

RISKY BUSINESS: TRADEOFFS BETWEEN NUTRITION, TOXICITY,
AND PREDATION BY A SPECIALIST MAMMALIAN HERBIVORE

by

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A thesis

submitted in partial fulfillment

of the requirements for the degree of

Master of Science in Biology

Boise State University

May 2016

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BOISE STATE UNIVERSITY GRADUATE COLLEGE

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of the thesis submitted by

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Thesis Title: Risky Business: Tradeoffs Between Nutrition, Toxicity, and Predation
by a Specialist Mammalian Herbivore

Date of Final Oral Examination: 26 January 2016

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DEDICATION

To the many outstanding teachers who have gotten me this far.

ACKNOWLEDGEMENTS

This work has only been possible with the help of many kind and patient individuals. First and foremost, I thank my advisory committee. My major advisor, Dr. Jennifer Forbey, is a remarkable scientist and mentor. Her drive and curiosity know no bounds and inspire me daily. Dr. Lisa Shipley welcomed me to Washington State University and guided me through every stage of working with captive animals. I am grateful for both her expertise and patience. Dr. Marie-Anne de Graaff challenged me to consider the implications of my work in new ways. Her perspective added much to my work. While not a formal member of my committee, Dr. Janet Rachlow provided me with invaluable advice in every stage of development of this work.

Every Boise State faculty member with whom I interacted was generous with their time, knowledge, and advice. I particularly want to thank Dr. Julie Heath, Dr. Benjamin Pauli, Dr. Kevin Feris, and Dr. James Belthoff. Similarly, the graduate students and undergraduate students with whom I have studied have greatly contributed to my experiences.

Many members of the Forbey lab, both past and present, were instrumental in any success I have achieved. Special thanks to Peter Olsoy, Marcella Fremgen, Zoe Tinkle, Dan Melody, Brecken Robb, Natasha Wiggins, Jenny Fothergill, Cody Wilhelm, Britt Pendleton, Karli Graski, Joel Velasco, and anyone I may have missed. Chelsea Merriman is a phenomenal technician adept at both field work in adverse conditions and finicky laboratory assay. Her efforts were Herculean.

During my time at the University of Idaho and Washington State University, I was fortunate to be welcomed by Amy Ulappa, Miranda Crowell, Meghan Camp, and Charlotte Wilson. In addition to being warm and gracious hosts, all four assisted me as I learned new techniques in the field and the captive research facility.

Funding for this research was provided by the National Science Foundation, DEB-1146194, and IOS-1258217.

Finally, I want to thank my wife, Naomi, for her near infinite patience and incredible support.

ABSTRACT

Animals must balance many risks and rewards when using resources and selecting habitats. Understanding how animals make these choices requires elucidating the functional significance and interactions among habitat features. The criteria an animal uses to determine the functional quality of a resource may differ from those traditionally measured in surveys of habitat quality. Similarly, the relative value of a particular resource may vary with an animal's physiology or behavior, or the unique combination of the resource's characteristics. Previous studies have identified a number of specific individual, measurable, habitat parameters that influence habitat selection of a sagebrush specialist, the pygmy rabbit (*Brachylagus idahoensis*). We used a combination of those parameters to test the hypothesis that pygmy rabbits evaluate habitats differently based on their intended use of those habitats. We measured seven potentially toxic plant secondary metabolites (PSMs) and crude protein levels in sagebrush at and around pygmy rabbit burrows, in addition to the proximity of each plant to the burrow entrance and the concealment from aerial predators offered by each plant. We also quantified two distinct types of habitat use by pygmy rabbits by counting foraging bite marks and fecal pellets. We used model selection to determine which combinations of habitat parameters best predicted each type of use. In general, parameters representing food quality (e.g., PSMs and protein) best predicted foraging (bite marks) and parameters representing safety (e.g., concealment and distance to refuge) best predicted resting and digestion (fecal pellets). These results suggest that pygmy rabbits use different criteria when evaluating habitats

for different potential uses. We also used captive feeding trials to evaluate the preference of pygmy rabbits and mountain cottontails (*Sylvilagus nuttallii*) for five single PSMs in sagebrush compared to a mixture of those same five PSMs. Pygmy rabbits generally showed little preference among single PSMs compared to mixed PSMs, whereas mountain cottontails—dietary generalists—exhibited strong preferences. These results suggest that specialists are better adapted to cope with both high concentrations of single PSMs and mixtures in the foods they regularly encounter than are generalists. We propose that preference for particular PSMs by an herbivore reflect faster detoxification capacity for that specific PSM. The particular parameters used by pygmy rabbits to evaluate their habitats and food resources are important to understand if sagebrush habitats are to be effectively assessed, conserved, managed, and restored. Furthermore, identifying preference for particular components of resources by animals and correlating them with diverse measurements of use may facilitate more nuanced descriptions of habitat selection across taxa.

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CHAPTER 1: EVALUATING TRADEOFFS IN HABITAT SELECTION FOR FORAGING VERSUS RESTING BY A SPECIALIST MAMMALIAN HERBIVORE

Introduction

Animals selecting habitat are expected to evaluate and optimize many parameters of their habitat (e.g., food, cover, Gotmark et al. 1995, Searle et al. 2007, Ulappa et al. 2011, 2014, Bjorneraas et al. 2012, Camp et al. 2012, Frye et al. 2013). Because of spatial and temporal variation across available habitats, any specific habitat choice is unlikely to offer ideal conditions across all parameters simultaneously. Animals must therefore make tradeoffs among different habitat parameters when selecting habitats that will best meet their needs (Brown 1999, McArthur et al. 2014, Camp et al. 2015, Crowell et al. in press). Changes in physiological states (e.g., estrus versus lactation) or behavioral strategies (e.g., foraging versus resting) may also change the objectives of habitat selection and therefore shift the processes by which habitats are evaluated and selected (Rosenzweig 1981, Alonzo 2002). Identifying which specific parameters animals choose and the changing criteria by which they judge them are two major challenges in describing of habitat selection.

Traditionally, studies of habitat selection have used standard measurements such as food density, food abundance, and vegetation cover as proxies for habitat quality (Vivas and Saether 1987, Gabler et al. 2001, Dussault et al. 2005, Bailey and Provenza 2008, Doherty et al. 2008, Carpenter et al. 2010). Although relatively straightforward to quantify, these measurements may not accurately represent the functional parameters by

which animals actually evaluate habitat. Understanding the functional roles of individual habitat features through controlled captive studies or field experiments can help investigators identify which parameters most directly influence selection of natural habitats by animals (Sorensen et al. 2005a, Shipley et al. 2006, Degabriel et al. 2009, Estell 2010, Kimball et al. 2012, Camp et al. 2012). For example, plant preferences among herbivores may be better explained by a complex combination of nutritional quality, bite size, digestion rates, and spatial distribution rather than abundance alone (Wright et al. 1998, Villalba and Provenza 1999). Likewise, an animal's flush distance when approached by a predator may be influenced more by the concealment from predators that vegetation provides rather than percent canopy cover (Camp et al. 2013). Measures of the functional qualities of habitats have provided increasingly accurate predictions of habitat selection (Pierce et al. 2004, Moore and Foley 2005, Moore et al. 2010, Ulappa et al. 2014).

Measurements of habitat use should also reflect the evaluation processes used by animals. Optimal foraging theory predicts that animals balance many fitness costs and benefits when selecting habitats (Brown 1988, Brown et al. 1999). These costs and benefits can change as the nutritional and energetic requirements or proximity of predators change for an animal (Gilliam and Fraser 1987, Ferguson et al. 1988, Orians and Wittenberger 1991). Simply measuring the presence or density of animals within different habitats does not provide information about what those habitats are used for. Finer scale measurements of behavior make it possible to predict different tradeoff strategies employed by animals over time to determine functional quality of resources and use by animals (Dennis et al. 2003, Johnson 2007). Activity budgets from direct

observation may provide detailed information on how animals use habitats but may be prohibitively time and resource intensive to collect (Wiens et al. 1986, Marzluff et al. 1997). Video analyses or the use of accelerometers can provide quantitative measurements of specific behaviors but may also prove too costly to employ widely (Naylor and Kie 2004, Scheibe et al. 2008). Ideal measurements of habitat use are those that are collected easily and unobtrusively, and allow for differentiation of distinct behaviors or physiological states. Understanding which parameters of a habitat are functionally important to animals, the process by which those parameters are assessed, and the conditions under which those processes change, is vital to predicting patterns of habitat selection and identifying critical habitats for management actions.

We assessed the functional quality of diet and security cover for a specialist mammalian herbivore engaged in two general behaviors during winter -- foraging and resting. We focused on a dietary specialist, the pygmy rabbit (*Brachylagus idahoensis*) that is also subject to high predation. Pygmy rabbits rely almost entirely on a single and abundant shrub, sagebrush (*Artemisia* spp.) for food and cover during winter (Green and Flinders 1980, Estes-Zumpf and Rachlow 2009). Previous captive and field-based studies have demonstrated clear preferences by pygmy rabbits for high levels of dietary protein (Shiple et al. 2006), low levels of potentially toxic plant secondary metabolites (PSMs, Ulappa et al. 2014, Camp et al. 2015), high concealment from predators (Camp et al. 2013, Crowell et al. 2016), and proximity to refugia in the form of burrows dug in loose, mounded soil (Camp et al. 2012, Crowell et al. 2016). As such, the nutritional, chemical, and structural attributes of sagebrush offer specific, measurable habitat parameters likely to influence perceived functional quality of food and cover. Pygmy rabbits also leave

relatively obvious evidence of habitat use in the form of bite marks that indicate foraging and fecal pellets that indicate resting (Larrucea and Brussard 2008, Ulappa et al. 2014). The reliance of pygmy rabbits on a single plant taxon for food, refuge, and concealment from predators, coupled with our ability to identify specific types of habitat use (foraging or resting) allow us to evaluate the relative value of different functional habitat parameters and limit the number of potentially confounding variables that influence habitat use.

We used the known ecology of pygmy rabbits to predict selection for habitat features. Although pygmy rabbits can consume higher quantities of sagebrush compared to other species (Shiple et al. 2006, 2012), sagebrush contains high concentrations of potentially toxic PSMs (Kelsey et al. 1982) that can limit intake (Dziba and Provenza 2008) and inhibit digestive enzymes (Kohl et al. 2015). Pygmy rabbits also have relatively large energy requirements for their size (Shiple et al. 2006). We hypothesized that pygmy rabbits would select sagebrush with relatively low concentrations of PSMs and high concentrations of crude protein while foraging (Ulappa et al. 2011, 2014, Utz 2012, Shiple et al. 2012). Pygmy rabbits are also prey to many predator species, with predation the most common cause of death (Sanchez 2007, Estes-Zumpf and Rachlow 2009, Crawford et al. 2010). We hypothesized that pygmy rabbits would select sagebrush that provide high levels of concealment from predators and relatively easy access to refugia in the form of burrows while resting (Price et al. 2010, Camp et al. 2012, 2013).

By simultaneously assessing where animals use habitat, what they are doing in that habitat (foraging or resting), and the specific attributes that affect the food quality

and safety of that habitat, we could evaluate the relative importance of specific parameters known to individually influence the physiology, behavior, and habitat selection of pygmy rabbits. Specifically, we predicted that foraging habitats would be predicted by measures of food quality--PSM and crude protein concentrations, and resting would be predicted by measures of safety from predation -- concealment and distance to burrows.

Methods

Study Area

This study was conducted between December 2012 and March 2013 in a study site of ~ 1000 ha in southern Blaine County, Idaho (43°14 N, 114°19' W; elevation: 1470 m, Figure 1.1). The study site was dominated by Wyoming big sagebrush (*Artemisia tridentata wyomingensis*), with some three-tip sagebrush (*A. tripartita*), low sagebrush (*A. arbuscula*), and grasses and forbs. The site's microtopography was characterized by mima mounds, which are small (~5 m diameter) mounds with large, dense sagebrush and relatively deep, loose soil in which pygmy rabbits dig burrows.

Field Methods

To locate habitats frequented by pygmy rabbits, in December of 2012, we identified 20 mima mounds with burrow systems containing evidence of pygmy rabbit presence (Sanchez et al. 2009). On top of each mound, we established a 3 x 3 grid with 2 m between each point. The sagebrush plant closest to each vertex of the grid was identified and marked, establishing nine on-mound plants at each burrow system. Because morphology (e.g., height) differed between plants located on and off of the mounds, we established three additional plants 8 m from the mound in each cardinal

direction, for a total of 12 off-mound plants. If a sagebrush plant was not present within ~1 m of expected locations, we did not establish a plant at that location. We then examined each plant for evidence of pygmy rabbit use. Pygmy rabbit foraging leaves characteristic 45° bite marks on stems averaging 2 mm in diameter (Figure 1.2) that allowed us to differentiate between foraging by pygmy rabbits and rodents or other lagomorphs. All pygmy rabbit fecal pellets within a 0.5 m radius of the plant's central stem were collected and removed. These treatments represented time zero for evaluating new browsing and fecal pellets at our established plants over the subsequent three months of winter.

In March of 2013, we re-examined the established plants (n=403) for evidence of use that had occurred since December. Counts of both fresh bite marks and fresh fecal pellets were made, and samples of each plant were collected. We clipped approximately five sprigs of each plant at stem diameters of 2 mm to mimic pygmy rabbit foraging. Samples were stored on ice in the field before being transferred to a -20° C freezer.

At the same time, we determined available concealment at each plant by photographic analysis. Four 30 × 30 cm target boards were placed adjacent to the main stem of each plant, one at each of the four cardinal directions. A photograph was then taken from a height of 1.5 m directly above the center of the plant. We digitally superimposed an 11 × 11 square grid on each target board, and calculated the number of internal vertices obscured by vegetation (of a total of 100). The average of all four target boards was determined to be the percent available aerial concealment for each plant. To determine the proximity of each plant to refuge, we measured the distance (m) from each plant's stem to the nearest open and active burrow entrance.

Laboratory Methods

To prepare collected sagebrush for laboratory-based nutritional and chemical analysis, we coarsely ground previously frozen samples in liquid nitrogen with a mortar and pestle. Sagebrush contains diverse PSMs, including monoterpenes, phenolics, and sesquiterpene lactones (Kelsey et al. 1982). Previous studies have suggested that phenolics do not predict the foraging behavior of sagebrush specialists (Frye et al. 2013, Ulappa et al. 2014). Additionally, we conducted a preliminary study that showed no relationships between concentrations of total phenolics or coumarin (a specific phenolic compound) and foraging by pygmy rabbit. The identification and quantification of sesquiterpene lactones requires thin layer chromatography (TLC) or high pressure liquid chromatography, which were unavailable for this project. Additionally, previous studies disagree on whether sesquiterpene lactone compounds or quantities vary between species and subspecies of sagebrush (Kelsey et al. 1973, 1976). We therefore limited analysis of PSMs to monoterpenes. Monoterpenes limit or reduce intake in a wide variety of taxa (Dziba and Provenza 2008, Kirmani et al. 2010, Shipley et al. 2012, Frye et al. 2013, Ulappa et al. 2014), possibly through the inhibition of digestive enzymes (Kohl et al. 2015). We determined monoterpene concentrations using headspace gas chromatography. All samples were analyzed using an Agilent 5890 series II gas chromatograph coupled with a Hewlett-Packard HP7694 headspace autosampler (Palo Alto, California, Appendix A). One mL of headspace gas from each sample was injected into a J&W DB-5 capillary column. Retention times of individual monoterpenes and individual areas under the curve (AUC) were quantified using Hewlett Packard ChemStation software version B.01.00 (Palo Alto, California). To qualify for analysis, individual peaks had to represent greater

than 1% of total area under the curve, and be present in >70% of all samples tested (Frye et al. 2013). Peaks meeting those criteria were identified using co-chromatography with known standards (Table 1.1). Samples were then dried at 60° C for 24 hours and monoterpenes were quantified as AUC per 100 µg of dry weight (DW). To determine the nutritional value of sagebrush, the nitrogen content of each plant was measured via combustion (Dairy One Forage Labs, Ithaca, NY) and multiplied by 6.25 to estimate crude protein concentrations (Robbins, 1983). Both protein digestibility and fiber content may influence the overall nutritional value of a plant, however protein digestibility is uniformly high among sagebrush (Ulappa et al. 2011). Additionally, due to limited availability of biomass, we were unable to quantify fiber. We therefore limited our analyses to crude protein.

Statistical Methods

To assess whether significant differences existed between on- and off-mound sagebrush plants, we first compared predictor variables between the two plant types using Wilcoxon signed-rank tests.

To determine how habitat parameters influence different kinds of habitat use, we used the total numbers of bite marks, fecal pellets, and both bite marks and fecal pellets together as measurements of the intensity of foraging, resting, and any habitat use, respectively. Based on the distributions of all three types of use, these became continuous response variables in negative binomial regression models (Appendix C). Initial analyses suggested that these models had little predictive power (Appendix C). Consequently, we also used the presence or absence of bite marks, fecal pellets in the absence of bite marks, and either bite marks or fecal pellets as binary response variables

in logistic regression models. Evidence suggests that sagebrush plant chemistry may be spatially correlated (Burke 1989, Karban et al. 2006, Pu et al. 2015). We therefore did not assume plants at the same mound to be independent, and included mound location as a random effect in all models.

Before fitting models, we removed correlated predictor variables ($|r| > 0.7$). We selected PSMs shown to individually influence foraging in captive experiments or from other field studies whenever possible (Shipley et al. 2012, Ulappa et al. 2014). Because on- and off-mound plants were defined by their distances from active burrows, the distance to burrow structural variable had a bimodal distribution across all plants. This variable was therefore excluded from models examining all plants in favor of a binary on/off-mound variable. Based on the *a priori* hypothesis that pygmy rabbits use on- and off-mound plants differently, we fit additional models for each response variable to subsets of the data containing only on-mound or off-mound plants. Total monoterpene concentrations were highly correlated with several individual PSMs, but have been shown to influence habitat selection among other vertebrate herbivores specializing on sagebrush (Frye et al. 2013). We therefore evaluated univariate models with total monoterpenes as the predictor variable in addition to multivariate models. Variables used in analyses included seven PSM predictor variables, one nutritional predictor variable, and three safety predictor variables (Table 1.2).

We used a two-stage information theoretic approach (Burnham and Anderson 2002, Frye et al. 2013) to first identify top variables from each class, and then determine which combinations of variables across classes best predicted different types (foraging, resting, or both) of pygmy rabbit habitat use. We compared models using Akaike's

Information Criterion adjusted for small sample size (AIC_c). During each stage, we considered models within two AIC_c units of the top model that were also ranked above a null (intercept-only) model to have inferential value (Appendix B). We included variables appearing in top models during stage one in global models to be compared in stage two. We used Aikake model weight and unconditional standard error to calculate model-averaged parameter estimates and 85% confidence intervals (Arnold 2010).

All statistical analyses were conducted using R version 3.2.0 (R Foundation for Statistical Computing 2015) and JMP Pro 11.0 (SAS Institute Inc. 2013).

Results

Differences Between On-Mound and Off-Mound Plants

Of the seven PSM variables examined (Table 1.3), only borneol differed significantly between on- and off-mound sagebrush plants ($W=15,026.5$, $p<0.0001$), with borneol concentrations 18% higher in on-mound plants than in off-mound (Figure 1.3). Crude protein levels were 22% higher in on-mound plants than off-mound ($W=7,463.5$, $p<0.0001$, Figure 1.4). Concealment was 12% higher under on-mound plants than off-mound plants ($W=13,459$, $p<0.0001$, Figure 1.5). By definition, off-mound plants were further from burrow entrances than on-mound plants, so distance was not compared.

Plants that showed evidence of any use also differed, with off-mound plants having higher levels of 1,8-cineole ($\bar{X} = 28.74$ AUC/100 $\mu\text{g} \pm 2.34$ SE) and lower crude protein ($\bar{X} = 10.22\% \pm 0.03$ SE), compared to used on-mound plants (1,8-cineole: $\bar{X} = 26.99$ AUC/100 $\mu\text{g} \pm 1.74$ SE, crude protein: $\bar{X} = 11.86\% \pm 0.23$ SE).

Habitat Parameters Predicting Any Use at Plants

Top models for that predicted “any use” (i.e., bite marks and fecal pellets indicating foraging or resting) by pygmy rabbits across on- and off-mound plants included three PSMs (1,8-cineole, β -pinene, borneol) and two safety variables (on- vs. off-mound location, and concealment, Table 1.4). When on-mound plants were analyzed separately, a single model containing one PSM (1,8-cineole) ranked above the null model (Table 1.5). For off-mound plants, top models included two PSMs (1,8-cineole, borneol), a single nutritional variable (crude protein), and two safety variables (distance to burrow and concealment, Table 1.6). The odds of use at an on-mound plant were 2.5 times greater than those at an off-mound plant. The odds of use decreased by 2% for every 1 AUC/100 μ g DW increase of 1,8-cineole, irrespective of on/off-mound location (Figure 1.6). At off-mound plants, the odds of use increased by 50% with every 1% increase in crude protein (Figure 1.7), and decreased by 25% with every additional meter from a burrow entrance (Figure 1.8). Confidence intervals for all other variables overlapped one (Table 1.13).

Habitat Parameters Predicting Foraging at Plants

Top models for foraging by pygmy rabbits (i.e., bite marks) across all plants included six PSMs (1,8-cineole, β -pinene, borneol, camphor, ρ -cymene, unknown 3.2 min), one nutrition variable (crude protein), and one safety variable (on/off-mound location, Table 1.7). Top models for on-mound plants only included a single PSM (unknown 3.2 min., Table 1.8). For off-mound plants only, top models included one nutrition variable (crude protein, Table 1.9). All plants were 1.5% less likely to have bite marks for every 1 AUC/100 μ g increase in 1,8-cineole (Figure 1.9), although on-mound

plants were 8.3 times more likely to have bite marks than off-mound plants. The odds of foraging at an off-mound plant increased by 58% for every 1% increase in crude protein (Figure 1.10). Confidence intervals for all other variables overlapped one (Table 1.14).

Habitat Parameters Predicting Resting at Plants

Across all plants, top models for resting by pygmy rabbits (i.e., fecal pellets) included one safety variable (on/off-mound location, Table 1.10). At on-mound plants only, top models included four PSMs (1,8-cineole, borneol, ρ -cymene, and unknown 3.2 min., Table 11). At off-mound plants only, top models included two safety variables (distance to burrow entrance and available concealment, Table 1.12). The confidence interval for on/off-mound location narrowly overlapped one, although on-mound plants were 77% less likely to have only fecal pellets than on-mound plants. The odds of resting at off-mound plants decreased by 31% for every 1.0 m increase in distance to the nearest burrow (Figure 1.11).

Discussion

As we hypothesized, pygmy rabbits selected different habitat features depending on the primary activity or purpose of the site. Sagebrush plants growing on mima mounds were higher in crude protein and provided more concealment cover, therefore they were more intensely used by pygmy rabbits for both foraging and resting. Pygmy rabbits selected sites for foraging based primarily on nutritional and chemical characteristics (i.e., PSMs and crude protein) of sagebrush leaves, their primary food source, but selected sites for resting based primarily based on features that would be expected to improve safety from predators (i.e., concealment and distance to burrow).

Sagebrush plants growing on mima mounds were 2.5 times more likely to be used by pygmy rabbits for either foraging or resting than sagebrush plants nearby that were growing off-mound. Pygmy rabbits likely selected plants on mounds because of their higher crude protein, higher level of concealment cover, and proximity to burrows dug in the deep, friable soils, despite the higher levels of the PSM borneol in sagebrush leaves relative to off-mound plants. Others have documented differences between pygmy rabbit burrow sites found on mounds and surrounding areas, including taller and denser shrubs (Green and Flinders 1980, Gabler et al. 2001), more diverse plants (Katzner and Parker 1997), and deeper and looser soil (Weiss and Verts 1984). Although often correlated with higher levels of use, these structural characteristics are not always analogous to the functional value of habitat features that influence animal fitness. For example, concealment tends to increase with shrub density but can vary, even at the same plant, depending on the location, height, and aspect of a potential predator (Camp et al. 2012, 2013, Olsoy et al. 2013). While shrubs in soils with sufficient nitrogen and other nutrients might be expected to have both relatively dense foliage and high levels of crude protein, we found no correlation between concealment or plant volume and crude protein levels. Additionally, captive trials have demonstrated preferences for both higher protein (Shiple et al. 2006) and greater concealment (Utz 2012) under conditions in which shrub structure, plant diversity, and soil characteristics are either identical or non-existent. Pygmy rabbits also exhibit lower perceptions of risk, as measured by flight initiation distance, when located on or near burrow systems (Camp et al. 2012). These data support the assertion that pygmy rabbits differentiate between habitats at a finer scale than estimates of home range and daily movements would suggest (Katzner and Parker 1997,

Heady and Laundré 2005, Lee et al. 2010). In addition to previously-noted differences in shrub height and density, we propose that differences in PSM and protein concentrations contribute to dependence on burrow systems by pygmy rabbits and may influence their use of micro-habitats regardless of distance from burrow.

Overall patterns of habitat use, regardless of use type and whether the plant was on or off-mound, showed that pygmy rabbits favored plants with lower levels of potentially toxic PSMs and higher levels of protein. Available concealment was also present in top models predicting use, however confidence intervals overlapped one. These results are consistent with the hypothesis that PSMs, nutrition, and safety combine to influence functional habitat quality, and support earlier studies of pygmy rabbits (Ulappa et al. 2014), and other herbivores such as koalas (*Phascolarctos cinereus*, Moore and Foley 2005) and greater sage-grouse (*Centrocercus urophasianus*, Frye et al. 2013). The location of the plant (i.e., on- or off- mound) was the strongest predictor of use, however, with on-mound plants 2.5 times more likely to be used by rabbits than off-mound plants. By examining plant locations separately, it becomes clear that tradeoffs between these parameters are dynamic. At on-mound plants, a single PSM variable (1,8-cineole) best predicted habitat use of any kind (foraging or resting), whereas at off-mound plants crude protein, and distance to burrow predicted use. The monoterpenes 1,8-cineole and borneol, along with concealment also appear in top models for off-mound plants despite having confidence intervals that overlap one (Table 1.13). Pygmy rabbits are often classified as central place foragers (Heady and Laundré 2005), and are consequently expected to seek higher quality food at increasing distances from refuge to compensate for increased foraging effort and higher risk of predation (Schoener 1971,

1979, Elliott 1988, Basey and Jenkins 1995). However, off-mound plants with any use had higher levels of 1,8-cineole and lower crude protein, compared to used on-mound plants. It is unlikely that pygmy rabbits seek out plants of lower functional quality for foraging or refuge. Instead, pygmy rabbits likely use off-mound plants primarily when moving among burrow systems and therefore rely on other plant attributes for this activity. Fine-scale radio telemetry or GPS studies could facilitate mapping pygmy rabbit movements among mounds and help quantify off-mound use along those routes.

Mapping the variability of habitat parameters and corresponding use across larger spatial extents, including inter-mound areas, could also test the hypothesis that movements among burrow systems become more frequent as resources become patchier (Katzner and Parker 1997). Finally, genetic analyses of fecal pellets could quantify the relatedness of rabbits moving among burrow systems (DeMay 2015), and determine whether larger scale movements are based on social cues. Habitats that appear to be homogenous when evaluated solely on the basis of plant distribution, canopy cover, or other structural metrics, could prove to be significantly heterogeneous from the perspective of a foraging or translocating animal.

When choosing foraging sites, pygmy rabbits selected on-mound plants over off-mound plants (Table 1.7). In addition to location, top models included six of seven PSM variables (1,8-cineole, β -pinene, borneol, camphor, cymene, unknown 3.2 minutes) and crude protein, with higher 1,8-cineole concentrations significantly decreasing the odds of foraging (Table 1.14). Like most herbivores, pygmy rabbits must invest much of their time foraging to meet their daily nutritional requirements. Preferentially foraging on plants with the highest levels of crude protein may reduce the time and effort allocated to

foraging (Ulappa et al. 2011). Limiting exposure to PSMs may increase the amount of food that can be safely ingested during each foraging bout (Sorensen et al. 2005a, McLean et al. 2007). Detoxification mechanisms may also be energetically costly, and diets lower in PSMs can translate to lower daily energy costs (Sorensen et al. 2005b). Our results suggest that pygmy rabbits select sites for foraging based on PSM and protein concentrations.

When selecting which plants on mounds to forage on, only a single model with one PSM variable (unknown 3.2 min) outperformed a null model (Table 1.8). The 85% confidence interval overlaps one, so even this variable may not explain the variation observed in foraging activity at on-mound plants. Pygmy rabbits may have PSM and protein thresholds, beyond which they consider plants simply acceptable and do not discriminate in foraging behavior. If on-mound plants tended to meet those thresholds, evidence of foraging should be expected to be more or less universal at mound locations. However, these results show that only 37% (61 of 165) of on-mound plants had evidence of browsing. An alternative explanation of on-mound browsing patterns is the influence of complex mixtures of PSMs or individual PSMs not included in our analyses. Consuming mixtures of PSMs can multiply deleterious effects beyond those expected from a similar quantity of a single compound (Dyer et al. 2003, Wen et al. 2006, Richards et al. 2010, 2012). Data from captive studies suggests pygmy rabbits may prefer higher concentrations of specific individual monoterpenes to a mixture (Chapter 2). We also examined only nine individual monoterpenes, of which three were excluded from analysis due to collinearity. Sagebrush contains many more PSMs in smaller amounts, including other monoterpenes, polyphenols, and sesquiterpene lactones (Kelsey et al.

1973, 1982, Bray et al. 1991, Wilt et al. 1992). Hierarchical habitat selection has been demonstrated in other sagebrush specialists (Frye et al. 2013), and pygmy rabbits may use different criteria for evaluating foraging patches versus individual plants. They may choose to forage primarily on mounds based on their proximity to burrows and average concentrations of several prominent monoterpenes, and make different plant-scale foraging decisions based on parameters not included in this study.

Foraging at off-mound plants, by comparison, seems to be strongly influenced by their protein concentrations (Figure 1.10). Borneol was the only monoterpene to differ significantly between on- and off-mound plants, with concentrations 18% higher at on-mound plants (Table 1.3). Mean concentrations of total monoterpenes and three individual monoterpenes were also higher in on-mound plants than off-mound (Table 1.3). Conversely, 1,8-cineole and unknown 3.2 min. were higher in off-mound plants than on-mound (Table 1.3), but not significantly so. If higher concentrations of certain monoterpenes are balanced or negated by lower concentrations of others, the functional quality as defined by PSMs may not differ significantly between on- and off-mound plants. The pattern of protein is clear, however, with off-mound plants averaging 22% less crude protein than on-mound plants. Pygmy rabbits' demonstrated preference for high protein amid complex variation in PSMs (Shipley et al. 2006, Ulappa et al. 2014) suggests two compatible hypotheses: 1) pygmy rabbits foraging at off-mound plants may prioritize a clear gain in nutrition over a complex tradeoff in exposure to PSMs, and 2) higher protein intake may facilitate more efficient detoxification and thereby increase tolerance to PSMs by rabbits (Au et al. 2013). Taken together, these possibilities

reinforce the idea that phytochemicals, nutritional and toxic, and interactions between them, must be carefully considered when attempting to explain foraging ecology.

Individual shrubs often showed evidence of both foraging in the form of bite marks, and resting or digestion in the form of fecal pellets. Specifically, nearly half (49 of 102) of the plants that had fecal pellets also had bite marks suggesting that rabbits rest at the same plants where they forage. Resting may serve to aid in thermoregulation, digestion, concealment from predators, or to conserve energy (Gehman 1983, Katzner et al. 1997). Detailed activity budgets of pygmy rabbits have not been produced, but mountain cottontail (*Sylvilagus nuttallii*), a sympatric species, spend more than 80% of active hours engaged in either foraging or resting behavior (Gehman 1983). Most rabbits require approximately 5% of their body weight in forage daily (Irlbeck 2001) and video evidence suggests pygmy rabbits forage frequently while above ground (Wiggins, unpublished data). It is therefore likely that plants with evidence of both foraging and resting were selected at least in part based on food quality. Because we were primarily interested in how pygmy rabbits may use different criteria to select sagebrush plants (or sites) for different behaviors, we defined plants used for resting or digestion as having only fecal pellets and no bite marks. A single variable model with on/off-mound location performed better than a null, with an 85% confidence interval that narrowly overlapped one (Table 1.10). Unlike undifferentiated use and foraging only, pygmy rabbits generally preferred off-mound plants for resting. This could be an artifact of high levels of foraging at on-mound (37.0%) versus off-mound plants (19.7%). Because we chose to examine plants with only fecal pellets, on-mound plants offering high levels of concealment could have been selected for resting on the basis of safety, but still be

excluded from analysis having earlier or subsequently been selected for foraging on the basis of food quality. Off-mound plants, by contrast, were much less likely to have been foraged upon and consequently less likely to be excluded from analysis. Indeed, if the presence of fecal pellets, regardless of foraging evidence, is used as a response variable, on/off-mound location again appears in top models, but pygmy rabbits were generally more likely to select on-mound plants for resting than off-mound.

Our findings from use of on-mound plants only further support the possibility that resting is likely to occur at plants that are also used for foraging. Top models for on-mound plants included four PSM variables (1,8-cineole, borneol, ρ -cymene, and unknown 3.1, Table 1.11). Only unknown 3.1 had 85% confidence intervals that did not overlap one (Table 1.15). Interestingly, the parameter estimate for this monoterpene was greater than one, with an on-mound plant 2.5% more likely to be used for resting for every 1 AUC/100 μ g DW increase in concentration of unknown 3.1 min. This is in contrast to the parameter estimate for foraging, where a 1 AUC/100 μ g DW increase in unknown 3.1 min. made use 0.6% less likely. Since a lack of foraging is a prerequisite for on-mound plants to be considered used only for resting, any increase in food quality will necessarily decrease the likelihood of a plant being used for resting as we defined it. In other words, it is likely that high levels of unknown 3.1 min. reduce the odds of foraging and do not have a direct influence on resting. Concealment does not appear to influence resting at on-mound plants. This could be because the higher levels of concealment generally available on mounds provides sufficiently continuous cover to make small-scale selection unnecessary. It could also result from pygmy rabbits'

preference for proximity to burrows to concealment (Crowell et al. in press), with flight being a more advantageous strategy than hiding (Camp et al. 2012).

At off-mound plants, resting is generally influenced only by safety variables (distance to burrow and concealment). An increase of 1 m in the distance of a plant from an active burrow entrance resulted in a 31% reduction in the odds of it being used for resting (Figure 1.11). This strong preference for proximity to burrows is further evidence that the apparent selection of off-mound plants for resting is an artifact of study design. While 85% confidence intervals narrowly overlapped one, a 1% increase in available concealment appeared to correspond to a 1% reduction in the odds of use for resting/digesting (Table 1.15). This is counter to our original prediction that pygmy rabbits would preferentially select plants with high concealment. However, off-mound plants with any sign of use were also approximately 1% less likely to be selected for every 1% increase in concealment. This preference for lower concealment could suggest a preference for high visibility. Previous examination of free ranging pygmy rabbits did not show a direct relationship between increased visibility and perceived predation risk, although preference for high concealment became less pronounced as visibility increased (Camp et al. 2012). Reduced access to refuge in rabbits resting at off-mound plants could also increase the importance of early predator detection. European rabbits (*Oryctolagus cuniculus*) resting at latrine sites show increased alertness and vigilance behavior compared to foraging rabbits (Sneddon 1991). Finally, while not synonymous, concealment is highly correlated with thermal refuge (Burrow et al. 2001). Despite being well-adapted to cold environments, pygmy rabbits may experience significant heat loss while above ground during the winter (Katzner et al. 1997). Pygmy rabbits resting in

sunny microhabitats with low concealment could reduce their energy requirements via radiative heat gain. Studies examining the thermal environments in which pygmy rabbits forage and rest could help to explain the seeming preference for off-mound plants with relatively low concealment.

Conclusion

Whether for foraging, resting, or both, our findings suggest that pygmy rabbits evaluate and select sagebrush plants on mima mounds that contain burrows using different criteria than sagebrush plants up to 8 m off mounds. Lower concealment and increased distance to burrows reduced the functional value in terms of safety, thus their use of off-mound plants. Furthermore, food quality, as defined by higher protein and lower concentrations of certain PSMs, also differed between on and off-mound plants. This difference may be a result of repeated foraging by pygmy rabbits that could spur new growth with increased nutritional quality (Craig 2010). Plants that have been repeatedly used by rabbits or other herbivores may also have access to increased levels of nitrogen and carbon in the form of feces and plant litter from foraging. This effective fertilization could increase the height and density (Hyder and Sneva 1961), increase nutritional quality (Barrett 1979), and decrease PSM concentrations of affected plants (Sneva et al. 1983). The effects of repeated browsing on PSM concentrations are less clear, but could result in relatively lower levels as plants prioritize new growth over defense (Orians et al. 2010), or could trigger increased PSM levels in browsed plants, neighboring plants, or both (Karban et al. 2006, Shiojiri and Karban 2008). Higher levels of protein and PSMs at burrows with long histories of occupancy (Ulappa et al. 2014) provide additional evidence that pygmy rabbits alter the phytochemistry of the plants on

which they browse. Pygmy rabbits' dependence on burrows has been previously documented (Heady and Laundré 2005), but it remains unclear whether on- and off-mound habitats are viewed as binary (i.e., acceptable vs. not acceptable), or whether as our results show, that habitat quality decreases along a continuum as distance from burrows increases. Further studies should evaluate how variation in protein, PSMs, concealment and distance among burrows influence selection by pygmy rabbits across larger spatial scales. The potential for pygmy rabbits to influence the functional quality of habitats they occupy may vary from individual plants, to mima mounds (patch scale), to habitat scale. Responses to these quality parameters by pygmy rabbits may similarly vary. Along with associated measurements of use and patterns of pygmy rabbit movements, further studies should aim to define the precise effects of burrow location on habitat quality.

The difference in habitat quality between on- and off-mound plants is complicated because pygmy rabbits seemed to select sites based on which type of use they intend for it. Use of any kind is far more likely at on-mound burrow locations and a combination of PSMs, nutrition, and safety variables therefore affects the evaluation of these important sites of activity. Off-mound foraging and resting behaviors, by contrast, seem to be driven by distinct habitat parameters. The reduced quality of off-mound habitat may increase the importance of selecting individual plants best suited for particular activities. Pygmy rabbits foraging at off-mound plants are strongly influenced by nutrition, whereas those resting are influenced by safety. Currently, assessments of sagebrush habitat quality depend upon measurements of canopy cover and structure, taxonomic distributions, diversity of vegetation, topographic features, and habitat connectivity

(Connelly et al. 2004, Chalfoun and Martin 2007, Pyke 2011, Homer et al. 2013).

Recently, the importance of plant chemistry and nutrition on habitat quality has been demonstrated for specialist herbivores reliant on sagebrush (Frye et al. 2013, Ulappa et al. 2014). While plant structure, abundance, and distribution are almost certainly related to food quality, toxicity, and safety, this and previous studies demonstrate that these parameters are not synonymous. Recent advances in unmanned aerial systems, remote sensing, and data analysis can facilitate assessments of these parameters across larger spatial scales (Moore et al. 2010, Mitchell et al. 2012, Anderson and Gaston 2013).

In conclusion, differentiating habitats by the activity for which they are used and defining their value to animals based on criteria specific to those types of use is complex and can be resource intensive. As conservation and restoration efforts become increasingly important to the long-term survival of wildlife, so too will the needs to identify habitats suitable for these efforts and assess their results. This study illustrates the importance of measuring habitat from the perspective of wildlife.

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Tables

Table 1.1 Retention times measured using headspace gas chromatography for individual monoterpenes quantified from sagebrush (*Artemisia* sp.) samples collected in Southern Blaine County, ID, in March 2013. Monoterpenes were identified using co-chromatography with known standards.

Monoterpene Name	Retention Time (minutes)
Unknown 3.1 min.	3.14
Unknown 12.55 min.	12.55
α -pinene	13.00
Camphene	13.58
β -pinene	14.70
ρ -cymene	16.56
1,8-cineole	16.81
Camphor	21.15
Borneol	21.50

Table 1.2 Variable classes and constituent explanatory variables included in models of habitat selection by pygmy rabbits (*Brachylagus idahoensis*) in Blaine County, ID, in March 2013. Total monoterpenes are collinear with several individual monoterpenes and were included only in univariate models.

Variable Class	Variable
PSM	Total monoterpenes ¹
	1,8-cineole ²
	β -pinene ²
	Borneol ²
	Camphor ²
	ρ -cymene ²
Nutrition	Unknown 3.1 min. ²
	Protein ³
	On/off ⁴
	Distance ⁵
Safety	Concealment ⁶

¹Total monoterpene concentration (cumulative concentration of all compounds with retention times ≤ 28.0 min. [area under the chromatogram curve/100 μg dry weight])

²Monoterpene (AUC/100 μg DW)

³Crude protein (% DW)

⁴Binary on- or off-mound designation of plant

⁵Distance of plant to nearest active burrow entrance (m)

⁶Aerial concealment available at base of plant (%)

Table 1.3 Means and standard errors (SE) of variables predicting use at on-mound and off-mound sagebrush (*Artemisia spp.*) plants by pygmy rabbits (*Brachylagus idahoensis*) in Blaine County, ID, in March 2013. Bold *p*-values denote significant differences ($\alpha = 0.05$) as determined by Wilcoxon signed-rank tests.

Variable Class	Variable	Off-mound mean (SE)	On-mound mean (SE)	<i>W</i>	<i>p</i>
PSM	Total monoterpenes ¹	243.0 (5.22)	249.0 (6.66)	18,823	0.48
	1,8-cineole ²	32.07 (1.19)	29.77 (1.35)	21,007	0.23
	β -pinene ²	6.96 (0.23)	7.06 (0.32)	19,558	0.95
	Borneol ²	2.76 (0.07)	3.26 (0.11)	15,026	<0.0001
	Camphor ²	116.7 (2.52)	122.43(3.31)	18,089	0.18
	ρ -cymene ²	3.00 (0.12)	3.34 (0.22)	18,635	0.38
	Unknown 3.2 min. ²	11.20 (1.56)	9.93 (1.37)	18,715	0.42
Nutrition	Protein ³	9.74 (0.07)	11.87 (0.17)	7464	<0.0001
Safety	Concealment ⁴	46.19 (1.37)	57.92 (1.58)	13,459	<0.0001

¹Total monoterpene concentration (cumulative concentration of all compounds with retention times ≤ 28.0 min. [area under the chromatogram curve/100 μg dry weight])

²Monoterpene (AUC/100 μg DW)

³Crude protein (% DW)

⁴Aerial concealment available at base of plant (%)

Table 1.4 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the final stage of models predicting use by pygmy rabbits (*Brachylagus idahoensis*) at all sagebrush (*Artemisia spp.*) plants. Only models with $\Delta AIC_c \leq 2.0$ plus a null model are presented.

Response Variable	Model	Log Likelihood	K	AIC_c	ΔAIC_c	w_i
Any Use	Borneol ¹ + 1,8-cineole ¹ + Protein ²	-185.2	4	380.6	0.00	0.28
	Location ⁴	-187.6	2	381.3	0.69	0.20
	Borneol ¹ + 1,8-cineole ¹ + Protein ² + Location ⁴	-184.6	5	381.4	0.82	0.18
	Borneol ¹ + 1,8-cineole ¹ + Protein ² + Concealment ³	-185.1	5	382.5	1.92	0.11
	Null	-222.4	1	448.9	68.29	0.00

¹Monoterpene (AUC/100 μ g DW)

²Crude protein (% DW)

³Aerial concealment available at base of plant (%)

⁴Binary on- or off-mound designation of plant

Table 1.5 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the final stage of models predicting use by pygmy rabbits (*Brachylagus idahoensis*) at all sagebrush (*Artemisia spp.*) plants. Only models with $\Delta AIC_c \leq 2.0$ plus a null model are presented.

Response Variable	Model	Log Likelihood	K	AIC_c	ΔAIC_c	w_i
Any Use	1,8-cineole	-79.5	2	165.1	0.00	0.51
	Null	-80.6	1	165.2	0.09	0.49

¹Monoterpene (AUC/100 μg DW)

²Crude protein (% DW)

³Aerial concealment available at base of plant (%)

⁴Distance of plant to nearest active burrow entrance (m)

Table 1.6 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the final stage of models predicting use by pygmy rabbits (*Brachylagus idahoensis*) at all sagebrush (*Artemisia spp.*) plants. Only models with $\Delta AIC_c \leq 2.0$ plus a null model are presented.

Response Variable	Model	Log Likelihood	K	AIC_c	ΔAIC_c	w_i
Any Use	Protein ³ + Distance ⁵	-106.4	3	220.9	0.00	0.29
	1,8-cineole ¹ + Protein ³ + Distance ⁵	-105.3	4	220.9	0.03	0.29
	1,8-cineole ¹ + Protein ³	-106.2	4	222.6	1.65	0.13
	1,8-cineole ¹ + Protein ³ + Concealment ⁴ + Distance ⁵	-105.1	5	222.6	1.72	0.12
	1,8-cineole ¹ + Borneol ¹ + Protein ³ + Distance ⁵	-108.4	2	222.9	1.93	0.11
	Null	-110.8	1	225.7	4.75	0.02

¹Monoterpene (AUC/100 μ g DW)

²Total monoterpene concentration (cumulative concentration of all compounds with retention times)

³Crude protein (% DW)

⁴Aerial concealment available at base of plant (%)

⁵Distance of plant to nearest active burrow entrance (m)

Table 1.7 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the final stage of models predicting foraging by pygmy rabbits (*Brachylagus idahoensis*) at all sagebrush (*Artemisia spp.*) plants. Only models with $\Delta AIC_c \leq 2.0$ plus a null model are presented.

Response Variable	Model	Log Likelihood	K	AIC_c	ΔAIC_c	w_i
Foraging	Borneol ¹ + 1,8-cineole ¹ + Location ³	-156.6	4	323.3	0.00	0.03
	Location ³	.158.7	2	323.5	0.24	0.03
	1,8-cineole ¹ + Location ³	-157.7	3	323.5	0.26	0.03
	1,8-cineole ¹ + Unknown 3.2 ¹ + Location ³	-156.8	4	323.7	0.40	0.03
	Borneol ¹ + Location ³	-157.8	3	323.8	0.49	0.03
	1,8-cineole ¹ + Protein ² + Location ³	-157.1	4	324.3	1.06	0.02
	ρ -cymene ¹ + Location ³	-158.1	3	324.4	1.11	0.02
	Protein ² + Location ³	-158.2	3	324.5	1.26	0.02
	Borneol ¹ + Camphor ¹ + Location ³	-157.3	4	324.7	1.50	0.02
	1,8-cineole ¹ + β -pinene ¹ +	-156.5	5	325.2	1.96	0.01

Borneol ¹ + Location ³					
Null	-189.0	1	382.0	58.76	0.00

¹Monoterpene (AUC/100 µg DW)
²Crude protein (% DW)
³Binary on- or off-mound designation of plant

Table 1.8 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the final stage of models predicting foraging by pygmy rabbits (*Brachylagus idahoensis*) at all sagebrush (*Artemisia spp.*) plants. Only models with $\Delta AIC_c \leq 2.0$ plus a null model are presented.

Response Variable	Model	Log Likelihood	K	AIC_c	ΔAIC_c	w_i
Foraging	Unknown 3.2 ¹	-92.9	2	192.0	0.00	0.56
	Null	-94.2	1	192.5	0.48	0.44

¹Monoterpene (AUC/100 μ g DW)

Table 1.9 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the final stage of models predicting foraging by pygmy rabbits (*Brachylagus idahoensis*) at all sagebrush (*Artemisia spp.*) plants. Only models with $\Delta AIC_c \leq 2.0$ plus a null model are presented.

Response Variable	Model	Log Likelihood	K	AIC_c	ΔAIC_c	w_i
Foraging	Protein ¹	-63.7	2	133.5	0.00	0.72
	Null	-65.6	1	135.3	1.84	0.28

¹Crude protein (% DW)

Table 1.10 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the final stage of models predicting resting by pygmy rabbits (*Brachylagus idahoensis*) at all sagebrush (*Artemisia spp.*) plants. Only models with $\Delta AIC_c \leq 2.0$ plus a null model are presented.

Response Variable	Model	Log Likelihood	K	AIC_c	ΔAIC_c	w_i
Resting	Location ¹	-148.1	2	302.2	0.00	0.31
	Null	-148.9	1	302.8	0.55	0.23

¹Binary on- or off-mound designation of plant

Table 1.11 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the final stage of models predicting resting by pygmy rabbits (*Brachylagus idahoensis*) at all sagebrush (*Artemisia spp.*) plants. Only models with $\Delta AIC_c \leq 2.0$ plus a null model are presented.

Response Variable	Model	Log Likelihood	K	AIC_c	ΔAIC_c	w_i
Any Use	Unknown 3.2 ¹	-67.6	2	141.4	0.00	0.23
	Borneol ¹ + Unknown 3.2 ¹	-67.3	3	142.8	1.49	0.11
	1,8-cineole ¹ + Unknown 3.2 ¹	-67.3	3	142.9	1.54	0.11
	ρ -cymene ¹ + Unknown 3.2 ¹	-67.5	3	143.2	1.80	0.09
	Null	-69.7	1	143.5	2.19	0.08

¹Monoterpene (AUC/100 μ g DW)

Table 1.12 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the final stage of models predicting resting by pygmy rabbits (*Brachylagus idahoensis*) at all sagebrush (*Artemisia spp.*) plants. Only models with $\Delta AIC_c \leq 2.0$ plus a null model are presented.

Response Variable	Model	Log Likelihood	K	AIC_c	ΔAIC_c	w_i
Resting	Distance ²	-80.1	2	166.2	0.00	0.58
	Concealment ¹ + Distance ²	-79.9	3	168.0	1.75	0.24
	Distance ²	-82.6	1	169.3	3.08	0.12
	Null					

¹Aerial concealment available at base of plant (%)

²Distance of plant to nearest active burrow entrance (m)

Table 1.13 Covariates, model-averaged parameter estimates, and 85% confidence intervals from top models predicting any use by pygmy rabbits (*Brachylagus idahoensis*) at all, on-mound, and off-mound sagebrush (*Artemisia spp.*) plants. Covariates with confidence intervals not overlapping 1 are presented in bold.

Response Variable	Plants	Covariate	Parameter estimate ¹	Lower 85% C.I. ¹	Upper 85% C.I. ¹
Any use	All plants	1,8-cineole	0.98	0.97	0.99
		Borneol	1.04	0.99	1.42
		β -pinene	1.00	0.96	1.08
		Distance	1.00	1.00	1.00
		Concealment	1.00	0.99	1.01
		Location	2.50	1.37	16.78
		Unknown 3.2 ¹	1.00	0.99	1.01
		On-mound	1,8-cineole	0.98	0.96
	Off-mound	1,8-cineole	0.98	0.96	1.00
		Borneol	1.10	0.85	1.43
		Protein	1.50	1.15	1.96
		Distance	0.75	0.61	0.93
		Concealment	0.99	0.98	1.01

¹Because the response variable is the log-odds of use, exponentiated parameter estimates and confidence intervals are reported.

Table 1.14 Covariates, model-averaged parameter estimates, and 85% confidence intervals from top models predicting foraging by pygmy rabbits (*Brachylagus idahoensis*) at all, on-mound, and off-mound sagebrush (*Artemisia spp.*) plants. Covariates with confidence intervals not overlapping 1 are presented in bold.

Response Variable	Plants	Covariate	Parameter estimate ¹	Lower 85% C.I. ¹	Upper 85% C.I. ¹	
Foraging	All plants	Borneol	1.20	1.00	1.45	
		1,8-cineole	0.98	0.97	0.99	
		Location	9.29	5.63	15.34	
		Unknown 3.2	0.99	0.97	1.01	
		Protein	1.10	0.97	1.25	
		ρ -cymene	0.94	0.85	1.04	
		Camphor	1.00	0.99	1.00	
		β -pinene	1.01	0.95	1.08	
		On-mound	Unknown 3.2	0.98	0.99	1.00
		Off-mound	Protein	1.58	1.12	2.23

¹Because the response variable is the log-odds of use, exponentiated parameter estimates and confidence intervals are reported.

Table 1.15 Covariates, model-averaged parameter estimates, and 85% confidence intervals from top models predicting resting by pygmy rabbits (*Brachylagus idahoensis*) at all, on-mound, and off-mound sagebrush (*Artemisia spp.*) plants. Covariates with confidence intervals not overlapping 1 are presented in bold.

Response Variable	Plants	Covariate	Parameter estimate ¹	Lower 85% C.I. ¹	Upper 85% C.I. ¹
Resting	All plants	Distance	0.86	0.71	1.04
		Location	0.23	0.05	1.01
		Unknown 3.2	1.02	1.00	1.05
	On-mound	Borneol	0.87	0.66	1.14
		1,8-ineole	0.99	0.97	1.01
		ρ -cymene	0.94	0.80	1.11
		Distance	0.69	0.55	0.87
	Off-mound	Concealment	0.99	0.98	1.01

¹Because the response variable is the log-odds of use, exponentiated parameter estimates and confidence intervals are reported.

Figures

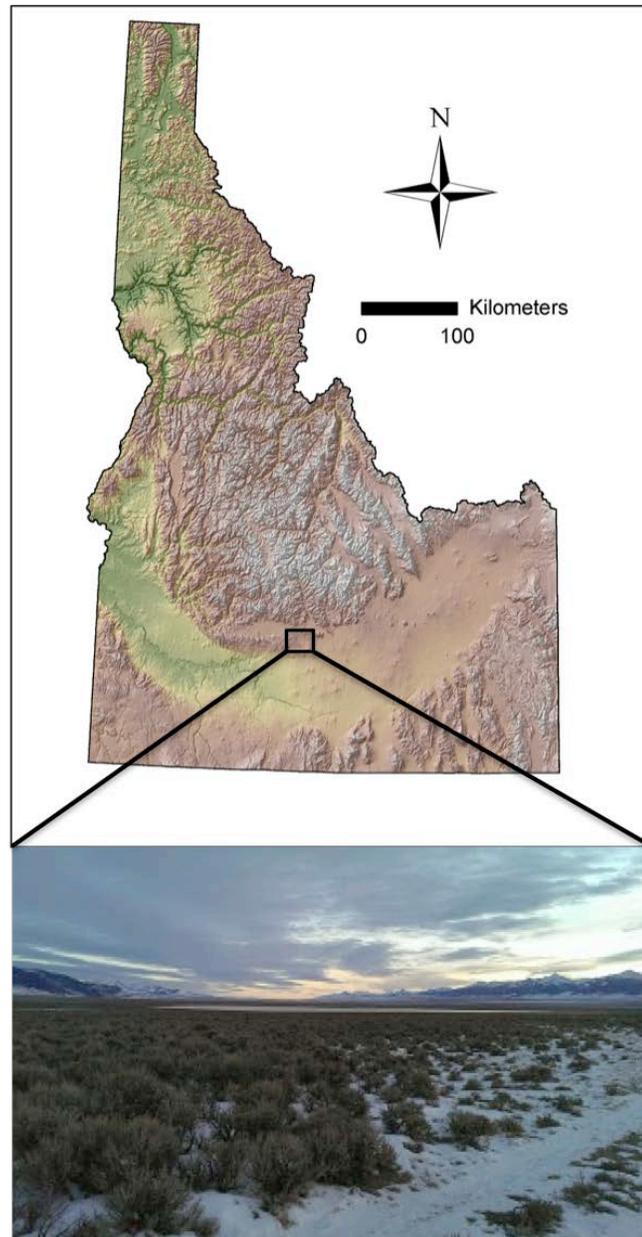


Figure 1.1 Study area for examining habitat selection by pygmy rabbits (*Brachylagus idahoensis*) within a 1000-ha area dominated by sagebrush (*Artemisia spp.*) in southern Blaine County, Idaho ($43^{\circ}14' N$, $114^{\circ}19' W$; elevation: 1470 m).



Figure 1.2 Evidence of pygmy rabbit (*Brachylagus idahoensis*) foraging on sagebrush (*Artemisia spp.*) is characterized by clean, 45-degree bite marks, and differentiated from those left by rodents or other lagomorphs by the diameter of the clipped branch and the lack of leaves left at the site.

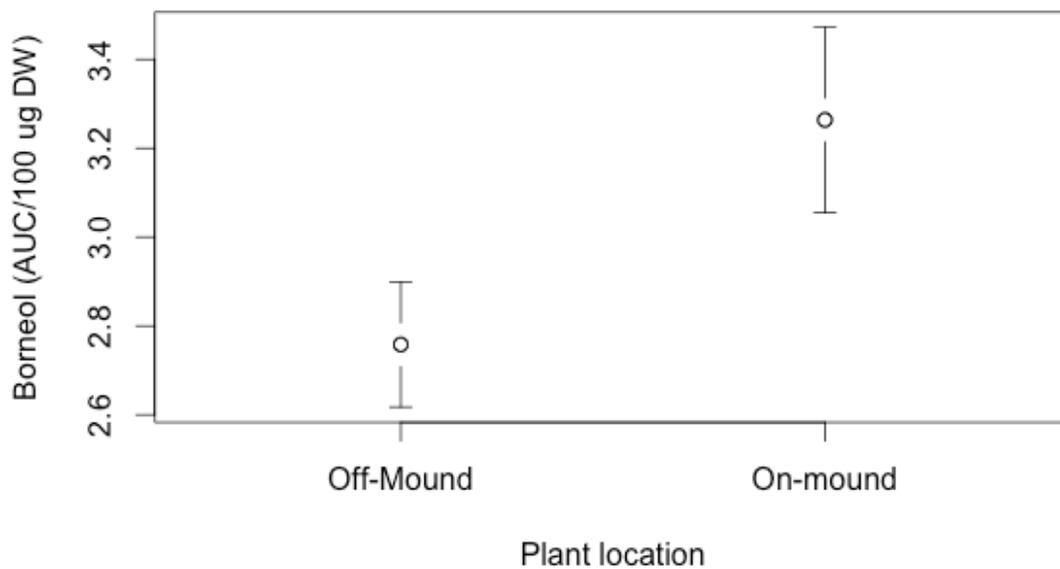


Figure 1.3 Mean borneol concentrations (with 95% confidence intervals) in off-versus on-mound sagebrush (*Artemisia spp.*) plants differ significantly ($W=15,026$, $p<0.0001$).

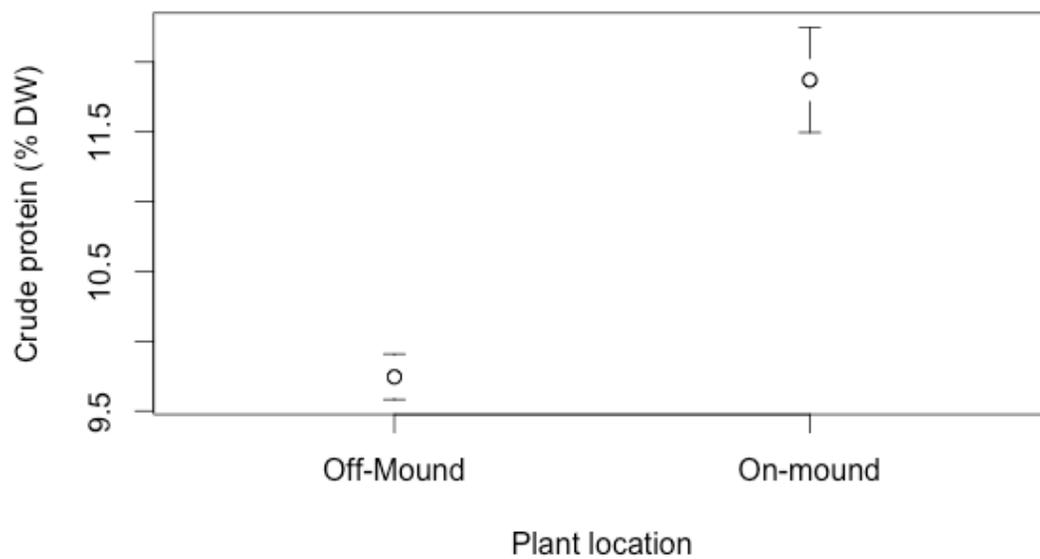


Figure 1.4 Crude protein concentrations (with 95% confidence intervals) in off- versus on-mound sagebrush (*Artemisia spp.*) plants differ significantly ($W=7,464$, $p<0.0001$).

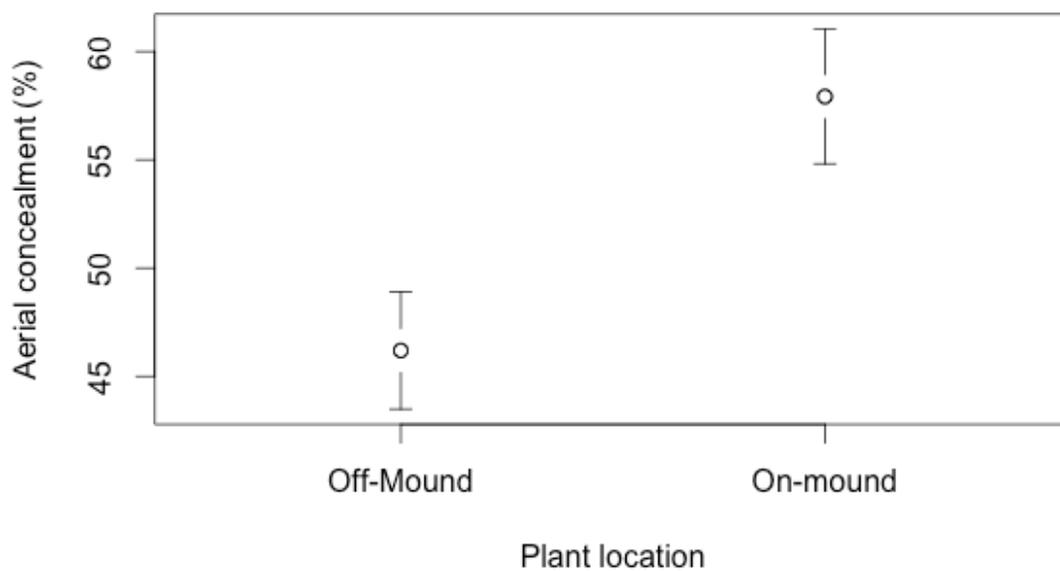


Figure 1.5 Aerial concealment (with 95% confidence intervals) in off- versus on-mound sagebrush (*Artemisia spp.*) plants differ significantly ($W=13,459, p<0.0001$).

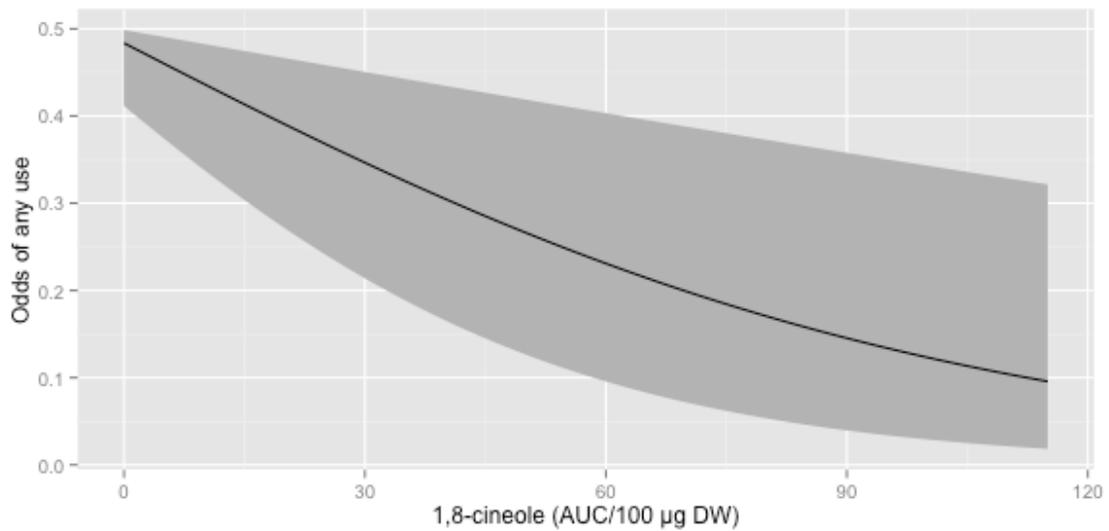


Figure 1.6 Logistic regression model of 1,8-cineole concentration (AUC/100 µg dry weight) as a predictor of the odds that pygmy rabbits (*Brachylagus idahoensis*) used a sagebrush (*Artemisia spp.*) plant either on- or off-mima mounds for either foraging (i.e., bite marks) or resting (i.e., fecal pellets). To develop odds of use, other predictor variables were held constant at their respective means across all plants. Gray band represents 85% confidence intervals.

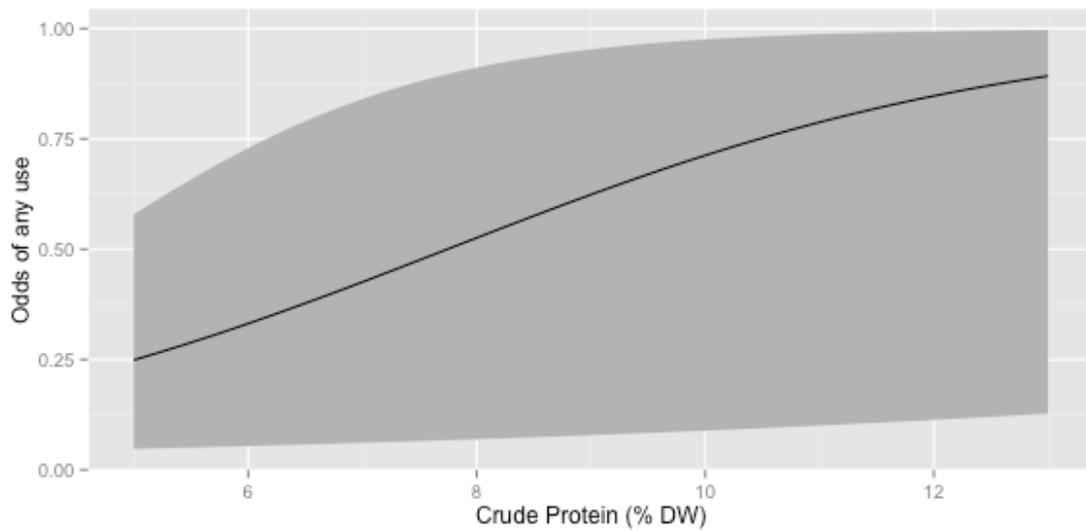


Figure 1.7 Logistic regression model of crude protein concentrations (% dry weight) as a predictor of the odds that pygmy rabbits (*Brachylagus idahoensis*) used an off-mound sagebrush (*Artemisia spp.*) plant for either foraging (i.e., bite marks) or resting (i.e., fecal pellets). To develop odds of use, other predictor variables were held constant at their respective means across all plants. Gray band represents 85% confidence intervals.

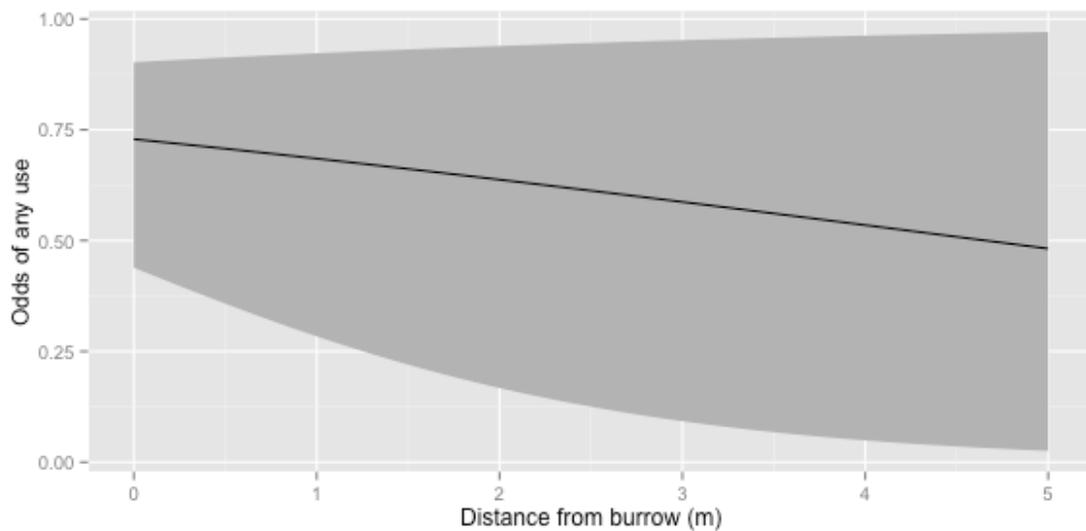


Figure 1.8 Logistic regression model of distance from burrow entrances as a predictor of the odds that pygmy rabbits (*Brachylagus idahoensis*) used an off-mound sagebrush (*Artemisia spp.*) plant for either foraging (i.e., bite marks) or resting (i.e., fecal pellets). To develop odds of use, other predictor variables were held constant at their respective means across all plants. Gray band represents 85% confidence intervals.

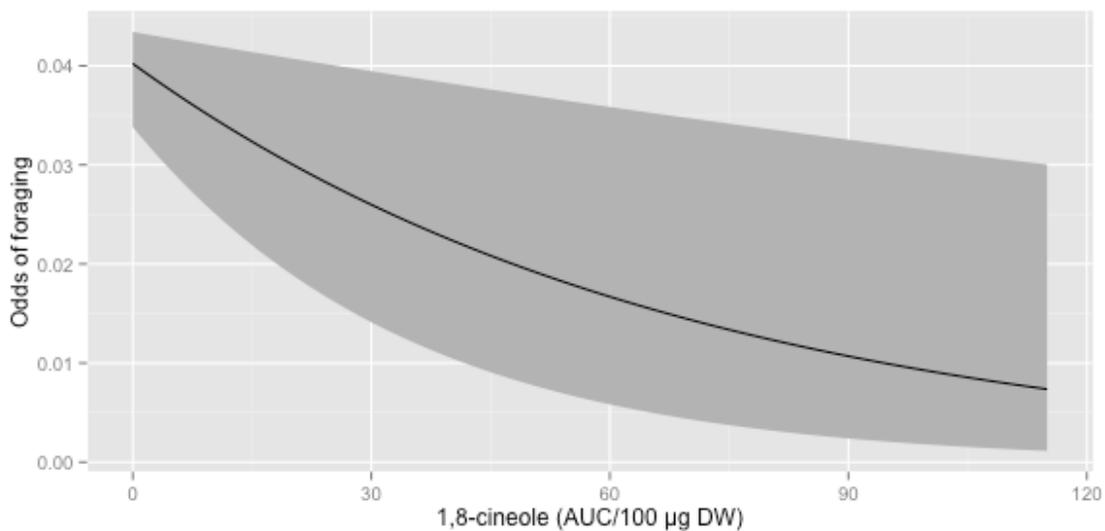


Figure 1.9 Logistic regression model of 1,8-cineole concentration (AUC/100 µg dry weight) as a predictor of the odds that pygmy rabbits (*Brachylagus idahoensis*) used a sagebrush (*Artemisia spp.*) plant either on- or off-mima mounds for foraging (i.e., bite marks). To develop odds of use, other predictor variables were held constant at their respective means across all plants. Gray band represents 85% confidence intervals.

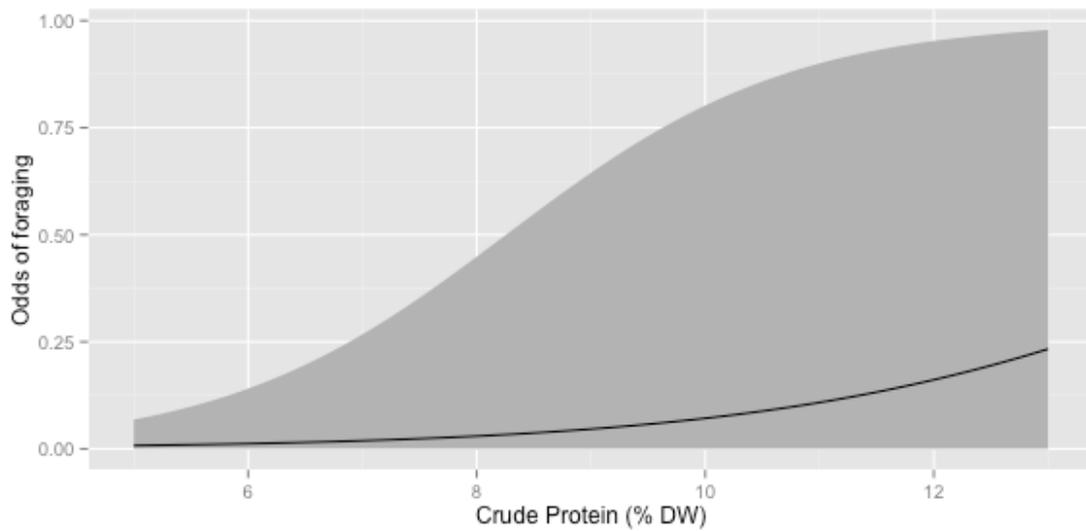


Figure 1.10 Logistic regression model of crude protein concentration (% dry weight) as a predictor of the odds that pygmy rabbits (*Brachylagus idahoensis*) used an off-mound sagebrush (*Artemisia spp.*) plant for foraging (i.e., bite marks). To develop odds of use, other predictor variables were held constant at their respective means across all plants. Gray band represents 85% confidence intervals.

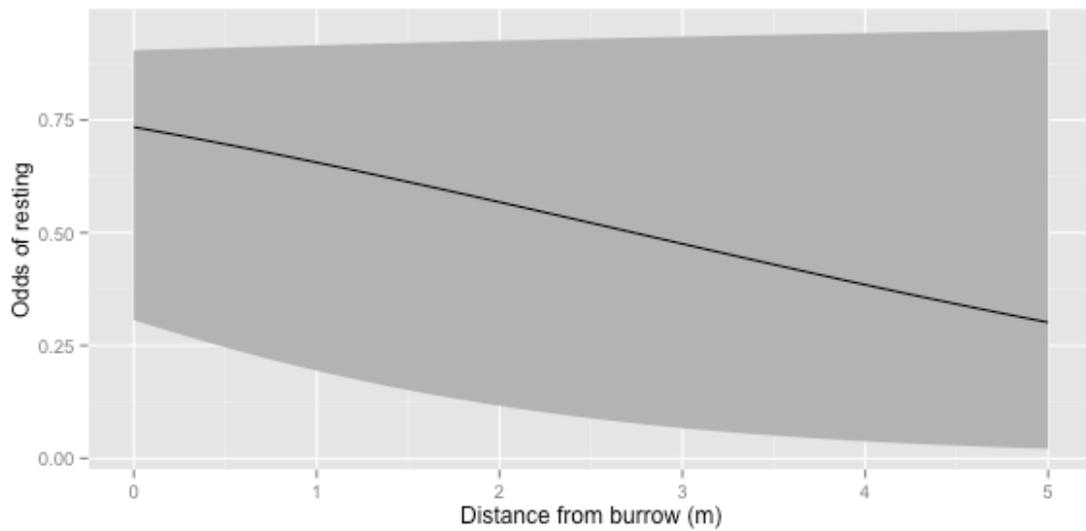


Figure 1.11 Logistic regression model of distance to burrow entrance (meters) as a predictor of the odds that pygmy rabbits (*Brachylagus idahoensis*) used an off-mound sagebrush (*Artemisia spp.*) plant for resting (i.e., fecal pellets). To develop odds of use, other predictor variables were held constant at their respective means across all plants. Gray band represents 85% confidence intervals.

CHAPTER 2: EVALUATING PREFERENCES FOR MIXTURES VERSUS
INDIVIDUAL PLANT SECONDARY METABOLITES IN A SPECIALIST AND
GENERALIST MAMMALIAN HERBIVORE

Introduction

Plant secondary metabolites (PSMs) influence the foraging behavior of herbivores and may affect patterns of habitat selection at multiple scales (Duncan and Gordon 1999, Lawler et al. 2000, Moore and Foley 2005, Frye et al. 2013, Ulappa et al. 2014, Chapter 1). High concentrations of PSMs often have deleterious effects on foraging herbivores (Guglielmo et al. 1996, Sorensen et al. 2005b, Degabriel et al. 2009, Estell 2010), and selective foraging can limit exposure to harmful compounds (Moore and Foley 2005, Wiggins et al. 2006, Frye et al. 2013, Ulappa et al. 2014). Plants often contain complex mixtures of PSMs, the identities and concentrations of which can vary among taxa, populations, and individual plants within populations (Julkunen-Tiitto 1986, Hemming and Lindroth 1995, Lawler et al. 1998, Nyman and Julkunen-Tiitto 2005, Thoss et al. 2007, O'Reilly-Wapstra et al. 2013, Frye et al. 2013, Ulappa et al. 2014, Richards et al. 2015). This diversity of PSMs has wide-ranging physiological effects on vertebrate herbivores including reduced digestion, interference with cellular processes, and compromised energy budgets and reproductive success (Guglielmo et al. 1996, Sorensen et al. 2005b, Degabriel et al. 2009, Estell 2010). Animals also cope with ingested PSMs via different detoxification strategies (Sorensen and Dearing 2006, Sorensen et al. 2006),

with specialist herbivores often able to consume relatively higher concentrations of PSMs from their host plant than generalists (Sorensen et al. 2005c, Shipley et al. 2012). The complexities of PSM mixtures in plants and responses by herbivores to these mixtures make it difficult to identify which specific compounds, combinations, and concentrations drive observed patterns in diet selection by herbivores.

Two general approaches have been used to investigate the relationships between PSMs and foraging behavior of herbivores. Field-based, observational studies maintain the complexity inherent in natural systems while sacrificing a degree of causality in the relationships observed. These studies often identify correlations between intake and individual PSMs, broad classes of PSMs, and even physical characteristics (e.g., near infrared reflectivity) thought to be influenced by PSMs (Duncan et al. 1994, Moore and Foley 2005, Moore et al. 2010, Frye et al. 2013, Ulappa et al. 2014). The patterns that emerge from these studies may help predict habitat selection and foraging behavior, but are correlative, and must be considered in light of other habitat parameters (e.g., nutritional quality, predation risk, microclimate) that may co-vary with PSMs in natural settings.

To address the mechanisms by which PSMs directly affect foraging, manipulative studies vary the intake of specific compounds and measure the responses of captive animals (Farentinos et al. 1981, Dziba and Provenza 2008, Kirmani et al. 2010, Kimball et al. 2012, Shipley et al. 2012). Although better suited to establish causal relationships between PSMs and foraging than field-based studies, captive studies often sacrifice natural chemical complexity by focusing on a single compound as an analog for the complex mixtures of PSMs found in whole plants (Wiggins et al. 2003, McLean et al.

2007, Kirmani et al. 2010, Shipley et al. 2012). Some captive studies that rely on artificial diets that contain whole plants or extracts from plants do maintain the chemical complexity of natural forage (McIlwee et al. 2001, Sorensen et al. 2005b, Kohl et al. 2015). However, they do not help identify which specific PSMs or combination of PSMs predict the foraging responses of herbivores. Additionally, many herbivores respond differently to diets containing single versus mixtures of several PSMs (Bernays et al. 1994, Dyer et al. 2003, Wiggins et al. 2003, Marsh et al. 2006, Richards et al. 2010, 2012). Generalist herbivores restricted to a single PSM may overload a specific detoxification pathway and consequently consume less food than when offered a diet containing a mixture of PSMs (Dearing and Cork 1999, Burritt and Provenza 2000, Wiggins et al. 2003). Specialist herbivores may show relatively higher tolerances for the PSMs they regularly encounter (Sorensen et al. 2004, 2005b, Shipley et al. 2012), but have reduced capacities to detoxify novel PSMs (Sorensen et al. 2005c). Captive feeding trials employing single compounds do not capture the additive or synergistic effects of consuming PSM mixtures. Likewise, trials employing artificial diets containing whole plants or plant extracts do not capture which combination or single compound explains foraging responses by herbivores.

Incorporating biologically relevant mixtures of PSMs into captive feeding trials can help bridge the gap between field approaches and captive trials. Providing captive herbivores with a mixture of PSMs that represents a simplified but realistic “plant” allows researchers to better assess how synergistic effects of multiple compounds influence foraging by herbivores. Controlling the identities, concentrations, and ratios of PSMs within this mixture eliminates the potentially confounding variation found within

plant populations. Lastly, comparing preferences by herbivores for this mixture relative to individual compounds that constitute the mixture facilitates the identification of specific PSMs most likely to influence foraging under natural conditions. Although a simplified mixture is incapable of representing the full complexity of PSMs produced by wild plants, the individual compounds identified using this method could be considered viable biomarkers to understand how PSMs influence diet selection in wild herbivores. In addition, *in vivo* experiments that reveal the pharmacokinetics (e.g., rates of detoxification, Sorensen et al. 2004, Sorensen and Dearing 2006, McLean et al. 2007, Shipley et al. 2012) and pharmacodynamics (e.g., mechanism of toxicity, Foley et al. 1995, Sorensen et al. 2005b, McLean et al. 2007) can provide a mechanistic understanding of why individual PSMs, specific doses, or mixtures do or do not influence foraging by herbivores.

We used this hybrid approach to provide causal insights into realistically complex relationships between monoterpenes, a class of PSMs, in sagebrush (*Artemisia tridentata* spp.) and the foraging behavior of a specialist (pygmy rabbits, *Brachylagus idahoensis*) and generalist (mountain cottontail, *Sylvilagus nuttallii*) mammalian herbivore. Sagebrush has relatively high levels of PSMs (Kelsey et al. 1982) that influence the foraging behavior of herbivores (Carpenter et al. 1979, Johnson and Hansen 1979). Monoterpenes are a class of volatile PSMs that comprise 1-4% of the dry weight (DW) of sagebrush (White et al. 1982). High concentrations of both total monoterpenes and specific individual monoterpenes have been correlated with reduced intake among a variety of free-ranging herbivores (Frye et al. 2013, Ulappa et al. 2014, Chapter 1) and captive herbivores (Lamb et al. 2004, Dziba and Provenza 2008, Kirmani et al. 2010,

Shiple et al. 2012). Specifically, monoterpenes from sagebrush may inhibit digestibility of protein, (Striby et al. 1987, Kohl et al. 2015), a critical nutrient for many herbivores. Pygmy rabbits rely almost exclusively on sagebrush for food (Green and Flinders 1980) and have a higher tolerance to sagebrush and specific monoterpenes than mountain cottontails (Shiple et al. 2012). However, foraging by pygmy rabbits is compromised, at least in part, by concentrations of monoterpenes (Ulappa et al. 2014, Shiple 2009). The prevalence of monoterpenes in sagebrush, their putative and differential effects on foraging by a variety of specialist and generalist herbivores (Lawler et al. 1998, Boyle et al. 1999, Wiggins et al. 2003, Shiple et al. 2012), and commercial availability of pure forms of monoterpenes make them an ideal class of PSMs for comparing the effects of individual versus mixtures of PSMs on foraging by herbivores.

We compared preference for a mixture of monoterpenes versus individual monoterpenes offered to the specialist pygmy rabbit (*Brachylagus idahoensis*), and the generalist mountain cottontail rabbit (*Sylvilagus nuttallii*). The mixture of monoterpenes was representative of the composition and ratio of monoterpenes quantified in Wyoming big sagebrush (*Artemisia tridentata* subsp. *wyomingensis*) from sites where both pygmy rabbits and mountain cottontails forage. We predicted that specialists and generalists would differ in their preferences for mixtures versus individual monoterpenes. Specifically, because toxins consumed individually could overwhelm any single detoxification pathway (Estell 2010), we predicted that generalists would show strong preferences for the mixture of monoterpenes which contained lower concentrations of any one monoterpene. We also predicted that specialists would have higher tolerances

than their generalist counterpart for both individual and mixtures of monoterpenes, and therefore not show clear preferences.

Methods

Animal Capture and Care

We captured adult pygmy rabbits from sagebrush-dominated sites in Blaine, Camas, and Lemhi Counties in Idaho (Idaho Department of Fish and Game collection permits 100310 and 01813) and Beaverhead County, Montana (Montana Department of Fish, Wildlife, and Parks scientific collection permit 2014-062). We captured mountain cottontails rabbits in Pullman, Washington (Washington Department of Fish and Wildlife Scientific Collection Permit #14-206). When not undergoing trials, all animals were housed indoors in individual 1.2 x 1.8 m mesh cages at the Small Mammal Research Facility at Washington State University (Boise State University Institutional Animal Care and Use Committee Protocol # 006-AC12-009, Washington State University Institutional Animal Care and Use Committee Protocol # 04513-001), and provided with pelleted commercial rabbit chow (Purina Professional Rabbit Chow, Purina Mills LLC, St. Louise, MO) and fresh water, both *ad libitum*, with approximately 15 g of fresh mixed greens and greenhouse-grown sagebrush. The rabbit chow was the same used throughout experimental trials and was similar in fiber (36%) and nitrogen (3.4%) to sagebrush leaves (30% fiber and 2.5-4.5% nitrogen, Camp et al. 2015). Rabbits were maintained at approximately 15° C throughout trials.

Identification of Monoterpenes

To create a diet that mimicked the natural concentration of monoterpenes in sagebrush, we first sampled 420 individual Wyoming big sagebrush plants in an ~ 1000-

ha area with evidence of foraging by both pygmy rabbits and mountain in southern Blaine County, Idaho (43°14' N, 114°19' W; elevation: 1470 m). In March 2013, we clipped ~ five sprigs of each plant 2-mm stem diameter to mimic rabbit foraging. Samples were stored on ice in the field before being transferred to a -20° C freezer.

Frozen sagebrush samples were coarsely ground in liquid nitrogen with a mortar and pestle. Relative concentrations of monoterpene from each sample (100 mg wet weight) were determined using headspace gas chromatography (Appendix A). All samples were analyzed using an Agilent 5890 series II gas chromatograph (Santa Clara, CA) coupled with a Hewlett-Packard HP7694 headspace autosampler (Palo Alto, CA). One mL of headspace gas from each sample was injected into an Agilent J&W DB-5 capillary column (Santa Clara, CA). Retention times of individual monoterpenes and individual areas under the curve (AUC) were quantified using Hewlett-Packard ChemStation software version B.01.00 (Palo Alto, CA). Peaks were identified using co-chromatography with known standards. Samples were then dried at 60° C for 24 hours and monoterpenes were quantified as AUC per 100 µg of DW of sagebrush. Relative concentrations (AUC/100 µg DW) of individual monoterpenes were then averaged across all plants and divided by the total amount of monoterpenes to obtain ratios among constituent compounds. We chose the top five most prevalent individual monoterpenes based on relative AUC, which together accounted for 87% of the total monoterpenes in sagebrush (Table 2.2), to create a monoterpene mixture that represented whole sagebrush. By preserving the ratios found in sagebrush we could treat the food pellets with a realistic 1% monoterpenes by weight without affecting the relative amounts of the constituent compounds.

Food Preparation

To create artificial diets offered to captive rabbits, we added monoterpenes to commercially available rabbit chow (Purina Professional Rabbit Chow, Purina Mills LLC, St. Louise, MO). The five most common monoterpenes identified (1,8-cineole, α -pinene, β -pinene, camphor, and camphene) in our Wyoming sagebrush samples were available commercially at 99% purity or greater (Sigma Aldrich, St. Louis, MO). To simulate the ratios of an average sagebrush plant, these five compounds were added to food in the same average proportions in which they occurred naturally (Table 2.2).

The monoterpene mixture was added to commercial pelleted rabbit chow at 1% of DW weight. Camphor and camphene are solids at room temperature and cannot be added homogenously to rabbit chow, whereas 1,8-cineole, α -pinene, and β -pinene are liquid and can be directly added to chow. Pure camphor (1.71 M) and camphene (1.82 M) were therefore dissolved together in methylene chloride ($\geq 99.8\%$ pure, Sigma Aldrich, St. Louis, MO). The mixture was thoroughly mixed with rabbit chow in a glass jar. The treated chow was then spread in a single layer in a fume hood for 6 hours to allow the highly volatile solvent to evaporate. With minimal evaporation of camphor and camphene, this resulted in the desired final concentrations of monoterpenes (Table 3). In a preliminary study, we determined that pygmy rabbits and mountain cottontails did not discriminate between control rabbit chow and chow that was spiked with methylene chloride only (no camphor and camphene) and allowed to evaporate for 6 hours (Appendix D). After the solvent was evaporated off, the remaining liquid monoterpenes were thoroughly mixed with the rabbit chow already treated with camphor and camphene in a glass jar. To prevent the volatilization of monoterpenes, all treated chow was stored at

-20° C until offered to rabbits. Samples of prepared food were saved in sealed scintillation vials at -20° C before being analyzed for concentrations of monoterpenes via gas chromatography (Table 3).

Feeding Trials

Before beginning feeding trials with monoterpene diets, all animals were acclimated to receiving commercial chow offered in equal portions at two feeding stations equal distances from a nest box over a period of 3 days. After acclimation, rabbits were offered a choice between chow treated with either 1% of each individual monoterpene or 1% monoterpene mixture by DW. This concentration represents the lower end of the range of monoterpene concentration by weight in sagebrush (Kelsey et al. 1982), and corresponds with concentrations at which individual monoterpenes reduce the intake of mountain cottontails (Shipley et al. 2012). Individual monoterpene treatments that were paired with the mixture were administered sequentially, but in a randomly-determined order. Animals were also given rest periods of 3 to 5 days between treatments to prevent habituation. Treatments were first offered on a randomly determined (coin flip) side of the pen, followed by alternating sides for three days to avoid directional bias (Utz 2012). We recorded the amount of food offered and remaining (orts) after 24 hours from each choice (single monoterpene versus mixture) in feeding trial (encompassing both diurnal and nocturnal intake), and corrected for dry weight by drying the orts and a sample of the food pellets offered at 100° C for ≥ 24 hrs. Five feeding trials were conducted, comparing the monoterpene mixture to each of the five individual monoterpenes.

Statistical Analysis

To determine preferences for or against single monoterpenes compared to a mixture, we divided the amount of each treatment consumed (i.e., single monoterpene versus mixture) by the total amount of food consumed from both choices each day. We then averaged these proportions of treatment consumed across the three days of each trial for each animal. Preferences for the mixture (compared to each individual compound) are reported as the mean proportion (\pm 95% confidence interval) of the total food consumed constituting the monoterpene mixture. Preferences were reported separately for each treatment comparison (n=5), and for each species (i.e. pygmy rabbits and mountain cottontails).

Animals consuming an equal proportion (0.50) from the feeding station with the monoterpene mixture and the feeding station with a single monoterpene were considered to have no preference between the treatments. To compare the proportion of mixture consumed to the individual monoterpene it was offered against, we created a mixed linear model with rabbit species and treatment (i.e., individual monoterpene offered), and the interaction of species and treatment as main effects, and with individual rabbit as a random effect. We then used a contrast statement to compare the proportion of mixture consumed to 0.50. We used two-sampled t-tests to compare proportions of mixture consumed in each trial between species.

All statistical analyses were conducted using R version 3.2.0 (R Foundation for Statistical Computing 2015) and JMP Pro 11.0 (SAS Institute Inc. 2013).

Results

The proportion of mixture consumed did not differ between species ($F_{1,96} = 0.03$, $p = 0.86$), but did differ with treatment ($F_{3,96} = 16.04$, $p < 0.0001$), and species \times treatment interaction ($F_{3,96} = 114.35$, $p < 0.0001$). When offered choices between mixed monoterpenes compared to five single monoterpenes, pygmy rabbits showed no preference when the mix was paired with α -pinene ($t_{14} = -2.075$, $p = 0.057$), β -pinene ($t_{14} = 1.488$, $p = 0.159$), or camphene ($t_{14} = -4.267$, $p = 0.68$). However, pygmy rabbits consumed a greater proportion of their daily intake from patches with camphor ($t_{14} = -4.250$, $p = 0.0008$) and 1,8-cineole ($t_{14} = -4.140$, $p = 0.001$) over the mixture (Fig. 5a). Pygmy rabbits consumed twice as much camphor ($67\% \pm 9\%$) as the monoterpene mixture ($33\% \pm 9\%$) and more than twice as much 1,8-cineole ($70\% \pm 10\%$) as the monoterpene mixture ($30\% \pm 10\%$) (Figure 2.1).

Similar to pygmy rabbits, mountain cottontails showed no significant preference between α -pinene and the monoterpene mixture ($t_{11} = 0.317$, $p = 0.52$). However, they showed significant preferences for both camphene ($t_{11} = -14.067$, $p < 0.0001$) and 1,8-cineole ($t_{11} = -25.204$, $p < 0.0001$), consuming $85\% (\pm 5\%)$ camphene versus $15\% (\pm 5\%)$ monoterpene mixture, and $96\% (\pm 4\%)$ 1,8-cineole versus $4\% (\pm 4\%)$ monoterpene mixture. Mountain cottontails preferred the monoterpene mixture over β -pinene ($t_{11} = 0.643$, $P < 0.0001$) and camphor ($t_{11} = 4.991$, $P = 0.0004$). They consumed $25\% (\pm 9\%)$ β -pinene compared to $75\% (\pm 9\%)$ monoterpene mixture and $31\% (\pm 8\%)$ camphor versus $69\% (\pm 8\%)$ monoterpene mixture (Figure 2.1).

Neither pygmy rabbits nor cottontails showed a significant preference for α -pinene compared to the monoterpene mixture, nor did their preferences differ

significantly from one another ($t_{25} = -1.40$, $p = 0.91$). Cottontails preferred the monoterpene mixture to β -pinene, and camphene offered singly to the mixture, whereas pygmy rabbits showed no preference for either. In both cases, the preferences between species differed significantly (β -pinene: $t_{25} = -4.30$, $p = 0.0006$; camphene: $t_{25} = 3.10$, $p < 0.0001$). Pygmy rabbits preferred camphor to the monoterpene mixture, and cottontails preferred the mixture to camphor offered singly, with the proportion consumed differing between species ($t_{25} = -4.24$, $p = 0.0005$). Both pygmy rabbits and cottontails preferred 1,8-cineole to the monoterpene mixture, but cottontails showed a significantly stronger preference ($t_{25} = 5.31$, $p < 0.0001$).

Discussion

Our results suggest that herbivores view single and mixed PSMs differently, and that further differences in preferences exist between specialists and generalists. Food preferences and intake have long been hypothesized to represent variations in the behavioral and physiological abilities of herbivores to cope with ingested PSMs (Freeland and Janzen 1974, Freeland 1991, Foley et al. 1999).

Demonstrated preferences for individual or mixtures of monoterpenes are likely functions of the dose-dependent pharmacological consequences of ingested PSMs (Forbey et al. 2011, Kohl et al. 2015). 1,8-cineole, for example, has been used as a representative monoterpene in numerous captive feeding trials (Wiggins et al. 2003, McLean et al. 2007, Kirmani et al. 2010, Shipley et al. 2012), but seemed to be well-tolerated by both pygmy rabbits and mountain cottontails. Pygmy rabbits consumed 2.3 times and cottontails consumed 24.0 times as much food treated with 1,8-cineole than that treated with a mixture containing 1,8-cineole plus four other monoterpenes.

Furthermore, captive pygmy rabbits did not reduce intake until 1,8-cineole concentrations in the diet increased beyond 5% (Shipley et al. 2012). The same study showed that mountain cottontails reduced their total food consumption at 1,8-cineole concentrations of only 1%. However, mountain cottontails continued to consume smaller portions of food treated with concentrations as high as 7% (Shipley et al. 2012). It is possible, therefore, that neither pygmy rabbits nor mountain cottontails in our study consumed sufficient doses of 1,8-cineole to deter feeding.

Despite the evidence that doses of 1,8-cineole were too small to reduce food preferences by pygmy rabbits or mountain cottontails, these concentrations were significantly higher than those an animal would encounter in wild sagebrush. Even sagebrush consisting of up to 4% monoterpenes by DW (Kelsey et al. 1982, White et al. 1982), would contain less than 0.5% 1,8-cineole by DW. Although a study of free-ranging pygmy rabbits found that 1,8-cineole was not a reliable predictor of foraging (Ulappa et al. 2014), these results contradict the findings from Chapter 1, in which 1,8-cineole concentrations significantly influenced the odds of both foraging a sagebrush plant and unspecified use of that sagebrush plant.

One possible explanation for this discrepancy is that 1,8-cineole itself does not have deleterious effects at the concentrations in which it occurs in sagebrush. Instead, 1,8-cineole may co-vary with another, more toxic, unmeasured PSM that is more difficult for pygmy rabbits to detect directly, thereby serving as a sensory cue. A similar arrangement has been proposed in *Eucalyptus* trees, in which monoterpene concentrations, including 1,8-cineole specifically, are positively correlated with concentrations of formylated phloroglucinol compounds (FPCs), which themselves

strongly discourage foraging by herbivores (Moore et al. 2004, Matsuki et al. 2011). Captive trials that dissociate the cue from the negative consequences of consuming the more toxic PSM might be expected to show herbivores demonstrating no or reduced preference against the cue (Matsuki et al. 2011).

Because pygmy rabbits did not demonstrate a preference for the mixture over any of the five constituent monoterpenes, it suggests that no single compound at these concentrations was consumed at a dose sufficient to deter foraging. In contrast, mountain cottontails avoided β -pinene and camphor at 1% in the diet in favor of the monoterpene mixture where these specific compounds were in lower concentrations (0.018% β -pinene by DW, 0.48% camphor by DW). Differences in the doses at which PSMs begin to influence foraging may represent differences in the abilities of pygmy rabbits and mountain cottontails to detoxify these compounds.

Quantifying detoxification capabilities requires comparing the pharmacokinetics (i.e., absorption, distribution, metabolism, and excretion) and pharmacodynamics (i.e., mechanism of action) of specific compounds. *In vivo* studies that quantify ingested versus excreted PSMs (Sorensen and Dearing 2003a, Shipley et al. 2012) allow comparisons of an herbivore's abilities to limit the absorption of different compounds, while *in vitro* assays of efflux transporters and their substrates (see Sorensen et al. 2006) facilitate the same comparisons among taxa. Evidence exists that dietary specialists can more effectively limit the absorption of PSMs than generalists. For example, specialist woodrats absorbed five times less of the most abundant monoterpene in juniper (α -pinene) than generalists counterparts after receiving identical doses (Sorensen and Dearing 2003b). Pygmy rabbits and greater sage-grouse are also able to excrete

unchanged PSMs (Forbey, in preparation). Quantifying rates of metabolism by liver enzymes (see Sorensen et al. 2006), provides another means of measuring herbivores' detoxification abilities. Preliminary data from *in vitro* assays suggest that pygmy rabbit enzymes detoxify monoterpenes significantly faster than those from mountain cottontails (Forbey, unpublished data). Although similar techniques have been used in the pharmaceutical industry for decades, their incorporation into investigations of plant-herbivore interactions is relatively recent (Forbey and Foley 2009). One significant barrier to their more widespread employment is the diversity of compounds herbivores encounter. Mixtures of PSMs isolated from whole plants may contain dozens or hundreds of individual compounds (Shafizadeh and Melnikoff 1970, Kelsey and Shafizadeh 1979, Welch and McArthur 1981), making it difficult or impossible to identify potential drivers of foraging. Our use of a simplified mixture makes it possible to narrow the search to a select few compounds that may play significant roles in foraging ecology and are more amenable to *in vitro* assays. The chemical and physiological effects of those specific compounds on herbivores can then be explored more thoroughly, potentially enabling their use as valuable biomarkers of palatability.

Generalist herbivores like cottontails are often thought to use a variety of pathways to enable the efficient detoxification of low doses of the diverse PSMs they consume eating a varied diet (Freeland and Janzen 1974, Dearing and Cork 1999, Dearing et al. 2000, Shipley et al. 2009). Diet mixing has been proposed as a mechanism by which generalists can avoid overwhelming a single detoxification pathway, and other generalist herbivores have been shown to consume less food when restricted to a single PSM than when offered a diet containing mixed PSMs (Dearing and Cork 1999, Burritt

and Provenza 2000, Wiggins et al. 2003). This pattern remains even when the diets are identical nutritionally (Bernays et al. 1994), supporting the hypothesis that saturated detoxification pathways can play a role in limiting intake (Freeland and Janzen 1974). Under this assumption, we expected mountain cottontails to prefer the monoterpene mixture under most conditions. Their preference for single compounds over the monoterpene mixture may indicate some deterrent of the mixture itself.

Synergistic interactions between compounds, defined as greater effects of mixtures of compounds than those expected given their individual effects (Nelson and Kursar 1999, Richards et al. 2015), could explain pygmy rabbits' demonstrated preference against, or indifference to, the monoterpene mixture. Synergistic effects of mixtures of PSMs have been repeatedly demonstrated in plant-insect systems (Dyer et al. 2003, Richards et al. 2010, 2012), including monoterpenes specifically (Hummelbrunner and Isman 2001, Pavela 2008). The pharmaceutical industry has again forged the way in developing methods for detecting and describing synergy, often referred to as drug-drug interactions (Prichard and Shipman 1990, White et al. 1996), but these methods have not been widely applied to ecological systems. Some early evidence suggests that PSMs in sagebrush may inhibit the proteins that regulate their absorption, and therefore the detoxification, of ingested compounds (Forbey, unpublished data). Our results demonstrate that not all compounds in sagebrush are likely to interact with one another, and those that do are likely to effect different animal species in different ways. Identifying combinations of compounds likely to deter foraging provides both a better understanding of foraging behavior in natural settings, and a road map for future researchers investigating the mechanisms of PSM mixtures.

Taken together, particularly potent individual PSMs and small combinations acting synergistically could serve as valuable biomarkers of plant palatability. Recent advances in remote sensing suggest that PSM concentrations can be assessed, *in situ*, across large spatial scales (Dury et al. 2001, Moore et al. 2010, Couture et al. 2013). Mapping compounds that exert significant influences on foraging behavior across habitats and landscapes could assist researchers and managers in identifying and conserving high quality food sources and habitats.

Conclusion

In summary, the indifference of captive pygmy rabbits to four out of five individual monoterpenes compared to the mixture further suggests that dietary specialists are better adapted than generalists such as mountain cottontails to consume PSMs they regularly encounter. Likewise, the significant preferences of mountain cottontails between four out of five single compounds versus a mixture (either for or against), suggests that generalists are sensitive to the effects of PSMs in the ratios commonly found in sagebrush. These differences in preference between single compounds and mixtures, as well as the differences between specialists and generalists, may help to explain observed patterns in foraging among free-ranging animals.

The preferences of both pygmy rabbits and mountain cottontails suggest that individual PSMs differ in their effects on herbivores. Preferences for single compounds or mixtures may reflect the dose-dependent effects of consuming those compounds, the synergistic effects of consuming PSM mixtures, or both. Differences in preference between the two rabbit species reinforce evidence in other systems that detoxification capabilities vary among herbivores, specifically between specialists and generalists

(Freeland and Janzen 1974, Boyle et al. 1999, Dearing and Cork 1999, Johnson 1999, Sorensen and Dearing 2003a), suggesting that pygmy rabbits are uniquely adapted to effectively deal with the most common PSMs in sagebrush.

The role of plant secondary metabolites in influencing patterns of foraging and habitat selection is slowly becoming better understood and more appreciated (Lawler et al. 1998, Moore and Foley 2005, Moore et al. 2010, Rosenthal and Berenbaum 2012, Denno 2012, Frye et al. 2013, Ulappa et al. 2014). These relationships may become even more important as climates change. In regions where rising temperatures and decreasing availability of water place additional stress on plants, plants may respond by increasing production of PSMs (Coley et al. 1985, Hobbie 1992, Forbey et al. 2013). Conversely, some sagebrush habitats are predicted to see seasonal changes in precipitation patterns including increasing spring rains (Klos et al. 2014) that could lead to faster growth (Germino and Reinhardt 2014) and reductions in PSM production (Coley et al. 1985, Coley 1998). Finally, many detoxification mechanisms employed by herbivores are metabolically costly and may be compromised in thermally stressed animals (McLister et al. 2004, Dearing 2012, Forbey et al. 2013). Attempts to manage, conserve, and restore chemically defended plants like sagebrush and the herbivores that rely on them must take into account the potentially complicating effects of changing climates.

The complexity of plant secondary chemistry and its diverse effects on the physiology and behavior of herbivores has made it difficult to identify the compounds and combinations of compounds most likely to drive complex patterns of foraging. When forced to choose at random from hundreds of potentially influential PSMs, chemical ecologists and physiologists have been hard pressed to narrow their focus and

determine mechanistic relationships between compounds and the animals that consume them. Field-based studies can be used to identify and quantify the most common PSMs thought to influence habitat selection. Those data in turn, can inform the hybrid approach we present in this paper, in which simplified mixtures of PSMs can identify the few compounds most likely to influence foraging, either singly or synergistically. *In vitro* studies can then identify and quantify the specific mechanisms by which those compounds influence herbivores. Together, this approach can help investigators better understand how complexity of natural habitats affects foraging and habitat selection.

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Tables

Table 2.1 Headspace gas chromatography retention times for the five most abundant individual monoterpenes quantified from Wyoming big sagebrush (*Artemisia tridentata* subsp. *wyomingensis*) samples collected in Southern Blaine County, ID in March 2013. Monoterpenes were identified using co-chromatography with known standards.

Monoterpene Name	Retention Time (minutes)
α -pinene	13.00
Camphene	13.58
β -pinene	14.70
1,8-cineole	16.81
Camphor	21.15

Table 2.2 The five most abundant monoterpenes quantified from Wyoming big sagebrush (*Artemisia tridentate* subsp. *wyomingensis*) samples collected in Southern Blaine County, ID in March 2013, and their proportional representations of the total monoterpenes quantified and proportion in the mixture created to represent whole sagebrush.

Monoterpene	Proportion of total monoterpenes (\pm S.E.)	Proportion of monoterpene mixture
α -pinene	2.2(\pm 0.2)%	2.5%
Camphene	19(\pm 0.8)%	22%
β -pinene	1.7(\pm 0.1)%	2%
1,8-cineole	7.5(\pm 0.6)%	8.5%
Camphor	56.5(\pm 1.7)%	65%
Total	87(\pm 2.9)%*	100%

*Total does not equal 100% because other monoterpenes comprise the remaining portion in whole sagebrush.

Table 2.3 The desired proportion of monoterpenes in artificial diets offered to captive pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontails (*Sylvilagus nuttallii*) based on actual concentrations in Wyoming big sagebrush (*Artemisia tridentata* subsp. *wyomingensis*) and the actual proportion measured in frozen diets consisting of commercial rabbit pellets treated with the mixture of monoterpenes. Concentrations were determined using co-chromatography with known standards.

Monoterpene	Desired proportion of mixture	Actual proportion of mixture \pm 95% C.I.
α -pinene	2.5%	1.6 \pm 0.3%
Camphene	22%	32.7 \pm 5.8%
β -pinene	2.0%	1.8 \pm 0.3%
1,8-cineole	8.5%	8.5 \pm 2.3%
Camphor	65%	55.4 \pm 5.4%
Total	100%	100%

Figures

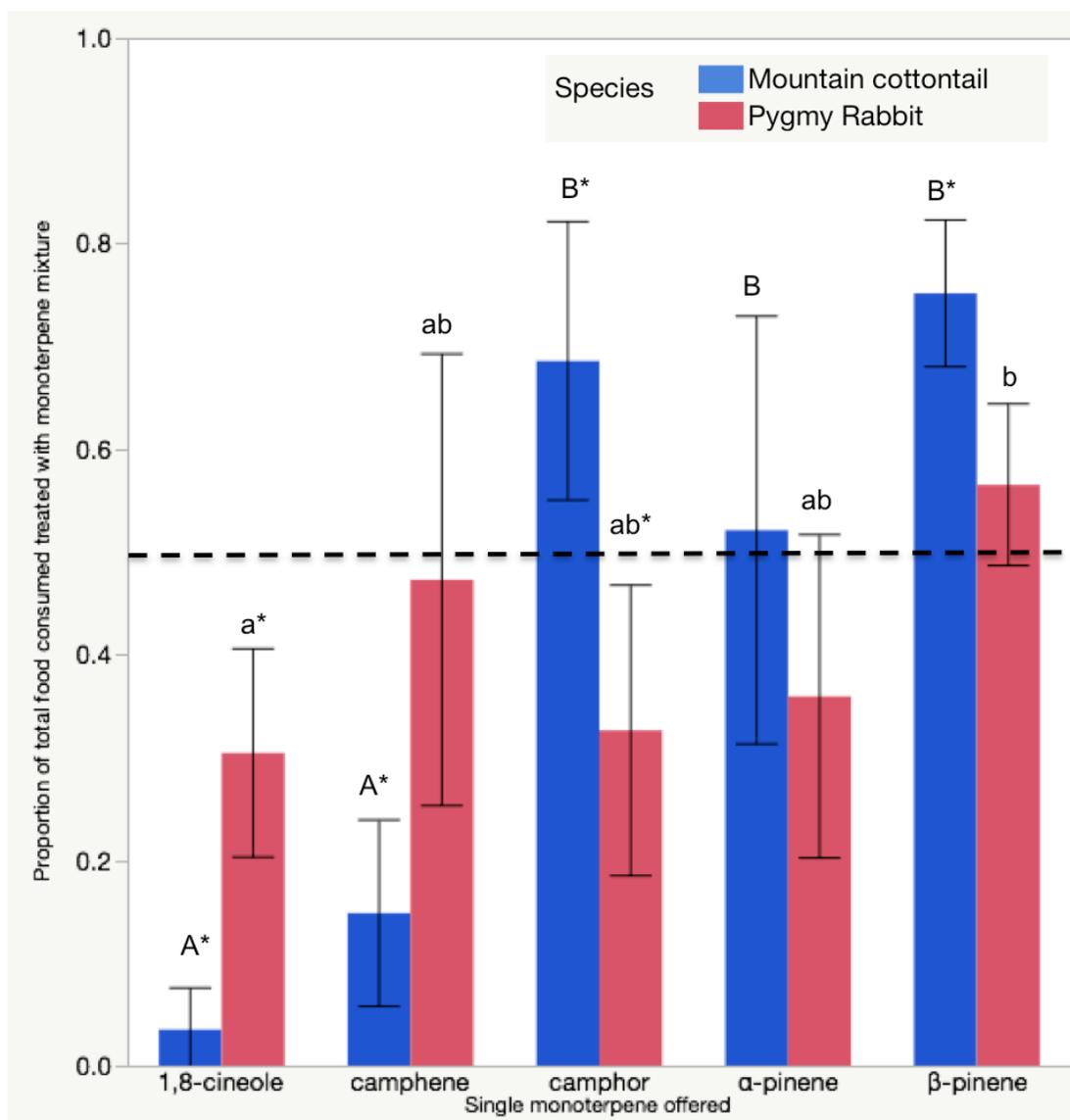


Figure 2.1 Mean proportions (\pm 95% confidence intervals) of total mass consumed by pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontails (*Sylvilagus nuttallii*) from a feeding station consisting of a diet of commercial rabbit pellets containing mixture of monoterpenes paired with a diet containing a single monoterpene. When the mixture constitutes a 0.50 proportion of total food consumed, rabbits are considered to have no preference. Lower case letters denote differences among specific single monoterpenes paired with the monoterpene mixture for pygmy rabbits, and capital letters denote significant differences for mountain cottontails. An asterisk denotes proportions that were significantly different from 0.5 for each species with $\alpha = 0.05$.

GENERAL CONCLUSION

The calculus by which herbivores assess and select habitats is complex. No habitat is ideal, and animals must make tradeoffs between food and safety, nutrition and toxicity, single and mixed PSMs. Understanding and describing these tradeoffs requires measuring the parameters of a habitat that matter to animals living and moving within it, differentiating uses of that habitat that vary spatially, temporally and with changing physiological and behavioral needs, and testing mechanistically the effects of habitat parameters on animals. While difficult, the integration of field- and laboratory-based studies can provide a blueprint for more thoroughly describing interactions between herbivores and their habitats.

By measuring habitat parameters suggested to influence habitat selection in conjunction with multiple metrics of use, we were able to match specific plant characteristics with distinct behaviors and identify potential tradeoffs pygmy rabbits make when choosing habitats for different purposes. Though these relationships were necessarily correlative, they help to simplify a complex system and provide narrowed objectives for future study. Assays of PSMs and nutrition are resource and labor intensive, but emerging remote sensing technologies may allow investigators to assess similar habitat parameters across larger spatial and temporal scales more easily. Combined with GPS or telemetry-based monitoring of animal behavior, these techniques could describe potential tradeoffs at landscape scales.

The putative relationships established between habitat parameters and distinct types of habitat use can be used to design captive or laboratory investigations of the mechanisms responsible for those relationships. While field work suggested that foraging pygmy rabbits evaluate habitat quality at least in part based on PSM concentrations, we used manipulative, captive feeding trials to demonstrate that individual PSMs are viewed differently when compared to one another singly and a mixture. In other words, we demonstrated that it is likely herbivores make even finer scale tradeoffs within habitat parameters. We propose that the same techniques can be used describe preferences and tradeoffs for other broad habitat characteristics (e.g., fiber and protein within nutrition, visibility and concealment within safety). These results can also inform *in vitro* assays that demonstrate clear mechanistic relationships between individual parameters and herbivores. For example, metabolic stability assays may show that pygmy rabbits have different rates of detoxification for different PSMs, thereby explaining observed variations in preference. These sorts of clear, causal relationships between specific, measurable habitat parameters and animal behavior and physiology can then be used to further inform field-based studies, and predict habitat selection *in natura*.

By mapping habitat parameters demonstrated to be of importance to herbivores across landscapes, subsequent investigations should be able to predict areas likely to be used or not used, used for different purposes, and perhaps even the intensity of use. Validating those predictions with actual measurements of use will facilitate the honing of predictive models and contribute to a vastly improved capacity for assessing the functional quality of habitats. This ability will be vital for resource managers tasked with conserving and restoring sagebrush habitats and the species that depend on them. We

believe this process can be applied to other systems as well, leading to more nuanced understandings of the processes animals use to select habitats and improved tools for identifying and managing critical habitats.

APPENDIX A

Gas Chromatograph Settings

To quantify monoterpene concentrations in sagebrush samples, we used an Agilent 7694 headspace sampler and an Agilent 6890N gas chromatograph. One ml of headspace gas was injected into a J&W DB-5 capillary column (30m x 250 μ m x 0.25 μ m).

Operating conditions for the headspace sampler were: oven temperature at 100°C, loop temperature at 110°C, transfer line temperature at 120°C, a vial equilibrium time of 20 min, a pressurization time of 0.20 min, a loop fill time of 0.50 min, a loop equilibrium time of 0.20 min, and an injection time of 0.50 min.

Operating conditions for the GC were: splitless injector at 250°C, flame ionization detector at 300°C, oven temperature at 40°C for 2 min, then increasing 3°C/min to 60°C, then increasing 5°C/min to 120°C, then increasing 20°C/min to 300°C, and held at 300°C for 7 min. The make-up gas was nitrogen and the carrier gas was helium. The inlet pressure was 80 KPa with a flow rate of 1.0 mL/min.

APPENDIX B

Stage One Model Selection Results

Tables

Table B.1 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the first stage of pygmy rabbit use models at all plants. Variables appearing in models above a null intercept-only model with $\Delta AIC_c \leq 2.0$ were included in global models (bold). For the PSM category, the top seven models and the null model are presented.

Response Variable	Predictor Category	Model	Log Likelihood	K	AIC_c	ΔAIC_c	w_i
Any Use	PSM	Borneol ¹ + 1,8-cineole ¹	-215.9	4	439.9	0.00	0.20
		Borneol + 1,8-cineole + ρ -cymene ¹	-215.3	5	440.8	0.92	0.12
		Borneol + β -pinene ¹ + 1,8-cineole	-215.6	5	441.4	1.57	0.09
		Borneol + 1,8-cineole + Unknown 3.2 ¹	-215.8	5	441.8	1.94	0.07
		Borneol + Camphor ¹ + 1,8-Cineole	-215.9	4	441.9	2.06	0.07
		Borneol + β -pinene + 1,8-cineole ¹ + ρ -cymene	-215.2	5	442.6	2.74	0.05
		Borneol + Camphor + 1,8-Cineole + ρ -cymene	-215.3	5	442.9	3.00	0.04
		Null		2	448.9	8.99	0.00

		-222.4				
Nutrient	Protein ²	-211.0	3	428.1	0.00	1.00
	Null	-222.4	2	448.9	20.75	0.00
Safety	Location ³	-189.6	3	385.3	0.00	0.72
	Location + Concealment ⁴	-189.5	4	387.2	1.87	0.28
	Concealment	-221.0	4	448.1	62.81	0.00
	Null	-222.4	2	448.9	63.55	0.00

¹Monoterpene (AUC/100 µg DW)

²Crude protein (% DW)

³Binary on- or off-mound designation of plant

⁴Aerial concealment available at base of plant (%)

Table B.2 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the first stage of pygmy rabbit use models at on-mound plants. Variables appearing in models above a null intercept-only model with $\Delta AIC_c \leq 2.0$ were included in global models (bold). For the PSM category, the top seven models and the null model are presented.

Response Variable	Predictor Category	Model	Log Likelihood	K	AIC_c	ΔAIC_c	w_i
Any Use	PSM	1,8-cineole ¹	-79.5	2	165.2	0.00	0.07
		Null	-80.6	1	165.3	0.09	0.07
		β -pinene ¹ + 1,8-cineole	-78.8	3	165.8	0.64	0.05
		Borneol ¹ + 1,8-cineole	-79.0	3	166.3	1.15	0.04
		Camphor ¹ + 1,8-cineole	-79.0	3	166.3	1.17	0.04
		β -pinene	-80.2	2	166.5	1.33	0.04
		Borneol	-80.3	2	166.7	1.54	0.03
	Total monoterpene ²	-80.3	2	166.7	1.57	0.03	
	Nutrient	Null	-80.6	1	165.3	0.00	0.73
		Protein ³	-80.6	2	167.3	2.01	0.27
	Safety	Distance ⁴	-187.6	2	381.3	0.00	0.71
		Concealment ⁵ + Distance	-187.5	3	383.1	1.80	0.29
		Concealment	-221.0	2	448.1	66.85	0.00
		Null	-222.4	2	118.9	67.59	0.00

¹Monoterpene (AUC/100 μ g DW)

²Total monoterpene concentration (cumulative concentration of all compounds with retention times ≤ 28.0 min. [area under the chromatogram curve/100 μ g dry weight])

³Crude protein (% DW)

⁴Distance of plant to nearest active burrow entrance (m)

⁵Aerial concealment available at base of plant (%)

Table B.3 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the first stage of pygmy rabbit use models at off-mound plants. Variables appearing in models above a null intercept-only model with $\Delta AIC_c \leq 2.0$ were included in global models (bold). For the PSM category, the top seven models and the null model are presented.

Response Variable	Predictor Category	Model	Log Likelihood	K	AIC_c	ΔAIC_c	w_i
Any Use	PSM	1,8-cineole ¹	-109.6	2	225.3	0.00	0.07
		Borneol ¹ + 1,8-cineole	-108.7	3	225.6	0.23	0.07
		Null	-110.8	1	225.7	0.33	0.06
		Borneol	-110.1	2	226.3	0.99	0.05
		Total monoterpenes ²	-110.4	2	226.8	1.46	0.04
		β -pinene ¹	-110.4	2	227.0	1.61	0.03
		β -pinene + 1,8-cineole	-109.5	3	227.3	1.93	0.03
	Nutrient	Borneol + β -pinene	-109.6	3	227.3	1.94	0.03
		Protein ³	-108.4	2	222.9	0.00	0.80
		Null	-110.8	1	225.7	2.82	0.20
	Safety	Null	-80.6	1	165.3	0.00	0.45
		Concealment ⁴	-80.2	2	166.5	1.22	0.25
		Distance ⁵	-80.4	2	166.9	1.67	0.20
		Distance + Concealment	-80.0	3	168.2	2.92	0.10

¹Monoterpene (AUC/100 μ g DW)

²Total monoterpene concentration (cumulative concentration of all compounds with retention times ≤ 28.0 min. [area under the chromatogram curve/100 μ g dry weight])

³Crude protein (% DW)

⁴Aerial concealment available at base of plant (%)

⁵Distance of plant to nearest active burrow entrance (m)

Table B.4 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the first stage of pygmy rabbit foraging models at all plants. Variables appearing in models above a null intercept-only model with $\Delta AIC_c \leq 2.0$ were included in global models (bold). For the PSM category, the top seven models and the null model are presented.

Response Variable	Predictor Category	Model	Log Likelihood	K	AIC_c	ΔAIC_c	w_i
Foraging	PSM	Borneol ¹ + 1,8-cineole ¹ + Unknown 3.1 ¹	-182.54	4	375.3	0.00	0.12
		Borneol + 1,8-cineole	-183.6	3	375.3	0.09	0.12
		Borneol + β -pinene ¹ + 1,8-cineole + Unknown 3.1	-182.2	5	376.6	1.39	0.06
		Borneol + β -pinene + 1,8-cineole	-182.4	4	376.9	1.65	0.05
		Borneol + Camphor ¹ + 1,8-cineole + Unknown 3.1	-182.3	5	377.1	1.87	0.05
		Borneol + 1,8-cineole + ρ -cymene ¹ + Unknown 3.1	-182.4	5	377.1	1.87	0.05
		Borneol + Camphor + 1,8-cineole	-183.5	4	377.1	1.90	0.05

	Null	-189.0	1	382.0	6.78	0.00
Nutrient	Protein ²	-176.6	2	359.3	0.00	1.00
	Null	-189.0	1	382.0	22.70	0.00
Safety	Location ³	-158.7	2	323.5	0.00	0.73
	Concealment ⁴					
	+ Location	-158.7	3	325.5	2.03	0.27
	Concealment	-187.6	2	381.2	57.7	0.00
	Null	-189.0	1	382.0	58.5	0.00

¹Monoterpene (AUC/100 µg DW)

²Crude protein (% DW)

³Binary on- or off-mound designation of plant

⁴Aerial concealment available at base of plant (%)

Table B.5 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the first stage of pygmy rabbit foraging models at on-mound plants. Variables appearing in models above a null intercept-only model with $\Delta AIC_c \leq 2.0$ were included in global models (bold). For the PSM category, the top seven models and the null model are presented.

Response Variable	Predictor Category	Model	Log Likelihood	K	AIC_c	ΔAIC_c	w_i	
Foraging	PSM	Unknown 3.2 ¹	-65.6	1	135.3	0.00	0.08	
		Null	-64.9	2	136.0	0.67	0.06	
		1,8-cineole ¹ + Unknown 3.2	-65.0	2	136.2	0.91	0.05	
		Borneol ¹ + Unknown 3.2	-65.1	2	136.2	0.91	0.05	
		Borneol	-65.1	2	136.3	1.03	0.05	
		1,8-cineole	-64.2	3	136.5	1.24	0.04	
		β -pinene ¹ + Unknown 3.2	-64.2	3	136.7	1.38	0.04	
		ρ -cymene ¹	-65.6	2	137.2	1.94	0.03	
		Nutrient	Null	-63.7	2	133.5	0.00	0.72
			Protein ²	-65.6	1	135.3	1.84	0.28
	Safety	Null	-65.6	1	135.3	0.00	0.53	
		Distance ³	-65.6	2	137.2	1.01	0.20	
		Concealment ⁴	-65.6	2	137.3	2.04	0.19	
		Distance + Concealment	-65.5	3	139.3	3.97	0.07	

¹Monoterpene (AUC/100 μ g DW)

²Crude protein (% DW)

³Distance of plant to nearest active burrow entrance (m)

⁴Aerial concealment available at base of plant (%)

Table B.6 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the first stage of pygmy rabbit foraging models at off-mound plants. Variables appearing in models above a null intercept-only model with $\Delta AIC_c \leq 2.0$ were included in global models (bold). For the PSM category, the top seven models and the null model are presented.

Response Variable	Predictor Category	Model	Log Likelihood	AIC_c	ΔAIC_c	w_i
Foraging	PSM	Null	-65.6	135.3	0.00	0.08
		1,8-cineole ¹	-64.9	136.0	0.67	0.06
		ρ -cymene ¹	65.0	136.2	0.91	0.05
		Borneol ¹	65.1	136.2	0.91	0.05
		Total monoterpenes ²	65.1	136.3	1.03	0.05
		Borneol + 1,8-cineole	64.2	136.5	1.24	0.04
		Borneol + ρ -cymene	64.2	136.7	1.38	0.04
	Nutrient	Camphor ¹	65.6	137.2	1.94	0.03
		Protein ³	63.7	133.5	0.00	0.72
		Null	65.6	135.3	1.84	0.28
	Safety	Null	65.6	135.3	0.00	0.53
		Distance ⁴	65.6	137.2	1.1	0.20
		Concealment ⁵	65.6	137.3	2.04	0.19
		Distance + Concealment	65.5	139.3	3.97	0.07

¹Monoterpene (AUC/100 μ g DW)

²Total monoterpene concentration (cumulative concentration of all compounds with retention times ≤ 28.0 min. [area under the chromatogram curve/100 μ g dry weight])

³Crude protein (% DW)

⁴Distance of plant to nearest active burrow entrance (m)

⁵Aerial concealment available at base of plant (%)

Table B.7 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the first stage of pygmy rabbit resting models at all plants. Variables appearing in models above a null intercept-only model with $\Delta AIC_c \leq 2.0$ were included in global models (bold). For the PSM category, the top seven models and the null model are presented.

Response Variable	Predictor Category	Model	Log Likelihood	AIC_c	ΔAIC_c	w_i
Resting	PSM	Null	-149.4	302.8	0.00	0.08
		Unknown 3.2 ¹	-148.5	303.1	0.27	0.07
		1,8-cineole ¹	-148.9	303.8	1.04	0.05
		ρ -cymene ¹	-148.9	303.9	1.08	0.05
		1,8-cineole + Unknown 3.2	-148.0	304.0	1.25	0.04
		1,8-cineole + ρ -cymene	-148.3	304.7	1.87	0.03
		Total monoterpenes ²	-149.3	304.7	1.87	0.03
		Camphor ¹	-149.4	304.8	2.00	0.03
	Nutrient	Null	-149.4	302.8	0.00	0.70
		Protein ³	-149.2	304.5	1.72	0.30
		Safety	Null	-149.4	302.8	0.00
	Location ⁴		-148.7	303.5	0.75	0.30
	Concealment ⁵		-149.3	304.7	1.96	0.16
	Concealment + Location		-148.7	305.6	2.79	0.11

¹Monoterpene (AUC/100 μg DW)

²Total monoterpene concentration (cumulative concentration of all compounds with retention times ≤ 28.0 min. [area under the chromatogram curve/100 μg dry weight])

³Crude protein (% DW)

⁴Binary on- or off-mound designation of plant

⁵Aerial concealment available at base of plant (%)

Table B.8 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the first stage of pygmy rabbit resting models at on-mound plants. Variables appearing in models above a null intercept-only model with $\Delta AIC_c \leq 2.0$ were included in global models (bold). For the PSM category, the top seven models and the null model are presented.

Response Variable	Predictor Category	Model	Log Likelihood	AIC_c	ΔAIC_c	w_i
Resting	PSM	Unknown 3.2 ¹	67.6	141.4	0.00	0.12
		Borneol ¹ + Unknown 3.2	67.3	142.8	1.49	0.06
		1,8-cineole ¹ + Unknown 3.2	67.3	142.9	1.54	0.05
		ρ -cymene ¹ + Unknown 3.2	67.5	143.2	1.80	0.05
		β -pinene ¹ + Unknown 3.2	67.6	143.5	2.10	0.05
		Camphor ¹ + Unknown 3.2	69.7	143.5	2.12	0.04
		Null	69.0	143.5	2.19	0.04
	Nutrient	ρ -cymene	69.2	144.2	2.86	0.03
		Null	69.7	143.5	0.00	0.72
		Protein ²	69.6	145.4	1.86	0.28
	Safety	Null	69.7	143.5	0.00	0.53
		Concealment ³	69.7	145.5	1.95	0.20
		Distance ⁴	69.7	145.6	2.05	0.19
		Distance + Concealment	69.7	147.6	4.03	0.07

¹Monoterpene (AUC/100 μ g DW)

²Crude protein (% DW)

³Aerial concealment available at base of plant (%)

⁴Distance of plant to nearest active burrow entrance (m)

Table B.9 Log likelihood, number of parameters (K), Akaike's Information Criterion with sample size bias adjustment (AIC_c), change in AIC_c from the top model (ΔAIC_c), and model weight (w_i) for the first stage of pygmy rabbit resting models at off-mound plants. Variables appearing in models above a null intercept-only model with $\Delta AIC_c \leq 2.0$ were included in global models (bold). For the PSM category, the top seven models and the null model are presented.

Response Variable	Predictor Category	Model	Log Likelihood	AIC_c	ΔAIC_c	w_i
Resting	PSM	Null	82.6	169.3	0.00	0.12
		β -pinene ¹	82.3	170.6	1.29	0.06
		1,8-cineole ¹	82.3	170.8	1.45	0.06
		Borneol ¹	82.5	171.0	1.70	0.05
		Unknown 3.2 ¹	82.5	171.2	1.87	0.05
		Camphor ¹	82.6	171.4	2.03	0.04
		p -cymene ¹	82.6	171.4	2.05	0.04
		Total monoterpenes ²	82.7	171.4	2.10	0.04
	Nutrient	Null	82.6	169.3	0.00	0.51
		Protein ³	81.7	169.4	0.10	0.49
	Safety	Distance ⁴	80.1	166.2	0.00	0.58
		Distance ⁴ + Concealment ⁵	79.9	168.0	1.75	0.24
		Null	82.6	169.3	3.08	0.13
		Concealment	82.6	171.3	5.02	0.05

¹Monoterpene (AUC/100 μ g DW)

²Total monoterpene concentration (cumulative concentration of all compounds with retention times ≤ 28.0 min. [area under the chromatogram curve/100 μ g dry weight])

³Crude protein (% DW)

⁴Distance of plant to nearest active burrow entrance (m)

⁵Aerial concealment available at base of plant (%)

APPENDIX C

**Negative Binomial Models of Pygmy Rabbit Undifferentiated Use, Foraging,
and Resting**

All model-averaged parameter estimates for variables predicting the numbers of bite marks, fecal pellets, and both bite marks and fecal pellets considered together, overlapped one (Tables C1, C2, and C3). We therefore excluded these models from further analysis. In general, however, top models for foraging included primarily dietary variables (i.e., PSMs and protein), whereas top models for resting included primarily safety variables (i.e., distance to refuge and concealment). We believe that similar models with continuous response variables are potentially useful predictors of the intensity of use, and should be carefully explored in future studies.

Tables

Table C.1 Covariates, model-averaged parameter estimates, and 85% confidence intervals from top negative binomial models of total pygmy rabbit use at all, on-mound, and off-mound plants.

Response Variable	Plants	Covariate	Parameter estimate ¹	Lower 85% C.I. ¹	Upper 85% C.I. ¹
Total use	All plants	1,8-cineole	1.00	0.96	1.04
		ρ -cymene	1.00	0.99	1.01
		Protein	1.00	0.88	1.14
		Concealment	1.00	0.96	1.04
	On-mound	Borneol	1.00	0.85	1.17
	Off-mound	Protein	1.20	0.06	23.77

Table C.2 Covariates, model-averaged parameter estimates, and 85% confidence intervals from top negative binomial models of total pygmy rabbit bite marks at all, on-mound, and off-mound plants.

Response Variable	Plants	Covariate	Parameter estimate ¹	Lower 85% C.I. ¹	Upper 85% C.I. ¹
Total bite marks	All plants	Total monoterpenes	1.00	0.99	1.01
		Camphor	1.00	0.97	1.02
		1,8-cineole	1.00	0.97	1.02
		ρ -cymene	1.00	0.97	1.03
		Location	0.79	0.47	1.31
		Concealment	1.00	0.98	1.02
	On-mound	Total monoterpenes	1.00	0.99	1.01
		1,8-cineole	1.00	0.97	1.02
		β -pinene	1.00	0.97	1.03
		ρ -cymene	1.00	0.98	1.02
		Unknown 3.2	1.00	0.97	1.02
		Camphor	1.00	0.99	1.01
	Off-mound	Distance	1.00	0.95	1.05
		Total monoterpenes	1.00	0.99	1.01
		1,8-cineole	1.00	0.98	1.02
		Borneol	1.04	0.86	1.27
		Camphor	1.00	0.96	1.04
		Unknown 3.2	1.00	0.98	1.02

Table C.3 Covariates, model-averaged parameter estimates, and 85% confidence intervals from top negative binomial models of total pygmy rabbit bite marks at all, on-mound, and off-mound plants.

Response Variable	Plants	Covariate	Parameter estimate ¹	Lower 85% C.I. ¹	Upper 85% C.I. ¹
Total fecal pellets	All plants	Protein	0.99	0.74	1.32
		Location	1.00	0.98	1.02
		Concealment	1.00	0.93	1.08
	On-mound	Borneol	0.99	0.83	1.18
	Off-mound	1,8-cineole	1.00	0.98	1.02
		β -pinene	0.98	0.86	1.12
		Camphor	1.00	0.97	1.04
		Borneol	1.00	0.94	1.07
		Protein	1.15	0.64	2.06
		Concealment	1.00	0.99	1.01

Figures

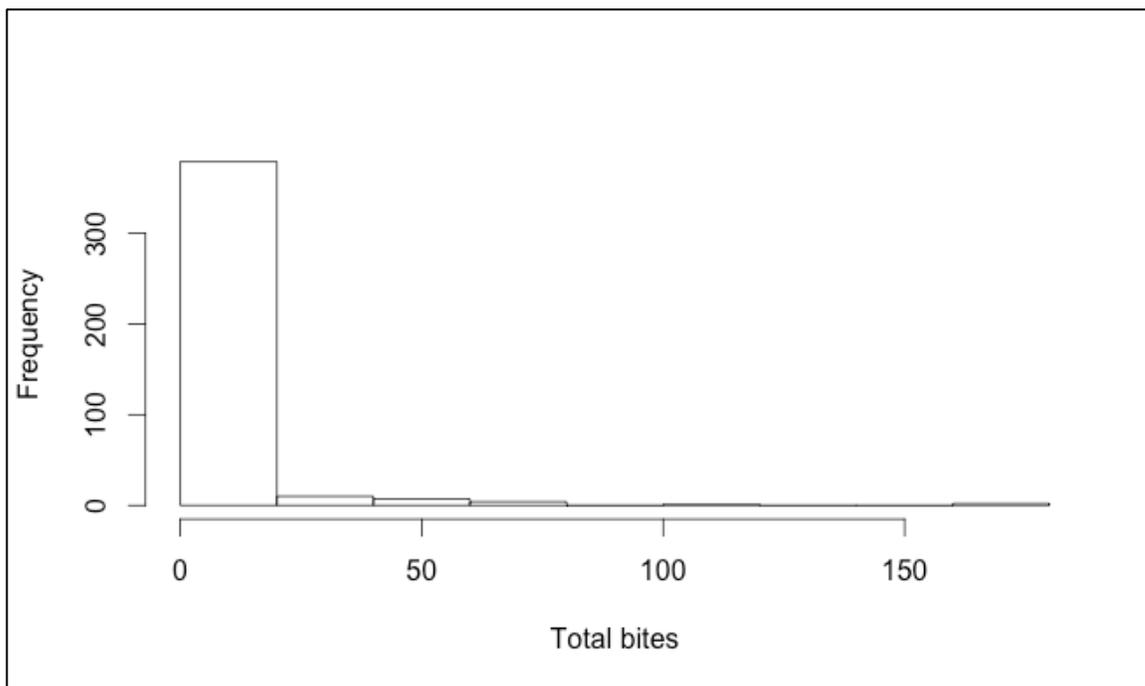


Figure C.1 Frequency histogram of foraging, as measured in total pygmy rabbit (*Brachylagus idahoensis*) bite marks at on- and off-mound sagebrush (*Artemisia spp.*) plants in Blaine County, ID in March 2013.

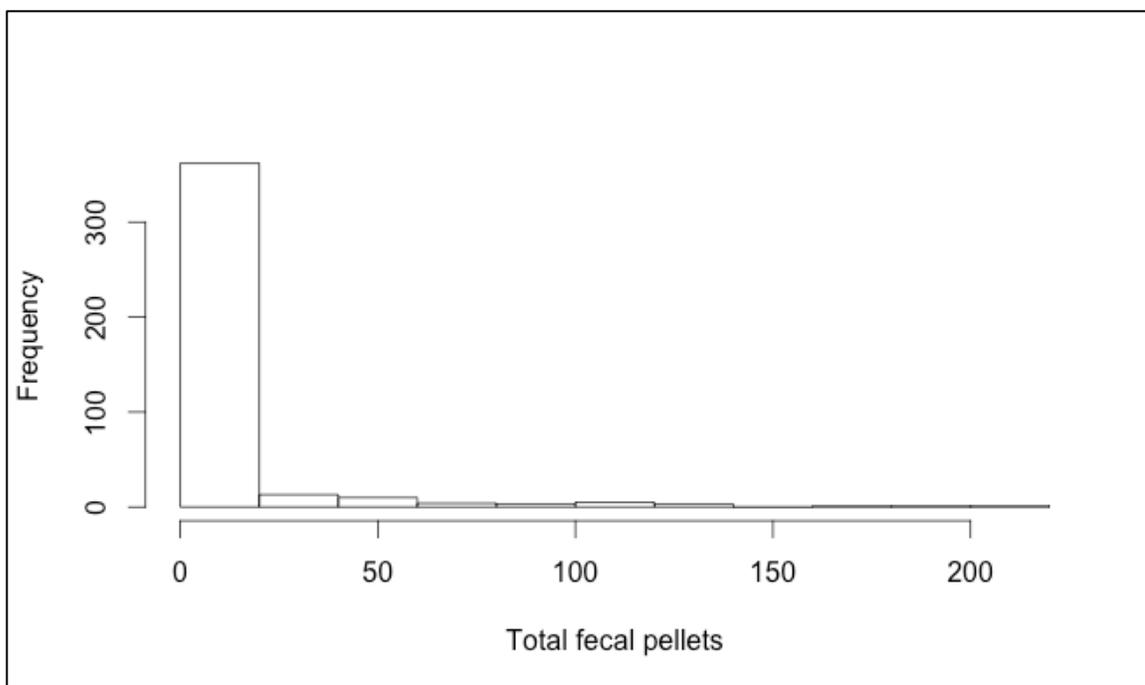


Figure C.2 Frequency histogram of resting, as measured in total pygmy rabbit (*Brachylagus idahoensis*) fecal pellets at on- and off-mound sagebrush (*Artemisia spp.*) plants in Blaine County, ID in March 2013.

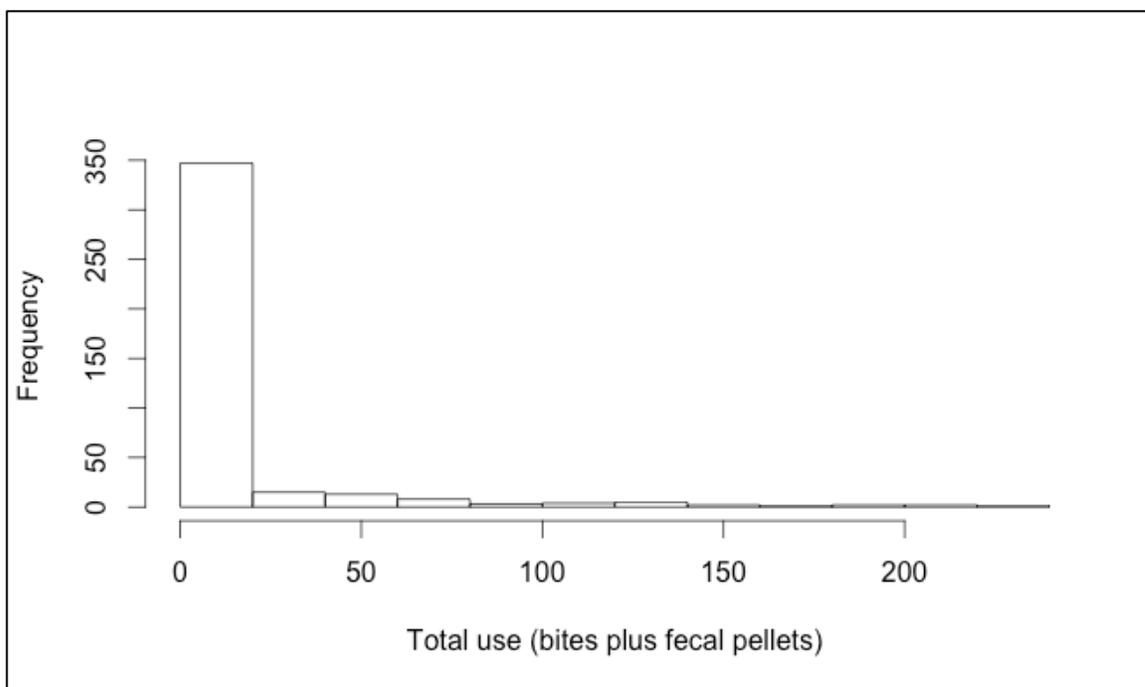


Figure C.3 Frequency histogram of total use, as measured in total pygmy rabbit (*Brachylagus idahoensis*) bite marks plus fecal pellets at on- and off-mound sagebrush (*Artemisia spp.*) plants in Blaine County, ID in March 2013.

APPENDIX D**Effects of Methylene Chloride on Pygmy Rabbit and Mountain Cottontail Intake**

To ensure any residual methylene chloride did not affect selection, an additional three-day choice trial was conducted in which all rabbits were offered equal amounts of either untreated pellets, or pellets treated with methylene chloride that was allowed to evaporate for six hours in the hood. Treatments were offered on alternating sides of the pen each day. Neither species showed a significant preference and total intake did not decline ($t_{2,16}=-1.21$, $P=0.25$).

APPENDIX E

Equivalence Point Trials Between 1,8-Cineole And Monoterpene Mixture

To assess whether increasing doses change pygmy rabbit and mountain cottontail preferences for or against single monoterpenes compared to a monoterpene mixture, We performed equivalence point trials wherein the dose of one treatment was altered until intake of both treatments equilibrated. These trials utilize the economic theory of marginal rates of substitution (Caraco 1979), in which different habitat attributes or resources can be evaluated using the common currency of utility. This approach has been used to compare both resources and risks as diverse as food density, predation risk, food toxicity and digestibility, vigilance behavior, and food handling time (Brown 1988, 1999, Schmidt 2000, Searle et al. 2008, Camp et al. 2015), and is described by Nersesian et al. (2011) and Camp et al. (2015). Despite both treatments, single and mixed monoterpenes, being quantifiable in identical units, in this case percent dry weight (DW) of food, it is possible or likely that they are evaluated differently by herbivores. This could result from differences in the inherent toxicity of different compounds (Rice and Coats 1994, Cornelius et al. 1997, Kohl et al. 2015), differences in the abilities of herbivores to detoxify different compounds (Boyle et al. 1999, Dearing and Cork 1999), or synergistic effects of mixtures of compounds (Pavela 2008, Richards et al. 2010, 2012).

To assess whether preferences for or against single compounds compared to a mixture are consistent or an artifact of the doses at which they are administered, We identified equivalence points between the two treatments at multiple concentrations. These are defined as the concentrations of each treatment at which pygmy rabbits or mountain cottontails consume an equal proportion of each. We performed only a single trial, comparing the monoterpene mixture to 1,8-cineole. Animal care, mixture preparation, treatment of food pellets with both the mixture and single monoterpene,

treatment offering, and determination of proportions consumed were identical to those in the choice trials described in Chapter 2, the only difference was the concentrations of monoterpene treatments added to food pellets. Animals were offered the choice between a fixed concentration of monoterpene mix and a varying concentration of 1,8-cineole. These concentrations ranged from 1% to 7% for mountain cottontails and 1% to 9% for pygmy rabbits. The proportion of the mixture consumed was plotted against the concentration of 1,8-cineole offered, and trials continued until a line regressed against the data for each animal fit with an R^2 value of at least 0.80 (Figure E1). We used the equation of that line to solve for the concentration of 1,8-cineole at which the proportion of mixture consumed was 0.50. After a three day break for all animals, the process was repeated with 2% and 3% monoterpene mixture being offered to mountain cottontails and pygmy rabbits respectively, followed by another break and 3% and 5% monoterpene mixture. By regressing a line against the averages of all three equivalence points for each species, rates of substitution between the treatments were created (Figure E2). Insights can be drawn based on the slopes and intercepts of this line.

Both species again demonstrated their preference for 1,8-cineole compared to the monoterpene mixture. The intercepts of the equivalence lines represent the concentration of 1,8-cineole equivalent to food not treated with the mixture at all -- that is, the minimum dose of 1,8-cineole needed to induce the consumption of food treated with any monoterpene mixture at all. Pygmy rabbits and mountain cottontails evaluated 0% monoterpene mixture as equivalent to 2.7% and 0.9% 1,8-cineole, respectively (Figure E2). The slopes of the lines represent the actual rates of substitution. A slope of one indicates that the single monoterpene and the mixture are viewed as essentially

equivalent, that is a given increase in the concentration of 1,8-cineole is considered equivalent to an identical increase in the concentration of the mixture. A slope greater or less than one indicates a disparity between the treatments, with a given increase in one viewed as either more or less significant than an identical increase in the other. Treating the concentration of the mixture as the independent variable and the concentration of 1,8-cineole as the dependent variable, both species demonstrated rates of substitution slightly greater than one, though not significantly so based on 95% confidence intervals (1.13 ± 1.06 and 1.29 ± 0.69 , respectively, fig). Regressing data for both species together with an interaction term for species demonstrates that these slopes are not significantly different ($t_2 = -2.70$, $p = 0.81$).

The combination of different intercepts but similar slopes between the two species raises questions about the relative ability of each to detoxify a single compound and a monoterpene mixture. Differing rates of detoxification have been proposed as causes of the observed differences in the abilities of herbivores to tolerate plant secondary metabolites (Sorensen et al. 2004, Sorensen and Dearing 2006, McLean et al. 2007, Shipley et al. 2012). However, equal rates of substitution for both pygmy rabbits and mountain cottontails suggests that as concentrations of 1,8-cineole increase, each species views the change as equal in comparison to another potential risk (i.e. the increase concentration of the mixture). Moreover, the slopes for each species are not significantly different from one. Taken together, these data could suggest that not only do pygmy rabbits and mountain cottontails detoxify 1,8-cineole at similar rates to one another, but also that each species detoxifies 1,8-cineole and a monoterpene mixture at similar rates.

Shiple et al. (2012) clearly showed that pygmy rabbits and mountain cottontails have different tolerances for and different abilities to detoxify 1,8-cineole. Likewise, the significant preferences of both species for 1,8-cineole over the mixture reported in Chapter 2 reinforce the assertion that the treatments are not viewed equally. Perhaps then, rates of detoxification do not play primary roles in determining the observed preferences of these two species. Woodrats specializing on juniper have been shown to consume more PSMs than their generalist counterparts (Sorensen et al. 2005b), however they do not show a difference in the speed with which each species detoxifies those compounds (Sorensen and Dearing 2003b).

The different thresholds at which pygmy rabbits and mountain cottontails consider 1,8-cineole equivalent to a monoterpene mixture could be evidence that ingesting similar concentrations of 1,8-cineole result in different effective doses for each species. The ability to regulate absorption has also been proposed as a driver of PSM tolerance (Sorensen and Dearing 2006). Proteins lining the gut may be capable of effluxing absorbed compounds back into the lumen, reducing the effective dose of ingested PSMs (Dietrich et al. 2003). Neither captive pygmy rabbits nor captive mountain cottontails seem to excrete unmetabolized 1,8-cineole in urine or feces, casting some doubt on the ability of efflux transporters to explain observed differences in preference, however it is possible that excreted 1,8-cineole is consumed in cecal droppings (Shiple et al. 2012). Regardless, these proteins can be quantified and are known to differ in prevalence among animal taxa (Sorensen and Dearing 2006). *In vitro* assessments of the presence and prevalence of efflux transporter proteins in each species

would be a valuable next step in describing the mechanisms by which pygmy rabbits and mountain cottontails cope with PSMs.

A third possibility beyond dose and detoxification rate is that eliminating 1,8-cineole is limited by substrates required for its conjugation and subsequent excretion. By relying more on conjugation than pygmy rabbits (Shiple et al. 2012), mountain cottontails may exhaust substrate stores at lower concentrations of ingested 1,8-cineole. Additional 1,8-cineole would then need to be detoxified by other pathways, potentially at similar rates, until more substrate became available.

Determining the exact mechanisms by which herbivores cope with ingested PSMs requires a combination of field-based, captive, and laboratory studies, and is labor and resource intensive. Comparing preferences for single and mixed compounds can help point to individual PSMs for further study. Equivalence point trials can go a step further and suggest specific mechanisms more likely than others to regulate the effects of those PSMs. Differences in exchange rates between species provide evidence that they either detoxify a compound at different rates or by entirely different mechanisms. For example, pygmy rabbits preferred camphor to the monoterpene mixture, while mountain cottontails preferred the mixture to camphor (Chapter 2). In a similar equivalence point trial with camphor, the two species would be expected to have different exchange rates with pygmy rabbits having a shallower slope and mountain cottontails having a steeper slope. Increasing or decreasing tolerance of a compound as concentration increases (i.e. an exchange rate significantly different than one) points to different capacities for single versus mixed compounds within the same species. Combining traditional choice trials

with equivalence point trials sheds further light on the manner in which PSMs influence herbivores and drive foraging.

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Figures

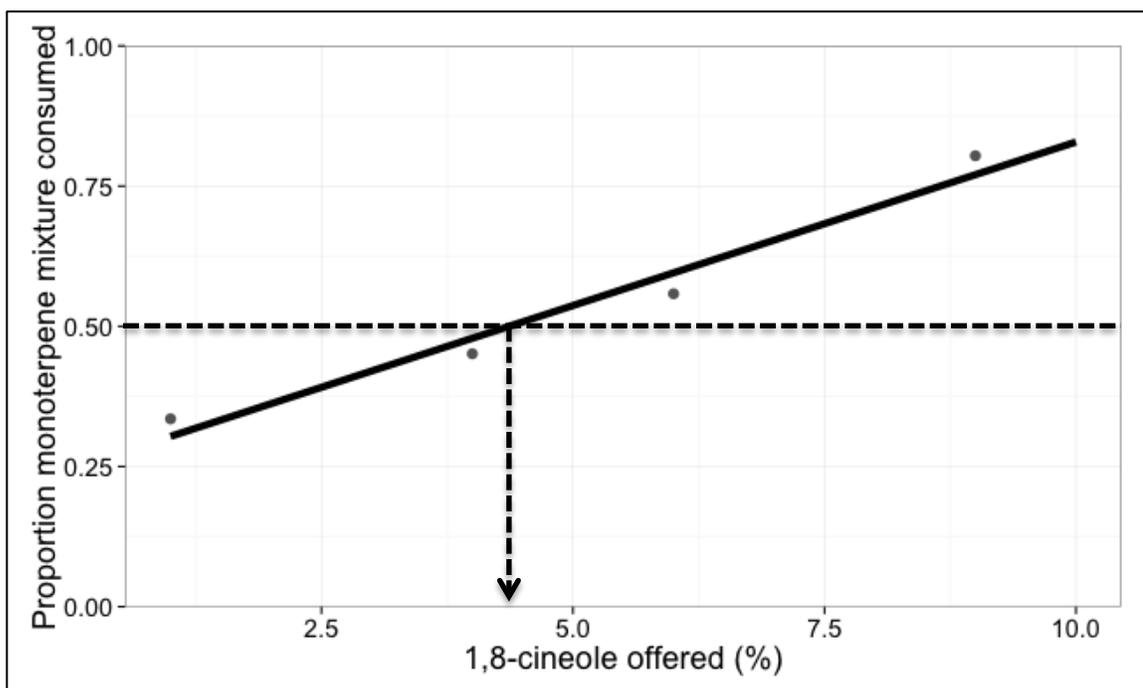


Figure E.1 Proportion consumed of a diet containing a 1% concentration of a mixture of 5 monoterpenes when paired with a diet containing increasing amounts of a single monoterpene, 1,8 cineole. The equivalence point (i.e., equal dry matter intake of both diets, dashed arrow) is the 1,8 cineole concentration where the proportion is 0.5 (dashed line, $X = 4.36\%$).

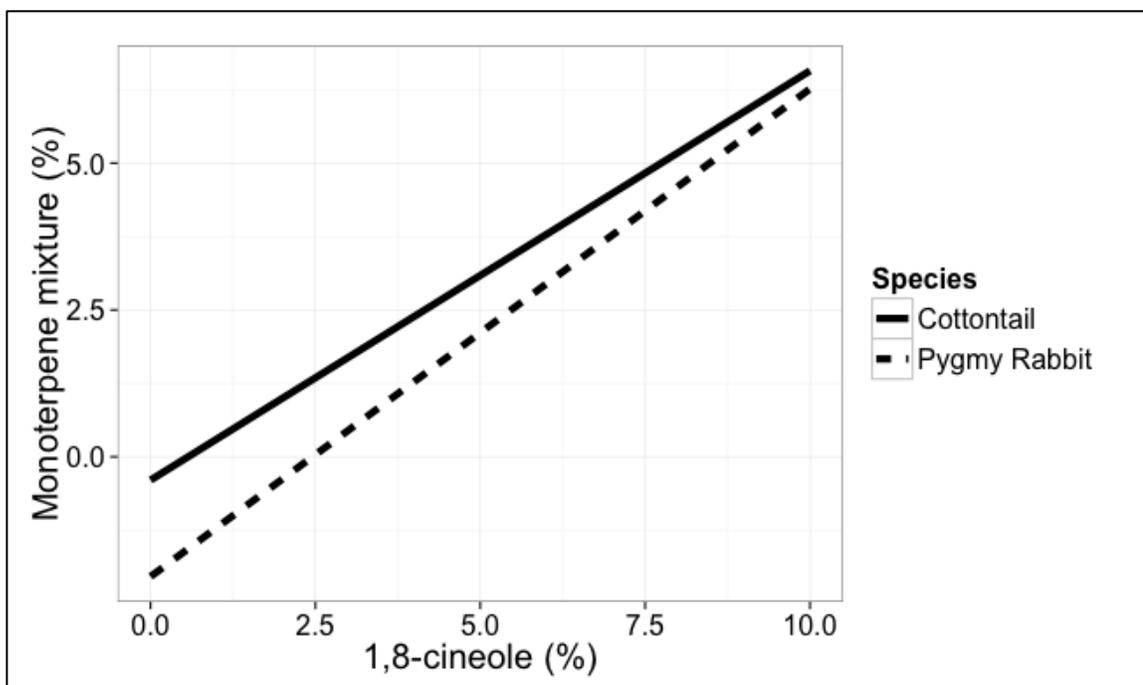


Figure E.2 Modeled equivalence curves created from equivalence points between diets containing 1,8-cineole and diets containing a mixture of 5 monoterpenes for pygmy rabbits (*Brachylagus idahoensis*) and cottontails (*Sylvilagus nuttallii*). Slopes do not differ significantly ($t_2=-2.70$, $p=0.81$).