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Florivory and nectar-robbing perforations in flowers of pointleaf manzanita *Arctostaphylos pungens* (Ericaceae) and their effects on plant reproductive success

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Abstract

Damage to petals may have varying effects on the reproductive success of the plant. The variation may depend on the kind of damage to the corolla. Whether the damage is limited to the corolla, as is usually the case with nectar-robbing perforations, or extending to the reproductive parts of the flower, as in the case of florivory holes, might determine the extent of the effect on the plant's reproduction. We examined the various perforations in the flowers of *Arctostaphylos pungens* and correlated their presence with fruiting success. We found that though florivory holes were highly associated with damage to reproductive parts, fruiting success did not differ significantly between flowers with the two kinds of damage. Although nectar-robbing perforations were not associated with reduced number of fruit produced, they were significantly correlated with reduced number of fruit that contained seemingly viable seeds. The implications of our findings are discussed in the context of pollination and antagonism.

Keywords

Florivory; Nectar robbing; Reproductive success; Floral damage; Pollination; Antagonism

Introduction

Many animal-pollinated plants have colorful, attractive flowers, advertising the presence of nectar and other rewards with varied shapes, colors, patterns, and scents (Willmer 2011). These same advertisement aids may attract animals that will not provide pollination services and instead may damage the flower, thus reducing the reproductive success of the plant. Plants, therefore, face the challenge of attracting pollinators while at the same time deterring exploiters (Irwin et al. 2004). Florivores, animals that consume flower parts (e.g.,

caterpillars), and nectar robbers, consumers of nectar through punctures in the flower's corolla (e.g., bees and wasps), are examples of such exploiters.

Florivores can affect reproductive success of plants directly, by feeding on pistil or anthers, or indirectly, by reducing the attractiveness of the plant to pollinators (McCall and Irwin 2006). Conversely, animals that feed on flowers may indirectly benefit plants by reducing the plant attractiveness to other antagonists (McCall and Irwin 2006), or by also serving as pollinators, as in the case of some thrips (Eliyahu et al. 2015; García-Fayos and Goldarazena 2008). Plants possess certain traits, such as for resistance and tolerance, that combat potentially harmful effects of florivores. For example, some plants may change their flowering phenology to avoid florivores (McCall 2006; Kawagoe and Kudoh 2010). Other plants may emit scents that are differentially attractive to pollinators but deterrent to florivores (Kessler et al. 2013). Some species with sex separation, whereby male and females parts are housed completely or partly separately (dioecy or gynodioecy, respectively), exhibit sexually dimorphic traits, with female flowers less preferred by florivores than male or hermaphrodite flowers (Tsuji and Sota 2010; McCall and Barr 2012; Vega-Frutis et al. 2013). In other cases, florivory may affect floral sex ratios, thus reducing the prevalence of male flowers (Wise and Hebert 2010).

Nectar robbers are another type of visitor that can negatively affect plants through floral tissue consumption. Specifically, they either perforate the corollas to access nectaries (primary nectar robbers) or take advantage of existing perforations (secondary nectar robbers). By doing so, they obtain nectar rewards while potentially circumventing contact with reproductive tissues, thereby not providing pollination services (Irwin et al. 2010). Often plants that are frequently robbed have flowers with tubular, or a variation of tubular, corollas. Thus, legitimate access to nectar is restricted to those particular visitors that are morphologically matched to provide pollination services. For example, there might need to be a match between corolla depth and tongue length. Perforations in such tubular corollas provide nectary access to a wider variety of visitors, including those that lack such morphologically matched traits (Barrows 1980). Similar to florivores' damage, perforation of the flower for larceny purposes may affect the plant's reproductive success negatively or positively. Meta-analyses found mostly negative effects of nectar robbing, caused through either direct or indirect mechanisms (Burkle et al. 2007; Irwin et al. 2010). Some recent studies have found positive effects of nectar robbing. The reduced volume of nectar resulted in shorter visits and more inter-plant movement of the bumble bee pollinator of *Aconitum napellus*, when nectar robbing was simulated (Mayer et al. 2014). A similar response of pollinators to nectar-robbled flowers was observed in *Tecomella undulata* (Bignoniaceae; Singh et al. 2014). Another way in which nectar robbing may affect plant reproductive success is by providing an entry to other animals, such as thrips (Eliyahu et al. 2015), that can potentially cause damage (Irwin et al. 2010). As in plants' response to florivory, some plants possess traits that minimize visitation by nectar robbers, while still attracting pollinators. For example, nectar guides may be used to facilitate legitimate visits (Leonard et al. 2013). Or, as in the case of *Ipomopsis aggregata*, the flowers produce diluted nectar to deter nectar-robbing bumble bees, but still attract pollinating hummingbirds (Irwin et al. 2004). More strikingly, the sunbird-pollinated tree tobacco *Nicotiana glauca* produces more

secondary plant metabolites in the nectar following a nectar-robbing event (Kaczorowski et al. 2014).

While a number of studies focused on florivory and nectar robbing separately, few have focused on both simultaneously. Interactions between florivores and nectar robbers will affect the plant in ways that cannot be seen when focusing on one of the actors. For example, if a florivore attacks a flower first, the flower may become unattractive to other antagonists or to legitimate pollinators. Furthermore, ignoring one player (e.g., the florivore) when focusing on the other (e.g., the nectar robber) may lead to attributing the effects of the former to the latter. Thus, to fully and more accurately understand the effect of any single floral–insect interaction, we need to know how it relates to other interactions.

Pointleaf manzanita, *Arctostaphylos pungens* (Ericaceae), is an ideal system for studying these aspects of insect–plant interactions. This southwestern shrub flowers during a time of the year when very few other plants are in bloom. The flowers are arranged in racemes that may carry as many as 26 individual flowers, at various stages of maturity (Richardson 1999), and each plant may carry many racemes during its short period of bloom, resulting in a very showy display of white–pink flower clusters. The plant serves as a very abundant resource for a variety of floral visitors, ranging from beneficial pollinators to antagonist florivores. In fact, Richardson and Bronstein (2012) identified no fewer than 46 such foraging visitor taxa. The globular flowers have a narrow opening, allowing only select visitors to access the nectaries. Some manzanita visitors have exclusively been observed in nectar-robbing behaviors, such as *Nomada* bees and two *Vespula* wasp species. These visitors also possess mouthparts that enable them to perforate the corolla. Other visitors, such as honey bees, may be facultative robbers, and though they might not be able to create a hole, lacking suitable mouthparts, will opportunistically use one if available (secondary nectar robbers; Richardson and Bronstein 2012). The flowers may also be punctured by florivores, most commonly caterpillars (personal observation), which often completely consume the reproductive parts of the flower, leaving a hollow corolla, making these florivory perforations distinguishable from those made by nectar robbers (Fig. 1).

Pointleaf manzanita and its multitude of visitors, including florivores and nectar robbers, were used for testing a number of hypotheses related to the interactions between the different types of floral visitors and the plant. Our first hypothesis was that florivory and nectar-robbing perforations are influenced by floral phenology and morphology. Specifically, we predicted that buds would be damaged primarily by florivores, because florivores do not need to make holes to enter open flowers. At the same time, nectar robbers might prefer open flowers, which are likely to contain more nectar than buds (Navarro 2001; Akamatsu et al. 2011). Secondly, we predicted a positive correlation between corolla length and nectar-robbing perforations, because visitors with shorter tongues would be more likely to get nectar illicitly. Our second hypothesis was that there is a negative interaction between florivores and nectar robbers, and we predicted that the presence of one type would exclude another. This prediction was based on the assumption that floral damage by one visitor would render flowers less attractive to subsequent visitors. Finally, we hypothesized that floral perforations reduce reproductive success. Specifically, we predicted that florivory would be more associated with damage to reproductive parts, and therefore, that its negative

effect on reproductive success of manzanita would be greater than that of nectar robbing. By testing these hypotheses and their specific predictions, we gain a deeper understanding of the interactions between different groups of floral visitors and the effects of each on the plant.

Methods

Study plant species

Pointleaf manzanita (*A. pungens*) is a perennial shrub that grows at elevations of about 1200–2500 m in chaparral habitats, including in the sky-island mountain ranges of Arizona. Plants can reach a height of 3 m and produce small pink urceolate (spherical/globular) flowers in racemes. Flowers contain ten stamens, with pollen dehiscence via pores in the anthers, and possess 5–10 ovules (Richardson and Bronstein 2012). The fruits are berries that produce an average of six and a maximum of eight seeds (unpublished data). Pointleaf manzanita is largely self-incompatible, whereby pollination using pollen from flowers of the same plant results in significantly fewer viable fruit than pollen from flowers from other plants (Richardson and Bronstein 2012). The plant blooms roughly from mid-January to mid-March, with peak season usually around mid-February. It is one of the only blooming plants at the beginning of its season. This, in conjunction with its producing hundreds of flowers per plant, leads to the attraction of many species of floral visitors (Richardson and Bronstein 2012). The fruits resulting from cross-pollination remain and dry on the plant and are edible as both fresh and dried fruit (personal observation) and thus may serve as a consistent food source for birds and mammals.

Study site

The study was conducted in the Santa Catalina Mountains, Arizona, USA (32.20°N, 110.33°W), at the Gordon Hirabayashi Recreation Site (1500 m elevation). At this site, *A. pungens* is a dominant shrub species, and the study plants are situated in the northern and western aspects of a hill, as well as its apex.

Florivory versus nectar-robbing perforations

During our 2012 collection and survey of perforations, it was observed that some perforations had a typical size and shape and often included a flap or remains of a flap (Fig. 1a). This means that the tissue was not consumed, only cut to provide an opening. Though we did not directly observe a nectar robber making these holes, we did observe similar holes being used for secondary robbing (i.e., we saw visitors inserting their mouthparts through the holes), so we categorized them as nectar-robbing perforations. We additionally found that the location of these perforations was typically at a distance from the base that corresponded with the location of nectaries (Fig. 2). A second type of perforation we observed varied extensively in both size and shape and did not include a flap, suggesting that the tissue was consumed to produce the opening (Fig. 1b). Perforations of this type were not restricted to a particular area on the corolla and were found anywhere from the very base to the very tip (Fig. 2). We categorized these as florivory holes. Fittingly, this type of perforation was more associated with damage to reproductive parts of the flower (see “Results” section below).

Survey of perforations

At the peak of flowering period in 2012 (Feb 22), we collected 15 haphazardly chosen racemes with at least one open flower from each of 17 haphazardly chosen *A. pungens* plants. The racemes were collected into small plastic cups that were immediately capped and kept in a -20°C freezer until further analysis. The analysis included measuring the diameter and length of flowers using a pair of digital calipers, as well as the location of holes, which was measured as the distance between the hole (the part closest to the base of the flower) and base of the flower. The data collected in 2012 prompted us to repeat and enhance the collection in a following year. Since 2013 proved a very short flowering season, we did not collect data for the survey, but conducted a more thorough collection in 2014. That year, we conducted three collections: one at the beginning of the flowering period (Jan 20), one at the peak of the period (Feb 5), and another at the end of the period (Feb 19). At each collection, five racemes from roughly the same 20–22 plants were chosen haphazardly. The flowers from all collections were later dissected, and the type and number of holes in each flower were recorded.

A Fisher's exact test was used to determine which type of perforation was more prevalent in open flowers and closed buds, and to determine what type of perforation was associated with reproductive organ damage. A Chi-square test was used for examining the likelihood that flowers with at least two perforations would have both nectar-robbing and florivory perforations. These analyses were performed separately for each flowering period. For relationships between floral perforations and floral dimensions, we analyzed only data from 2012. We used negative binomial regression models due to the large variance in the number of holes per flower. To test the effects of plant identity and corolla dimensions on hole number per flower, we used a generalized linear mixed model (glm.nb function in R). The number of perforations, produced by either nectar robbers or florivores, was selected as the dependent variable, and plant identity and inflorescence nested within plant were specified as two of the independent variables. We did not identify plant as a random variable because we were specifically interested if plant identity had an effect on the number of perforations. Other independent variables included corolla maximum length and corolla maximum width. All analyses were performed using R (R Core Team 2013; v. 3.0.3 “Warm Puppy”).

Effect of perforation type on fruiting success

We conducted two separate studies to look at the relationship between holes and fruiting success. In an observational study throughout the flowering periods of 2012 and in 2013, we marked open flowers in five haphazardly chosen racemes per plant and noted whether those flowers had any holes in them. We considered only those racemes that had either no flowers with holes (391 and 514 in 2012 and 2013, respectively) or all flowers with holes (73 and 124 in 2012 and 2013, respectively). We ignored racemes that included both intact and perforated flowers because we could not always determine which of those produced fruit. We followed the development of the fruit and compared numbers and dry weight of fruit between those flowers that had perforations and those that did not. To determine whether the proportion of flowers with holes affected fruit set in 2012 and 2013, we ran a Wilcoxon rank-sum test on the proportion of flowers setting fruit versus the status of holes in the racemes. We used a nonparametric test because the arcsine-transformed proportions did not

meet the homoscedasticity assumption of a t test. We measured dry weight because it was found to be associated with cross-fertilization of manzanita flowers (Richardson and Bronstein 2012). A Fisher's exact test was used to determine whether there is a significant difference in fruit production and fruit weight between flowers that had floral perforations and flowers that did not. In this analysis, we did not distinguish between perforations caused by nectar robbers or by florivores.

On February 9, 2014, up to five racemes were haphazardly chosen and tagged on ten plants. Only plants that possessed both open flowers and floral buds were chosen. Flowers in each raceme were marked, using a sharpie, as having open flowers with either no perforation, a nectar-robbing hole, or a florivory hole; 3 days later, the racemes were covered with drawstring mesh bags for protection from biotic and abiotic factors, such as florivores, frugivores, and wind. Fruits were harvested when mature (Apr 12), counted for each category, and then their seeds were dissected to determine which fruit had seemingly viable seeds. For this, seeds were soaked in water for at least 30 min to soften the outer shell and dissected to reveal the embryo. Seeds that did not appear to have an embryo (revealing empty space where the embryo should have been) were considered inviable. It is important to note here that most of the fruit we analyzed had seeds of fairly uniform shape and size, and because we could not dissect each and every seed in a fruit, we assumed that if some of the seeds did not contain an embryo, the others in the same fruit were also lacking.

To analyze the total fruit number and the proportion of those fruits that were seemingly viable, we used two separate GLMMs (generalized linear mixed models) with a binomial distribution and the logit link function. For the proportion of flowers setting fruit, we performed a binomial regression with the proportion of flowers setting fruit as the dependent variable, treatment as a fixed independent variable, and plant as a random independent variable. Similarly, we tested the proportion of flowers that produced viable fruit using a binomial regression with treatment as a fixed independent variable and plant as a random independent variable. If the tests showed a significant overall treatment effect, they were followed by post hoc Wald tests to determine which treatment levels were significantly different from each other. All analyses were performed using R (R Core Team 2013; v. 3.0.3 “Warm Puppy”).

Results

Influence of floral phenology and morphology on perforation type and prevalence

In 2014, florivory holes were more prevalent than nectar-robbing perforations, especially earlier in the season. However, in 2012, the opposite was true (see Tables 1, 2). In buds, florivory holes were much more prevalent than nectar-robbing holes (depending on the season, between 4 and 53 % of the flowers with florivory holes were buds compared to 0–12.2 % of flowers with nectar-robbing holes; Table 2).

Corolla dimensions had significant effects on the number of perforations per flower as seen in the data from 2012. Maximum corolla length was significantly correlated with the number of nectar-robbing perforations (negative binomial regression; $z = 1.99$, $P = 0.046$), which only appeared in flowers that were at least 6 mm long (Fig. 3a). On the other hand,

maximum width was related to the number of florivory perforations ($z = 5.03$, $P < 0.0001$), such that flowers of medium width had the highest numbers of florivory holes (Fig. 3b).

In 2012, plant identity had a significant effect on the number of both robbing ($z = 2.58$, $P = 0.01$) and florivory holes ($z = 3.72$, $P = 0.0002$).

Interactions between nectar robbers and florivores

In flowers that had at least two perforations, it was more likely to see a single kind of perforation, either florivory or nectar-robbing perforations, than to see both kinds (Table 3). This observation was made for all collections, although it was less obvious during the end of the flowering season in 2014.

Damage to reproductive parts and effect of perforations on fruiting success

Nectar-robbing perforations, for the most part, were not associated with damage to reproductive parts of the flower. Florivory holes, on the other hand, were highly associated with the presence of damage to reproductive parts (Table 1; Fig. 4).

Fruit production by intact flowers was not significantly higher than by flowers that had at least one hole (though holes were not distinguished by type). In 2012, on average, 40.3 ± 1.8 % of flowers set fruit on intact racemes, and 43.4 ± 4.6 % of flowers set fruit on racemes where every flower had holes (Wilcoxon rank-sum test, $z = 0.45$, $P = 0.65$); in 2013, 40.7 ± 1.8 % of flowers set fruit on intact racemes, while 41.8 ± 3.6 % of flowers set fruit on racemes with holes (Wilcoxon rank-sum test, $z = 0.13$, $P = 0.90$). Similarly, the fruits in the two categories (setting from either intact or flowers with holes) were not significantly distinguishable by weight (0.122 ± 0.005 and 0.122 ± 0.012 g/fruit for intact flowers and flowers with holes, respectively, for 2012, $t = 0.024$, $df = 462$, $P = 0.98$; 0.096 ± 0.004 and 0.107 ± 0.011 g/fruit for intact flowers and flowers with holes, respectively, for 2013, $t = 1.041$, $df = 636$, $P = 0.298$).

In the bagging study in 2014, we did not find a significant difference in the percentage of flowers setting fruit between intact flowers, flowers with florivory holes, or flowers with nectar-robbing perforations (38.9 ± 0.11 (1SE) for intact flowers; 73.3 ± 0.13 (1SE) for flowers with florivory holes; and 62.9 ± 0.08 (1SE) for flowers with nectar-robbing perforations; binomial regression, $\chi^2 = 4.11$, $df = 2$, $P = 0.13$ for treatment effect). However, there was a significant effect of hole presence on the percent of flowers with viable fruit such that flowers with nectar-robbing holes set fewer viable fruit than intact control flowers (27.8 ± 6.9 (1SE) for control intact flowers; 6.67 ± 7.5 (1SE) for flowers with florivory holes; and 2.9 ± 4.9 (1SE) for flowers with nectar-robbing perforations; binomial regression, $\chi^2 = 6.06$, $df = 2$, $P = 0.048$ for overall treatment effect; Wald test, $z = 2.25$, $P = 0.025$, for comparing nectar-robbing holes with intact flowers; Wald test, $z = 1.45$, $P = 0.15$, for comparing florivory holes with intact flowers; Wald test, $z = 0.61$, $P = 0.54$, for comparing florivory holes with nectar-robbing holes).

Discussion

Our data suggest that perforations found in corollas of manzanita flowers vary greatly in shape and position depending on their origin. Perforations caused by florivory were more often associated with further damage to reproductive parts of the flower, and could be located anywhere on the corollas. Nectar-robbing perforations, on the other hand, were usually limited to damage to the corollas and were located within a distinct range of distance from the base, in the vicinity of the nectaries. Furthermore, nectar-robbing perforations were found, for the most part, in flowers with long corollas (longer than 6 mm), suggesting that primary robbers might have mouthparts that are shorter than 6 mm long. Indeed, the main primary robbers in this system, members of the *Nomada*, *Augochlorella*, and *Vespula* genera (Richardson and Bronstein 2012) might have shorter tongues than other manzanita floral visitors. For example, some *Nomada* species (Apidae) have shorter tongues than most bees in this family (Armbruster and Guinn 1989; Moisset and Buchmann 2010). *Augochlorella* (Halictidae) belongs to a family that is known to possess shorter tongues (Moisset and Buchmann 2010). Nevertheless, this positive correlation between corolla length and nectar-robbing perforations was not found in a previous analysis of manzanita plant–insect interactions (Richardson 1999). Instead, only an association with corolla width, whereby narrower flowers were more associated with corolla perforations, was established. This apparent contradiction to our findings could be explained by the fact that Richardson (1999) did not distinguish between the different types of perforations.

Interestingly, the width of the corolla in our study was associated with the presence of florivores, which tended to prefer flowers of medium width. Larger flowers are sometimes more attractive to florivores, as in the case of *Cistus ladanifer* (Teixido et al. 2011). In the case of manzanita, the widest flowers tended to have a very different proportion than most—their width-to-length ratio was larger than one, whereas most manzanita flowers are narrower than they are long. All of these widest flowers, all of which were wider than they were long, contained either maggots or damage caused by these maggots (data not shown). The intermediate flower width, which seemed to be associated with florivores most, could therefore be an indication of unoccupied flowers (i.e., not already occupied by maggots) that are large enough to allow room for development, and possibly have more ovules and/or larger anthers for florivore consumption. For example, in *I. aggregata*, the size of the corolla is positively correlated with the number of ovules, which in turn affects oviposition choice by females of a seed predator, such that large flowers tended to have more eggs (Brody 1992). Similarly, in *Raphanus sativus*, there is a positive correlation between the size of the flower and the weight of anthers and number of pollen grains (Stanton and Preston 1988).

Plant identity was found to have significant effect on both florivory and nectar-robbing perforations. Width and length of corollas could have been affected by plant identity (Goodwillie et al. 2006), though our data do not provide this kind of information. Indeed, the effects of plant identity could have been due to environmental influences, such as location of the plant within the site, or physical attributes, such as the size of the plant at the time of the study. These aspects relating to the effects of plant identity on the presence of florivores and nectar robbers could be explored in future studies.

Buds were more likely to have florivory holes than nectar-robbing perforations. This phenomenon could be explained by the fact that florivores, most of which were caterpillars of various unidentified lepidopteran species, made the perforation in order to access the inside of the bud. Caterpillars could also access an open flower without damaging the petals, or they could enter through a nectar-robbing hole. Our results, however, indicate these happened rarely, as damage to reproductive parts of flowers with only nectar-robbing perforations was not common. When it did occur, it suggests that indeed nectar-robbing holes may serve as entrances to florivores, especially if entry occurred prior to the flower's opening, though we cannot confirm this based on our data. At the same time, it could be that nectar robbers avoided buds unless they were the most common flowering stage (as in the beginning of the flowering season, Fig. 4). There are very few studies that consider relationship between buds and nectar robbing. If indeed nectar robbers avoided buds, it could be explained by limited nectar production in buds as was found in other Ericaceae flowers (Navarro 2001) though this has not been studied in *A. pungens*.

Interestingly, with the exception of the end of the flowering period in 2014, very few flowers possessed both florivory and nectar-robbing perforations (Table 3). If florivory damage indeed affected visitation rates by pollinators in manzanita as it does in other systems (Botto-Mahan et al. 2011; Cardel and Koptur 2010; Cascante-Marin et al. 2009; Ferreira and Torezan-Silingardi 2013; Liao et al. 2013; Sober et al. 2010; Zangerl and Berenbaum 2009), and since in manzanita nectar robbers and pollinators are not necessarily systematically distinguishable (Richardson and Bronstein 2012), it is possible that nectar robbers could be deterred by florivory damage. Since the prevalence of floral perforation was much higher during the end of the flowering season, it is not surprising that it was that time when flowers were more likely to bear both kinds of perforations.

It is surprising that flowers with perforations had no significant reduction in fruit production, especially when considering the damage to reproductive parts normally seen in flowers with florivory holes. Flowers with perforated corollas still produced fruit at a similar rate to intact flowers, and this was the case in both observational and bagging studies. Upon closer inspection, however, we found that flowers with nectar-robbing perforations tended to produce more fruit with inviable seeds than did intact flowers. A similar trend was observed in the flowers with florivory holes, but, possibly due to a smaller sample size, this reduction was not statistically significant. It is important to note that we did not record the type of perforations in the flowers in the observational study, so the holes could be the result of either nectar robbing or florivory. The reduction in fruits with viable seeds in flowers with either type of perforation (though not a significant reduction in the case of florivory holes) suggests that we might also see a difference in apparent seed viability between fruits of intact flowers and those with perforations.

The fact that nectar-robbing damage affected reproductive success of flowers as much as, if not more than, florivory damage, suggests that indirect effects on pollinators may also play a role [though we cannot rule out possible direct effects of nectar robbing suggested by Irwin et al. (2010)]. Indirectly, nectar robbers may reduce reproductive success by reducing pollinator visits through reduced nectar volumes. Pollinators may spend less time on flowers that are robbed due to reduced nectar volume (Irwin et al. 2010). Either way, the outcome

would be similar in that the interaction would not result in successful pollination. Since self-fertilized flowers are more likely to produce lighter fruits (Richardson and Bronstein 2012), suggesting their inviability, we might assume that the majority of the fruit produced from damaged flowers, with their inviable seeds, were perhaps only partially crossed-fertilized, or completely self-fertilized.

Another indirect effect of nectar robbing on cross fertilization could be through damaged corollas. The question of whether pollinators prefer to consume nectar illicitly in the presence of nectar-robbing holes is currently being addressed with honey bees and preliminary results suggest that indeed these bees, when visiting legitimately, avoid perforated corollas (Barker et al. unpublished data). Honey bees may or may not be effective pollinators of manzanita, but currently they are the most abundant bees at our site and have been observed visiting flowers both legitimately and illicitly. Whether pollinators are deterred by damage to manzanita flowers is yet to be examined. In other systems, such as the butterfly pea, *Centrosema virginianum* (Fabaceae) and *Banisteriopsis* spp. (Malpighiaceae) floral damage caused by florivores indeed reduced pollinator visitations (Cardel and Koptur 2010; Ferreira and Torezan-Silingardi 2013). In the alpine herb, *Pedicularis gruin*a (Orobanchaceae), bees not only avoided flowers that were damaged by florivores, they also avoided the plant with the damaged flowers (Liao et al. 2013). Similarly, damage by nectar robbers to flowers of *I. aggregata* (Polemoniaceae) reduced pollinator visitation and, as a consequence, seed production (Irwin 2009).

Thus, although nectar-robbing perforations differ from those resulting from florivory in manzanita flowers in many different ways—location on the corolla, damage to the reproductive organs, prevalence in open flowers, etc.—the resulting effect seems to be similar. Although no reduction in total fruit production is evident, there is reduction in the viability of the seeds of these fruits (although not statistically significant in the case of florivory holes). Whether or not these reductions in viable seeds translate into reduced reproductive success of manzanita plants—robust perennial shrubs, each with many inflorescences and many flowers in each raceme—is yet to be determined.

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Fig. 1. Floral perforations in pointleaf manzanita. **a** Nectar-robbing perforations. Note the “flap” in both holes and position on the corolla. **b** An extreme case of florivory perforation, including damage to reproductive parts (*arrow* pointing to hole)

Fig. 2.

Relative location of hole from the base of the flower. Holes are sequentially arranged along the x -axis (from 1 to n). *Circles* represent florivory holes. *Triangles* represent perforations that possessed a flap and are considered as nectar-robbing holes. Nectar-robbing holes are more likely to be at a distance from the base that corresponded with the location of nectaries. This distance (0.345 ± 0.006 mm, mean $\pm$ SEM) is significantly smaller than the average distance of florivory holes (0.424 ± 0.007 mm, mean $\pm$ SEM) from the base ($\chi^2 = 26.72$, 1 df , $P < 0.0001$). The variation in position of robbing holes is also much lower than that of florivory holes (0.068 and 0.18 SD for robbing and florivory holes, respectively; O'Brien [0.5] $F = 93.27$, 1 df , $P < 0.0001$), which are not likely to be found anywhere specific

Fig. 3. Number (*bars*) and percentage (*line*) of perforations as a function of corolla dimensions. **a** Number of nectar-robbing holes with relation to corolla length; **b** number of florivory holes with relation to corolla maximum width. *Gray bars* represent flowers with perforations, and *white bars* represent intact flowers

Fig. 4. Portion of flowers whose reproductive parts are damaged (*hatched bars*) out of total number of perforated flowers at different points throughout the flowering season: 2012 peak season (*white*); beginning of season in 2014 (*light gray*); peak of season in 2014 (*dark gray*); end of season in 2014 (*black*)

Table 1

Damage to reproductive parts is significantly more associated with florivory holes than with nectar-robbing holes

Time during the flowering period	Total number of flowers	Number of flowers with holes (%)	Flowers with florivory holes	Flowers with robbing holes	Damaged flowers with florivory holes	Damaged flowers with robbing holes	Fisher's exact test <i>P</i> value (two-tailed)
Peak 2012	1719	406 (23.6)	145 (8.4)	275 (16.0)	74	23	$P < 0.0001$
Beginning 2014	710	290 (40.8)	215 (30.2)	98 (13.8)	148	3	$P < 0.0001$
Peak 2014	674	289 (42.9)	236 (35.0)	59 (8.7)	188	4	$P < 0.0001$
End 2014	474	325 (68.6)	215 (45.3)	179 (37.8)	93	6	$P < 0.0001$

Table 2

Floral buds with perforations are associated with florivory more than with nectar robbing

Time during the flowering period	Buds with florivory holes (% ^a)	Buds with robbing holes (% ^b)	Fisher's exact test <i>P</i> value (2-tailed)
Peak 2012	48 (33.1)	2 (0.7)	<i>P</i> < 0.0001
Beginning 2014	114 (53.0)	12 (12.2)	<i>P</i> < 0.0001
Peak 2014	42 (17.8)	0 (0)	<i>P</i> < 0.0001
End 2014	10 (4.7)	5 (2.8)	<i>P</i> = 0.79

^aPercent of buds with florivory holes of all flowers with florivory holes (Table 1, column 4)

^bPercent of buds with nectar-robbing holes of all flowers with nectar-robbing holes (Table 1, column 5)

Table 3

Likelihood of flowers with at least two perforations to have both florivory and nectar-robbing holes is smaller than their likelihood of having only one type of holes

Time during the flowering period	Number of flowers with at least two holes	Flowers with only one kind of holes	Flowers with both kinds of holes	Chi-square value and <i>P</i> value (2-tailed)
Peak 2012	157	112	45	$\chi^2 = 21.8$; $P < 0.0001$
Beginning 2014	94	70	24	$\chi^2 = 23.3$; $P < 0.0001$
Peak 2014	60	54	6	$\chi^2 = 39.4$; $P < 0.0001$
End 2014	168	98	70	$\chi^2 = 4.4$; $P = 0.0367$