phytoseiids were offered two different diets (*T. urticae* eggs (T) and *T. urticae* eggs + Pollen (TP)) at optimal (OC; 25 ºC – 70 %RH) and climate change conditions (CCC; 30 ºC – 50 %RH). For each combination of competition, Pearson's chi-squared test (x^2) were used to compared predation obtained between predation expected of each combination with the percentage of reductions (%) for each combination. Within each column of observed predation, values followed by the same letter are not significantly different (Bonferroni post hoc test at P < 0.05)

6.2.3 Oviposition

Oviposition of three different combinations of phytoseiids included in this thesis were significantly affected either directly or through their interactions by all factors considered (Table 6.11). Therefore, we further analyzed the oviposition rate of each phytoseiid combination separately.

Factors	*E. stipulatus*	*N. californicus*	*P. persimilis*
Model	76.47; 23; < 0.001	198.87; 21;< 0.001	212.60; 21; < 0.001
Combination	0.36; 2; 0.549	31.14; 2; < 0.001	41.19; 2; < 0.001
Diet*Combination	33.88; 11; < 0.001	161.16; 11; < 0.001	135.91; 11; < 0.001
Climate*Combination	18.94; 5; 0.002	53.75; 5; < 0.001	65.52; 5; < 0.001
Diet*climate*Combination	55.01; 23; < 0.001	234.01; 21; < 0.001	142.01; 21; < 0.001

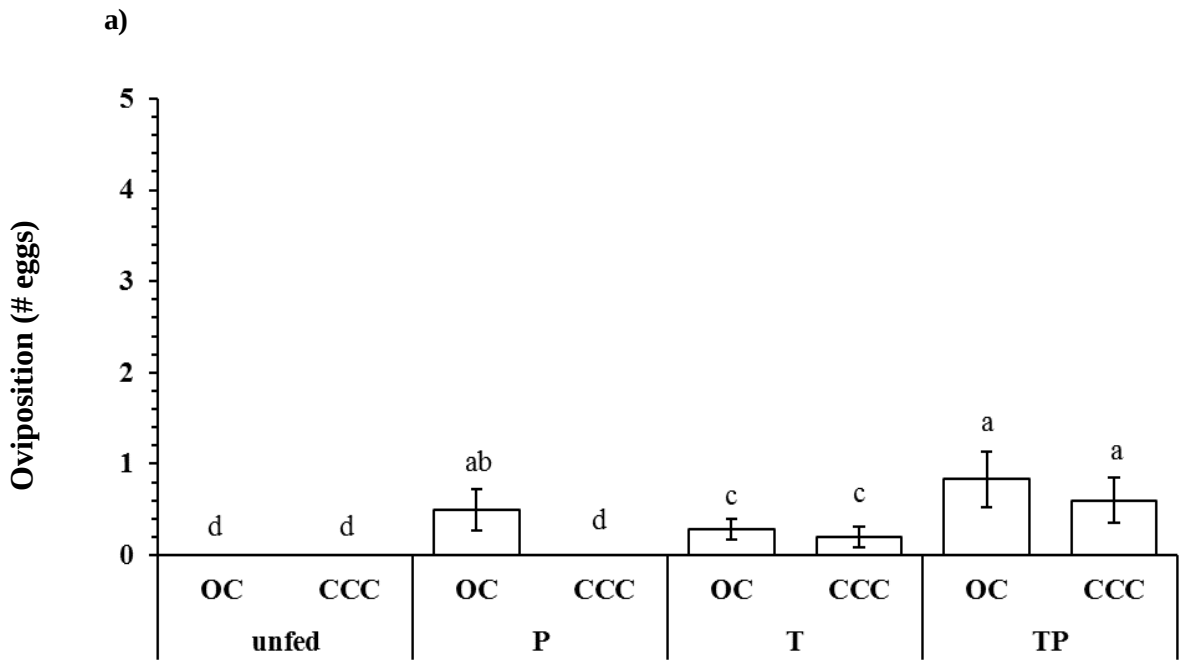
Table 6.11. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to oviposition (Poisson) for *E. stipulatus*, *N. californicus*, and *P. persimilis* considering the factor combination and their interactions with diet, climate as explanatory variables for each phytoseiid species.

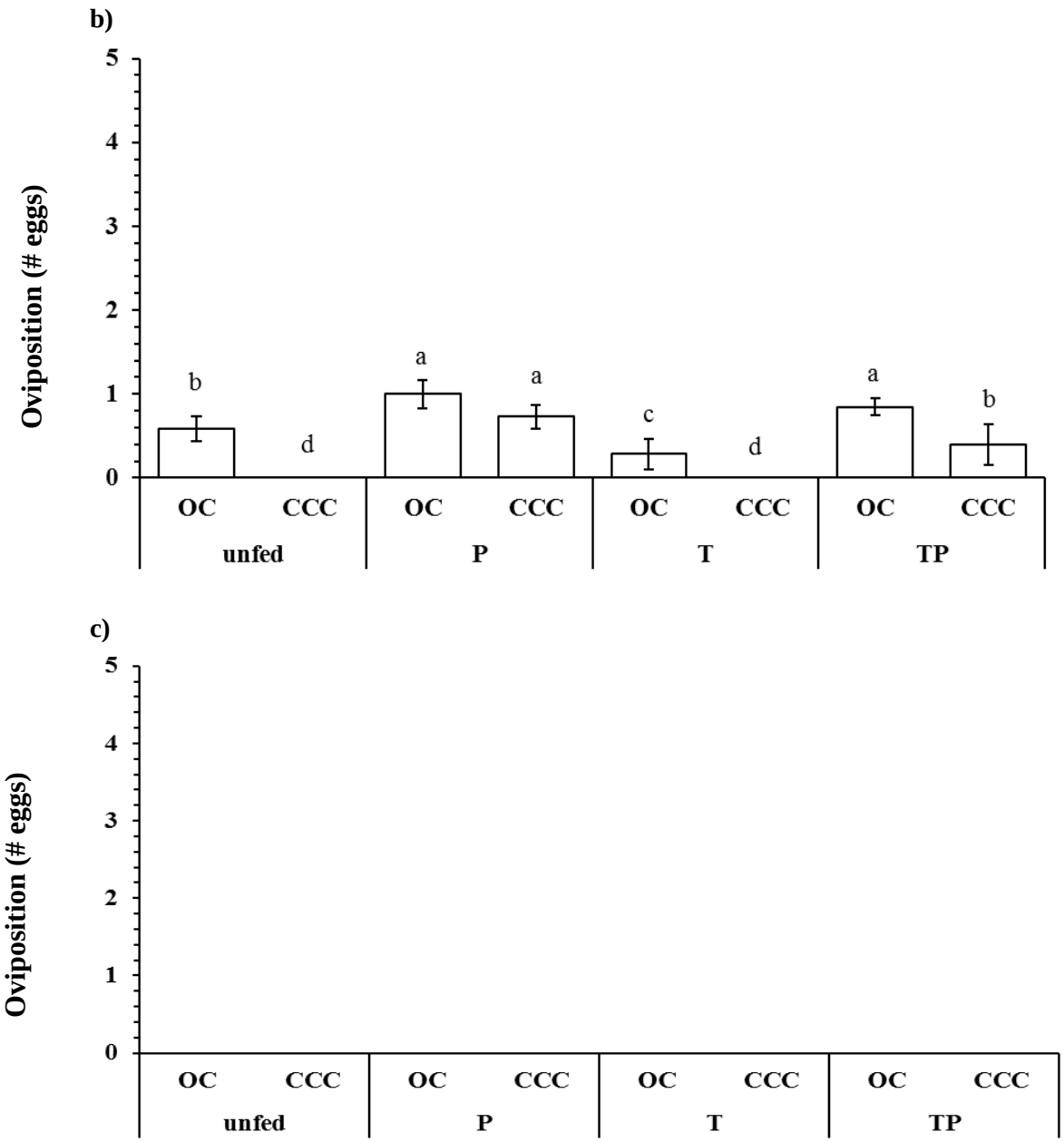
Oviposition of *E. stipulatus* when challenged alone depended on diet and the interaction of diet and climate (Table 6.12). No eggs were observed when no food was available and oviposition rate remained below one egg per female even when it had access to its preferred food source (pollen) (Figure 6.6). The addition of a competing peer changed this situation. While oviposition still depended on diet and climate when competing with *N. californicus* (Table 6.11), the effect of *P. persimilis* became independent of these factors as it resulted in no eggs of *E. stipulatus* left (Figure 6.6). This lack of eggs can be attributed to predation from *P. persimilis* and remarkably, pollen could not change this trend. When competing with *N. californicus*, no eggs were observed at Climate Change conditions for diets not including pollen. However, pollen supply allowed *E. stipulatus* to oviposit and these eggs, at least partly, could survive likely predation from *N. californicus* even at Climate Change conditions. Therefore, pollen addition in this case allowed *E. stipulatus* to better deal with competition from *N. californcius* but not from *P. persimilis*.

Table 6.12. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to oviposition (Poisson) for *E. stipulatus* either alone, in competition with *N. californicus* and *P. persimilis* considering the factors diet, climate, and their interactions as explanatory variables.

Factors	Combination		
	***E. stipulatus* alone**	**+ *N. californicus***	**+ *P. persimilis***
Model	18.28; 7; 0.011	17.22; 7; 0.016	-
Diet	10.17; 3; 0.017	23.71; 2; <0.001	-
Climate	0.23; 1; 0.631	18.93; 1; <0.001	-
Diet * Climate	16.01; 7; 0.007	39.01; 7; <0.001	-

Figure 6.6. Oviposition of *E. stipulatus*, when the phytoseiid was challenged either (a) alone, competing with (b) *N. californicus* (and with (c) *P. persimilis* . Phytoseiids were offered four different diets (unfed, P = pollen, T = *T. urticae* eggs, TP = *T. urticae* eggs + pollen) at optimal (OC; 25 ºC – 70 %RH) and climate change (CCC; 30 ºC – 50 %RH) conditions. For each figure, bars with the same letter are not statistically different (Bonferroni P<0.05).





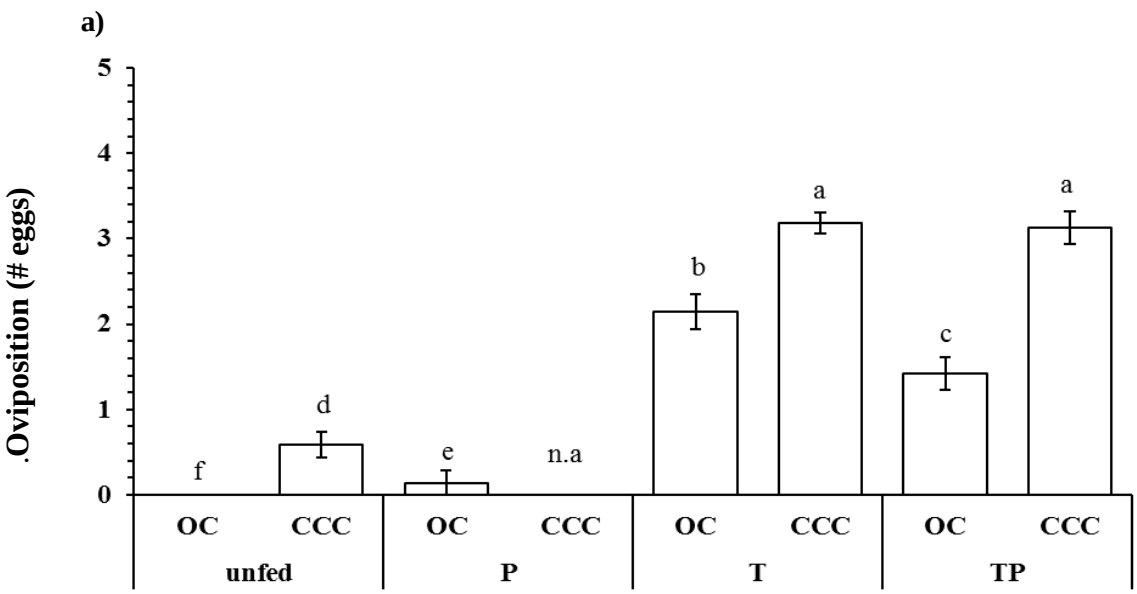
In the case of *N. californicus* diet and climate affected oviposition irrespective of this predator being alone or competing with the other two phytoseiids (Table 6.13). When it was alone, oviposition was not affected by the interaction of those two factors and, same as observed in Chapter 4, more eggs could be recovered at Climate Change conditions and when the diet included *T. urticae* eggs (6.7a). In this situation, the addition of pollen increased oviposition both at optimal and Climate Change conditions. The addition of *E. stipulatus* in the system decreased oviposition (Figure 6.7b) and importantly, the addition of pollen (comparison of *T. urticae* eggs only with mixed diet) did not compensate this decrease at both climatic conditions studied. In the case of *P. persimilis*, oviposition

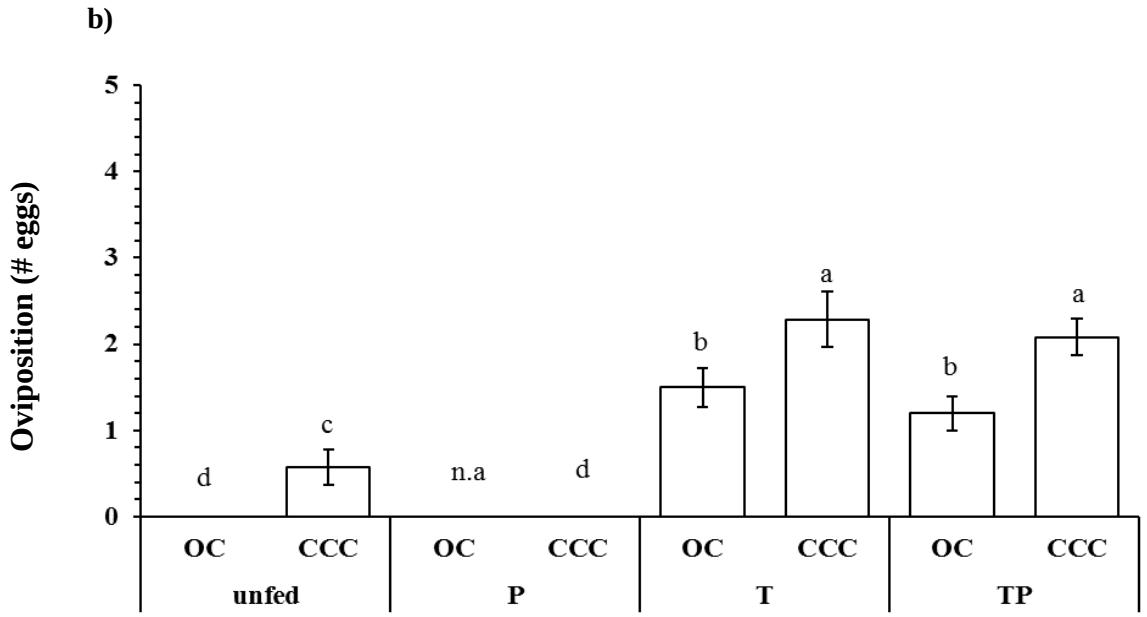
was more drastically reduced and oviposition occurred at Climate Change conditions only when *T. urticae* eggs were available (Figure 6.7c). Same as before, the addition of pollen did not change this result. Therefore, we can conclude the number of *N. californicus* eggs is reduced because of competition with peers, especially with *P. persimilis*, and that this situation cannot be mitigated by the addition of pollen.

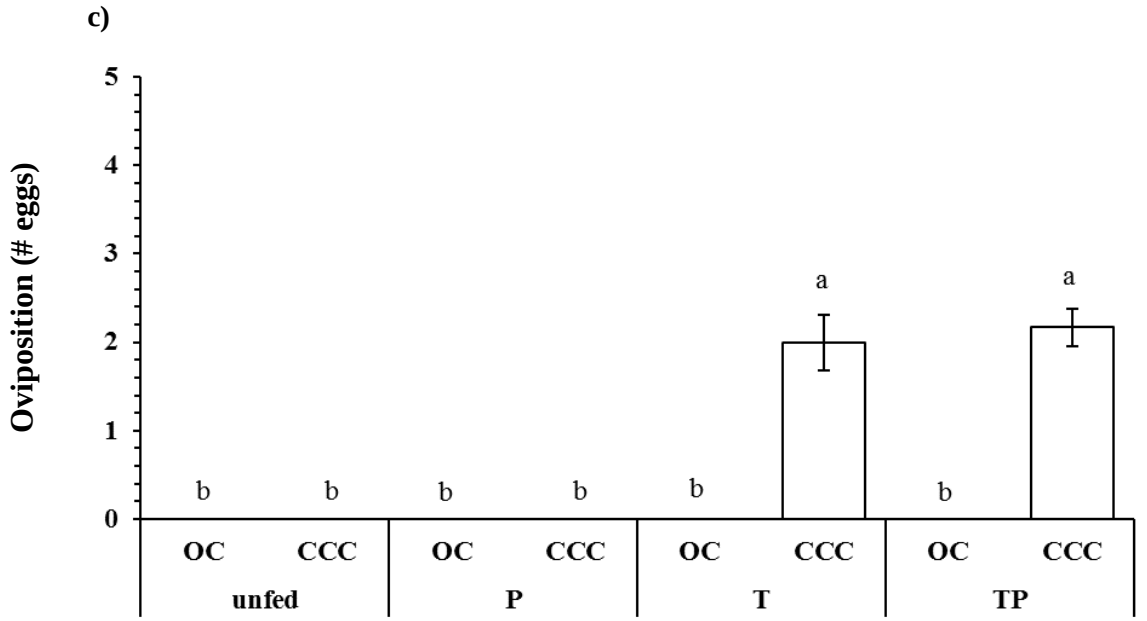
Table 6.12. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to oviposition (Poisson) for *N. californicus* either alone, in competition with *E. stipulatus* and *P. persimilis* considering the factors diet, climate, and their interactions as explanatory variables.

Factors	Combination		
	***N. californicus* alone**	**+ *E. stipulatus***	**+ *P. persimilis***
Model	87.11; 6; < 0.001	13.58; 6; 0.035	74.78; 7; < 0.001
Diet	25.72; 3; < 0.001	45.59; 3; < 0.001	0.78; 3; 0.987
Climate	9.96; 1; 0.002	7.03; 1; 0.008	0.14; 1; 0.994
Diet * Climate	1.18; 1; 0.290	61.1; 6; < 0.001	35.99; 7; < 0.001

Figure 6.7. Oviposition of *N. californicus*, when the phytoseiid was challenged either (a) alone, competing with (b) *E. stipulatus* and with (c) *P. persimilis*. Phytoseiids were offered four different diets (unfed, P = pollen, T = *T. urticae* eggs, TP = *T. urticae* eggs + pollen) at optimal (OC; 25 ºC – 70 %RH) and climate change (CCC; 30 ºC – 50 %RH) conditions. For each figure, bars with the same letter are not statistically different (Bonferroni P<0.05).







Oviposition by *P persimilis* was affected by diet and climate and when competing with *E. stipulatus* by the interaction of these two factors, as well (Table 6.15). When this species was alone, no eggs could be recovered when offered pollen only. However, a few eggs could be observed when no food was available (Figure 6.8a) and in this case, no live mites were found at the end of the assay (Figure 6.3a). Therefore, this may be taken as indicative of the occurrence of cannibalism in the pollen treatment. Oviposition increased for treatments including *T. urticae* eggs in the diet and at Climate

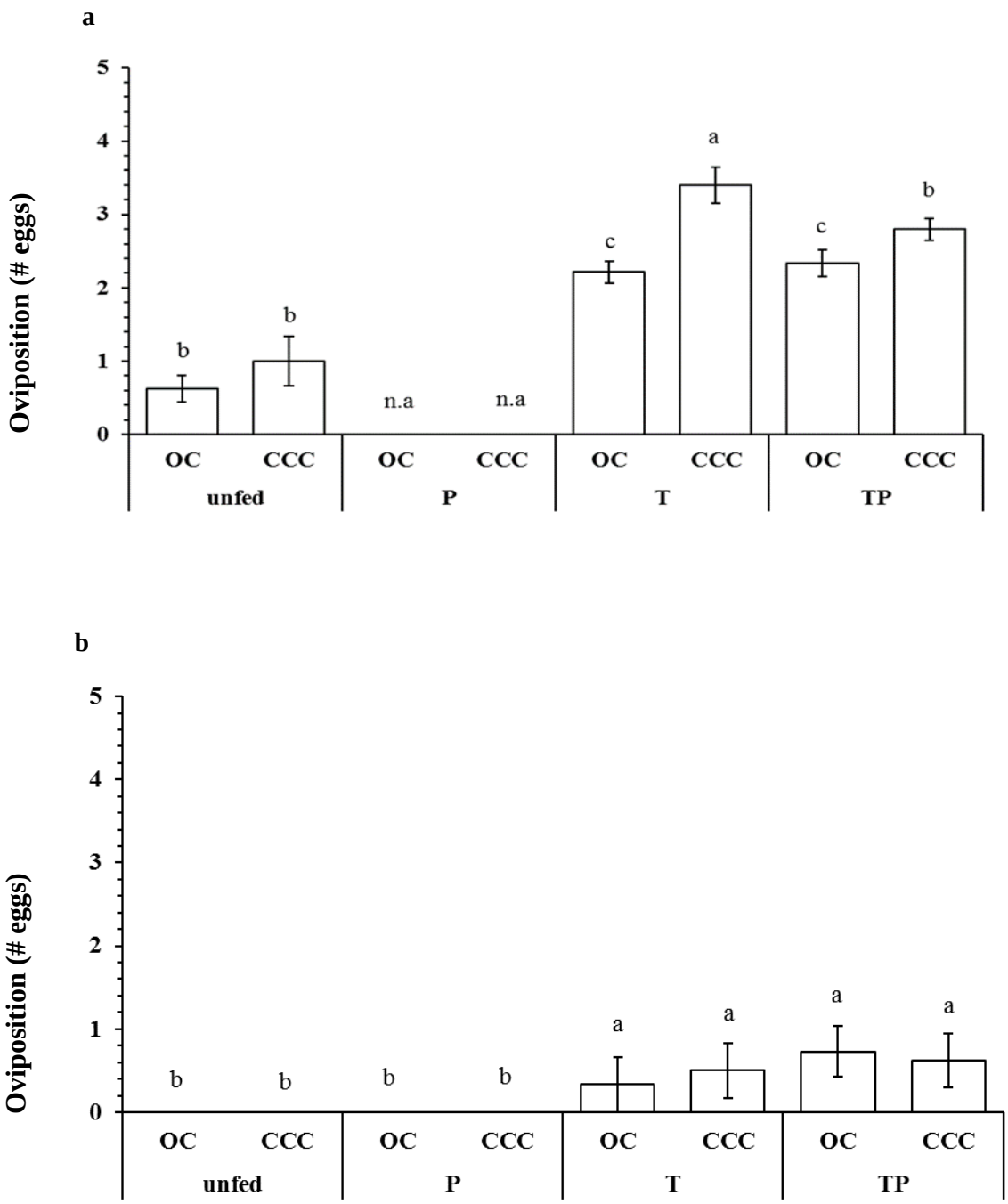
Change conditions (Figure 6.8a). The addition of *N. californicus* in the system (Figure 6.8b) significantly affected the number of eggs recovered. Values below one egg per female were found at both climatic conditions for those diets including *T. urticae* eggs. In this case, the addition of pollen to the system did not change the results. Finally, the addition of *E. stipulatus* resulted in increased oviposition in those treatments including *T. urticae* eggs but, contrary to what happened when competing with *N. californicus*, in this case the addition of pollen increased oviposition at both climatic conditions (Figure 6.8c).

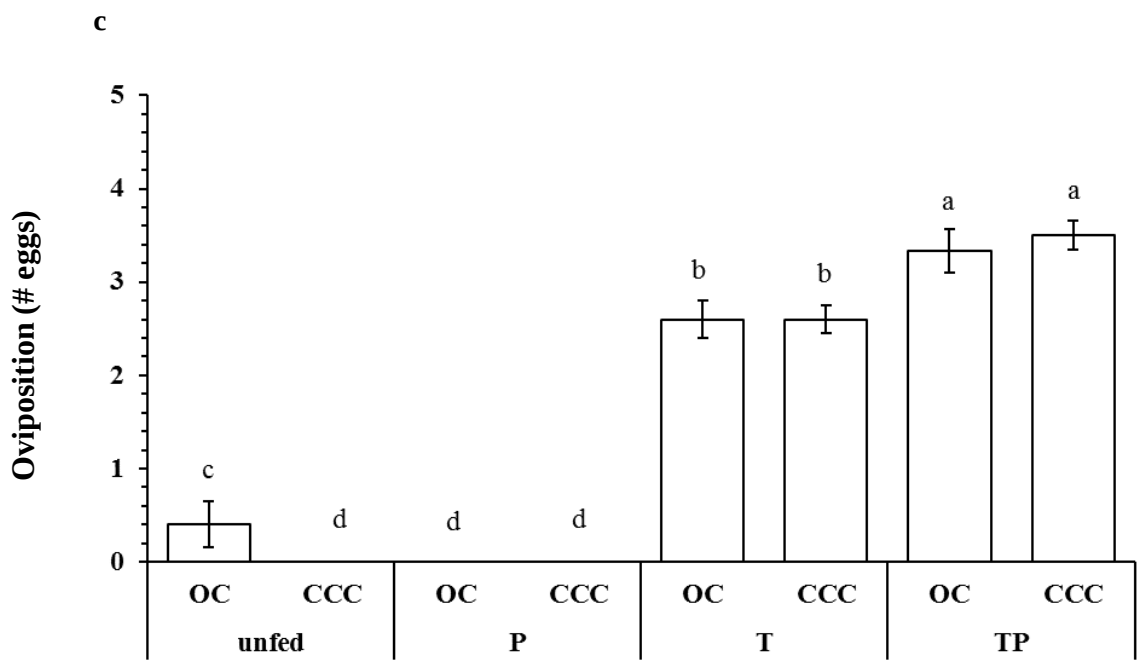
In brief, we have seen that competition has an impact on oviposition of the three phytoseiids considered in this study. In general, it seems that both *P. persimilis* and *N. californicus* take advantage of the eggs deposited by their competitors. However, specific assays targeting this issue would be required to distinguish predation from competition. Only in the combinations including *E. stipulatus*, the addition of pollen may compensate these negative effects even at Climate Change conditions.

Table 6.12. Statistics (Wald X^2; *df*; P-value) of the different GLM models adjusted to oviposition (Poisson) for *P. persimilis* either alone, in competition with *E. stipulatus* and *N. californicus* considering the factors diet, climate, and their interactions as explanatory variables.

Factors	**Combination**		
	***P. persimilis* alone**	**+ *E. stipulatus***	**+ *N. californicus***
Model	18.51; 5; 0.002	39.24; 5; <0.001	35.83; 7; <0.001
Diet	16.74; 2; <0.001	39.24; 5; <0.001	17.64; 3; <0.001
Climate	1.06; 2; 0.304	26.94; 1; <0.001	0.60; 1; 0.807
Diet * Climate	21.43; 5; 0.001	62.01; 5; <0.001	19.01; 7; 0.001

Figure 6.8. Oviposition of *P. persimilis*, when the phytoseiid was challenged either (a) alone, competing with (b) *N. californicus* and with (c) *E. stipulatus*. Phytoseiids were offered four different diets (unfed, P = pollen, T = *T. urticae* eggs, TP = *T. urticae* eggs + pollen) at optimal (OC; 25 ºC – 70 %RH) and climate change (CCC; 30 ºC – 50 %RH) conditions. For each figure, bars with the same letter are not statistically different (Bonferroni P<0.05).





6.3 Discussion

Our results can be taken as evidence of a direct effect of climate change on competition interactions between phytoseiids through survival, predation and oviposition under laboratory conditions. In fact, *E. stipulatus* is the species most at risk from competition from the other two predators and *N. californicus* is the most successful competitor, with *P. persimilis* occupying an intermediate position. The provision of pollen in a mixed diet may help *E. stipulatus* to better survive when competing with *P. persimilis* but this is not the case when the competitor is *N. californicus* (Figure 6.1).

Euseius stipulatus has been considered as a stronger phytoseiid competitor compared with *N. californicus* and *P. persimilis,* in semifield conditions in citrus at 25 ºC and 70 % RH (Abad-Moyano et al. 2010c). However, these authors did not check what could happen at harsher conditions typical of Climate Change and, importantly, used a commercial strain of *P. persimilis,* which may greatly differ from the locally adapted one used in our assays. In line with results from González-Fernández et al. (2009), we have shown that the combination of *N. californicus* and *E. stipulatus* supplied with an

alternative food source would boost phytoseiid populations. Nevertheless, Montserrat et al., (2013a) observed that although pollen supply promoted phytoseiid populations, under extreme abiotic conditions biological control of *Oligonychus perseae* Tuttle, Baker and Abbatiello was disrupted. In our case, the addition of pollen reduced competition measured in terms of predation between *E. stipulatus* and *N. californicus* at Climate Change conditions but not at optimal conditions (Table 6.9). Co-occurrence of alternative food and the target pest may promote predator abundance and pest control through apparent competition (Holt 1977), and this as has been reported in some studies (Messelink et al., 2008; González-Fernández et al. 2009; Nomikou et al. 2010; Aguilar-Fenollosa et al. 2011c; Maoz et al. 2011). Our results prove that these results also depend on environmental conditions and, indeed, the opposite to what we have observed for *N. californicus* and *E. stipulatus* is true for *N. californcius* and *P. persimilis* (Table 6.9). Therefore, the lifestyle of the second predator involved may be key for the final outcome of these interactions.

In agreement with the results of obtained in our field assay (chapter 3), highest predation rates observed in the laboratory for the *E. stipulatus-P. persimilis* combination (at both optimal and Climate Change conditions) correspond to better field regulation of *T. urticae* than the other phytoseiid combinations both in spring and summer (Table 3. 4). Similarly, the disruption of natural regulation of *T. urticae* observed for combinations including *N. californicus* when comparing spring and summer conditions are in agreement with the negative impact on predation found in pairs including *N. californicus*. These results could be explained by the high reproductive performance of the *N. californicus* strain used in our studies (previously characterized by Toyoshima and Hinomoto 2004), which suggests that it has the potential to displace both native populations of the same species and different competing species. Our field results, though, do not support this conclusion as *P. persimilis*, was always the most abundant species recovered at the end of the assays independently of the pair released (Table 3.3). Furthermore, our laboratory results point at both *P. persimilis* and *N. californicus* preying on the eggs of their competitors. Therefore, the occurrence of reciprocal Intraguild predation (Schausberger and Croft 2000; Montserrat et al. 2008, 2012) and cannibalism (Walzer and Schausberger 1999; Schausberger 2003) cannot be excluded and only in the combinations including *E. stipulatus*, the

addition of pollen could partly compensate these negative effects of competition, even at Climate Change conditions.

Our results are not in complete agreement with the hypothesis that generalist phytoseiids are more predisposed to compete than are specialists (Croft et al., 1998; McMurtry and Croft, 1997; Monetti and Croft, 1997). We have proven that this depends on the environmental conditions and the type of specialization (lifestyle) of the competing pair. The results obtained, study exhaustively the effect of climate change conditions in each biological parameter of each phytoseiid predator of *T. urticae* and this new data is important for the future biological control strategies.

In a nutshell, we have shown that community structure could be affected by the abiotic conditions (optimal versus Climate Change) and food availability (pollen supply) and this is achieved through their effect on interspecific relationships including competition, predation, as well as symbiosis, parasitism, mutualism, etc. (Dunson & Travis 1990). The combination of predation and competition between phytoseiid mites can lead to the displacement of a species (Yao and Chant 1989; Zhang and Croft 1995; MacRae and Croft 1997; Schausberger 1997), and the provision of an alterative food item may partly mitigate these effects but may be insufficient to maintain the efficacy of biological control of relevant pestiferous arthropods, including *T. urticae*.

7

Discusión General

En la presente tesis doctoral, se ha demostrado que las condiciones abióticas que emulan las próximas predicciones de cambio climático (IPCC, 2014), han afectado de manera significativa a la mayoría de los parámetros biológicos de los principales depredadores de *T. urticae* en cítricos, *E. stipulatus, N. californicus* y *P. persimilis*, así como al propio fitófago. Existe una elevada cantidad de estudios que describen como la temperatura y la humedad relativa pueden afectar significativamente sobre el ciclo de vida, supervivencia, tasa intrínseca de crecimiento, eclosión, puesta, depredación, etc. de distintos depredadores fitoseideos (Mori and Chant 1966; Ferragut et al. 1987; Morewood 1992; De Courcy Williams et al. 2004; Ahn et al. 2010; El Taj and Jung 2012; Ozawa et al. 2012; Ghazy et al. 2013, 2016; Vangansbeke et al. 2015b; Guzmán et al. 2016a). Nuestros resultados en condiciones de campo, indican que las condiciones de cambio climático pueden alterar la regulación natural de *T. urticae*, como ya han descrito otros autores en sistemas similares al nuestro (Montserrat et al. 2013a, b; Guzmán et al. 2016b). En nuestro experimento de campo, se evaluó la regulación de *T. urticae* ejercida por los tres depredadores fitoseidos estudiados en distintas situaciones (cadena trófica, competencia y efectos prioritarios), durante primavera (aproximación de las condiciones óptimas) y verano (aproximación de las condiciones de cambio climático). A continuación, se decidió bajo condiciones de laboratorio estudiar los módulos de cadena trófica (capitulo 4) y competencia (capitulo 5). Además, también se estudió el efecto que podría tener la adición de polen al sistema en estos dos módulos comunitarios (capítulos 6 y 7). Por lo tanto, bajo condiciones de laboratorio controladas se evaluaron minuciosamente los parámetros biológicos de cada depredador individualmente, con el objetivo de poder entender y explicar con más detalle los resultados obtenidos en campo. Estos modelos biológicos, que simularon las condiciones de cambio climático.

Cuando las sueltas de fitoseidos se realizaron individualmente (cadena trófica) en condiciones de laboratorio se observó como el efecto del cambio climático afectó negativamente a los parámetros biológicos de *E. stipulatus*, disminuyendo la supervivencia, depredación y puesta. Por el contrario, para *N. californicus* y *P. persimilis* la supervivencia y la depredación se mantuvieron al mismo nivel. No obstante, hubo un incremento en la puesta en condiciones de cambio climático (30 ºC – 50 % humedad relativa). Sin embargo, cuando se añadió polen al sistema, se observó una mejora en todos los

parámetros evaluados del omnívoro palinófago *E. stipulatus*, un nulo efecto en *N. californicus* y, curiosamente, un efecto negativo para *P. persimilis*. Otros autores (Musser and Shelton 2003; Spellman et al. 2006; Desneux and O'Neil 2008) también han observado un efecto negativo en el control biológico de plagas, tras la adición de alimento alternativo al sistema. Estos resultados obtenidos bajo condiciones de laboratorio, concuerdan con los que se obtuvieron en condiciones de campo durante el verano, donde se observó una nula eficacia del depredador *E. stipulatus* en la regulación de *T. urticae*. No obstante, tanto *N. californicus*, como *P. persimilis* incrementaron su eficacia significativamente en esta estación (71 y 98 %) en comparación con las que se obtuvieron en primavera (52.5 %, ambas). Palevsky y colaboradores en 2008, observaron resultados similares a los nuestros sobre la misma raza de *N. californicus* que utilizamos en nuestros estudios, procedente de Koppert Biological Systems. Sin embargo, distintos estudios (Skirvin and Fenlon 2003; Walzer et al. 2007) han catalogado a *P. persimilis* como un depredador óptimo de *T. urticae* hasta temperaturas no superiores a 25 ºC. Por el contrario, en nuestros experimentos, tanto en condiciones de laboratorio, como de campo, *P. persimilis* presentó una mejor respuesta biológica en condiciones de cambio climático, incrementando todos sus parámetros biológicos. Estos resultados podrían justificarse, por la utilización de una raza autóctona de *P. persimilis*. Ya que este depredador fue capturado de campo, para la realización de este trabajo, donde puede haber conseguido adaptarse a las temperaturas elevadas y humedades bajas. Existen estudios (Castagnoli and Simoni 1999; Walzer et al. 2007) que han confirmado que la raza de fitoseido utilizada puede tener una influencia directa sobre los distintos parámetros biológicos obtenidos en algunos los distintos depredadores.

Durante la primavera en campo, cuando los depredadores de *T. urticae* se liberaron al mismo tiempo en competencia, las combinaciones *E. stipulatus-N. californicus* y *N. californicus-P. persimilis* mantuvieron su eficacia sobre *T. urticae*, cuando comparamos los resultados en las sueltas individuales, sin aparentes signos de competencia entre estas especies de depredadores. La eficacia mostrada por la combinación formada por *E. stipulatus-P. persimilis* fue significativamente mayor que cuando se evaluaron los depredadores por separado durante la primavera, incrementando significativamente el control de la araña roja. Sin embargo, en verano la eficacia en el control de *T. urticae* disminuyó

dramáticamente en las otras dos combinaciones de depredadores estudiadas (*E. stipulatus-N. californicus* y *P. persimilis-N. californicus*), siendo *N. californicus* la especie más competitiva de las tres. En cambio, cuando se evaluó la combinación formada por *E. stipulatus- P. persimilis* mantuvo su eficacia sobre las poblaciones de *T. urticae* durante el verano. En los experimentos en condiciones de laboratorio, la combinación formada por *E. stipulatus-N. californicus* fue en la que mostró mayor competencia interespecífica. Aunque *N. californicus* demostró preferencia por las condiciones de cambio climático, cuando estuvo en competencia con *E. stipulatus*, sus parámetros biológicos se vieron negativamente afectados. Sin embargo, *E. stipulatus* mejoró sus parámetros biologicos y disminuyó el nivel de competencia ejercido sobre *N. californicus* cuando se añadió polen al sistema, mejorando la supervivencia, la puesta y la depredación de *N. californicus*. En la combinación de *N. californicus-P. persimils*, también se observaron claros signos de competencia, ya que tanto la depredación, como la puesta de ambos fitoseidos se vio afectada negativamente. En estos experimentos, cabe remarcar la dramática disminución de la puesta de ambos fitoseidos, probablemente dada por depredación intragremial recíproca (Schausberger and Croft 2000; Montserrat et al. 2008, 2012) o también por canibalismo (Walzer and Schausberger 1999; Schausberger 2003).

En nuestros experimentos, la adición de polen al sistema no resultó tener ningún tipo de beneficio para *N. californicus* y *P. persimilis*, confirmando la elevada competencia ejercida por el depredador *N. californicus* vista en los experimentos de campo. En cambio, esta estrategia en períodos de escasez de presa, puede contribuir a mantener la abundancia de *E. stipulatus y N. californicus* aunque no el control biológico, debido a la competencia aparente que se genera entre el herbívoro y el alimento alternativo (Montserrat et al. 2013b). La combinación *P. persimilis-E. stipulatus* mantuvo la eficacia sobre la regulación de *T. urticae* en campo durante el verano. Además, tampoco presentó una disminución en los niveles de depredación esperados, en condiciones de cambio climático bajo condiciones de laboratorio. En esta combinación de competencia *E. stipulatus* fue el fitoseido más débil, ya que sus parámetros de supervivencia disminuyeron y la puesta fue nula en combinación con *P. persimilis*, presentando claros signos de depredación. Sin embargo, la adición de polen al sistema

aumentó significativamente la puesta en combinación con este depredador omnívoro, disminuyendo los signos de competencia entre estos dos fitoseidos.

En general, los resultados obtenidos en el presente trabajo servirán a la comunidad científica para mejorar la GIP en *T. urticae* en cítricos situados próximos a la cuenca Mediterránea, en un futuro próximo. Ya que se ha pronosticado un incremento entre 1,5 y 2 ºC en las temperaturas de invierno y verano respectivamente y un decrecimiento alrededor del 5 % en las precipitaciones (Giorgi and Lionello, 2008; Gualdi et al., 2012; Navarra y Tubiana, 2013). De acuerdo con otros autores (O'Neill et al., 2017), es necesaria más investigación para evaluar mejor los distintos riesgos que puede producir estos futuros escenarios. Concretamente, en nuestro sistema se necesita más investigación para poder determinar el comportamiento biológico de esta comunidad depredador-presa-cítricos bajo estas condiciones de cambio climático.

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Conclusiones

1. Las poblaciones de *T. urticae* sobre clementino aumentan de manera superior a lo que cabría esperar al cambiar las condiciones de primavera a verano, y este aumento podría estar mediado por los efectos de las condiciones abióticas típicas de esas estaciones sobre la planta huésped (clementino). Si se considera el verano como proxy de las condiciones de cambio climático, podemos esperar mayor incidencia de este fitófago, en proporciones superiores a las predichas en ensayos de laboratorio a condiciones controladas, donde el destino de *T. urticae* es independiente de la temperatura y la humedad relativa y su puesta aumenta con la temperatura (hasta 35ºC).

2. El control ejercido por los los fitoseidos más abundantes en los cítricos valencianos (*E. stipulatus*, *N. californicus* y *P. persimilis*) sobre *T. urticae* depende de las condiciones climáticas, y estos efectos son distintos en función de la/s especie/s implicadas y de su secuencia en el sistema. En general, parece que el fitoseido *E. stipulatus* es el que más acusa estos cambios, que resultan en un control deficiente de *T. urticae*. Puesto que esta es la especie de fitoseido predominante en los cítricos valencianos (un orden de magnitud más abundante que cualquier otra), estos efectos podrían traducirse tanto en una mejora del control del tetraníquido al quedar la comunidad dominada por fitoseidos considerados más eficaces en el control de *T. urticae* (*N. californicus* y *P. persimilis*), como en un empeoramiento del mismo, por desaparecer esa especie del sistema, que podría jugar un papel de presa alternativa para los otros dos fitoseidos. De hecho, dado que *N. californicus* en combinación con otros fitoseidos suele conllevar un peor control biológico en condiciones de cambio climático, y esta especie parece la mejor adaptada a las condiciones de Cambio Climático, habría que tener en cuenta este segundo escenario en futuras predicciones.

3. Los depredadores *E. stipulatus* y *P. perimilis* fueron la combinación que mejor reguló en condiciones de campo *T. urticae*. Además, bajo condiciones de laboratorio se confirmó la compatibilidad entre estos dos fitoseidos. Sin embargo, *N. californicus* en combinación con cualquiera de estos dos depredadores resultó ejercer un elevado nivel de competencia interespecífica, afectando dramáticamente a los parámetros biológicos de *E. stipulatus* y *P. persimilis*.

4. Como era de esperar, la adición de polen al sistema podría compensar en parte los efectos negativos de las altas temperaturas y las bajas humedades relativas sobre el palinófago *E. stipulatus*. Puesto que el polen no parece favorecer a *N. californicus* y mucho menos a *P. persimilis*, que se muestran como competidores superiores a *E. stipulatus* en condiciones de cambio climático y con acceso a una dieta mixta de polen y huevos de *T. urticae*, parece probable que la adición de polen al sistema lograra mantener en el mismo a *E. stipulatus*, lo que podría ayudar a mantener también el control de *T. urticae* en esas condiciones más extremas.

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