

Exploring insect specialization on *Artemisia tridentata* and *Artemisia rothrockii*

Naomi Charlet¹, Elise Cypher¹, Anupama Sethuraman², Tamar Viz³

¹University of California, Santa Barbara; ²University of California, San Diego;

³University of California, Berkeley

ABSTRACT

Invertebrates have been shown to specialize to different host environments, both with and without geographic isolation. Some plants emit volatile organic compounds (VOCs) to deter invertebrate herbivory. Specialist invertebrates have been shown to adapt to host plants regardless of VOC deterrents. Sagebrush (*Artemisia spp.*) is a plant which exhibits this damage-induced resistance. In the White Mountains, *Artemisia tridentata* and *Artemisia rothrockii* occupy the same general areas and are closely related yet differ in their chemistry and morphology. Invertebrate preference and specialization on specific *Artemisia* taxa has not been fully explored. In our study we investigated invertebrate specialization on *A. tridentata* and *A. rothrockii*. We conducted a paired-design study as well as an insect-choice experiment to examine the invertebrate communities, herbivory, and parasitism on *A. rothrockii* and *A. tridentata*. There was greater predator abundance, total abundance, and herbivory damage on *A. rothrockii* and greater gall abundance on *A. tridentata*. Our experiment revealed that *Miridae* insects preferred their plant of origin. Our study demonstrates that invertebrate communities specialize on closely related sagebrush taxa, which may signify the beginnings of co-evolution.

Keywords: sagebrush, *Artemisia tridentata*, *Artemisia rothrockii*, plant-invertebrate interactions, insect specialization

INTRODUCTION

Organisms adapt and specialize to their environments in order to survive and coexist. Specifically, insects have been shown to specialize to different host environments, both with and without geographic isolation (Mayr 1947; Futuyma and Moreno 1988). Different kinds of invertebrates, including those which are herbivorous, predatory, and parasitic, can develop adaptations to their host plants.

This invertebrate specialization can eventually lead to adaptive divergence and sexual isolation in which speciation can occur between different insect communities on different host plants (Futuyma and Moreno 1988; Ehrlich and Raven 1964).

In particular, parasitic insects, like gall wasps (*Cynipidae*), can specialize by detecting small differences between host oak species. In fact, these galls, or parasitic growths of plant tissue, can be used as a tool

to resolve plant systematics (Abrahamson 1998). Specialization can also occur with predatory insects, who often distinguish between plants to find their prey (Schuman et al. 2013). Furthermore, herbivorous insects, who use their host plants for food, can specialize and drive patterns of evolution (Bruce 2014). For example, stick insects (*Timema cristinae*) have demonstrated speciation without geographic isolation, exhibited by their specialization on specific host plants (Nosil et al. 2002, 2008). They have shown morphological shifts depending on their host plant, and consistently choose to mate with insects from their same host plant, displaying how insect specialization can lead to sexual isolation (Nosil et al. 2002).

In addition to morphological adaptations, there are also insects who can distinguish between plants or recognize their host plant based on chemistry (Jaeger et al. 2016). As a reaction to herbivory, plants have adapted to contain defense mechanisms which involve secretion of chemicals such as volatile organic compounds (VOCs) (De Moraes et al. 2001). However, herbivores have adapted to use VOCs to distinguish between plants or recognize their host plant based on chemistry (Jaeger et al. 2016). Herbivores, including insects, can facilitate adaptive selection and incur species divergence by specializing to their host plant's chemistry (Conchou et al. 2019). By studying herbivorous insect communities on plants, one can better understand the mechanisms of insect specialization.

One such plant which contains chemical herbivory defense is Sagebrush (*Artemisia spp.*). Sagebrush is an abundant and widespread plant across the Great Basin, and many organisms utilize it, including mammals, birds, insects, fungi, and parasitic

plants (Welch and Criddle 2003). In the White Mountains of California, two sagebrush species *Artemisia rothrockii* and *Artemisia tridentata* occupy the same general areas (Shultz 2012) and are closely related (Garcia et al 2011). *A. rothrockii* and *A. tridentata* differ in their chemistry and morphology, with *A. tridentata* containing more toxic terpenes than *A. rothrockii* (C. Hobbs, personal communication, August 2, 2021; Shultz 2012). Both *A. rothrockii* and *A. tridentata* demonstrate damage-induced resistance to herbivory and release VOCs to deter herbivory (Karban 2007). While it is known that organisms such as pygmy rabbits (*Brachylagus idahoensis*) and the greater sage grouse (*Centrocercus urophasianus*) prefer foraging on specific taxa of Sagebrush and can recognize their VOCs (Ulappa et al. 2014; Frye et al. 2013), insect preference and specialization on specific *Artemisia* taxa has not been fully explored.

In our study, we wanted to know if *A. tridentata* and *A. rothrockii* harbor different invertebrate communities, and to what extent invertebrates are specialized to each species. We predicted that due to the difference in chemical breakdowns of the two sagebrush species and morphologies, they would have different species compositions. In addition, since *A. tridentata* contains more toxic terpenes, we predicted that *A. rothrockii* would have higher community abundance and richness. We also predicted that given a choice between *A. rothrockii* and *A. tridentata*, invertebrate individuals will favor the species they originate from, due to possible specialization on their host plant.

METHODS

2.1 Study System

Our study was conducted from August 2 to 6, 2021 at the White Mountains Research Center, Crooked Creek Field Station (3,090 m, 37°29'57"N / 118°10'19"W) which is a site within the University of California Natural Reserve System located in Inyo National Forest. The White Mountains features sagebrush steppe habitat containing several different species of *Artemisia*, with *A. tridentata* and *A. rothrockii* as the most locally abundant.

2.2 Sampling Design

We sampled 25 *A. tridentata* plants and 25 *A. rothrockii* plants at four sites which varied in elevation, aspect, sunlight exposure, moisture availability, and substrate; we accounted for these environmental variables by using a paired design. For each plant, we measured the overall dimensions (height, width, and length), the diameter of the largest bud, and the length of the longest leaf. Recording the bud diameter and leaf length of each plant enabled us to use the morphological differences between the two to confirm our identification of the two species (*A. rothrockii* had bud diameters ranging from 1.8 mm to 3.0 mm; *A. tridentata* had bud diameters ranged from 1.0 mm to 1.8 mm). Two people observed opposite sides of the plant for 60 seconds, actively searching for signs of herbivory on leaves, likely resulting from *Miridae* and other invertebrates. To build a more complete picture of invertebrate activity on each sagebrush, we recorded the number of galls and the invertebrate community on each plant, including both herbivores and

their predators (i.e. arachnids and ants). To do so, we sweep-netted and then observed each plant for 180 seconds and actively searched for galls and invertebrates, which were both later identified in the laboratory. The invertebrates were also classified as being herbivorous or predatory.

For the experimental portion of our study, we conducted herbivore choice trials. We collected 16 insects of the family *Miridae* from each sagebrush species in sweep nets. These herbivores were present in roughly equal amounts on both *A. rothrockii* and *A. tridentata* plants. The arenas for the choice trials were 90 mm diameter petri dishes with equal amounts of bud and leaf segments of *A. tridentata* and *A. rothrockii* separated on opposite sides of each dish. Each insect was placed in the center of a petri dish and observed for 30 minutes. We first recorded the insect's immediate choice after being placed into the arena. To account for variability in the insect's initial orientation, we observed *Miridae* at 15 minutes and 30 minutes in addition to the first choice. At each of these recording periods, if the herbivore was clearly on one side of the petri dish but not touching the plant, we made note of this as on a specific side but not touching. If the herbivore was touching the plant, then this was noted as a specific choice. If the herbivore was in or close to the center of the dish, this was recorded as "no choice." We repeated this process for 16 choice trials with herbivorous true bugs (*Miridae*) that were sweep-netted from *A. rothrockii* plants.

2.3 Statistical Analysis

We used JMP Statistical Software Version 16.0 Pro for all our analyses. To analyze the relationships between *Artemisia* species and

herbivore richness and abundance, predator abundance, number of galls, gall richness, and incidences of herbivory damage, we ran paired t-tests. To analyze *Artemisia* size, we created a size index using a principal components analysis on *Artemisia* height, width, and length. We used an ANCOVA model to examine the relationship between *Artemisia* species on invertebrate richness and abundance, while accounting for size. Furthermore, we ran a discriminant analysis to quantify the differences between insect communities on different *Artemisia* species plants. Lastly, for the herbivore choice experiment, we conducted a chi-square analysis to discern the relationship between insect preference and host plant. After recording the insects' choice three times, the ratio of choosing *A. rothrockii* and *A. tridentata* was calculated and converted into a percent. Two-sample t-tests were run to analyze these choice ratios with the insect's host *Artemisia* species.

RESULTS

A discriminant analysis was able to correctly identify 25 out of 25 *A. tridentata* plants and 20 out of 25 *A. rothrockii* plants, showing that the invertebrate communities are distinct. Larger sized *Artemisia* plants had higher invertebrate species richness and total abundance (Table 1). However, when considering their smaller size, *A. rothrockii* plants had higher invertebrate species richness and total abundance overall (Table 1).

Table 1. *Artemisia* size and species type results. We used an ANCOVA model to test the effect of *Artemisia* species, size, and an interaction between size and species on total invertebrate richness and abundance. Size was calculated by creating an index of sagebrush height, width, and length. The sample size N = 50.

Total Invertebrate Richness	Variable	t	P
	Sage Species	2.72	0.0091*
	Size	2.93	0.0053*
	Size*Species	0.58	0.56
Total Abundance			
	Sage Species	3.06	0.0037*
	Size	2.84	0.0068*
	Size*Species	0.83	0.41

Additionally, there were 13 types of galls found in the field, including *Rhopalomyia medusirrasa* and other species on various parts of the stems and leaves of the plants. *A. tridentata* plants had a higher abundance and richness of galls than *A. rothrockii* (Table 2; Fig. 1). Specifically, *A. tridentata* had a higher abundance of both stem galls (*Eutreta spp.*) and leaf galls (*Rhopalomyia spp.*) than *A. rothrockii* (Table 2).

Table 2. Paired *A. rothrockii* and *A. tridentata* t-test results. The differences between herbivore richness¹, herbivore abundance¹, predator abundance¹, instances of herbivory damage¹, gall abundance¹, gall richness², # of leaf galls², and # of stem galls² between *A. rothrockii* and *A. tridentata* were analyzed using dependent t-tests between the two species of *Artemisia*. ¹N = 25; ²N = 20

Variable	DF	t	P
Herbivore Richness	24	1.31	0.20
Herbivore Abundance	24	0.65	0.52
Predator Abundance	24	2.20	0.0371*
Instances of Herbivory Damage	24	2.99	0.0064*
Gall Abundance	24	3.22	0.0036*
Gall Richness	19	4.28	0.0004*
# of Leaf Galls	19	2.36	0.029*
# of Stem Galls	19	2.88	0.0096*

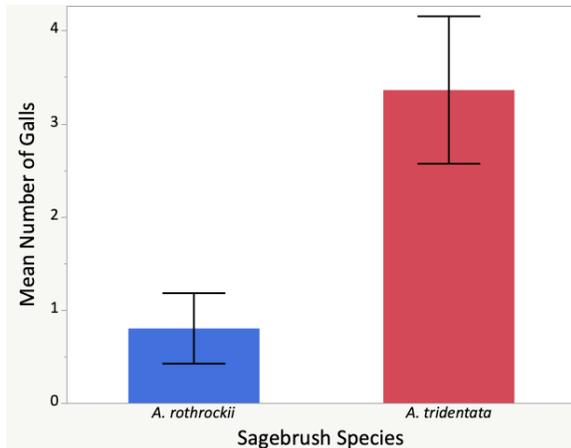


Figure 1. Higher gall abundance on *A. tridentata* than on *A. rothrockii*. As a result, gall specialization may be taking place on *A. tridentata*. Galls were counted during the span of three minutes for each plant. The differences in gall abundance on the two sagebrush species *A. rothrockii* and *A. tridentata* were analyzed using a paired t-test (DF = 24, SE = 0.08, t = 3.22, P = 0.0036*). Standard error bars are represented vertically in gray.

A. rothrockii plants had more herbivory damage than *A. tridentata* plants (Table 2; Fig. 2). *A. rothrockii* had marginally higher total invertebrate abundance than *A. tridentata* (Table 2). *A. rothrockii* plants had a greater abundance of predatory insects than *A. tridentata* plants (Table 2). We found no relationship between *Artemisia* species and herbivore richness and abundance.

In the experimental study, *Miridae* insects were more likely to first choose the *Artemisia* species they originated on (Table 3). This held true for the entire 30-minute period as well: insects originally found on *A. rothrockii* plants preferred *A. rothrockii* plants and insects originally found on *A. tridentata* plants preferred *A. tridentata* plants in the experimental arena (Table 3; Fig. 3).

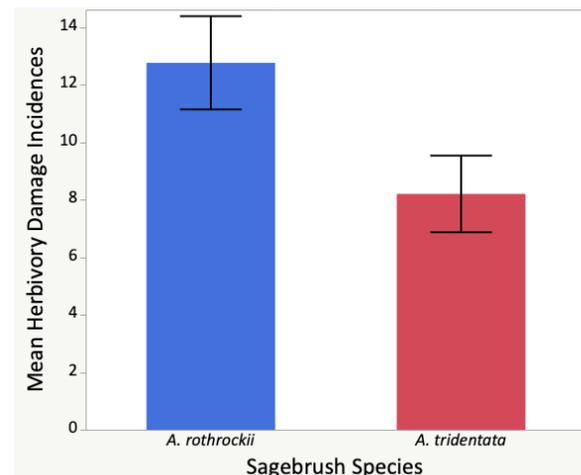


Figure 2. More herbivory damage on *A. rothrockii* than on *A. tridentata*. Herbivory damage was measured by counting the number of incidences on each plant within one minute. The differences in herbivory damage between the two sagebrush species *A. rothrockii* and *A. tridentata* were analyzed using a paired t-test (DF = 24, SE = 1.52, t = 2.99, P = 0.0064*). Standard error bars are represented vertically in gray.

Table 3. Insect-choice experiment results. 32 insects from the family *Miridae* were collected for the experiment. % Choice *A. rothrockii* or *A. tridentata* represents the frequency an insect chose a certain species. These analyses were conducted using a two-sample t-test. A chi-square analysis was also conducted to analyze the insects' first choice.

Variable	Estimate	SE	t	P
% Choice <i>A. Rothrockii</i>	0.15	0.06	2.52	0.0167*
% Choice <i>A. Tridentata</i>	0.49	0.07	1.99	0.0551
			χ^2	P
First Choice			4.5	0.034*

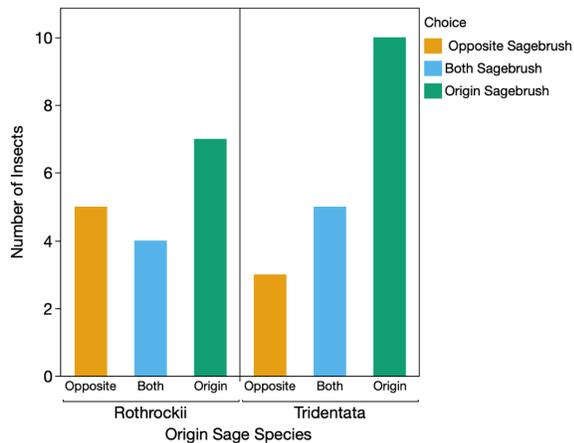


Figure 3. *Miridae* insects prefer their *Artemisia* species of origin. (*A. rothrockii*: Estimate = 0.15, SE = 0.06, $t = 2.52$, $P = 0.0167^*$; *A. tridentata*: Estimate = 0.49, SE = 0.07, $t = 1.99$, $P < 0.0551^*$). *Miridae* insects found on both *A. rothrockii* and *A. tridentata* were given the choice between both species for a total of 30 minutes. Insects found on *A. rothrockii* preferred *A. rothrockii*, as can be seen by the green bar. This effect was marginal for insects found on *A. tridentata*. The insect's choice is displayed as 'origin,' or the same species as their host plant, 'opposite,' or the opposite species as their host plant, or 'both' in which they chose both species.

DISCUSSION

Overall, we found that *A. tridentata* and *A. rothrockii* differed in their invertebrate communities. Our discriminant analysis supported this claim as it was able to correctly distinguish between the two *Artemisia* species, based on the invertebrate community breakdown of each plant. Furthermore, *A. rothrockii* plants, when accounting for their smaller size, tended to have higher abundance as well as richness of invertebrates. This implies that *A. rothrockii* may be able to support a larger and more diverse invertebrate community than *A. tridentata*, although future studies are needed to affirm this.

Additionally, our paired tests revealed more gall richness and abundance on *A.*

tridentata than on *A. rothrockii*, including two gall species which exclusively parasitize *A. tridentata*. Galls typically highly specialize to their host plant (Abrahamson 1998), indicating that *A. tridentata* may contain different specialized invertebrates than *A. rothrockii*. On the other hand, *A. rothrockii* contained more herbivory damage than *A. tridentata*. Moreover, though we found no difference between the herbivorous invertebrate abundance and richness on either sagebrush species, *A. rothrockii* contained higher predator abundance than *A. tridentata*. These two findings are in line with studies which have found that increased herbivory damage cause *Artemisia* spp. to release VOCs, consequently attracting predators (Karban 2007; Takahashi and Huntly 2010).

Lastly, our insect-choice experiment provided further support for invertebrate specialization on *Artemisia* species. Namely, *Miridae* insects were more likely to first choose and continue to choose the *Artemisia* species that they originated on. To elaborate, the insects could tell the difference between the two species and had a strong preference for their host plant. This reinforces the fact that *A. tridentata* and *A. rothrockii* differ in their chemical breakdowns and could suggest possible adaptations of different *Miridae* insects to different sagebrush species.

Herbivory damage on the two sagebrush species differed, signaling that they may demonstrate their own herbivory-tolerance strategies. Although *A. rothrockii* contained more herbivory damage, which is often associated with plant weakness (Belsky 1986), more herbivores could signify greater plant health. To be specific, herbivores may support plant health in numerous ways including improving nutrient uptake and

plant herbivory defense (Poveda et al. 2005; Wiesser and Siemann 2008). Though some studies suggest plants with more herbivory are able to sustain fewer invertebrates (Belsky 1986), *A. rothrockii* sustained higher numbers of herbivory damage as well as larger invertebrate communities (Table 1). Therefore, *A. rothrockii* may be more tolerant of herbivory.

Since *A. rothrockii* and *A. tridentata* differ in the composition of their damage-induced VOCs (C. Hobbs, personal communication, August 2, 2021), it is possible that their distinct blends correspond to their specific invertebrate communities or relate to different herbivory tactics. It has been documented that monoterpenes and other specific compounds which make up *Artemisia* VOCs are associated with phenomena such as herbivory resistance, predator attraction, and pheromone release (Maffei 2010). Because *A. rothrockii* and *A. tridentata* displayed different herbivory types and rates, this may suggest further that their VOCs are highly specialized and correlated with their specific invertebrate communities. Invertebrates, including herbivores, herbivore enemies, and parasites may also discriminate between different VOC mixtures, indicating specialization and possibly even adaptive divergence (McCormick et al. 2012).

Our study was able to provide significant support for *A. rothrockii* and *A. tridentata* containing distinct invertebrate communities and specific herbivory types and strategies. However, while there is a clear ecological relationship between invertebrates on *Artemisia spp.* and their host plant, there is not enough information to say that there is divergence and ultimately community co-evolution between the two. In order to demonstrate co-evolution, a

reciprocal process between plants and invertebrates, sexual isolation and parallel phylogenies, in addition to specialization and adaptations, should be measured (Ehrlich and Raven 1964). Future studies which show close interactions and reproductive isolation may be able to suggest co-evolution between *Artemisia spp.* and invertebrates, such as *Miridae*. These studies may also include further observation of chemical (VOC) specialization between invertebrates and *Artemisia spp.* as well as morphological adaptations (Nosil et al. 2002).

In all, our results reveal distinct communities on *A. rothrockii* and *A. tridentata* in which invertebrates that associate with one species intentionally chose that species. Our study shows that one can distinguish between *A. rothrockii* and *A. tridentata* by examining their invertebrate communities, namely herbivorous, predatory, and parasitic species, in addition to their chemical makeups. The effects of insect herbivory may influence the adaptations of sagebrush, and this in turn can change the communities that depend on them. Examining interactions between plants and their invertebrate communities could signify specialization and ultimately co-evolution between closely related taxa.

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