

Entrapped carrion increases indirect plant resistance and intra-guild predation on a sticky tarweed

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ABSTRACT

Many plants employ indirect defenses against herbivores; often plants provide a shelter or nutritional resource to predators, increasing predator abundance, and lessening herbivory to the plant. Often, predators on the same plant represent different life stages and different species. In these situations intraguild predation (IGP) may occur and may decrease the efficacy of that defense. Recently, several sticky plants have been found to increase indirect defense by provisioning predatory insects with entrapped insects (hereafter: carrion). We conducted observational studies and feeding trials with herbivores and predators on two sticky, insect-entrapping asters, *Hemizonia congesta* and *Madia elegans*, to construct food webs for these species and determine the prevalence of IGP in these carrion-provisioning systems. In both systems, intraguild predation was the most common interaction observed. To determine whether IGP was driven by resource abundance, whether it reduced efficacy of this indirect defense and whether stickiness or predator attraction was induced by damage, we performed field manipulations on *H. congesta*. Carrion supplementation led to an increase in predator abundance and IGP. IGP was asymmetric within the predator guild: assassin bugs and spiders preyed on small stilt bugs but not vice versa. Despite increased IGP, carrion provisions decreased the abundance of the two most common herbivores (a weevil and a mealybug). Overall seed set was driven by plant size, but number of seeds produced per fruit significantly increased with increasing carrion, likely because of the reduction in the density of a seed-feeding weevil. Observationally and experimentally, we found that carrion-mediated indirect defense of tarweeds led to much intraguild predation, though predators effectively reduced herbivore abundance despite the increase in IGP.

Keywords: intraguild predation, indirect defense, sticky plants, mutualisms, true bugs

INTRODUCTION

Trophic interactions have typically been described in simplified form because of the conceptual (Hairston et al. 1960) and analytical (Oksanen et al. 1981) ease of thinking about discrete trophic levels. However, food web complexity in the forms of intraguild predation (IGP), scavenging, omnivory, and cannibalism, appears to be the norm (Polis and McCormick 1987, Polis et al. 1989, Eubanks and Denno 1999, Coll and Guershon 2002, Law and Rosenheim 2011, Krimmel and Pearse 2013). Indeed, increased and more sophisticated sampling of trophic interactions within foodwebs uncovers more complexity of interactions than were previously recognized (Gagnon et al. 2011). Predatory organisms occupy less discrete trophic positions than herbivores or producers (Thompson et al. 2007), probably because IGP, when predators eat one another, is particularly common (Polis et al. 1989, Sih et al. 1998). Nevertheless, discrete trophic levels are central to many core concepts in ecology, such as trophic cascades, a common mechanism underlying indirect defense of plants.

Intraguild predation (IGP) is widespread and has been shown to have important impacts on herbivore populations and plant fitness and productivity (Rosenheim et al. 1993, Finke and Denno 2004, Cardinale et al 2003). The effects of IGP on herbivore suppression can be particularly apparent in agricultural systems, potentially because these systems tend to have simplified food webs (Rosenheim et al. 1993, Letourneau et al 2009; Martin et al. 2013). In agricultural systems, natural enemies introduced for biological control of herbivores can promote IGP to the extent that they also suppress populations of native predators (Yasuda et al. 2004, Carvalheiro et al. 2008). While less-studied, evidence suggests that intraguild predation is both common and ecologically important in many natural systems, as well (Polis and McCormick 1987, Finke and Denno 2004, Schmitz 2007, Barton and Schmitz 2009, Gagnon et al. 2011).

In combination, these studies have highlighted the need to understand IGP as an important component of plant-herbivore-predator systems at a variety of scales.

Plants commonly engage in indirect defense where they manipulate predator interactions with herbivores in order to decrease herbivore pressure (Kessler and Heil 2011). Plants employ indirect defense by providing predators with resources of food, shelter or information about prey (Turlings et al. 1990, Agrawal and Karban 1997, Palmer et al. 2010, Kessler and Heil 2011; Krimmel and Pearse 2013). Indirect defense is common for many plant-herbivore-predator systems including classic cases of ant-acacia interactions mediated by extrafloral nectary secretions (Ness et al. 2009), predatory mite interactions with plant domatia (Agrawal and Karban 1997, Weber et al. 2012), and parasitoid attraction to herbivore-induced plant volatiles (Turlings et al. 1990). These provisions or cues boost predator abundance around plants with herbivores (Kessler and Heil 2011) and this increase in predators may decrease damage to the plant either consumptively (e.g. consuming herbivores) or nonconsumptively (e.g. decreasing oviposition or reducing feeding) (LoPresti et al 2015).

In cases where multiple predators consume the herbivores of an individual plant, resources provided by the plant could increase the prevalence of IGP. In this case, IGP may partially negate the benefits of these resources to the plant because IGP can decrease the ability of predators to suppress herbivore populations (Mooney et al 2010; Vance-Chalcraft et al 2012). Indeed, IGP should be expected to be particularly important in mediating the efficacy of indirect defense. Habitats that have few places for IG prey to hide tend to promote IGP (Langellotto and Denno 2004), and spatial aggregation of predators, as occurs in indirect defense systems, may alter the frequency and consequences of IGP on predator interactions (Reader et al. 2006) and could affect the plant's fitness as well.

Other aspects of plant life histories have been shown to alter the foraging habits of predators. Omnivorous thrips that feed on both cotton plants and mites disproportionately fed on herbivorous mites when cotton defenses were induced by previous damage (Agrawal et al. 1999). Likewise, the ability of two omnivorous predators to suppress a shared host was altered by the presence of plant-derived resources in the form of floral nectar (Maselou et al. 2015). However, IGP has not been studied explicitly in the context of indirect defense systems.

A recently tested indirect defense system occurs in sticky plants which entrap insects on their surfaces. These entrapped insects (hereafter: carrion) have been experimentally shown to supplement predator populations, decrease herbivore abundance, and increase plant fitness (Romero et al. 2008, Krimmel and Pearse 2013, LoPresti et al. 2015; first hypothesized in Thomas 1988). In tarweeds, this indirect defense is only expressed in plants that flower late in the season when herbivores are present, suggesting that herbivore interactions may be a key selective driver of the defense (Krimmel and Pearse 2014, 2016). During the course of investigations into these sticky plant systems, we noticed frequent intraguild predation and sought to investigate the structure and consequences of this interaction. Carrion-mediated interactions are amenable to field manipulations by adding or removing carrion available to predators on the plant surface and we felt these systems would be ideally-suited for experimentally testing the effect of resource provisioning on IGP, the three trophic levels, and the overall outcome of the mutualism for the plant.

We conducted two manipulative experiments on a common tarweed *Hemizonia congesta* to assess the efficacy of a carrion mediated indirect defense and to determine the effect of carrion and previous damage on intraguild predation. In the first experiment, we manipulated entrapped carrion abundance on *H. congesta*, an annual

tarweed, while also manipulating defensive induction of this plant. We directly observed the rate of IGP among the two most abundant predators (stilt bugs and assassin bugs) on these plants by censusing the number of corpses of each of these predator species that remained stuck to the sticky plants. Because indirect defense causes an aggregation of predators, we expected to find increased IGP on plants with more carrion resources. We also tested the effect of mechanical damage and any resulting induction on these interactions; tarweeds induce both eglandular and glandular trichome production after mechanical damage (Gonzales et al 2008). Consistent with Agrawal et al (1999), we expected that induction might lead to greater IGP because the “predators”, many of which are omnivores and scavengers, would be less able to rely on (reduced) plant resources and more may be present because of a volatile cue (Kessler and Heil 2011). In experiment 2, we damaged a second, larger, set of plants more extensively to minimize the chance of missing a smaller induction effect. To complement these experimental approaches, we gathered observational data for several years on trophic interactions in two tarweed systems (*H. congesta* and *M. elegans*) to better represent the full food web of arthropods that interact with those plants and each other.

MATERIALS AND METHODS

Observations on food web assembly

We noted all instances of trophic interactions involving predators or scavengers on *Madia elegans* (Krimmel & LoPresti) and *Hemizonia congesta* (LoPresti) while running experiments (Krimmel and Pearse 2013, 2016; Krimmel, unpublished; *Hemizonia* studies below) and observing these species during the years 2012-2015. For those instances where we did not directly observe a pairwise interaction between two species, but suspected them based on trophic positions, we forced interactions between these species in either 59mL deli containers (Dart Industries) or Petri dishes. We added the

organisms and a length of tarweed stem for a period of 24-48 hours and checked occasionally for any consumptive interactions. We do not consider these laboratory results as robust as the direct field observations, although we believe these rare interactions are likely to occur in the field as well.

Carrion tracking observations

To determine whether predator numbers correlated positively with carrion density in a natural population of *Hemizonia congesta*, we censused predators, herbivores and carrion on all bolting individuals ($n = 80$) in a small roadside population (~ 10 meters in length, along North side of road at 38.862419 N, 122.430074 W) on 7-8 June, 2015. As many predators are easily perturbed and flee, we censused each plant first from a short distance, then went through the plant leaf-by-leaf to accurately count carrion and small predator nymphs (which do not fly off as many of the adults do). We measured plant height as a covariate upon finishing the arthropod censuses. Early in the season, pedicels are small and height is a reasonable proxy for the size of the plant as arthropod habitat. This population was near the experimental populations (< 500 m), but bolted earlier in the season.

Experiment 1: Hemizonia congesta carrion and damage manipulation

To test the effects of carrion provisioning and plant damage on arthropod communities, interactions, and plant fitness, we manipulated both damage and entrapped carrion factorially. On 12 June, 2015, we marked 150 plants in a small serpentine meadow (< 1 km²; >1,000 plants) at the UC-Davis McLaughlin Reserve (38.864539 N, 122.427574 W) and alternated applying six treatments. The first three were non-damaged plants (ND) that had all carrion removed (“no carrion”, NC), carrion adjusted to five dead arthropods per plant (“low carrion”, LC), and carrion adjusted to ten dead arthropods per plant (“high carrion”, HC). These carrion levels are appropriate for the site and plant

size (LoPresti, pers. obs.). Adjustments were made by removing natural carrion or adding freshly frozen fruit flies; they are similar in size to most of the small flies and parasitoids entrapped and have been used in similar studies (e.g. Krimmel and Pearse 2013, supplementary information in LoPresti et al 2015). We repeated the three carrion treatments (NC, LC, HC) for damaged plants (D) on which we clipped two flower buds per plant with small surgical scissors (sterilizing the buds by damaging the inner reproductive structures) to mimic the feeding of *Heliothis* or *Heliothodes* caterpillars on tarweeds (and as done in Gonzalez et al 2008 in a tarweed, which triggered inductive effects) and handled the others, but left them unclipped as controls. We applied the same carrion treatments on 22-June and on 5-July (damage treatments were imposed just once). We adjusted the carrion numbers to higher values of 0/10/20 [NC/LC/HC] to reflect the natural increase of carrion over the season, on treatment reapplications on 12-July, 19-July, 25-July and 8-August. On each date when carrion was adjusted, we recorded carrion number (prior to adjustment), number and identity of all arthropods, number and damage of reproductive structures, and seed numbers from all mature fruit. We were able to observe arthropods that were killed by other arthropods while on the plant because their corpses remained attached to the sticky surface of the plant (any which were questionable whether an exuvia or a corpse were inspected with a 20x loupe). Specifically, we observed numerous examples of predation of stilt bugs (*Hoplinus eschinatus*) and some examples of predation of assassin bugs (*Pseliopus spinicollis*), and recorded these data on each observation date. We cannot rule out other causes of mortality, including senescence, pathogens, starvation, or entrapment in sticky resins; however, based on our extensive direct observations of these species on these and other sticky plants and IGP between these species we see no reason to suspect these potential causes accounted for most mortality. We also cannot rule out cannibalism (a

specific case of IGP); however, as most of the recorded *H. eschinatus* corpses were adults, we consider this unlikely in that species. It is quite likely that cannibalism was the cause of observed *P. spinicollis* mortality, as they cannibalize readily when in captivity (Krimmel & LoPresti, pers. obs.), and all corpses found were dead juveniles. We collected seed set data on senescent plants on 2-September, 13-September, 5-October and 23-October.

Experiment 2: Simple damage manipulation

To increase power to detect a small effect size and test whether early-season damage increased carrion entrapment rate, IGP, or predator recruitment we marked 100 pre-flowering, equally-sized *Hemizonia congesta* plants in a nearby serpentine meadow on 13-June-2015. We alternated between two treatments, the first clipping five flower buds per plant (compared to two in Experiment 1) and the second treatment handled as the first but without clipping. On 21-June, 3-July, 11-July, 20-July, 26-July, 6-August, 3-September, 13-September, 6-October and 23-October, we censused all plants. We recorded number of reproductive structures intact and damaged, number of entrapped carrion (until mid-August when stickiness declines), and number and identity of all arthropods. *H. congesta* fruit mature and dehisce very slowly, we were able to collect all fruit and count seeds. One flag marking a control plant disappeared the first week and we excluded the plant from all analyses.

Statistical Analysis

We analyzed observational data of carrion – predator relationships as a linear model with carrion treatment and plant size as interacting numerical predictors of predator abundance. We tested for heteroscedasticity with a Breush-Pagan test and overdispersion and corrected with a negative binomial error distribution.

In Experiment 1, we analyzed the interactive effect of carrion amount (3 levels) and bud removal (2 levels) on the summed abundance of predators, herbivores, IGP events as observed by predator corpses attached to plants, matured fruit, and seeds. In preliminary models, we included number of reproductive structures (a better proxy for plant size than height in reproductive individuals) as a covariate for each response variable. We then used a stepwise deletion approach with likelihood ratio tests to determine the best-fitting model and avoid overfitting. Plant size and its interaction with carrion treatment improved model fit for models of fruit, seeds and herbivores but not for predators or IGP events, and the term was removed from models where it did not improve model fit. Fruit and seed sets as well as herbivore abundance were compared among treatments using a Poisson error distribution. The summed number of predator observations or IGP events was compared between treatments using a generalized linear model with a negative binomial error distribution to account for the presence of zeros (or overdispersion) in the data. *Per capita* IGP rates were calculated for *H. eschinatus* as dead *H. eschinatus* / total *H. eschinatus*, and were modeled as a GLM with a quasipoisson error distribution. For the linear model of seeds per fruit, assumptions of homoscedasticity and normality of residuals were satisfied. Experiment 2, we compared means of the control and damaged plants in various community (predator, herbivore abundance) and performance (seed number, seeds per fruit) metrics. We compared these metrics both across the season (to examine long-term inductive effects) and on the first check only (to determine the transient effects of damage).

All analyses were conducted and all figures made in R version 3.1.3 (R Foundation for Statistical Computing) using packages *lme4*, *lmtest*, and *MASS* (Venables and Ripley 2002, Bates et al. 2015).

RESULTS

Observations and food web links

Both *Hemizonia* and *Madia* tarweed systems had a complex web of interactions with two basal resources, the plant itself and the carrion entrapped on the plant surfaces (Figure 1). We observed traditional “predators” including assassin bugs (*Pseliopus spinicollis*), stilt bugs (*Hoplinus eschinatus*) and damselflies (*Enallagma* c.f. *civile*) scavenging entrapped insects in the field; lynx spiders (*Peucetia viridens*) readily consumed dead fruit flies in the lab, while crab spiders (*Mecaphesa schlingeri*) were the only predators to eschew carrion completely. Omnivorous tree crickets (*Oecanthus* sp.) scavenged carrion, but also nibbled leaves and damaged plants with oviposition scars along the stem. Normally herbivorous caterpillars *Heliothodes dimunitiva* and *Heliothis phloxiphaga* also scavenged small arthropods (LoPresti & Krimmel, in review). Spur-throated grasshoppers (*Melanoplus* spp.) scavenged readily in the lab, but were not observed doing so in the field, though they consumed small carrion so rapidly, the chance of field observation was low. (Grasshoppers are well-known to scavenge arthropod carrion: Lavigne and Pfadt 1964; Richardson et al 2012).

On *H. congesta* (2014-5), we directly observed 16 interactions involving living arthropod visitors: 25% scavenging, 25% predation on herbivores, 44% IGP and the remaining 6% parasitism (Fig. 1A). On *M. elegans* (2012-5), we directly observed 50 interactions: 30% scavenging, 32% predation on herbivores, 32% IGP, 4% predation on pollinators and 2% parasitism (Fig. 1B).

In the censused population of *H. congesta*, we observed a strong positive correlation between carrion abundance and predator abundance (predators: *H. eschinatus*, *P. spinicollis*, *P. viridens*, *M. schlingeri*), explaining 22% of the variance in predator number (Figure 2). Using plant height as a covariate did not improve the fit of the a negative-binomial model (LR test, $X^2 = 2.90$, $df = 1$, $p = 0.09$) (Supplementary table S2-

1). Herbivore number correlated positively with plant size and a model incorporating carrion number and plant size fit no better than one with plant size alone (Likelihood ratio test, $X^2 = 0.52$, $df = 1$, $p = 0.47$).

Carrion manipulation affects predator abundance, herbivores, and intraguild predation (Experiment 1)

Carrion supplementation had large effects on most predator and herbivore species (Figure 3). Carrion supplementation significantly increased predator abundance (summed predators) on the high carrion treatment compared to the no carrion treatment (Figures 3 & 4; Table 1). Carrion supplementation significantly decreased herbivore abundance on high carrion treatments compared to no carrion treatments (Figures 3 & 4, Table 1). The reduced herbivore effect was driven by decreased abundance of the two primary herbivores at higher carrion levels: a weevil (tribe Smicronychini), an important seed predator of *H. congesta*, and a sap-feeding mealybug (Pseudococcidae: unidentified sp.), which together made up 93% of all herbivore observations ($n = 924$) during this experiment. Both predators and herbivores tracked plant size positively and the plant size covariate was retained (additively) in each best-fitting model (Table 1).

Intraguild predation of *Hoplinus eschinatus* (as inferred by corpses) was greater on plants with more carrion (negative binomial GLM, $\chi^2 = 19.39$, $P < 0.0001$; Figure 5).

On plants in the high carrion treatment, predation of *H. eschinatus* was 97% more common than on plants in the middle carrion treatment (Tukey's HSD, $P = 0.002$) and 756% more common than on plants with all carrion removed (Tukey's HSD, $P < 0.0001$). On plants in the intermediate carrion treatment, *H. eschinatus* predation was 330% more common than on plants with all carrion removed (Tukey's HSD, $P = 0.02$).

IGP was observed to be asymmetric in this system with several predators using *H. eschinatus* as the IGP prey. Predation of *Pselliopus spinocollis* was rare and

unaffected by carrion treatments (negative binomial GLM, $\chi^2 = 0.81$, $P=0.66$), and may have been all, or primarily, cannibalism. Predation of *Mecaphasa schlingeri* and *Peucetia viridens* were not observed at all. We observed a positive correlation between live *P. spinocollis* and dead *H. eschinatus*, but not between live *H. eschinatus* and dead *P. spinocollis* (Table 2). Spider abundance did not correlate with abundance of living or dead *H. eschinatus* (Spearman's rho = 0.04; -0.01 respectively).

IGP can be defined based on *per capita* attack rates of the IGP prey, such that a greater incidence of IGP events that was directly proportional to an increase in IGP prey abundance would not indicate greater *per capita* predation risk for IGP prey. In our study, the abundance of dead *H. eschinatus* was greater on plants with more live *H. eschinatus* (Table 2). Likewise, carrion increased the number of living *H. eschinatus* by 107% (no carrion – high carrion; negative binomial GLM, $\chi^2 = 8.73$, $P = 0.012$), which reflected an overall increase in predator abundance in the presence of carrion (Fig. 4 & 5). The effect of carrion on dead *H. eschinatus* was not simply caused by a greater abundance of live *H. eschinatus*, because greater carrion levels caused a greater *per capita* incidence of *H. eschinatus* IGP (quasipoisson GLM, $\chi^2 = 9.14$, $P = 0.010$). Carrion treatment had no effect of the observed number of *P. spinocollis* (negative binomial GLM, $\chi^2 = 0.343$, $P = 0.842$) or spiders (negative binomial GLM, $\chi^2 = 1.81$, $P = 0.709$).

The effect of carrion manipulation on plant fitness (Experiment 1)

In experiment 1, increased carrion increased the average seeds per fruit significantly from the no carrion to the high carrion group (Figure 4: HC-NC: $p = 0.03$) and the best-fitting model included only carrion level (Table 1). Seeds per fruit is a negative proxy for larval weevil damage, as they feed on developing fruit, consuming the seeds. External damage to the plants (almost all from grasshoppers) did not differ

between treatments in the factorial experiment, and models incorporating carrion level and artificial damage fit no better than a null model. Plant size, proxied by average number of reproductive structures per check, was the only significant predictor of overall seed set, and adding damage or carrion level did not improve model fit (Table 1).

Previous damage does not alter carrion capture, predator abundance, or IGP (Experiment 1)

Damage of *H. congesta* plants with mechanical damage did not significantly affect predator abundance, herbivore abundance, total seeds, or seeds per fruit (Table 1). The damage manipulation did have a marginal negative effect on intraguild predation ($\chi^2 = 3.41$, $P=0.06$), contrasting results from a different system (Agrawal et al. 1999).

The effect of artificial damage on carrion entrapment, predators, and plant fitness (Experiment 2)

We found no evidence for any inductive effect in the simple damage manipulation. Artificially damaged (D) and undamaged (C) plants ($n = 50$ & 50 , respectively) did not significantly differ in any measured characteristic: total seeds (C: 30.7 ± 3.7 SE, D: 34.0 ± 4.6 SE; two-tailed t-test, $t = .57$, $df = 97$, $p = 0.57$), total fruit (C: 18.2 ± 1.5 , D: 19.1 ± 2.1 ; $t = 0.35$, $df = 97$, $p = 0.72$), average seeds per fruit (C: 1.57 ± 0.1 , D: 1.60 ± 0.1 ; $t = 0.20$, $df = 96$, $p = 0.84$), total carrion entrapped (C: 6.8 ± 0.9 , D: 7.4 ± 0.7 ; $t = 0.56$, $df = 97$, $p = 0.58$), average carrion per check (C: 1.25 ± 0.15 , D: 1.4 ± 0.11 ; $t = 0.66$, $df = 97$, $p = 0.51$), total predators (C: 0.76 ± 0.14 , D: 0.74 ± 0.16 ; $t = 0.07$, $df = 97$, $p = 0.94$) or total herbivores (C: 6.9 ± 0.9 , D: 6.4 ± 0.8 ; $t = 0.40$, $df = 97$, $p = 0.69$). The lack of any difference in total seeds or total fruit may suggest compensatory growth or production of reproductive structures after the experimental damage. Plants in the control and damaged treatments averaged virtually

equal average dead *H. eschinatus* per check (C: $0.065 + 0.020$; D: $0.061 + 0.020$; $t = 0.06$, $df = 97$, two-tailed t-test, $p = 0.95$), suggesting no direct effect of damage on IGP.

To determine if an induction effect on predators, carrion, or herbivores might be expressed in a transitory way immediately after damage, we also analyzed the first check separately. Again, the plants in each treatments did not significantly differ in any characteristic: carrion entrapped (C: $2.5 + 0.3$, D: $2.9 + 0.2$ SE; two-tailed t-test $t = 1.23$, $df = 96$, $p = 0.22$), total predators (C: $0.24 + 0.09$, D: $0.22 + 0.07$; $t = 0.17$, $df = 96$, $p = 0.86$) or total herbivores (C: $1.32 + 0.2$, D: $1.25 + 0.1$; $t = 0.37$, $p = 0.71$). Neither experiment produced a significant effect of induction on any of the measured transient or seasonal variables.

DISCUSSION

Intraguild predation was the most commonly observed interaction over several years on *Madia elegans* and *Hemizonia congesta*. Predator density correlated positively with increasing carrion in both the observational and experimental studies. This aggregation of predators led to increased IGP. IGP between predators on *H. congesta* was asymmetric; large assassin bugs (*P. spinocollis*) and spiders (*P. viridens* and *M. schlingeri*) commonly killed small stilt bugs (*H. eschinatus*), but not vice versa. Increasing the reward (carrion) to predators increased the incidence of IG predation on *H. eschinatus* (Fig. 4).

Despite the negative interactions between predators as a consequence of carrion provisioning, the abundance of herbivores, including seed-feeding weevils and mealybugs was significantly reduced in higher carrion treatments. The reduction in herbivores, especially the seed-feeding weevil, was likely the mechanism behind the increased seed number per fruit seed found in the factorial experiment. This experimental design (as an open field experiment) did not permit us to evaluate a benefit

of carrion entrapment in the absence of IGP; however, we have two hypotheses for the benefit to the plant despite rampant IGP: (1) the herbivore reduction may be behaviorally-mediated – they may avoid the higher numbers of predators found on higher carrion plants (using direct observation of predators or carrion as a proxy) (2) *Hoplinus eschinatus* seems to be an effective predator of caterpillar eggs and small larvae (e.g. LoPresti et al 2015); however, as caterpillars caused little of the damage during this experiment (in other years they have been far more common and destructive), it is possible the fitness effect of reducing *H. eschinatus* was negligible given the lack of caterpillars.

The increase in seed set was a smaller and more conditional fitness gain than found experimentally on *M. elegans* (Krimmel and Pearse 2013) and *Aquilegia eximia* (LoPresti et al. 2015), but the plant still benefited from increased carrion provisioning. Overall seed set was driven by plant size; randomly marked small plants at the beginning of the season had much variance in size by the end of the season, likely driven by water and nutrient availability in the harsh dry serpentine soils, and this may have obscured any overall fitness effect of the treatment.

When might we expect IGP to occur in association with indirect defense (or resistance), and when might it have a negative impact on plant fitness via the efficacy of that defense? We suggest that an increase in IGP may be a common consequence of provisioning to mutualists, because these provisions or refuges, by definition, cause an aggregation of predators on or around the plant. Moreover, multiple predators are commonly involved in any indirect defense. For example, insectivorous birds can be involved in indirect defense of plants where they are attracted to many of the same damage cues as predatory insects (Mantyla et al. 2008). Because insectivorous birds probably consume both herbivorous and predatory arthropods, they act as an intraguild

predator and both reduce herbivorous insects and predators (Mooney et al 2010; Martin et al 2013).

A potentially analogous situation occurs in ant-acacia protective mutualisms. Negative interactions between predators may play a key role in long-term dynamics of ant-protected plants. For example, *Acacia drepanolobium* is colonized by one of four ant species (Stanton et al. 1999, Stanton and Palmer 2011). Ant colonies of these different species compete for tree resources and displace each other through colony takeovers in the process. Because ant species differ both in their protective benefits and associated costs to plants, the long-term consequence of the indirect defense for the plant depends on interactions among these predators (Stanton and Palmer 2011). The interactions between ant species in this system are best characterized as competition for food and shelter resources, but they demonstrate the effect of predator interactions in determining the fitness environment experienced by the plant.

Additionally, positive interactions between herbivores and predators can result in indirect defense for a plant, and IGP may be a common component of these systems. For example, the net consequence of aphids on cotton plants was shown to be positive because the strong association between herbivorous aphids and predatory fire ants, which also consume other herbivores that are more destructive to cotton than aphids (Kaplan and Eubanks 2005). Fire ants also consume most other predatory arthropods associated with cotton, but the consequence of IGP for the plant in this case is minimal because fire ants offer better protection from herbivores than all other predators on cotton combined.

In sticky plants, the degree of stickiness filters out potential interactions by physically excluding various species, including both predators and herbivores. Both *Hemizonia congesta* and *Madia elegans* are moderately sticky and have extensive food

webs with many interacting partners (Figure 1). The columbine *Aquilegia eximia* is far stickier, and while it grows in close proximity to *H. congesta* (often within 10 meters) and other sticky plants, the arthropod community on it is comprised of only a subset of the other plants' arthropod communities; its extreme stickiness filters out many possible interactors (LoPresti et al 2015; LoPresti, unpublished data). McLellan and Boecklan (1993) and Krimmel and Wheeler (2014) both detail analogous cases where established interactions between ants and Homoptera are disrupted by host plant stickiness in a *Boerhavia* (Nyctaginaceae) and an *Erica* (Ericaceae), respectively.

In other cases, indirect defenses may decrease IGP when the indirect defense increases habitat complexity. Leaf domatia (hair tufts) on pepper (Solanaceae: *Capsicum*) and coffee (Rubiaceae: *Coffea*) plants act as an indirect defense by housing predatory mites, which attack herbivorous mites on those plants (Ferreira et al. 2011). IGP among predatory mites increased when mites were not allowed access to domatia, probably because predatory mites that were intraguild prey had few places to escape from the intraguild predator. In general, habitat complexity has been shown to decrease IGP by decreasing encounter rates among predators and by providing the intraguild prey with refuges from their intraguild predators (Langellotto and Denno 2004).

Based on these limited examples, we can hypothesize situations in which IGP might be an important consequence of plant provisioning to predators. First, IGP should increase with the number of generalist predators attracted to the provisions. Second, IGP should be greater in protective mutualisms that do not increase habitat complexity. Third, IGP should be expected in systems where predators differ in size at any point (which may be stage-specific and lead to seasonal pulses of IGP). Finally, IGP might be expected to increase in indirect defenses in which the plant-provided reward does not

satiates a predator, a situation that is expected of most indirect defenses (Ness et al. 2009).

Carrion entrapment as an indirect defense satisfies at least the first two of these criteria. Multiple predators were attracted to *H. congesta* in the current study. Multiple predators were also attracted to *Madia elegans*, *Mimulus bolanderi* and *Aquilegia eximia* in previous demonstrations of carrion mediated indirect defense and overall predator abundance tracks carrion density in these systems (Krimmel and Pearse 2013, LoPresti et al. 2015; LoPresti and Toll 2017). Concerted sampling of less common interactions in the tarweed systems reveals even more complexity to the arthropod food webs than previously documented (Fig. 1). Carrion entrapment does not change habitat complexity in an obvious way. It is currently unclear at what point predators become satiated by carrion rewards, though theoretical models, observations, and empirical data suggest that satiation probably occurs in sticky plant systems in nature (LoPresti and Pearse, unpublished data).

In the current study, artificial damage to a plant did not affect any detectable aspect of the plant-based food web in either experiment. Carrion entrapment, predator abundance, herbivore abundance, plant fitness and IGP were all unaffected by previous damage to the plant. This contrasts with previous work on cotton plants that demonstrated a change in diet of omnivorous thrips in response to plant damage (Agrawal et al. 1999). While there is some overlap in arthropod communities between tarweed and cotton, there are also several differences between these systems that may affect the consequences of past damage. Studies have demonstrated that cotton induces a suite of defenses such as terpenoid aldehydes in response to herbivore damage (Bezemer et al. 2004); however the inducible defensive response of tarweeds is far less clear (but see Gonzáles et al 2008). Indeed, in our study, there was no evidence for

increased carrion entrapment or attraction of predators to damaged *H. congesta*. On cotton, omnivorous thrips include both plant tissue and live mites in their diet (Agrawal et al. 1999) (thrips are generally rare on sticky plants, except as carrion). Omnivory of this sort also occurred in tarweed systems; however, most arthropods, whether typically thought of as herbivores, predators, or omnivores, also feed extensively on carrion (Mansfield and Hagler 2016), which is probably not the case in domesticated cotton that does not entrap carrion.

Lastly, LoPresti and Toll (2017) proposed three conditions that are necessary for a carrion-provisioning system to operate. The first and second condition – presence of scavenging predators and their positive correlation with carrion provisions – are satisfied in this system (and probably many others, see also Mansfield and Hagler 2016). The third condition – that these predators can control the herbivores on the plant – is satisfied for the weevils but not for grasshoppers, due to their large body size. The late-instar grasshoppers that feed in the late summer on *H. congesta* are larger than all of the predators except adult lynx spiders (*P. viridans*), and only early-instar lynx spiders were found in our study. The previous experimental tests of carrion provisioning all had herbivores that were potential prey of the scavenging predators. The herbivores on *H. congesta* that were in the size range of consumption (weevils) decreased in abundance with increasing carrion – though whether this was a behavioral (e.g. avoidance or dispersal) or trophic (e.g. predation) response is not known. Grasshoppers were not affected, nor was their external feeding damage. Furthermore, on the serpentine barren where we performed this experiment, *H. congesta* individuals differed drastically in size due to microclimate. The limited nature of the carrion-mediated protection in this system (e.g. herbivore reduction and increase in seeds per fruit, but no

increase in total seed set due to increased carrion) makes sense based on the natural history and these criteria.

Conclusion

We found evidence that IGP is increased by plant provisions to predators. Because predator communities associated with plants having indirect resistance traits can be complex, we suggest that IGP may be an important consequence of indirect defenses. Carrion mediated indirect resistance in this and other systems attract multiple generalist predators, omnivores, and (by definition) scavengers; IGP may be of particular relevance in these systems. Nevertheless, carrion additions reduced abundance of both primary herbivores, complementing similar results in other plant taxa (Romero et al. 2008, Krimmel and Pearse 2013, LoPresti et al. 2015), and increased seeds per fruit. These benefits to the plant were found despite IGP being a common and potentially disadvantageous consequence of carrion attached to plants in all these systems.

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Statement on authorship: All authors designed and performed the studies and wrote the paper, EL & IP analyzed data.

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FIGURE LEGENDS

Figure 1: Food webs of arthropods associated with two tarweed species (A) *H. congesta* and (B) *M. elegans*. Numerous organisms scavenge on entrapped carrion in both tarweed systems (black arrows). Intraguild predation (red arrows) is also a common interaction in these systems. Direct observations supporting food web links are provided in Supplementary Table S1.

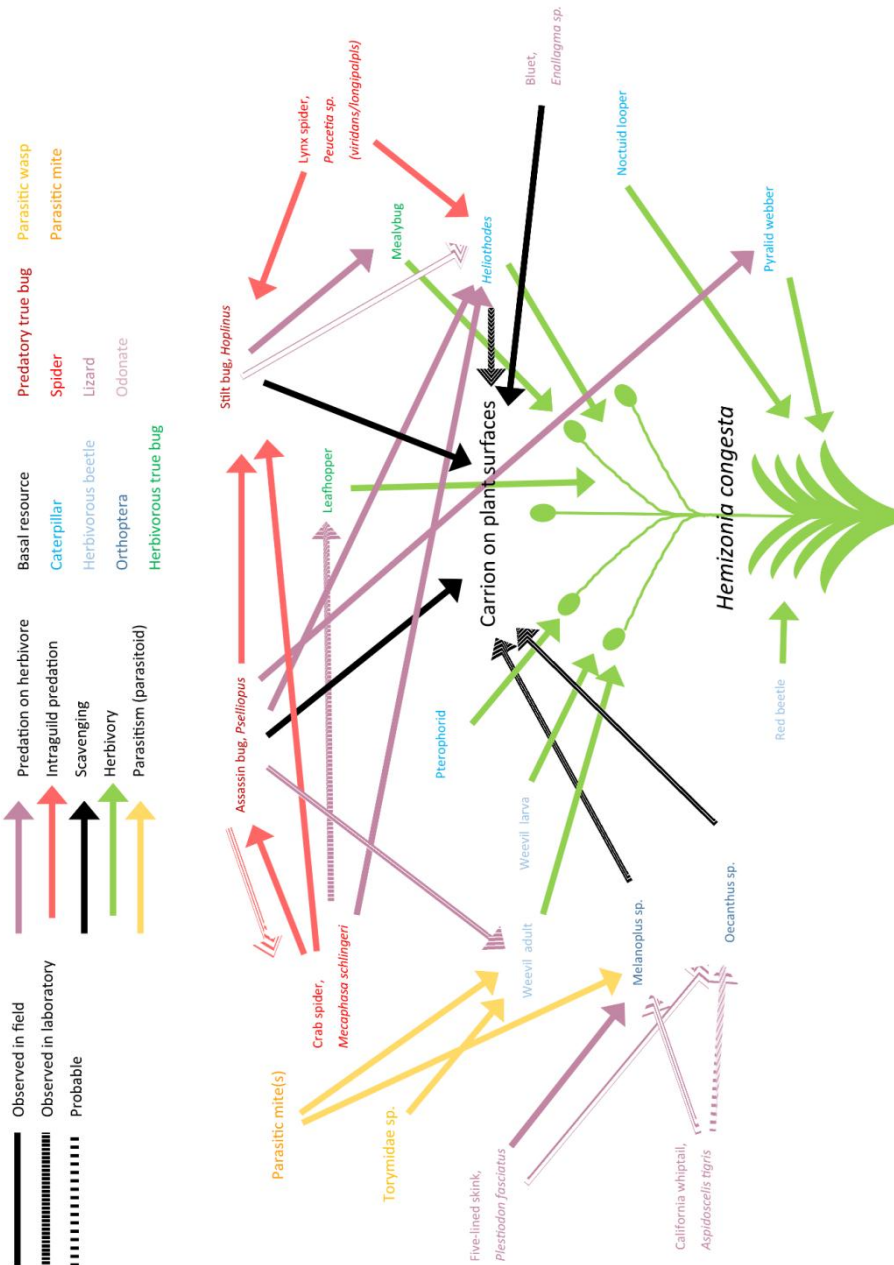


Figure 2: A positive correlation between carrion abundance and predator abundance on unmanipulated *H. congesta* plants (predictions for a mean plant size, model in Supplementary table S2-1; dotted lines indicate 95% CI of the mean).

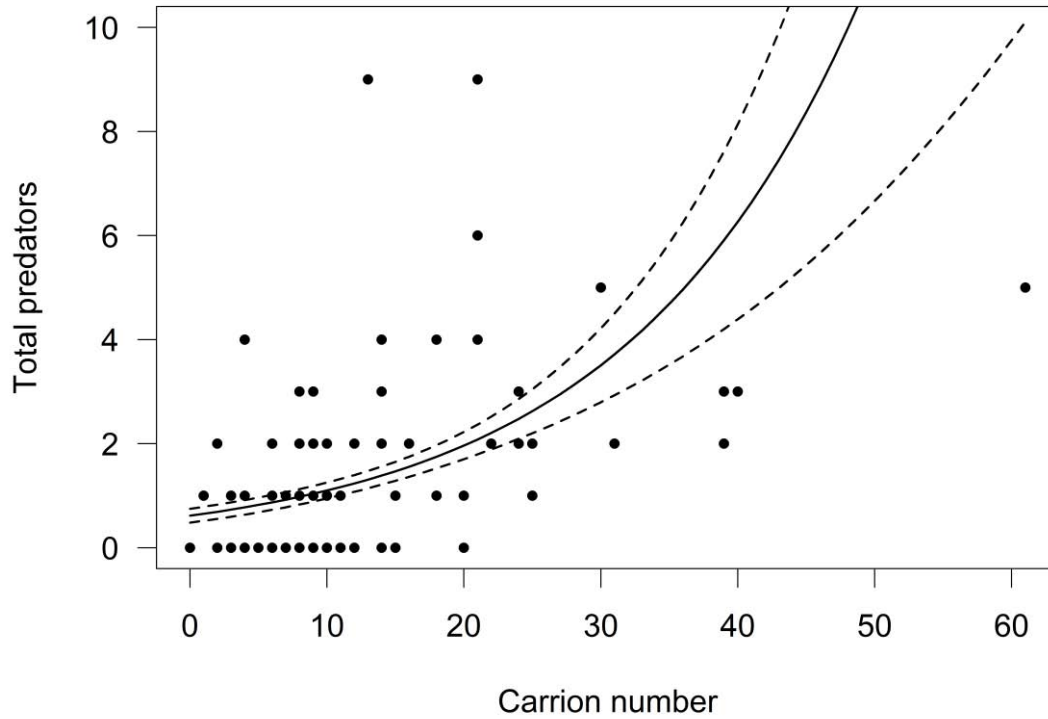


Figure 3: Per plant average observations (after treatments applied) of the four most common predators (left column and top middle) and five most common herbivores (right column and center and bottom middle). Unlisted predators were unidentified spiders (13 observations). Unlisted herbivores were an unidentified chrysomelid (6 observations), an unidentified aphid (5 observations). Of uncertain trophic position were tree crickets (7 observations) and unknown true bug nymphs (7 observations). All photos by author.

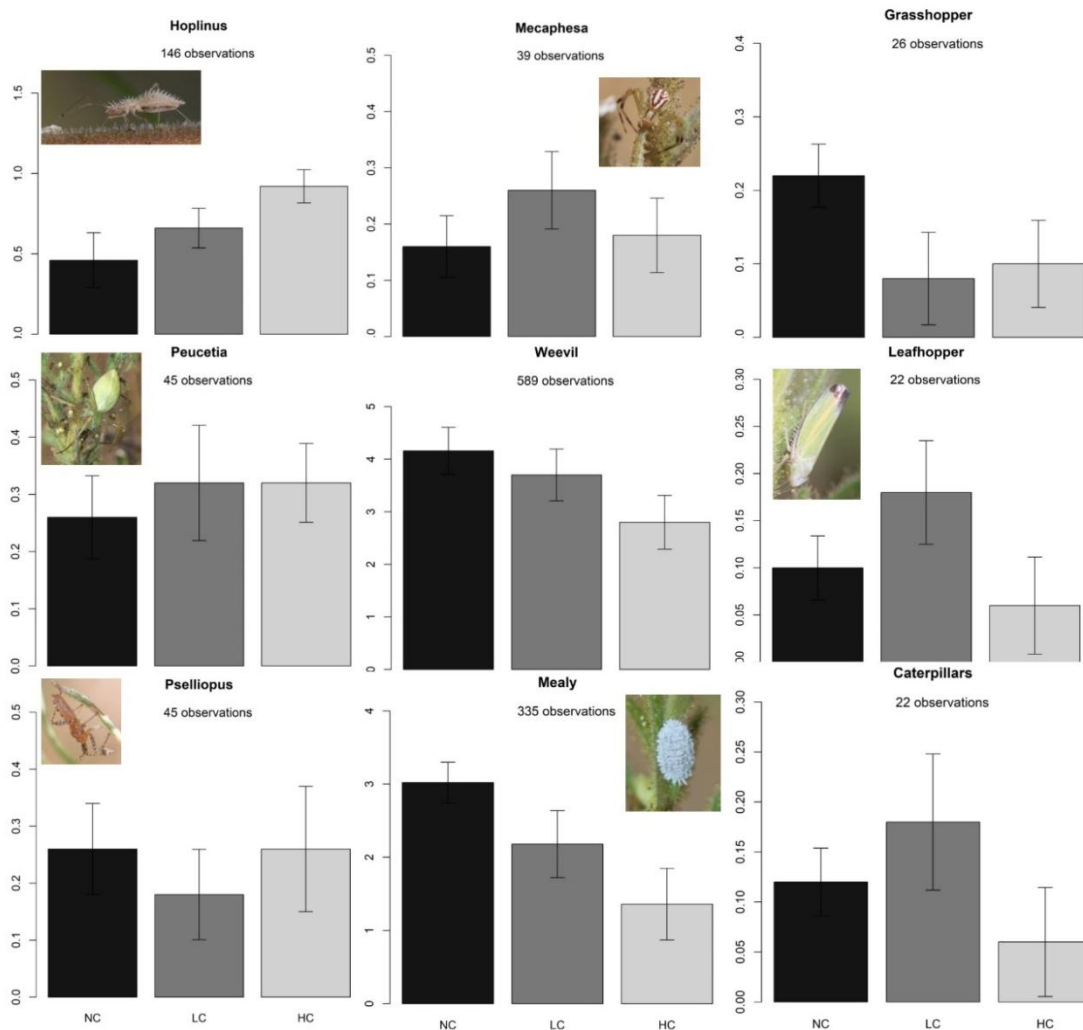


Figure 4: Effect of carrion manipulation on predators, herbivores, and seed set. Top left: average predators observed per plant, significant differences with Tukey's comparison of means on the best-fitting poisson model (HC-NC, $p = 0.03$). Bottom left: Average herbivores observed per plant, significant differences with Tukey's comparison of means on the best-fitting negative binomial model (HC-NC, $p < 0.01$). Top right: average seeds produced per plant. Bottom right: average seeds per fruit per plant, significant differences with Tukey's comparison of means on the best-fitting linear model HC-NC, $p = 0.03$). Bars and points are means \pm standard error.

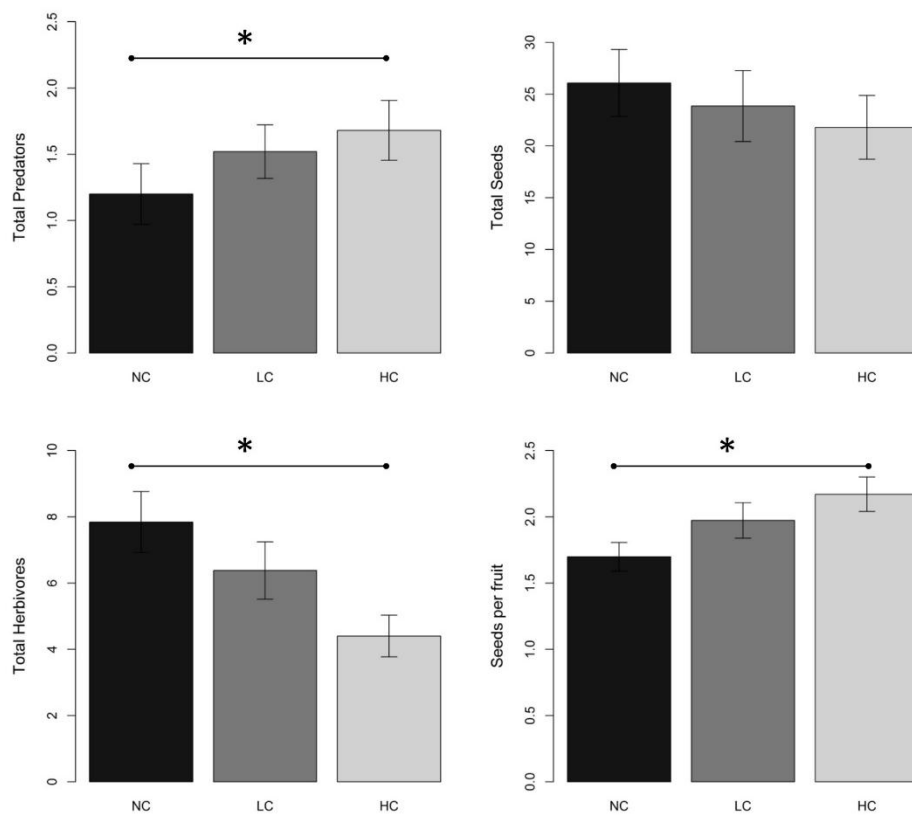


Figure 5: Carrion manipulation, but not previous damage, affected the rate of predation of intraguild prey *H. eschinatus*. Unique letters indicate differences in means established with Tukey's HSD test.

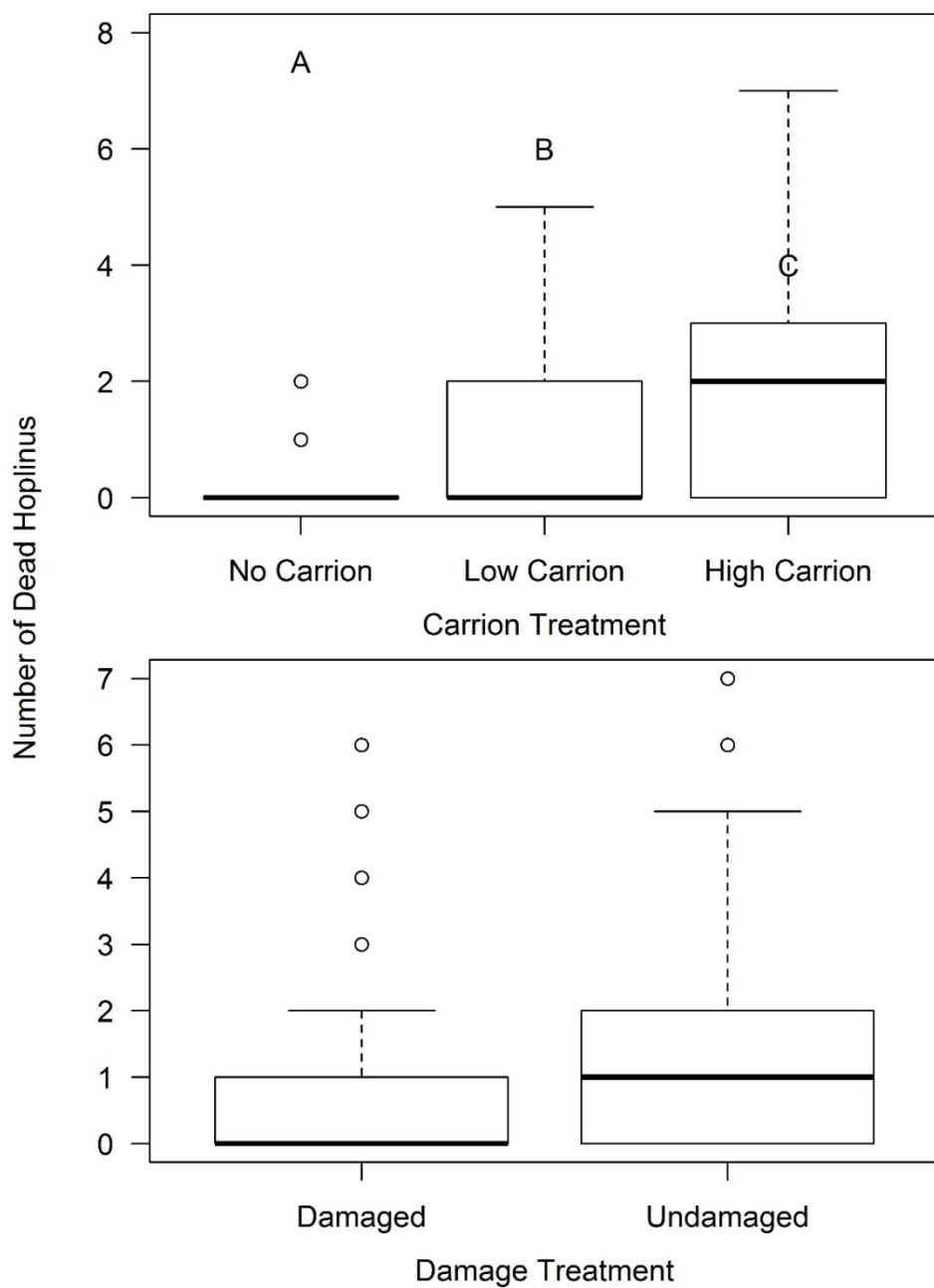


TABLE LEGENDS**Table 1:** Tables for model selection results of Experiment 1.

Predators

	log likelihood	X ²	p =	
Treatment*Plant Size	-242.07	0.91	0.63	compared to additive alone
Plant Size	-253.13	22.12	<0.01*	deleting this term from additive model
Treatment	-245.30	6.45	0.04*	deleting this term from additive model
Damage	-241.29	1.56	0.21	compared to treatment + size model

Herbivores

	log likelihood	X ²	p =	
Treatment*Plant Size	-414.86	5.31	0.07	compared to additive alone
Plant Size	-429.93	24.83	<0.01*	deleting this term from additive model
Treatment	-422.42	9.80	0.01*	deleting this term from additive model
Damage	-416.88	1.28	0.26	compared to treatment + size model

Total Seeds

	log likelihood	X ²	p =	
Treatment*Plant Size	-601.93	3.74	0.15	compared to additive alone
Plant Size	-627.97	48.34	<0.01*	deleting this term from additive model
Treatment	-604.29	0.98	0.61	deleting this term from additive model
Damage	-604.29	0.82	0.36	compared to size model

Seeds per fruit

	log likelihood	X ²	p =	
Treatment*Plant Size	-174.40	1.17	0.56	compared to additive alone
Plant Size	-175.33	0.71	0.40	deleting this term from additive model
Treatment	-178.39	6.82	0.03*	deleting this term from additive model
Damage	-174.68	1.3	0.25	compared to treatment model

Table 2: Spearman rank correlations of dead and living predatory insects: assassin bugs (*P. spinicollis*) and stilt bugs (*H. eschinatus*) that each feed on carrion, herbivores, and each other on *H. congesta* plants.

	Dead Assassin Bug	Dead Stilt Bug	Alive Assassin Bug
Dead Assassin Bug	-	-	-
Dead Stilt Bug	0.045	-	-
Alive Assassin Bug	-0.011	0.202*	-
Alive Stilt Bug	0.003	0.252**	0.236**

*P<0.05; **P<0.01
