3-22-2018

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In brood pollination mutualisms, predation of developing fruit can have large negative repercussions for both plant and pollinator population dynamics. The Sonoran Desert rock fig Ficus petiolaris and its highly-coevolved wasp pollinator are subject to frequent attack by lepidopteran larvae that consume fig fruit and the developing seeds and larval pollinators they contain. We used generalized linear mixed models to investigate how the phenology, quantity, and spatial distribution of fig fruits is associated with variation in lepidopteran damage intensity on individual trees at nine geographic locations spanning a 741 km latitudinal transect along Mexico's Baja California Peninsula. We found lepidopteran damage to be strongly positively associated with more synchronous fig crops and larger trees, and only weakly associated with local host tree density. These results imply that fruit production that is asynchronous within trees and spread out over time, as observed in several fig species, benefits female and male components of fitness (pollen disperser and seed production, respectively) by reducing pre-dispersal predation by frugivores.

Disciplines

Ecology and Evolutionary Biology | Entomology | Plant Sciences

Comments

This is a manuscript of an article published as Piatscheck, Finn, Justin Van Goor, Derek D. Houston, and John D. Nason. "Ecological factors associated with pre-dispersal predation of fig seeds and wasps by fig-specialist lepidopteran larvae." *Acta Oecologica* (2018). doi: 10.1016/j.actao.2018.03.001. Posted with permission.

Ecological Factors Associated with Pre-dispersal Predation of Fig Seeds and Wasps by Fig-Specialist Lepidopteran Larvae

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Abstract

In brood pollination mutualisms, predation of developing fruit can have large negative repercussions for both plant and pollinator population dynamics. The Sonoran Desert rock fig *Ficus petiolaris* and its highly-coevolved wasp pollinator are subject to frequent attack by lepidopteran larvae that consume fig fruit and the developing seeds and larval pollinators they contain. We used generalized linear mixed models to investigate how the phenology, quantity, and spatial distribution of fig fruits is associated with variation in lepidopteran damage intensity on individual trees at nine geographic locations spanning a 741 km latitudinal transect along Mexico's Baja California Peninsula. We found lepidopteran damage to be strongly positively associated with more synchronous fig crops and larger trees, and only weakly associated with local host tree density. These results imply that fruit production that is asynchronous within trees and spread out over time, as observed in several fig species, benefits female and male components of fitness (pollen disperser and seed production, respectively) by reducing pre-dispersal predation by frugivores.

1. Introduction

The majority of flowering plants rely on animals for pollination services while pollinators, in turn, obtain valuable plant resources (Ollerton et al., 2011). The success of this mutualistic interaction depends on numerous ecological factors, as the plant-pollinator partners may be differentially affected by both biotic and abiotic environments. For example, numerous studies have investigated climate variation effects on plant phenology as well as temporal mismatches between plants and pollinators that their negatively impacts on mutualistic outcomes (Harrison, 2000; Wall et al., 2003; Memmott et al., 2007; Burkle et al., 2013; Rafferty et al., 2013; Rafferty et al., 2015). Theoretical and empirical studies of optimal foraging also indicate plant density and per-plant production of floral resources to be positively associated with pollinator attraction, floral visitation and, ultimately, seed production (Platt et al., 1974; Silander, 1978; Klinkhamer and de Jong, 1990; Kunin, 1993, 1997), while decreases in both the density and spatial continuity of natural plant populations negatively impact pollinator visitation rates and plant reproductive success (Nason and Hamrick, 1997; Tscharntke and Brandl, 2004; Aguilar et al., 2006; Potts et al., 2010). Such studies are of growing importance since the global-scale increase in average temperature is causing a temporal shift towards earlier flowering (Fitter and Fitter, 2002; Walther et al., 2002; Cleland et al., 2007; Franks et al., 2007; Cook et al., 2012) and human-mediated landscape fragmentation is already advanced and increasing (Vitousek et al., 1997; Achard et al., 2002; Hansen et al., 2013), which together are driving concerns about both temporal and spatial mismatches in plant-pollinator interactions.

In addition to mutualistic interactions with insect pollinators, flowering plants are ubiquitously subject to antagonistic interactions from a diverse assemblage of phytophagous insects (including herbivores, florivores, seed predators, and frugivores), which may also be subject to environmentally-driven spatial and temporal mismatches with host plants (Thompson and Gilbert, 2014). A number of studies have investigated host plant density and frequency effects on insect herbivory (Root, 1973; Bach, 1980, 1988; Fagan et al., 2005) and phenological mismatches in plant-antagonist interactions (Augspurger, 1981; Brody, 1997; Wisser et al., 2001; Singer and Parmesan, 2010; Yang and Rudolf, 2010; Liu et al., 2011; Rafferty et al., 2013). Few studies, however, have jointly investigated spatial and phenological effects on plant-pollinator-herbivore interactions (but see Parsche et al., 2011). Concerning phenology, the theoretical study by Fabina et al. (2010) indicates that changes in

phenological overlap in these interactions can alter the relative population densities and persistence of all three symbionts in ways not predicted solely from their pair-wise interactions. This suggests that temporal and spatial variation in climate may simultaneously affect the phenological overlap/mismatch of pollinators, phytophagous insects, and a shared host plant, and, consequently, the outcome of their species interactions.

Researchers considering the consequences of changes in flowering phenology have focused on the distribution of flowering time among individuals within and among populations. Virtually unstudied is the variance in flowering phenology *within* individual plants and, in turn, its effects on antagonistic interactions with phytophagous insects. While more asynchronous flowering may potentially ameliorate environmentally-driven phenological mismatches with pollinators, it may similarly benefit antagonists and, thus, have unappreciated costs for mutualism. Flowering phenology, resource availability (flower or fruit production), and plant density might thus be factors potentially affecting the plant-phytophagous insect dynamic. We expect that 1) within-plant asynchronous flowering will extend the phenological overlap between plant resources and insect antagonists leading to greater costs to the plant, 2) larger crops of resources will experience greater damage than smaller ones, and 3) fruit damage will increase with local host plant density. We tested these predictions by investigating interactions between a native fig and an associated pre-dispersal fruit-feeding insect in Baja California, Mexico.

Figs (*Ficus*, Moraceae) are diverse (750+ species) and widely distributed across tropical and subtropical environments worldwide (Berg, 1989). *Ficus* is typically one of the most speciose genera in tropical forests, with members functioning as keystone species because their aseasonal fruit production provides food vital to the survival of many vertebrate frugivores, especially during seasons when fruiting activity of other plant species is low (Terborgh, 1986; Lambert and Marshall, 1991; Shanahan et al., 2001; Serio-Silva et al., 2002; Harrison, 2005; Kissling et al., 2007). Contributing to reproductive isolation where they co-occur, each fig species typically has a species-specific relationship with a single pollinating chalcidoid wasp species (family Agaonidae; superfamily Chalcidoidea) that develops within galled fig seeds before mating within host inflorescences (syconia, commonly called fig fruits). The reproductive phenology of figs is unusual in that flowering is generally highly synchronized within trees and yet highly aseasonal at the population level. Moreover, the production of male and female flowers is separated by several weeks so that to interbreed

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trees must be phenologically out of phase with each other. In some species, however, trees exhibit substantial within-crown flowering asynchrony and may simultaneously bear syconia at various stages of development (Smith and Bronstein, 1996; Gates and Nason, 2012). This asynchrony may be of substantial benefit to the reproductive success of figs and pollinators by increasing overlap in flowering times and, hence, opportunities for pollinator and pollen transfer between trees (Gates and Nason, 2012). It is expected to be particularly beneficial where host populations are small and spatially isolated (Bronstein et al., 1990; Anstett et al., 1995; Anstett et al., 1997; Gates and Nason, 2012).

In addition to providing an excellent model system for studying obligate mutualism, figs are exploited by a diversity of non-pollinating insects. The best known of these are non-pollinating fig wasps (multiple chalcidoid families) that reproduce within fig syconia (West et al., 1996; Weiblen, 2002; Borges, 2015). These non-pollinating wasps exploit the mutualism in various ways and are generally associated with a decrease in pollinator production, and to a lesser extent seed production (West and Herre, 1994; Kerdelhue et al., 2000). Less well studied are the myriad of other animals that are antagonist of the fig-pollinator mutualism, including moths. In the Sonoran Desert rock fig, *Ficus petiolaris*, an undescribed moth species (family Crambidae) lays eggs on or under the cuticle of the fig syconium, with later instar caterpillars boring into syconia and consuming developing seeds and wasps. Although few cases of fig boring lepidopteran caterpillars have been reported in the literature (New World: Janzen, 1979; Bronstein, 1988; Jandér, 2015; Old World: Sugiura and Yamazagi, 2004), we observed lepidopteran damage to the syconia of *F. petiolaris* to not only be common but to sometimes be drastically high, destroying all syconia with their developing seeds and pollinators within a tree. *Ficus petiolaris* thus provides a useful and interesting biological system to study the effects of flowering phenology, crop size and plant density on levels of attack by a phytophagous insect that has significant consequences on host fitness

For this study we first obtained samples of the moth antagonist of *F. petiolaris* from nine sites spanning its range in Baja California, Mexico, and employed mitochondrial sequences and phylogenetic tools to confirm its species delimitation and determine its taxonomic placement within the Crambidae. We then used generalized linear mixed models to test whether within- and among-site variation in fig damage caused by the moth larvae are related to measures of asynchronous flowering, crop size and/or local fig tree density. These

models enabled us to test our *a priori* hypotheses, namely that each of the three predictor variables will be positively associated with levels of caterpillar damage and fruit loss.

2. Materials and methods

2.1. The biological system

Ficus petiolaris is an evergreen rock-strangling fig tree occurring within Mexico from Oaxaca in the south to the Sonoran Desert environments of Sonora and Baja California in the north. Because *F. petiolaris* grows on rocks, cliffs or canyon walls, it forms small natural population patches on rocky landscapes. Tree size varies greatly and can reach 20+ meters in height (Finn Piatscheck, pers. obs.). As a member of subgenus *Urostigma* (section *Americana*), this species is monoecious with syconia containing both female and male flowers. In contrast to most other *Americana* species, however, syconia production in *F. petiolaris* is often asynchronous within individual trees (Smith and Bronstein, 1996; Gates and Nason, 2012). *Ficus petiolaris* is pollinated by a single species of chalcid wasp (*Pegoscapus sp.*, Agaonidae), with this mutualism being exploited by several obligately-associated insects, including eight species of non-pollinating chalcidoid wasps and one species of crambid moth. Extensive surveys of *F. petiolaris* trees and surrounding vegetation have located larvae of this moth only on fig trees (J. Nason, pers. obs.), consistent with it being a fig specialist. Moth larva typically bore into syconia near the peduncle and an individual late-instar caterpillar consumes the interior of several syconia – including seeds and wasp larvae - during its development. Indeed, after consuming the inside of one syconium, a caterpillar will move to another syconium (often the nearest one), chew its way inside, and use latex produced by the fig mixed with other material to then close the entry hole (F. Piatscheck, pers. obs.). In addition, a caterpillar will often feed within a pair of adjacent syconia connected via a tunnel it constructs from latex material, an unusual behavior further suggesting fig specialization. Oviposition by female moths and movement of larvae between syconia is exclusively nocturnal, and fifth instar caterpillars drop by night on silken threads from *F. petiolaris* trees to pupate in the soil. Any fig damaged by a caterpillar will abort with the loss of all remaining developing seeds and pollinators. The total damage to a tree can be substantial as we have in some cases observed fruit crops of *F. petiolaris* in which 100% of syconia were consumed by the caterpillars.

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2.2. Site distribution and sampling

Field data were collected from nine sites distributed along a 741 km transect on Mexico's Baja California peninsula (Fig. 1, Table 1). At each site, we established a census population of geo-referenced *F. petiolaris* trees. Field data, including measures of reproductive activity and lepidopteran damage per tree, were obtained during three extended field trips: during the dry season in spring (May-June) 2013 and 2014, and during the wet season in fall (November-December) 2013. Southern sites were relatively mesic with more summer rainfall, whereas northern sites are climatically more extreme with less rainfall and greater seasonal temperature variation. Due to natural variation in reproductive activity, the number of flowering trees censused for lepidopteran damage varied by site and trip.
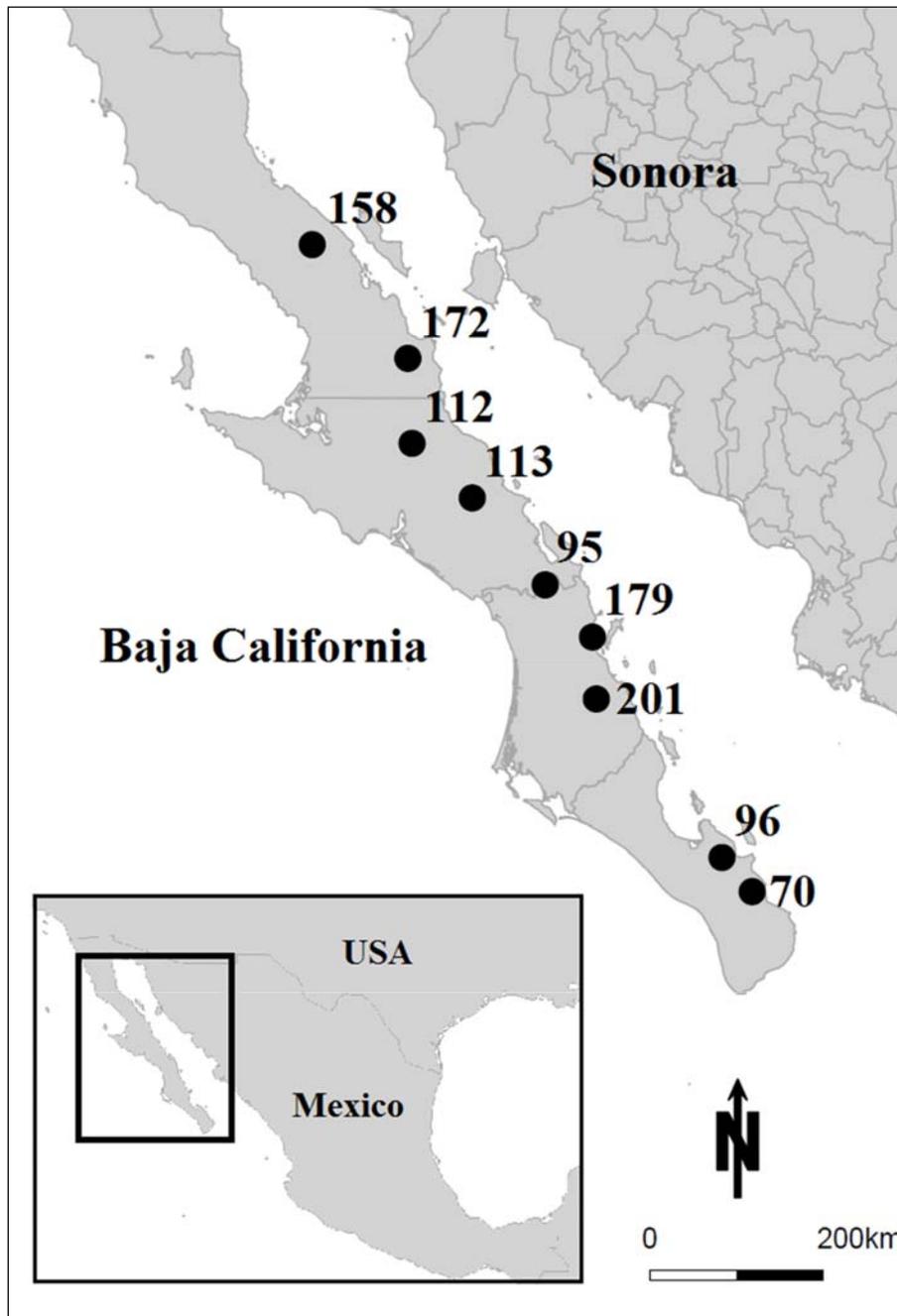
Fig. 1. Locations of the nine *Ficus petiolaris* study sites located along the Baja California peninsula, Mexico. The numbers correspond to the site designations described in Table 1.

Table 1

Site information from north to south, including numbers of mapped trees and incidence of *Omiodes* damage on flowering trees by study season (S denotes spring [May-June], F denotes fall [November-December]).

Site	Latitude, longitude	No. mapped trees	No. damaged trees/no. flowering trees		
			S2013	F2013	S2014
Site 158: Cañon Aguajito Higuera	29,2628391, -114,021134	103	8/19 (42%)	10/18 (56%)	17/20 (85%)
Site 172: Bahia San Francisquito	28,26775278, -113,1095672	70	9/14 (64%)	6/14 (43%)	7/15 (47%)
Site 112: Sierra San Francisco	27,56043406, -113,0671864	75	9/15 (60%)	7/21 (58%)	10/41 (23%)
Site 113: La Higuera	27,0995915, -112,4968451	76	15/20 (75%)	13/24 (54%)	11/30 (37%)
Site 95: Fig Canyon	26,3598798, -111,8040866	101	N/A	14/26 (54%)	9/29 (31%)
Site 179: Nopolo	25,91341244, -111,3514215	38	N/A	4/13 (31%)	2/19 (11%)
Site 201: El Ranchito	25,37795264, -111,3125629	42	N/A	4/7 (57%)	2/16 (13%)
Site 96: La Paz Summit	24,0459034, -110,1344447	337	11/18 (61%)	14/29 (48%)	3/39 (8%)
Site 70: San Bartolo	23,73747426, -109,8275927	105	24/37 (65%)	14/37 (38%)	14/34 (41%)
TOTAL		947	76/123 (62%)	86/189 (46%)	75/243 (31%)

2.3. Lepidopteran caterpillar identification

Caterpillars were collected from infested syconia across the nine studied sites. We selected 14 caterpillars (at least one per site) and sequenced the mitochondrial gene cytochrome c oxidase subunit I (COI). We used primers LCO1490 (GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO20198 (TAA ACT TCA GGG TGA CCA AAA AAT CA) (Vrijenhoek, 1994) resulting in a total targeted mDNA region of 696 nucleotides. The targeted region was amplified by polymerase chain reaction with 35 cycles following this profile: 95°C for 30 seconds, 48°C for 30 seconds and 72°C for 90 seconds. We used NCBI BLAST (NCBI, R. C., 2016) to obtain a first approximation of closely related crambid taxa, finding that the lepidopteran antagonist of *F. petiolaris* clusters with members of the genus *Omiodes* (Guenée, 1854). To determine the position of our species within this genus, we constructed a COI phylogeny of *Omiodes* from our sequences and publicly available data from NCBI, including 3 sequences from *O. stigmosalis* associated with fig fruit in Costa Rica (D. Jansen, per. comm.), 1 sequences from *O. stigmosalis* collected by an amateur collector in Florida, United State (J. Hayden, per comm.) and 36 sequences from non-fig associated *Omiodes* (Haines and Rubinoff, 2012). Crambid species *Eurrhypara hortulata* and *Tetridia caletoralis* were included as outgroups, resulting a total of 46 sequences. After alignment using MAFFT (Katoh and Standley, 2013) and trimming using MEGA7.0.21 (Kumar et al., 2016), we obtained a final alignment of 604 nucleotides per individual. We built a phylogeny from this alignment using MrBayes (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The appropriate model of sequence evolution was determined using FindModel (http://hiv.lanl.gov/content/sequence/findmodel/findmodel.html). After setting the model to generalized time-reversible with gamma-distributed rate variation across sites and a proportion of invariable sites (GTR+Γ+I),, two simultaneous runs with 4 chains were executed for 10,000,000 generations, with a sampling realized each 1000 generations. Posterior probabilities were calculated with a discard of the first 1000 samples as burn in.

2.4. Lepidopteran damage data

Syconia attacked by lepidopteran larvae are easily recognized from the damage they cause. First instar larvae (3-6 mm in length) are aggregated in small groups (presumably siblings) on syconia where they generate a cluster small diameter holes chewed vertically into the fruit wall. Later instar larvae disperse to other syconia where they inflict increasingly

greater damage, with fifth instar larvae (1.5-2 cm in length) consuming the contents of entire syconium. To obtain a tree-level measure of lepidopteran damage we sampled 15 independent syconia from each of four separate branches for a total of 60 fig fruits per tree. For smaller or less reproductive trees, we sampled as many syconia as were available. Each syconium was recorded as damaged or not damaged resulting in binary data set for each tree.

2.5. Measurement of fig reproductive phenology

To measure the phenological synchrony/asynchrony of syconia production within trees, we sampled 10 fruits from each of four branches per tree per trip. The syconia were opened and the developmental phase recorded. Seven development phases were recognized, two more than the A-E phases previously described by Galil and Eisikowitch (1968): pre-female phase (A phase: pistillate and staminate flowers immature), receptive female phase (early B phase: pistillate flowers mature, syconia attracting pollinating fig wasps), post-pollination female phase (late B phase: pistils decaying, syconia no longer attracting pollinators), early inter-floral phase (early C phase: early development of wasps and seeds), late inter-floral phase (late C phase: late development of wasps and seeds, pigmentation of larvae), male phase (D phase: staminate flowers and seeds mature, adult fig wasps present within the syconium), and post male phase (E phase: syconium expanding, softening, and sweetening, wasps have exited via hole chewed by male fig wasps). We measured reproductive asynchrony within the crowns of individual trees using the inverse of Simpson's diversity index, which is a function of the observed proportion of syconia of each phase divided by the number of fig phases (Heip et al., 1998):

$$I = \frac{1}{\lambda} = \frac{1}{\sum_{i=1}^{R} p_i{}^2},$$

where I is evenness and our measure of reproductive asynchrony, λ is Simpson's index and p_i is the proportional abundance of flowering phase i. With seven phases recorded, the closer I is to one the more synchronous is the tree's reproduction (all the syconia sampled had the same developmental phase), and the closer the index is to seven the more asynchronous is the flowering (all flowering phases in equal abundance). Nonetheless, any $I \geq 1$ is indicative of within-crown asynchrony, while the maximum $I = 7$ requires perfectly even asynchrony that is unlikely to be observed in nature.

The size of a tree's crop was estimated from its level of reproductive activity and the volume of its crown. We followed Gates & Nason (2012) and estimated a tree's reproductive activity relative to its maximum fig production: 0, $\leq 5\%$, $5 - 25\%$, $25 - 50\%$, $50 - 75\%$, $75 - 95\%$, or $\geq 95\%$. The following formula was used to calculate the volume of an ellipsoid to estimate the size of a tree's crown:

$$V = \left(\frac{\pi}{6}\right) * a * b * c$$

with V being the volume of the crown, a the height of the tree, b the width, and c the length. We multiplied a tree's crown volume by reproductive activity to obtained a measure of its crop size.

2.6. Spatial aggregation of lepidopteran attack

If female Lepidoptera disperse locally to lay their eggs, then lepidopteran larvae and the associated damage of fig are expected to be spatially aggregated. We performed spatial autocorrelation analysis to investigate the spatial aggregation of lepidopteran fruit damage levels across trees within sites. Distance intervals (bins or lags) were determined for each site based on the local density of censused trees and ranged from 50 to 250 m. For each of our 24 site by study season data sets (Table 1), we then measured the mean correlation in the proportion of damaged fruit for each distance interval and plotted the mean for successive distance intervals as a spatial autocorrelogram. The significance of the mean correlation at each distance interval was determined by 1000 permutation replicates. These analyses were conducted in R (R Core Team, 2013) using the correlog function in the R package ncf (Bjornstad, 2009). Spatial aggregation is expected to result in significant positive correlations at smaller distance intervals followed fluctuating positive and negative values at larger spatial scales (Legendre and Fortin 1989). Consequently, in interpreting spatial autocorrelograms we focused on significant positive correlations at the smallest distance interval as evidence of spatial aggregation. Given our 24 site by season data sets, we conducted a total of 24 tests of damage correlation at the smallest distance. Assuming a Type I error rate of 0.05, under the null hypothesis of no spatial aggregation of lepidopteran fruit damage levels, we expected $0.025 \times 24 = 0.6$ of these tests to result in false significant positive correlations (and 0.6 tests to result in false significant negative correlations; 1.2 false significant tests total). Consequently, an observed number of significant positive correlations exceeding 0.6 was considered a global test of spatial aggregation of lepidopteran fruit damage across fig trees.

*2.7. Modeling the ecological correlates of lepidopteran damage*

We used Generalized Linear Mixed Models (GLMM) with binomial errors and a logit link function (logistic regression) to investigate the relationship between the odds of lepidopteran attack (binary counts: damaged/non-damaged fruits) and predictor variables *trees*, *seasons*, the interaction of *trees* and *seasons*, *sites*, *reproductive asynchrony*, *crop size*, and *tree density*. This analysis was conducted using the *glmer* function in the lme4 package (Bates et al., 2014) in R. The predictor variables *trees* and *trees by seasons* were treated as random effects because of the over dispersion of lepidopteran damage levels observed between trees within sites and because some of the same trees were sampled across field seasons. Two measures of *F. petiolaris* density were obtained, (i) the number of geo-referenced trees within a radius of 0.25 km, 0.5 km, 1 km and 2 km, and (ii) the distance to the 5th, 10th, 20th, and 30th nearest neighbor. The relative quality of alternative logistic regression models was evaluated by comparison of Akaike information criterion (AIC) scores, with an AIC difference of 10 or more considered a substantial difference in model support.

3. Results

3.1. Phylogenetic placement of lepidopteran fruit predator

Using NCBI Blast, COI sequences from the lepitopteran seed predator of *F. petiolaris* always matched best with *Omiodes stigmosalis*, most samples of which were obtained from fig fruit in Costa Rica. The sequences were 93% to 98% identical with *O. stigmosalis*, with lower scores likely due to ambiguous base calls in some sequences. Although the consensus phylogenetic tree contained some nodes with low support, three clades received high support: a Hawaiian clade, as seen in Haines and Rubinoff (2012), a clade comprised of two samples of *Omiodes diemenalis*, and a clade including and all neotropical plus a few Australasian *Omiodes* (Fig. 2). Within this latter clade, all fig-associated samples (from *F. petiolaris* and Costa Rica) form a single, well-supported monophyletic lineage. Further, based on posterior probabilities the *F. petiolaris* samples form a distinct lineage nested among previously sequenced *O. stigmosalis* samples, suggesting that *O. stigmosalis* is a single widespread species or closely-related set of species, in either case incorporating phylogeographic or host fig-associated genetic structure. We conclude from this phylogenetic analysis (and the natural

history described above) that the lepidopteran predator of *F. petiolaris* is a fig specialist, and assume for the remainder of the paper that it is a genetically distinct population of *O. stigmosalis*.
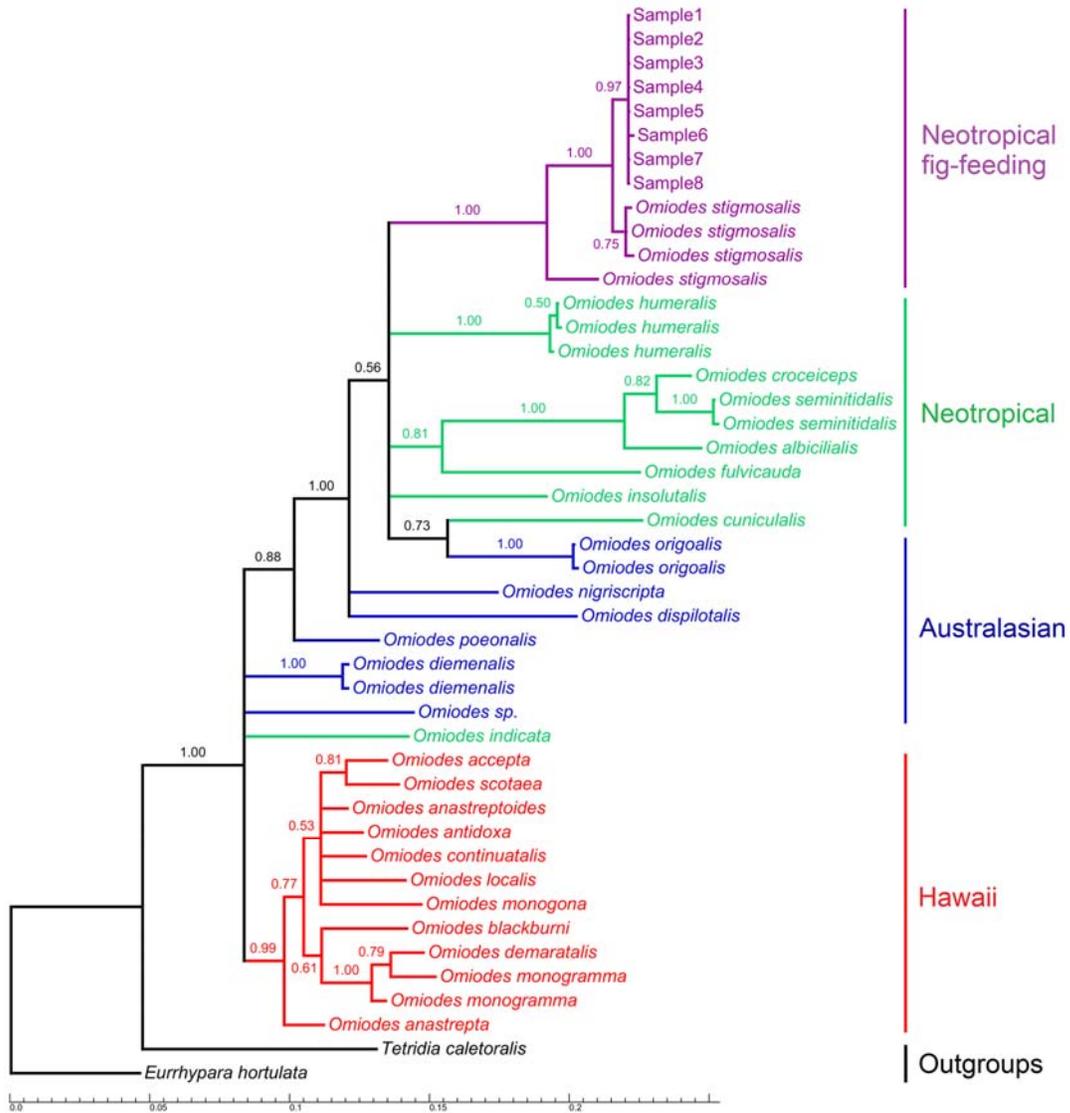
Fig. 2. Consensus tree from Bayesian phylogenetic inference of the pre-dispersal predator of *F. petiolaris* seeds and wasps, *O. stigmosalis* reared from Costa Rican figs, and 38 *Omiodes* sequences from Haines and Rubinoff (2012). Values above the nodes represent posterior probabilities. Values below 0.5 are not shown and associated nodes are collapsed.

3.2. Lepidopteran damage of figs

During the spring 2013 field season, 76 of 123 (62%) reproductively-active trees were attacked by *Omiodes* larvae (Table 1), with 14% of syconia being consumed (Supplementary Table S1). The following two field seasons showed a reduction in the proportion of damaged trees and syconia, with 86 of 189 (46%) of trees and 6% syconia attacked in fall 2013 and 73 of 236 (31%) of trees and 5% of syconia attacked in spring 2014. Lepidopteran larvae and damaged syconia were observed at every site during each season, however, most trees had little or no damaged syconia while only a few trees had high levels of damage, though in some cases with 100% of the fruit crop destroyed (Fig. 3). Site- and season-level data on the proportion of *Omiodes* damaged syconia are presented in Table S1. The proportion of damaged syconia varied substantially across sites and seasons, ranging from nearly 0% to 40% (Fig. 4). The highest damage was observed during the first field season (spring 2013) at Site 113 with an average 40% damaged syconia per tree and with 100% of syconia damaged on some trees. In spring 2014, in contrast, only 5% of syconia were damaged at this site, illustrating the high variation observed between seasons at most sites (Fig. 4).
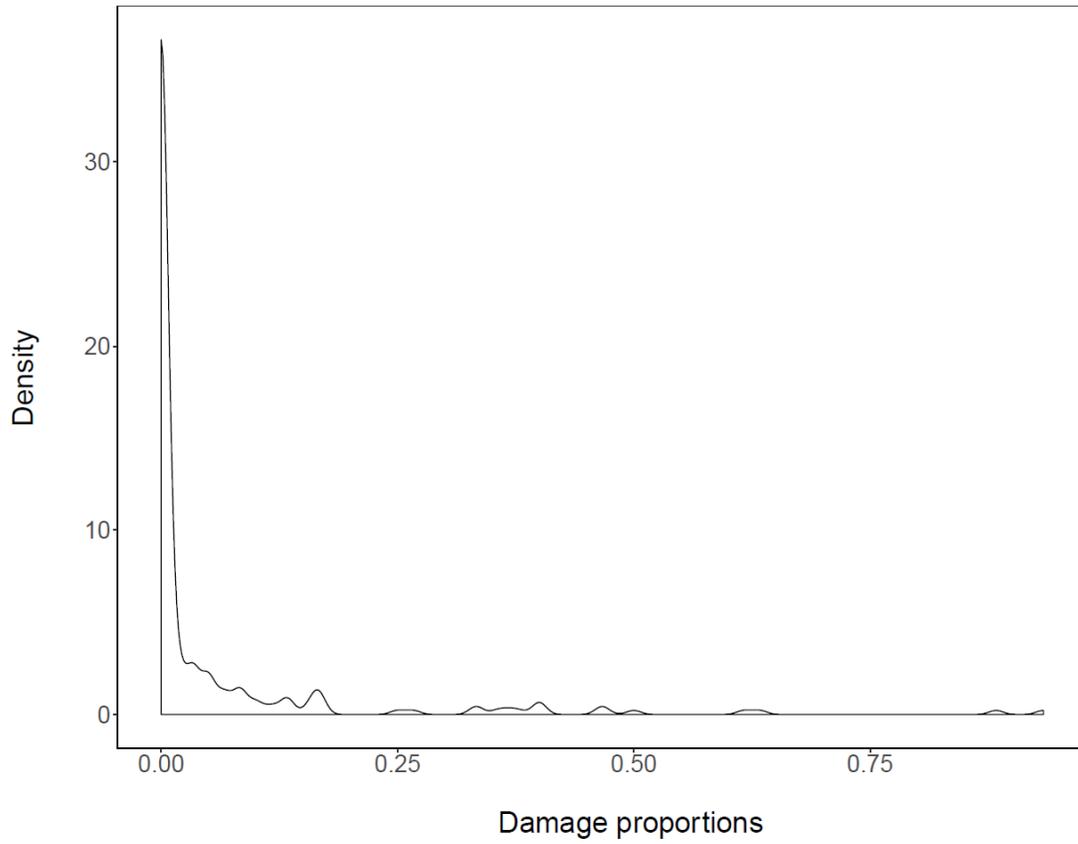
Fig. 3. Density plot of the proportion of *Omiodes*-damaged syconia, with data pooled across trees, sites, and seasons.
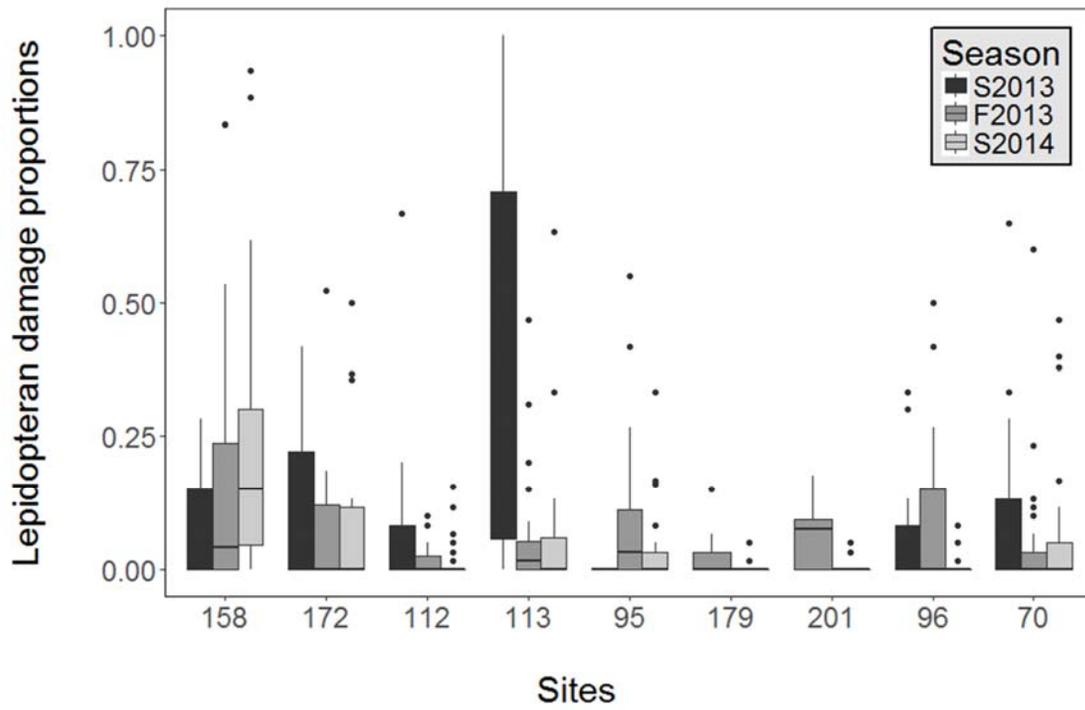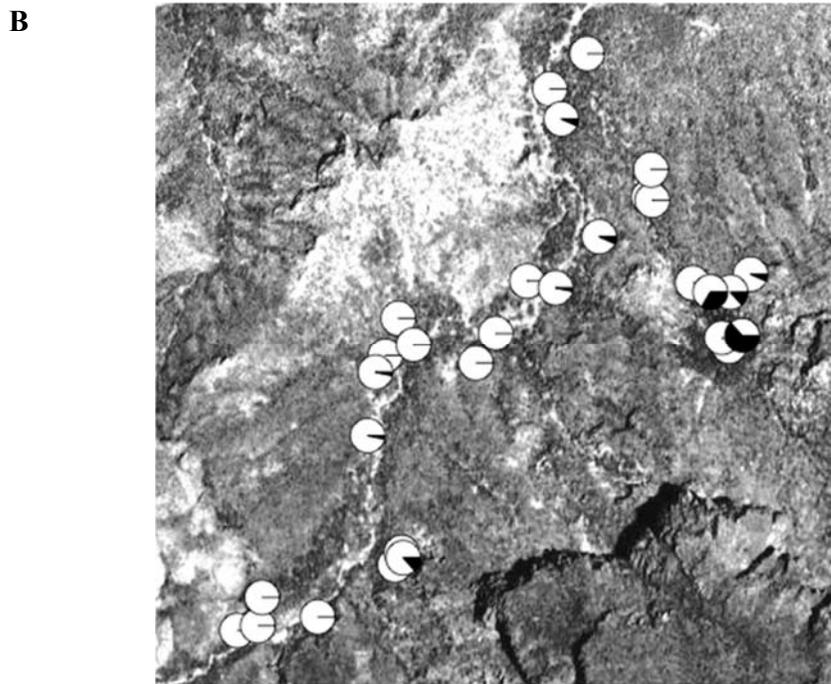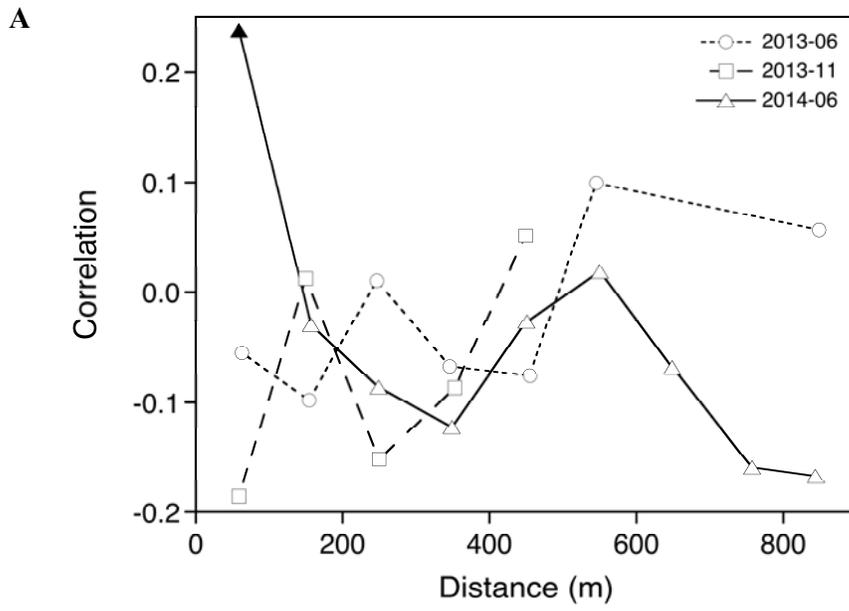
Fig 4. The proportion of *Omiodes* -damaged fig syconia across sites and seasons. Sites are designated as in Table 1 and are ordered from north (158) to south (70).

3.3. Fig reproductive phenology

Measurements of reproductive phenology are presented in Supplementary Table S2. Across sites and seasons, the average percentage of reproductively active, fig-bearing trees was 42%, with more fruiting trees observed during the dry season (39% and 49% during spring 2013 and 2014) than during the wet season (38% during fall 2013). Our measure of within-crown reproductive asynchrony, *I*, averaged 2.06-2.35 over seasons, with a minimum of 1.29 at Site 179 in Fall 2013 and a maximum of 3.23 at Site 96 in Spring 2013. Though less than the maximum possible $I = 7$, asynchrony was ubiquitous and, indeed, 90% of the reproductive trees had syconia in two or more developmental phases. Reproductive trees varied greatly in estimated crown volume (347 m^3 with standard error 16.41) and, consequently, variation in crop size was large.

3.4. Spatial aggregation of lepidopteran attack

Spatial autocorrelation analysis of *Omiodes* fruit damage across trees was conducted for each site and season combination. Results of the 24 tests of autocorrelation at the smallest distance interval are presented in Supplementary Table S3. Of these tests, five were significantly positive, which is substantially more than the 0.6 tests expected to be false positives under the null hypothesis of no spatial aggregation lepidopteran damage. Only a single test was significantly negative (Site 158, November 2013), which is biologically insignificant given the expected 0.6 false-negative results. The five significant positive correlations were observed for different sites and seasons, with significant heterogeneity of spatial autocorrelation results observed across seasons within sites. Results for Site 113 in central Baja are presented as an example of the spatial aggregation of *Omiodes* damage and its variation across seasons (Fig. 5).

A

B

Fig 5. Spatial aggregation of *Omiodes* damaged *F. petiolaris* syconia at Site 113. A- Spatial autocorrelograms of damage at 100 m distance intervals for three seasons (significant correlation indicated by filled symbols). B- The proportion of damaged (black) and undamaged (white) syconia mapped to trees at Site 113 for May 2014, the season with significant autocorrelation of damage at the smallest distance interval, as shown in panel A. Map image is 1 km square.

3.5. Ecological correlates of lepidopteran damage

Based on comparison of AIC scores, the preferred logistic regression model of *Omiodes* fruit damage included as fixed effects *seasons*, *sites*, as random effects *trees* and *trees by seasons*, and as covariates reproductive *asynchrony*, *crop size* and *tree density*. We tested the several measurements of *tree density* and obtained better fits with the distance to the 20th nearest neighbor, which was used in all subsequent analyses. The AIC score for this model was 1559 and the results indicated a significant effect of the *crop size* (slope 0.77, p = 0.0071) and *tree density* (slope 0.14, p = 0.0296). The effect of *asynchrony* was marginally significant (slope -0.35, p = 0.0503). In an exploratory model, we replaced *reproductive asynchrony* with the *proportion of interphase syconia* (early + late) which had a significant positive association with fruit damage and resulted in substantially better support with an AIC score of 1548. This result makes biological sense as the syconia on which the larvae feed are primarily in interphase, and trees that do not have these syconia will experience little damage. Finally, we fit a model of *Omiodes* damage decomposing *crop size* into *crown volume* and *reproductive activity* as two separate predictor variables. This last, preferred, model had better support (AIC = 1542) and identified the *proportion of interphase syconia* (slope 1.72, p = 0.0003) and *crown volume* (slope 0.60, p = 0.0005) as having highly significant positive effects, and tree *density* (slope 0.12, p = 0.061) and *reproductive activity* (slope 0.86, p = 0.091as having only marginally significant positive effects.

4. Discussion

The main goal of this study was to identify the ecological factors influencing pre-dispersal damage of *F. petiolaris* syconia by the larvae of *O. stigmosalis*, an abundant and widespread lepidopteran species. To address this goal we measured fig tree reproductive characteristics, density, and fig damage rates at nine geographic locations spanning the range of the host species in Baja California. Before discussing the inferred relationship between predictor and response variables, we consider evidence supporting the phylogenetic placement of *F. petiolaris'* lepidopteran frugivore within the genus *Omiodes*.

4.1. Phylogenetic placement of lepidopteran fruit predator

Bayesian phylogenetic analysis incorporating publicly available *Omiodes* mitochondrial COI sequences and closely related crambid outgroups (Haines and Rubinoff, 2012), indicates that the lepidopteran predator of *F. petiolaris* fruits in Baja California forms a well-supported monophyletic clade. In turn, this clade is nested within a well-supported clade of *O. stigmosalis* reared from the fruit of Costa Rican *Ficus* (D. Janzen, pers. comm.) and from Florida (J. Hayden, pers. comm.). Given our taxon sampling, this suggests that fig-feeding *Omiodes* are a distinct Neotropical lineage. The moths reared from *F. petiolaris* differ in wing scale characters from described *O. stigmosalis* (Janzen, 1989; D. Janzen and A. Solis pers. comm.), suggesting they and *O. stigmosalis* may represent sister species whose biology is very similar. Further morphological and genetic studies would be necessary to confirm this hypothesis. Interestingly, we found that *O. stigmosalis* and the *F. petiolaris*-associated samples nested within the genus *Omiodes* with high support. This contradicts the suggestion of D. Janzen and A. Solis (pers. comm.) that *O. stigmosalis* is misplaced within *Omiodes* and that future taxonomic revision would place it in a related crambid genus. Further phylogenetic analyses employing multiple loci and detailed morphological comparisons should help to resolve this issue. We also expect such analyses to resolve whether the *F. petiolaris* fig predator and *O. stigmosalis* represent a single species or closely-related species. For now, our working hypothesis is that *Omiodes* attacking the fruit of New World figs are all *O. stigmosalis*.

4.2. Lepidopteran damage of figs

Across sites and seasons, damage of syconia by *Omiodes* larvae varied greatly, occurring in 8 to 85% of *F. petiolaris* trees (Table 1) and in 0 to 40% of fruits (Table S1). Overall, damage tended to decrease across the seasons, but with some exceptions (Fig. 4). Site 158, for example, exhibited an increase in the level of damage over seasons, suggesting different lepidopteran population dynamics at the northern limits of the *F. petiolaris* range. *Omiodes* damage was observed on trees at all sites and censuses, mostly at low levels, though a few trees suffered complete crop failure due to pre-dispersal predation (100% damaged syconia). Thus, substantial spatiotemporal variation of fig damage was observed at the tree, site and season levels. Similar results have previously been reported for plant pre-dispersal seed predator systems (Ehrlén, 1996; Sperens, 1997; Leimu et al., 2002). As we report below, although the variation in fig damage does include a substantial stochastic component, it is

associated with several predictor variables that, in turn, identify key ecological processes influencing interactions between *F. petiolaris* and *Omiodes* adults and larvae.

Bronstein (1988) reported unidentified moth larvae (likely *O. stigmosalis*) attacking up to 20% of syconia per crop of *F. pertusa* in Costa Rica. We observed a similarly low mean *O. stigmosalis* attack rate of 5-14% across seasons (Table S1) in *F. petiolaris*, however we also occasionally observed outbreak dynamics in which 100% of syconia within a crop were infested. The difference in the incidence of these outbreaks between Bronstein's and our study may be a consequence of sample size, with 555 fruit crops sampled from *F. petiolaris* versus 14 from *F. pertusa*. As we have observed for *O. stigmosalis* associated with *F. petiolaris*, Bronstein also reported larvae moving from fruit to the ground, where they likely pupated in the soil. More recently, Sugiura and Yamazagi (2004) reported larvae of two moth species (*Pachybotys spissalis* [Crambidae] and *Stathmopoda sp.* [Oecophoridae]) infesting 0-38.5% of syconia sampled from six *Ficus* species (1-3 trees per species) on Iriomote Island, Japan. Moth larvae attacking figs are thus not limited to North American *Ficus* and may have broad-scale implications for the stability and fitness of fig-pollinator mutualisms. In the following section, we discuss ecological factors statistically associated with patterns of *Omiodes* damage in *F. petiolaris*.

4.3. Lepidopteran damage response to tree density and reproductive characteristics

Logistic regression analyses indicate that attack of *F. petiolaris* fruits by *Omiodes* larvae is positively associated with the production of larger, more synchronous fruit crops and, to a lesser extent, the local density of fig trees. The positive effect of *crop size* is consistent with our hypothesis that the larger the number of syconia on a tree, the more it attracts ovipositing moths and the more fruit damage it experiences. An additional explanation for the positive effect of *crop size* is that larger crops increase the likelihood that larvae will successfully locate successive syconia, as required for their development. As a consequence, larger crops may experience greater fruit damage not because they host more *Omiodes* larvae, but because individual larvae are able to destroy more syconia. While we found a significant relationship between *crop size* - the estimated number of syconia on trees - and *Omiodes* damage, this estimate was obtained as the product of *crown volume* and *reproductive activity*, and when analyzed separately, *crown volume* was significant whereas *reproductive activity* was not. These observations suggest that tree size, and not just fruit production, may be important in attracting ovipositing *Omiodes* and supporting larval development.

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Predator satiation is an anti-predator adaptation in which prey (or fruit) occur at high density only sporadically, reducing the probability of individual prey being eaten (Janzen, 1971; Silvertown, 1980; Kelly and Sork, 2002; Shibata et al., 2002; Espelta et al., 2008). Satiation of seed predators is often associated with population-scale masting, however sporadic reproduction can also occur at the individual tree level and locally satiate predators (Nilsson and Wastljung, 1987). Although we find fruit damage to be positively associated with *F. petiolaris* crop size, predator satiation is suggested by only a minority of available syconia being attacked by *Omiodes* larvae. Moreover, our observation that most reproductive trees have low levels of syconium damage suggests that the sizes of *Omiodes* populations associated with *F. petiolaris* are not limited by the availability of fig syconia.

In contrast to the large majority of fig species in the moist and wet tropics that bear highly synchronized fruit crops within trees, 90% of the reproductively active *F. petiolaris* trees we censused had syconia in two or more developmental phases. That attack of *F. petiolaris* fruits by *Omiodes* larvae is negatively associated with within-tree reproductive asynchrony does not support our hypothesis that asynchronous crops and greater phenological overlap of syconia and insect antagonists increase the likelihood of moth oviposition and, consequently, larval damage of fruit. A potential explanation for this unexpected observation is that greater asynchrony decreases the relative abundance of interphase syconia, which is the developmental stage most susceptible to *Omiodes* attack (F. Piatscheck, pers. obs). Also relevant is the behavior of *Omiodes* larvae to damage several syconia during their development, consuming the content of an interphase syconia and then moving to a new healthy one by night. When fruit are produced asynchronously within a tree, these susceptible syconia are fewer and located farther apart, which may decrease the odds of their being located and predated. Consistent with this explanation, our favored logistic regression model revealed a significant positive relationship between the level of *Omiodes* damage within a crown and the proportion of developing syconia specifically in interphase.

Plant density has been associated with increased herbivory (Bach, 1988), decreased herbivory (Platt et al., 1974) or found to have no effect on herbivore dynamic (Bach, 1980). In *F. petiolaris*, *tree density* was marginally significantly ($p = 0.061$) associated with greater *Omiodes* damage, which may help explain the spatial aggregation of fig damage observed in some sites and seasons (Fig. 5). These two results emphasize the need to examine ecological phenomena at a range of spatial scales. Because of the association of *F. petiolaris* with rocky substrates, its populations are patchily distributed and often have high local tree densities, both of which contrast with figs typical of moist and wet tropical environments. Given this

unusual population structure, it is possible that the positive association between *Omiodes* damage and *F. petiolaris* density will not generalize to the majority of Neotropical fig species.

Other, unmeasured ecological factors may also affect lepidopteran activity. For example, Jandér (2015) examined the effects of ants on *F. obtusifolia* and its associated pollinator, finding that ants reduce fig predation by an unidentified lepidopteran caterpillar. Ants have also been observed on *F. petiolaris* and could play an as yet unappreciated role in limiting in *Omiodes* damage. That said, on *F. petiolaris*, ants are largely absent at night when *Omiodes* oviposit and larvae disperse to locate new fruit.

4.4. Fig reproductive phenology

We observed substantial variation in *F. petiolaris* reproductive phenology across the nine study sites and three extended field trips spanning two seasons. Despite this variation, fig production was observed at each site during each visitation, consistent with the pattern of year-round fruiting that is characteristic of fig species. More interesting is that most trees exhibited within-crown asynchrony in reproductive phenology, contrasting with the highly reproductive synchrony characteristic of fig trees in moist and wet Neotropical forests (Windsor et al., 1989). Within-tree asynchrony was initially thought to be an adaptation to extreme seasonality enabling pollinator generations to cycle within the natal tree and figs to reproduce via selfing (Bronstein, 1989). Bronstein (1992) later suggested that this asynchrony may also be an adaptation promoting the overlap of reproductive activity and outcrossing between trees, which was demonstrated to be the case in *F. petiolaris* (Gates and Nason, 2012). Smith and Bronstein (1996) reported within-tree reproductive asynchrony in *F. petiolaris* at two locations in mainland Sonora, Mexico. In our study of nine locations in Baja California, we found reproductive asynchrony to independent of tree size, which was also independent of reproductive effort; we observed small trees with heavy crops, large tree with light crops, and vice versa. Our results also generalize the distribution of asynchrony in *F. petiolaris*, revealing it to be very common across the range of the species in Baja California, including more mesic areas to the south and more xeric areas to the north. *Ficus petiolaris* adds to the list of fig species exhibiting within-tree asynchrony (Bronstein and Patel, 1992; Cook and Power, 1996; Yu et al., 2006; Lin et al., 2008; Yang et al., 2014). Yang et al. (2014) demonstrated that *F. microcarpa* trees from the same genetic group (clone) had similar flowering characteristics, including within-tree asynchrony, suggesting for the first time a genetic basis for within-tree asynchrony in *Ficus*. Further studies would be useful to

understand the degree to which genetic relatedness, spatial proximity, and similarity of microsite conditions influence variation in patterns of *F. petiolaris* flowering and successful pollinator and pollen dispersal between trees.

5. Conclusion

In this study, we provide a survey of lepidopteran pre-dispersal seed predation across sites and seasons and identify ecological factors that influence lepidopteran damage at the tree level in *F. petiolaris*. We found the syconia borne by larger trees and in denser patches to be more susceptible to attack by *Omiodes* larvae, which in turn can have severe consequences for the production of seeds and mutualist fig wasp pollinators, occasionally including the loss of entire fruit crops. In contrast, we found within-tree reproductive asynchrony to be negatively associated with *Omiodes* damage. Within-tree asynchrony and small fruit crops are common in *F. petiolaris*, though unusual of fig species in general. It has previously been suggested that asynchronous fruiting at the tree level and year-round fruiting at the population level may be adaptations to extreme environments, such as deserts, that favor the maintenance of obligate pollinator populations and fig reproductive success (Janzen, 1979; Bronstein, 1989; Nason and Gates, 2012). As revealed by this study, however, tree-level asynchronous fruiting also appears to benefit the *F. petiolaris*-pollinator mutualism by decreasing predation of seeds and larval pollinators by *Omiodes* larvae. A better understanding of the adaptive significance of reproductive synchrony/asynchrony in figs will be provided by future studies disentangling the relationship between variation in this trait and different sources of fig and fig wasp fitness.

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Appendix A. Supplementary data

Supplementary Table S1

Season- and site-level data on the numbers of sampled and damaged figs proportion (with 95% confidence interval).

Season and site	Total fruits sampled	Damaged fruits	Damage proportion [95% CI]
Spring 2013			
Site 158	1140	79	0.07 [0.005, 0.085]
Site 172	840	113	0.13 [0.112, 0.159]
Site 112	960	86	0.09 [0.072, 0.109]
Site 113	1180	470	0.40 [0.370, 0.427]
Site 95	N/A	N/A	N/A
Site 179	N/A	N/A	N/A
Site 201	N/A	N/A	N/A
Site 96	1020	73	0.07 [0.057, 0.089]
Site 70	2220	207	0.09 [0.081, 0.106]
Total	7420	1029	0.14 [0.131, 0.147]
Fall 2013			
Site 158	845	109	0.13 [0.107, 0.153]
Site 172	651	31	0.05 [0.033, 0.067]
Site 112	1018	13	0.01 [0.007, 0.022]
Site 113	1263	82	0.06 [0.052, 0.080]
Site 95	1560	137	0.09 [0.074, 0.103]
Site 179	725	14	0.02 [0.011, 0.032]
Site 201	283	16	0.06 [0.033, 0.090]
Site 96	1430	134	0.09 [0.079, 0.110]
Site 70	2075	66	0.03 [0.025, 0.040]
Total	9850	602	0.06 [0.056, 0.066]
Spring 2014			
Site 158	1155	269	0.23 [0.209, 0.258]
Site 172	855	85	0.10 [0.080, 0.121]
Site 112	2025	27	0.01 [0.009, 0.019]
Site 113	1721	90	0.05 [0.042, 0.064]
Site 95	1545	29	0.02 [0.013, 0.027]
Site 179	960	4	0.00 [0.001, 0.011]
Site 201	930	5	0.01 [0.002, 0.013]
Site 96	2340	9	0.00 [0.002, 0.007]
Site 70	1960	123	0.06 [0.052, 0.074]
Total	13491	658	0.05 [0.045, 0.053]

Supplementary Table S2

Season- and site-level data on the proportion of reproductive trees and average within-tree reproductive synchrony/asynchrony.

Season and site	Proportion of reproductive trees	Average synchrony/asynchrony
Spring 2013		
Site 158	0.29 (19/65)	2.28
Site 172	0.33 (14/42)	2.67
Site 112	0.3 (15/50)	1.60
Site 113	0.47 (20/43)	2.23
Site 95	NA	NA
Site 179	NA	NA
Site 201	NA	NA
Site 96	0.31 (18/58)	3.23
Site 70	0.65 (37/57)	2.16
Total	0.39 (123/315)	2.35
Fall 2013		
Site 158	0.25 (18/71)	1.51
Site 172	0.23 (14/61)	2.13
Site 112	0.34 (21/62)	1.78
Site 113	0.37 (24/65)	1.82
Site 95	0.54 (26/48)	2.33
Site 179	0.54 (13/24)	1.29
Site 201	0.41 (7/17)	1.72
Site 96	0.34 (29/85)	2.42
Site 70	0.54 (37/69)	2.16
Total	0.38 (189/497)	2.06
Spring 2014		
Site 158	0.31 (20/64)	1.56
Site 172	0.29 (15/52)	1.79
Site 112	0.68 (41/60)	1.59
Site 113	0.59 (30/51)	2.08
Site 95	0.48 (29/61)	2.00
Site 179	0.61 (19/31)	2.12
Site 201	0.42 (16/38)	2.19
Site 96	0.51 (39/77)	2.45
Site 70	0.53 (34/64)	3.07
Total	0.49 (243/498)	2.15

Supplementary Table S3

Results of spatial autocorrelation analysis of lepidopteran damage of fig fruit by site and season. Bolded rows represent the five site and season combinations in which significant evidence of spatial aggregation of lepidopteran damage was observed at the smallest distance interval. The lone significant negative spatial association is indicated in italics.

Site	Season	Distance interval (m)	No. of pairs	Mean distance (m)	Correlation	*p*-value
70	2013-06	150	62	63.28	-0.14	0.13
70	**2013-11**	**150**	**63**	**69.47**	**0.17**	**0.02**
70	2014-06	150	55	68.54	0.07	0.18
96	2013-06	150	21	82.14	-0.18	0.26
96	2013-11	150	44	83.85	-0.18	0.12
96	2014-06	150	74	85.29	-0.05	0.46
201	2013-11	250	5	135.37	-0.04	0.38
201	2014-06	250	21	136.56	-0.18	0.11
179	2013-11	150	12	63.45	0.22	0.08
179	2014-06	150	23	83.40	-0.15	0.21
95	**2013-11**	**150**	**43**	**62.27**	**0.18**	**0.04**
95	2014-06	150	65	70.06	0.04	0.17
113	2013-06	100	22	63.20	-0.06	0.47
113	2013-11	100	20	58.89	-0.19	0.24
113	**2014-06**	**100**	**44**	**58.86**	**0.24**	**0.03**
112	**2013-06**	**250**	**30**	**147.80**	**0.15**	**0.03**
112	2013-11	250	36	124.56	-0.13	0.30
112	2014-06	250	94	128.26	-0.08	0.27
172	2013-06	100	29	67.82	-0.14	0.37
172	2013-11	100	14	64.19	-0.05	0.46
172	2014-06	100	19	68.21	-0.10	0.47
158	2013-06	50	14	25.84	-0.11	0.48
158	*2013-11*	*50*	*6*	*23.51*	*-0.61*	*0.04*
158	**2014-06**	**50**	**12**	**34.72**	**0.30**	**0.05**

Author Contribution Statement

Study conception and design: Piatscheck and Nason

Acquisition of data: Piatscheck, Van Goor, Houston, and Nason

Analysis and interpretation of data: Piatscheck and Nason

Drafting of manuscript: Piatscheck, Van Goor, Houston, and Nason

Critical revision: Piatscheck and Nason

All of the authors have approved the final manuscript and have provided true contributions to this manuscript.