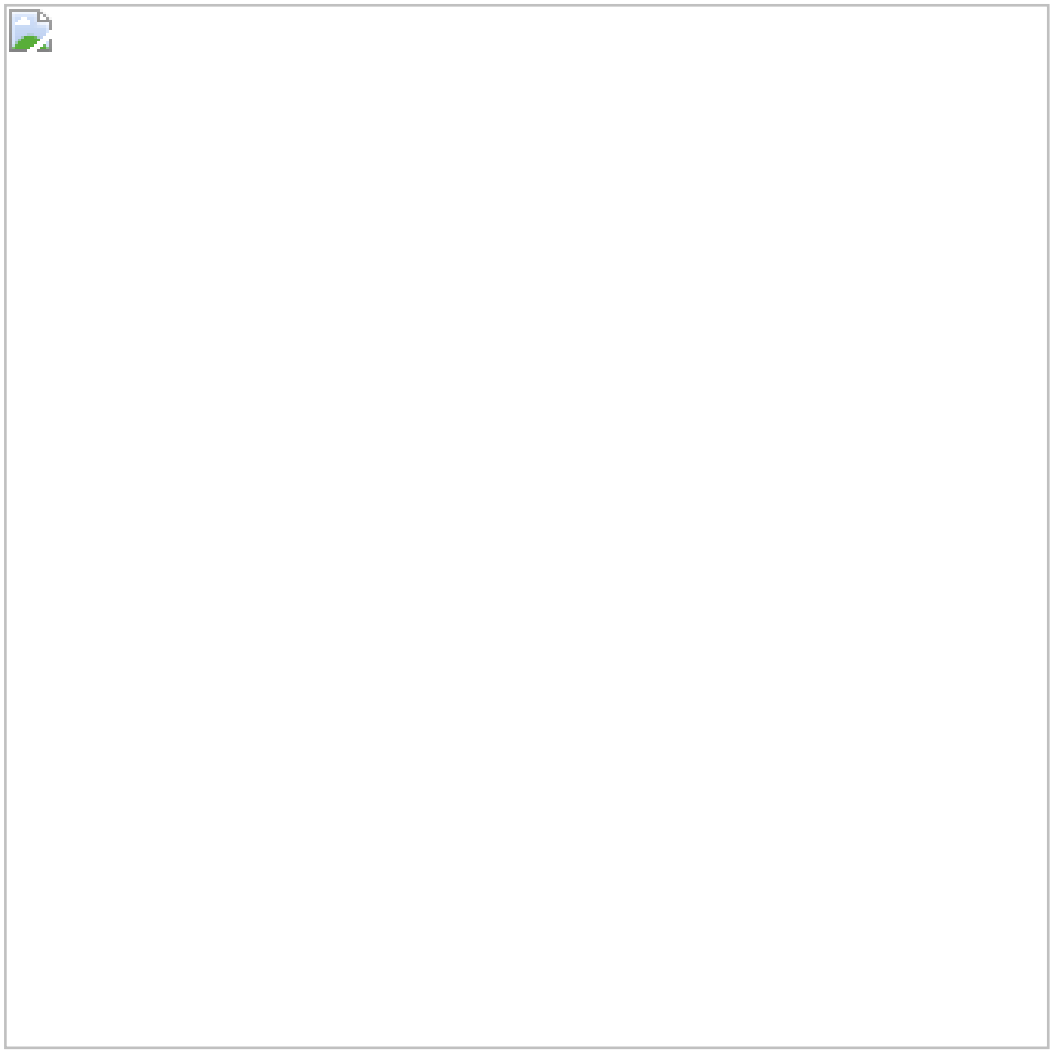

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ORIGINAL RESEARCH

Nectar for Plant Defense: the Feeding of the Non-Native Coccinellid Beetle, *Curinus coeruleus*, on Extra-Floral Nectaries of Hawaiian Native *Hibiscus Brackenridgei*

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Abstract: The interaction between the non-native coccinellid beetle, *Curinus coeruleus* Mulsant, and the Hawaiian native plant *Hibiscus brackenridgei* A. Gray, was investigated on Kauai, HI. The presence of extra floral nectar appears to maintain the beetle presence on the plant. Because coccinellid beetles are predators on insects that are damaging to plants, beetle presence may increase plant fitness. Beetles were found feeding heavily on the extra floral nectaries of the *Hibiscus*. An examination of the beetle mouth parts with scanning electron microscopy revealed no structures specifically adapted for the consumption of nectar. The sensory ability of the coccinellids was tested to determine if they respond to visual or olfactory cues to detect the nectar. Studies with an eight-armed air-flow olfactometer concluded there was no olfactory cue. Tracing the pathways of beetles in laboratory experiments yielded results that suggest a visual cue. The extra floral nectaries are concluded to be a potential mechanism to maintain beetle presence on a plant to provide defense against herbivores.

Keywords: extra-floral nectaries, coccinellids, Malvaceae, insect-plant interactions, Hawaii, nectar

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Introduction

Extra-floral nectaries are effective in attracting predators that reduce herbivory, resulting in increased fitness for the plant.[1] Although the majority of studies of extra-floral nectaries look at the association of ants and extra-floral nectaries,[2] there are extra floral nectaries on plants not associated with ants.[3] Lady beetles, while primarily predators on invertebrates, are found on extra-floral nectaries. Pemberton and Vandenberg[4] reported a summary from the literature of 41 species in 19 genera, representing five of the six coccinellid subfamilies, observed feeding on the extra-floral nectaries of 32 plant species in 15 families. Nectar functions mainly as an attractant for pollinators, and therefore is usually produced inside the flower of the plant. But extra-floral nectaries are glands that are found outside of the reproductive parts of a plant, usually on leaves.[5] Extra-floral nectaries may function as a defense mechanism to attract insect or avian predators of herbivores.[6,7] Around 93 plant families throughout the world are known to have extra-floral nectaries[4] and are more common on plants in warm climates.[8]

The common examples of interactions between insects and extra-floral nectaries illustrate mutualisms that are the result of the co-evolutionary relationship between the species.[2,4] However, mutualistic interactions are also observed between native and non-native species. Although non-native plant species are usually detrimental to the survival of native plant species,[9] non-native insects can, in some cases, successfully form mutualistic interactions with native plants.[10–12] In the summer of 2004 at the National Tropical Botanical Gardens (NTBG) on Kauai, *Curinus coeruleus* Mulsant (Coleoptera: Coccinellidae) beetles, which are not native to Hawaii, were found in abundance feeding on the extra-floral nectaries of the native *Hibiscus brackenridgei* A. Gray (Malvaceae). *Curinus coeruleus* was not among the beetles listed previously as common plant extra-floral nectary feeders.[4]

C. coeruleus, an Australian lady beetle, was introduced to the Hawaiian Islands to control plant pests, particularly those on commercial crop plants.[13] These pests continue to infest the Kauai native and introduced flora, making the lady beetles desirable biocontrol agents.[14] One of the most common pests is a spider mite that is a concern for the plant species of Kauai.[15] Mites suck plant juices, and where they bite, infection enters the plant, often resulting in the death of the plant.[16] Pathogens can have major effects on both the survival and reproductive success of a plant.[17] The lady beetle family is often used in controlling mite populations, their effectiveness having been estimated at each beetle eating an average of 2400 spider mites during its lifespan.[15] Thus, the maintenance of lady beetle populations on plants is highly desirable.

Currently mechanisms of attraction for coccinellids to extra-floral nectaries and the feeding habits of the coccinellids on the extra-floral nectaries are not known. The nectar from the extra-floral nectaries may play a role in attracting and maintaining populations of coccinellids that also consume the plant's herbivores. The coccinellid beetle has been described as a "blundering idiot" that encounters food sources by unguided roaming behaviors.[18] However, other studies report that coccinellid beetles use specific scent cues to find food sources.[19–21] Understanding the mechanisms of attraction to extra-floral nectaries and the feeding habits of coccinellids on these alternate food sources is important to understanding this facultatively mutualistic interaction between a native and non-native species.

The objective of this study was to document the interaction between the lady beetle and *H. brackenridgei,* and to better understand the mechanisms of attraction to the extrafloral nectary and the feeding behavior of the beetles. The following research questions were addressed: 1) What are the feeding habits, including length of time and amount of nectar consumed, of *C. coeruleus* on *H. brackenridgei* extra-floral nectaries? 2) How does *C. coeruleus* find the extra-floral nectaries, are they "blundering idiots" or are they following visual or scent cues? 3) How does the morphology of the mouthparts of *C. coerulus* and the *H. brackenridei* extra-floral nectaries impact this species interaction? 4) Is the nectar of the *H. brackenridei* a potential food source for *C. coeruleus*?

Methods and Materials

Study species and study site

Hibiscus brackenridgei is native to Hawaii. It is a shrub or small tree with solitary yellow flowers that open in the afternoon and are one-day flowers.[22] The leaves are 5–15 cm long and are deeply lobed.

The flowers are 4–8 cm long, monoecious, and often have a maroon spot at the base of the petals.[23] Known as "Ma'o hau hele", it is the state flower of Hawaii. It is rare and found mostly in dry forest and shrub lands at 130–800 m elevation and is found on all the Hawaiian Islands except Ni'ihau and Kaho'olawe.[23] Like many Malvaceae species, *H. brackenridgei* possesses extra-floral nectaries which are located on the dorsal sides of the leaves, at the base of the mid-vein.[24,25]

Curinus coeruleus, an Australian lady beetle, was introduced to the Hawaiian Islands around 1895 by the entomologist Albert Koeble.[26,27] Koeble introduced many species of Coccinellidae to Hawaii to control the detrimental plant pests such as plant lice, scale insects, mealybugs, mites, and other insects that damage commercially important plants.

This study was conducted at the National Tropical Botanical Garden (NTBG) on the tropical island of Kauai, Hawaii. The coccinellid beetles were collected from the native *Hibiscus* species, labeled *H. brackenridgei* at the NTBG outdoor nursery. The 32 plants of *H. brackenridgei* had extra-floral nectaries on the mid-vein on the dorsal side of the leaves, and the plants were not flowering at the time of the study. No observable mite damage or mite presence could be seen on any of the plants. The plants were watered and placed in direct sunlight, but not receiving any pesticide treatment. Observations and experiments took place between 1 June 2004 and 15 July 2004. Environmental Scanning Electron Microscope (ESEM) images of beetles were collected in August 2004. Vouchers of both the *Hibiscus* plant species and the *C. coeruleus* beetle were collected and deposited with the Brigham Young University Bean Museum.

Insect observations

To document the feeding habits of *C. coeruleus* beetles, individual *C. coeruleus* beetles that were timed to see how long they spent feeding on *H. brackenridgei* extra-floral nectaries either in a petri dish or in the field. We conducted hourly counts of the number of beetles on extra-floral nectaries and the number of beetles cruising on the leaves of the *H. brackenridgei.* Individual beetles were clocked for four hours at a time, to determine the amount of time a beetle spent on an extra-floral nectary. Beetles designated as "on" were feeding on extra-floral nectaries. Beetles designated as "off" were roaming over the leaf surfaces, not feeding on the extra floral nectaries. Beetles that rested in folds of leaves were not counted as feeding or roaming.

We measured the feeding time under laboratory conditions by placing a coccinellid beetle in a petri dish with a sugar droplet. The sugar droplet was replenished as needed. Finally, coccinellid beetles were starved for 24 hours, weighed using a Fisher Scientific A-200D scale, and then fed sugar water consisting of droplets of sucrose, glucose, and fructose at concentrations of 0.5 to 3.0 ppm. The amount of time spent feeding, the amount of sugar water replenished, and the number of fecal pellets excreted by the beetle were recorded. The beetles were then weighed again at the end of the feeding to record weight gain.

Olfactometer studies

Olfactometer studies were conducted to determine if the coccinellids were finding the extra-floral nectaries from scent cues. The olfactometer experiment protocols and the olfactometer design were similar to those reported by Hamilton et al.[20] Testing took place between 9 June 2004 and 29 June 2004 at the NTBG field lab. The olfactometer had eight arms each terminating in a scent chamber with a recessed exposure chamber piston in the middle. A vacuum pump and oxygen flow meter pulled air through the olfactometer during each test run. For each run, one arm was randomly assigned to hold the treatment in its scent chamber and that arm was then designated the active arm. The four treatments were droplets of sucrose, glucose, and fructose at concentrations of 0.5 to 3.0 ppm and *H. brackenridgei* nectaries. Each treatment was replicated five times. The olfactometer was oriented with arm one to the north, and with the same light exposure on each run. Runs were conducted at mid mornings and afternoons when the beetles had been observed to be active.

Prior to each run, the upper part of each arm of the olfactometer was coated with vaseline to prevent the beetles from climbing the sides. Five active, adult beetles that had been field collected from the experiment site on *H. brackenridgei* and starved for 24 hours were placed in the exposure chamber. Adult beetles are defined as those beetles with fully developed elytra. The acrylic lid, lined with vaseline to create an airtight seal, was placed on top. The exposure chamber piston was raised to release the beetles into

the olfactometer. A run lasted 45 minutes. The number of entrances to each arm was recorded, as well as the final number of beetles in each chamber at the end of the run. A beetle that exited an arm, and then re-entered it past the halfway mark of the arm was counted as another entrance to that arm. Each beetle was used only once to prevent any learning bias. The beetles were returned to the host plants after the run. After each run, the olfactometer was dismantled, washed with warm soapy water, and air-dried.

Tracing experiments

In order to examine if the way beetles locate nutrition sources has a visual cue, tracing experiments were conducted. Coccinellids were collected from the study site and starved for 24 hours. After the experiments, the beetles were returned to the *H. brackenridgei* populations. Tracing experiments consisted of recording the search pattern of coccinellid beetles and the amount of time to contact an odor source. Protocols as described in Acar et al[21] were used. A filter paper (9 cm diameter) was inserted on the inside of a petri dish and the stimulus placed at the center of the filter paper. Fourteen stimulus sources were tested on beetles that had been collected randomly from both *Hibiscus* species. The fourteen stimuli were: droplets of sucrose, glucose, and fructose at concentrations of 0.5 to 3.0 ppm. at 10 µl and 50 µl, 50 µl droplets of sucrose, glucose and fructose on a 1 cm diameter piece of *H. brackenridgei* leaf tissue, *H. brackenridgei* nectaries, *H. brackenridgei* vein tissue, a water droplet on a 1 cm in diameter piece of *H. brackenridgei* leaf tissue, and spider mites. Once the stimulus was in place a randomly selected adult beetle that had been starved for 24 hours was released at the inside edge of the petri dish. The dish was covered and a transparency placed over the top. The path the beetle followed to the stimulus was traced on the transparency, and the length of time it took the beetle to find the stimulus recorded. Beetles that took longer than 6 min were recorded as "not found". The category of mites as a visual feeding cue was removed from the analysis data set due to low trial runs and confounding biological variables. The *C. coeruleus* beetles did not exhibit any response in the one sample of mites presented to them, most likely because the mites were adults, not larvae.

ESEM Studies

An Environmental Scanning Electron Microscope (ESEM) was used to study the *C. coeruleus* morphology to determine if and how the structures of the coccinellid beetle mouthparts relate to the structure of the extra-floral nectary of the *H. brackenridgei* species. *H. brackenridgei* leaves were stored in 70% EtOH for transport from Kauai. The extra-floral nectaries were dissected and brought through an EtOH series to 100% EtOH. They were then subjected to three repetitions of acetone wash and Critical Point dried.[28] The nectaries were mounted on stubs and gold-coated. All ESEM observations took place under low vacuum conditions. The nectaries were examined for any evidence of beetle presence such as bite marks. They were examined for presence of nectar and sooty fungus. The beetles collected at the field sites were placed in ethyl acetate activated killing jars and then placed in vials of 70% EtOH for transport. The beetles were air-dried, mounted on a stub and gold coated. All ESEM observations were at low vacuum conditions. The mouth parts of the beetles were examined for nectary drinking morphology, dried nectar, and presence of sooty fungus.

Collection and gas chromatography and mass spectrometry (GC-MS) of nectar

For nectar collection, the extra floral nectaries were bagged with netting and tagged. Twenty-four hours later a 10 µl capillary tube were used to collect liquid from the nectary. The total volume of nectar produced was recorded. Using a hand-held Belingham and Stanley (0%–50%) refractometer, the Brix reading of total percent sugars were determined. The nectar was then transferred to filter paper for later analysis and frozen to prevent fungus contamination.

To determine the type of sugars present an Agilent 6890 GC equipped with a Agilent MSD 5973 detector with an electron impact mode of 70 eV was used. To prepare the samples for the GC, the dried nectar on filter paper was first cut out and placed in a test tube. Three ml of 70% EtOH were added and the tube was sonicated for 3–5 minutes to leach out and dissolve all sugars present. The solution and filter paper were then placed in a syringe and filtered into a small 10 ml test tube. The solution was evaporated to 1.0 ml with

nitrogen. This remaining solution was pipetted into vials and dried.

The GC-MS was equipped with a column of HP-1, 25 m × 0.32 mm and a 0.17 um film thickness. The initial temperature was 145 C and initial time of 1.00 min. Temperature ramps were 1 C to 170 C, then 15 C to 190 C, and finally 5 C to 270 C for two minutes. The split injection volume was 1 µl, the inlet temperature was 250 C, and the MSD transfer line heater was 280 C. Soluble carbohydrates were identified using a mass spectrum Wiley 275 library. For sylilation the dried sample was dissolved in 50 µl of DFM (Dimethyl Formamide) and derivalized in 50 µl BSTFA + 1% TMCS in a ventilation oven at 75 C for 15 minutes.

Numerous attempts were made to collect the nectar from the extra-floral nectaries. The sample size is only n = 2 due to several complications. The first problem was the persistence of the beetles, despite the netting. Second, heavy rains knocked off the netting or caused it to press against the nectary, absorbing any nectar.

Data analysis

The statistical analysis of olfactometer data began with a natural logarithmic transformation of the number of entrances the beetles made into each arm to equalize the variances. The data were then analyzed using a mixed model analysis of variance (ANOVA) based on an unbalanced split plot design, with runs as whole units and treatment chambers as subunits.[20] Observations showed that the beetles had a preference for entering certain arms, whether they were active or inactive. Terms were included in the model to account for this. Data were transformed back to counts for reporting.

To determine if the beetles showed a visual preference in the tracing experiments, we used an analysis of variance (ANOVA) to compare all the treatment groups.[29] The treatment groups were compared individually, and then collapsed and compared as treatments with color and treatments without color.

Results

Insect observations

Counts of beetles on and off the nectaries on each *H. brackenridgei* plant were pooled for each time of day for four days (June 17, June 23, June 29, June 21). Both plant species were not blooming, were similar in height and leaf size, have the same chemical composition of nectar and had beetles on every leaf. The mean number of beetles per plant was plotted as a function of time of day (Fig. 1). Across all time periods the mean number of beetles on the nectaries was 3.78 ± 3.08, and the mean of the number of beetles roaming the leaves was 4.38 ± 3.14.

In both the wild and lab conditions, the beetles exhibited similar feeding times on the extra-floral nectaries. The average time spent by a beetle (n = 10) on an extra-floral nectary was 139.7 ± 4.9 minutes, and 9.1 ± 1.9 minutes off the nectary roaming the leaf. Average feeding time of a beetle (n = 10) in the lab on a sugar based source was 174.4 ± 8.9 minutes, with 32.4 ± 4.2 minutes off the sugar source. Beetle weights after feeding increased over 50% (Table 1).

Olfactometer studies

Olfactometer tests (n = 248 beetles) showed that *C. coeruleus* beetles did not use olfactory cues to locate a sugar source. The mixed model analysis showed no significant differences in beetle preference for an active arm of any of the treatments. A test of fixed effects showed that the beetles had a preference for arms four, five, and six ($F = 9.13$; $df = 7, 231$; $P = 0.0001$), regardless of the treatment in the arm. Therefore, terms were added to the model to incorporate the bias. There was no significant difference in the mean number of beetle entrances into the active vs. the inactive arms for each treatement, or when comparing the active arms of the four treatments (Table 2).

Tracing experiments

The tracing experiments indicated that *C. coeruleus* beetles do use visual cues to locate a nutrition source (Table 3). No difference was seen between the treatment groups individually. When treatment groups without color were collapsed and compared to those with treatments that had color, such as sugar solution on a leaf or a nectary, we get a significant difference ($F = 19.5$; $df = 1,11$; $P = 0.002$) (Figs. 2 and 3).

ESEM studies

The ESEM showed the extra-floral nectaries of *H. brackenridgei* were heavily tomentose, and had

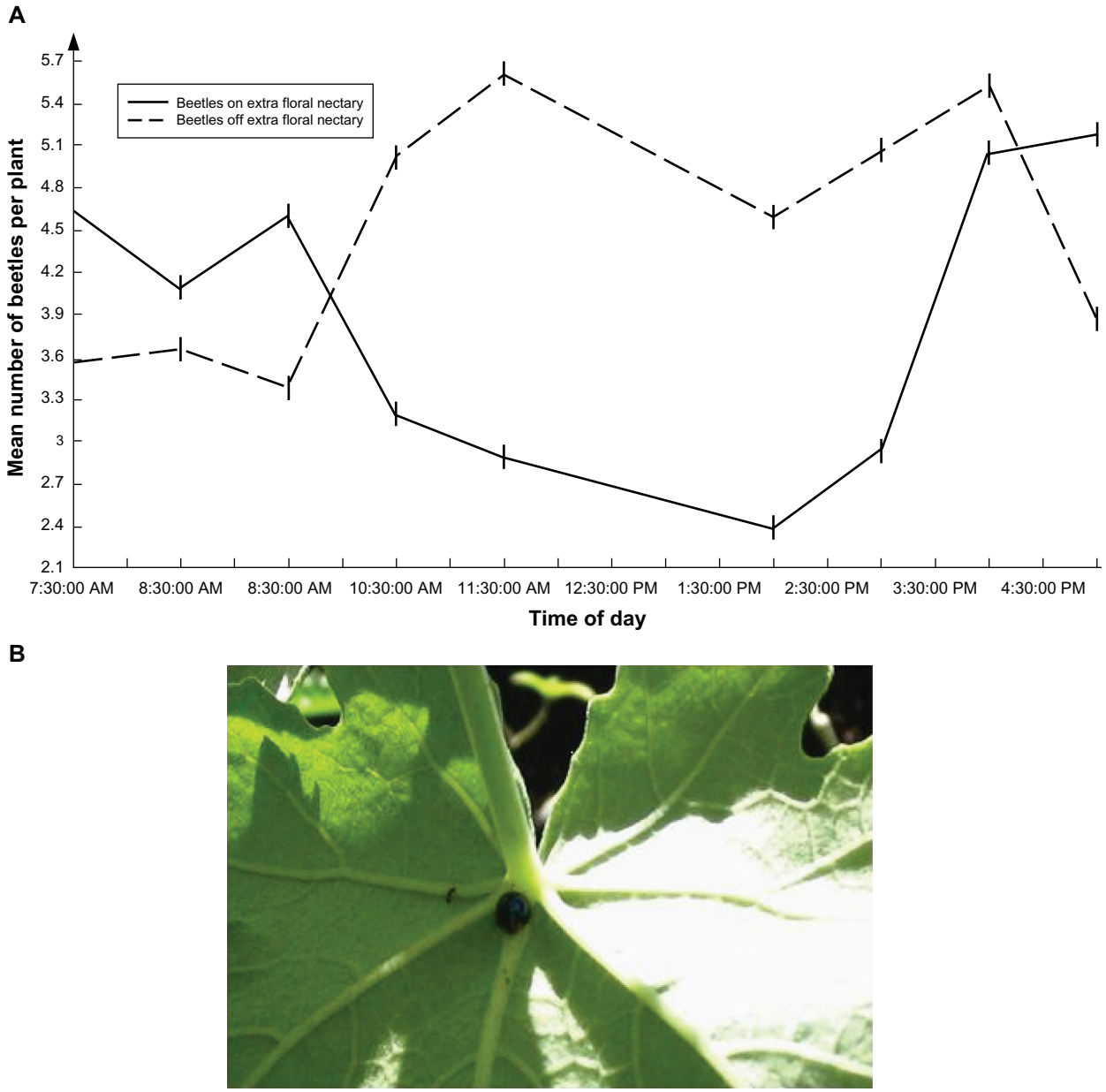

Figure 1. A) Mean number of *Curinus coeruleus* observed on or off extra-floral nectaries of *Hibiscus brackenridgei* at study site. **B**) *Curinus coeruleus* beetle on a *Hibiscus brackenridgei* extra-floral nectary.

dried nectar around the entrance of the nectaries (Fig. 4A). Sooty-fungus was seen growing on the outside of the nectaries, presumably on the nectar sugars (Fig. 4B). The fungus was not dense, and did not obscure the nectary in any way. The fungus was not seen elsewhere on the plant or detected in the opening of the extra-floral nectary. Mouthparts of the *C. coeruleus* beetle are mandibulate which are typical for predaceous beetles; hence, there was no apparent morphological structure for sucking the nectar

Table 1. *Curinus coeruleus* weight before and after feeding on sugar water (n = 2 beetles), and total time coccinellid beetles spent feeding.

Beetle (n)	Pre-weight	Final weight	Weight gain	% Gain	Total time
1	0.009 g	0.014g	0.005 g	56.00%	3 hrs 53 mins 14 sec
2	0.011 g	0.017g	0.006 g	54.50%	3 hrs 37 mins 50 sec

Table 2. Response of *Curinus coeruleus* exposed to five treatments using an eight-armed olfactometer (n = 248).

Treatment	Mean of beetle entrances into active arms†	95% CI for mean no. of entrances into active arm		Mean of beetle entrances into nonactive arms‡	95% CI for mean no. of entrances nonactive arms		*P*-values*
		Lower limit	Upper limit		Lower limit	Upper limit	
Fructose (50 µl)	3.65	2.04	6.54	3.77	3.03	4.69	0.78
Glucose (50 µl)	5.07	2.73	9.41	4.08	3.23	5.15	0.52
Sucrose (50 µl)	5.8	3.25	10.35	5.32	4.28	6.61	0.92
H. brackenridgii	3.02	1.33	6.84	2.95	2.17	4.02	0.91

Notes: †Active arm (1) refers to the olfactometer arm in which the treatment was placed; ‡Inactive arm (7) refers to the olfactometer arms that were left empty during each treatment; *No significant difference means of active and nonactive arms, or between the active arms of the four treatments.

Table 3. Percentage of *Curinus coeruleus* that located a treatment source in tracing experiments.

Treatment	n	Percentage of beetles that found source
Sucrose (50 µl) on leaf	15	73.33%
Sucrose (50 µl)	10	50.00%
Sucrose (10 µl)	5	0.00%
Glucose (50 µl) on a leaf	15	46.67%
Glucose (50 µl)	10	50.00%
Glucose (10 µl)	5	0.00%
Fructose (50 µl) on a leaf	15	46.67%
Fructose (50 µl)	10	30.00%
Fructose (10 µl)	5	40.00%
Vein tissue	9	55.56%
H. brackenridgei nectary	15	60.00%
Water on *H. brackenridgei* tissue	10	70.00%

(Fig. 4C). No bite marks or evidence of chewing by the beetles was seen on the extra-floral nectaries or surrounding tissue. Dried nectar was visible on the beetle (Fig. 4D).

Collection and GC-MS of nectar

The nectar from the extra-floral nectaries of *H. brackenridgei* showed the same four main sugars. A 1 µl volume nectar sample has 24.45% fructose, 15.90% mannose, 22.23% glucose, 26.0% sucrose, and .91% maltose. Due to low sample size, these results are only an indication that sugars are present and similar. Amino acids were detected as well, but more testing is needed to confirm the number and amount. Further testing is needed to determine replenishment rates, composition, and production levels of the extra-floral nectaries.

Discussion

This study uses an example of a potential facultative mutualism between the native plant *H. brackenridgei*

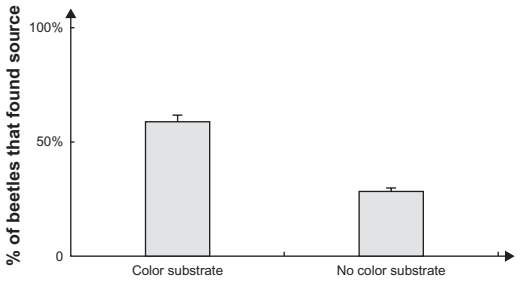

Figure 2. Comparison of percentage of *Curinus coeruleus* that found the source treatments with color (58.7% ± 11.3%) and those treatments without color (30% ± 7.1%) in the tracing experiments.

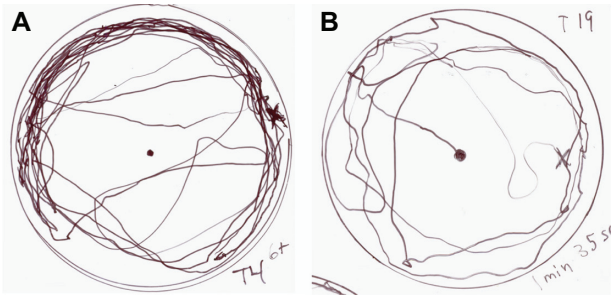

Figure 3. A sample of tracing patterns on the transparencies from the tracing experiments with *Curinus coerulus*. **A**) Beetle tracing pattern when treatment had no color component. **B**) Beetle tracing pattern when treatment source had a color component.

and the lady beetle, *C. coeruleus,* a non-native insect to examine the feeding behaviors of lady beetles. In this plant-beetle interaction, *H. brackenridgei* is likely protected from herbivores, and *C. coeruleus* receives a non-protein source of nutrition from the extra-floral nectar to supplement the traditional coccinellid diet. The feeding on non-protein sources of nutrition is not a new development since 35-million year old fossil impressions of extra-floral nectaries and coccinellid beetles have been found.[30]

Ladybeetles are what Bentley[31] charmingly called the "pugnacious bodyguards" that benefit plants by consuming herbivores. Measurements of *C. coeruleus* species prey consumption rates were not comprehensively documented in this study, however *C. coeruleus* predatory habits have been measured for many pests, showing it to be an effective predator of plant

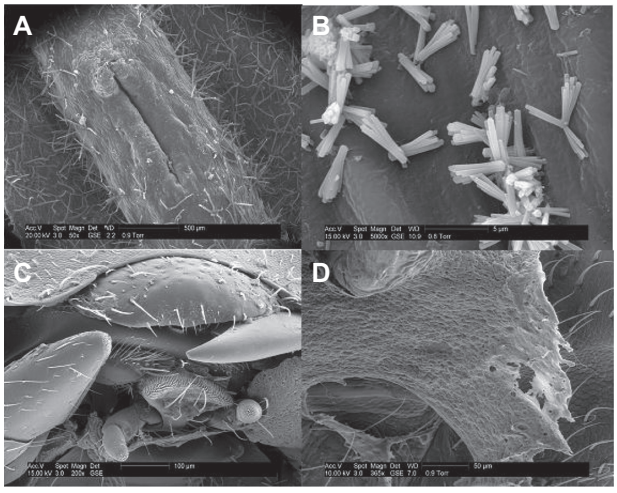

Figure 4. ESEM Micrographs of *Hibiscus brackenridgei* and *Curinus coeruleus*. **A**) *H. brackenridgei* extra-floral nectary. **B**) Sooty fungus growing on *H. brackenridgei* extra-floral nectary. **C**) Mandibulate mouth parts of *C. coeruleus* beetle. **D**) *H. brackenridgei* extra-floral nectar dried on *C. coeruleus* beetle.

herbivores such as plant lice, psyllids, and mites.[32–36] Studies have found that plants with extra-floral nectaries do have reduced insect herbivory.[8]

Our results showed a significantly higher percentage of beetles on the nectaries in the morning and late afternoon (Fig. 1). Beetle roaming behavior was prevalent mid-day. This could indicate that during the mid-day, *C. coeruleus* is more often roaming for prey, and nectary feeding is a secondary feeding preference. Roaming behavior was consistent even in the absence of mites or other arthropods which suggests that this is not beetle behavior induced by the presence of prey. This roaming behavior may be triggered by temperature or light exposure, and needs further study. Beetles were observed chasing away other beetles that attempted to feed on the nectary they were on. Aggressive and territorial behavior by *C. coeruleus* beetles on extra-floral nectaries has not been documented and needs further study.

Feeding on herbivorous arthropods and extra-floral nectar may not be the only benefit to the coccinellid beetles. Our observations also noted beetles not feeding or roaming, but resting in the curls of the *Hibiscus* leaves. Refuge is another possible reason for beetles' presence on a plant.[37]

Pemberton and Vandenberg[4] report the feeding episodes of ladybeetles on sugar based sources as brief, however we found that the average feeding time of a beetle on a sugar based source was between two and three hours. The time a beetle spent feeding on a nectary was consistent both under lab conditions and as observed on the plants at the study site. Beetle weights after feeding increased over 50% after consumption episodes (Table 1). Apparently, beetles will consume nectar as a main food source in the absence of prey.

Nectar in extra-floral nectaries is produced from photosynthesis in the parenchyma and not from stored starch.[6] Nectar of the *H. brackenridgei* extra-floral nectaries has both carbohydrates and amino acids. Our GC-MS analysis of the *H. brackenridgei* extra-floral nectar found four main sugars, fructose, mannose, glucose, and sucrose, as well as amino acids. The presence of both amino acids and carbohydrates in the nectar gives evidence of the *H. brackenridgei* nectar's nutritional value to beetles,[38] however our results are preliminary and further testing is needed.

The ESEM studies did not show any special mouth part for the consumption of nectar (Fig. 4C), which is expected for a beetle that is primarily a predator. However, the *C. coeruleus* beetles fed consistently on the *H. brackenridgei* extra-floral nectaries. The beetle would rest on the nectar droplet with its head fully in contact with, even buried in, the liquid (Fig. 4D). It may be that the beetles draw liquid into their mandibulate mouthparts by capillary action or by rapid movement of the maxillary palpi. The feeding on extra-floral nectaries of the *H. brackenridgei* by the *C. coeruleus* showed no damage, such as bite marks, to the plant.

The sensory ability of coccinellids to detect both prey and non-prey nutrition sources by visual or olfactory cues is equivocal. Once referred to as "blundering idiots",[18] lady beetles of varying species have been shown in repeated studies to find their prey by olfactory stimuli.[19–21] Our study looked at how lady beetles detect nectar and other non-prey sources of nutrition, specifically the nectar of extra-floral nectaries. Non-prey related odors have been found to attract coccinellids. Hamilton et al[20] also found that the ladybeetle *Hippodamia convergens* was significantly attracted to the odor of radish leaves without the presence of prey on them. Earlier work by Kesten[39] found that the ladybeetle *Anatis ocellata* L. was attracted to the scent of pine needles. Although sugar is not the main diet of the predatory lady beetle, some studies have reported an olfactory attraction to carbohydrates by coccinellids. For instance, Shands et al[40] conducted olfactometer experiments with *C. septempuncutata* and found they were attracted to strawberry Jello. Additionally, artificial honeydews have been sprayed on agricultural crops to attract coccinellids.[41] Our olfactometer experiments showed that *C. coeruleus* ladybeetles do not sense non-protein or non-prey sources by olfaction (Table 3). All the sources in the active olfactometer arms, both the sugar water and plant nectaries, had no effect on beetle behavior. Our findings agree with the work of Da Silva[42] for *C. coeruleus* in that he found that the lady beetles did not detect their prey through olfactory stimulation, but rather visually or by physical contact.

Many beetles use visual cues for finding flowers and plants in general.[37,43] Other studies have shown that visual cues play a crucial role in prey detection by lady beetles.[20,44–46] In this study, we found that *C. coeruleus* beetles responded to visual cues for non-prey nutrition sources. The tracing experiments suggest that the beetles find their nutrition source, in this case nectar or sugar water, visually. The nectar forms a clear bubble on the green nectary. When the source was only a clear droplet, the beetles rarely, if ever found the source. However, a significant difference ($P = 0.002$) could be seen in the higher percentage of beetles that found color, in this case the green of the nectary, based sources compared to non color sources (see Fig. 2).

The tracing data also showed no discernable pattern in the *C. coeruleus* finding or roaming behavior (Fig. 3). Beetles tended to circle the edge of the petri dish, apparently seeking a way out. Only when oriented towards the source did they move to the source. Once on the food source, beetles stayed and fed for extended periods of time. Lady beetles do drink water from the leaves of plants in the wild,[4] and this may be the attraction to the nectar. There may be no specific mechanism for finding nectar, and like water, it is simply happened upon during roaming behavior. Because nectar is a suspected secondary nutrition source, the expectation is that *C. coeruleus* do not possess an evolved sensory mechanism for nectar.

The ESEM studies of the extra-floral nectaries showed the presence of sooty fungus growing around the edges of the nectary (Fig. 4B). This is not unexpected because of the presence of the sugary nectar. The presence of sooty fungus on nectaries may be a visual or olfactory attractant to the sugar secretion.[8] Our study showed no observable interaction between the coccinellids and the fungus; however, it has been suggested that mites eat fungus.[47] This is expected, since nectar is likely to be exploited by several different prey items. In his study of *Gossypium thurberi*, Keeler[48] suggests that the diversity of insects attracted to extra floral nectaries indicates a complex series of interactions that are not well understood, including the role of extra-floral nectaries in terrestrial food webs. The possibility of a complex interaction between sooty fungus, mites, coccinellids, and extra-floral nectary needs further study.

What are the historical insect interactors with *H. brackenridgei*? Extra floral nectaries have evolved repeatedly in angiosperms in response to insect herbivores.[30] Recent studies have demonstrated how insect activity and presence produces a vari-

able response in extra-floral nectar production;[1,7,49,50] and that Malvaceae species with extrafloral nectaries benefit from protection from herbivores.[7] This indirect defense could be a selective force in the evolution of extra-floral nectaries.[50] However, the Hawaiian Islands have no native coccinellid beetles. We do not know the selective force for this Hawaiian native *H. brackenridgei*, and it may not have been a coccinellid species. There is no indication that the structure or nectar content of the extrafloral nectaries of *H. brackenridgei* is specialized to coccinellids. In addition, coccinellids are highly generalized and are found on numerous plant species. Despite not being historical co-evolutionary partners; currently, this non-native and native species interaction is an example of a potential facultative mutualism where the *H. brackenridgei* is protected against predatory arthropods by the *C. coeruleus*, and the *C. coeruleus* receive nectar as a nutrition source.

In conclusion, the extra-floral nectaries of *H. brackenridgei* appear to be a mechanism to maintain the presence of ladybeetles, *C. coeruleus,* on the plant as a possible protection against herbivorous insect populations. Whether the nectar attracts the beetles directly or indirectly, the presence of the extra-floral nectar, once detected visually by the beetle, appears to maintain the beetles' presence. Since coccinellid presence may increase the fitness of a plant, these findings will be useful to insect and pest management programs. Further study is needed as to the chemical make-up of extra-floral nectar, if the lady beetles have a preference for certain types of extra-floral nectar, and what the impact of increased beetle presence is to the plants fitness.

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Disclosures

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