THE EFFECTS OF ARTIFICIAL CRAB SPIDERS (THOMISIDAE: *Misumenops* spp.) ON *Piper* POLLINATOR BEHAVIOUR IN COSTA RICA: DIFFERENCES BETWEEN INSECT ORDERS

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ABSTRACT

Communication drives ecosystem interactions, including the visual signals of plants used to attract pollinators. However, the effectiveness of flower color in attracting pollinators increases dishonest signalling by pollinator predators exploiting floral inflorescences as hunting niches. Negative pollinator associations with spiders on flowers may cause avoidance of specific plants, thus reducing plant fitness from decreased pollination, but this remains understudied. In this study, we used clay models to simulate crab spiders (*Misumenops* spp.) on *Piper* plants in a paired design (spider vs. no spider) on the same plant to further investigate the role of pollinator predators on pollinator behaviour. Using this design, we compared avoidance and visitation of *Piper* plants, identified pollinators to order (and to insect, if possible, such as the case with bees, wasps, and ants in Hymenoptera), and observed individual insect behaviour towards spiders. *Piper* pollinators were more likely to be aware of spider treatments *vs.* controls and were also 5.01 times as likely to avoid spider treatments (p < 0.001). Visitation on controls was also higher, but this was not found to be significant (p = 0.800). Overall, nine orders of pollinators were observed visiting *Piper* plants, with Hymenopterans, Dipterans, and Coleopterans found to be the dominant orders observed with differences in visitation and avoidance, using Poisson distributions in generalized linear model analyses. This study provides a framework for investigating non-consumptive effects of predators on plant fitness.

Keywords: clay models; food webs; insect-plant interactions; plant fitness.

Flowers utilize bright colours to attract visually-oriented insect pollinators through communicating food rich reward signals that culminate in increased pollination and plant fitness (Tepedino 1979, Suttle 2003, Brechbühl *et al.* 2010). However, the visual signal of plant colour can be intercepted by other organisms as well, resulting in a top-down cascade, in which a pollinator-predator can indirectly reduce plant fitness (Letourneau & Dyer 1998, Schmitz *et al.* 2000, Suttle 2003, Muñoz and Arroyo 2004, Romero *et al.* 2011). While signal interceptors, such as plant-predators, can physically damage a plant directly through folivory (Marquis 1992, Schmitz *et al.* 2000), plant fitness may also be impacted by indirect, or non-consumptive, costs as well (Letourneau & Dyer 1998, Gastreich 1999, Gonçalves-Souza *et al.* 2008). Just as plant colour may serve as an effective attractant for certain groups of pollinators, these signals may also attract pollinator predators, like spiders (Heiling *et al.* 2003).

Crab spiders (Thomisidae) are ambush predators of floral bloom pollinators, utilizing crypsis in two distinct ways (Bhaskara *et al.* 2009, Llandres *et al.* 2013). Firstly, crab spiders exhibit a number of colourful phenotypes (*e.g.*, brown, green, yellow, and white), which are preferentially used to match plant colour (Chittka 2001, Heiling & Herberstein 2004, Heiling *et al.* 2005, Bhaskara *et al.* 2009, Llandres *et al.* 2013, Anderson & Dodson 2015). Secondly, crab spiders may use UV-reflectance/contrast for camouflage or to attract UV-visually oriented pollinators, such as bees or butterflies (Chittka 2001, Heiling & Herberstein 2004, Thery *et al.* 2005). While this utilization of crypsis may increase predation success, insect pollinators may also learn to avoid plants or areas inhabited by spiders through their recognition of certain shapes (*e.g.*, rounded abdomen) or structural features (*e.g.*, forelimbs) of spider predators through the development of spider-specific search images (Heiling & Herberstein 2004, Gonçalves-Souza *et al.* 2008, Ings *et al.* 2012). Indeed, because crab spiders

initiate attacks with their forelimbs, spider recognition and avoidance of forelimbs would appear to be an essential learned association necessary for pollinator survival that would likely cause avoidance of a certain area or plant (Dukas & Morse 2003). Therefore, failed spider attacks may represent a true measure of pollinator risk assessment and learning.

In this study, crab spider clay models (modelled after *Misumenops* species) were used to assess predator avoidance (*i.e.*, adaptive learning) and plant visitation behaviour in insect pollinators of *Piper* plants (pepper plants; Piperaceae) in Costa Rica, namely *Piper cenocladum*. While previous studies have also used similar models to assess adaptive learning in plant pollinators (*e.g.*, Gonçalves-Souza *et al.* 2008, Ings *et al.* 2012, Wang *et al.* 2013), to our knowledge, no study has investigated the effects of spider models on *Piper* insect pollinator behaviour at both the hierarchical level of order and at the individual level for Hymenopterans. Using a paired design of control *Piper* flowers lacking spider models and experimental *Piper* flowers containing spider models, we hypothesized that both avoidance and visitation would differ between the two experimental treatments, with increased avoidance and decreased visitation in experimental flowers relative to control flowers. We further hypothesized that visitation and avoidance would differ between insect orders, since pollinators may behave differently in response to spider predators (Dukas & Morse 2003, Gonçalves-Souza *et al.* 2008). In addition, we hypothesized that visitation would be highest in the morning as has been shown previously (Fleming 1985, Kikuchi *et al.* 2007).

This study was conducted in the lowland tropical forests of Costa Rica, near La Selva Biological Station (LSBS; 10°25'53.14" N; 84°0'10.51"W) from 2-8 March 2013. The study site was approximately 100 meters in elevation and receives about 3962 mm of rainfall annually (Letourneau & Dyer 1998). Although March is part of the Costa Rican dry season, this study was conducted during an unseasonably wet period, which coincides with the stimulus (*i.e.*, rain) for flowering in tropical *Piper* plants (Fleming 1985).

There are more than 90 species of *Piper* L. in Costa Rica, with plants often inhabiting riparian zones of tropical lowland forests or along forest edges (Burger 1971, Fleming 1985). *Piper* species are tall shrub-like plants, commencing flower production between 1-15 m in height (Letourneau & Dyer 1998). They are characterized by possessing white, spike-like inflorescences that have very small flowers in which the anther and stigma are in close proximity, ensuring an act of visitation is also likely an act of pollination (Fleming 1985).

Artificial spider clay models were handmade with white, non-toxic Sculpey clay (Polyform Products Company, Elk Grove Village, IL, USA; reflectance spectra 300-700 nm) in a similar fashion to artificial spider models in other studies (*e.g.*, Gonçalves-Souza *et al.* 2008). Briefly, model body weight (< 1 g) and body (. 1.5 cm), forelimb (. 3 cm total), and leg length (. 2 cm total; Figure 1) were standardized proportionally to resemble *Misumenops* spp. (Thomisidae) (Gonçalves-Souza *et al.* 2008), notably *M. pallens* (Keyserling; body length . 1 cm), which is found throughout central and South America (World Spider Catalog 2017). Two bent paper clips were inserted into the model to resemble raptorial forelimbs. Since the objective of this study was to understand if visually-oriented pollinators recognized spider models, olfactory cues of models were not considered. However, models were placed outside for approximately 1 month in an attempt to account for clay scent.

Fifty-seven *Piper* (identified to genus) plants were used in this study. All plants were . 1 m in height, spaced > 1 m apart, similar in colour (reflectance spectra 300-400 nm), in bloom (*i.e.*, capable of attracting pollinators), and randomly distributed across various microhabitats along edges of designated trails at LSBS. Microhabitats included areas close to water or buildings near the station, plantation regions, or secondary growth forest. At 30 minute intervals 2 times/day (morning and afternoon), three individual experimenters individually observed a flowering *Piper* plant. Each plant (N = 57), used only once, included a pair-wise comparison of a control inflorescence with no-spider model and an experimental inflorescence containing the artificial spider model (placed at start of observation). The two treatment inflorescences were spaced > 30 cm apart (Gonçalves-Souza *et al.* 2008), at approximately the same height, and on the

same plant per trial in order to prevent potential inter-plant variations that could confound results. Experimenters stood at a distance of approximately 1 m (from the plant) to observe pollinators either avoiding or visiting the two inflorescent treatments. *A priori*, avoidance behaviour was defined as the approach of a pollinator for $ 3 seconds near an inflorescence without walking or landing on the inflorescence, whereas visitation was defined as any walking or landing on one of the two inflorescences for a timed period of $ 3 seconds (Gonçalves-Souza *et al.* 2008). In the event of avoidance or visitation, insect pollinators were identified to order using general characteristics (see Gillot 2005).

All statistical analyses were performed in R (Version i386 3.2.2., 2015). Number of visitations and avoidances by pollinators, respectively, along with total number of pollinators (*i.e.*, avoidances + visitations) served as response variables. Since data for visitation, avoidance, and total number of pollinators were counts, general linear models with Poisson distributions were conducted for all analyses. Model treatment (spider *vs*. no spider) served as the main fixed effect in analysis. In avoidance and visitation analyses, to account for variation, observer bias, plant number (based on observer), and location served as random effects, whereas time (of day) served as a random effect in avoidance-visitation analyses and as an independent variable in analyses for examing time of day effects on the number of pollinator visitors. Nesting of appropriate variables (*i.e.*, blocking larger factors, such as time of day, within smaller factors, such as treatment) was also used in broader analyses, as well as the observance of possible interactions between treatments and insects on numbers of avoidances and visitations.

All odds ratios (β) and confidence limits (CLs) were created using coefficients of variation, taking appropriate values from analysis to the exponential power. Because count data analysed via Poisson distributions are expected to be non-normal, CLs (β ± 2×standard error) with 95% confidence to account for the expected asymmetry in the results were used rather than confidence intervals. To demonstrate the effect of spider models on pollinator visitation and avoidance, βs: 95% CLs are provided below. A β > 1 indicates that an outcome is more likely to occur, whereas a β < 1 indicates an outcome is less likely to occur. A 5% significance level was chosen to evaluate fixed effects.

Figure 1. Representative model spider made of white clay.

Overall, there were a total of 345 pollinators (268 avoidances and 77 visitations) observed near *Piper* inflorescences in this study, distributed among nine insect orders (Table 1). Pollinators significantly avoided spider inflorescences more often than control inflorescences (Odds ratio: confidence limit, 5.01: 3.60-6.98; $p < 0.001$) and visited control inflorescences more often (0.944:0.59-1.50; $p = 0.800$) than spider treated ones, supporting our hypothesis.

Hymenopterans were the only insect order that were significantly more likely to both avoid (2.31: 1.19-4.50; $p = 0.012$) and visit (2.20: 0.98-4.91; $p = 0.050$) spider models more often than controls, contrary to our original hypothesis. To further investigate this, we compared behaviors between ants, bees, and wasps (Figure 2). Bees significantly avoided (2.26: 1.51-3.0; $p = 0.030$) both treatments more often than ants, but visited spider models less than ants (0.12: 1.92-2.16; $p < 0.001$). Because ants live on *Piper* plants and gain food from extra-floral nectaries, they may exhibit increased territoriality or aggression to perceived threats to their home or food source (Letourneau 1998), accounting for their increased visitation. In fact, we observed ants both attacking spider models and exhibiting behavioural displacement (see Gastreich 1999). Bees, however, likely avoided spiders due to the chance of being predated upon.

Both Dipterans (7.77: 4.83-12.50; $p < 0.001$) and Coleopterans (3.12: 1.45-6.71; $p = 0.003$) significantly avoided spider models more often than controls. Since crab spiders are well-documented predators of Dipterans, and Coleopterans (Jennings 1974, Heiling & Herberstein 2004, Brechbühl *et al.* 2010),

experienced individuals from these prey groups may possess spider search images and consequently, avoided models. Even though all other insect orders avoided spider treatments more than controls (Figure 2; Table 1), this was not significant. Lepidopterans were previously shown to be a dominant leaf forager of *Piper* plants at La Selva (see Marquis 1992), but only 7 individuals were observed in this study. Since Lepidopterans rely heavily upon olfaction, especially for foraging and mating (*e.g.*, Yoshida *et al.* 2015), sensory aversion to models may account for the low numbers observed. Ants may have also influenced Lepidopteran behaviour through the emission of olfactory-alarm cues to suspected predators (*i.e.*, the spider model; Attygale & Morgan 1984).

Due to other studies illustrating pollinator proclivity for early morning *Piper* pollination in the tropics (Fleming 1985, Kikuchi *et al.* 2007), we hypothesized that pollinators would be most active during the morning. In contrast, however, there were significantly (1.37: 1.10-1.71; $p = 0.005$) more pollinators observed in the afternoon than in the morning (Table 1). Peak pollination (mostly by Dipterans) was observed between 1500-1600 hours. Interestingly though, pollinators avoided *Piper* treatments more (1.40: 1.09-1.81; $p = 0.007$) in the afternoon than in the morning.

This study coincided with unseasonably wet weather. Therefore, it is possible that pollinator behaviour was affected by precipitation. Rain can affect photoperiod, olfactory cue transmission, and UV reflectance (see Bowen & Janzen 2005), all of which could influence pollinator behaviour and perception of

Table 1. Taxonomical avoidance and visitation (Spider *vs*. No spider) by *Piper* pollinators at different times of the day.

Pollinator Order	Spider Treatment		No Spider Treatment		Total Pollinators	Morning Pollinators	Afternoon Pollinators
	Avoidances	Visitations	Avoidances	Visitations			
Diptera	155	9	20	14	198	71	127
Hymenoptera	28	21	10	9	68	32	36
Coleoptera	28	4	9	9	50	24	26
Lepidoptera	6	0	1	0	7	2	5
Hemiptera	6	1	0	5	12	2	10
Araneae	4	2	0	0	6	2	4
Orthoptera	1	0	0	1	2	1	1
Phasmida	0	0	0	1	1	0	1
Chilopoda	0	0	0	1	1	0	1
Total	228	37	40	40	345	134	211
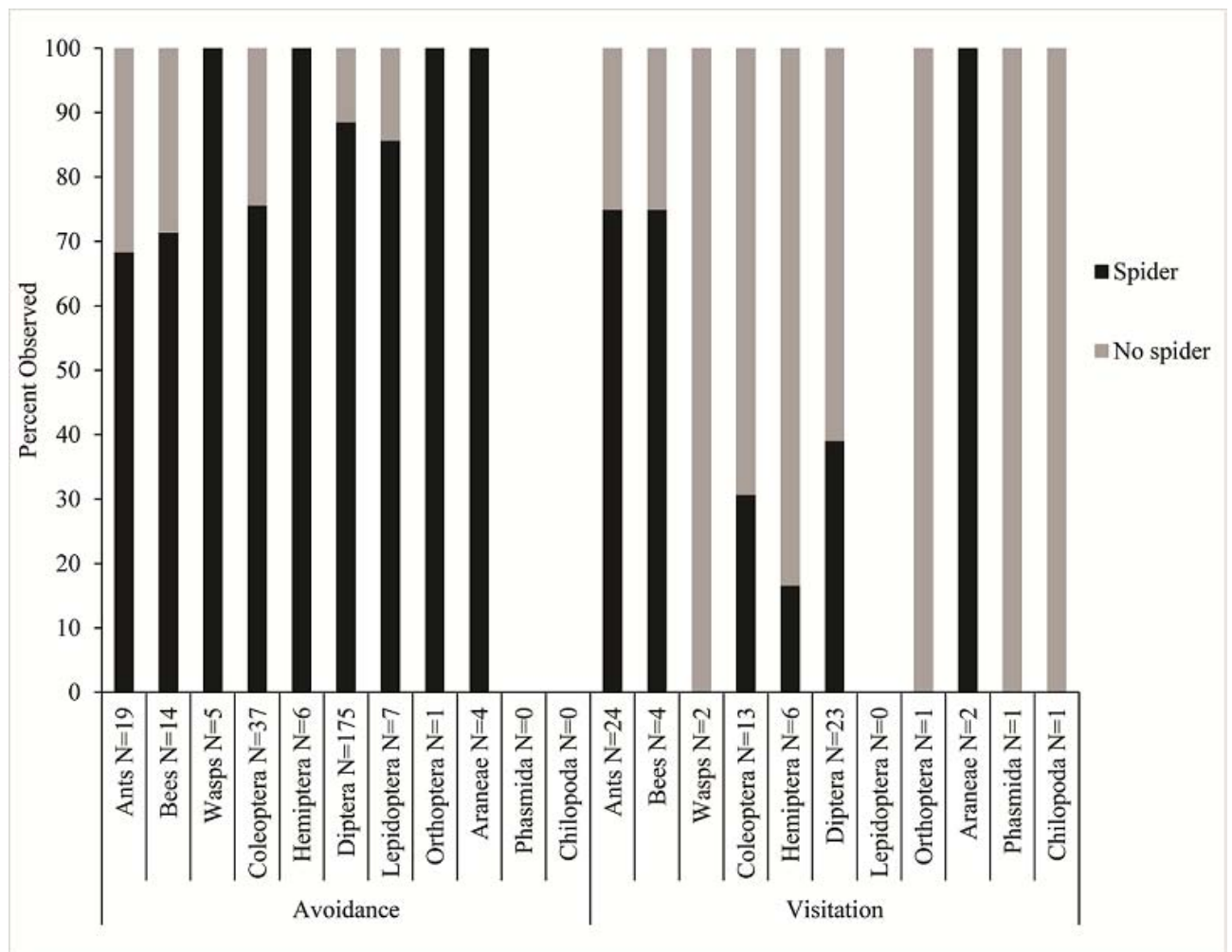
Figure 2. Ratios of avoidance and visitation among the nine observed pollinator orders organized by treatment (spider *vs*. no spider). Sample sizes (N; *i.e.*, the total number of avoidances of visitations) are provided for each pollinator. Note that no values are provided for avoidance of Phasmida and Chilopoda, along with Lepidoptera visitation, due to no representative individuals observed. Solid bars indicate 100% visitation or avoidance due to one particular treatment.

spider predators. Additionally, rain may have indirectly affected insect behaviour by affecting the plants themselves. Since rain can cause reversals in *Piper* phenology (see Stiles 1977), this may have induced unseasonal flowering of certain *Piper* plant species in the study area. The unseasonably wet weather observed during the study period may represent an obvious confounder of our results, which necessitates further exploration.

In conclusion, we used spider models to investigate Piperacae pollinator behaviour. To our knowledge, this is one of the first studies to investigate, in an entirely open field study, how different orders of arthropod pollinators of *Piper* plants may be influenced by spider predators. Our results suggest that pollinators were more likely to avoid and less likely to visit spider models, indicating that spiders may influence pollinator behavior. Future studies further investigating the effects of pollinator predators on plant fitness are necessary.

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