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Ingestion of plant secondary compounds causes diuresis in desert herbivores

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Abstract Plant secondary compounds are recognized deterrents and toxins to a variety of herbivores. The effect of secondary compounds on water balance of herbivores is virtually unexplored, yet secondary compounds could potentially cause a decrease in an animal's ability to maintain water balance. We investigated the effects of secondary compounds, alpha-pinene and creosote resin, on water balance in three species of herbivorous woodrats (*Neotoma stephensi, N. albigula, N. lepida*). In separate experiments, we measured the effect of these secondary compounds on voluntary water consumption, urine volume and urine osmolarity. In both experiments, water intake and urine volume increased and urine osmolarity decreased compared to controls. Water balance of specialist or experienced woodrats was less affected than generalists and woodrats with less prior experience with particular secondary compounds. Our results suggest that secondary compounds have diuretic-like effects on herbivores. Woodrats live in arid habitats with limited access to freestanding water; thus an increase in water requirements may have profound consequences on foraging behavior and fitness.

Keywords Diuretics · *Neotoma* · Plant secondary compounds · Water balance · Woodrat

Introduction

The ingestion of plant secondary metabolites can exact a multitude of physiological consequences on a herbivore. These effects include but are not limited to toxic effects, disturbance of acid-base homeostasis, emetic stimulation and mineral wasting (Dearing 1997; Foley and Hume 1987; Foley and McArthur 1994; Iason and Murray 1996; Iason and Palo 1991; Lindroth and Batzli 1984; Lindroth et al. 1986; McArthur and Sanson 1993; McArthur et al. 1991; McLean et al. 1993; Meyer and Karasov 1989; Thomas et al. 1988). In addition, numerous secondary compounds are classified as deterrents that reduce or eliminate consumption of particular species of plants (Pass and Foley 2000). In many cases, the specific causes of deterrent effects of secondary compounds are unknown. The proposed mechanisms underlying deterrence may include any of the physiological consequences listed above and as yet undescribed mechanisms (Pass and Foley 2000).

One potential consequence of secondary metabolite ingestion that has been largely ignored is the effect on water balance. Yet, many secondary compounds are potent diuretics in humans and laboratory animals – that is they increase urine volume and/or decrease osmolarity of urine (Adsersen and Adsersen 1997; Beaux et al. 1999; Johnson et al. 1999; Hardman and Limbird 1996; Navarro et al. 1994). Caffeine from coffee and black tea is probably the most familiar diuretic agent from plants (Neuhauser et al. 1997). However, caffeine is not exceptional. Over 100 species of plants from various families contain compounds that produce diuresis in humans and laboratory rats (Dearing et al. 2001). In many cases the diuretic action is more substantial than that produced by caffeine. Given the diversity of plants with diuretic effects, it seems plausible that wild herbivores may encounter plants containing secondary compounds that could negatively impact water balance.

In a previous study, we demonstrated that water balance of herbivorous woodrats was significantly altered when animals consumed diets containing juniper foliage (Dearing et al. 2001). Juniper is present in the diets of woodrats in nature and the foliage contains numerous secondary compounds. Consumption of juniper foliage by woodrats increased water intake and urine output and

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decreased osmolarity of the urine compared to a control diet. Moreover, woodrats that specialize on juniper (*Neotoma stephensi*) were less affected with respect to water balance than a generalist species (*N. albigula*). The results suggest that plant secondary metabolites in juniper have a diuretic effect on woodrats. Since woodrats have limited access to free water in nature, diuretic plant compounds could present a significant physiological challenge.

To test more specifically whether secondary metabolites are diuretics, we conducted experiments with crude extracts of secondary compounds as well as an individual secondary metabolite. We tested three hypotheses: (1) plant secondary compounds act as diuretics by increasing urine volume or decreasing urine osmolarity; (2) ingestion of plant secondary compounds increases water intake; (3) extensive prior experience with toxins lessens the impact of toxin ingestion on water intake. Animals that specialize on particular toxins should exhibit less diuresis than animals that do not specialize or have no prior experience with a compound because specialists should have detoxification machinery that reduces the physiological impacts of toxins to a greater extent than that of generalist herbivores.

Natural history of the study system

We used two systems to test these hypotheses. The first consisted of a specialist-generalist pair of woodrats, *N. stephensi* and *N. albigula*. These two species occur in sympatry in the southwestern United States (Dial 1988). *N. stephensi* is a juniper specialist; 65–95% of its year round diet consists of foliage of one-seeded juniper, *Juniperus monosperma* (Dial 1988; Vaughn 1982). *N. albigula* is a generalist that also consumes juniper, but juniper never comprises more than 35% of the diet (Dial 1988). In the laboratory, *N. stephensi* can tolerate higher doses of juniper and juniper toxins than *N. albigula* (Dearing et al. 2000). The secondary compound we used with this specialist-generalist pair was alpha-pinene, which is the most common monoterpene in juniper. Alpha-pinene is a known toxin to mammals, causing CNS depression, kidney and liver lesions (Hedenstierna et al. 1983; Koppel et al. 1981; Levin et al. 1992).

The second system consisted of two populations of *N. lepida* from distinct habitats and with disparate diets (Mangione et al. 2000). One population, from the Mojave desert, feeds extensively on creosote bush, *Larrea tridentata*. The other population, from the Great Basin desert, consumes primarily *J. osteosperma*, Utah juniper. The secondary metabolites used in this experiment were a complex mixture of phenolic resins extracted from the leaves of creosote, *L. tridentata* (Mabry et al. 1977). The Great Basin population had no prior ecological or evolutionary experience with creosote resin whereas the Mojave population had both ecological and evolutionary experience with creosote.

Materials and methods

Collection of woodrats

Woodrats were captured using Tomahawk and Sherman live traps baited with peanut butter and oats. Pieces of cotton batting were placed in traps as nesting material. Traps were opened late in the afternoon and checked immediately after sunrise. While in the laboratory, animals were maintained on diets of high-fiber rabbit chow (Harlan Teklad) and occasionally offered apple slices.

N. stephensi and *N. albigula* were trapped on Woodhouse Mesa, AZ (35°30′N 111°27′W) in March 1996. This site was the same as used by Dial (1988) for a 4-year study of the diets of these species. We trapped at many of the exact locations as indicated by trap markers. Additional *N. albigula* were trapped in Castle Valley, UT (38°38;N109°18′W). Vegetation in both trapping areas was similar; see Dearing et al. (1998) for a detailed description. We confirmed that *N. stephensi* was consuming more juniper than *N. albigula* by analyzing feces from trapped woodrats for juniper fragments using microscopic techniques (Williams 1969). The average percentage of microscope fields containing juniper stomata was 16% for feces from *N. stephensi* and 7% for *N. albigula*. Individuals of both species participated in a 35-day digestibility trial of a toxin free chow with nitrogen and fiber contents similar to juniper and also a 6-day feeding trial with juniper (Dearing et al. 2000) prior to the experiment described here.

N. lepida from the Mojave desert were trapped near Beaver Dam, Utah (37°06′N, 113°58′W). *N. lepida* from the Great Basin were trapped in two locations 70 km apart at Jericho, Utah (39°57′N 112°22′W) and Dugway, Utah (40°19′N 112°57′W). Trapping was conducted in April and May 1996. There is no creosote present at either of the Great Basin sites. For a detailed description of the habitats see Dearing et al. (1998). We confirmed that Mojave woodrats ate creosote foliage and Great Basin woodrats did not by analyzing feces from both populations (Williams 1969). The percentage of microscope fields containing creosote fragments found in feces of wild caught woodrats was 21.2% for Mojave woodrats and 0% for Great Basin woodrats. From here on we refer to the Mojave woodrats as "experienced" and Great Basin woodrats as "na" to indicate their previous experience with creosote resin.

Prior to the onset of experiments, all woodrats were screened for hantavirus. Three individuals from the Great Basin population tested positive for Sin Nombre hantavirus (Dearing et al. 1998) and were removed from the colony prior to the onset of experiments. The hantavirus screening was completed 6 months from the time of initial capture (March 1996).

Alpha-pinene experiment

To determine whether the primary monoterpene in juniper, alpha-pinene, is a diuretic, we fed woodrats diets with and without alpha-pinene. The base diet was a low-nitrogen and high-fiber formulation similar to that found in *J. monosperma* (Dearing et al. 2000). The ingredients and proportions were Harlan Teklad ground rabbit chow [50%, formula 8630; 17.5% crude protein, 22.02% acid detergent fiber (ADF), 2.5% fat and 7.5% water], Amersham Life Science Cellufil cellulose (12%), microencapsulated corn oil (3%), vitamin mix (0.5%) Teklad mineral mix TD 79055 (1.75%), cornstarch (13%), pectin (7%) methionine (0.055%) and sucrose 13%.

For the treatment diets, we added microencapsulated alpha-pinene to the base diet. Microencapsulation was necessary to reduce volatilization of alpha-pinene during the feeding trials. Alpha-pinene from Sigma was microencapsulated in gelatin-gum arabic capsules by complex coacervation (Clancy et al. 1992; Usher et al. 1989), which causes the formation of gelatin and gum arabic microcapsules around oil droplets (in this case either corn oil or alpha-pinene). Microcapsules range in diameter from about 30 to 80 μm. The gelatin-gum arabic solution forms solid capsules around oil when subjected to temperature and pH changes. Cap-

sules were hardened using a 25% glutaraldehyde solution as a cross-linking catalyst. Residual glutaraldehyde was rinsed from the capsules with deionized water. Capsules were suspended in water, yielding a slurry of ~30% capsules. To further control for any effect of microcapsules in the treatments, the control treatment contained microencapsulated corn oil. The only difference between the microencapsulated corn oil and microencapsulated alpha-pinene was the oil encapsulated, i.e., corn oil versus alpha-pinene. The coacervation process and reagents used were identical for encapsulation of corn oil and alpha-pinene. Control, low and intermediate diets contained ~1.3% microcapsules per gram wet mass of diet; the high treatment was ~3.1% microcapsules (described below). Preliminary feeding trials with microencapsulated corn oil verified that a diet of ~4.8% corn oil microcapsules had no effect on food intake.

Eight *N. stephensi* and 10 *N. albigula* were fed treatments in the following sequence for 3 days each: control (0% alpha-pinene/g d.w.), low (2.3 µl alpha-pinene/g d.w.), intermediate (18.5 µl alpha-pinene/g d.w.) and high (23.3 µl alpha-pinene/g d.w.). The sequential design was necessary to provide an adequate amount of time for complete induction of detoxification enzymes, which can take 24–48 h after exposure to a toxin. Furthermore, because diuretics are dose dependent, we wanted to test animals at concentrations representative of the highest they could encounter in nature while minimizing significant changes in food intake and body masses that can occur with a sudden shift to a drastically different diet. Concentrations of alpha-pinene in the diets were determined using gas chromatography (Hewlett Packard 5860). Concentrations of alpha-pinene in treatments correspond to the amount of alpha-pinene that would be consumed in diets of ~10%, 80% and 100%, *J. monosperma,* respectively. These predicted concentrations of alpha-pinene in the diet of *J. monosperma* are based on the total oil yield (3.73% oil by dry weight) from steam distillation of samples of juniper from the study site, and measurements of alpha-pinene in juniper oil (63%) by gas chromatography. Diet treatments were prepared and leftovers collected daily.

Animals were allowed to acclimate to each diet for 3 days prior to the collection of urine. Food intake and body mass stabilized during this time frame with the exception of the generalist on the highest treatment. Other experiments with juniper diets suggested that a 3-day treatment was a sufficient time period for a response. On day 3 of each treatment, woodrats were confined to a portion of their cage (16×19×20 cm) that allowed for the separate collection of urine and feces. Previous experiments demonstrated that confinement to a section of the cage did not affect food intake (Mangione et al. 2000). We collected urine during the last 24 h of each treatment. The collection vial for urine was embedded in a frozen pack to keep urine cold and decrease evaporation. Temperature of urine in the coolers ranged from 0 to 5°C. Urine was stored at –20°C until analyzed. As part of another experiment, we measured body mass and food intake daily (Dearing et al. 2000). Urine volume was measured in graduated cylinders. The osmotic pressure of the urine was measured with a Wescor 510B Vapor Pressure Osmometer. The concentration of the samples exceeded the capacity of the osmometer. Therefore, samples were diluted with distilled water (1:1 or 1:2 urine:water depending on concentration of sample) prior to the measurement.

Water intake was determined on day 3 of each treatment by summing free water consumption with the amount of free water ingested in food. Free water consumption was estimated from the daily change in weight of the water bottles and corrected for spillage and evaporation by using control water bottles (<0.5 ml). The amount of water in food (control and treatment diets) was estimated gravimetrically by drying a known mass of food in an oven at 50°C and weighing after drying. Food water intake was the product of the proportion of water in the food and daily food intake.

Water intake, urine volume and urine concentration were compared in separate repeated measures ANOVAs with woodrat species as the main effect and diet (control, low, medium and high alpha-pinene) as the repeated measure. Paired *t*-tests were used to compare differences between treatments within a species. Tukey's tests were performed to compare differences between woodrat species within a treatment. For cases where urine volume was significantly greater on the treatment versus the control, we examined the effect of water intake on urine volume with analyses of covariance (ANCOVA) for each species with water intake as the covariate and diet treatment (control versus a single treatment) as the main effect.

Creosote resin experiment

We examined the effect of creosote resin on water intake of na and experienced *N. lepida*. Nine experienced and seven na woodrats were given a control diet of 0% resin and then switched sequentially to 1, 2, 3 and 5% resin in the chow. The resin concentrations used were approximately equal to diets of 8, 14, 24 and 40% creosote foliage given the concentration of resin in the leaves (12.4%). Each treatment including the control was given for 6 days. We chose 6-day intervals instead of 3-day intervals used in the previous experiment because we had no previous experiments with resin on which to base the interval length and wanted to allow substantial time for acclimation. The control diet consisted of rabbit chow (Harlan Teklad 8630, Wisconsin). Food and water were offered ad libitum. During all treatments, food was replaced daily and any leftovers were collected, dried at 50°C and weighed. Food intake and body mass were monitored daily as part of another experiment (Mangione et al. 2000). Animals were removed from the experiment if mass loss exceeded 15% of initial mass.

Creosote resin was extracted from leaves of creosote collected at the Mojave site near the trapping areas. A mixture of young and mature foliage was clipped from twigs not bigger than 0.3 cm in diameter. Foliage was transported on dry ice to the University of Utah. Resin was extracted from creosote bush foliage by soaking the leaves in diethyl ether for 45 min (1:5, wet leaf mass/volume). The extract was filtered (Whatman no. 4 paper) and evaporated under vacuum at 38–39°C for 2–4 h. The resin concentration from the leaves we used was ~12.4% of the dry weight.

The diets were treated with creosote resin by dissolving a known amount of resin in a volume of ethanol (95%) equal to 25% of the mass of the ground rabbit chow used in the treatment. The ethanol-resin mixture was added to ground rabbit chow and thoroughly mixed. The chow was then pelleted. Any remaining ethanol was evaporated by leaving the pellets in a hood at room temperature for 24 h. Creosote resin does not require microencapsulation because the phenolic compounds of interest are not volatile. Control diet was prepared by adding ethanol only to the ground rabbit chow and pelleting.

Animals were allowed to acclimate to the diet for 6 days prior to measurements of water intake and urine output. Water consumption and urine output was measured on day 6 of each treatment as described in the alpha-pinene experiment. Urine volume and osmotic pressure were measured as described above.

Water intake, urine volume and urine concentrations were analyzed with repeated measures ANOVA with population as the main effect and diet as the repeated measure. In all cases values are expressed as mean ± one standard error. Paired *t*-tests were used to compare differences between treatments within a species. Tukey's tests were performed to compare differences between woodrat species within a treatment. To compare differences in urine volume between treatments while controlling for water intake, we performed ANCOVA for each population with water intake as the covariate and diet treatment (control versus a single treatment) as the main effect.

Results

Alpha-pinene experiment

The data for food intake are presented elsewhere (Dearing et al. 2000). In summary, food intake, body

mass and water intake stabilized by day 3 of each treatment with the exception of the generalist on the highest dose treatment. There was no difference in food intake for the specialist on any treatment. The generalist decreased food intake by 22% on the highest alpha-pinene treatment compared to the control. On the highest alpha-pinene treatment the specialist and generalist ingested approximately equal doses of alpha-pinene.

There was a significant effect of alpha-pinene ingestion on total water consumption (Table 1, Fig. 1a). Both species of woodrats significantly increased water consumption on the high alpha-pinene treatment compared to the control (Fig. 1a generalist: paired t=2.5, df=9, P=0.03; specialist: paired t=3.2, df=7, P=0.01). There was no difference in total water consumption between the specialist and generalist on control (Tukey's HSD, P>0.05) or the high alpha-pinene treatment (Tukey's HSD, P>0.05; Fig. 1a).

The addition of alpha-pinene to the diet significantly affected urine output (Table 1, Fig. 1b). The generalist produced 1.6× more urine on the high alpha-pinene treatment versus the control (paired t=3.4, df=9, P=0.008). The volume of urine excreted by the specialist was 27% higher on the high alpha-pinene treatment than the control; however, this increase was not statistically significant (paired t=1.1, df=7, P=0.31).

Urine concentration decreased with increasing alpha-pinene in the diet (Table 1, Fig. 1c). There was no main effect of species on urine concentration. However, there was a significant interaction between species and diet treatment. The generalist produced more dilute urine on the high alpha-pinene treatment diet compared to the control (paired t=2.9, df=9, P=0.02). Urine concentration did not differ between diet treatments for the specialist (paired t=0.7, df=7, P=0.51).

The relationship between urine volume and water intake was similar for specialist and generalist on the control and high alpha-pinene treatment (Table 2; Fig. 2). For both the specialist and generalist there was a significant relationship between urine volume and water intake with no effect of diet. Sample size was reduced by 1 for the specialist due to the removal of an extreme outlier.

Creosote resin experiment

Na woodrats were excluded from the experiment after the 3% resin treatment due to excessive weight loss. All between-population comparisons are for resin concentrations of 3% and lower.

Data for food intake are reported elsewhere (Mangione et al. 2000). In brief, food intake was stable for all treatments with the following exceptions. From 0–3% resin, food intake decreased significantly for the na woodrats (~19%) but did not change for the experienced population. Experienced woodrats did not exhibit a significant reduction in food intake until the 5% resin treatment. On all other treatments, food intake, water intake, and body mass stabilized during the 6 days of the treatment.

Table 1 Repeated measures ANOVA results for the experiment comparing *Neotoma stephensi* and *N. albigula* on diets with alpha-pinene. Significant differences are in bold

Alpha-pinene experiment	*F*	*df*	*P*
Total water intake			
Species	0.14	1, 16	0.71
Diet	10.5	3, 48	**0.02**
Interaction	0.19	3, 48	0.90
Urine volume			
Species	0.08	1, 16	0.8
Diet	8.7	3, 48	**0.0001**
Interaction	0.75	3, 48	0.50
Urine concentration			
Species	0.98	1, 16	0.34
Diet	3.2	3, 48	**0.03**
Interaction	3.6	3, 48	**0.03**

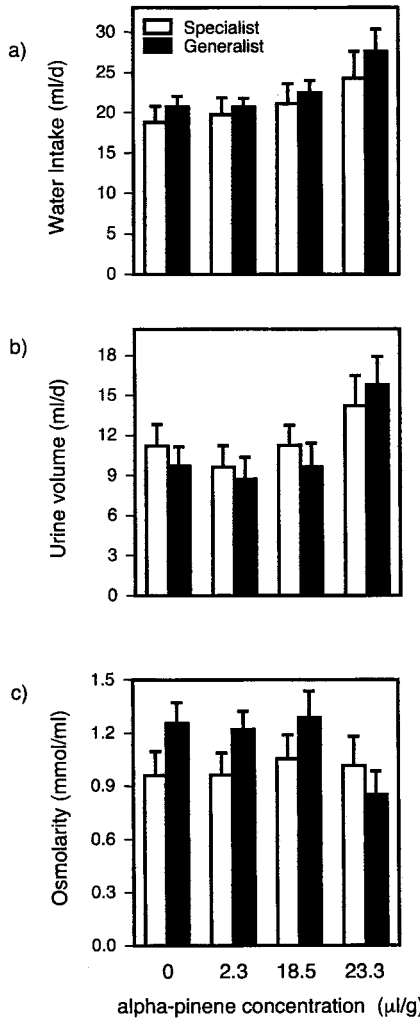

Fig. 1 Water intake (**a**), urine output (**b**) and urine concentration (**c**) of specialist and generalist woodrats on control and alpha-pinene diets. *Error bars* are 1 SE

Table 2 Results of analysis of covariance for urine output by control and alpha-pinene treatment with water intake as the covariate

Alpha-pinene experiment	*F*	*df*	*P*
Specialist			
Diet	2.3	1, 11	0.16
Covariate	195.8	1, 11	**0.0001**
Interaction	1.8	1, 11	0.21
Generalist			
Diet	0.4	1, 16	0.62
Covariate	33.6	1, 16	**0.0001**
Interaction	0.2	1, 16	0.68

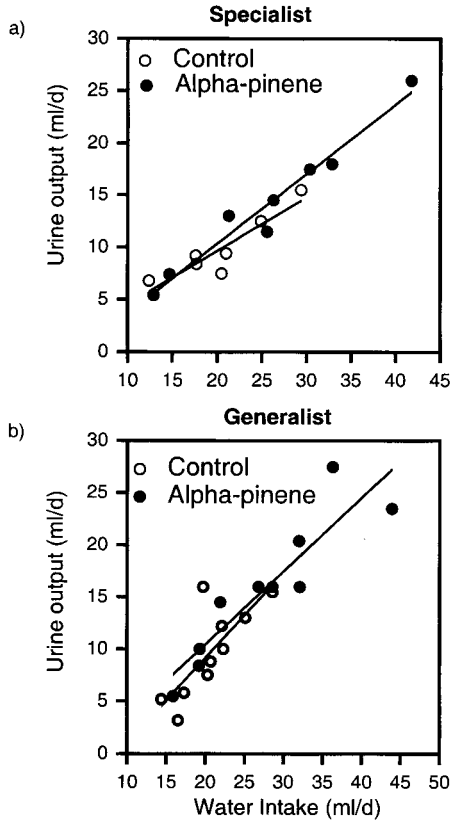

Fig. 2 Urine volume against water intake for **a** specialist and **b** generalist woodrats on control and high alpha pinene diet treatments. Regression lines are plotted for each treatment. There was no difference in slope of the regression lines for either the specialist or the generalist (Table 2)

Resin had a significant effect on water consumption (Table 3, Fig. 3a). At resin concentrations less than 3%, water intake increased significantly for na woodrats but not experienced woodrats. Water intake of na woodrats on a 3% resin diet was 60% higher than the control diet (paired t=2.6, df=6, P=0.04) and 64% higher than experienced woodrats on the same diet treatment (Tukey's HSD, P<0.05; Fig. 3a). Experienced woodrats showed an increase water intake compared to the control at the 5% resin treatment (paired t=3.2, df=6, P=0.02).

Table 3 Repeated measures ANOVA results for the experiment with populations of *N. lepida* consuming diets with creosote resin. Significant differences are shown bold

Creosote resin	*F*	*df*	*P*
Water intake			
Population	7.9	1, 14	0.014
Diet	7.5	3, 42	**0.0001**
Interaction	1.7	3, 42	0.17
Urine volume			
Population	11.24	1, 14	**0.005**
Diet	5.02	3, 42	**0.005**
Interaction	3.04	3, 42	0.039
Urine concentration			
Population	16.6	1, 14	**0.001**
Diet	6.8	3, 42	**0.001**
Interaction	1.2	3, 42	0.32

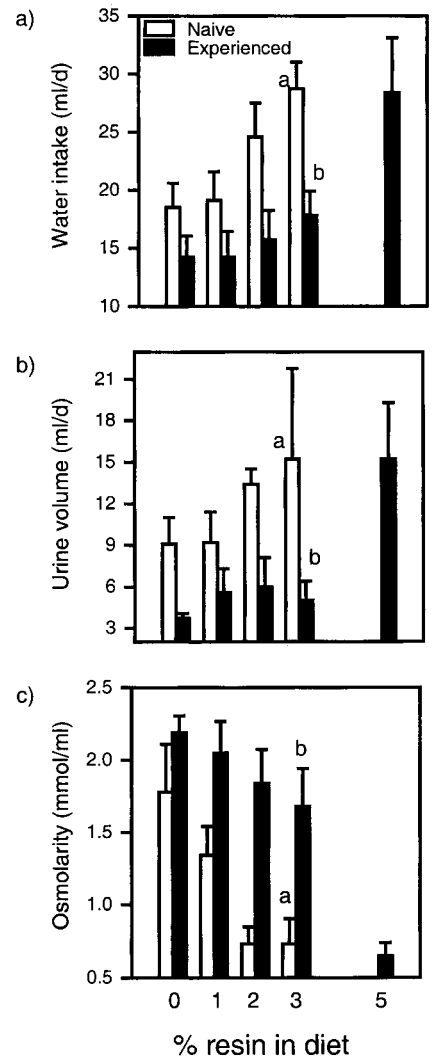

Fig. 3 Water intake (**a**), urine output (**b**) and urine concentration (**c**) of experienced and na woodrats (*Neotoma lepida*) on control and creosote diets. Error bars are 1 SE. Different letters indicate significant differences between populations within a treatment (Tukey's HSD). Na woodrats were removed from the experiment after the 3% resin treatment due to excessive weight loss

Table 4 Analysis of covariance of urine output with water intake as the covariate and diet treatment as the independent variable

Creosote	*F*	*df*	*P*
Experienced 3% resin			
Diet	0.002	1, 13	0.96
Covariate	9.2	1, 13	**0.001**
Interaction	1.2	1, 13	0.30
Experienced 5% resin			
Diet	4.3	1, 11	**0.06**
Covariate	108.7	1, 11	**0.0002**
Interaction	34.1	1, 11	**0.009**
Naive 3% resin			
Diet	0.008	1, 11	0.92
Covariate	24.9	1, 11	**0.0004**
Interaction	0.01	1, 11	0.90

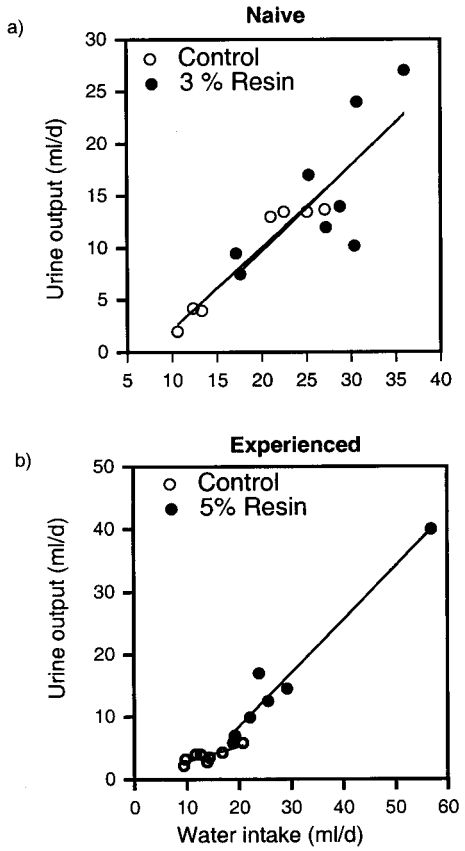

Fig. 4 Urine volume against water intake for **a** na woodrats and **b** experienced woodrats on control and creosote diet treatments. Data for experienced woodrats on 3% creosote are not shown but are similar to that of the na woodrats on the 3% treatment. Regression lines are plotted for each diet treatment. There was a no significant difference in slopes of lines for either experienced or na woodrats on the 3% resin treatment. There was a significant difference in slopes for the experienced woodrats on 5% resin versus control (Table 4). Na woodrats were removed from the experiment prior to the 5% resin treatment due to excessive weight loss

Across all treatments, urine volume increased with increasing resin concentration for na woodrats, but not experienced woodrats as indicated by the significant interaction between population and treatment (Table 3, Fig. 3b). At 3% resin in the diet, urine output of na woodrats was nearly 2× greater than that on the control treatment (paired t=2.6, df=6, P=0.042) and 3× greater than experienced woodrats on the 3% resin diet (Tukey's HSD, P<0.05; Fig. 3b). Urine volume was significantly greater for the experienced woodrats on the 5% resin treatment compared with the control (paired t=2.81, df=6, P=0.03).

Urine concentration decreased significantly with increasing resin in the diet of na woodrats but not experienced woodrats (Table 3, Fig. 3c). Urine concentration of na woodrats was lower than experienced woodrats on the 3% resin treatment (Tukey's HSD, P<0.05; Fig. 3c). The urine of na woodrats was marginally less concentrated at 3% resin than at 0%, (paired t=2.4, df=6, P=0.06).

The relationship between urine volume and water intake was dependent on the concentration of creosote in the diet and the population. There was no difference in the amount of urine relative to the amount of water ingested for either the experienced or na woodrats on the 3% resin diet versus the control (Table 4, Fig. 4 a). However, experienced woodrats on the 5% resin treatment produced more urine per unit water consumed than on the control (Table 4, Fig. 4b).

Discussion

In this study, we examined whether secondary compounds produced diuresis in woodrats and whether specialist and experienced woodrats exhibit less diuresis than generalists and na woodrats. Woodrats indeed consumed more water, excreted more urine and produced more dilute urine when plant toxins were incorporated into the diets compared to toxin-free diets. In one case, the experienced woodrat on 5% creosote, urine output relative to intake was greater than predicted based on the same parameters on control diets. Moreover, woodrats with greater experience with a toxin exhibited less diuresis than woodrats with no or less experience with that toxin.

Diuretic compounds are defined pharmacologically as compounds that elevate urine excretion or decrease urine osmolarity compared to control levels (Hardman and Limbird 1996). Ingestion of either alpha-pinene or creosote resin resulted in increased urine output and decreased urine concentration in woodrats. Thus, both alpha-pinene and creosote resin meet the pharmacological criteria to be classified as diuretics. Moreover, for both secondary compounds, the response was contingent on secondary compound concentration. Threshold doses below which there was no effect of the secondary compound existed for each woodrat population. The concept of threshold is a fundamental principle in pharmacology applicable to diuretic compounds and pharmaceuticals in general (Hardman and Limbird 1996).

The increase in water intake cannot be attributed to an increase in food intake. In neither the creosote nor the alpha-pinene experiment did animals increase food intake (Dearing et al. 2000; Mangione et al. 2000). Food intake tended to decrease for both the na and generalist woodrats whereas it remained relatively constant at comparable toxin concentrations for the experienced and specialist woodrats. Thus, all changes in water intake were due to an increase in water consumed from water bottles.

The increase in water intake associated with the consumption of secondary compounds has serious implications for wild herbivores such as desert dwelling ones with limited access to free water. Woodrats, for example, obtain nearly all of their free water from leaves (Dial 1988). If a plant secondary compound ingested as part of the diet resulted in a net loss of water, woodrats would need to obtain additional water from other dietary sources lacking diuretic compounds so as not to exacerbate water imbalance.

Possible mechanisms

Most diuretics, both synthetic and plant-derived, act by interfering with sodium reabsorption in the kidney and other epithelial tissues that results in elevated sodium loss and increased urine production (Hardman and Limbird 1996). The net loss of water can result in dehydration and thus stimulate thirst leading to increased water intake. Chronic net loss of sodium results in death by cardiovascular collapse.

Increased urine production may also be the result of increased water intake caused by properties of the secondary compounds that affect the sensory system of the herbivore. The discomfort sensed by humans consuming spicy foods is caused by stimulation of the trigeminal nerve (Silver 1987). This irritation is temporarily eliminated through the consumption of water. Herbivores also detect secondary compounds via the trigeminal nerve. For example, the ruffed grouse *(Bonasa umbellus)* detects the plant secondary compound, coniferyl benzoate, with the trigeminal nerve (Jakubas and Mason 1991). Moreover, grouse increase water intake and, thus, urine output when consuming diets with coniferyl benzoate (Jakubas et al. 1993).

Our study did not distinguish between these two possible mechanisms for increased urine production on toxic diets. Increased urine production caused by elevated sodium excretion clearly confers far greater physiological consequences on a herbivore than that caused by irritation of the trigeminal nerve. If secondary compounds disturb sodium reabsorption, we predict a relative increase in urine volume per milliliter of water ingested. Changes in sodium reabsorption in the nephron could alter the volume of urine produced per unit water ingested due to physiological impairment at the cellular level. In one case, that of the experienced woodrat on the 5% creosote diet, there appeared to be an increase in the ratio of urine produced to water consumed on the treatment compared to the control as indicated by the significant treatment and interaction terms (Table 4). This result should be interpreted cautiously given the lack of overlap between the values for the treatments. Alternatively, if the toxin is an irritant that causes increased water intake, then the relative amount of urine excreted should not change. Such a pattern was found for the generalist and specialist on the high alpha-pinene diets and the na woodrat on 3% creosote. Further investigation is necessary to distinguish between possible causes of elevated urine loss.

Effect of experience

We predicted that herbivores with previous experience with a secondary compound would be less impacted than herbivores with less experience. The results of the experiment with *N. lepida* populations unequivocally support this prediction. The Great Basin population of *N. lepida* that had no previous ecological or evolutionary experience with creosote resin showed marked diuresis at lower concentrations of resin than the experienced population from the Mojave. The results from the specialist-generalist comparison also supported this prediction. The generalist had significantly greater urine output on the highest alpha-pinene treatment relative to the control diet, whereas urine output of the specialist did not change significantly with alpha-pinene concentration.

Secondary compounds as diuretics

We propose that secondary compounds have the potential to act as diuretics and that such diuretic compounds may be widespread. Furthermore, we suggest that diuresis may be a common consequence for mammalian herbivores consuming plants with secondary compounds.

Two separate lines of evidence suggest that secondary compounds with diuretic properties may be common in the plant kingdom. First, the secondary compounds used in this study were not closely related either chemically or phylogenetically. Creosote resin is a complex mixture of phenolics, which are aromatic rings with hydroxyl groups and are typically hydrophilic (Mabry et al. 1977). Nordihydroguaiaretic acid, the most abundant phenolic compound in creosote resin, exemplifies these properties (Mabry et al. 1977). Alpha-pinene, in comparison, is a lipophilic terpene with a bicyclic ring structure and no hydroxyl groups (Gershenson and Croteau 1991). Despite their chemical differences, alpha-pinene and creosote resin produced diuresis in woodrats. These results are consistent with the observation that commercially available diuretics exhibit diversity in chemical structures (Hardman and Limbird 1996). Second, ethnopharmacological studies have documented over 85 species of plants with diuretic action (Dearing et al. 2001).

Could mammalian herbivores other than woodrats be subject to diuresis when ingesting secondary com-

pounds? The structure and function of the mammalian kidney is similar among species. Compounds that cause diuresis in laboratory rats also produce diuresis in humans (Dearing et al. 2001). Moreover, there are at least six distinct physiological pathways and sites in mammals through which diuretics act (Hardman and Limbird 1996). Thus, we suggest that given the prevalence of secondary compounds with diuretic properties, diuresis in mammalian herbivores may be a frequent outcome of ingestion of secondary compounds, even chemically unrelated compounds. Future studies are needed to evaluate this hypothesis.

We are not suggesting that diuresis is a universal trait of plant secondary compounds. Many secondary compounds may have no effect or even a positive effect on water balance. For example, Campbell (1999) documented a positive effect of secondary compounds on water balance. White-tailed deer consuming guajillo had reduced water intake and urine output compared to deer on control diets.

Plant secondary compounds with diuretic properties pose an obvious challenge for herbivores in environments such as deserts where water is limited. Diuretic plant compounds may also be an obstacle for herbivores in mesic environments. Sodium is scarce in wet habitats (Robbins 1993). If diuretic plant compounds act by decreasing sodium reabsorption, the loss of sodium in sodium-limited habitats may present a significant physiological cost.

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