Pre-dispersal seed predation by weevils (*Curculio spp.*): The role of host-specificity, resource availability and environmental factors

Harold Arias-LeClaire

Pre-dispersal seed predation by weevils (*Curculio spp.*)

The role of host-specificity, resource availability and environmental factors

HAROLD
ARIAS-LECLAIRE

Barcelona, 2018

Pre-dispersal seed predation by weevils (*Curculio spp.*): The role of host-specificity, resource availability and environmental factors

Pre-dispersal seed predation by weevils (*Curculio spp.*): The role of host-specificity, resource availability and environmental factors

Harold Arias-LeClaire

Barcelona, 2018

This page intentionally left blank

UNIVERSITAT DE
BARCELONA

Pre-dispersal seed predation by weevils (*Curculio spp*.): The role of host-specificity, resource availability and environmental factors

Harold Arias-LeClaire

Thesis

to be eligible for the Doctor degree and submitted in fulfillment
of the requirements of Academic Doctoral program in Biodiversity

with the supervision and approval of
Dr. Josep Maria Espelta Morral
Dr. Raúl Bonal Andrés

Universitat de Barcelona

Barcelona, 2018

Facultat de Biologia

Department de Biologia Evolutiva, Ecologia i Ciències Ambientals

Pre-dispersal seed predation by weevils (*Curculio spp*.):
The role of host-specificity, resource availability and environmental factors

Research memory presented by Harold Arias-LeClaire for the degree of *Doctor*
at the Universitat de Barcelona Biodiversity Program with the approval of
Dr. Josep Maria Espelta Morral and Dr. Raúl Bonal Andrés thesis advisors
and Dr. Santiago Sabaté Jorba supervisor

Harold Arias-LeClaire
Doctoral student

Dr. Josep Maria Espelta Morral

Thesis advisor
Researcher at Centre de Recerca Ecològica i Aplicacions Forestals

Dr. Raúl Bonal Andrés

Thesis advisor
Researcher at Departamento Ingeniería Medio Agronómico y Forestal
Universidad de Extremadura

Dr. Santiago Sabaté Jorba

Thesis supervisor
Researcher at Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals
Universitat de Barcelona

Barcelona, 2018

Arias-LeClaire, Harold (2018). Pre-dispersal seed predation by weevils (*Curculio spp*.): The role of host-specificity, resource availability and environmental factors

Doctoral thesis. Academic Doctoral program in Biodiversity conducted under the emphasis in Biodiversity conservation and management. Universitat de Barcelona, Barcelona, Catalunya.

Key words: *Corylus avellana*, Granivory, Mediterranean oak forests, plant–animal interactions, *Quercus spp*., rainfall stochasticity, resource pulses.

This thesis has been conducted in the Center for Ecological Research and Forestry Applications (Centre de Recerca Ecològica i Aplicacions Forestals CREAF, UAB Campus de Bellaterra, Barcelona) with the support of NOVFORESTS project and scholarship as work permit by Universidad Estatal a Distancia (UNED, Mercedes de Montes de Oca, Costa Rica).

CREAF

UNED
UNIVERSIDAD ESTATAL A DISTANCIA

This page intentionally left blank

Acknowledgment

“La Vita è Bella”

I place on record, my sincere thanks you to my advisors Dr. Espelta - Tete and Dr. Bonal - Raul, I take this opportunity to express my genuine gratitude for their invaluable help, outstanding patience, and encouragement. I am thankful and indebted to them for sharing remarkable expertise and valuable direction. Their advice and guidance helped me in my research and writing of this thesis. Also, my thanks to Dr. Santiago Sabaté for his instruction and work as supervisor. Thanks to Marcos, Enrique, and Alberto for all their work as coauthors and friendship shared.

I wish to express my thanks to CREAF for providing me access to the laboratory and research facilities. My sincere thanks also go to the projects NOVFORESTS (CGL2012-33398) and BEEMed (014 SGR 913430), particularly to Dr. Joan Pino and the research group, without this support it would not have been possible to conduct this research. Thanks to Marta and Magda for all their help. I thank all the fellows in the lab and field mates, Pau, Iraima, Marina, Guille, Ana, David and Helena for the discussions and for the shared work. My sincere thanks also to Daniel García, partner in field campaigns, coauthor and very good friend. Also, I thank the Biology Faculty (UB) professors for their helpful and inspiring support, Dra. Mª Jose Lopez, Dr. Pedro Moral, Dr. Gustavo Llorente and Dra. Emilia Gutierrez. I place on record, my sense of gratitude to all, who directly or indirectly, have lent their hand in this venture. To Dra. Dolors Vinyoles thank you very much for the support and encouragement in the final stage.

I must express my very profound and heartfelt gratitude to my family for supporting me throughout writing this thesis and my life in general. My sincere thanks to my brother Edgar for the valuable grammatical revision in the final versions of this thesis. Particularly I am deeply grateful to my wife Nancy, supportive partner in this journey as always on so many ventures we have gone through side by side. She is a lighthouse in my life, thank you for providing me with unfailing support and continuous encouragement throughout these years and through the process of researching and writing this thesis. This accomplishment would not have been possible without you. I also thank my Mother and Stepfather for their unceasing help, advice and supporting me spiritually, as well thanks to my Parents in law for constant encouragement and attention.

Thank you, God, for blessing me with what is necessary to complete my Doctoral study!

And finally, last but by no means least, I would like to dedicate this thesis to my kids Oriana, Daniel, David, Josué and Joel but specially on behalf of the loving memory of my brother and my father. Adrián and Papa, I deeply wish you were here to share with you this achievement.

Sincerely *“gracias totales”*
HAL

…Live long and prosper!

Table of contents

Chapter I.

Published Research Articles

Chapter II.

Chapter III.

Chapter IV.

This page intentionally left blank

Summary

Pre-dispersal seed predation (PDSP, hereafter) significantly reduces plant reproductive output. The negative effects on plant fitness have triggered the development of different strategies to protect the seeds and/or reduce the impact of PDSP. These strategies, in turn, have promoted insect trophic specialization by means of morphological, physiological and behavioral adaptations. The close relationship between specialist insects and their host plants conditions insect community assemblage and population dynamics. Specialization would favour multi-species co-occurrence according to the Competitive Exclusion Principle, as different species cannot use the same limited resources. At the same time, specificity makes these species strongly dependent on a particular trophic resource, so that host plant population dynamics may lead to bottom-up forces influencing insect numbers. In this Thesis, I have studied the consequences of trophic specialization on species assemblage and demography in the most prevalent pre-dispersal predators of oak *Quercus* spp., chestnuts *Castanea sativa* and hazelnut *Corylus avellana* seeds, namely the weevils of the genus *Curculio* (Coleoptera: Curculionidae). Using DNA barcoding I could identify larvae infesting the seeds to the species level and hence assess resource partitioning among *Curculio* spp. in oak-hazelnut mixed forests. These forests were distributed along a latitudinal gradient in which the degree of overlap in the timing of seeding between the two species differed. The results showed that there was a strict host-based segregation, as the species found in hazelnuts was never recorded on oak acorns and vice versa. Contrary to other studies, segregation of seed parasites was not driven by seed size, as the seeds of both plants were large enough to host the larvae of any species. Rather, co-existence was more likely modulated by the combination of time partitioning, and probably by dissimilarities in dispersal-dormancy strategies among weevils. The timing of oogenesis differs among *Curculio* spp. and does the timing of seeding between oaks and hazelnuts. Early maturing hazelnuts are thus exploited only by *Curculio nucum*, as its eggs mature earlier too. Such specialization on a patchily distributed host plant conditioned its population genetics, as gene-flow between populations showed restrictions undetected in the other *Curculio* spp. that fed on the widespread oaks. Regarding the bottom-up effects of food availability on insect numbers we assessed that, as expected, irregular seed crops (masting) conditions weevil population dynamics and certainly help reducing acorn predation in Mediterranean oaks. However, we found that the effects of rainfall stochasticity on the success of weevil emergence from the soil (i.e. rain is needed to soften the soil) contributed to decrease seed predation in a similar magnitude to masting. The present Thesis stresses the need of introducing the time/phenology component (i. e. egg maturation, timing of seeding) to assess the mechanisms underlying host plant-specialist insect associations. Also, it shows that, despite their specificity, other environmental variables apart from food availability condition weevil numbers. This result must be considered in further studies on the significance of oak masting as a strategy to reduce pre-dispersal seed predation. Lastly, the results provide an insight into the potential consequences of Global Change on the communities of these specialist insects linked to oaks. The populations of a narrow specialist like the hazelnut feeding *C. nucum* will be very vulnerable to forest fragmentation, which will reduce more severely inter-population gene-flow and lead to population bottlenecks. In turn, climate change (e.g. temperature rising, rainfall decrease) could disrupt the matching phenologies of insects and their host plants and reduce insect population size.

Resumen

La depredación de semillas predispersión (PDSP, de aquí en adelante) reduce significativamente el desempeño reproductivo de las plantas. Los efectos negativos han desencadenado el desarrollo de diferentes estrategias para proteger las semillas y/o reducir el impacto de PDSP. Estas estrategias, han promovido la especialización trófica de insectos a través de adaptaciones morfológicas, fisiológicas y de comportamiento. La estrecha relación entre los insectos especialistas y sus plantas hospedantes condiciona el ensamblaje de las comunidades de insectos y la dinámica poblacional. La especialización favorecería la coexistencia de múltiples especies de acuerdo con el Principio de Exclusión Competitiva, ya que estas no pueden usar los mismos recursos limitados. Al mismo tiempo, la especificidad hace que estas especies dependan fuertemente de un recurso trófico particular, de modo que la dinámica poblacional de plantas hospedadoras puede conllevar a que las fuerzas “bottom-up” influyan en el número de insectos. En esta Tesis, he estudiado las consecuencias de la especialización trófica en el ensamblaje de especies y la demografía en los depredadores pre-dispersión más prevalentes de *Quercus spp*., castañas *Castanea sativa* y avellanas *Corylus avellana*, principalmente los gorgojos del género *Curculio* (Coleoptera: Curculionidae). Usando técnicas de secuenciación de ADN pude identificar las larvas que parasitan las semillas a nivel de especie y, por lo tanto, evaluar la segregación de recursos entre especies de *Curculio spp*. en bosques mixtos de roble y avellana. Estos bosques se distribuyeron a lo largo de un gradiente latitudinal en el cual el grado de superposición de su distribución espacial difería entre las dos especies hospederas. Los resultados mostraron que hubo una estricta segregación basada en el hospedador, ya que la especie encontrada en avellanas nunca se registró en bellotas de roble y viceversa. Contrariamente a otros estudios, la segregación de los parásitos de las semillas no se debió al tamaño de la semilla, ya que las semillas de ambas plantas eran lo suficientemente grandes como para albergar las larvas de cualquier especie. Por el contrario, la coexistencia fue modulado con mayor probabilidad por la combinación de la partición de tiempo, y las diferencias en las estrategias de latencia y dispersión entre los gorgojos. El periodo de oogénesis de *Curculio spp* difiere con el periodo producción de semillas en robles y avellanas. Las avellanas de maduración temprana son explotadas solo por *Curculio nucum*, ya que sus huevos también maduran antes. Dicha especialización en una planta hospedera distribuida en forma dispersa condicionó su genética poblacional, ya que el flujo genético entre poblaciones mostró restricciones no detectadas en las otras especies de *Curculio spp*. que se alimentaban de los robles con distribución continua. Con respecto a los efectos “bottom-up” de la disponibilidad de alimentos sobre el número de insectos, evaluamos que, como era de esperar, la producción irregular de semillas (masting) condicionan la dinámica poblacional de los gorgojos y ciertamente contribuyen a reducir la depredación de las bellotas en los robles del Mediterráneo. Sin embargo, encontramos que los efectos de la estocasticidad de las lluvias sobre el éxito de la emergencia del gorgojo desde el suelo (la lluvia es necesaria para ablandar el suelo) contribuyeron a disminuir la depredación de semillas en una magnitud similar a la del masting. La presente Tesis enfatiza la necesidad de introducir el componente tiempo/fenología (es decir, la maduración del óvulo, el momento producción de semillas) para evaluar los mecanismos que subyacen a las asociaciones de insectos especialistas en plantas hospederas. Además, muestra que, a pesar de su especificidad, otras variables ambientales aparte de la disponibilidad de alimentos condicionan el número de gorgojos. Este resultado debe considerarse en estudios posteriores sobre la importancia de la producción irregular de semillas de roble como una estrategia para reducir la depredación de semillas pre-dispersión. Por último, los resultados proporcionan una idea de las posibles consecuencias del cambio global en las comunidades de estos insectos especializados vinculados a los robles. Las poblaciones de un especialista, como *C. nucum* que se alimenta de la avellana, será muy vulnerables a la fragmentación de los bosques, lo que reducirá el flujo genético entre las poblaciones y provocará cuellos de botella en la población. A su vez, el cambio climático (por ejemplo, aumento de la temperatura, disminución de las precipitaciones) podría alterar las coincidentes fenologías de los insectos y sus plantas hospederas y reducir el tamaño de la población de insectos.

General introduction

Interactions between plants and insects are amazingly diverse and complex and reach over numerous environments.

This page intentionally left blank

Plant-insect interactions: predispersal seed predation.

Granivory (i.e. seed predation) significantly reduces plant fitness by decreasing plant effective reproductive output and natural recruitment (Janzen 1971, Hulme & Benkman 2009, Schoonhoven *et al.* 2005, Bonal *et al.* 2007, Espelta *et al.* 2009a, Boivin *et al.* 2017). Insects are major seed consumers and in many cases prey on seeds still attached to the mother plant, what is called pre-dispersal seed predation (PDSP hereafter) (Crawley 2000, 2014, Ramírez & Traveset 2010, Beckman & Muller-Landau 2011). Seeds constitute a rich source of concentrated proteins, oils and minerals, with generally higher caloric and nutrient values than other plant tissues (Jordano 2014). Moreover, they represent a resource commonly aggregated in space with high local abundance, despite only accessible for a transitory period due to usual seasonal patterns of plant reproduction (Boivin *et al.* 2017). Insects may feed on seeds at all life stages, but they commonly do it during the larval period. Owing to their rich nutrient composition and the presence of a protective coat, seeds are a suitable place for laying eggs and thus the larvae of many granivorous insects complete their development within them, consuming the nutrient-rich kernel (Fenner & Thompson 2005, Ramírez & Traveset 2010, Schowalter 2016).

Plants have evolved a series of strategies to protect their seeds from predators or at least reduce the negative consequences of granivory on their fitness. Resistance mechanisms include physical barriers that prevent consumption (Janzen 1971, Kelrick *et al.* 1986, Hulme and Benkman 2009, Travers-Martin & Müller 2008). Defenses may also be chemical, such as toxic or deterrent compounds (Janzen 1971, Hulme & Benkman 2009, Kergoat *et al.* 2005, Schowalter 2016). Physical and chemical defenses are effective but costly, as they require large investments of resources (Schoonhoven *et al.* 2005, Agrawal & Fishbein 2006, Crawley 2014), nonetheless, they are not the only mechanisms to reduce the negative consequences of PDSP. Some species try to "escape" by producing seeds when predators are either absent or in low numbers (Pilson 2000, Elzinga *et al.* 2007, Boivin *et al.* 2017). Other plants exhibit a "masting" behavior (sensu Janzen 1971) combining the synchronous and erratic production of large and nil seed crops to satiate and starve predators (Espelta *et al.* 2008) or abort part of their seeds to trap predators and reduce their population size (Peguero *et al.* 2014). Indeed, these mechanisms do not prevent predation, but they help to "tolerate" it by assuring that a fraction of the seed crop will survive and be dispersed (Crawley 1997, 2014). Tolerance may also occur at the seed level, as larger seeds may better satiate predators saving a certain portion of cotyledons and still producing viable seedlings (Sousa *et al.* 2003, Bonal *et al.* 2007, Espelta *et al.* 2009a). Interestingly these strategies to reduce the negative impact of seed predation may co-occur in the same species varying in their importance across environmental gradients (e.g. seed abortion vs. masting through an altitude gradient in Peguero *et al.* 2014).

Trophic specificity and assembly of a granivorous insect community: Resource or time partitioning?

Plant strategies to avoid predation have triggered the development of morphological, physiological and behavioral adaptations by phytophagous insects and promoted their trophic specialization (Strauss & Zangerl, 2009, Yguel *et al.*, 2011). This defense-counter defense dynamics (also called arms race) has promoted outstanding examples of local adaptations (Toju & Sota 2005, 2006) and coevolution (Jordano *et al.* 2003, Herrera & Pellmyr 2009, Schowalter 2016, Cheplick 2015). The small-size, short life span and rapid reproductive rate of insects promotes a tight association with their host plants (Strauss & Zangerl, 2009, Boivin *et al.* 2017). The complexity of some plant defensive strategies makes that trophic specialists are either the only species able to feed on the host plants or at least more efficient than generalists. Insects are in fact a paradigmatic example of trophic specialization, going from those that feed on many plants, plants of the same family or genus to extreme specialists that exploit only one species (reviewed in Barrett & Heil, 2012).

Trophic specificity rules the life histories of specialist insects to a great extent, conditioning community assemblage and population dynamics. Specialization would favour inter-specific partitioning of the trophic resources among species, guaranteeing that each species acquires a portion and ensuring their co-existence. According to the Competitive Exclusion Principle (Hardin, 1960), natural selection would maintain specialization because different species cannot use the same limited resources indefinitely. However, more recently the Co-existence

Theory (Chesson, 2000 a,b) has proposed mechanisms to explain that competing species can sustainably maintain an overlapping trophic niche, although it does not deny that specialization favours niche partitioning.

Regarding population dynamics, specificity enhances the specialist fitness when feeding on the preferred food source. However, it also reduces its trophic versatility, as many species cannot feed on alternative plants when the preferred host is not available (Espelta *et al.* 2009a, Bonal *et al.* 2016). Hence, trophic specialization implies a strong influence of the host plant over insect populations (Ylioja *et al.* 1999). Plant population dynamics (i.e. the spatial-temporal variation of resource for the insects) may thus lead to bottom-up forces influencing insect numbers by means of stochastic demographic variability (Lande 1988, Kolb *et al.* 2007). Such effects may ultimately affect insect population genetics, with small and isolated populations of specialists being more prone to bottlenecks and genetic drift (Gaete-Eastman *et al.* 2004) compared with generalists with no host restrictions (Newman & Pilson 1997, Peled *et al.* 2016).

In this Thesis we have assessed how trophic specificity and environmental conditions affects species assemblage and population dynamics in one of the most prevalent groups of specialist seed feeding insects, namely *Curculio spp*. Weevils of the genus *Curculio* (Coleoptera: Curculionidae) are the most common pre-dispersal predators of many Fagaceae species, such as oaks (*Quercus sp*.) (Bonal *et al.* 2007, Peguero *et al.* 2017, Yu *et al.* 2015), hazels (*Corylus avellana*) (Ali Niazee 1998, Saruhan & Tuncer 2000, Guidone *et al.* 2007) and sweet chestnuts (*Castanea sativa*) (Desouhant *et al.* 2000). After eggs are laid in the seeds, the weevil larvae complete their growth within a single seed by feeding on the cotyledons (Desouhant *et al.* 2000, Bonal *et al.* 2007, Bonal & Muñoz 2009). Small seed size constrains larval growth and fitness prospects (Desouhant *et al.* 2000, Bonal & Muñoz 2008, Espelta *et al.* 2009b), hence, oviposition takes place only when the seeds have reached a minimum size (Espelta *et al.* 2009a, Bonal *et al.* 2011). Adult phenology differs depending on the species, for instance, in some species adults emerge from their underground refuges in spring after diapause, whereas in others they do not pop out until late summer (Bonal *et al.* 2011). After emerging and mating, females drill an oviposition puncture through the seed coat with their specialized rostrum while the seeds are still attached to the branch (Desouhant *et al.* 2000, Bonal *et al.* 2007) and they usually lay one egg per seed.

Different studies have analyzed the role of morphological diversification, phenology and life cycles on niche partitioning among species in this striking group of insects. Regarding morphological diversification, the closely related *Curculio camelliae* constitutes probably one the most outstanding examples of trophic adaptation. In this species, the geographical variability of the seed coat thickness of its host plant (*Camellia japonica*) has promoted a disproportionate rostrum enlargement in certain weevil populations (Toju & Sota 2005, 2006, Toju 2008, Toju *et al.* 2011). By contrast, this has not occurred in weevils predating upon acorns despite the large variability in the size of these seeds (Espelta *et al.* 2009a, Bonal *et al.* 2016).

Macroevolutionary comparative studies have shown that shifts to exploit larger seeds could promote a body size increase, as a larger seed would allow a larger larval size with the subsequent fitness advantages (Desouhant *et al.* 2000, Hughes & Vogler 2004, Bonal *et al.* 2011). However, even though rostrum length differs among species predating upon oaks, it does proportionately to the rest of the body (Bonal *et al.* 2011). Interestingly, contrary to Camellia seeds, coat thickness does not vary as much in acorns, which probably explains the lack of such a disproportionate rostrum development. By means of applying molecular techniques, the role of body size on trophic niche partitioning has been assessed in natural conditions. Genetic analyses (i. e. DNA barcoding) allow the unequivocal identification of larvae, something generally impossible based on morphological traits (see Pinzón-Navarro *et al.* 2010). This technique allows to know the plant host and the different seed sizes exploited by each weevil species. Doing so, it has been found that the effect of niche partitioning according to body size is mediated by seed size. In Mediterranean oak forests with different sized *Curculio spp.* (e.g. the smaller *Curculio glandium* and the bigger *C. elephas*), small-sized ones can exploit small acorns that do not allow the development of larger species larvae (Espelta *et al.* 2009a, Bonal *et al.* 2011). In America, such a niche partition also exists, being more marked in tropical weevils than in those that inhabit temperate regions (Peguero *et al.* 2017).

Niche partitioning could also be independent of body/seed size relationships but linked to digestive adaptations to feed on certain plant species. This might be the case observed when larvae of a particular weevil species grow unrestricted within large acorns of different oak species, but they attain a different final mass (Muñoz *et al.* 2014, Peguero *et al.* 2017). The mechanisms underlying such differences remain unknown, yet some studies have shown that the species composition of the endosymbiont bacteria linked to *Curculio spp*. differs according to the preferred host plant (Toju & Fukuatsu 2011). However, even considering that a certain degree of specificity at this level could occur, it is far from being the norm.

Large-scale studies, including different species of *Curculio* and many species of oaks, have found no differences in larval performance among *Quercus spp*. (Bonal *et al.* 2016). In temperate forests of Europe and western North America, all weevil species may feed on any *Quercus spp*., with no strict specialists (Govindan *et al.* 2012, Bonal *et al.* 2018 in press). The few reports from tropical oak forests show specificity at the subgenus level: weevils that feed on white oak (*Leucobalanus*) very rarely prey on red oak (*Erithrobalanus*) acorns, and vice versa (Peguero *et al.* 2017). However, differences at the subgenus level coincide with differences also in seed sizes, making it difficult to identify the ultimate cause of such trophic segregation.

Besides niche partitioning based on host specificity and/or acorn size, other studies have pointed at time partitioning as the mechanism allowing the co-existence of these species sharing a common resource (sensu Chesson 2000a,b). Such time partitioning could occur within the same year and between years. Time partitioning within the same year would result from inter-specific differences in the timing of reproduction. This will depend not only on adult emergence phenology, but also on the maturation of eggs. In some species, females have mature eggs at the onset of their adult life (proovigenic), whereas in others it requires a certain period (synovigenic) (Jervis *et al.* 2008, Richard & Casas 2009, Péllison *et al*. 2012). Within a given year, early reproducing species would infest most available seeds in lean years, whereas late reproducing ones would outcompete them in highly productive years in which their larvae would grow larger within full sized acorns (Espelta *et al.* 2009a, Venner *et al.* 2011, Pélisson *et al.* 2013a, b). Time partitioning between years would be related to inter-specific differences among weevils in diapause length (i.e. from one to four years depending on the *Curculio* species), combined with the stochasticity of seed crops (Espelta *et al.* 2008). Interestingly, variable diapause will promote that unpredictable large seed crops will not always benefit the same species but promote their co-existence (Venner *et al.* 2011).

Weevil population dynamics and seed predation extent: A matter of resource availability or environmental stochasticity?

Ecomorphological adaptations and trophic specificity may allow the co-existence of different *Curculio* species and their ability to intensively predate upon seeds. Many Fagaceae trees including oaks show synchronous and irregular patterns of seed production across years, so-called masting (Kelly & Sork 2002). Masting is expected to impact on the population dynamics of insects like weevils, with specialized seed-feeding habits, limited mobility, and short life span encompassed within the seeding season (Shibata *et al.* 1998, Kelly *et al.* 2000, Maeto & Ozaki 2003). Although the ultimate causes underlying masting may be multiple (Kelly 1994, Kelly *et al.* 2000, Yu *et al.* 2003, Koenig & Knops 2005, Espelta *et al.* 2008, Fukumoto and Kajimura 2011, Archibald *et al.* 2012, Moreira *et al.* 2016), one of the hypotheses, namely the most supported is the predator satiation hypothesis (PSH hereon) that proposes precisely that masting may be a reproductive mechanism to control the seed predator populations (Espelta *et al.* 2008, 2009b, Bogziewicz *et al.* 2018).

Satake *et al.* (2004) proposed that predator satiation at the population level by means of irregular large and nil seed crops would occur through a combination of “functional satiation” and “numerical satiation”. Functional satiation will occur as not all seeds can be consumed during an unpredictable massive crop as it surpasses the feeding capacity of predators (Ims 1990), and numerical satiation takes place as predator populations collapse during poor crop years, so the existing number of predators may be too low to consume many seeds (Crawley & Long 1995). However, although specialist insect populations are clearly conditioned by seed availability, such principle of PSH might be too simplistic, as changes in population size in insects have been also observed to be directly related to meteorological conditions or to disturbances (Robinet & Roques 2010, Schowalter 2016).

In the specific case of *Curculio* weevils, adult emergence failure due to the lack of rainfall has been reported both in sweet chestnut *Castanea sativa* orchards (Menu 1993) and oak *Quercus spp*. stands (Bonal *et al.* 2010, 2015). Adults need to dig up their way to the surface from their underground refuges after diapause, and they may die without achieving it if the soil is too hard owing to the lack of precipitation. Thus, an independent effect of meteorology on insect populations might disrupt the crop size/infestation rates relationship. Specifically, even if there is a bumper crop of seeds ready to be consumed, negative environmental conditions may impede adult weevils to predate upon it. Unfortunately, few studies have simultaneously tested whether environmental factors influencing masting (e.g. temperature in Schauber *et al.* 2002, Kelly *et al.* 2013; precipitation in Pérez-Ramos *et al.* 2010, Fernández-Martínez *et al.* 2015 or even "weather packages" in Fernández-Martínez *et al.* 2017) may also be directly involved in controlling seed predators and thus contribute to safeguard part of the seed crop. This is a plausible hypothesis, as we know that rainfall amount and its seasonal distribution may influence oviposition from granivorous insects (Bonal *et al.* 2010) by affecting the number of adults emerging from the soil (Schraer *et al.* 1998), or even altering their sex ratio (Bonal *et al.* 2015).

This page intentionally left blank

Research Objectives

The main goal of this Thesis is to assess how trophic specificity affects species assemblage in the specialist *Curculio spp*. weevils and to what extent their population dynamics is driven by resource pulses (seed availability) or environmental stochasticity (rainfall). The use of molecular techniques has allowed identifying larvae to the species level and to establish unequivocal host plant-weevil species relationships. In addition, it has allowed for the first time to assess the genetic signature left by the degree of specialization of some species in this group of insects considering the spatial distribution of their hosts (i.e. continuous or fragmented). Moreover, by means of the analyses of midterm databases I have been able to disentangle the contribution of masting (i.e. extreme resource variability) and environmental stochasticity (seasonal pattern of rainfall distribution) in the dynamics and predation intensity of these granivorous insects. These aims have been addressed in two studies (chapters) already published in international journals.

-In the first study, host specificity among *Curculio spp*. have been assessed in a system with three potential host plants: hazelnut tree *Corylus avellana* and two oaks *Quercus ilex* and *Quercus humilis* by means of DNA analyses. The three hosts species differ in the size of their seeds and in the timing of their maturation. As an additional novelty, specificity patterns have been analyzed along a geographical gradient, in which the degree of overlap among host plants in the timing of seeding and seed size may differ owing to climate variation (i.e. higher overlap of seeding phenology in Norther sites owing to a shorter vegetative season and more extended seed production timing in the south). In this context I have assessed whether host specificity and weevil co-existence is a matter of resource or time partitioning: i.e. seed size vs. egg maturation in females. Lastly, I have assessed whether narrow specialist feeding on a host with a patchier distribution is more likely to suffer population bottlenecks and gene flow restrictions.

-In the second study, we have tried to disentangle whether weevil population dynamics and its impact on seed predation is mostly controlled by variability in the resource (seed production by hosts) or by the direct impact of environmental conditions (i.e. rainfall amount and distribution) on the abundance of adult weevils. By contrast, some previous studies have highlighted the paramount influence of the extreme interannual variability in seed production (masting) to reduce the size of the weevil populations and their damage to crops. On the other hand, others have emphasized the role of stochastic adverse events (e.g. drought) in blocking adult emergence form the soil after diapause and thus in reducing population numbers. Yet, this is the first time that both factors are jointly analyzed.

In addition to shedding light on how trophic specificity, resource availability and environmental stochasticity may affect species assemblage and population performance in communities of *Curculio spp*., my thesis may help to understand how increasing habitat loss and fragmentation owing to land-use change and new climatic scenarios arising from climate change may impact on these insects and this plant-animal interaction.

General introduction references

Agrawal, A. A., & Fishbein, M. (2006). Plant defense syndromes. Ecology, 87(sp7), S132-S149.

AliNiazee, M. T. (1998). Ecology and management of hazelnut pests. Annual Review of Entomology, 43(1), 395-419.

Archibald, D. W., McAdcuram, A. G., Boutin, S., Fletcher, Q. E., & Humphries, M. M. (2012). Within-season synchrony of a masting conifer enhances seed escape. The American Naturalist, 179(4), 536-544.

Barrett, L. G., & Heil, M. (2012). Unifying concepts and mechanisms in the specificity of plant–enemy interactions. Trends in plant science, 17(5), 282-292.

Beckman, N. G., & Muller-Landau, H. C. (2011). Linking fruit traits to variation in predispersal vertebrate seed predation, insect seed predation, and pathogen attack. Ecology, 92(11), 2131-2140.

Bogdziewicz, M., Espelta, J. M., Muñoz, A., Aparicio, J. M., & Bonal, R. (2018). Effectiveness of predator satiation in masting oaks is negatively affected by conspecific density. Oecologia, 186(4), 983-993.

Boivin, T., Doublet, V., & Candau, J. N. (2017). The ecology of predispersal insect herbivory on tree reproductive structures in natural forest ecosystems. Insect science, 00, 1 -17. DOI 10.1111/1744-7917.12549.

Bonal, R., & Muñoz, A. (2008). Seed growth suppression constrains the growth of seed parasites: Premature acorn abscission reduces *Curculio elephas* larval size. Ecological Entomology 33(1), 31–6.

Bonal, R., Muñoz, A. (2009). Seed weevils living on the edge: Pressures and conflicts over body size in the endoparasitic *Curculio* larvae. Ecological Entomology 34(3), 304–9.

Bonal, R., Munoz, A., & Díaz, M. (2007). Satiation of predispersal seed predators: the importance of considering both plant and seed levels. Evolutionary Ecology, 21(3), 367-380.

Bonal, R., Muñoz, A., & Espelta, J.M. (2010). Mismatch between the timing of oviposition and the seasonal optimum. The stochastic phenology of Mediterranean acorn weevils. Ecological Entomology, 35(3), 270-278.

Bonal, R., Espelta, J. M., & Vogler, A. P. (2011). Complex selection on life-history traits and the maintenance of variation in exaggerated rostrum length in acorn weevils. Oecologia, 167(4), 1053-1061.

Bonal, R., Hernández, M., Espelta, J. M., Muñoz, A., & Aparicio, J. M. (2015). Unexpected consequences of a drier world: evidence that delay in late summer rains biases the population sex ratio of an insect. Royal Society open science, 2(9), 150198.

Bonal, R., Espelta, J. M., Muñoz, A., Ortego, J., Aparicio, J. M., Gaddis, K., & Sork, V. L. (2016). Diversity in insect seed parasite guilds at large geographical scale: the roles of host specificity and spatial distance. Journal of biogeography, 43(8), 1620-1630.

Bonal, R., Muñoz, A., Aparicio, J.M., Santoro, M., & Espelta, J.M. (2018). Filogeografía, factores históricos y especificidad parásito-hospedador: estudio comparativo de las comunidades de insectos depredadores de bellotas (*Curculio spp.*) en la Península ibérica y California. Revista Ecosistemas (in press).

Cheplick, G. P. (2015). Approaches to plant evolutionary ecology. Oxford University Press, USA. 312 pp.

Chesson, P. (2000a). General theory of competitive coexistence in spatially-varying environments. Theoretical population biology, 58(3), 211-237.

Chesson, P. (2000b). Mechanisms of maintenance of species diversity. Annual review of Ecology and Systematics, 31(1), 343-366.

Crawley, M. J. (1997). Plant–herbivore dynamics. (13), 401–474. In Crawley M. J. Editor. Plant Ecology. 2nd ed. Cambridge, MA: Blackwell Scientific 717pp.

Crawley, M. J. (2000). Seed predators and plant population dynamics. (7), 167-182. In Fenner, M., Editor. Seeds: the ecology of regeneration in plant communities. 2nd ed. CABI London UK. 410 pp.

Crawley, M.J. (2014). Seed predators and plant population dynamics (3), 94–110. In Gallagher, R.S. Editor. Seeds: The Ecology of Regeneration in Plant Communities. 3er ed. CABI London, UK. 320 pp.

Crawley, M., & Long, C. R. (1995). Alternate bearing, predator satiation and seedling recruitment in *Quercus robur* L. Journal of Ecology, 683-696.

Desouhant, E., Debouzie, D., Ploye, H., & Menu, F. (2000). Clutch size manipulations in the chestnut weevil, *Curculio elephas*: fitness of oviposition strategies. Oecologia, 122(4), 493-499.

Elzinga, J. A., Atlan, A., Biere, A., Gigord, L., Weis, A. E., & Bernasconi, G. (2007). Time after time: flowering phenology and biotic interactions. Trends in Ecology & Evolution, 22(8), 432-439.

Espelta, J. M., Cortés, P., Molowny-Horas, R., Sánchez-Humanes, B., & Retana, J. (2008). Masting mediated by summer drought reduces acorn predation in Mediterranean oak forests. Ecology, 89(3), 805-817.

Espelta, J. M., Bonal, R., & Sánchez-Humanes, B. (2009a). Pre-dispersal acorn predation in mixed oak forests: interspecific differences are driven by the interplay among seed phenology, seed size and predator size. Journal of Ecology, 97(6), 1416-1423.

Espelta, J. M., Cortés, P., Molowny-Horas, R., & Retana, J. (2009b). Acorn crop size and pre-dispersal predation determine inter-specific differences in the recruitment of co-occurring oaks. Oecologia, 161(3), 559-568.

Fenner, M., & Thompson, K. (2005). The ecology of seeds. Cambridge University Press. Cambridge UK. 260 pp.

Fernández-Martínez, M., Garbulsky, M., Peñuelas, J., Peguero, G., & Espelta, J. M. (2015). Temporal trends in the enhanced vegetation index and spring weather predict seed production in Mediterranean oaks. Plant ecology, 216(8), 1061-1072.

Fernández-Martínez, M., Vicca, S., Janssens, I. A., Espelta, J. M., & Peñuelas, J. (2017). The North Atlantic Oscillation synchronises fruit production in western European forests. Ecography, 40(7), 864-874.

Fukumoto, H., & Kajimura, H. (2011). Effects of asynchronous acorn production by co-occurring *Quercus* trees on resource utilization by acorn-feeding insects. Journal of forest research, 16(1), 62-67.

Gaete-Eastman, C., Figueroa, C. C., Olivares-Donoso, R., Niemeyer, H. M., & Ramírez, C. C. (2004). Diet breadth and its relationship with genetic diversity and differentiation: the case of southern beech aphids (Hemiptera: Aphididae). Bulletin of entomological research, 94(3), 219-227.

Govindan, B. N., Kéry, M., & Swihart, R. K. (2012). Host selection and responses to forest fragmentation in acorn weevils: inferences from dynamic occupancy models. Oikos, 121(4), 623-633.

Guidone, L., Valentini, N., Rolle, L., Me, G., & Tavella, L. (2007). Early nut development as a resistance factor to the attacks of *Curculio nucum* (Coleoptera: Curculionidae). Annals of applied biology, 150(3), 323-329.

Hardin, G. (1960) The Competitive Exclusion Principle. Science, 131, 1292–1297.

Herrera, C. M., & Pellmyr, O. (Eds.). (2009). Plant animal interactions: an evolutionary approach. 2nd ed. Blackwell Science Ltd. Oxford, UK. 328pp.

Hughes, J., & Vogler, A. P. (2004). Ecomorphological adaptation of acorn weevils to their oviposition site. Evolution, 58(9), 1971-1983.

Hulme, P.E., & Benkman, C.W. (2009) Granivory. (5), 77–106. In. Herrera, C. M., & Pellmyr, O. (Eds.). (2009). Plant animal interactions: an evolutionary approach. 2nd ed. Blackwell Science Ltd. Oxford, UK. 328pp.

Janzen, D. H. (1971). Seed predation by animals. Annual review of ecology and systematics, 2(1), 465-492.

Jervis, M. A., Ellers, J., & Harvey, J. A. (2008). Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. Annual Review of Entomology 53, 361–85.

Jordano, P. (2014). Fruits and frugivory. (1), 18-61. In Gallagher, R.S. Editor. Seeds: The Ecology of Regeneration in Plant Communities. 3er ed. CABI London, UK. 320 pp.

Jordano, P., Bascompte, J., & Olesen, J. M. (2003). Invariant properties in coevolutionary networks of plant–animal interactions. Ecology letters, 6(1), 69-81.

Kelly, D. (1994). The evolutionary ecology of mast seeding. Trends in ecology & evolution, 9(12), 465-470.

Kelly, D., Geldenhuis, A., James, A., Penelope Holland, E., Plank, M. J., Brockie, R. E., Cowan, P.E., Harper, G.A., Lee, W.G., Maitland, M.J., Mark, A.F., Mills, J.A., Wilson, P.R. & Byrom, A.E. (2013). Of mast and mean: differential-temperature cue makes mast seeding insensitive to climate change. Ecology Letters, 16(1), 90-98.

Kelly, D., Harrison, A. L., Lee, W. G., Payton, I. J., Wilson, P. R., & Schauber, E. M. (2000). Predator satiation and extreme mast seeding in 11 species of *Chionochloa* (Poaceae). Oikos, 90(3), 477-488.

Kelly, D., & Sork, V. L. (2002). Mast seeding in perennial plants: why, how, where?. Annual Review of Ecology and Systematics, 33(1), 427-447.

Kelrick, M. I., MacMahon, J. A., Parmenter, R. R., & Sisson, D. V. (1986). Native seed preferences of shrub-steppe rodents, birds and ants: the relationships of seed attributes and seed use. Oecologia, 68(3), 327-337.

Kergoat, G. J., Delobel, A., Fédière, G., Le Rü, B., & Silvain, J. F. (2005). Both host-plant phylogeny and chemistry have shaped the African seed-beetle radiation. Molecular phylogenetics and evolution, 35(3), 602-611.

Koenig, W. D., & Knops, J. M. (2005). The mystery of masting in trees: Some trees reproduce synchronously over large areas, with widespread ecological effects, but how and why?. American Scientist, 93(4), 340-347.

Kolb, A., Ehrlen, J., & Eriksson, O. (2007). Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. Perspectives in Plant Ecology, Evolution and Systematics, 9(2), 79-100.

Lande, R. (1988). Genetics and demography in biological conservation. Science, 241(4872), 1455-1460.

Maeto, K., & Ozaki, K. (2003). Prolonged diapause of specialist seed-feeders makes predator satiation unstable in masting of *Quercus crispula*. Oecologia, 137(3), 392-398.

Menu, F. (1993). Strategies of emergence in the chestnut weevil *Curculio elephas* (Coleoptera: Curculionidae). Oecologia, 96(3), 383-390.

Moreira, X., Abdala-Roberts, L., Zas, R., Merlo, E., Lombardero, M. J., Sampedro, L., & Mooney, K. A. (2016). Masting behaviour in a Mediterranean pine tree alters seed predator selection on reproductive output. Plant Biology, 18(6), 973-980.

Muñoz, A., Bonal, R., & Espelta, J. M. (2014). Acorn–weevil interactions in a mixed-oak forest: Outcomes for larval growth and plant recruitment. Forest ecology and management, 322, 98-105.

Newman, D., & Pilson, D. (1997). Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. Evolution, 51(2), 354-362.

Peguero, G., Bonal, R., & Espelta, J. M. (2014). Variation of predator satiation and seed abortion as seed defense mechanisms across an altitudinal range. Basic and applied ecology, 15(3), 269-276.

Peguero, G., Bonal, R., Sol, D., Muñoz, A., Sork, V. L., & Espelta, J. M. (2017). Tropical insect diversity: evidence of greater host specialization in seed-feeding weevils. Ecology, 98(8), 2180-2190.

Peled, E., Shanas, U., Granjon, L., & Ben-Shlomo, R. (2016). Connectivity in fragmented landscape: Generalist and specialist gerbils show unexpected gene flow patterns. Journal of Arid Environments, 125, 88-97.

Pélisson, P. F., Bernstein, C., Francois, D., Menu, F., & Venner, S. (2013a). Dispersal and dormancy strategies among insect species competing for a pulsed resource. Ecological entomology, 38(5), 470-477.

Pélisson, P. F., Bel-Venner, M. C., Giron, D., Menu, F., & Venner, S. (2013b). From income to capital breeding: when diversified strategies sustain species coexistence. PloS one, 8(9), e76086.

Pélisson, P. F., Bel-Venner, M. C., Rey, B., Burgevin, L., Martineau, F., Fourel, F., ... & Venner, S. (2012). Contrasted breeding strategies in four sympatric sibling insect species: when a proovigenic and capital breeder copes with a stochastic environment. Functional ecology, 26(1), 198-206.

Pérez-Ramos, I. M., Ourcival, J. M., Limousin, J. M., & Rambal, S. (2010). Mast seeding under increasing drought: results from a long-term data set and from a rainfall exclusion experiment. Ecology, 91(10), 3057-3068.

Pilson, D. (2000). Herbivory and natural selection on flowering phenology in wild sunflower, Helianthus annuus. Oecologia, 122(1), 72-82.

Pinzón-Navarro, S., Barrios, H., Múrria, C., Lyal, C. H., & Vogler, A. P. (2010). DNA-based taxonomy of larval stages reveals huge unknown species diversity in neotropical seed weevils (genus *Conotrachelus*): relevance to evolutionary ecology. Molecular phylogenetics and evolution, 56(1), 281-293.

Ramírez, N., & Traveset, A. (2010). Predispersal seed-predation by insects in the Venezuelan Central Plain: overall patterns and traits that influence its biology and taxonomic groups. Perspectives in Plant Ecology, Evolution and Systematics, 12(3), 193-209.

Richard, R., & Casas, J. (2009). Stochasticity and controllability of nutrient sources in foraging: host-feeding and egg resorption in parasitoids. Ecological Monographs, 79(3), 465-483.

Robinet, C., & Roques, A. (2010). Direct impacts of recent climate warming on insect populations. Integrative Zoology, 5(2), 132-142.

Saruhan, İ., & Tuncer, C. (2000). Population densities and seasonal fluctuations of hazelnut pests in Samsun, Turkey. 495-502p. In V International Congress on Hazelnut. 556pp.

Satake, A., N. Bjørnstad, O., & Kobro, S. (2004). Masting and trophic cascades: interplay between rowan trees, apple fruit moth, and their parasitoid in southern Norway. Oikos, 104(3), 540-550.

Schauber, E. M., Kelly, D., Turchin, P., Simon, C., Lee, W. G., Allen, R. B., Payton, I.J., Wilson, P.R., Cowan, P.E., & Brockie, R. E. (2002). Masting by eighteen New Zealand plant species: the role of temperature as a synchronizing cue. Ecology, 83(5), 1214-1225.

Schoonhoven, L. M., Van Loon, J. J., & Dicke, M. (2005). Insect-plant biology. 2nd ed. Oxford University Press. New York, USA. 440 pp.

Schowalter, T. D. (2011). Insect ecology: an ecosystem approach. 3rd ed. Academic Press. Oxford, UK. 633 pp.

Schowalter, T. D. (2016). Insect ecology: an ecosystem approach. 4th ed. Academic Press. Oxford, UK. 762 pp.

Schraer, S. M., Harris, M., Jackman, J. A., & Biggerstaff, M. (1998). Pecan weevil (Coleoptera: Curculionidae) emergence in a range of soil types. Environmental entomology, 27(3), 549-554.

Shibata, M., Tanaka, H., & Nakashizuka, T. (1998). Causes and consequences of mast seed production of four co-occurring *Carpinus* species in Japan. Ecology, 79(1), 54-64.

Sousa WP, Kennedy PG, Mitchell BJ. (2003) Propagule size and predispersal damage by insects affect establishment and early growth of mangrove seedlings. Oecologia 135:567–575

Strauss, S. Y., & Zangerl, A. R. (2009). Plant-insect interactions in terrestrial ecosystems. (3), 77-106. In. Herrera, C. M., & Pellmyr, O. (Eds.). (2009). Plant animal interactions: an evolutionary approach. 2nd ed. Blackwell Science Ltd. Oxford, UK. 328pp.

Toju, H. (2008). Fine-scale local adaptation of weevil mouthpart length and camellia pericarp thickness: altitudinal gradient of a putative arms race. Evolution, 62(5), 1086-1102.

Toju, H. & Fukatsu, T. (2011) Diversity and infection prevalence of endosymbionts in natural populations of the chestnut weevil: relevance of local climate and host plants. Molecular Ecology, 20, 853–868.

Toju, H., & Sota, T. (2005). Imbalance of predator and prey armament: geographic clines in phenotypic interface and natural selection The American Naturalist 167 (1), 105-117

Toju, H., & Sota, T. (2006). Phylogeography and the geographic cline in the armament of a seed-predatory weevil: effects of historical events vs. natural selection from the host plant. Molecular Ecology, 15(13), 4161-4173.

Toju, H., Ueno, S., Taniguchi, F., & Sota, T. (2011). Metapopulation structure of a seed–predator weevil and its host plant in arms race coevolution. Evolution: International Journal of Organic Evolution, 65(6), 1707-1722.

Travers-Martin, N., & Müller, C. (2008). Matching plant defence syndromes with performance and preference of a specialist herbivore. Functional Ecology, 22(6), 1033-1043.

Venner, S., Pélisson, P.F., Bel-Venner, M.C., Debias, F., Rajon, E. & Menu, F. (2011) Coexistence of insect species competing for a pulsed resource: toward a unified theory of biodiversity in fluctuating environments. PLoS ONE, 6, e18039.

Yguel, B., Bailey, R., Tosh, N. D., Vialatte, A., Vasseur, C., Vitrac, X., Jean, F. & Prinzing, A. (2011). Phytophagy on phylogenetically isolated trees: why hosts should escape their relatives. Ecology Letters, 14(11), 1117-1124.

Ylioja, T., Roininen, H., Ayres, M. P., Rousi, M., & Price, P. W. (1999). Host-driven population dynamics in an herbivorous insect. Proceedings of the National Academy of Sciences, 96(19), 10735-10740.

Yu, F., Shi, X., Wang, D., Yi, X., Fan, D., Guo, T., & Lou, Y. (2015). Effects of insect infestation on *Quercus aliena* var. *acuteserrata* acorn dispersal in the Qinling Mountains, China. New forests, 46(1), 51-61.

Yu, X., Zhou, H., & Luo, T. (2003). Spatial and temporal variations in insect-infested acorn fall in a *Quercus liaotungensis* forest in North China. Ecological Research, 18(2), 155-164.

This page intentionally left blank

Directors reports

Impact factor of published articles

Here by Dr. Josep Maria Espelta and Dr. Raúl Bonal, directors of the doctoral thesis of Harold Arias-LeClaire, submit the following report on the impact factor of the published articles that are part of this Doctoral thesis.

Arias-LeClaire, H., R. Bonal, D. García-López and J.M. Espelta. 2018. The role of seed size, phenology, oogenesis and host distribution in the specificity and genetic structure in seed weevils (Curculio spp.) in mixed forests. Integrative Zoology 13(3), 267-279. DOI10.1111/1749-4877.12293

Accepted on November 2017 and published on May 2018
Integrative Zoology, Impact Factor: 1.856, ISI Ranking: 2017: 40/166 (Zoology), Online ISSN: 1749-4877.

Espelta, J. M., H. Arias-LeClaire, M. Fernández-Martínez, E. Doblas-Miranda, A. Muñoz, and R. Bonal. 2017. Beyond predator satiation: Masting but also the effects of rainfall stochasticity on weevils drive acorn predation. Ecosphere 8(6): e01836.10.1002/ecs2.1836 DOI10.1002/ecs2.1836

Accepted and published on June 2017
Ecosphere, Impact Factor: 2.671, ISI Ranking 2017: 55/158 (Ecology), Online ISSN: 2150-8925.

The articles part of the doctoral thesis of Harold Arias LeClaire have been subjected to scientific journals of international scope contained in the Science Citation Index (SCI).

Barcelona September 2018

Josep Maria Espelta Morral, Dr. Centre de Recerca Ecològica i Aplicacions Forestals Universitat Autònoma de Barcelona	Raúl Bonal Andres, Dr. Departamento Medio Agronómico y Forestal Universidad de Extremadura

Contribution of the doctorate in the published articles

Here, Dr. Josep Maria Espelta and Dr. Raúl Bonal as supervising Directors of Harold Arias LeClaire during his studies, present this report to state the doctorate contribution on each published article part of his Doctoral Thesis.

- Arias-LeClaire H, Bonal R, García-López D, Espelta JM. 2018. Role of seed size, phenology, oogenesis and host distribution in the specificity and genetic structure of seed weevils (Curculio spp.) in mixed forests. Integrative Zoology 13, 267–79. DOI10.1111/1749-4877.12293

Doctorate contribution: H. Arias-LeClaire participated in the experimental design; conducted the field samplings and laboratory procedures for data collection, analyzed the data and led the writing, discussing, and editing of the manuscript.

Co-authors contribution: D. García-López shared part of his field data, R. Bonal and J.M. Espelta participated in the experimental design, data analysis process and supervised the writing, discussing, and editing of manuscript.

- Espelta, J. M., H. Arias-LeClaire, M. Fernández-Martínez, E. Doblas-Miranda, A. Muñoz, and R. Bonal. 2017. Beyond predator satiation: Masting but also the effects of rainfall stochasticity on weevils drive acorn predation. Ecosphere 8(6): e01836.10.1002/ecs2.1836 DOI10.1002/ecs2.1836

Doctorate participation: H. Arias-LeClaire led the data analysis process and contributed with writing, discussing, and editing of the manuscript.

Co-authors contribution: J. M. Espelta and R. Bonal designed the study, M. Fernández-Martínez, E. Doblas-Miranda and A. Muñoz, participated in the field data gathering, data analysis process and writing, discussing, and editing of manuscript.

Barcelona September 2018

Josep Maria Espelta Morral, Dr.
Centre de Recerca Ecològica i Aplicacions Forestals
Universitat Autònoma de Barcelona

Raúl Bonal Andres, Dr.
Departamento Medio Agronómico y Forestal
Universidad de Extremadura

Published research articles

Plant–animal interactions are a powerful evolutionary force sustaining trophic fluxes and diversity in terrestrial ecosystems.

This page intentionally left blank

The role of seed size, phenology, oogenesis and host distribution in the specificity and genetic structure in seed weevils (Curculio spp.) in mixed forests.

Arias-LeClaire H, Bonal R, García-López D, Espelta JM (2018). Role of seed size, phenology, oogenesis and host distribution in the specificity and genetic structure of seed weevils *(Curculio spp.)* in mixed forests. Integrative Zoology 13, 267–79. DOI10.1111/1749-4877.12293

Accepted on November 2017 and published on May 2018
Integrative Zoology, Impact Factor: 1.856, ISI Ranking: 2017: 40/166 (Zoology), Online ISSN: 1749-4877.

http://onlinelibrary.wiley.com/doi/10.1111/1749-4877.12293/full

This page intentionally left blank

Integrative Zoology 2018; 13: 267–279 doi: 10.1111/1749-4877.12293

ORIGINAL ARTICLE

Role of seed size, phenology, oogenesis and host distribution in the specificity and genetic structure of seed weevils (*Curculio* spp.) in mixed forests

Harold ARIAS-LECLAIRE[1,2] Raúl BONAL,[3,4] Daniel GARCÍA-LÓPEZ[5] and Josep Maria ESPELTA[1]

[1]CREAF, Centre for Ecological Research and Forestry Applications, Cerdanyola del Vallès, 08193, Spain, [2]Exact and Natural Sciences School, State Distance University of Costa Rica, UNED, Mercedes de Montes de Oca, San José, Costa Rica, [3]Forest Research Group, INDEHESA, University of Extremadura, Plasencia, Spain, [4]DITEG Research Group, University of Castilla-La Mancha, Toledo, Spain and [5]Zoology Department, Autonomous University of Barcelona, Cerdanyola del Vallès, Spain

Abstract

Synchrony between seed growth and oogenesis is suggested to largely shape trophic breadth of seed-feeding insects and ultimately to contribute to their co-existence by means of resource partitioning or in the time when infestation occurs. Here we investigated: (i) the role of seed phenology and sexual maturation of females in the host specificity of seed-feeding weevils (*Curculio* spp.) predating in hazel and oak mixed forests; and (ii) the consequences that trophic breadth and host distribution have in the genetic structure of the weevil populations. DNA analyses were used to establish unequivocally host specificity and to determine the population genetic structure. We identified 4 species with different specificity, namely *Curculio nucum* females matured earlier and infested a unique host (hazelnuts, *Corylus avellana*) while 3 species (*Curculio venosus*, *Curculio glandium* and *Curculio elephas*) predated upon the acorns of the 2 oaks (*Quercus ilex* and *Quercus pubescens*). The high specificity of *C. nucum* coupled with a more discontinuous distribution of hazel trees resulted in a significant genetic structure among sites. In addition, the presence of an excess of local rare haplotypes indicated that *C. nucum* populations went through genetic expansion after recent bottlenecks. Conversely, these effects were not observed in the more generalist *Curculio glandium* predating upon oaks. Ultimately, co-existence of weevil species in this multi-host-parasite system is influenced by both resource and time partitioning. To what extent the restriction in gene flow among *C. nucum* populations may have negative consequences for their persistence in a time of increasing disturbances (e.g. drought in Mediterranean areas) deserves further research.

Key words: *Corylus avellana*, *Curculio* spp., genetic structure, *Quercus* spp., trophic breadth

Correspondence: Josep Maria Espelta, CREAF, Cerdanyola del Vallès, Spain.
Email: josep.espelta@uab.cat

INTRODUCTION

Seed predation by insects may play a crucial role in plant population dynamics, by reducing the reproductive output (Bonal *et al.* 2007; Espelta *et al.* 2008) and constraining the regeneration process (Espelta *et al.* 2009b). Trophic breadth and specificity of seed-feeding insects is often explained by differences among plant hosts in chemical or morphological traits (Bernays & Graham 1988; Forister *et al.* 2015). Differences in phytochemistry (mainly nitrogen-based defensive compounds) have been observed to be highly relevant for the diversification of phytophagous insects and their diet breadth (Kergoat *et al.* 2005). Concerning other seed features, size is a trait claimed to influence ecomorphological diversification in many endophytic insects (e.g. body size and rostrum shape), promoting differences in their trophic niche, ecological adaptations and species radiation (Hughes & Voegler 2004a; Bonal *et al.* 2011; Peguero *et al.* 2017). In addition to chemical and morphological differences, seeding phenology and stochasticity in the availability of this resource have also been suggested as key factors influencing the guild of insect species predating upon a particular plant host (Espelta *et al.* 2008, 2009b; Coyle *et al.* 2012; see also Pélisson *et al.* 2013a). As insects are short-living organisms, synchronization of their life-cycle with the resources upon which they depend is critical (Bale *et al.* 2002, 2007; Hood & Ott 2010). Therefore, processes such as adult emergence (Espelta *et al.* 2017) and oogenesis (Trudel *et al.* 2002; Son & Lewis 2005) have to be tightly connected with the presence of seeds for oviposition (Bonal *et al.* 2010). In particular, oogenesis (i.e. egg maturation in females) has been predicted to differ depending on the stochasticity of seeds availability. Thus, proovigenesis (i.e. females have already mature eggs at the onset of their adult life) would be favored in species predating upon hosts that regularly produce seeds while synovigenesis (i.e. females start their adult life with immature eggs) would be advantageous for species exposed to more random fluctuations of seed production (Jervis *et al.* 2008; Richard & Casas 2009), as they can better adjust the amount of energy invested in reproduction to the amount of seeds (but see Pélisson 2013b). Ultimately, the co-existence of the different seed consumers in a multi-host community could be mediated by resource partitioning (e.g. insects predate preferentially upon different species according to different seed traits; see Espelta *et al.* 2009a), time-partitioning (e.g. insects exhibit differences in life span and the timing of seed predation; see Pélisson *et al.* 2012) or the trade-off among dispersal versus dormancy ability to cope with resource scarcity (Pélisson *et al.* 2012). Yet, the importance of the interplay among seed size, seeding phenology and oogenesis in driving the guild of insects predating upon seeds of different hosts in multi-specific systems has been seldom explored.

The breadth of the trophic niche of seed-feeding insects (specialist *vs* generalist) may influence the number of species that predate upon different seeds and it has consequences for the dynamics of the community of hosts (Espelta *et al.* 2009b). However, beyond the effects on plant fitness, differences in the trophic niche may also influence the population dynamic of the seed consumers (Ylioja *et al.* 1999) depending on life-history traits such as dispersal ability and landscape attributes (i.e. abundance and spatial distribution of hosts). Spatial connectivity among plant-hosts has been shown to be especially relevant for insects with low dispersal ability (Thomas *et al.* 2001; Kruess 2003), resulting in a stronger genetic structure and reduced gene flow in the insect populations located on more isolated hosts. In the long run, host isolation may even result in colonization credits for some insect species, especially those with a narrower diet breadth (Ruiz-Carbayo *et al.* 2016) and poor dispersal ability (Pélisson *et al.* 2013b; Heineger *et al.* 2014). Conversely, generalist species may show a more continuous distribution in the landscape, benefiting from the spatial overlap of the different host plants upon which they feed (Newman & Pilson 1997), and show no genetic structure differences among populations owing to gene flow. Interestingly, for seed-feeding insects a comparison of the genetic structure of their populations and the spatial structure of their potential hosts could provide strong evidence about differences of the trophic niche breadths. Moreover, the use of molecular techniques (DNA barcoding) may help to detect cryptic speciation and trophic niche segregation among morphologically similar species (Peguero *et al.* 2017), and also to establish species specificity in an unequivocal way in comparison to classifications based on the presence or absence of a species on a particular plant, especially when the lack of morphological differences at certain stages (e.g. larvae) make species identification impossible otherwise (Govindan *et al.* 2012). Unfortunately, this combination of landscape ecology (i.e. host connectivity) and population genetics when studying the breadth of the trophic niche and dispersal ability of phytophagous insects remains largely unexplored.

The main aims of this study have been to investigate in a multi-host and multi-seed-predator system the role of seed size, seed phenology and ooegenesis in the host specificity of seed-parasite weevils (*Curculio* spp.) and to analyze the consequences that potential differences in trophic specialization and host distribution may have in the genetic structure of weevil populations. *Curculio* spp. (Coleoptera: Curculionidae) are seed parasites that differ in their dispersal ability (Venner *et al.* 2011), diapause duration (Pélisson *et al.* 2013a,b), oogenesis (with both proovigenic and synovigenic species; Pélisson *et al.* 2013a) and the breadth of their trophic niche (Muñoz *et al.* 2014; Bonal *et al.* 2015; Peguero *et al.* 2017). We conducted this study in Catalonia (northeast Spain) in mixed forests including oaks (*Quercus ilex*, *Quercus pubescens*) and common hazel trees (*Corylus avellana*) with 4 different weevil species present (*Curculio nucum* Linnaeus, *Curculio glandium* Marsham, *Curculio venosus* Gravenhorst and *Curculio elephas* Gyllenhaal). Interestingly, in this region oaks show a much more continuous distribution and later seeding, while hazels often appear in more discontinuous patches and have an earlier production of fruits (Gracia *et al.* 2004). Concerning weevils, the 4 species overwinter underground, but they differ in the duration of their diapause, the phenology of emergence, oogenesis and dispersal ability. Adults of *C. glandium*, *C. venosus* and *C. nucum* emerge in spring 2 years after larvae buried into the soil, while *C. elephas* exhibits variable diapause and adults emerge in early autumn (Bonal *et al.* 2010; Espelta *et al.* 2017) for up to 3 years (Pelisson *et al.* 2013b). Concerning oogenesis, in *C. glandium*, *C. venosus* and *C. nucum* females are reproductively immature (synovigenic) and ovarian development is accomplished after 1 or 2 months of the feeding period (Bel-Venner *et al.* 2009), while *C. elephas* females are proovigenic and food intake is not required for ovarian development (Pélisson *et al.* 2012). Regarding host selection, previous studies have suggested that *C. nucum* is highly specialized in hazelnuts (Bel-Venner *et al.* 2009), while the other weevils depredate upon several oak species (Muñoz *et al.* 2014). However, these results have not been confirmed by means of DNA analyses as no study has been conducted in mixed hazel–oak forests. Considering the traits of the species involved in this multi-host and multi-predator system and the spatial distribution of hosts, we hypothesize that: (i) seed size and the synchronization of seeding phenology and oogenesis will be responsible for the guild of weevils predating upon the different plants; and (ii) the narrower trophic breadth of *C. nucum* and the more patchy distribution of hazels in comparison to the more generalist habit of the other weevils and the continuous distribution of oaks will result in differences in the genetic structure of weevil populations of these species.

MATERIALS AND METHODS

Study area and species

The study was carried out in mixed forests with the presence of oaks (*Q. ilex* and *Q. pubescens* and common hazel trees (*Co. avellana*) in Catalonia (north-east Spain, Fig. 1). The evergreen *Q. ilex* and the winter-deciduous *Q. pubescens* are extensively distributed in pure and mixed forests in all the western rim of the Mediterranean basin (Espelta *et al.* 2008), while the common hazel (*Co. avellana*) often appears in scattered groups in mixed deciduous forests or cultivated in monospecific stands (AliNiazee 1998). Acorns in *Quercus* spp. and hazelnuts in *Co. avellana* mature in 1 year and both are subjected to intense pre-dispersal seed predation by weevils (*Curculio* spp.), a group of granivorous insects extensively distributed in the northern hemisphere (Hughes & Voegler 2004a). In Catalonia, the most common weevil species predating upon acorns are *C. glandium* and *C. elephas* (Espelta *et al.* 2009b), the latter also depredating upon chestnuts (*Castanea* spp.), while in hazelnuts the unique species described up to now has been *C. nucum*, a hypothesized highly specific seed parasite (Guidone *et al.* 2007; Bel-Venner *et al.*

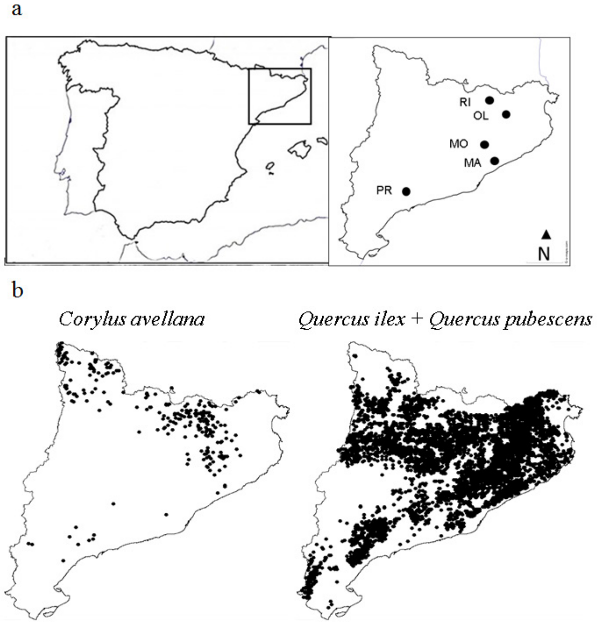


Figure 1 (a) Location of study sites in Catalonia (north-east Spain). (b) Distribution of *Corylus avellana*, *Quercus ilex* and *Quercus pubescens* according to the presence of this species in plots inventoried in the Catalan Forest Inventory (Gracia *et al.* 2004). RI, Ripoll (5 plots); OL, Olot (4 plots); MO, Montseny (4 plots); MA, Maresme (5 plots); PR, Prades (5 plots).

2009). However, it must be highlighted that except for the weevil species predating upon oaks, ascription of weevil species to a plant host is based on the observation of adults in the foliage of that particular species, but no study has addressed this issue comprehensively (e.g. identifying by means of molecular techniques the species of the larvae inside chestnuts or hazelnuts).

During early summer on hazelnuts (AliNiazee 1998) and early autumn on acorns (see Bonal & Muñoz 2009) female weevils perforate the seed cover with their snout and oviposit commonly a single egg so the larvae develop feeding on the seed kernel. At the middle of summer in *C. nucum* (Bel-Venner *et al.* 2009) or late autumn in *C. elephas* and *C. glandium* (Espelta *et al.* 2009a), larvae exit the seed and bury into the ground to overcome the diapause period and undergo full metamorphosis.

Sampling design

In 2013 we established a total of 23 sampling plots grouped into 5 geographical clusters (Sites) in a north to south latitudinal gradient (see Fig. 1). This sampling procedure was selected to account for the possible effects of latitude on the duration of the vegetative season and, thus, on the seeding phenology of oaks and hazelnuts, their overlap and the overlap among these host species and the weevils predating upon their seeds. Presumably a tighter vegetative season in northern and colder sites would lead to more similar patterns of seed production while these could be more relaxed and longer in southern and warmer places. Ultimately, this could lead to differences in the guild of weevils predating upon these plants. Plots were selected by searching for the presence of trees of *Co. avellana* and *Q. ilex* or *Q. pubescens* based on the Catalan Forest Inventory (Gracia *et al.* 2004) and field observations of their reproductive status (i.e. presence of seeds). From late July (end of hazel seeding season) to early October (end of the acorn crop) we carried out 3 sampling campaigns: (i) late July to early August; (ii) late August to early September; and (iii) late September to early October to account for possible differences in the phenology of seed infestation by the different weevil species present. In every plot and in each sampling period we randomly collected a minimum of 100 seeds from each species (*Co. avellana* and *Quercus* spp.) under the canopies of several randomly selected trees. Seeds were taken to the laboratory and classified as sound or infested to assess infestation rates per species and sampling period. Infested seeds are easily recognizable by the presence of female oviposition scars. We calculated the volume of both sound and infested seeds by measuring the length and width to the nearest 0.01 mm with a digital caliper (see Espelta *et al.* 2009a). Infested seeds were placed individually in plastic trays for individual monitoring. Each seed was checked daily to register the emergence of larvae, which were immediately transferred to 2 mL Eppendorf with 96% alcohol. Once larvae stop emerging (approximately 3 weeks after seeds were collected) seeds were dissected to check for the presence of non-emerged larvae. From hazelnuts only *Curculio* larvae emerged, while for acorns the 6% of larvae corresponded to the *Cydia* spp. moth.

In parallel, during the abovementioned field campaigns adult weevil were captured by shaking the canopy and collecting the fallen individuals in an inverted umbrella held beneath the foliage for species identification at the laboratory. To establish whether females were sexually mature, they were dissected under a microscope to observe abdominal segments and ovary maturity. We considered the presence of eggs as a sign of female ready for oviposition and the absence of eggs as females that were still immature or had already oviposited (Pélisson *et al.* 2013a).

DNA barcoding and larval species identification

A total of 1657 *Curculio* larvae emerged from hazelnuts and acorns. In order to establish unequivocal trophic relationships between insects and their host plants we used molecular techniques (DNA barcoding) as larvae cannot be determined according to morphological characters. Therefore, from 342 larvae selected randomly among the ones emerged in the laboratory from the 3 hosts we extracted DNA from a small piece of larval tissue (approximately 2-mm long) using the NucleoSpin-Tissue kit according to the manufacturer’s instructions (MACHEREY-NAGEL GmbH, Düren, Germany; www.mn-net.com). We amplified a fragment (826 bp) of the mitochondrial cytochrome oxidase subunit 1 (cox1) using primers Pat and Jerry (please see Hughes & Vogler [2004b] for details on primer sequences and PCR protocols). We chose this fragment of cox1 due to the availability of many reference sequences from correctly determined adults of European *Curculio* spp. For comparison (Hughes & Vogler 2004b). Sequencing was performed using Big-Dye (Perkin-Elmer) technology and an ABI3700 sequencer. Sequence chromatograms were assembled and edited using Sequencher 4.6 (Gene Codes, Ann Arbor, MI, USA). For species identification we discarded those sequences that after edition were shorter than 500 base pairs. Edited sequences were aligned using CLUSTALW supplied via http://align.genome.jp, with default gap open and gap extension penalties. The alignment sets were collapsed into unique haplotypes and each of this compared to the *Curculio* spp. reference sequences available at GenBank. We applied the most conservative limit used in DNA barcoding, which states a maximum genetic divergence (number of different nucleotides by the total number of compared nucleotides) of 1% with respect to the reference sequence for an unambiguous identification to the species level (Ratnasingham & Herbert 2007).

Data analysis

To evaluate the occurrence of the different weevil species in the 3 potential hosts *Co. avellana*, *Q. ilex* and Q. *pubescens*) across the 5 study sites, we conducted a Pearson's χ^2-test. Similarly, we used χ^2-tests for the comparison of the presence of male and female weevil proportion, as well as that of immature and mature females among sampling periods. A generalized linear mixed model (GLMM), following a binomial distribution, was used to test for the effects of the study site (RI, Ripoll; OL, Olot; MO, Montseny; MA, Maresme; PR, Prades), sampling period (1, 2, 3) and host species (*Co. avellana*, *Q. ilex* and *Q. pubescens*) on the seed predation rate by weevils. The factor "plot" was included as a random effect in the GLMM analyses to account for the repeated nature of the measurements and other unexplained variation. Analyses of deviance Type II Wald χ^2-tests were performed to establish the significance of each different independent variable in the model. A general linear mixed model was applied to test for the effects of host species, sampling period and seed condition (sound or infested) on seed size (volume in mm^3) with the factor "plot" included as a random effect.

For population genetic analyses we chose those species in which there were a minimum of 10 individuals per population with sequences longer than 750 bp; namely, *Curculio glandium* and *Curculio nucum*. We used ARLEQUIN software (Excoffier *et al.* 2005) to calculate standard molecular diversity indices (gene diversity and nucleotide diversity) and to perform analyses of the molecular variance (AMOVAs). Signatures of population demographic changes (bottlenecks or expansions) were examined by Tajima's *D* (Tajima 1989) and Fu's *F* (Fu 1997) as implemented in ARLEQUIN software. We also tested whether there was any geographic pattern in the population genetic structure using SAMOVA 1.0 (Dupanloup *et al.* 2002). This method identifies the optimal grouping option (K) that maximizes the among-group component (FCT) of the overall genetic variance. We defined the number of populations (K) and ran 100 simulated annealing processes. We simulated different numbers of populations, ranging from $K = 2$ to $K = 4$, to determine the best population clustering option.

RESULTS

Molecular analyses allowed the identification of the larvae emerged from the seeds of the 3 host species (*Co. avellana*, *Q. ilex* and *Q. pubescens*) as all sequences showed a divergence below 1% with respect to *Curculio* spp. reference sequences from GenBank. This divergence was much lower than inter-specific differences, which in all cases exceeded 8%. All larvae corresponded to 4 species; namely, *C. elephas*, *C. glandium*, *C. nucum* and *C. venosus*. As shown in Figure 2, weevil species were not randomly distributed among hosts; that is, *C. nucum* was exclusively present in hazelnuts while the other 3 weevils emerged uniquely from acorns ($\chi^2_6 = 263.9$, $P < 0.001$). *C. glandium* and *C. elephas* were more abundant in *Q. ilex* (respectively, $\chi^2_{14} = 91.8$, $P < 0.001$, and $\chi^2_8 = 23.3$, $P < 0.001$) while there were not significant differences in the presence of *Curculio venosus* between the 2 oak species ($\chi^2_5 = 7.47$, $P > 0.05$). The different presence of larvae of the 4 weevil species

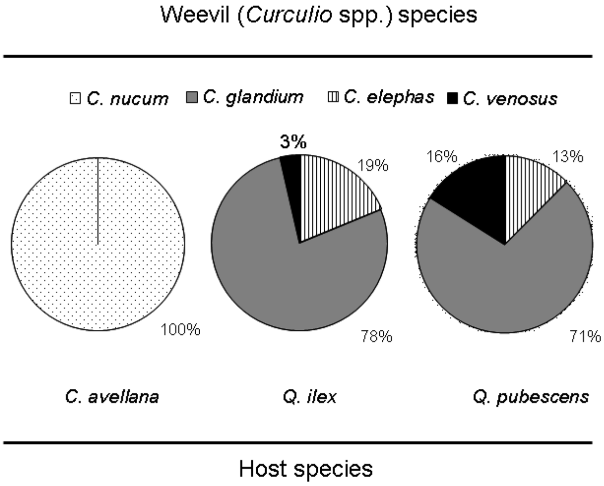


Figure 2 Proportion of the different weevil species infesting the seeds of the 3 host species (*Corylus avellana*, *Quercus ilex* and *Quercus pubescens*) according to the DNA analyses of the larvae emerging from the seeds.

in the 3 hosts, especially among hazelnut and the 2 oaks, was not due to the lack of a particular species in a given site as we captured adult specimens of all weevil species along the entire gradient. Moreover, as hazelnuts were bigger than acorns during almost the entire seeding season (Table 1 and Fig. 3), the abovementioned differences in weevil specificity among these 2 groups of hosts does not seem to be due to the exclusion of certain weevil species from hazelnuts by a too small seed size.

Male and female weevils occurred in nearly the same frequency with no significant variation along the sampling periods ($\chi^2_2 = 2.28$, $P > 0.05$). Yet the proportion of females with presence of eggs and without eggs showed significant differences through the season ($\chi^2_2 = 33.7$, $P < 0.001$) and for the different weevil species. As shown in Figure 4, through the season the presence of females with eggs was earlier in *C. nucum*, followed by *C. glandium* and *C. elephas*. In the 2 synovigenic species, *C. nucum* had a decreasing pattern ($\chi^2_2 = 10.5$, $P < 0.01$) in the presence of females with eggs, while *C. glandium* exhibited an increasing pattern ($X^2_2 = 35.3$, $P < 0.001$). In the proovigenic *C. elephas* we did not find females on the very first sampling period but as soon as they appeared during the second and third sampling dates they were already sexually mature ($\chi^2_2 = 16.5$, $P < 0.001$). Consistently with the seasonal patterns of the presence of females ready to oviposit, we found that infestation rates showed significant variation among study sites ($\chi^2_4 = 16.5$, $P < 0.001$), sampling periods ($\chi^2_1 = 5.5$, $P < 0.05$) and host species ($\chi^2_2 = 6.4$, $P < 0.05$). Overall, infestation was higher in northern localities and it increased as the seeding season progressed (see coefficients for the different effects in Table 2). Concerning host species, infestation rates showed contrasting temporal patterns in hazelnut versus oaks (Table 2, Fig. 5), in agreement with host seeding phenology and oogene-

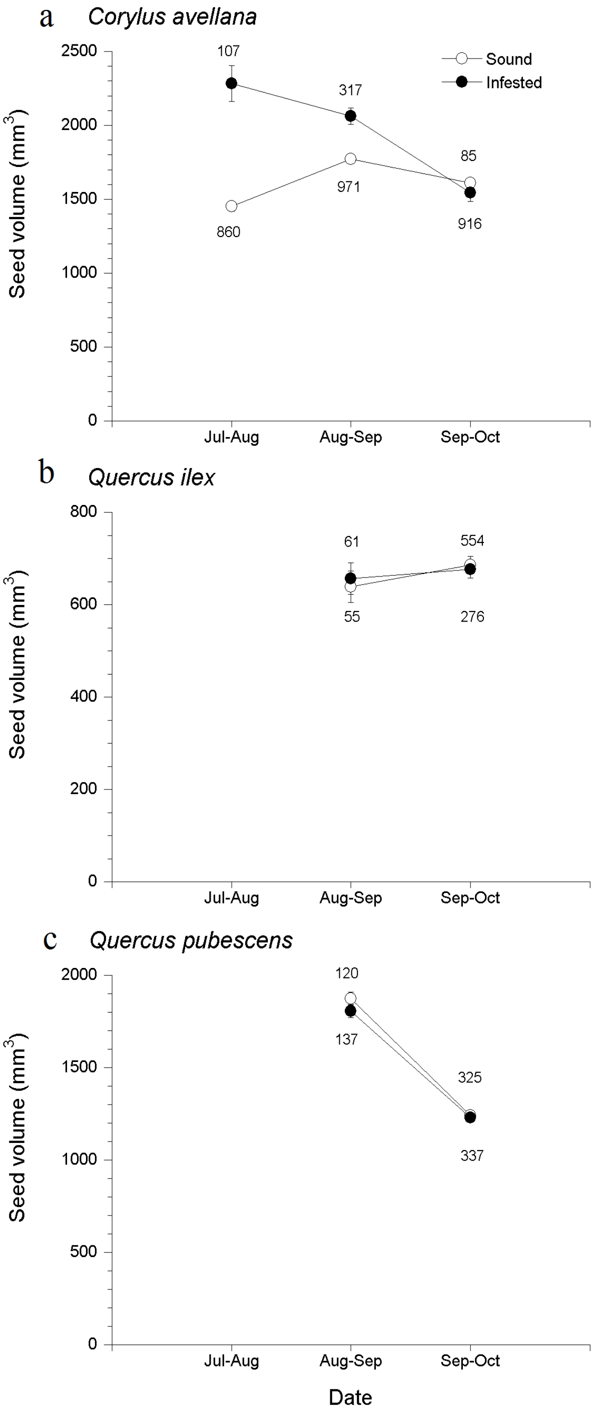


Figure 3 Proportion of females with eggs (black column) and without eggs (white columns) for the 3 weevil species (*Corylus avellana*, *Quercus ilex* and *Quercus pubescens*) captured in the 3 sampling periods during the seeding season: Jul–Aug = from the end of July to the beginning of August; Aug–Sep = from the end of August to the beginning of September; Sep–Oct = from the end of September to the beginning of October.

Table 1 Estimates for the significant effects of tree host, sampling period and seed condition (sound or infested) over seed size (volume in mm^3) according to the linear mixed model

Effects	Estimate	Standard error	*t*-value
Intercept	3.199	0.0278	153.912***
Date	0.01695	0.004249	3.990***
Host, *Q. pubescens*	−0.1876	0.008793	−21.336***
Host, *Q. ilex*	−0.4808	0.009984	−48.159***
Seed condition, sound	−0.03143	0.006962	−4.515***

**P* < 0.05, ***P* < 0.01 and ****P* < 0.001.

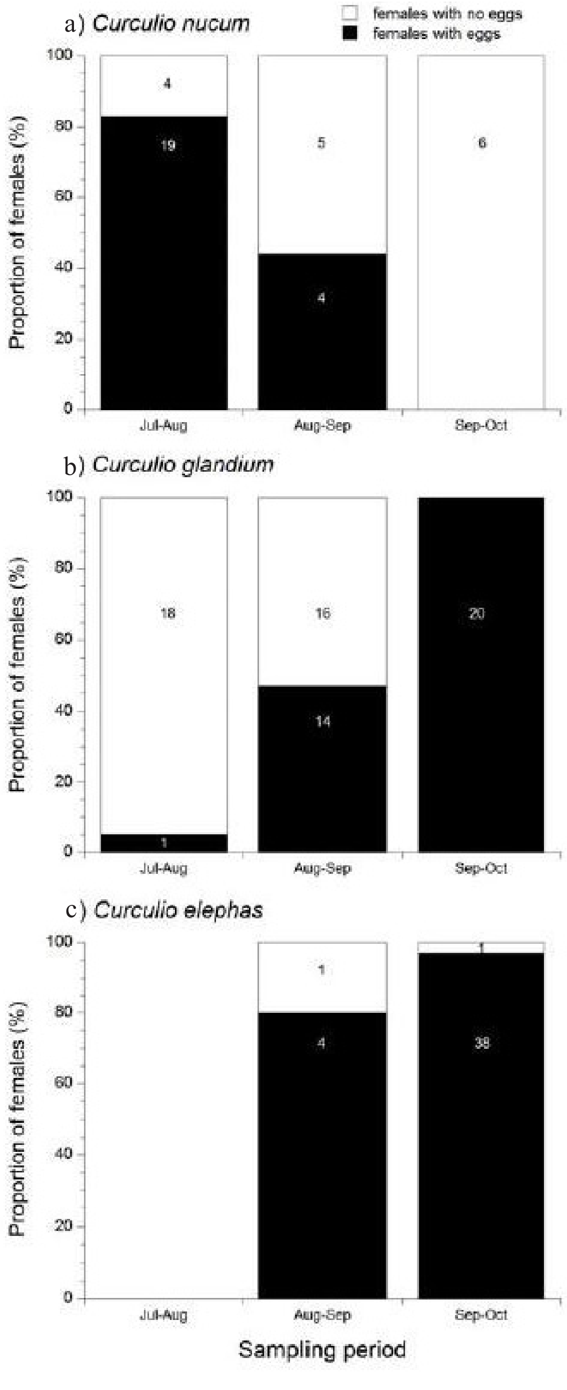


Figure 4 Mean ± SE volume (mm^3) of sound (open dots) and infested (black dots) seeds of the 3 host species (*Corylus avellana*, *Quercus ilex* and *Quercus pubescens*) along the sampling dates. Jul–Aug = from the end of July to the beginning of August; Aug–Sep = from the end of August to the beginning of September; Sep–Oct = from the end of September to the beginning of October. Notice the difference in the scale of the *y*-axis for the 3 host species.

Table 2 Estimates for the significant effects of study site, tree host and sampling period on weevil infestation rates according to the generalized linear mixed model

Effects	Estimate	Standard error	*z*-value
Intercept	−2.8280	0.5462	−5.177***
Site Olot	1.0830	0.4776	2.268*
Site Ripoll	1.5611	0.4788	3.260**
Host, *Q. pubescens*	0.9615	0.3810	2.523*
Date	0.4634	0.1973	2.349*

*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

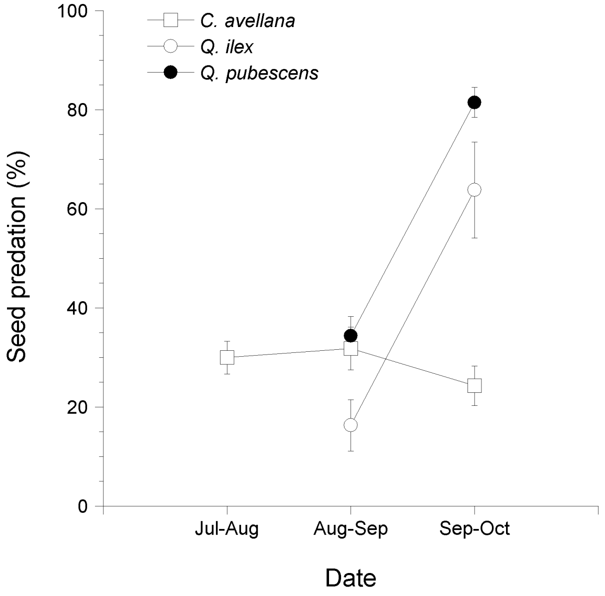


Figure 5 Mean ± SE seed infestation rate of the 3 host species (*Corylus avellana*, *Quercus ilex* and *Quercus pubescens*) along the sampling dates. Jul–Aug = from the end of July to the beginning of August; Aug–Sep= from the end of August to the beginning of September; Sep–Oct = from the end of September to the beginning of October.

sis in females; that is, in hazelnuts infestation occurred earlier and slightly decreased through the season, while it was absent during the first sampling date in the 2 oak species, and progressively increased towards the end of the season (Fig. 5).

The population genetic analyses showed marked differences between *C. nucum* and *C. glandium*. Mean genetic diversity was higher in *C. nucum* (Table 3), mainly due to the higher number of distinct haplotypes; that is, an ANOVA in which the population was included as

Table 3 Values of gene diversity, nucleotide diversity, Tajima's *D* and Fu's *F* recorded at each population for *Curculio nucum* (a) and *Curculio glandium* (b)

	Gene diversity	Nucleotide diversity	Tajima's *D*	Fu's *F*
(a) *Curculio nucum*				
Ripoll	0.87	0.0015	−1.63*	−4.54***
Olot	0.75	0.0024	−1.96**	−8.34***
Montseny	0.89	0.0032	−1.40*	−6.87***
Maresme	0.88	0.0034	−0.41	−1.20
Prades	0.59	0.0012	−1.69**	−5.27***
(b) *Curculio glandium*				
Ripoll	0.71	0.0012	−1.10	−2.61*
Olot	0.57	0.0009	−0.46	−0.84
Montseny	0.69	0.0013	−1.22	−2.61*
Maresme	0.69	0.0013	−0.75	−1.95
Prades	0.63	0.0034	−1.79	1.46

$^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

a random factor showed that mean gene diversity was significantly higher in *C. nucum* ($F_{1,4} = 9.40$; $P = 0.03$). A total of 31 haplotypes were retrieved from the 118 sequences of *C. nucum* included in the analyses versus just 13 from 96 sequences in *C. glandium* (see Tables 4 and 5). In *C. nucum*, 48% of the individuals had the most common haplotype but there were many rare haplotypes sometimes found in just one individual and/or at a single population (Table S1). In the case of *C. glandium*, there were much fewer rare haplotypes and the two most frequent ones were found in 80% of the individuals (Table S2). The high proportion of rare haplotypes in *C. nucum* suggests population expansion after recent bottlenecks and, accordingly, both Tajima's D and Fu's F had negative and significant values in all populations except one. In the case of *C. glandium* only for Fu's test were the values significant in two populations, thus showing that most populations were in equilibrium (Tajima 1989; Fu 1997). The AMOVA revealed a more restricted gene flow between populations in the case of *C. nucum*, in which differentiation among populations explained 5.02% of the total molecular variance (degrees of freedom = 4; $P < 0.01$), whereas in *C. glandium* inter-population differences were not significant. The results of the SAMOVA were marginally significant for *C. nucum* (FCT = 0.08; degrees of freedom = 1; $P = 0.08$) and defined two clusters within the geographical range of our study, the first grouping the nearby populations of Montseny (MO) and Maresme (MA) and another one including the rest (see Fig. 1). No significant geographical pattern of molecular variance was found in *C. glandium*.

DISCUSSION

Seed infestation by weevils did not occur randomly but with 2 opposite breadths of host specificity; namely, the highly specialized *C. nucum* infested a unique host (hazelnuts), while up to 3 species (*C. glandium*, *C. elephas* and *C. venosus*) predated almost indistinctively upon 2 oaks (*Q. ilex* and *Q. pubescens*). These differences in trophic specificity coupled with differences in the geographical distribution of the hosts resulted in 2 distinct patterns concerning the genetic characteristics of weevils' populations; that is, we only found a significant genetic structure among the populations in the highly specialist *C. nucum*. Ultimately, the results of these genetic analyses confirmed the specialist or generalist trophic breadth of the different weevil species according to the identification of the larvae found in the seeds and they stress how molecular techniques may help to establish unequivocal trophic relations for seed feeding insects.

Previous studies have suggested that seed size has been a relevant trait promoting ecomorphological adaptations in the genus *Curculio* and driving species diversification (Hughes & Vogler 2004a; see also Peguero *et al.* 2017). In the end, a tight relationship between seed and weevils' body size would result in differences in the ability of larger and smaller weevils to infest seeds of different size (differences in trophic breadth); that is, small species would be able to infest both small and large seeds while species with a larger body size would be restricted to larger seeds to obtain enough resources to complete larvae development (see Bonal & Muñoz 2008; Espelta *et al.* 2009a; Bonal & Muñoz 2011; Peguero *et al.* 2017). Yet, this does not seem to be the case in our study system where hazels, the species infested by a single species (*C. nucum*), showed the largest seeds during most of the season (Fig. 3) and experienced the lower infestation rate (see Table S3). Instead of an influence of seed size, our results suggest that the exclusive infestation of hazelnuts by *C. nucum* could be more related to a different pattern of sexual maturation of females among the 2 weevil species emerging from the soil in spring, specifically an earlier maturation in *C. nucum* in comparison to *C. glandium* (Fig. 4). These differences could be due to differences between the 2 weevils in the requirements of resource acquisition as it has been demonstrated that sexual maturation in females of these synovigenic species critically requires some feeding at adulthood before reproductive development takes place (Bel-Venner *et al.* 2009; Pélisson *et al.* 2012). The early maturation in *C. nucum* would be advantageous to oviposit in hazelnuts before the hardening of the nutshell, as this is a fast process occurring during seed growth and the main mechanism in hazels to avoid infestation (Guidone *et al.* 2007). Moreover, oviposition of *C. nucum* would be expected to occur soon after mating as weevils do not adjust laying eggs to the moment of highest seed availability, but they oviposit as soon as females have mature eggs (Bonal *et al.* 2010). This behavior is probably linked to the temporal unpredictability of seed crop size (Bonal & Muñoz 2008; Espelta *et al.* 2008) and other constraints they have to cope with, such as the need of rainfall episodes to soften the soil and allow the emergence of adults (Bonal *et al.* 2010, 2015; Espelta *et al.* 2017). Only during the 2 earlier samplings, females of *C. nucum* seemed to preferentially choose bigger seeds, a behavior related with the need to select a minimum seed size to ensure larvae development and also owing to the availability of more seeds for oviposition (see Espelta *et al.* 2009a).

In comparison to the extreme host–parasite specificity of *C. nucum*, the other 3 weevils (*C. venosus*, *C. glandium* and *C. elephas*) predated indistinctively upon the 2 oaks with no evidence of a strategy in the partitioning of this resource according to the identity of the host species or to seed size. The avoidance of competitive exclusion among these species could be explained by several mechanisms contributing to stabilize their coexistence; that is, time partitioning (Pélisson *et al.* 2012; see also Espelta *et al.* 2009a) and/or diversification of dispersal-dormancy strategies (Pélisson *et al.* 2012). On the one hand, time partitioning in breeding activity can exist when 2 competing species differ in the speed of energy acquisition to be allocated to reproduction by females and the duration of their lifespan; that is, one species acquires resources faster and it is able to oviposit earlier on seeds, but it is exposed to a higher risk of seed abortion, while the other oviposits later but has a longer life span allowing it to lay eggs during a larger time frame (see Pelisson *et al.* 2012 for *C. pellitus* and *C. glandium*). On the other hand, stabilization can be reached by means of different dispersal versus dormancy strategies with some species relying on a high dispersal ability and others depending on dormancy strategies (e.g. variable diapause) to cope with seed scarcity. This seems to be the case for *C. glandium* and *C. elephas*; that is high dispersal ability (up to 11 km) in the former species and an extended diapause (up to 3 years) in the later (see Venner *et al.* 2011; Pélisson *et al.* 2012). Yet, other factors not covered in this study, such as the risk of parasitism or survival of larvae during diapause, may also help equalize their success to infest (Bonal *et al.* 2011). Similarly, future studies with more intense and appropriate sampling schemes should address the relationship between the number of adults of the different species and the number of larvae to disentangle the different predation rates upon each species and the influence of other environmental factors.

Ultimately, differences in the trophic breadth leave a contrasting genetic signature in the populations of the 2 species of weevils. A much higher number of local rare haplotypes were found in the monophagus *C. nucum*, along with a marginally significant genetic structure among populations, contrary to the more generalist *C. glandium* (see, for a similar example in aphids, Gaete-Eastman *et al.* 2004). Inter-specific differences in genetic characteristics of phytophagous insects could arise from differences in their dispersal ability or in the spatial distribution (isolated *vs* continuous) of the host (Peterson & Denno 1998; Kubish *et al.* 2014). Unfortunately, in comparison to the precise information about the dispersal ability of *C. glandium* (approximately 10 km in Pélisson *et al.* 2012), we lack detailed knowledge about the dispersal range of *C. nucum*, except some evidence of weevils moving away from local sites to feed during adulthood and prior to mating (Bel-Venner *et al.* 2009). Yet the fact that *C. nucum* and *C. glandium* are sister species (Hughes & Voegler 2004a) and they share many similar life-history traits, such as ecomorphological adaptations and body size, adult emergence in spring, synovigenic females and a fixed diapause of 2 years (see Hughes & Vogler 2004a; Bel-Venner *et al.* 2009; Pélisson *et al.* 2012a,b), make us consider that they may have a similar dispersal ability. Therefore, the differences we observed in their genetic characteristics would be probably due to their different diet breadth and the more patchy and discontinuous distribution of hazels in comparison to the more abundant and constant presence of oaks (Gracia *et al.* 2004; see also Fig. 1), along the geographical gradient sampled (approximately 225 km from Ripoll to Prades).

Connectivity may be critical for population survival (Fahrig & Merrian 1985; Fahrig & Paloheimo 1988) and metapopulation dynamics (Levins 1970), especially in front of a disturbance: for example, the negative impact of severe drought episodes for the emergence of adult weevils (Bonal *et al.* 2015; Espelta *et al.* 2017). In that sense, our molecular data show that such disturbances may have occurred and left their signature in *C. nucum* population genetics. The significant negative values retrieved in the neutrality tests (Tajima's D and Fu's F) indicate that most of the *C. nucum* populations sampled went through population expansion after recent bottlenecks. Almost half of the individuals had the same haplotype and there was an excess of rare haplotypes that differed little from the most common one. The lower gene flow between populations (marginally significant genetic structure among populations) in *C. nucum* would favor such bottlenecks as the patchy distribution of hazel trees would complicate the arrival of immigrants. None of this happened in the case of *C. glandium* feeding on the widespread oak trees. Yet the interpretation of the results for *C. nucum* must be cautious as the shallow genetic structure observed suggests that a fair amount of gene flow still occurs, enough to overcome drift. Moreover, in addition to the current distribution of hazelnuts, other abiotic environmental conditions could also be involved in the genetic structure observed in *C. nucum* (e.g. geological barriers or altitude for *Trichobaris soror* in De la Mora *et al.* 2015)

CONCLUSION

The use of molecular analyses allowed us to precisely identify the weevil species depredating upon the various potential hosts in these mixed deciduous forests and to unequivocally confirm the high specificity of the hazelnut *C. nucum* and the more flexible and wider trophic breadth of the rest of the weevils (*C. venosus*, *C. glandium* and *C. elephas*) depredating upon acorns. In this multi-host and multi-parasite system, co-existence of the various weevil species seems to be mediated by a combination of extreme resource partitioning (i.e. among *C. nucum* and the rest of species) and a combination of time partitioning and differences in dispersal-dormancy strategies among the 3 species depredating upon oaks. Interestingly, although sometimes suggested, differences in seed size did not have any effect in driving host specificity or the trophic breadth of the weevil species present. Moreover, our results highlight that differences in specificity in trophic breadth and in the spatial distribution of hosts at a large geographical scale may result in the presence of genetic structure among the populations of highly specific parasites (*C. nucum*) depredating upon patchily distributed hosts (*Co. avellana*). To what extent this restriction in gene flow (dispersal) may have negative consequences for the persistence of the populations of these highly specialized seed-feeding pests in a time of increasing disturbances (e.g. drought events in Mediterranean areas) is a fascinating question that deserves further research.

ACKNOWLEDGMENTS

This research was supported by the projects FOR-ASSEMBLY (CGL2015-70558-P) and PLAGANADO (AGL2014-54739-R) of the Spanish Ministry of Economy, and the projects BEEMED (SGR913) (Generalitat de Catalunya) and PII1C09-0256-9052 (Regional Government of Castilla-La Mancha and the European Social Fund). R.B. was funded by a contract of the Program Atracción de Talento Investigador (Gobierno de Extremadura).

REFERENCES

AliNiazee MT (1998). Ecology and management of hazelnuts pests. *Annual Review of Entomology* **43**, 395–41.

Bale JS, Masters GJ, Hodkinson ID, Awmack *et al*. (2002). Herbivory in global climate change research: Direct effects of rising temperature on insect herbivores. *Global Change Biology* **8**, 1–16.

Barat M, Tarayre M, Atlan A (2007). Plant phenology and seed predation: Interactions between gorses and weevils in Brittany (France). *Entomologia Experimentalis et Applicata* **124**, 167–76.

Bel-Venner MC, Mondy N, Arthaud F *et al.* (2009). Ecophysiological attributes of adult overwintering in insects: Insights from a field study of the nut weevil, *Curculio nucum*. *Physiological Entomology* **34**, 61–70.

Bernays EA, Graham M (1988). On the evolution of host specificity in phytophagous arthropods. *Ecology* **69**, 886–92.

Bonal R, Muñoz A (2008). Seed growth suppression constrains the growth of seed parasites: Premature acorn abscission reduces *Curculio elephas* larval size. *Ecological Entomology* **33**, 31–6.

Bonal R, Muñoz A (2009). Seed weevils living on the edge: Pressures and conflicts over body size in the endoparasitic *Curculio* larvae. *Ecological Entomology* **34**, 304–9.

Bonal R, Muñoz A, Díaz M (2007). Satiation of predispersal seed predators: The importance of considering both plant and seed levels. *Evolutionary Ecology* **21**, 367–80.

Bonal R, Muñoz A, Espelta JM (2010). Mismatch between the timing of oviposition and the seasonal optimum. The stochastic phenology of Mediterranean acorn weevils. *Ecological Entomology* **35**, 270–78.

Bonal R, Espelta JM, Vogler AP (2011). Complex selection on life-history traits and the maintenance of variation in exaggerated rostrum length in acorn weevils. *Oecologia* **167**, 1053–61.

Bonal R, Hernández M, Ortego J, Muñoz A, Espelta, JM (2012). Positive cascade effects of forest fragmentation on acorn weevils mediated by seed size enlargement. *Insect Conservation and Diversity* **5**, 381–8.

Bonal R, Hernández M, Espelta JM, Muñoz A, Aparicio JM (2015). Unexpected consequences of a drier world: Evidence that delay in late summer rains biases the population sex ratio of an insect. *Royal Society Open Science* **2**, 150198.

Coyle DR, Mattson WJ Jr, Jordan MS, Raffa KF (2012). Variable host phenology does not pose a barrier to invasive weevils in a northern hardwood forest. *Agricultural and Forest Entomology* **14**, 276–85.

Dupanloup S, Schneider I, Excoffier L (2002). A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology* **11**, 2571–81.

Espelta JM, Cortés P, Molowny-Horas R, Sánchez-Humanes B, Retana J (2008). Masting mediated by summer drought reduces acorn predation in Mediterranean oak forests. *Ecology* **89**, 805–17.

Espelta JM, Bonal R, Sánchez-Humanes B (2009a). Pre-dispersal acorn predation in mixed oak forests: interspecific differences are driven by the interplay among seed phenology, seed size and predator size. *Journal of Ecology* **97**, 1416–23.

Espelta JM, Cortés P, Molowny-Horas R, Retana J (2009b). Acorn crop size and pre-dispersal predation determine inter-specific differences in the recruitment of co-occurring oaks. *Oecologia* **161**, 559–68.

Espelta JM, Arias-LeClaire H, Fernández-Martínez M, Doblas-Miranda E, Muñoz A, Bonal R (2017). Beyond predator satiation: Masting but also the effects of rainfall stochasticity on weevils drive acorn predation. *Ecosphere* **8**, e01836.

Excoffier L, Laval G, Schneider S (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**, 47–50.

Fahrig L, Merriam G (1985). Habitat patch connectivity and population survival. *Ecology* **66**, 1762–8.

Fahrig L, Paloheimo J (1988). Effect of spatial arrangement of habitat patches on local population size. *Ecology* **69**, 468–75.

Forister ML, Novotny V, Panorska AK *et al.* (2015). The global distribution of diet breadth in insect herbivores. *PNAS* **112**, 442–7.

Fu YX (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics **147**, 915–25.

Gaete-Eastman C, Figueroa CC, Olivares-Donoso R, Niemeyer HM, Ramírez CC (2004). Diet breadth and its relationship with genetic diversity and differentiation: The case of southern beech aphids (Hemiptera: Aphididae). *Bulletin of Entomological Research* **94**, 219–27.

Govindan BN, Kéry M, Swihart RK (2012). Host selection and responses to forest fragmentation in acorn weevils: inferences from dynamic occupancy models. *Oikos* **121**, 623–33.

Gracia C, Ibàñez JJ, Burriel JA, Mata T, Vayreda J (2004). *Inventari Ecològic i Forestal de Catalunya*. CREAF, Bellaterra.

Guidone L, Valentini N, Rolle L, Me G, Tavella L (2007). Early nut development as a resistance factor to the attacks of *Curculio nucum* (Coleoptera: Curculionidae). *Annals of Applied Biology* **150**, 323–9.

Jervis MA, Ellers J, Harvey JA (2008). Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. *Annual Review of Entomology* **53**, 361–85.

Heiniger C, Barot S, Ponge JF *et al*. (2014). Effect of habitat spatiotemporal structure on collembolan diversity. *Pedobiologia* **57**, 103–17.

Hood GR, Ott JR (2010). Developmental plasticity and reduced susceptibility to natural enemies following host plant defoliation in a specialized herbivore. *Oecologia* **162**, 673–83.

Hughes J, Vogler AP (2004a). Ecomorphological adaptation of acorn weevils to their oviposition site. *Evolution* **58**, 1971–83.

Hughes J, Vogler AP (2004b). The phylogeny of acorn weevils (genus *Curculio*) from mitochondrial and nuclear DNA sequences: The problem of incomplete data. *Molecular Phylogenetics and Evolution* **32**, 601–15.

Kergoat GJ, Delobel A, Fédière G, Le Rü B, Silvain JF (2005). Both host–plant phylogeny and chemistry have shaped the African seed-beetle radiation. *Molecular Phylogenetics and Evolution* **35**, 602–11.

Kruess A (2003). Effects of landscape structure and habitat type on a plant–herbivore–parasitoid community. *Ecography* **26**, 283–90.

Levins R (1970). Extinctions. Some mathematical questions in biology. In: Gerstenhaber R, ed. *Lectures on Mathematics in Life Sciences 2*. The American Mathematical Society, Providence, RI, pp. 77–107.

Kubisch A, Holt RD, Poethke HJ, Fronhofer EA (2014). Where am I and why? Synthesizing range biology and the eco–evolutionary dynamics of dispersal. *Oikos* **123**, 5–22.

Merville A, Venner S, Henri H *et al*. (2013). Endosymbiont diversity among sibling weevil species competing for the same resource. *BMC Evolutionary Biology*, **13**, 28.

Muñoz A, Bonal R, Espelta JM (2014). Acorn–weevil interactions in a mixed-oak forest: Outcomes for larval growth and plant recruitment. *Forest Ecology and Management* **322**, 98–105.

Newman D, Pilson D (1997). Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. *Evolution* **51**, 354–62.

Peguero G, Bonal R, Sol D, Muñoz A, Sork VL, Espelta JM (2017). Tropical insect diversity: Evidence of greater host specialization in seed-feeding weevils. *Ecology* **98**, 2180–90.

Pélisson PF, Bel–Venner M, Rey B *et al*. (2012). Contrasted breeding strategies in four sympatric sibling insect species: When a proovigenic and capital breeder copes with a stochastic environment. *Functional Ecology* **26**, 198–206.

Pélisson PF, Bel-Venner MC, Giron D, Menu F, Venner S (2013a). From income to capital breeding: When diversified strategies sustain species coexistence. *PloS One* **8**, e76086.

Pélisson PF, Bernstein C, Francois D, Menu F, Venner S (2013b). Dispersal and dormancy strategies among insect species competing for a pulsed resource. *Ecological Entomology* **38**, 470–77.

Peterson MA, Denno RF (1998). The influence of dispersal and diet breadth on patterns of genetic isolation by distance in phytophagous insects. *The American Naturalist* **152**, 428–46.

Ratnasingham S, Hebert PDN (2007). Bold: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* **7**, 355–64.

Richard R, Casas J (2009). Stochasticity and controllability of nutrient sources in foraging: Host–feeding and egg resorption in parasitoids. *Ecological Monographs* **79**, 465–83.

Ruiz–Carbayo H, Bonal R, Espelta JM, Hernández M, Pino J (2017). Community assembly in time and space: The case of Lepidoptera in a *Quercus ilex* L. savannah–like landscape. *Insect Conservation and Diversity* **10**, 21–31.

Solar A, Stampar F (2011). Characterisation of selected hazelnut cultivars: Phenology, growing and yielding capacity, market quality and nutraceutical value. *Journal of the Science of Food and Agriculture* **91**, 1205–12.

Son Y, Lewis EE (2005). Effects of temperature on the reproductive life history of the black vine weevil, *Otiorhynchus sulcatus*. *Entomologia experimentalis et applicate* **114**, 15–24.

Tajima F (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**, 585–95.

Thomas CD, Bodsworth EJ, Wilson RJ *et al*. (2001). Ecological and evolutionary processes at expanding range margins. *Nature* **411**, 577–81.

Toju H, Fukatsu T (2011). Diversity and infection prevalence of endosymbionts in natural populations of the chestnut weevil: Relevance of local climate and host plants. *Molecular Ecology* **20**, 853–68.

Trudel R, Lavallee R, Guertin C (2002). The effect of cold temperature exposure and long–day photoperiod on the termination of the reproductive diapause of newly emerged female *Pissodes strobi* (Coleoptera: Curculionidae). *Agricultural and Forest Entomology* **4**, 301–8.

Venner S, Pélisson PF, Bel-Venner MC, Débias F, Rajon E, Menu F (2011). Coexistence of insect species competing for a pulsed resource: toward a unified theory of biodiversity in fluctuating environments. *PloS One* **6**, e18039.

Ylioja T, Roininen H, Ayres MP, Rousi M, Price PW (1999). Host-driven population dynamics in an herbivorous insect. *PNAS*, 10735–40.

SUPPLEMENTARY MATERIALS

Additional supporting information may be found in the online version of this article.

Table S1 Number of *Curculio nucum* individuals bearing each haplotype in the 5 study populations

Table S2 Number of *Curculio glandium* individuals bearing each haplotype in the 5 study populations

Table S3 Mean ± SE density of host plants and the percentage of sound and infested seeds per location and host plant Density of host plants was calculated as the mean of the nearest inventoried plots included in the Catalan Forest Inventory (Gracia *et al.* 2004).

Cite this article as:

Arias-LeClaire H, Bonal R, García-López D, Espelta JM (2018). Role of seed size, phenology, oogenesis and host distribution in the specificity and genetic structure of seed weevils (*Curculio* spp.) in mixed forests. *Integrative Zoology* **13**, 267–79.

Table S1 Number of *Curculio nucum* individuals bearing each haplotype in the five study populations

Haplotype	Maresme	Montseny	Olot	Prades	Ripoll
1					2
2					1
3	1	1		1	
4		1			
5					1
6					1
7	2	3			
8		1		1	
9		1			
10	1				
11	1	7	13	28	4
12	3	4		1	
13	1	1	1		
14		1			
15		1	1		
16		1			
17		1			
18			1		2
19	1		1		
20			1		
21		1	2		
22			1		
23			1	4	
24			1		
25			2		
26				6	
27				2	
28				1	
29				1	
30			1		
31					1

Table S2 Number of *Curculio glandium* individuals bearing each haplotype in the five study populations

Haplotype	Maresme	Montseny	Olot	Prades	Ripoll
1	11	15	11	4	6
2	1				1
3			1		1
4	8	9	5	7	8
5	1	3		1	1
6		1			
7		1			
8			1		
9	1				
10	2				
11		1			
12		1			1
13	1				

Table S3 Mean ± SE density of host plants and the percentage of sound and infested seeds per location and host plant. Density of host plants was calculated as the mean of the nearest inventoried plots included in the Catalan Forest Inventory (Gracia *et al.* 2004).

	Q. pubescens			*Q. ilex*			*C. avellana*		
	Density (stems ha^{-1})	Sound (%)	Infested (%)	Density (stems ha^{-1})	Sound (%)	Infested (%)	Density (stems ha^{-1})	Sound (%)	Infested (%)
Ripoll	778 ± 64	25 ± 5	75 ± 9	-	-	-	341 ± 41	60 ± 4	40 ± 7
Olot	1021 ± 131	29 ± 11	71 ± 7	456 ± 33	45 ± 8	55 ± 11	441 ± 39	75 ± 8	25 ± 9
Montseny	678 ± 64	55 ± 9	41 ± 5	714 ± 81	60 ± 7	40 ± 5	456 ± 64	76 ± 11	24 ± 10
Maresme	512 ± 131	50 ± 8	50 ± 6	915 ± 44	59 ± 11	41 ± 6	501 ± 73	89 ± 12	11 ± 4
Prades	455± 96	64 ± 5	36 ± 7	875 ± 74	67 ± 6	33 ± 9	315 ± 84	76 ± 11	24 ± 8

This page intentionally left blank

Beyond predator satiation: Masting but also the effects of rainfall stochasticity on weevils drive acorn predation.

Espelta J M, Arias-LeClaire H, Fernández-Martínez M, Doblas-Miranda E, Muñoz A, Bonal R (2017). Beyond predator satiation: Masting but also the effects of rainfall stochasticity on weevils drive acorn predation. Ecosphere 8(6): e01836.10.1002/ecs2.1836 DOI10.1002/ecs2.1836

Accepted and published on June 2017
Ecosphere, Impact Factor: 2.671, ISI Ranking 2017: 55/158 (Ecology), Online ISSN: 2150-8925.

http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1836/full

This page intentionally left blank

esa ECOSPHERE

Beyond predator satiation: Masting but also the effects of rainfall stochasticity on weevils drive acorn predation

Josep Maria Espelta,[1,†] Harold Arias-LeClaire,[1,2] Marcos Fernández-Martínez,[1,3]
Enrique Doblas-Miranda,[1] Alberto Muñoz,[4] and Raúl Bonal[5,6]

[1]*CREAF, Cerdanyola del Vallés, 08193 Catalonia, Spain*
[2]*Escuela de Ciencias Exactas y Naturales, Universidad Estatal a Distancia, Mercedes de Montes de Oca, San José 474-2050 Costa Rica*
[3]*CSIC, Global Ecology Unit, CREAF-CSIC-UAB, Cerdanyola del Vallés, 08193 Catalonia, Spain*
[4]*Departamento de Didáctica de las Ciencias Experimentales, Facultad de Educación, Universidad Complutense de Madrid, 28040 Madrid, Spain*
[5]*Forest Research Group, INDEHESA, University of Extremadura, 10600 Plasencia, Spain*
[6]*Grupo de Investigación de la Biodiversidad Genética y Cultural, Instituto de Investigación en Recursos Cinegéticos CSIC-UCLM-JCCM, 13005 Ciudad Real, Spain*

Citation: Espelta, J. M., H. Arias-LeClaire, M. Fernández-Martínez, E. Doblas-Miranda, A. Muñoz, and R. Bonal. 2017. Beyond predator satiation: Masting but also the effects of rainfall stochasticity on weevils drive acorn predation. Ecosphere 8(6):e01836. 10.1002/ecs2.1836

Abstract. Escaping seed predation is a classic "economy of scale" hypotheses (*predator satiation hypothesis, Psh*) to explain the selection for the synchronous production of massive and nil seed crops (masting) in plants. The *Psh* postulates that predator satiation occurs through a combination of (1) "functional satiation," as not all seeds can be consumed during a massive crop, and (2) "numerical satiation," as predator populations collapse during poor crop years. Many studies advocate for the *Psh*, but few have investigated the importance of masting compared to other factors for the control of predation extent. Namely, environmental cues prompting masting could also determine predator's success and, ultimately, influence directly and independently seed predation intensity. We explored this question in Mediterranean oaks, as they exhibit strong masting behavior; acorns are heavily predated upon by weevils; and rainfall stochasticity drives masting and the emergence of adult weevils from the soil. Results of two mid-term studies (4 and 11 yr) showed that acorn production and predation were highly variable across years, while the abundance of adult weevils was positively related to autumn rainfall and to the number of infested acorns the previous years. Ultimately, acorn predation was negatively influenced by inter-annual fluctuation of seed production (masting) yet, mainly and positively, prompted by autumn rainfall and acorn crop size (only in one site). Our results highlight the relevance of masting to reduce seed predation. Yet evidences that rainfall stochasticity directly determines the success of weevils, and it independently influences seed predation extent, indicate that environmental cues prompting masting may also fine-tune the output of this reproductive behavior. Additionally, local differences suggest that the relevance of masting may change with tree characteristics (low vs. high seed production) and landscape structure (isolated vs. dense forests). We also discuss what can be the effects of increasing drought in Mediterranean areas for this antagonistic interaction, triggered by rainfall.

Key words: *Curculio* spp.; extended diapause; granivory; Mediterranean oak forests; plant–animal interactions; pre-dispersal seed predation; *Quercus ilex;* resource pulses.

Received 27 April 2017; accepted 28 April 2017. Corresponding Editor: T'ai Roulston.

† **E-mail:** josep.espelta@uab.cat

INTRODUCTION

The synchronous and intermittent production of large and nil seed crops (often termed *masting*) is a taxonomically and geographically widespread reproductive behavior among plants (Kelly and Sork 2002). The superabundance of seeds in certain years combined with periods of almost no reproduction not only influences the temporal patterns of seedling recruitment in these species (De Steven and Wright 2002, Oddou-Muratorio et al. 2011) but also results in an erratic pulse of resources triggering a cascade of "bottom-up" effects across trophic levels: seed consumers (Selva et al. 2012), predators of seed consumers (Ritchie and Johnson 2009), parasites (Jones et al. 1998), and even parasitoids (Satake et al. 2004).

Evolutionary hypotheses for the advantages of masting as a reproductive strategy are based on an "economy of scale" principle: A single but extraordinary reproductive episode should be more beneficial than continuous but moderate reproductive events (Kelly and Sork 2002). In this sense, the advantage of masting as a mechanism to reduce seed predation (*the seed predation satiation hypothesis* sensu Janzen 1971, see also Silverstown 1980) has probably been one of the hypotheses receiving greatest attention (Kelly et al. 2000, Yu et al. 2003, Espelta et al. 2008, Fukumoto and Kajimura 2011, Archibald et al. 2012, Moreira et al. 2017). As proposed by Satake et al. (2004), the synchronous, irregular production of large and nil seed crops would lead to predator satiation by combining two different mechanisms: (1) "functional satiation" (large seed crops surpass the feeding capacity of predators, see also Ims 1990) and (2) "numerical satiation" (the current number of predators may be too low to consume many seeds if the previous year's crop was small, Bonal et al. 2012). Certainly, masting is expected to be especially effective in controlling predators with specialized seed-feeding habits, limited mobility, and a short life span encompassed within the seeding season (Shibata et al. 1998). This is the case of many preeminent seed consumers such as granivorous insects (e.g., weevils in Mulder et al. 2012, Muñoz et al. 2014, bruchids in Peguero et al. 2014).

Evidences of the benefits that extreme interannual variability in seed production may play in reducing seed predation have been extensively suggested (Satake et al. 2004, Espelta et al. 2008, Peguero et al. 2014, Moreira et al. 2017). However, few studies have tested whether environmental factors (proximate causes) influencing masting might also be directly involved in controlling seed predator's populations and thus also contribute to escape seed predation (but see Poncet et al. 2009). Therefore, while the relevance of environmental conditions for synchrony in reproduction and variability in seed crops has been thoroughly investigated (temperature in Schauber et al. 2002, Kelly et al. 2013, rainfall in Pérez-Ramos et al. 2010, Fernández-Martínez et al. 2015, or even "weather packages" in Fernández-Martínez et al. 2016, see also Koenig et al. 2016), their potential direct impact on the predators that consume these seeds has often been neglected. Temperature and precipitation may certainly have an effect on predation extent by determining food resource (crops) variability, but they may also directly influence the population size or the performance of seed consumers. For example, meteorological conditions are known to directly affect the population size and activity of rodents (e.g., squirrels in Kneip et al. 2011, wood mice in Wróbel and Bogdziewicz 2015, Sunyer et al. 2016) and ungulates (Servanty et al. 2009). Similarly, rainfall amount and its seasonal distribution may influence oviposition by granivorous insects (Bonal et al. 2010) by affecting the number of adults emerging from the soil (Schraer et al. 1998), or even altering their sex ratio (Bonal et al. 2015).

Disentangling the issue as to whether seed predation is mainly controlled by variability in seed production or by the direct impact of meteorological conditions on predators may help in shedding light on the evolution of this reproductive behavior in plants as well as to understand its relevance under new climatic scenarios arising from climate change. Mediterranean oaks (*Quercus* spp.) offer a worthwhile opportunity to examine this question since they show clear masting behavior (Koenig and Knops 2000) and acorns are largely consumed by seed predator specialists such as acorn weevils (*Curculio* spp.; Bonal et al. 2007). Moreover, rainfall stochasticity, including the occurrence of severe drought episodes, influence masting in these species (Espelta et al. 2008, Pérez-Ramos et al. 2010, Fernández-Martínez et al. 2012) and the emergence of adult weevils from the soil after diapause (Bonal et al. 2010). Thus, the main aim of this study was to elucidate the contribution of the inter-annual variability in acorn crop size and the direct effects of precipitation (i.e., conditioning the likelihood of acorn weevil emergence from the soil) for the extent of acorn predation. To test this, we used an intensive four-year monitoring of acorn production, rainfall variability, adult weevil emergence from the soil after diapause, and acorn predation in isolated *Quercus ilex* trees in a savannah-like landscape, and also a database (11 yr) of acorn production, rainfall variability, and acorn predation by weevils in 15 forest stands. We specifically assessed (1) the inter-annual variability of acorn production and acorn predation, (2) the effect of rainfall on adult emergence from the soil and thus on the abundance of adult weevils, and (3) the dependence of acorn predation rates on current crop size, inter-annual variability in acorn crop sizes, and rainfall amount, as a surrogate of the abundance of adult weevils. We hypothesize that, apart from the reported effects of rainfall stochasticity in determining masting in Mediterranean oaks (Espelta et al. 2008, Fernández-Martínez et al. 2012), it may play a relevant role in acorn infestation through its direct effect on the number of adult weevils present, thus fine-tuning the expected effects of masting on predation.

MATERIALS AND METHODS

Study area and species

This study was conducted in two different areas of Spain: Huecas (Toledo, central Spain) and Collserola (Barcelona, northeast Spain; Fig. 1) where the reproductive patterns of *Quercus ilex* and acorn predation by weevils (*Curculio*

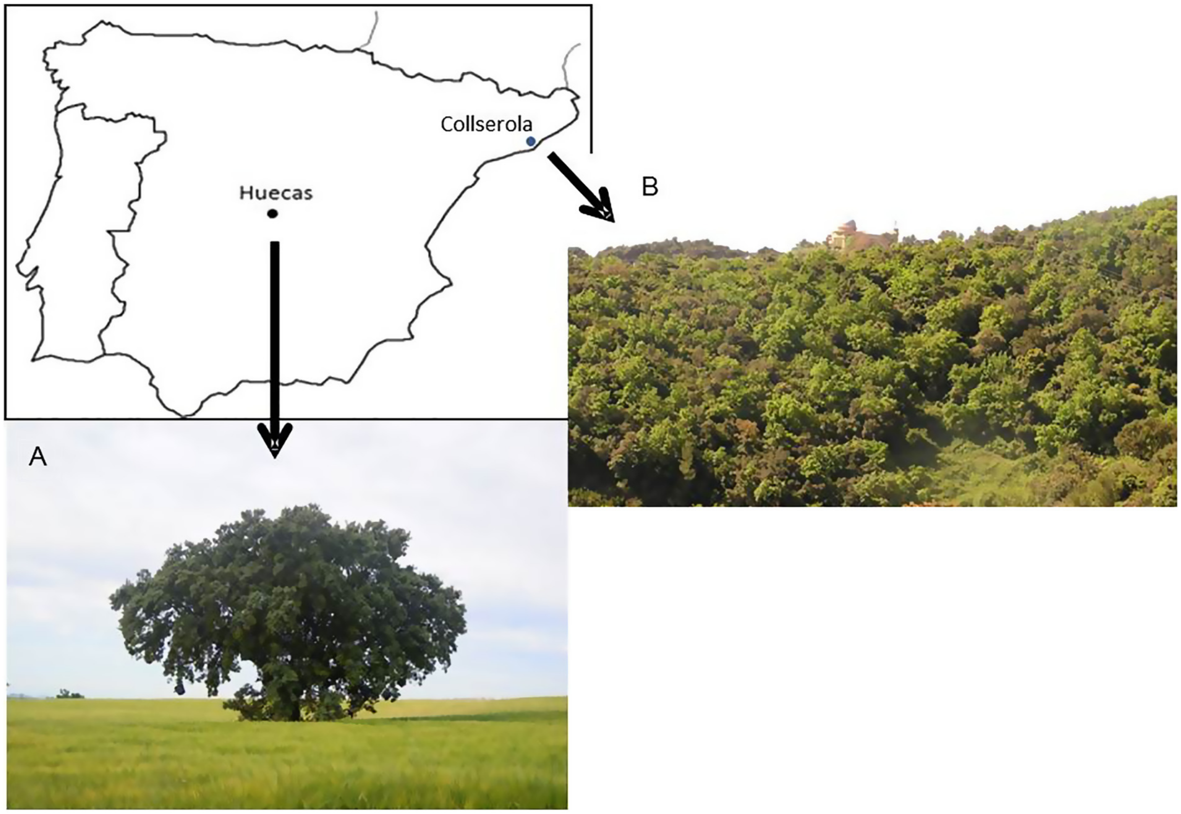


Fig. 1. Location of the study sites (Huecas and Collserola) in Peninsular Spain. Notice that Huecas (A) corresponds to a savannah-like landscape (“dehesa”) with scattered oaks, while Collserola (B) is an old-coppiced oak forest. Photograph A courtesy of Helena Ruíz-Carbayo.

spp.) have been thoroughly studied (Bonal et al. 2007, 2010, Espelta et al. 2008, 2009). Huecas is a savannah-like landscape with scattered *Q. ilex* trees (from 1 to 100 trees/ha, up to 2 km apart) embedded in a cropland matrix (Fig. 1A). Climate is continental Mediterranean with cold winters, extremely hot, dry summers, and rain concentrated in spring and autumn. Mean annual temperature ranges from 14.6° to 16°C, and mean annual precipitation ranges from 350 to 450 mm per year. The landscape structure allows the intensive monitoring of separate individual trees that are like "islands" for insects' specialists on oaks (Ruíz-Carbayo et al. 2017). For the purpose of this study, we selected 24 focal trees and monitored acorn production and seed predation rates over four years (2009, 2010, 2011, and 2012) and adult weevil emergence from the soil beneath each tree over five years (2008, 2009, 2010, 2011, and 2012). Rainfall patterns from 2008 to 2012 were obtained from a local weather station. Collserola Natural Park is a coastal massif covered by dense, old-coppiced *Q. ilex* forests (see Fig. 1B). Climate is typically Mediterranean, with 614 mm of mean annual precipitation and monthly temperatures with a maximum of 24.3° ± 0.7°C in August and a minimum of 8.5° ± 0.6°C in January. Summer is the warmest, driest season, while most rainfall occurs in spring and autumn (Fernández-Martínez et al. 2012). For the purposes of this study, we used the data collected from 15 *Q. ilex* trees in 15 forest stands (~225 trees) where acorn production and seed predation were monitored from 1998 to 2009 (see Espelta et al. 2008 for methodological details). The annual pattern of rainfall per plot from 1998 to 2009 was obtained from the meteorological database developed in the MONTES project (M. Ninyerola and M. Batalla).

Quercus ilex acorns mature in one year and seed production is subjected to strong inter-annual fluctuations (Espelta et al. 2008). In Collserola, acorns are subjected to pre-dispersal predation by two weevil species, *Curculio glandium* and *Curculio elephas* (Espelta et al. 2009), while *C. elephas* is the prevalent acorn parasite in Huecas (Bonal et al. 2007). *Curculio glandium* is widely distributed in Europe associated with oak trees (*Quercus* spp.), while *C. elephas* is present in central and southern Europe, also associated with oaks (*Quercus* spp.) and chestnuts (*Castanea sativa*). In the two study areas, both weevils can only infest acorns since there are no chestnuts around. Both *C. glandium* and *C. elephas* overwinter underground, but the two species present different phenologies: *C. glandium* emerges earlier, mostly in spring (Pélisson et al. 2013), while the emergence of *C. elephas* is restricted to autumn (Espelta et al. 2009, Bonal et al. 2012). After emerging, adult weevils climb to the crown for mating. This is the time when acorn predation also takes place (Bonal and Muñoz 2009, Espelta et al. 2009), after females drill a tiny hole in the seed cover with their rostrum and generally deposit a single egg in the developing acorn using their oviscapt. The two weevil species also differ in their dispersal ability and dormancy strategy. Adults of *C. glandium* emerge from the soil two years after larvae development and are able to fly up to 10 km, while *C. elephas* emerges in early autumn and has a much lower dispersal ability (Venner et al. 2011, Pélisson et al. 2013). In fact for *C. elephas*, genetic differences and restricted gene flow among populations are observed beyond 300 m (H. Ruiz-Carbayo, *unpublished manuscript*). This species also has the ability to spread the emergence of adults up to three years (~66% emerging the first year, 30% in the second, and 4% during the third year; see Venner et al. 2011, Pélisson et al. 2013).

Sampling design

In Huecas, acorns were collected using buckets (0.12 m^2 × 0.5 m) hung from the lower branches of every tree to prevent predation by large ungulates. The number of traps per tree was proportional to its canopy surface, and covered at least 1.5–2% of the canopy (see Bonal and Muñoz 2009 for details). Seed traps were sampled periodically and, after the first infested acorns were collected, traps were checked every 10 d until acorn fall ceased. On each revision, seeds were taken to the laboratory to identify sound and infested ones (Bonal and Muñoz 2009). In addition, to estimate the population size of acorn weevils and the phenology of their emergence from the ground, a survey of adult emergence was carried out from August 2008 to October 2012, using emergence traps attached to the trunks of focal trees. Each trap consisted of a cone of mosquito netting attached to the tree trunk with a closed bottle on top. After emerging from the soil, the weevils climbed up the trunk and were trapped in the bottle (see Bonal et al. 2012 for further details). Distance between trees and their location in a hostile media for weevils (croplands) make successful dispersal of *C. elephas* among trees almost impossible (Bonal et al. 2012). Traps were checked on a daily basis from August to late October in order to record the number of individuals that had emerged. At the same time, weekly precipitation was measured at a local weather station for the same period in order to determine the possible influence of rainfall on the emergence of adults.

In Collserola Natural Park, acorn production and predation rates were recorded in 15 plots established in oak forests in 1998. At each plot, 15 trees were randomly selected (225 trees in total) from among those with most of their crowns exposed to full sun and with similar dbh. Each tree was tagged and four branches of similar size (~2–3 cm in diameter) were randomly chosen from different sections of the canopy (see Espelta et al. 2008 for further details). From 1998 to 2009, the number of acorns produced and infested was counted on these branches at the peak of the acorn crop in the area (usually in early to mid-September, Espelta et al. 2009). This sampling protocol was selected because the intermingling of tree branches precludes the possibility of individual monitoring of seed production per tree by means of seed traps (see Fig. 1B). Similarly, surveys of adult emergence were not conducted owing to the difficulties of performing them in these dense old-coppiced forests (~1500–2000 individuals/ha).

For each tree, we calculated the number of sound acorns produced per year, the number of parasited acorns, the inter-annual variability in acorn production (CV, coefficient of variation of seed production across years), and synchrony in the pattern of seed production. Synchrony was calculated as the Pearson's coefficient of correlation (r) of non-log-transformed data of each tree with the rest of trees in Huecas and with all trees in the same plot in Collserola (see Espelta et al. 2008 for further details).

Data analysis

Generalized linear mixed models (GLMMs), following a binomial distribution, were used to test for the effects of several variables on acorn predation rate (i.e., the ratio of infested acorns to the total number of acorns in year t). Seed predation rate is the variable commonly used to investigate whether temporal variability in seed production (masting) may influence seed predation extent (see Satake et al. 2004, Bonal et al. 2007, Espelta et al. 2008, Moreira et al. 2017), as the use of the crude number of seeds depredated can be tightly linked to the number of seeds available. The rationale behind the inclusion of the different independent variables in the model and their expected impact (positive or negative) on acorn predation is listed below:

1. *Acorn crop size in year t*.—This variable may have a negative effect on predation if functional satiation takes place (Satake et al. 2004). Conversely, it could have a positive effect, especially in Collserola where the weevil with the highest dispersal ability is found (*C. glandium*), if weevils are attracted by more productive trees. Therefore, differences between the two study sites Huecas and Collserola may be expected due to their extremely different tree density.
2. *Number of infested acorns in year*$_{t-1}$ *and year*$_{t-2}$.—The number of infested acorns during previous years can be assumed to be an estimate of the current size of the weevils' population in year t, especially if environmental conditions do not affect their success (no effects of rain). We added infested acorns in the previous two years, as the weevil species present both in Collserola and in Huecas (*C. elephas*) exhibits extended diapause (i.e., 96% of adults emerging in two years; Venner et al. 2011), and the other species present in Collserola (*C. glandium*) emerges after two years (Venner et al. 2011). This variable is expected to increase acorn predation (Bonal et al. 2010).
3. *Inter-annual fluctuation on seed production (i.e., ratio of mature acorn crop size in year t to that in year*$_{t-1}$ *and year*$_{t-2}$*)*.—Inter-annual fluctuation in seed production is the variable often used to explore the potential advantages of masting for escaping seed predation by combining the numerical and functional satiation of predators (see Satake et al. 2004, Espelta et al. 2008, Tachiki and Iwasa 2013, Moreira et al. 2017). This variable is the ratio of the

size of the current acorn crop available to previous year crops, as the proxy of the potential number of adult weevils present in year *t*. This ratio is expected to be negatively related to acorn predation: A high ratio (a large acorn crop size in year *t* and a low seed production in previous years) would mean the presence of few adult weevils and a high number of acorns in year *t*, thus resulting in low predation rates.

4. *Rainfall*.—As rainfall amount is suggested to be a crucial factor to allow the emergence of adult weevils from the soil (Alverson et al. 1984), and this may directly affect acorn predation, we included in the analyses the values of total rainfall for the periods when the emergence of weevils has been observed in our study areas in previous studies: from September to October for *C. elephas* in Huecas and Collserola (Espelta et al. 2009, Bonal et al. 2010) and from May to June for *C. glandium* in Collserola (Espelta et al. 2009). In the two sites, we expected rainfall to have a positive effect on acorn predation as the greater the amount of rainfall, the more weevils would be able to emerge from the soil (Schraer et al. 1998) and potentially infest acorns.

The "plot" and the "tree" factors in Collserola and the "tree" factor in Huecas were included as random effects in the GLMM analyses to account for the repeated nature of the measurements and other unexplained variation. Selection of the most adequate model was done by using the dredge function of the MuMIn package in R (Barton 2015). Comparison of sets of alternative models was done by using differences in the second-order (or corrected) Akaike Information Criterion ($\mathrm{AIC_c}$) and contrasting models by using χ^2 tests. Inclusion of a variable in the model required a significant χ^2 test ($P = 0.05$) and a difference between $\mathrm{AIC_{cs}}$ (delta: $\Delta\mathrm{AIC_c}$) of at least of four units compared to the same model excluding that variable. Pseudo-coefficients of determination (R^2) were used to estimate the contribution (in %) of fixed effects (R^2_{marginal}) and both fixed and random effects ($R^2_{\mathrm{conditional}}$) in explaining the variability of acorn predation.

To test for the main factors accounting for the abundance of adult weevils in a given year in the site where we monitored their emergence (Huecas), we ran a GLM including the number of weevils emerging per tree and year as the dependent variable and rainfall during the season of emergence (from September to October) and the number of acorns infested during year_{t-1} and year_{t-2} per tree as the independent factors. In addition, we analyzed the relationship between rainfall and emergence of adult weevils from the soil at a fine-grained temporal scale by plotting the number of emerged weevils and the amount of rainfall per week. We then ran correlation analyses comparing rainfall accumulated in one or two weeks and the number of emerged weevils one week later, since a minimum rainfall threshold is needed to soften the soil enough for weevil emergence (Mulder et al. 2012 and references therein).

Data analyses were performed using the R statistical software program, version 3.1.1 (R Development Core Team 2014).

RESULTS

Annual acorn production varied in both study sites with years of abundant and low seed crops, although this pattern was much more evident in Collserola than in Huecas (Fig. 2, CV = 1.97 ± 0.07 in Collserola and 0.94 ± 0.11 in Huecas). In Collserola, oaks usually exhibited very low acorn production (422 ± 139 $\mathrm{acorns \cdot tree^{-1} \cdot yr^{-1}}$), and there were high pulses of production in only three out of 11 yr (Fig. 2A): remarkable peaks in 2002, 2004, and 2008 and almost no mature acorn production in 2000, 2001, and 2005. In Huecas, trees were more productive (5281 ± 1608 $\mathrm{acorns \cdot tree^{-1} \cdot yr^{-1}}$), yet similar to Collserola, and despite a shorter time series, there was a year with a remarkable crop size (2012) preceded by some with lower values (Fig. 2B). Synchrony among trees in their inter-annual pattern of seed production was also higher in Collserola than in Huecas (Pearson's *r*: 0.67 ± 0.05 and 0.36 ± 0.09, respectively). Parallel to the large variation observed in seed production, the proportion of acorns depredated by weevils exhibited large variability over the years in both sites, with a pattern of low values in years of high seed production and more variable rates (both low and high) in years of lower acorn crops (Fig. 2). In Collserola, the higher predation rates were observed in 2001, 2003, 2006, and 2009 as opposed to 2000,

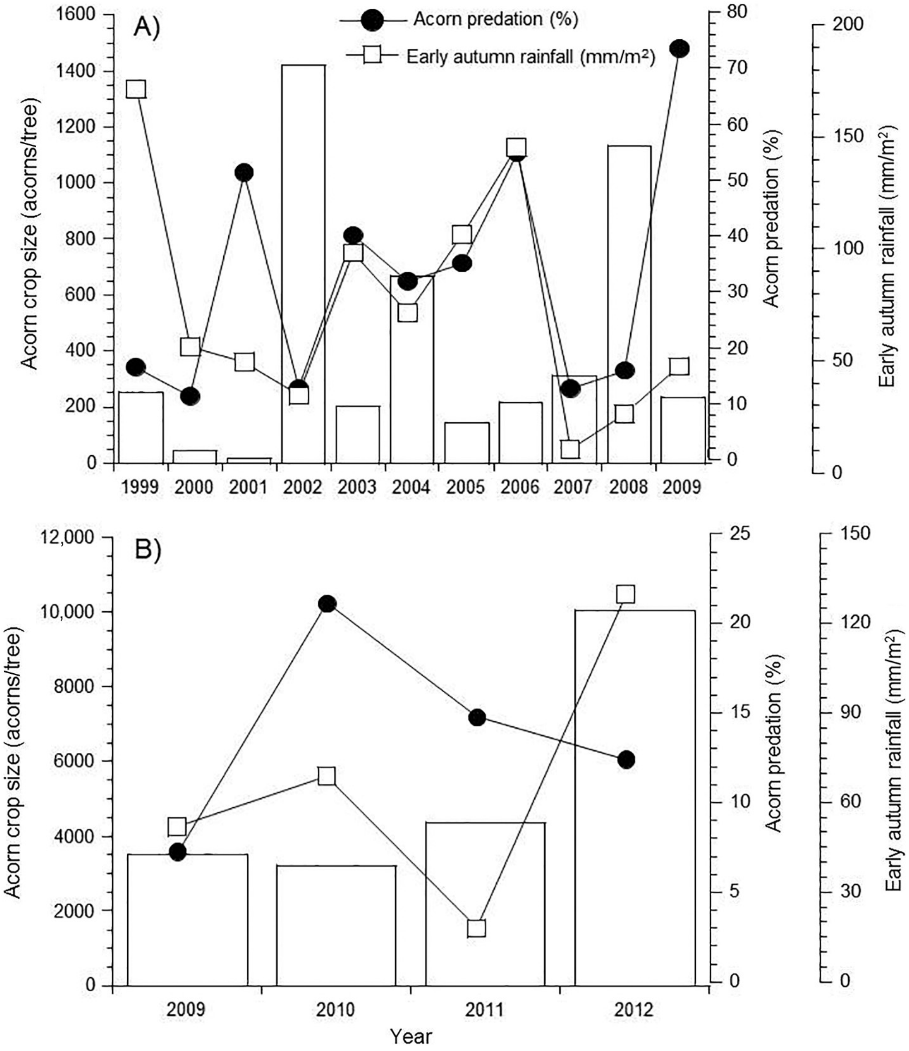


Fig. 2. Mean acorn production per tree (columns), mean acorn predation percentage per tree (black dots), and cumulative rainfall (empty squares) in early autumn (September and October) in Collserola (A) and Huecas (B).

2002, and 2007 (Fig. 2A), while in Huecas (Fig. 2B) 2009 and 2012 showed lower predation rates than 2010 and 2011.

The detailed ("per tree") monitoring of adult weevil emergence conducted in Huecas revealed that the number of adult weevils present in a given year was significantly and positively related to the amount of autumn rainfall that year ($F = 12.61$, $P < 0.001$) and to the number of infested acorns—a proxy of the number of larvae—the two previous years ($F = 24.9$, $P < 0.001$). This relationship between the numbers of adult weevils present (emerged from the soil) and rainfall amount in early autumn is also supported by the weekly patterns of rain accumulated and the number of weevils emerged and trapped (Fig. 3). In the four years included in the study, emergence of adult weevils from the soil started in early September and peaked some weeks later, roughly at the end of October, concurrent with rainfall accumulation patterns (see Fig. 3). In fact, emergence was positively correlated ($r = 0.53$, $P < 0.001$) with the amount of rainfall in the previous two weeks, probably because some time is needed to moisten and soften the soil enough to facilitate the emergence process.

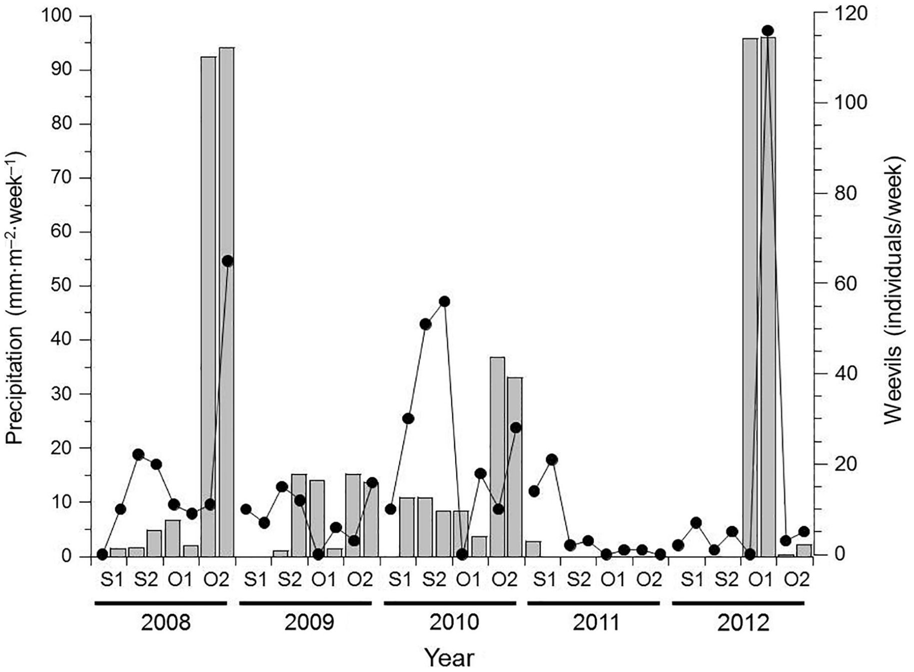


Fig. 3. Weekly precipitation (gray columns) and adult weevil emergence (black dots) from early September to late October for the years 2008 to 2012 in Huecas. S = September; O = October; 1 = first half month; 2 = second half month.

As shown in Table 1, in Collserola, acorn predation per tree was significantly influenced by the size of the current acorn crop, rainfall amount in early autumn, and inter-annual fluctuation of seed production (ratio of acorn crop t to acorn crop $t-1$ and $t-2$). In the model, acorn crop size and autumn rainfall had a positive effect on acorn predation, while inter-annual fluctuation in seed production (masting) had a negative effect. Similarly, in Huecas, the proportion of infested acorns per tree was significantly affected by inter-annual fluctuation in seed production and by autumn precipitation (Table 1). As observed in Collserola, rainfall had a positive effect on acorn predation, while inter-annual fluctuation had a negative effect (Table 1). In both sites, considering the relative importance of all variables included in the model, early autumn rainfall was the main source of acorn predation variability followed by inter-annual fluctuation in seed production (see Table 2 and also Fig. 2 for the similarity in the patterns of early autumn rainfall amount and acorn predation).

Table 1. Coefficients of the significant effects in the generalized linear mixed models on the effects of crop size$_t$, crop size$_{t-1 \text{ and } t-2}$, inter-annual variability in crop size (ratio of crop year$_t$ to crop year$_{t-1 \text{ and } t-2}$, hereafter crop ratio), autumn rainfall, and spring rainfall on acorn predation rate per tree in year t (see the Data analysis section for further details about these variables).

Site	Effect	Estimate	Std. error	*z*-Value
Huecas	Intercept	−0.56682	0.16953	3.277**
	A. Rainfall	1.02438	0.14032	7.155***
	Crop ratio	−0.44428	0.14161	3.075**
Collserola	Intercept	−3.9772	0.2330	16.091***
	Crop$_t$	0.7540	0.2210	3.284***
	A. Rainfall	1.8931	0.2617	6.915***
	Crop ratio	−1.3791	0.2687	5.572***

Notes: A. rainfall, autumn rainfall. Predictors are ordered by relative importance established by the dredging process according to maximum likelihood and the model average function.
$^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

DISCUSSION

Our results demonstrate that early autumn rainfall stochasticity directly affects the emergence of adult weevils from the soil, and this

Table 2. Relative contribution of the different variables explaining acorn predation calculated by comparing the best model and alternative models with an identical fixed-effects structure, but ignoring one variable at a time.

Site	Model	$\mathrm{df}_{\mathrm{mod.}}$	$\mathrm{AIC_c}$	$\Delta\mathrm{AIC_c}$	$\mathrm{df}_{\mathrm{test}}$	χ^2	R^2 Marg.	R^2 Condit.
Huecas	Best model	6	374.3				0.205	0.385
	A. Rainfall	4	408.6	34.2	2	39.0***	0.015	0.398
	Crop ratio	4	378.7	4.4	2	9.1*	0.175	0.395
Collserola	Best model	8	6417.3				0.167	0.910
	A. Rainfall	6	6504.2	68.9	2	74.0***	0.068	0.900
	Crop ratio	6	6495.5	33.1	2	37.2***	0.123	0.912
	Crop_t	7	6437.7	9.3	1	10.9***	0.152	0.903

Note: $\mathrm{df}_{\mathrm{mod}}$: degrees of freedom of the model; $\mathrm{df}_{\mathrm{test}}$: degrees of freedom of the test; marg.: marginal R^2, proportion of variance explained by the fixed factors alone; condit: conditional R^2, proportion of variance explained by both the fixed and random factors; $\mathrm{AIC_c}$, Akaike Information Criterion.

$^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

effect may become as important as the inter-annual variation in acorn crop size (masting) for acorn predation extent. Interestingly, as masting in Mediterranean oaks is also driven by inter-annual differences in rainfall amount, mostly in spring and summer (see Espelta et al. 2008, Fernández-Martínez et al. 2012, 2015, Koenig et al. 2016), rainfall stochasticity appears as the trigger point of this complex plant–animal interaction and its final outcome: acorn predation. Indeed, our results do not falsify the predator satiation hypothesis as an ultimate cause for the selection of masting. Yet they highlight that proximate environmental causes involved in this reproductive phenomenon may also directly and independently influence seed predation extent, thus fine-tuning the effects of inter-annual seed variability. To what extent this role may change under new ecological scenarios arising from climate change (e.g., increasing drought in Mediterranean areas) remains a challenging question to be further investigated.

Early autumn rainfall influenced the emergence of adults of *Curculio elephas* from the soil, and this is the most likely explanation for the positive influence of precipitation during this season on acorn predation extent. Previous studies, both experimental (Alverson et al. 1984, Schraer et al. 1998) and observational (Bonal et al. 2010), had already shown a positive relationship between precipitation and adult weevil abundance, with the need of a certain rainfall amount threshold to enhance their emergence (Fig. 3, see also Mulder et al. 2012). Yet for the first time, we demonstrate that this effect may influence seed predation extent. Rainfall in early autumn certainly would benefit the emergence of *C. elephas*, the weevil with a later phenology, and the only one present in Huecas, but not *Curculio glandium*, the second species present in Collserola that emerges in spring. The lack of significant effects of spring rainfall we observed for acorn predation in this site may be due to the fact that moister conditions during late winter and initial spring observed in the soils of the study area (Sánchez-Costa et al. 2015) make rainfall not to be such a crucial factor for the emergence of *C. glandium* as it is for *C. elephas* after summer.

The effect of rainfall stochasticity on the emergence of some weevil species becomes crucial to explain why predation rates do not always match the *functional* and *numerical satiation* effects presumably associated with inter-annual crop variability. For example, as shown in Fig. 2A, acorn predation in Collserola in 2007 was as low as the values observed during the two masting events (2002 and 2008), although the crop size in 2007 was much lower (poor *functional satiation* expected) and very similar to that of the previous year 2006 (poor *numerical satiation* expected). Interestingly, in 2007, rainfall in September–October was extremely low (Fig. 2A) and this could constrain weevil emergence from the soil and reduce predation intensity. Similarly, in Huecas, predation in 2011 was much lower than that in 2010, even though the number of acorns produced in these two years was very similar (Fig. 2B), probably owing to the extremely dry autumn in 2011 (see Fig. 3).

Our results demonstrate that aside from acorns, weevils require perforce a certain amount of rainfall at a very precise time of the year (autumn) in order to be able to emerge from the soil and successfully infest the acorns. Interestingly, this environmental requirement is uncoupled with the ones driving acorn production as this mostly depends on rainfall in spring and early summer (Espelta et al. 2008, Fernández-Martínez et al. 2012, 2015), with barely any effects of autumn rain, when acorns have almost fully developed (Espelta et al. 2009). Therefore, in a year with a large acorn crop available after optimal conditions in spring and summer, weevils could not benefit from it, if a dry autumn occurs and it blocks their emergence. This suggests that extended diapause in weevils is indeed a complex phenomenon that might have evolved not only to cope with the extreme inter-annual variability in seed crop size but also to cope with the effects of climate uncertainty (see also Venner et al. 2011, Pélisson et al. 2013). At the same time, it is challenging whether masting promoted variable diapause or it was the unpredictable emergence of weevil adults, prompted by rainfall stochasticity, what made masting advantageous for oaks. In any case, theoretical models developed by Satake and Bjørnstad (2004) suggest that, whatever the origin for extended diapause in a predator, extremely high temporal variability in seed production (masting) would benefit the host to buffer its effects.

The relevance of rainfall for controlling seed predation by weevils also challenges which will be the outcome of this antagonistic interaction in new climatic scenarios arising from climate change. In Mediterranean-type areas, the predicted increase in the intensity and length of drought events will certainly affect the patterns of acorn production, reducing the size of acorn crops, as has been already tested in experiments of rainfall exclusion (Sánchez-Humanes and Espelta 2011). Yet extended drought may also directly affect weevil populations by limiting the success of their emergence (Bonal et al. 2010) or even altering the sex ratio of populations (Bonal et al. 2015). This raises the question as to whether inter-annual variability in seed prediction or direct rainfall shortage effects on weevils will increase in importance for controlling acorn predation as climate change progresses (see also McKone et al. 1998, Poncet et al. 2009).

The observed effect of inter-annual crop variability to reduce acorn predation supports *predator satiation* as one of the evolutionary hypotheses based on a plant–animal interaction to explain masting in oaks. Certainly, it could be argued that there are other animals also feeding on acorns such as rodents, birds, or ungulates not included in this study. However, weevils are one of the most important pre-dispersal acorn consumers, being able to destroy up to 60% of the crop in a given year (Leiva and Fernández-Alés 2005), and the only ones that comply with the three characteristics suggested by Shibata et al. (1998) to make a predator highly sensitive to inter-annual seed variability: short life span, reduced mobility, and high specificity. In addition to these effects, we cannot discard that the production of extraordinary large crops may also increase the chances of successful seed dispersal and recruitment in these species both by increasing the attraction of avian seed dispersers (Pesendorfer and Koenig 2016) and by reducing the removal of cached seeds during masting years (dispersal satiation hypothesis in vander Wall 2010, see also Zwolak et al. 2016).

In contrast to some previous studies, we did not observe a higher proportion of seeds consumed at low levels of seed availability, the so-called type II functional response of seed consumers (sensu Holling 1959, see also Moreira et al. 2017), indicating that the effects of masting may be somewhat idiosyncratic. In fact, although the effects of masting and early autumn rain on acorn predation were similar in the two localities, they differed in the intensity of these effects and the importance of other variables: greater importance of masting in Collserola and of rainfall in Huecas and significant effect of the current acorn crop sizes in the former site (see Tables 1 and 2). Moreover, in Collserola, the high value of the R^2 conditional (~0.90), which accounts for the variability explained by random factors, suggests a major importance of the factor "plot" that may be due, among others, to differences in acorn production among forest stands (see also Espelta et al. 2008). Considering the size of crops produced in the two sites, it seems reasonable that masting had more importance in a site like Collserola where trees produce smaller crops (~10% of the values recorded in Huecas, see Fig. 1 and Espelta et al. 2008), forests are more continuous, and adult weevils of *C. glandium* are much more able to disperse from one tree to another. The production of moderate-low seed crops and the intermingling of trees may explain why weevils disperse and may concentrate on relatively more productive trees, a pattern suggested by the positive effect we observed of the current acorn crop size on predation (Table 1). This scenario would give masting a greater importance to control seed predation (see also Maeto and Ozaki 2003). Conversely, isolated trees in the savannah-like landscape of Huecas are bigger, less resource limited, and may produce larger acorn crops regularly (Morán-López et al. 2016). In this situation, although masting remains still important, the direct effects of rainfall stochasticity on weevils may become more relevant to control acorn predation extent. Altogether, these local differences suggest that the relevance of masting may be somewhat context dependent and change with the size and productivity of trees and the landscape structure (see also Espelta et al. 2008, Yamauchi et al. 2013), especially in long-lived tree species such as oaks, a question that has been barely addressed up to now and requires further research.

CONCLUSION

The main contribution of our study has been to demonstrate that irregular seed crops (masting) certainly help escaping acorn predation in Mediterranean oaks, yet the direct effects of rainfall stochasticity on the success of weevil populations may be as important as in reducing seed predation extent. Furthermore, our results suggest that the importance of masting to mitigate seed predation may change with individual characteristics (e.g., seed production) and spatial attributes (i.e., isolation). Our findings also question whether the increase in more severe and long-lasting drought events in Mediterranean areas owing to climate change will have a positive or negative impact in the outcome of this plant–animal interaction.

ACKNOWLEDGMENTS

This research was supported by the projects NOVFORESTS (CGL2012-33398), FORASSEMBLY (CGL2015-70558-P), and PLAGANADO (AGL2014-54739-R) of the Ministerio de Economia y Competitividad (Spain), and the projects BEEMED (SGR913) of the Generalitat de Catalunya and PII1C09-0256-9052 of the Regional Government of Castilla-La Mancha and the European Social Fund. R.B. was funded by a contract of the Programa Atracción de Talento Investigador del Gobierno de Extremadura. M.F.M. was funded by the Generalitat de Catalunya FI-2013 grants. We thank Susan Rutherford (SER Translations) for the English revision and Miquel Ninyerola and Meritxell Batalla for providing the meteorological data for the Collserola plots obtained during the MONTES (Consolider-Ingenio Montes CSD2008-00040) project.

LITERATURE CITED

Alverson, D. R., M. K. Harris, C. E. Blanchard, and W. G. Hanlin. 1984. Mechanical impedance of adult pecan weevil (Coleoptera: Curculionidae) emergence related to soil moisture and penetration resistance. Environmental Entomology 13:588–592.

Archibald, D. W., A. G. McAdam, S. Boutin, Q. E. Fletcher, and M. M. Humphries. 2012. Within-season synchrony of a masting conifer enhances seed escape. American Naturalist 179:536–544.

Barton, K. 2015. MuMIn: Multi-Model Inference. R Package Version 1.15.1. http://CRAN.R-project.org/package=MuMIn

Bonal, R., M. Hernández, J. M. Espelta, A. Muñoz, and J. M. Aparicio. 2015. Unexpected consequences of a drier world: evidence that delay in late summer rains biases the population sex ratio of an insect. Royal Society Open Science 2:150198.

Bonal, R., M. Hernández, J. Ortego, A. Muñoz, and J. M. Espelta. 2012. Positive cascade effects of forest fragmentation on acorn weevils mediated by seed size enlargement. Insect Conservation and Diversity 5:381–388.

Bonal, R., and A. Muñoz. 2009. Seed weevils living on the edge: pressures and conflicts over body size in the endoparasitic *Curculio* larvae. Ecological Entomology 34:304–309.

Bonal, R., A. Muñoz, and M. Díaz. 2007. Satiation of predispersal seed predators: the importance of considering both plant and seed levels. Evolutionary Ecology 21:367–380.

Bonal, R., A. Muñoz, and J. M. Espelta. 2010. Mismatch between the timing of oviposition and the seasonal optimum. The stochastic phenology of Mediterranean acorn weevils. Ecological Entomology 35: 270–278.

De Steven, D., and J. Wright. 2002. Consequences of variable reproduction for seedling recruitment in three neotropical tree species. Ecology 83:2315–2327.

Espelta, J. M., R. Bonal, and B. Sánchez-Humanes. 2009. Pre-dispersal acorn predation in mixed oak forests: Interspecific differences are driven by the interplay among seed phenology, seed size and predator size. Journal of Ecology 97:1416–1423.

Espelta, J. M., P. Cortés, R. Molowny-Horas, B. Sánchez-Humanes, and J. Retana. 2008. Masting mediated by summer drought reduces acorn predation in Mediterranean oak forests. Ecology 89:805–817.

Fernández-Martínez, M., J. Belmonte, and J. M. Espelta. 2012. Masting in oaks: disentangling the effect of flowering phenology, airborne pollen load and drought. Acta Oecologica 43:51–59.

Fernández-Martínez, M., M. Garbulsky, J. Penuelas, G. Peguero, and J. M. Espelta. 2015. Temporal trends in the enhanced vegetation index and spring weather predict seed production in Mediterranean oaks. Plant Ecology 216:1061–1072.

Fernández-Martínez, M., S. Vicca, I. A. Janssens, J. M. Espelta, and J. Peñuelas. 2016. The North Atlantic Oscillation synchronises fruit production in western European forests. Ecography. https://doi.org/10.1111/ecog.02296

Fukumoto, H., and H. Kajimura. 2011. Effects of asynchronous acorn production by co-occurring *Quercus* trees on resource utilization by acorn-feeding insects. Journal of Forest Research 16:62–67.

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

Ims, R. A. 1990. On the adaptive value of reproductive synchrony as a predator-swamping strategy. American Naturalist 136:485–498.

Janzen, D. H. 1971. Seed predation by animals. Annual Review of Ecology and Systematics 2:465–492.

Jones, C. G., R. S. Ostfeld, M. P. Richard, E. M. Schauber, and J. O. Wolff. 1998. Chain reactions linking acorns to gypsy moth outbreaks and Lyme disease risk. Science 279:1023–1026.

Kelly, D., A. L. Harrison, W. G. Lee, I. J. Payton, P. R. Wilson, and E. M. Schauber. 2000. Predator satiation and extreme mast seeding in 11 species of *Chionochloa* (Poaceae). Oikos 90:477–488.

Kelly, D., and V. L. Sork. 2002. Mast seeding in perennial plants: Why, how, where? Annual Review of Ecology, Evolution, and Systematics 33: 427–447.

Kelly, D., et al. 2013. Of mast and mean: Differential-temperature cue makes mast seeding insensitive to climate change. Ecology Letters 16:90–98.

Kneip, E., D. H. Van Vuren, J. A. Hostetler, and M. K. Oli. 2011. Influence of population density and climate on the demography of subalpine golden-mantled ground squirrels. Journal of Mammalogy 92:367–377.

Koenig, W. D., and J. M. H. Knops. 2000. Patterns of annual seed production by northern hemisphere trees: a global perspective. American Naturalist 155:59–69.

Koenig, W. D., et al. 2016. Is he relationship between mast-seeding and weather in oaks related to their life-history or phylogeny? Ecology. https://doi.org/10.1002/ecy.1490

Leiva, M. J., and R. Fernández-Alés. 2005. Holm-oak (*Quercus ilex* subsp. *Ballota*) acorns infestation by insects in Mediterranean dehesas and shrublands. Its effect on acorn germination and seedling emergence. Forest Ecology and Management 212:221–229.

Maeto, K., and K. Ozaki. 2003. Prolonged diapause of specialist seed-feeders makes predator satiation unstable in masting of *Quercus crispula*. Oecologia 137:392–398.

McKone, M., D. Kelly, and W. G. Lee. 1998. Effect of climate change on mast-seeding species: frequency of mass flowering and escape from specialist insect seed predators. Global Change Biology 4:591–596.

Morán-López, T., A. Forner, D. Flores-Rentería, M. Díaz, and F. Valladares. 2016. Some positive effects of the fragmentation of holm oak forests: attenuation of water stress and enhancement of acorn production. Forest Ecology and Management 370:22–30.

Moreira, X., I. M. Pérez-Ramos, L. Abdala-Roberts, and K. A. Mooney. 2017. Functional responses of contrasting seed predator guilds to masting in two Mediterranean oak species. Oikos. https://doi.org/10.1111/oik.03722

Mulder, P. G., K. Harris, and R. A. Grantham. 2012. Biology and management of the pecan weevil (Coleoptera: Curculionidae). Journal of Integrated Pest Management. https://doi.org/10.1603/IPM10027

Muñoz, A., R. Bonal, and J. M. Espelta. 2014. Acorn–weevil interactions in a mixed-oak forest: outcomes for larval growth and plant recruitment. Forest Ecology and Management 322:98–105.

Oddou-Muratorio, S., E. K. Klein, G. G. Vendramin, and B. Fady. 2011. Spatial vs. temporal effects on demographic and genetic structures: the roles of dispersal, masting and differential mortality on patterns of recruitment in *Fagus sylvatica*. Molecular Ecology 20:1997–2010.

Peguero, G., R. Bonal, and J. M. Espelta. 2014. Variation of predator satiation and seed abortion as seed defense mechanisms across an altitudinal range. Basic and Applied Ecology 15:269–276.

Pélisson, P. F., C. Bernstein, D. Francois, F. Menu, and S. Venner. 2013. Dispersal and dormancy strategies among insect species competing for a pulsed resource. Ecological Entomology 38:470–477.

Pérez-Ramos, I. M., J. M. Ourcival, J. M. Limousin, and S. Rambal. 2010. Mast seeding under increasing drought: results from a long-term data set and from a rainfall exclusion experiment. Ecology 91: 3057–3068.

Pesendorfer, M. B., and W. D. Koenig. 2016. The effect of within-year variation in acorn crop size on seed harvesting by avian hoarders. Oecologia 181: 97–106.

Poncet, B. N., P. Garat, S. Manel, N. Bru, J. M. Sachet, A. Roques, and L. Despres. 2009. The effect of climate on masting in the European larch and on its specific seed predators. Oecologia 159:527–537.

R Development Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ritchie, E. G., and C. N. Johnson. 2009. Predator interactions, mesopredator release and biodiversity conservation. Ecology Letters 12:982–998.

Ruíz-Carbayo, H., R. Bonal, J. M. Espelta, M. Hernández, and J. Pino. 2017. Community assembly in time and space: the case of Lepidoptera in a *Quercus ilex L.* savannah-like landscape. Insect Conservation and Diversity 10:21–31.

Sánchez-Costa, E., R. Poyatos, and S. Sabaté. 2015. Contrasting growth and water use strategies in four co-occurring Mediterranean tree species revealed by concurrent measurements of sap flow and stem diameter variations. Agricultural and Forest Meteorology 207:24–37.

Sánchez-Humanes, B., and J. M. Espelta. 2011. Increased drought reduces acorn production in *Quercus ilex* coppices: Thinning mitigates this effect but only in the short term. Forestry 84:73–82.

Satake, A., and O. N. Bjørnstad. 2004. Spatial dynamics of specialist seed predators on synchronized and intermittent seed production of host plants. American Naturalist 163:591–605.

Satake, A., O. N. Bjørnstad, and S. Kobro. 2004. Masting and trophic cascades: interplay between rowan trees, apple fruit moth, and their parasitoid in southern Norway. Oikos 104:540–550.

Schauber, E. M., D. Kelly, P. Turchin, C. Simon, W. G. Lee, R. B. Allen, I. J. Payton, P. R. Wilson, P. E. Cowan, and R. E. Brockie. 2002. Masting by eighteen New Zealand plant species: the role of temperature as a synchronizing cue. Ecology 83:1214–1225.

Schraer, S. M., M. Harris, J. A. Jackman, and M. Biggerstaff. 1998. Pecan weevil (Coleoptera: Curculionidae) emergence in a range of soil types. Environmental Entomology 27:549–554.

Selva, N., K. A. Hobson, A. Cortés-Avizanda, A. Zalewski, and J. A. Donázar. 2012. Mast pulses shape trophic interactions between fluctuating rodent populations in a primeval forest. PLoS ONE 7:e51267.

Servanty, S., J. M. Gaillard, C. Toïgo, S. Brandt, and E. Baubet. 2009. Pulsed resources and climate-induced variation in the reproductive traits of wild boar under high hunting pressure. Journal of Animal Ecology 78:1278–1290.

Shibata, M., H. Tanaka, and T. Nakashizuka. 1998. Causes and consequences of mast seed production of four co-occurring *Carpinus* species in Japan. Ecology 79:54–64.

Silverstown, J. W. 1980. The evolutionary ecology of mast seeding in trees. Biological Journal of the Linnean Society 14:235–250.

Sunyer, P., A. Muñoz, M. J. Mazerolle, R. Bonal, and J. M. Espelta. 2016. Wood mouse population dynamics: interplay among seed abundance seasonality, shrub cover and wild boar interference. Mammalian Biology-Zeitschrift für Säugetierkunde 81:372–379.

Tachiki, Y., and Y. Iwasa. 2013. Coevolution of mast seeding in trees and extended diapause of seed predators. Journal of Theoretical Biology 339:129–139.

Venner, S., P. F. Pélisson, M. C. Bel-Venner, F. Débias, E. Rajon, and F. Menu. 2011. Coexistence of insect species competing for a pulsed resource: toward a unified theory of biodiversity in fluctuating environments. PLoS ONE 6:e18039.

vander Wall, S. B.. 2010. How plants manipulate the scatter-hoarding behavior of seed-dispersing animals. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 365:989–997.

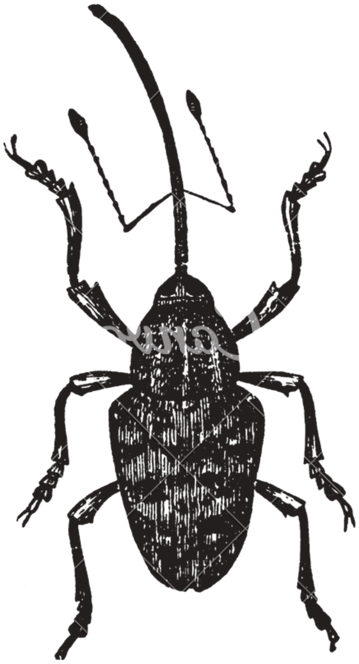
Wróbel, A., and M. Bogdziewicz. 2015. It is raining mice and voles: Which weather conditions influence the activity of *Apodemus flavicollis* and *Myodes glareolus*? European Journal of Wildlife Research 61:475–478.

Yamauchi, A., Y. Shirahama, and Y. Kobayashi. 2013. Evolution of masting with intermittence and synchronization under the enhancements of fertility and survival. Theoretical Ecology 6:505–518.

Yu, X., H. Zhou, and T. Lou. 2003. Spatial and temporal variations in insect-infested acorn fall in a *Quercus liaotungensis* forest in North China. Ecological Research 18:155–164.

Zwolak, R., M. Bogdziewicz, A. Wróbel, and E. E. Crone. 2016. Advantages of masting in European beech: Timing of granivore satiation and benefits of seed caching support the predator dispersal hypothesis. Oecologia 180:749–758.

This page intentionally left blank



General discussion

The ecological and evolutive principles underlying plant-insect interactions are still largely unknown.

This page intentionally left blank

Scope of General discussion

Throughout this Thesis several determinants and ecological drivers of granivory have been explored in Mediterranean forests. In a multi-host (*Quercus spp*., *Corylus avellana*) and multi-seed-predator (*Curculio spp*.) system, specificity and diet breadth of weevils was evaluated to understand the mechanisms allowing the coexistence of the different weevil species: i.e. resource partitioning (specificity based on host species membership, seed size variability) or time partitioning (specificity based on the combination of seed phenology and female ooegenesis). Our results showed that co-existence is not driven by differences in seed size but modulated by the combination of time partitioning and probably dissimilarities in dispersal-dormancy strategies among weevils. Differences in weevil specificity and in the spatial distribution of the hosts studied resulted in the presence of genetic structure among the populations of the more specific weevil depredating upon the more patchily distributed host. Additionally, two oak forest scenarios (savannah-like landscape with scattered oaks in Huecas and an old-coppiced oak forest in Collserola) were used to assess the influence of inter-annual variability in acorn crop size and precipitation during weevil emergence from the soil, for acorn predation. The results showed that irregular seed crops (masting) certainly helps reducing acorn predation in Mediterranean oaks. However, the direct influence of rainfall stochasticity on the success of weevil emergence from the soil also contributed to decrease seed predation in a similar magnitude. Beyond the results obtained and the issues discussed in both papers, some integrative ideas are discussed and exposed in this chapter, to broaden the perspectives of the research conducted as well as to suggest some possible new research lines.

Curculio spp. coexistence: Resource or time partitioning?

Ecological communities are limited membership assemblages in which interspecific biotic interactions and abiotic conditions are the primary factors influencing composition and diversity (Chase & Myers 2011). Species coexistence requires mechanisms such as partitioning factors that reduce fitness inequalities (Chesson 2000). Several types of factors allow species coexistence by partitioning, i.e. space, food, and time (Schoener 1974, Loreau 1992). For example, in discontinuous forest landscapes, seed weevil occupancy was related to the differential suitability of hosts as resources for each weevil species as long as this creates a differential spatial storage effect that, coupled with a temporal storage effect induced by differences in diapause, facilitates species coexistence (Govinda & Swihart 2015). In a different case, beta diversity of a Lepidoptera community in Mediterranean holm oaks (*Q. ilex*) in a savannah-like landscape was mostly explained by spatial distance between trees, supporting the importance of assemblages of species based on differences in dispersal. In that case community assembly was also driven by the interaction of connectivity with tree age and size, two relevant factors that contributed to differences in species abundance, richness, and diversity (Ruíz-Carbayo *et al*. 2016). Similarly, on studies of bee and bumblebee communities, coexistence has been observed to be driven by several factors, i.e. interspecific differences in spatial resource utilization, depending on foraging ranges according to body and colony sizes (Westphal *et al*. 2006) and morphological differences in tongue length (Heinrich 1976). Concerning *Curculio spp.*, several factors have been invoked to explain species coexistence in these granivorous weevil communities, including differential use of resources (seed size preferences, Espelta *et al*. 2009, Bonal & Muñoz 2009, Peguero *et al*. 2017) and diversification of dormancy strategies and dispersal abilities (Venner *et al*. 2011, Pélisson *et al*. 2012, 2013a,b; Ruíz-Carbayo *et al*. 2018). In my research, in a multi-host-multi-phytophagous study system, differences in host specificity among weevils were not driven by differential preferences according to seed size but by time partitioning (i.e. differences in female sexual maturation, among *C. nucum* and the rest of species, and a combination of time partitioning and differences in dispersal-dormancy strategies among the three species depredating upon the same oak species, *C. venosus*, *C. glandium and C. elephas*).

Time dimension has been observed to facilitate niche partitioning between co-occurring organisms and allow coexistence (Kronfeld-Schor & Dayan 2003, Castro-Arellano & Lacher 2009). Difference between the time scales of consumer and resource dynamics is a critical factor for the coexistence of consumers that occupy different temporal niches in a seasonal environment (Loreau 1992) in insects but also in mammals. For instance, in two rodent communities in a tropical semideciduous forest and a cloud forest, their temporal activity showed a non-random niche segregation (Castro-Arellano & Lacher 2009). Similarly, time partitioning favors the coexistence and reduces the competition of two sympatric crab-eating foxes (*Credocyon thous* and *Lycalopex gymnocercus*) with very similar body size and food habits, with distributional ranges that overlap extensively in South America (Di Bitetti et al. 2009). According to the results of my research, differences in the patterns of sexual maturation of females (i.e. synovigenic vs. provigenic species), could be a key factor for segregation in time of the different weevil species and their co-existence.

Tolerance of seed predation: the effects of resource variability (masting) and abiotic factors stochasticity.

Oaks show a strong "masting" reproductive behavior: namely, the synchronous and intermittent production of large and nil acorn crops over wide areas (Bonal *et al*. 2007, Espelta *et al*. 2008, Koenig *et al*. 2016). Weather conditions play a major role as proximate cues for synchrony and variability in reproduction (e.g. rainfall in Pérez-Ramos *et al*. 2010, or even "weather packages" in Fernández-Martínez *et al*. 2017, see also Koenig *et al*. 2016). Concerning the consequences of masting, there is an extensive consensus on the role of this bizarre reproductive behavior to reduce pre-dispersal acorn predation (see for granivorous insects Bonal *et al*. 2007; Espelta *et al*. 2008; Xia *et al*. 2016) yet, prior to this thesis, barely any study had analysed the importance of masting compared to other factors regarding infestation rates. In particular if the abovementioned environmental cues prompting masting could also determine predator's success and, ultimately, influence independently and directly the seed predation extent. Indeed, many studies have shown that environmental conditions, rainfall variation in particular, could directly influence abundance, demographic components, and population fluctuations of phytophagous species (see Schraer *et al*. 1998, Servanty *et al*. 2009, Bonal *et al*. 2010, Kneip *et al*. 2011, Bonal *et al*. 2015, Wróbel & Bogdziewicz 2015, Sunyer *et al*. 2016), yet they have not assessed whether this had an effect on the extent of seed predation (but see Poncet *et al*. 2009). In that sense, my results confirmed the important effect of rainfall amount and distribution for the emergence of adult weevils from the soil (see also Bonal *et al*. 2010) and ultimately its impact on the intensity of acorn predation. This provides some new perspectives on the nature of this plant-animal interaction. On the one hand, rainfall appears as a trigger environmental factor of this complex plant–animal interaction and its outcome (acorn predation) by influencing both the dynamics of seed crops and weevil population sizes. On the other hand, this effect of rainfall stochasticity coupled with the local differences among the two study sites (Collserola and Huecas) suggest that the relevance of masting to escape seed predation may be somewhat context dependent and change with the patterns of rainfall distribution in autumn and its effects on weevil emergence, tree characteristics (low vs. high seed production), landscape structure (isolated vs. dense forests). Indeed, several studies have highlighted that interactions among herbivore communities and host plants are driven by 'contingent' abiotic and biotic factors but are highly context dependent (Pringle 2016). For example, Bogdziewicz *et al*. (2018) on an eight-year study evaluating seed production, spatiotemporal patterns of weevil seed predation, and abundance of adult weevils in a holm oak (*Q. ilex*) covering a continuum of conspecific density found that the effects of temporal variation in seed production on pre-dispersal seed predation was highly influenced by the spatial arrangement of trees (i.e. differences among more or less dense areas). The context dependency of masting reported in my study may help to explain why, although many studies have emphasized that this curious reproduction pattern should follow an "economy of scale" principle: i.e. the production of infrequent bumper crop episodes should be more beneficial than moderate and continuous reproductive events (Kelly & Sork 2002) and increase regeneration success, we still lack empirical evidence of its ultimate positive effect on oak recruitment. Clearly, future research should be aimed to elucidate this point.

Effects of fragmentation and rainfall stochasticity: Insights of potential global change impacts on granivorous insects.

Global Change is threatening biodiversity conservation and exerting other pervasive impacts on biotic networks and interactions (Steffan-Dewenter *et al*. 2002, Tylianakis *et al*. 2008, Gilman *et al*. 2010, DeLucia *et al*. 2012, Forrest 2015). Effects of global change over plant–insect interactions have been shown to be variable at spatial and temporal scales but ultimately to jeopardize relevant ecosystems services (e.g. pollination in Byers 2017). In that context, this thesis provides some insights on the potential effects for granivorous weevils of two of the most common problems involved in global change, such as the fragmentation of habitats (chapter II) and the consequences of climate change (chapter III).

Habitat fragmentation may be a major a risk for plant insect interactions owing to the negative effects of habitat loss and isolation (Brudvig *et al*. 2015, Valiente-Banuet *et al*. 2015, Rossetti *et al*. 2017, Grass *et al*. 2018). Indeed, it has been observed that net habitat loss and associated changes in spatial configuration may cause drastic declines in pollinator populations and species richness (Steffan-Dewenter *et al*. 2002), ultimately decreasing reproductive output and increasing inbreeding depression in plant remnant habitat patches (see Young *et al.* 1996, Lennartsson 2002, Steffan-Dewenter *et al.* 2002). In the same line, a decrease in frugivorous seed dispersers in fragmented landscapes may lead to recruitment losses among plants (Asquit *et al*. 1997, Guariguata *et al*. 2000) and consequently cascading effects on community structure (Byrnes *et al*. 2006). Even though the specific aim of the study presented in chapter II was not to analyze the effects of landscape fragmentation on the granivorous weevils, our results highlight that the patchy distribution of a host (*Corylus avellana*) and the specificity of the granivorous weevil feeding on their seeds (*Curculio nucum*) may have consequences for the genetic structure of the seed predator. This result agrees with other studies conducted on other *Curculio* spp. that have observed that isolation of forest patches may result on restricted gene flow among weevil populations (see Ruíz-Carbayo 2018, for *Curculio elephas*). In summation, all these observations warn about the fact that the potential increase in the scattered distribution of host plants owing to land use changes may increase genetic drift in insect populations, reducing gene flow and increasing genetic erosion odds (Ingvarsson & Olsson 1997, Peterson & Denno 1998, Peled *et al*. 2016). This scenario may be particularly negative for highly specialized insects (Futuyma & Moreno 1988, Hernández-Vera *et al*. 2010), with low dispersal ability (Peterson & Denno 1998, Kubish *et al*. 2014) such as *Curculio spp*. weevils. Then, connectivity may be critical for population survival (Fahrig & Merrian 1985, Fahrig & Paloheimo 1988) and metapopulation dynamics (Levins 1970). In that sense, to what extent the low gene flow among sites and the population bottlenecks observed for the highly specific *C. nucum* may threaten the continuity of these populations if isolation of hazelnut patches increases, should deserve further research.

In addition to habitat loss and fragmentation, climate change is also a major threat for plant-insect interactions (Tylianakis et al. 2008, 2010, Gilman *et al*. 2010, Byers 2017), owing both to indirect effects such as a disruption in the matching of the phenologies of plants and insects (Forrest & Miller-Rushing 2010, Forrest 2015) or direct negative effects in any of the two components of the interaction. Mismatches in the phenology of plant-insect interactions caused by climate change and their negative consequences have been extensively reported (e. g. see for pollination Gordo & Sanz 2005, Kudo & Ida 2013), and once again the consequences are expected to be more negative for highly specialized insects than for polyphagous ones. The latter may switch and expand their feeding range as they are not constrained by a particular host, while narrow diet breadth phytophagous insect would be unable to track changing climate or shift to a new host. (Braschler & Hill 2007, De Lucia *et al*. 2012). Warming temperatures or changes in the precipitation regime my also directly impact growth, survival, and reproduction of plants and insects (Forrest 2015). For example, weevil oogenesis and egg maturation process depend on temperature (Guidone *et al*. 2007) so it is expected that extreme temperature variation could alter oviposition. Interestingly, the paramount importance of rainfall amount and distribution for the emergence of adult weevils observed in chapter III of this thesis emphasizes the dramatic consequences that the predicted decrease in precipitation and increased stochasticity owing to climate change in Mediterranean regions (Peñuelas *et al*. 2018) may have for this insect group. Indeed, extended drought may negatively affect weevil populations by altering the sex ratio (Bonal *et al*. 2015) and limit the success of their emergence or favor it out of time once seeds have fallen (Bonal *et al*. 2010). Considering that the predicted increase in drought episodes will reduce the size of acorn crops, as it has been already tested in experiments of rainfall exclusion (Sánchez-Humanes & Espelta 2011), these results challenge which will be the outcome of this antagonistic interaction in new climatic scenarios arising from climate change in Mediterranean-type areas.

New research perspectives for Plant-Phytophagous interactions.

After previous reflections, here I would like to present some areas of interesting perspectives that I personally consider deserve attention and in some extent beyond those exposed in both published papers. Indubitably, the research conducted on this thesis leaves behind interrogatives in community ecology, context dependency and the effects of global change for plants-phytophagous insect interactions. For instance, drivers of phytophagous insect coexistence in multi host set-ups, ecological specialization, underlying evolutive mechanisms, PSh in contingent scenarios, among others are still issues in the inkwell.

According to community ecology, specialization can be based upon the assumption that generalist species should co-occur with many different species across sites, whereas specialists should co-occur with relatively few species (Devictor *et al*. 2010). This attribute could be measured from species co-occurrence patterns along environmental gradients or contrasting scenarios and indirectly quantify specialization as it reflects the species response to environmental heterogeneity. Concomitantly with host species, co-occurrence patterns could help to understand the drivers of species coexistence stabilization mechanisms, host selection and phytophagous insect specialization determinants. Considering contingency of biotic and abiotic effects over plant-phytophagous insect interactions (Kolb et al. 2007, Maron et al. 2014, Moreira et al. 2016), experiments across broad gradients would determine whether the outcome of plant-phytophagous insect interactions varies with changing conditions (Moreira et al. 2016). An auspicious path of future research is also exploring evolutionary consequences of ecological dynamics driven by resource pulses and climatic cues, and how changes in species functional responses underlie such dynamics. Particularly interesting would be to evaluate rainfall stochasticity effects on plant-phytophagous insect interactions in subtropical and tropical deciduous forests as well in arid and desertic habitats.

On the other hand, precise predictions of biotic responses to global change are crucial for conserving natural and human-influenced ecosystems and cannot be considered comprehensive without a deep understanding of the widespread relevance of plant-phytophagous insect interactions (Gilman *et al*. 2010). Both host plants and phytophagous insect communities have been found to be changing because of several global change effects in their phenologies, reproductive performance, genetic structure and adaptive capabilities (De Lucia *et al.* 2012, Forrest 2015, Byers 2017). Considering species, interactions and community process levels, studies are needed to assess changes on phytophagous species co-occurrence, reproductive alterations on host and phytophagous insects and adaptative responses like host shifting. Studying the influence of dispersal capacity, flight activity and differences in diapause and dormancy trends as key factors in ecological diversification would be also relevant facing environmental changes. Other factors not enclosed on these studies such as the risk of parasitism, survival of larvae during diapause, natural enemy resistance (Oliver *et al*. 2003, Haine *et al*. 2008), differences in endosymbiont communities (Merville *et al*. 2013), could help understand the effects of global change.

Furthermore, gene flow restrictions and genetic consequences of fragmented habitats specially over phytophagous species deserve attention. For instance, assessment of restriction in landscape functional connectivity, could help understand consequent gene flow reduction and its negative aftereffects for the persistence of specialized herbivore populations compared to generalist species. Similarly, genetic variation erosion due to habitat fragmentation (Young *et al*. 1996) is interesting owing to possible long-term evolutionary consequences on host plants and herbivore species and its direct effect on individual fitness, short-term viability of remnant populations and resilience capacity on drastically changing scenarios.

General Conclusions

1- DNA analyses revealed that *Curculio nucum* shows a complete specificity on hazelnut (*Corylus avellana*) while the other weevil species (C. *venosus, C. glandium and C. elephas*) show more flexible and wide trophic breadth depredating upon acorns of different oak species (*Quercus ilex, Q. pubescens*) **(Chapter II).**

2- Seed-size does not influence host specificity by *Curculio* spp. Coexistence of the different weevil species seems to be intermediated by the combination of time partitioning driven by female sexual maturation (synovigenic vs. proovigenic species) and diversification of dispersal (high vs. low) and dormancy (fixed vs variable diapause) strategies. **(Chapter II).**

3- The avoidance of competitive exclusion among the weevil species emerging in spring could be explained by a different pattern of sexual maturation of females, specifically an earlier maturation in *C. nucum* in comparison to *C. glandium*. As well other ecological inter-specific differences are contributing to stabilize weevil coexistence on those that rely on acorn infestation; as *C. glandium* and *C. elephas*; that is high dispersal ability in the former species and an extended diapause in the later. **(Chapter II).**

4- Differences in weevil specificity in their trophic breadth and the spatial distribution of hosts (continuous vs. scattered) results in the presence of genetic structure among the populations of the highly specific weevil (*C. nucum*) depredating upon a single patchily distributed host (*C. avellana*) but not in (*C. glandium*) predating indistinctively in the various and more continuously distributed oaks (*Quercus ilex, Q. pubescens*). **(Chapter II).**

5- Oaks in Collserola and Huecas exhibited a strong masting behaviour with the production of highly synchronous and variable seed crops across years (**Chapter III**)

6- Rainfall amount and seasonality influenced the emergence of adult weevils from the soil in Huecas. **(Chapter III).**

7- Masting contributed to the reduction of seed predation in the two monitored sites (Collserola dand Huecas) and supports the Predator satiation hypothesis. Yet precipitation did also influence acorn predation, probably via its effects on the emergence of adult weevils (**Chapter III**).

8- Differences among the two studied sites (Collserola and Huecas) in the importance of masting to moderate seed predation suggest that the effects of this reproductive behaviour are highly context dependent and change with local conditions (e.g. seed production amount) and spatial location of trees (i.e., isolation). (**Chapter III**).

General discussion references

Asquith, N. M., Wright, S. J., & Clauss, M. J. (1997). Does mammal community composition control recruitment in neotropical forests? Evidence from Panama. Ecology, 78(3), 941-946.

Bogdziewicz, M., Espelta, J. M., Muñoz, A., Aparicio, J. M., & Bonal, R. (2018). Effectiveness of predator satiation in masting oaks is negatively affected by conspecific density. Oecologia, 186(4), 983-993.

Bonal, R., & Muñoz, A. (2008). Seed growth suppression constrains the growth of seed parasites: premature acorn abscission reduces *Curculio elephas* larval size. Ecological entomology, 33(1), 31-36.

Bonal, R., & Muñoz, A. (2009). Seed weevils living on the edge: pressures and conflicts over body size in the endoparasitic *Curculio* larvae. Ecological Entomology, 34(3), 304-309.

Bonal, R., Hernández, M., Espelta, J. M., Muñoz, A., & Aparicio, J. M. (2015). Unexpected consequences of a drier world: evidence that delay in late summer rains biases the population sex ratio of an insect. Royal Society open science, 2(9), 150198.

Bonal, R., Muñoz, A., & Díaz, M. (2007). Satiation of predispersal seed predators: the importance of considering both plant and seed levels. Evolutionary Ecology, 21(3), 367-380.

Bonal, R., Muñoz, A., & Espelta, J.M. (2010). Mismatch between the timing of oviposition and the seasonal optimum. The stochastic phenology of Mediterranean acorn weevils. Ecological Entomology, 35(3), 270-278.

Braschler, B., & Hill, J. K. (2007). Role of larval host plants in the climate-driven range expansion of the butterfly *Polygonia c-album*. Journal of Animal Ecology, 76(3), 415-423.

Brudvig, L. A., Damschen, E. I., Haddad, N. M., Levey, D. J., & Tewksbury, J. J. (2015). The influence of habitat fragmentation on multiple plant–animal interactions and plant reproduction. Ecology, 96(10), 2669-2678.

Byers, D. L. (2017). Studying plant–pollinator interactions in a changing climate: A review of approaches. Applications in plant sciences, 5(6), 1700012.

Byrnes, J., Stachowicz, J. J., Hultgren, K. M., Randall Hughes, A., Olyarnik, S. V., & Thornber, C. S. (2006). Predator diversity strengthens trophic cascades in kelp forests by modifying herbivore behaviour. Ecology letters, 9(1), 61-71.

Castro-Arellano, I., & Lacher, T. E. (2009). Temporal niche segregation in two rodent assemblages of subtropical Mexico. Journal of Tropical Ecology, 25(6), 593-603.

Chase, J. M., & Myers, J. A. (2011). Disentangling the importance of ecological niches from stochastic processes across scales. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 366(1576), 2351-2363.

Chesson, P. (2000). Mechanisms of maintenance of species diversity. Annual review of Ecology and Systematics, 31(1), 343-366.

De Lucia, E., Nabity, P., Zavala, J., & Berenbaum, M. (2012). Climate change: resetting plant-insect interactions. Plant Physiology, pp-112.

Devictor, V., Clavel, J., Julliard, R., Lavergne, S., Mouillot, D., Thuiller, W., Venail, P., Villéger, S., & Mouquet, N. (2010). Defining and measuring ecological specialization. Journal of Applied Ecology, 47(1), 15-25.

Di Bitetti, M. S., Di Blanco, Y. E., Pereira, J. A., Paviolo, A., & Pírez, I. J. (2009). Time partitioning favors the coexistence of sympatric crab-eating foxes (*Cerdocyon thous*) and pampas foxes (*Lycalopex gymnocercus*). Journal of Mammalogy, 90(2), 479-490.

Espelta, J. M., Bonal, R., & Sánchez-Humanes, B. (2009). Pre-dispersal acorn predation in mixed oak forests: interspecific differences are driven by the interplay among seed phenology, seed size and predator size. Journal of Ecology, 97(6), 1416-1423.

Espelta, J. M., Cortés, P., Molowny-Horas, R., Sánchez-Humanes, B., & Retana, J. (2008). Masting mediated by summer drought reduces acorn predation in Mediterranean oak forests. Ecology, 89(3), 805-817.

Fahrig, L., & Merriam, G. (1985). Habitat Patch Connectivity and Population Survival. Ecology, 66(6), 1762-1768.

Fahrig, L., & Paloheimo, J. (1988). Effect of spatial arrangement of habitat patches on local population size. Ecology, 69(2), 468-475.

Fernández-Martínez, M., Vicca, S., Janssens, I. A., Espelta, J. M., & Peñuelas, J. (2017). The North Atlantic Oscillation synchronises fruit production in western European forests. Ecography, 40(7), 864-874.

Forrest, J. R. (2015). Plant–pollinator interactions and phenological change: what can we learn about climate impacts from experiments and observations? Oikos, 124(1), 4-13.

Forrest, J. R., & Miller-Rushing, A. J. (2010). Toward a synthetic understanding of the role of phenology in ecology and evolution. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1555), 3101-3112

Futuyma, D. J., & Moreno, G. (1988). The evolution of ecological specialization. Annual Review of Ecology and Systematics, 19(1), 207-233.

Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G. W., & Holt, R. D. (2010). A framework for community interactions under climate change. Trends in ecology & evolution, 25(6), 325-331.

Gordo, O., & Sanz, J. J. (2005). Phenology and climate change: a long-term study in a Mediterranean locality. Oecologia, 146(3), 484-495.

Govindan, B. N., & Swihart, R. K. (2014). Community structure of acorn weevils (*Curculio*): inferences from multispecies occupancy models. Canadian Journal of Zoology, 93(1), 31-39.

Grass, I., Jauker, B., Steffan-Dewenter, I., Tscharntke, T., & Jauker, F. (2018). Past and potential future effects of habitat fragmentation on structure and stability of plant–pollinator and host–parasitoid networks. Nature ecology & evolution, DOI: 10.1038/s41559-018-0631-2.

Guariguata, M. R., Arias-LeClaire, H. & Jones, G. (2002). Tree seed fate in a logged and fragmented forest landscape, northeastern Costa Rica1. Biotropica, 34(3), 405-415.

Guidone, L., Valentini, N., Rolle, L., Me, G., & Tavella, L. (2007). Early nut development as a resistance factor to the attacks of *Curculio nucum* (Coleoptera: Curculionidae). Annals of applied biology, 150(3), 323-329.

Haine, E. R., Moret, Y., Siva-Jothy, M. T., & Rolff, J. (2008). Antimicrobial defense and persistent infection in insects. Science, 322(5905), 1257-1259.

Heinrich, B. (1976). Resource partitioning among some eusocial insects: bumblebees. Ecology, 57(5), 874-889.

Hernández-Vera, G., Mitoviĉ, M., Joviĉ, J., Toŝevski, I., Caldara, R., Gassmann, A., & Emerson, B. C. (2010). Host-associated genetic differentiation in a seed parasitic weevil *Rhinusa antirrhini* (Coleptera: Curculionidae) revealed by mitochondrial and nuclear sequence data. Molecular Ecology, 19(11), 2286-2300.

Ingvarsson, P. K., & Olsson, K. (1997). Hierarchical genetic structure and effective population sizes in Phalacrus substriatus. Heredity, 79(2), 153-161.

Kelly, D., & Sork, V. L. (2002). Mast seeding in perennial plants: why, how, where? Annual Review of Ecology and Systematics, 33(1), 427-447.

Kneip, É., Van Vuren, D. H., Hostetler, J. A., & Oli, M. K. (2011). Influence of population density and climate on the demography of subalpine golden-mantled ground squirrels. Journal of Mammalogy, 92(2), 367-377.

Koenig, W. D., Alejano, R., Carbonero, M. D., Fernández-Rebollo, P., Knops, J. M. H., Marañón, T., Padilla-Díaz, C.M., Pearse, I. S., Pérez-Ramos, I. M., Vázquez-Piqué, J., & Pesendorfer, M. B. (2016). Is the relationship between mast-seeding and weather in oaks related to their life-history or phylogeny? Ecology, 97(10), 2603-2615.

Kolb, A., Ehrlen, J., & Eriksson, O. (2007). Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. Perspectives in Plant Ecology, Evolution and Systematics, 9(2), 79-100.

Kronfeld-Schor, N., & Dayan, T. (2003). Partitioning of time as an ecological resource. Annual Review of Ecology, Evolution, and Systematics, 34(1), 153-181.

Kubisch, A., Holt, R. D., Poethke, H. J., & Fronhofer, E. A. (2014). Where am I and why? Synthesizing range biology and the eco-evolutionary dynamics of dispersal. Oikos, 123(1), 5-22.

Kudo, G., & Ida, T. Y. (2013). Early onset of spring increases the phenological mismatch between plants and pollinators. Ecology, 94(10), 2311-2320.

Lennartsson, T. (2002). Extinction thresholds and disrupted plant–pollinator interactions in fragmented plant populations. Ecology, 83(11), 3060-3072.

Levins, R. (1970). Extinction. In ‘Some Mathematical Problems in Biology. (Ed. M. Gerestenhaber.) pp. 75–107. American Mathematical Society: Providence, Rhode Island, USA.

Loreau, M. (1992). Time scale of resource dynamics and coexistence through time partitioning. Theoretical Population Biology, 41(3), 401-412.

Maron, J. L., Baer, K. C., & Angert, A. L. (2014). Disentangling the drivers of context-dependent plant–animal interactions. Journal of Ecology, 102(6), 1485-1496.

Merville, A., Venner, S., Henri, H., Vallier, A., Menu, F., Vavre, F., Heddi, A., & Bel-Venner, M. C. (2013). Endosymbiont diversity among sibling weevil species competing for the same resource. BMC evolutionary biology, 13(1), 28.

Moreira, X., Abdala-Roberts, L., Zas, R., Merlo, E., Lombardero, M. J., Sampedro, L., & Mooney, K. A. (2016). Masting behaviour in a Mediterranean pine tree alters seed predator selection on reproductive output. Plant Biology, 18(6), 973-980.

Oliver, K. M., Russell, J. A., Moran, N. A., & Hunter, M. S. (2003). Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. Proceedings of the National Academy of Sciences, 100(4), 1803-1807.

Peguero, G., Bonal, R., Sol, D., Muñoz, A., Sork, V. L., & Espelta, J. M. (2017). Tropical insect diversity: evidence of greater host specialization in seed-feeding weevils. Ecology, 98(8), 2180-2190.

Peled, E., Shanas, U., Granjon, L., & Ben-Shlomo, R. (2016). Connectivity in fragmented landscape: Generalist and specialist gerbils show unexpected gene flow patterns. Journal of Arid Environments, 125, 88-97.

Pélisson, P. F., Bel-Venner, M. C., Giron, D., Menu, F., & Venner, S. (2013b). From income to capital breeding: when diversified strategies sustain species coexistence. PloS one, 8(9), e76086.

Pélisson, P. F., Bel-Venner, M. C., Rey, B., Burgevin, L., Martineau, F., Fourel, F., Lecuyer, C., Menu, F., & Venner, S. (2012). Contrasted breeding strategies in four sympatric sibling insect species: when a proovigenic and capital breeder copes with a stochastic environment. Functional ecology, 26(1), 198-206.

Pélisson, P. F., Bernstein, C., Francois, D., Menu, F., & Venner, S. (2013a). Dispersal and dormancy strategies among insect species competing for a pulsed resource. Ecological entomology, 38(5), 470-477.

Peñuelas, J., Sardans, J., Filella, I., Estiarte, M., Llusià, J., Ogaya, R., ... & Peguero, G. (2017). Assessment of the impacts of climate change on Mediterranean terrestrial ecosystems based on data from field experiments and long-term monitored field gradients in Catalonia. Environmental and Experimental Botany 152, 49–59.

Pérez-Ramos, I. M., Ourcival, J. M., Limousin, J. M., & Rambal, S. (2010). Mast seeding under increasing drought: results from a long-term data set and from a rainfall exclusion experiment. Ecology, 91(10), 3057-3068.

Peterson, M. A., & Denno, R. F. (1998). The influence of dispersal and diet breadth on patterns of genetic isolation by distance in phytophagous insects. The American Naturalist, 152(3), 428-446.

Poncet, B. N., Garat, P., Manel, S., Bru, N., Sachet, J. M., Roques, A., & Despres, L. (2009). The effect of climate on masting in the European larch and on its specific seed predators. Oecologia, 159(3), 527-537.

Pringle, E. G. (2016). Orienting the Interaction Compass: Resource Availability as a Major Driver of Context Dependence. PLoS biology, 14(10), e2000891.

Rossetti, M. R., Tscharntke, T., Aguilar, R., & Batáry, P. (2017). Responses of insect herbivores and herbivory to habitat fragmentation: a hierarchical meta-analysis. Ecology letters, 20(2), 264-272.

Ruíz-Carbayo, H. (2018). The role of landscape and species attributes in insect community assembly, population genetics and plant-insect interactions in expanding Quercus ilex forests. Doctoral Thesis in Terrestrial Ecology. Ecological and Forestry Applications Research Centre (CREAF). Autonomous University of Barcelona (UAB). Cerdanyola, Barcelona, 162 pp.

Ruíz-Carbayo, H., Bonal, R., Espelta, J. M., Hernández, M., & Pino, J. (2017). Community assembly in time and space: the case of Lepidoptera in a Quercus ilex L. savannah-like landscape. Insect Conservation and Diversity, 10(1), 21-31.

Ruíz-Carbayo, H., Bonal, R., Pino, J., & Espelta, J. M. (2018). Zero-sum landscape effects on acorn predation associated with shifts in granivore insect community in new holm oak (Quercus ilex) forests. Diversity and Distributions, 24(4), 521-534.

Sánchez-Humanes, B., & Espelta, J. M. (2011). Increased drought reduces acorn production in Quercus ilex coppices: thinning mitigates this effect but only in the short term. Forestry, 84(1), 73-82.

Schoener, T. W. (1974). Resource partitioning in ecological communities. Science, 185(4145), 27-39.

Schraer, S. M., Harris, M., Jackman, J. A., & Biggerstaff, M. (1998). Pecan weevil (Coleoptera: Curculionidae) emergence in a range of soil types. Environmental entomology, 27(3), 549-554.

Servanty, S., Gaillard, J. M., Carole, T., Serge, B., & Eric, B. (2009). Pulsed resources and climate-induced variation in the reproductive traits of wild boar under high hunting pressure. Journal of Animal Ecology, 78(6), 1278-1290.

Steffan-Dewenter, I., Münzenberg, U., Bürger, C., Thies, C., & Tscharntke, T. (2002). Scale-dependent effects of landscape context on three pollinator guilds. Ecology, 83(5), 1421-1432.

Sunyer, P., Muñoz, A., Mazerolle, M. J., Bonal, R., & Espelta, J. M. (2016). Wood mouse population dynamics: Interplay among seed abundance seasonality, shrub cover and wild boar interference. Mammalian Biology-Zeitschrift für Säugetierkunde, 81(4), 372-379.

Tylianakis, J. M., Didham, R. K., Bascompte, J., & Wardle, D. A. (2008). Global change and species interactions in terrestrial ecosystems. Ecology letters, 11(12), 1351-1363.

Tylianakis, J. M., Laliberté, E., Nielsen, A., & Bascompte, J. (2010). Conservation of species interaction networks. Biological conservation, 143(10), 2270-2279.

Valiente-Banuet, A., Aizen, M. A., Alcántara, J. M., Arroyo, J., Cocucci, A., Galetti, M., García, M. B., García, D., Gómez, J., Jordano, P., Medel, R., Navarro, L., Obeso, J. R., Oviedo, R., Ramírez, N., Rey, P. J., Traveset, A., Verdú, M., & Zamora, R. (2015). Beyond species loss: the extinction of ecological interactions in a changing world. Functional Ecology, 29(3), 299-307.

Venner, S., Pélisson, P.F., Bel-Venner, M.C., Debias, F., Rajon, E. & Menu, F. (2011) Coexistence of insect species competing for a pulsed resource: toward a unified theory of biodiversity in fluctuating environments. PLoS ONE, 6, e18039.

Westphal, C., Steffan-Dewenter, I., & Tscharntke, T. (2006). Bumblebees experience landscapes at different spatial scales: possible implications for coexistence. Oecologia, 149(2), 289-300.

Wróbel, A., & Bogdziewicz, M. (2015). It is raining mice and voles: which weather conditions influence the activity of *Apodemus flavicollis* and *Myodes glareolus*?. European Journal of Wildlife Research, 61(3), 475-478.

Xia, K., Harrower, W. L., Turkington, R., Tan, H. Y., & Zhou, Z. K. (2016). Pre-dispersal strategies by *Quercus schottkyana* to mitigate the effects of weevil infestation of acorns. Scientific Reports, 6, 37520.

Young, A., Boyle, T., & Brown, T. (1996). The population genetic consequences of habitat fragmentation for plants. Trends in ecology & evolution, 11(10), 413-418.

This page intentionally left blank

Epilogue

Plant-insect interaction: the final frontier.

These are the voyages of the starship “Doctoral Thesis”. Its mission: to explore strange new hypotheses, to seek out new interactions and new conclusions, to boldly go where no phytophagous insect or plant host has gone before…

…A not so long time ago in a galaxy not so far away…It is a dark time for plant-insect interactions. Although the Oak masting has been functional, Weevil troops have been driven away by Rainfall Stochasticity forces from their hidden base and pursued them across the galaxy. Evading the dreaded Global Change, a group of freedom weevils led by Curculio has established a new secret base on the remote world of new Plant Hosts. The evil lord "Competitive Exclusion", obsessed with finding young Curculio, has dispatched thousands of DNA analysers into the far reaches of space....

…Thanks for sharing my journey into the secret life of plants and insects and having very close encounters of the biological interactions kind. Here I tried to explore plant-insect vicissitudes in wonderland or maybe somewhere in time. To find out that they are constantly living on the edge and weevils like zombies arise from the ground with the rain. On this trip, from The Shire to Mordor and back home the extremely amazing weevils from the segregating fellowship wander in winding paths on a thrilling odyssey full of contingent, pulsed and stochastic outcomes. Finally, this epiphanies and wonder tales of weevils riding seeds on the storm will be remembered between brainwaves and reflections in the ancient books of the dead peak-oilers society.

…I dedicate this epilogue in loving memory to all those weevils, adults and larvae, that lie in a 1.5 ml eppendorf on the lab in the name of science progress. Now you are one with the force and the force is one with you ...May the force be with you always…

This is the end
Beautiful friend
This is the end
My only friend
the end
Of our elaborate plans, the end
Of everything that stands, the end
No safety or surprise, the end
I'll never look into your eyes again

The Doors 1967

This page intentionally left blank

BARCELONA | 2018

PRE-DISPERSAL SEED PREDATION

Arias-LeClaire, Harold (2018).
Pre-dispersal seed predation by weevils (Curculio spp.): The role of host-specificity, resource availability and environmental factors

Top: adult female weevil Curculio sp.
Middle top: acorn infested by Curculio sp. larvae
Middle bottom: weevil larvae emerging from a hazelnut.
Bottom:field sampling (Prades, Catalunya)

Pictures by N. Gamboa-Badilla and H. Arias-LeClaire