

# Induction of the sticky plant defense syndrome in wild tobacco

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*Abstract.* Many plants engage in protective mutualisms, offering resources such as extrafloral nectar and shelters to predatory arthropods in exchange for protection against herbivores. Recent work indicates that sticky plants catch small insects and provide this carrion to predators who defend the plants against herbivores. In this study, we investigated whether wild tobacco, *Nicotiana attenuata*, fits this sticky plant defense syndrome that has been described for other sticky plants. We developed a bioassay for stickiness involving the number of flies that adhered to flowers, the stickiest tissues. In surveys conducted over three field seasons at four sites, we found that the number of carrion that adhered to a plant was positively correlated with the number of predators that we observed foraging over its surfaces. The number of predators was positively correlated with the number of seed capsules that the plant produced, a measure of lifetime female reproductive success. Structural equation modeling indicated strong support for the causal path linking carrion numbers to predator numbers to capsule production. We investigated whether stickiness was an inducible trait and examined two potential cues. We found that experimental clipping of rosette leaves induced greater stickiness, although clipping of neighboring sagebrush leaves did not. Damage to leaf tissue is likely to be a more reliable predictor of risk than is damage to a neighboring plant. The sticky plant defense syndrome is a widespread protective mutualism; its strength and ecological relevance can adjust as risk of herbivory changes.

*Key words:* herbivory; induced defense; plant fitness; predators; protective mutualism; structural equation modeling.

## INTRODUCTION

Many plants engage in protective mutualisms with animals; the plant provides resources and the animals protect the plant from damaging herbivores and pathogens. This interaction is termed indirect plant defense. The animals in these mutualisms, often ants, are attracted and retained by rewards that the plant provides (Janzen 1966, Bronstein 1998, Kessler and Heil 2011). The outcomes of these protective mutualisms for the plants are strongly context dependent and are influenced by many factors about the mutualists (e.g., their density, the species involved, their nutritional state, etc.) as well as many characteristics of the environment (e.g., abiotic conditions, the identity and density of potential herbivores, the identity and density of other enemies of the herbivores, etc.; Cushman and Addicott 1991, Bronstein 1998, Kessler and Heil 2011). Although context-dependent benefits to the plants are well documented, less well understood are costs to the plants of providing resources and associating with the animal partner; both

the benefits and costs to the presumed animal mutualist are also poorly known.

The most commonly studied rewards offered to the arthropod “bodyguards” of plants include extrafloral nectar, protein bodies, as well as shelters (see references already mentioned). Another potential reward that plants may offer to arthropods in exchange for protection from herbivores is insect carrion stuck on the plant surface. Many plants (20–30% of all vascular plants) have glandular trichomes (epidermal hairs), which often produce adhesive exudates (Duke 1994). Glandular trichomes have been found to provide direct defense against many herbivores, as small insects cannot move easily over these surfaces. The trichomes exude chemicals that deter herbivores, and make herbivores less able to reach nutritious tissues, and more prone to desiccation and other forms of morbidity and mortality (Levin 1973). In some cases, large numbers of small insects become stuck on the glandular trichomes of plants that are not carnivorous and become carrion that is much richer in protein than plant tissues (Darwin 1889, Spomer 1999).

Glandular trichomes may provide indirect defenses for sticky plants by catching carrion, which then attracts predators that consume both carrion and living prey.

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Specialized guilds of true bugs exploit sticky plants of many diverse plant species (Krimmel and Pearse 2013, LoPresti et al. 2015, 2018, Wheeler and Krimmel 2015). These specialized bugs thrive on sticky plants by avoiding contact with the adhesive exudates. They are able to walk on the tips of their long slender legs. Pretarsal claws and a nonsticky covering facilitate grooming away any exudates that happen to contact their bodies (Voigt and Gorb 2008). Lynx spiders are another group of predators with long legs that are adept at moving across sticky surfaces and consuming carrion (Romero et al. 2008). The spiders reduce the numbers of herbivores and the damage that herbivores cause on leaves and inflorescences. Similarly, experimental augmentation of carrion to tarweed plants increased the abundance of predators, decreased damage caused by herbivores, and increased plant fitness (Krimmel and Pearse 2013). Carrion-removal and -addition experiments involving another tarweed species produced similar results; plants with more carrion had fewer herbivores, more predators, and produced more seeds per fruit (LoPresti et al. 2018). Sticky columbine plants have also been found to attract insects that are nearby (LoPresti et al. 2015). These insects became stuck on the plant surface, and were associated with an increase in the density of predators and a reduction in plant damage. In these examples, both deterrence of herbivores and consumption of herbivores by predators are likely mechanisms. Collectively, these studies suggest that sticky plants may provision predators with carrion, similar to the better-studied mutualisms in which plants provide ants and other predators with nutritious resources (Janzen 1966, Bronstein 1998, Kessler and Heil 2011).

Many plant defenses are inducible, expressed exclusively or more strongly following cues that indicate elevated risk of damage (Karban and Baldwin 1997). Inducible defenses allow plants to conserve resources when they are not needed and to match their defenses to specific situations. Effective use of induced defenses requires that plants can recognize reliable cues that accurately predict future risks (Karban 2015). The most widely used cue is actual damage to plant tissues, perhaps because it is the most reliable indicator of

subsequent risk. However, plants are able to perceive diverse cues that predict future risk, including rupturing of trichomes by insects walking over leaf surfaces (Peiffer et al. 2009), chemicals associated with insect eggs (Hilker and Fatouros 2016), mating pheromones of specialist herbivores (Helms et al. 2013), and volatile cues from damaged neighbors (Karban et al. 2014).

Several of the plant provisions that are important in attracting predators that provide indirect defense have been found to be inducible. For example, ants recruited more vigorously to leaves that had been experimentally damaged (Agrawal 1998, Heil et al. 2001). Numerous studies have found that the volume and concentration of nectar in extrafloral nectaries of ant-plants increased in response to damage to plant tissues, the presence of herbivores, or cues of herbivores (Agrawal and Rutter 1998, Heil et al. 2001). However, it is not known whether plants become stickier in response to damage or cues of herbivores and whether such enhanced stickiness can protect them.

In summary, correlational and experimental studies in other systems have documented many of the links in a sticky plant defense (links d–g in Fig. 1). Added carrion attracted predators, reduced herbivores and their associated damage to plants, and increased plant fitness (see references already mentioned). Is stickiness a plastic plant trait that responds to appropriate environmental cues (links a and b) and results in more carrion on the plant surface (link c)?

Wild tobacco (*Nicotiana attenuata*) has been a model system for studying induced plant responses and communication between plants. Glandular trichomes of *N. attenuata* produce acyl sugars, nicotine, and other natural products that contribute to resistance against herbivory (Weinhold et al. 2011). These sugars are sticky and entrap small insects. The putative defensive chemicals present in the glandular trichomes are rapidly produced and released following damage (Laue et al. 2000) and protect *N. attenuata* from herbivores (Baldwin 1998). Damaged wild tobacco plants also have been found to emit volatiles that attract predators, particularly *Geocoris pallens*, benefiting from an indirect defense provided by these predators (Kessler and

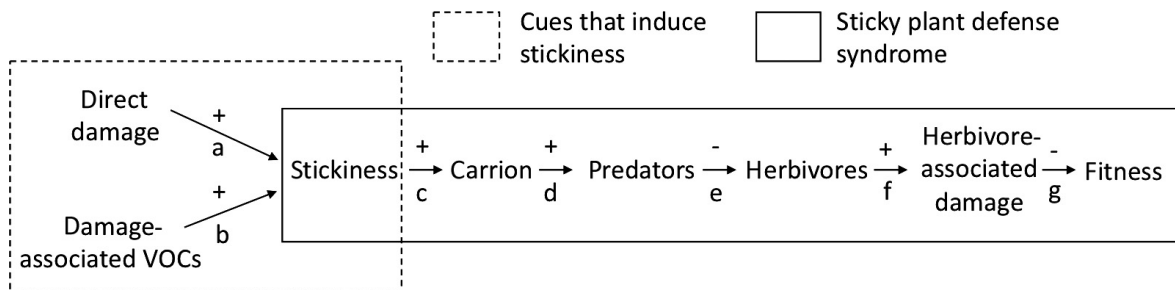


FIG. 1. Path diagram indicating the hypothesized model connecting cues of risk, stickiness, number of carrion, predators, herbivores, damage to plants, and number of capsules. The signs indicate the direction of the hypothetical relationship and the letters refer to links discussed in the text.

Baldwin 2001). Volatiles emitted by neighboring sagebrush plants (*Artemisia tridentata*) that have been experimentally damaged caused individuals of *N. attenuata* to become more resistant to their herbivores (Karban et al. 2000) and to experience increased fitness as a result (Karban and Maron 2002).

We conducted field observations and manipulative field experiments to examine relationships between stickiness, entrapped carrion, predators, and plant fitness (links c to g in Fig. 1). We were unable to measure number of herbivores and their damage (links e and f) in this study. We examined whether early-season experimental damage to tobacco or cues from clipped sagebrush neighbors affected stickiness (links a and b). We asked the following questions: (1) Does the amount of entrapped carrion predict the number of specialized predators? (2) Does variation in carrion affect plant fitness via increases in predators? These first two questions ask whether carrion on *N. attenuata* fits the sticky plant defense syndrome that has been described for other plants. (3) In addition, we asked whether experimental damage or volatile cues from neighboring sagebrush increased stickiness of tobacco? (4) Do stickier plants trap more insect carrion?

#### METHODS

This study was conducted using naturally occurring individuals of *N. attenuata* growing in the Great Basin in the rain shadow of the Sierra Nevada range in eastern California. This native species has an annual life history, germinating during snowmelt in early summer and producing seed-bearing capsules in late summer. It often grows in sites that have been burned or otherwise disturbed, although a small population can be found predictably at our primary study site, west of the Page Center at the Sierra Nevada Aquatic Research Lab (SNARL) of the Valentine Eastern Sierra Reserve (37.6136° N, 118.8312° W). Other populations are ephemeral in this region, appearing in some years, and remaining in the seedbank in others.

We evaluated the relationship between the number of carrion stuck on plant surfaces and the number of predators that were foraging on those plants for two populations during the summer of 2016. We recorded the number of carrion and the number of predators on 35 individuals southeast of the junction of US 395 and California 120 (37.89° N, 119.09° W) and 40 individuals near the junction of California 120 and the Benton Crossing Road (37.79° N, 118.57° W) on 22 July 2016.

We expanded our observations in 2017 to include the numbers of carrion, predators, and capsules (fruit). We marked 128 rosettes on 21 June 2017 at SNARL and recorded the number of carrion and predators that we observed on these plants on 2 July, 17 July, and 2 August. The majority of the predators that we observed were specialist stilt bugs (*Hoplinus echinatus* and *Jalysus wickhami*, Heteroptera: Berytidae), damsel bugs

(Heteroptera: Nabidae), and especially late in the season, predaceous leaf bugs (*Dicyphus* sp., Heteroptera: Miridae). These bugs are specialists on sticky plants, although they are omnivores that feed on live prey, carrion, and plant tissues. Several are considered important biological control agents of herbivorous insects and have been used in commercial agriculture. We also observed generalist big-eyed bugs (*Geocoris* sp., Heteroptera: Geocoridae), minute pirate bugs (*Orius* sp., Heteroptera: Anthracoridae), and ants on the plants. These generalists were considered as predators if they were observed moving on the plant surface and as carrion if they were observed stuck on the plant surface. In addition, green lynx spiders were found on the experimental plants (*Peucetia viridans*, Araneae: Oxyopidae). Jackrabbits also ate the flowering tops of several plants, and a virus affected seven individuals.

We repeated this experiment in 2018 and surveyed our experimental plants far more thoroughly. We marked 80 rosettes on 16 July 2018 and assigned them to four treatments: (1) a control, (2) clipped rosette leaves, (3) sagebrush volatiles, and (4) carrion removal. Control plants received no further manipulations. A second group of clipped plants had three basal rosette leaves cut with scissors through the midvein; this technique induces a strong plant response (Baldwin 1988). Each cutting event was repeated 3–4 times so that the number of damaged cells was maximized, although the amount of leaf tissue removed was minimized. A third group of plants received volatiles from experimentally clipped neighboring sagebrush (*Artemisia tridentata* subsp. *vaseyana*). The sagebrush branch was cut from a nearby shrub and the branch was immediately placed in a floral water pick filled with water. The water pick was inserted into the ground so that the sagebrush foliage was within 3 cm of the marked tobacco plant. The distal edges of half of the leaves on the sagebrush branch were clipped with scissors. This technique has been found to induce putative defenses in neighboring tobacco leaves (Farmer and Ryan 1990, Karban et al. 2000), and these two species commonly co-occur in close proximity (Wells 1959). A fourth group of plants had all carrion removed from their surfaces with fine forceps every fifth day throughout the course of the experiment. These plants with removed carrion received no other experimental cues indicating risk of herbivory.

We measured plant stickiness by dipping plant tissue into a vial containing dead *Drosophila pseudoobscura*. These flies were of roughly similar size and weight to the majority of the carrion that accumulated on *N. attenuata* surfaces. We found that the stickiest tissues were the sepals that surrounded the bases of the corolla tubes. The corolla and the stem of the inflorescence (peduncle) were also relatively sticky. For each plant we selected one fully expanded flower and, gripping the distal end of the corolla, we dipped the proximal end (where it would connect to the peduncle) into a vial of flies. The number of flies that adhered to the sepals was counted. Stickiness

was assessed on different days over the summer for different plants in order to standardize the developmental stage at which all plants were tested (fully open flowers).

We measured the number of carrion on each plant every fifth day from 16 July to August 30. Most carrion were found on the inflorescences once these structures elongated in August. All carrion observed on plants in the carrion-removal treatment were picked off with forceps. We also recorded the number of predators on all plants every fifth day. The predator community was similar to the one that we observed in 2017.

This annual plant produces flowers throughout the summer and these mature into seed-bearing capsules. Once the capsules are mature, they split open and the seeds shake out. As such, it was not possible to collect all the seeds. However, the number of capsules that a plant produces is probably a reliable estimate of relative lifetime female fitness (Baldwin 1998, Karban and Maron 2002). The plants in our study varied in size and larger plants had more carrion, more predators, and produced more capsules. We also measured plant size and included size in our models. We measured the diameter of the main stem with calipers just below the first basal leaves on 2 August 2017 and 8 August 2018.

#### *Statistical analyses*

*The sticky plant defense syndrome.*—Hypothetical relationships between the number of carrion stuck to plants and the number of predators that recruited to them were evaluated using standard least-squares models (Fit Model, JMP Pro 14.0). In 2016, we only measured carrion and predator numbers at one time. In 2017 and 2018 these variables were measured multiple times. For these seasons, we used the mean number of carrion and predators in our analyses because these are the functionally relevant measures of how well a plant is defended against insect herbivores at any given time; herbivores may attack tobacco plants anytime throughout the season. Relationships between the mean number of predators observed on each plant and the number of capsules that were produced in 2017 and 2018 were evaluated using standard least-square models.

We were able to evaluate the causal relationships between carrion, predators, and plant performance with more power with the use of structural equation modeling (SEM) for our data from 2017 and 2018. The specific causal path that we hypothesized was that plants with more carrion would attract more predators and that these predators would reduce losses to insect herbivores and result in greater lifetime plant fitness (seed capsules). SEM allowed us to test explicitly the hypothesis that predators serve as the causal link between carrion and seed capsule production by evaluating if predators explained covariation between carrion and capsules. To maximize statistical power and generality, we constructed one SEM model using data from both years. We used piecewise SEM (Lefcheck 2016) with a linear mixed

model of log mean number of predators per survey for the first submodel and a negative binomial mixed model of capsules for the second submodel. Both submodels included stem size as a covariate and treatment as a random effect. Structural equations were conducted in R (R Core Team 2018 v. 3.5.1) using the package piecewiseSEM (Lefcheck 2016), and glmmTMB for mixed effects submodels (Brooks et al. 2017).

*Is stickiness inducible?*—We evaluated the effects of experimental leaf clipping and exposure to volatiles from clipped sagebrush on stickiness using a standard least-square model. Because plants that had carrion removed were not exposed to additional cues of herbivory, we lumped the carrion-removal treatment with the control to increase sample size. We compared stickiness of clipped plants vs. controls and plants exposed to sagebrush volatiles vs. controls using a priori contrasts.

We evaluated the relationship between stickiness and the number of carrion that were attached to each plant using a standard least-squares model. Stickiness was measured as described above as the number of fruit flies that stuck to the sepals of a fully expanded flower. For this analysis, we considered the mean number of carrion per survey that were stuck onto each plant for the period between bolting and the end of the study when plants were maturing capsules.

## RESULTS

### *The stickiness plant defense syndrome*

The number of carrion attached to plants was a positive predictor of the number of predators that we observed at both sites in 2016 (Junction 395 and 120:  $R^2 = 0.48$ ,  $n = 35$ ,  $P < 0.001$  [Fig. 2A], Benton Crossing:  $R^2 = 0.20$ ,  $n = 40$ ,  $P = 0.004$  [Fig. 2B]). Similarly, the number of carrion explained much of the variation in the number of predators on plants at SNARL in 2017 ( $R^2 = 0.74$ ,  $n = 50$ ,  $P < 0.001$  [Fig. 2C]) and in 2018 ( $R^2 = 0.43$ ,  $n = 66$ ,  $P < 0.001$  [Fig. 2D]).

The mean number of predators observed on each plant was a positive predictor of the number of seed capsules that were produced (2017:  $R^2 = 0.30$ ,  $n = 50$ ,  $P < 0.001$  [Fig. 3A]; 2018:  $R^2 = 0.22$ ,  $n = 66$ ,  $P < 0.001$  [Fig. 3B]). These strong relationships were maintained regardless of how the models were constructed and whether covariates were included (see also the following discussion).

In 2017 and 2018 we were able to test the causal model linking carrion to plant defense. Shipley's d-separation test (Shipley 2000) indicated that the model structure was correctly specified (Fig. 4, Fisher's  $C = 1.75$ ,  $df = 4$ ,  $P = 0.78$ ). This suggests that there were no spurious correlations that could confound or invalidate our interpretation of the causal model. Larger plants were found to have more predators ( $\beta = 0.196$ ,  $z = 4.289$ ,  $P < 0.001$ ). Plants with more carrion had more



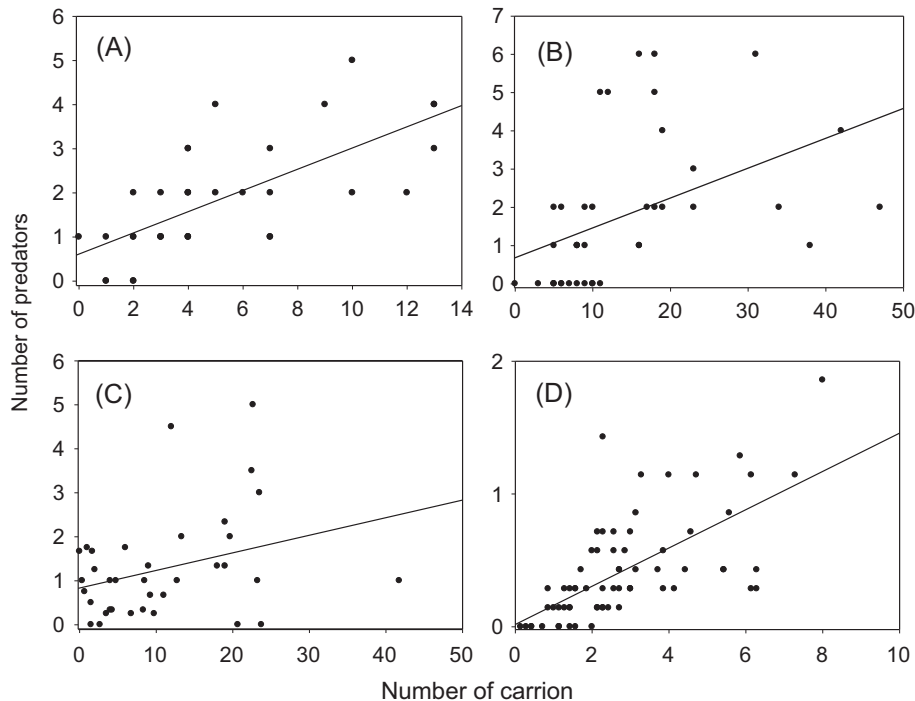


FIG. 2. The relationship between the mean number of carrion and the mean number of predators observed per survey in 2016 near (A) the junction of US 395 and California 120 and (B) between California 120 and Benton Crossing Road, and in (C) 2017 at Sierra Nevada Aquatic Research Lab (SNARL), and (D) in 2018 at SNARL.

predators ( $\beta = 0.01$ ,  $z = 6.63$ ,  $P < 0.001$ ; link d in Fig. 1). Plants with more predators produced more capsules ( $\beta = 1.059$ ,  $z = 6.27$ ,  $P < 0.001$ ; links e–g in Fig. 1). These relationships were not different in 2017 and 2018 (interaction year \* carrion  $z = -0.29$ ,  $P = 0.77$ ; interaction year \* predators  $z = -0.81$ ,  $P = 0.42$ ), although more capsules were produced per plant in 2018 than 2017 ( $\beta = 0.67$ ,  $z = 2.42$ ,  $P = 0.02$ ). The positive relationship between predators and capsules was found for plants of all sizes (size was not a significant predictor of capsule production beyond its effect on predators;  $z = 0.29$ ,  $P = 0.77$ ).

#### *Cues that induce stickiness*

The treatments providing cues of damage had a marginally significant overall effect on stickiness (Fig. 5,  $F_{2,62} = 3.01$ ,  $P = 0.056$ ). More specifically, plants that had had their leaves experimentally clipped became stickier than controls (a priori contrast  $F_{1,62} = 5.99$ ,  $P = 0.02$  [link a in Fig. 1]). This effect was responsible for much of the overall treatment effect. Plants that had been exposed to volatiles from clipped neighboring sagebrush shoots were not stickier than controls (a priori contrast  $F_{1,62} = 0.37$ ,  $P = 0.54$  [link b in Fig. 1]).

Plants that our bioassay characterized as stickier caught more carrion, although this relationship was noisy and explained a relatively small proportion of the

variation in carrion number ( $R^2 = 0.06$ ,  $n = 64$ ,  $P = 0.05$  [Fig. 6, link c in Fig. 1]).

#### DISCUSSION

Our results were consistent with the hypothesis that sticky plants trap small insects that serve as food for specialized predators and ultimately increase plant fitness (Fig. 1). At three different field sites, over three different field seasons, we observed a strong positive relationship between the number of carrion stuck on plant surfaces and the number of predators patrolling those plants (Fig. 2). Our study was conducted during conditions ranging from unusually dry (2016 and 2018) to unusually wet (2017). The plants varied considerably in size and larger plants had both more carrion and more predators, although the strong positive relationship between carrion and predators was found to be independent of this correlation (Fig. 3; size did not influence this link in the SEM, Fig. 4). In the 2 yr for which we measured capsule production, an estimate of lifetime fitness, there was a strong relationship between the number of predators and the number of capsules (Fig. 3). The SEM gave strong support for our causal hypothesis that the relationship between carrion and lifetime capsule production was mediated by the number of predators that recruited to the plants. This is because predator numbers effectively explained the observed covariance between carrion and capsules without the need for a direct link

between carrion and capsule production (Fig. 4). Nonetheless, we were forced to infer the causal links between more predators and reductions in herbivore numbers or their damage and capsule production (links e–g in Fig. 1); that is, herbivores were an unmeasured

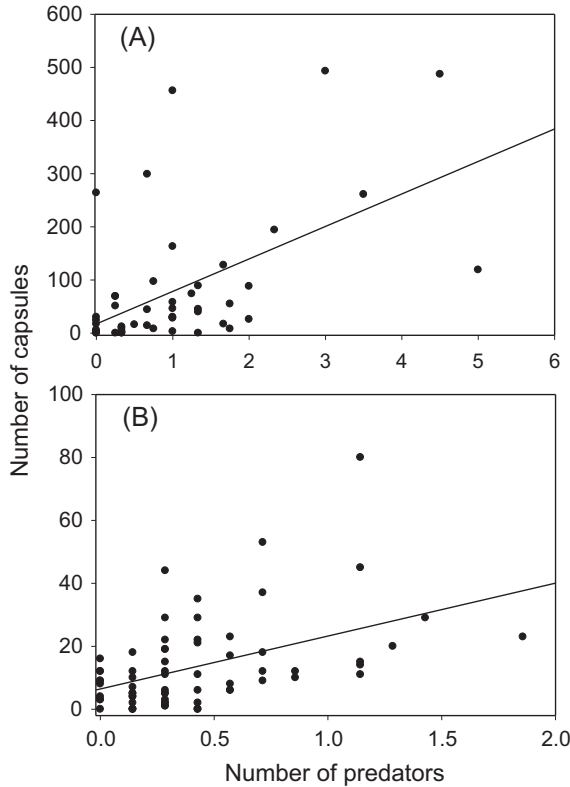


FIG. 3. The relationship between the mean number of predators per survey and the total number of seed capsules on plants in (A) 2017 and (B) 2018.

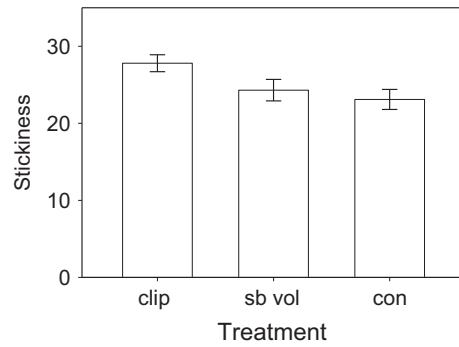


FIG. 5. Stickiness measured as the number of fruit flies that adhered to the sepals of a fully expanded flower of each plant. Bars show the mean  $\pm$  1 SE for plants that had leaves clipped (clip), were exposed to the volatiles from clipped sagebrush (sb vol), or controls (con).

intermediary variable in the parlance of SEM (Shipley 2000). In 2017 we attempted to measure numbers of herbivores, and in 2018 we attempted to measure herbivore damage, although neither of these efforts proved to be useful. Unlike the predators that resided on the plants, the herbivores were generally ephemeral and difficult to observe (see the following discussion). Attempts to measure herbivore damage was also unsatisfactory because chewing damage to rosette leaves was easy to measure but was not strongly correlated with damage to reproductive tissues, and the latter was difficult to estimate (personal observation). However, the high levels of herbivory experienced by these plants (~50% of leaves damaged) make it likely that supporting more predators will translate into higher plant fitness (Karban and Maron 2002, Kessler and Baldwin 2004).

Carrion attract and keep a variety of specialists on sticky plants, and these omnivores are likely to consume a wide range of small insect prey, including herbivores that

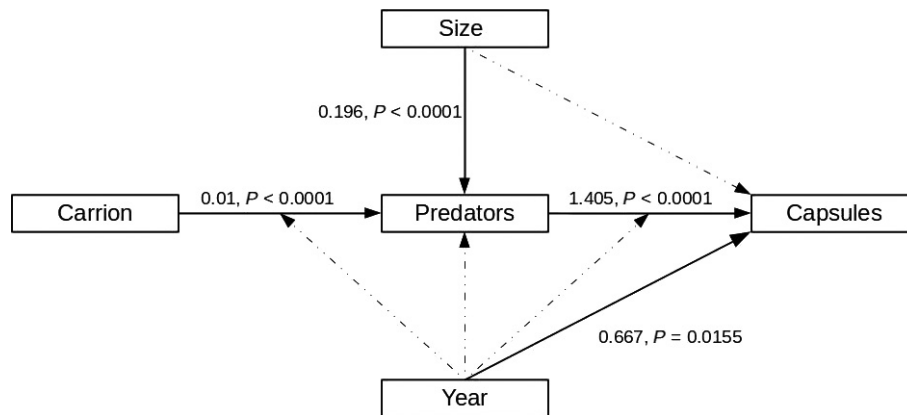


FIG. 4. Piecewise structural equation model of the effect of carrion on the number of seed capsules (lifetime fitness) via predators. Variables are indicated by boxes, and arrows show hypothetical causal links between variables. Significant paths are indicated by solid arrows and nonsignificant paths by dashed arrows. An interaction between variables is represented by an arrow that intersects (points to) the path between those two variables. Unstandardized coefficients and *P* values are shown for significant paths. The model includes data from 2017 and 2018. Predator numbers and links between all variables are on a log scale.

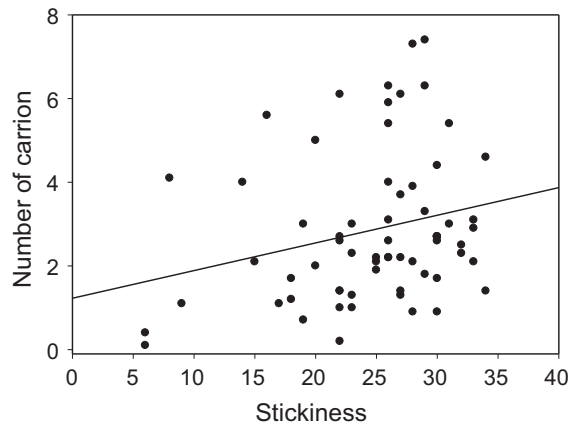


FIG. 6. The relationship between our assay of stickiness (flies adhered to sepals) and the mean number of carrion observed on plants during surveys after plants bolted.

damage reproductive tissues of this plant species. Hornworms (*Manduca quinquemaculata*) are voracious herbivores that can consume entire *N. attenuata* individuals (Wells 1959, Karban 1997, Kessler and Baldwin 2002). They move up from Mexico to our study sites in early summer and exhibit wide year-to-year variation in numbers (personal observation). Adult females prefer to oviposit on inflorescences and caterpillars pose considerable risk to inflorescence stalks, flowers, and capsules of *N. attenuata* (Kessler and Baldwin 2002). However, most *M. quinquemaculata* eggs and early instars are consumed by predators before they cause much plant damage. *Corimelaena extensa* (Heteroptera:Thyreocoridae) is another important florivore that feeds on the flowers and capsules, causing seed mortality (Wells 1959, Lung and Goeden 1982, Stanton et al. 2016). Although it is likely that these bugs will be vulnerable to attack by the guild of predators associated with sticky plants, we were not able to observe predation events, because nymphs of this seed feeder live primarily inside the capsules. Several species of cutworms are common herbivores of *N. attenuata*; although they fed more commonly on rosette leaves than on flowers or fruit, they were observed more often being taken by ants than by the other specialist predators.

We found that the reproductive tissues were stickier than vegetative tissues, although measures of stickiness were correlated among the various tissues of different individuals (data not shown). High stickiness of reproductive tissues is in keeping with predictions of optimal defense theory, which posits that those tissues that are most valuable for plant fitness and most vulnerable to herbivory should be most chemically defended (McKey 1974). A meta-analysis of optimal defense theory found broad support for predictions that younger leaves have more chemical defenses than older ones but failed to find much support for the prediction that flowers are better defended than vegetative tissues (McCall and Fordyce 2010). These authors suggested that the assumption that flowers are more valuable than leaves may be incorrect or

that lack of statistical power may be responsible for this negative result. Several other studies that have examined indirect defenses mediated by extrafloral nectar rewards have found that reproductive tissues are constitutively defended, whereas vegetative tissues have presumably cheaper induced defenses (Wackers and Bonifay 2004, Holland et al. 2009). These findings were interpreted as supporting optimal defense theory.

In our system, sepals became stickier when rosette leaves had been experimentally clipped (Fig. 5). Stickiness of reproductive tissue (sepals) was correlated with the number of carrion that were stuck to the entire plant, although this relationship was not strong (Fig. 6). Previous work in this system showed that experimental leaf damage increased levels of nicotine in floral tissues (Baldwin and Karb 1995, Euler and Baldwin 1996) and reduced levels of damage to flowers and capsules (McCall and Karban 2006). Our current finding that leaf damage increased stickiness of sepals is in accordance with these earlier results.

We previously found that tobacco plants consistently became more resistant to herbivory when they were exposed to volatile cues from clipped sagebrush neighbors that were rooted in the ground (Karbon et al. 2000). However, sepals showed no evidence of becoming stickier when tobacco plants received volatiles from a nearby clipped sagebrush branch, in contrast to leaf damage to the tobacco itself (Fig. 5). There are several possible explanations for this negative result, which was contrary to our a priori expectation. Cues from sagebrush branches that have been clipped may be weaker than those emitted by sagebrush that is rooted, although Farmer and Ryan (1990) found that cues from excised sagebrush branches were sufficient to induce resistance in tomato plants under lab conditions. It makes sense that tobacco plants will be more responsive to cues that are produced when their own rosette leaves have been clipped compared to cues from neighboring sagebrush leaves. Damage to their own rosette leaves is likely to be a better indicator of increased risk to reproductive tissues, because herbivores have already discovered the plant and demonstrated that they will consume it. As such, damage to rosette leaves is probably a more reliable cue than volatiles from neighboring sagebrush. It is surprising that tobacco leaves have given no indication that they perceive and respond to volatile cues from experimentally clipped tobacco neighbors (Karbon et al. 2003). Instead, systemic induced resistance within a tobacco plant appears to be coordinated via internal vascular cues that are more private.

## CONCLUSIONS

Our strong and consistent results linking carrion that accumulated on sticky plant surfaces to resident predators to increases in plant fitness add to the growing evidence that the stickiness provides an important and widespread indirect defense against herbivory (Romero

et al. 2008, Krimmel and Pearse 2013, LoPresti et al. 2015, 2018, Wheeler and Krimmel 2015). This sticky plant defense syndrome is likely to be more effective in arid and Mediterranean environments where the adhesive materials do not wash off plant surfaces during rainstorms (LoPresti 2017). In these environments, sticky plants that offer carrion as a reward to predators may well be more common than plants that offer extrafloral nectar. The costs associated with sticky surfaces are not known, although this trait is quite variable and can be induced by damage to leaves. In many cases, damage to leaf tissues probably represents a reliable cue of increased risk to valuable reproductive tissues.

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