

Plant Secondary Compounds as Diuretics: An Overlooked Consequence¹

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SYNOPSIS. Plant secondary compounds are deterrents and toxins to a variety of herbivores. The effect of secondary compounds on water balance of herbivores is virtually unexplored, yet many secondary compounds are renowned for their diuretic effects in humans and laboratory rats. We review data from the ethnopharmacological literature on plants with diuretic effects. We also present our data from experiments on water intake of specialist (*Neotoma stephensi*) and generalist woodrats (*N. albigula*) consuming plant secondary compounds from their natural diet. We measured effects of dietary secondary compounds on voluntary water consumption, urine volume and urine osmolarity. Ingestion of secondary compounds increased water intake and urine output and decreased urine osmolarity in both species. However, the generalist was more impacted by dietary secondary compounds than the specialist. Our results combined with that from the literature suggest that diuresis may be a prevalent consequence of ingestion of secondary compounds. Many herbivores live in arid habitats with limited access to free-standing water, thus an increase in the desire for water may have profound consequences on foraging behavior and fitness.

INTRODUCTION

The ingestion of plant secondary compounds by a herbivore can result in a multitude of physiological consequences. Outcomes of secondary compound ingestion include but are not limited to numerous toxic effects, disturbance of acid-base homeostasis, emetic stimulation and mineral wasting (Foley and Hume, 1987; Foley and McArthur, 1994; Foley *et al.*, 1995; Iason and Murray, 1996; Iason and Palo, 1991; Lindroth and Batzli, 1984; Lindroth *et al.*, 1986; McArthur and Sanson, 1993; McLean *et al.*, 1993; Meyer and Karasov, 1989; Thomas *et al.*, 1988). In addition, various secondary compounds have been documented as feeding deterrents that reduce or eliminate consumption of particular species of plant (Reichardt *et al.*, 1990*a, b*, 1984; Sinclair *et al.*, 1988; Snyder, 1992; Vaughn and Czaplewski, 1985). The proposed mechanisms underlying deterrence include any of the physiological conse-

quences listed above as well as yet undescribed ones (Pass and Foley, 2000).

Theoretically, consumption of secondary compounds could drastically affect the water balance of herbivores. Two separate lines of evidence suggest that ingestion of secondary compounds detrimentally impact water balance and that this effect may be a prevalent one. First, a multitude of plants or specific secondary compounds has been documented to negatively effect water balance in vertebrates, particularly humans (*e.g.*, Adsersen and Adsersen, 1997). Second, herbivores consuming secondary compounds exhibit elevated sodium excretion (Iason and Palo, 1991; Perhson, 1983). Sodium excretion, or natriuresis, almost inevitably increases urinary excretion (Hardman and Limbird, 1996). Therefore, the water balance of animals excreting increased quantities of sodium may have been severely altered.

The effect of plant secondary compounds on water balance of herbivores has been largely ignored by ecologists. In this paper, we review the literature on diuretic effects of plant secondary compounds on humans and laboratory animals such as rats. We also present data on water intake, urine out-

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TABLE 1. *The 6 classes of commercial diuretic drugs, their mechanism and site of action.*

Class	Mechanism	Site
Carbonic anhydrase inhibitors	Abolition of NaHCO ₃ reabsorption through inhibition of carbonic anhydrase	Proximal tubule
Mineralocorticoid receptor inhibitors	Decrease production or block effects of aldosterone	Distal tubule and collecting duct
Potassium sparing	Inhibit renal epithelial Na ⁺ channels	Late distal tubule
Benzothiadiazides	Inhibit Na ⁺ -Cl ⁻ Symport	Distal tubule
High-ceiling diuretics	Inhibit Na ⁺ -K ⁺ -2Cl ⁻ Symport	Thick ascending limb of loop of Henle
Osmotic diuretics	Osmotic effect in tubules; Reduction of medullary tonicity	Loop of Henle, Proximal tubule

put and concentration of herbivores consuming plants with secondary compounds. The results suggest that plant secondary compounds act as diuretics on herbivores and that diuresis may be a general consequence of the ingestion of secondary compounds irrespective of the particular class of compound.

PSC as diuretics

Diuretics are defined as compounds that increase urine flow (Hardman and Limbird, 1996). A primary therapeutic use of diuretic compounds is to lower hypertension by decreasing blood volume through the excretion of water in the urine (Hardman and Limbird, 1996). In general, diuretics decrease Na⁺ reabsorption in the kidney and other epithelial tissues thereby leading to increased urine output. There are approximately 6 different modes of action by which diuretics increase urine volume (Table 1, Hardman and Limbird, 1996). Most diuretic drugs typically act through a single route (Hardman and Limbird, 1996).

The most efficacious diuretics known are compounds that produce diuresis by acting on sodium reabsorption in the ascending limb of the loop henle (Hardman and Limbird, 1996). Compounds acting in this manner are referred to as "high ceiling" or "loop diuretics." There are at least 8 drugs classified as high-ceiling diuretics (Hardman and Limbird, 1996). They exhibit little chemical similarity and can not be classified on the basis of a particular functional group (Hardman and Limbird, 1996). Furosemide (LASIX) represents an example of a high-ceiling diuretic commonly used in the USA.

Although the concept of plant secondary compounds as diuretics is a novel one in the field of plant-herbivore interactions, the diuretic effects of plant secondary compounds on humans have been documented for millennia (Adrsersen and Adrsersen, 1997; Beaux *et al.*, 1999; Johnson *et al.*, 1999; Navarro *et al.*, 1994). Myriad plants are employed worldwide as medicines to control hypertension by reducing blood volume through diuresis. The compounds in these plants are typically administered to the patient orally as an infusion of leaves in hot water. A few are administered through direct consumption of leaves. With the popularity of bioprospecting for natural compounds and the ongoing search for diuretic drugs that do not increase potassium excretion, many "traditional medicines" with proposed diuretic effects have recently been evaluated under experimental conditions. Here we review data from ethnopharmacological literature on plants typically used as diuretics. Although the literature is replete with information on plant compounds proposed to cause diuresis, we present only data for which there were controlled studies.

There were 4 studies that each evaluated a number of plant species available in a particular region such as India, for reputed hypertension or diuretic pharmacological activity (Table 2). Three of these studies evaluated diuretic activity using an *in vitro* system that tests for the ability of a plant extract to inhibit angiotension converting enzyme, ACE (Adrsersen and Adrsersen, 1997; Nyman *et al.*, 1998; Somanadhan *et al.*, 1999). A result of 50% inhibition is cor-

TABLE 2. Results of 4 regional studies on plant extracts with proffered diuretic effects.

Citation	Region	No. species surveyed	No. species w/ diuretic effect	System
Nyman <i>et al.</i> , 1998	India (Gujarat, Rajasthan, Kerala)	75	16	<i>In vitro</i>
Somanadhan <i>et al.</i> , 1999	South India	73	22	<i>In vitro</i>
Adersen and Adersen, 1997	Reunion Island, Indian Ocean	38	17	<i>In vitro</i>
Ribeiro <i>et al.</i> , 1988	Sao Paulo, Brazil	32	28	Rats

related with diuresis in whole animal systems. One study investigated diuretic effects by orally administering plant extracts and measuring urine volume (Ribeiro *et al.*, 1988). Concentration of the extract was roughly equivalent to 5 g fresh leaf material/kg body mass of the animal. The results of all 4 studies were similar in that a large proportion of the plants produced diuretic effects (Table 2). Some plants produced diuretic effects comparable to the high-ceiling diuretic furosemide (Ribeiro *et al.*, 1988). The areas from which the plants were collected were geographically distinct (India, South America and Reunion Island). Moreover, plants with diuretic effects were from a huge range of species and families. For example in these studies, plants with diuretic effects were found in the Asteraceae, Brassicaceae, Erythroxaceae, Flacourtiaceae, Graminaceae, Leaceae, Rubeaceae, Zygophyllaceae and Zingiberaceae, as well as other families.

In addition to these large-scale studies using primarily *in vitro* assays, we found several studies that investigated the diuretic potential of one or a few species of plant in whole animal systems, humans and rats. Nearly all these studies measured sodium excretion as well as urine output. Ten species of plants from 8 families increased urine output in whole animals (Table 3). Nine of these species simultaneously increased sodium excretion. The amounts of plant material in effective doses were quantities that could be ingested by a herbivore in the course of day or even a single meal. For example, in many cases, ingestion of ~2 g of plant leaves by an animal the size of a rat caused significant diuresis. This amount corresponds to 10–15% of the total daily food intake of an animal the size of a rat. Many of these plant extracts were as effective as commercial diuretic drugs.

Of all the species in Table 3, *Othrosiphon aristatus* has received the most attention. This plant from southeast Asia has been used as a tea in Indonesia to treat hypertension and diabetes (Matsubara *et al.*, 1999). Water extracts of the whole plant cause diuresis and naturesis in rats similar in extent to that produced by the high-ceiling diuretic, furosemide. Three compounds in this species have been found thus far to elicit a diuretic effect (Table 3).

Humans regularly ingest as part of their daily diet, a variety of plant compounds with diuretic properties. Coffee, black tea and chocolate contain compounds that are potent diuretics in humans. Simple water extracts of coffee and black tea contain quantities of caffeine great enough to cause water imbalance in humans. For example, if a human ingests ~2 liter/day of fluid with 900 mls of this amount as coffee (~ equal to (2) 16 oz cups), severe negative water balance will result (Neuhaser-Berthold *et al.*, 1997).

Plant secondary compounds with documented diuretic effects occur across the major classes of plant compounds (Table 3). Diuretics can be found in terpenes, phenolics and alkaloids. No one functional group or class of compounds appears to be correlated with a diuretic effect. This result is consistent with the diversity of chemical structures present among commercial diuretic drugs (Hardman and Limbird, 1996). Thus, a wide variety of chemical compounds can produce diuretic effects.

The diuretic effects may be far more pronounced in herbivores that consume the entire leaf rather than a water extract of the plant. In the majority of the studies we reviewed, the material administered to the animal was the dried filtrate of a water extract that had been prepared from dried leaves. The drying process drives off many of the

TABLE 3. *Plants with diuretic effects and specifics of experiments.**

Species/Family	C. No.	U.V.	Na ⁺	Dose	Organism	Route	Positive Control	Plant secondary compounds
<i>Othrosiphon stamineus</i> Lamiaceae	1	↑	↑	50 mg/kg	Rat	IP	HCT*	Sinensein, 3'-hydroxy-5,6,7,4'-tetra methoxy-flavone, methyliriparinochomene
<i>Hieracium pilosella</i> Asteraceae	1	↑	N.C.	50–200 mg/kg	Rat	IP	HCT*	
<i>Sambucus nigra</i> Caprifoliaceae	1	↑	↑	50 mg/kg	Rat	IP	HCT*	
<i>Arctostaphylos uva ursi</i> Ericaceae	1	↑	N.C.	50 mg/kg	Rat	IP	HCT*	Phenolic glycosides, iridoid glycosides
Coffee Rubeaceae	2	↑	↑	8 mg caffeine/kg	Human	Oral	No	Caffeine
<i>Euphorbia hirta</i> Euphorbeaceae	3	↑	↑	50–100 mg/kg	Rat	IP	A* F	Phenolics
<i>Phyllanthus sellowianus</i> Euphorbeaceae	4	↑	↑	400 mg/kg	Rat	Oral	HCT*	Phyllanthol, flavones
<i>Phyllanthus amarus</i> Euphorbeaceae	5	↑	↑	420 mg/kg	Human	Oral	No	Phenolics, gallic acid
<i>Eugenia uniflora</i> Myrtaceae	6	↑	↓	15–120 mg/kg	Rat	Oral	AM~	Flavonoids, triperpinoids, monoterpenes
<i>Lepidium latifolium</i> Brassicaceae	7	↑	N.C.	50–100 mg/kg	Rat	Oral	No	Polyphenols, sulfurated essences

* C. No. refers to the citation number, 1 = Beaux, *et al.*, 1999; 2 = Neuhäuser-Berthold *et al.*, 1997; 3 = Johnson *et al.*, 1999; 4 = Hnatysyn *et al.*, 1999; 5 = Srividya and Perwal 1995; 6 = Consolini *et al.*, 1999; 7 = Navarro, *et al.*, 1994. U.V. stands for urine volume and Na⁺ stands for sodium excretion. Arrows indicate the direction of change relative to the control group. Route describes the way in which compounds were administered. Oral via gavage, IP = intraperitoneally. "Plant secondary compounds" are secondary compounds mentioned by authors. These refer to possible compounds responsible for diuretic effects. Abbreviations for diuretic drugs used as positive controls are A = acetazolamide, AM = Amelotide, HCT=hydrochlorozamide, F = furosemide.

volatile secondary compounds and inactivates other types of compounds. Extraction with water selects for compounds that are hydrophilic. Both of these processes could reduce the concentration of diuretic compounds present in leaves.

In summary, the ethnopharmacological literature indicates that many species of plants produce diuretic effects. These effects are not restricted to a particular family or group of plants. In our review of the literature, we found examples of over 85 species from an extremely diverse set of families that exhibited diuretic effects. The secondary compounds present in these plants represent all major classes of compounds such as terpenes, phenolics and alkaloids (Table 3). This result is consistent with the diversity of chemical structures present among commercial diuretics (Hardman and Limbird, 1996). Thus, a wide variety of plant chemical compounds produce diuresis in mammals.

Studies on natural plant-herbivore systems

To investigate the effects of ingestion of secondary compounds on water intake, we conducted experiments with two species of mammalian herbivores, *N. stephensi*, Stephen's woodrat and *N. albigula*, whitethroat woodrat. *N. stephensi* and *N. albigula* occur sympatrically in Great Basin ecotones in Arizona. *N. stephensi* and *N. albigula* consume one-seeded juniper, *Juniperus monosperma*. One-seeded juniper contains a number of secondary compounds, particularly monoterpenes and phenolics, which are toxic to mammals. *Juniperus communis*, a close relative of one-seeded juniper, has been reported to have diuretic properties (de Medina *et al.*, 1994).

We tested 3 specific hypotheses to determine whether juniper compounds affected various components of water balance of woodrats. Hypothesis 1: Ingestion of plant secondary compounds increases water intake. We fed woodrats control diets (no toxins) and treatment diets (with juniper toxins) and monitored voluntary water intake. We predicted that if plant secondary compounds influence water consumption, water intake should increase on toxic diets compared with controls.

Hypothesis 2: Plant secondary compounds act as diuretics. For a compound to be considered a diuretic, urine volume per ml of water consumed must increase. This relationship suggests that urine volume should be analyzed with total water intake as a co-variate. To determine whether the plant secondary compounds acted as a diuretic, we measured urine production and osmolarity of urine of woodrats on control and toxic diets. If secondary compounds act as diuretics, we predicted that urine volume would increase and urine osmolarity would decrease on the toxic diets.

Hypothesis 3: Extensive prior experience with specific toxins lessens the impact of toxin ingestion on water intake. We compared the performance of a specialist-generalist pair comprised of *N. stephensi* and *N. albigula*. *N. stephensi* is a juniper specialist, 65–95% of its year round diet consists of juniper. *N. albigula*, also consumes juniper, but it never comprises more than 35% of its diet. In the laboratory, *N. stephensi* can tolerate higher doses of juniper toxins than *N. albigula* (Dearing *et al.*, 2000). Because the specialist regularly ingests higher quantities of juniper toxins than the generalist, we predicted that the specialist should be physiologically more adept at consuming juniper toxins than the generalist and that water intake of the specialist should be less impacted than the generalist.

METHODS AND MATERIALS

Collection of woodrats and plants

Neotoma stephensi and *N. albigula* were trapped on Woodhouse Mesa, AZ (35°30'N; 111°27'W). This site was the same as used by Dial (1988); we trapped at many of the exact locations as indicated by trap markers. Additional *N. albigula* were trapped in Castle Valley, UT (38°38'N; 109°18'W). Woodrats were captured using Tomahawk and Sherman live traps baited with peanut butter and oats. Pieces of cotton batting were placed in all traps to keep animals from freezing. Traps were opened late in the afternoon and checked immediately after sunrise. Vegetation in both trapping areas was similar, see Dearing *et al.* (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.

Juniper (*J. monosperma*) used in the experiment was collected from the Woodhouse Mesa study site. Foliage was collected randomly from several trees (*ca.* 10) and combined. Foliage was kept frozen (-20°C) until the day of the experiment and was thawed prior to presentation to woodrats. Woody stems were removed from juniper branches such that woodrats were given only terminal tips, the portion they consume in nature.

Juniper experiment

We compared the water intake of specialist, *N. stephensi*, and generalist, *N. albigula* woodrats on control and juniper diets to determine whether the addition of whole juniper to the diet altered water intake. We fed 8 *N. stephensi* and 10 *N. albigula* control, acclimation and juniper treatments. Each treatment was fed for 3 days and in the following sequence control, acclimation, and juniper diets. The control treatment consisted of chow whose N (1.25%) and fiber (23% ADF) levels were similar to that of juniper (1% N; 24% acid detergent fiber; Dearing *et al.*, 2000). For a complete description of the diet formulations see Dearing *et al.*, 2000. The control was followed by a 3 day acclimation period where control chow and fresh juniper were provided *ad lib*. Three days was given as the acclimation period as this is the amount of time required for induction of the detoxification system (Dearing *et al.*, 2000). Following the acclimation period, woodrats were provided with juniper *ad lib*. and a reduced amount of control chow, equal to 15% of each animal's food intake on the control diet. It was necessary to provide this minimal amount of chow in the juniper treatment because some woodrats on juniper alone lose weight too quickly to remain in the experiment for 3 days. We provided 50 g of freshly thawed juniper daily during the acclimation and juniper treatments. This amount is approximately twice the juniper that woodrats require to maintain weight

(Vaughn, 1982). Food was replaced daily and any leftovers were collected, dried at 45°C and weighed. We monitored food intake and body mass daily as part of another study (Dearing *et al.*, 2000).

Total water consumption included water consumed from free water *i.e.*, that in the water bottle plus that contained in the food. Free water consumption was estimated from the daily change in weight of the water bottles. Three control water bottles did not show significant change in water (100 $\mu\text{l/day}$) through evaporation or spillage, therefore, we did not adjust for loss through evaporation or spillage. We measured the amount of water consumed in the food by multiplying the dry weight amount of food consumed by the amount of water per unit dry weight of food. Dry weight of food consumed was estimated daily as the amount of dry weight offered minus the dry weight of leftovers. Dry weight estimates of food consumed are more accurate than wet weights as wet weights can vary significantly during the experiment due to the loss of water through evaporation. The amount of water in all foods (juniper and chow) was estimated daily by drying a known mass of food in an oven at 45°C and weighing after drying.

On day 3 of each treatment, woodrats were confined to a portion of their cage (16 \times 19 \times 20 cm) that allowed for the separate collection of urine and feces. We collected urine during the last 24 hr of each treatment. The collection vial for urine was embedded in a frozen freezer pack to keep the urine cold and decrease evaporation. Temperature of urine in the coolers ranged from $0-5^{\circ}\text{C}$. Urine was stored at -20°C until it was analyzed.

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 and therefore were diluted with distilled water (1:1 or 1:2 urine:water depending on concentration of sample) prior to the measurement.

Water intake, urine volume and urine concentration was compared in separate repeated measures ANOVAs with woodrat

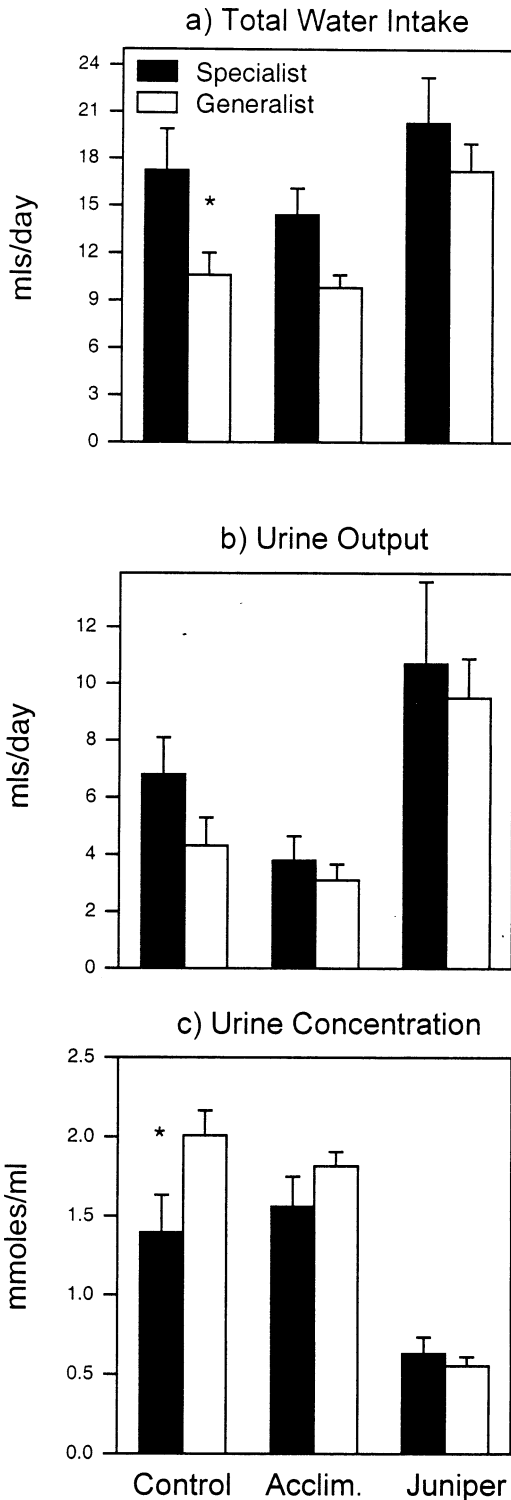


FIG. 1. a) Total water intake, b) urine output and c) urine concentration of woodrats on control, acclima-

species as the main effect and diet treatment as the repeated measure. Data from the acclimation treatment for juniper were not included in the statistical analysis as the acclimation diet was not an experimental treatment but rather a period to allow the animals to adapt to the consumption of juniper. Tukey's tests were performed to compare differences between woodrat species within a treatment. Differences between treatments within a species were compared with paired *t*-tests. To compare differences in urine volume between treatments while controlling for water intake, we performed analyses of covariance with water intake as the co-variate and species or treatment as the main effect.

RESULTS

On the juniper treatment, food intake by the specialist was $1.6\times$ that of the generalist (data in Dearing *et al.*, 2000). Neither the specialist nor the generalist consumed 100% of their maintenance food intake on the juniper treatment. On the juniper treatment, the specialist consumed $66\% \pm 7$ of maintenance whereas the generalist consumed only $41\% \pm 1$ of maintenance. Both species consumed all of the control food offered during the juniper treatment. The generalist lost significantly more weight than the specialist ($\sim 4\%$ vs. 8%) on the juniper treatment (Dearing *et al.*, 2000).

There was a significant effect of diet treatment on total water intake (Fig. 1a). The generalist increased its total water intake by $1.7\times$ on juniper treatment diet compared to the control (paired *t*-test, $t = 3.6$, $P = 0.004$). There was a marginal increase in water intake of the specialist on the juniper treatment versus the control (paired *t*-test, $t = 2.3$, $P = 0.06$). In the repeated measures analysis (RMA), there was not a significant difference between species of woodrats nor was the interaction term significant (Table 4).

Urine volume output increased on the

tion and juniper diets. The specialist is *N. stephensi* and the generalist is *N. albigula*. Asterisks indicate significant differences within a treatment (Tukey's HSD).

TABLE 4. Results of repeated measures analysis of variance for total water intake, urine volume and urine concentration. Significant results are indicated in bold.

	F	df	P
Total water intake			
Species	1.7	1,13	0.21
Diet	19.7	1,13	0.0007
Interaction	1.7	1,13	0.22
Urine output			
Species	2.0	1,15	0.18
Diet	19.3	1,15	0.0005
Interaction	2.5	1,15	0.13
Urine concentration			
Species	2.8	1,14	0.12
Diet	57.0	1,14	0.0001
Interaction	5.4	1,14	0.04

treatment diet (Table 4, Fig. 1b). There was no significant effect of species nor was the interaction term significant. However, even though there was not a significant species effect, the specialist and generalist responded differently to the treatment with respect to urine output. The generalist produced 2× the volume of urine on the treatment diet versus the control (paired *t*-test, *t* = 3.4, *P* = 0.009). Urine output did not change significantly for the specialist on the control versus treatment diet (paired *t*-test, *t* = 1.0, *P* = 0.33).

There was a significant effect of diet treatment on osmotic pressure of the urine. Osmolarity of the urine declined significantly with the addition of juniper to the diet (Table 4, Fig. 1c). There was a significant interaction between species and treatment. The generalist produced a significantly more concentrated urine than the specialist on the control diet only. On the treatment diet, there was no difference between species in urine concentration; both species produced urine approximately 2× as dilute as urine produced during the control diet.

Specialists differed significantly from generalists in urine output when water intake was statistically controlled using an ANCOVA (Table 5). Because of this difference, we performed separate ANCOVAs for each species to investigate urine volume on control and treatment diets. The specialist excreted significantly more urine per ml of water ingested on the treatment diet than

TABLE 5. Results of ANCOVAs on urine volume with water intake as the covariate. Significant results are indicated in bold.

	F	df	P
Both species			
Species	4.2	1,32	0.05
Covariate	69.0	1,32	0.0001
Interaction	3.6	1,32	0.07
Specialist			
Diet	7.5	1,12	0.02
Covariate	65.6	1,12	0.001
Interaction	12.3	1,12	0.005
Generalist			
Diet	0.37	1,16	0.55
Covariate	33.5	1,16	0.001
Interaction	0.18	1,16	0.67

that predicted by the control diet as indicated by significantly different slopes in an ANCOVA (Table 5, Fig. 2). The generalist did not exhibit this pattern. There was no significant effect of treatment or treatment by water intake for generalists on the control and treatment diets.

DISCUSSION

A central goal of this experiment was to determine whether the addition of plant secondary compounds to the diet affects water use of herbivores. The results are consistent with this hypothesis. In the generalist herbivore, *N. albigula*, water intake and urine volume increased while urine osmolarity decreased when plant secondary compounds in the form of whole juniper were added to the diet. The specialist, *N. stephensi*, excreted significantly more urine per ml of water ingested on the diet containing secondary compounds compared to the control. Thus, the addition of juniper to the diets of two species of herbivore altered water use.

We attribute the change in water use parameters of woodrats on the control versus juniper treatment to the secondary compounds in the juniper treatment. The primary difference between the two treatments was the presence of secondary compounds. The control diet was free of secondary compounds whereas the juniper treatment being primarily juniper foliage contained the miscellany of compounds present in fresh juniper. The nitrogen and fiber con-

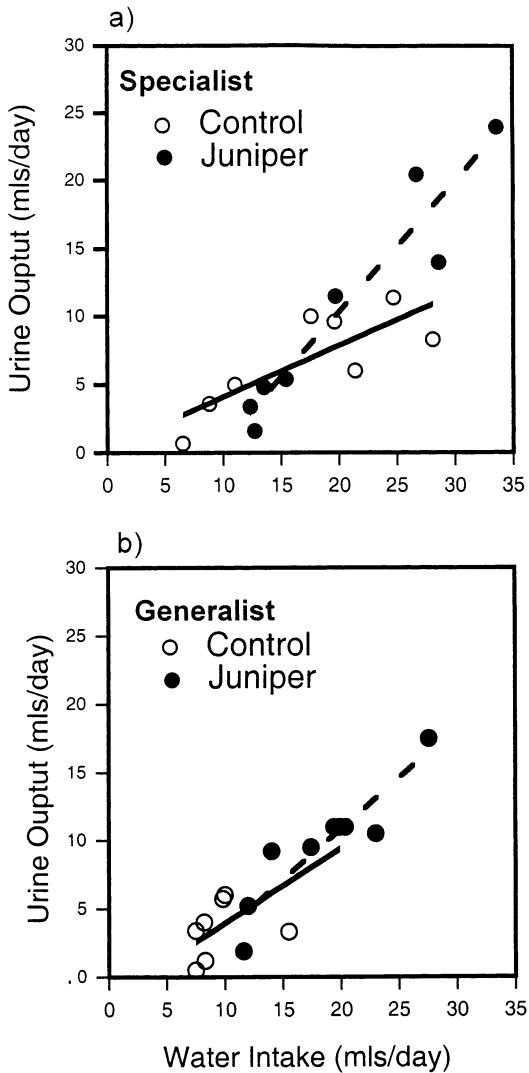


FIG. 2. Urine output *versus* water intake for woodrats on the control and juniper diets. The a) specialist and b) generalist are plotted separately because there was a significant species effect in the ANCOVA (Table 5). a) For a given water intake, the specialist excreted more urine on the juniper treatment ($P = 0.0002$; $r^2 = 0.90$) *versus* the control ($P = 0.02$; $r^2 = 0.58$; Table 5). b) In the generalist, the relationship between urine volume and water intake was similar between diet treatments (Table 5; pooled regression for control and treatment: $P = 0.0001$; $r^2 = 0.78$).

tents of the control diet were similar to that of juniper foliage (Dearing *et al.*, 2000). Therefore, it is unlikely that nutritional differences between the control and treatment

diet were responsible for the differences in water balance.

We do not know which secondary compounds in juniper caused the diuretic effects. The secondary compounds of juniper are diverse. Juniper contains 2 different classes of secondary compounds, terpenes and phenolics (Adams *et al.*, 1981; Dearing *et al.*, 2000; Holchek *et al.*, 1990). Examples of diuretic compounds are known from both of these classes (Consolini *et al.*, 1999; Matsubara *et al.*, 1999). The phenolics in juniper are a mixture of simple phenolics, hydrolyzable tannins and condensed tannins. The structures of these compounds and their quantities have not been evaluated. Thirty-five monoterpenes in juniper have been identified (Adams *et al.*, 1981). The primary monoterpene is alpha-pinene, which comprises approximately 60% of the terpene content and $\sim 2\%$ of the dry weight of the plant (Adams *et al.*, 1981). Alpha-pinene is a documented central nervous system depressant but has not been evaluated for diuretic effects (Adams *et al.*, 1981; Eriksson and Levin, 1990; Hedestierna *et al.*, 1983; Koppel *et al.*, 1981). Given its abundance and pharmacological activity on other systems, alpha-pinene may significantly contribute to the diuretic effects of juniper.

Another objective of this experiment was to contrast the effects of plant secondary compounds on water use of specialist and generalist herbivores. The prediction was that the specialist, whose diet consists of juniper foliage, should be less impacted with respect to water use when consuming juniper than the generalist. In some respects, the data support this prediction. The specialist did not significantly increase water intake or urine output with the addition of juniper to the diet, whereas the output for both of these parameters nearly doubled in the generalist. Moreover, even though both species exhibited a decrease in urine concentration on the juniper diet, they had similar urine concentrations on this treatment. This result is surprising given that diuretics act in a dose dependent fashion and that the specialist was consuming 55% more juniper toxins compared to the generalist. Thus, even though its toxin load was

greater the specialist performed the same as the generalist with respect to urine concentration.

However, the water use of the specialist was not unaltered by the ingestion of juniper. The results of the ANCOVA revealed that the specialist excreted significantly more urine per ml of water consumed on the treatment diet than that predicted from its performance on the control (Fig. 2). The generalist did not exhibit this pattern. These results could be interpreted two ways. First, the generalist may respond differently to the diuretic effects of juniper toxins than the specialist. Conversely, the dose of juniper toxins ingested by the generalist may have been below the threshold dose necessary to produce a diuretic effect. Minimum doses to produce diuresis have been documented for a number of secondary plant compounds. The specialist consumed $1.6\times$ the dose of juniper toxins as the generalist, which may have been above the threshold necessary to generate elevated urine excretion per ml of water consumed. More studies set in a phylogenetic context are needed to adequately compare the water use efficiencies of specialist and generalist herbivores on toxic diets.

The data presented in this paper represent a preliminary investigation of possible diuretic effects of secondary compounds on herbivores. A few caveats should be considered in evaluation of these data. First, we did not experimentally control water intake. Rather, we statistically controlled for differences in water intake when evaluating urine output through ANCOVA. In the case of the specialist, the interpretation of the data is straightforward because there were significant differences between urine output per ml of water consumed on the treatment versus the control diets. The specialist excreted $2.5\times$ more urine per ml of water consumed on the treatment diet than on the control. This result supports the hypothesis that juniper compounds act as a diuretic. However, it is impossible to distinguish decisively between two alternatives in the absence of significant differences in slope of urine output and water intake, as exemplified by the result from the generalist. The increased urinary output of the generalist

on the juniper treatment could have been a function of the increased water intake with no diuretic effect of juniper. It is possible that the addition of juniper to the diet caused the generalist to consume more water (*e.g.*, a response to different tastes) which in turn resulted in an increase in urine output. Alternatively, juniper consumption could have increased urine excretion and the animal could have compensated by the increasing water intake to match urine output. The only means to discriminate between these alternatives is to experimentally control water availability.

We also did not control for differences in food intake. On the treatment diet, food intake and urine concentration decreased significantly for both the specialist and generalist. Because osmolarity of urine is affected by osmolyte load in the diet the decreased osmolarity of the urine may have resulted from the decreased in food intake. However, the relationship between food intake and urine concentration can not completely explain the urine concentration results on the treatment diet. There was no difference in urine concentration between the specialist and generalist on the treatment diet yet the specialist was consuming 30% more food.

Because the mechanism of action of most diuretic drugs is through the blocking of sodium reabsorption, such compounds typically result in large losses of sodium. Could the loss of sodium be more important to the herbivore than the loss of water? The relative importance of the loss of water and sodium should be commensurate with the abundance of these compounds in the environment. The herbivores we studied in this experiment are desert dwellers that acquire the majority of water from their diet (Dial, 1988). They have access to free standing water only during the rainy season. In the desert environment, sodium is abundant in the soil. Thus for desert dwellers, the loss of water may have a far greater impact than the loss of sodium.

In contrast, herbivores that inhabit alpine and mesic areas may be much more impacted by the sodium loss associated with diuretic plant compounds rather than loss of water. Soils in most mesic and alpine hab-

itats are deplete of sodium to the extent that sodium availability is thought to be a primary force driving diet selection and animal distribution (Robbins, 1993). For example, diet selection by moose is governed by selection for plants with high concentrations of sodium. Thus, a diuretic plant compound could severely impact a herbivore in the desert through increased water loss but could also significantly affect animals where water is abundant through excess sodium loss.

In summary, we suggest that a general effect of many secondary compounds could be to negatively impact the water use efficiency of herbivores. The ethnopharmacological data suggest that diuretic compounds in plants are widespread and that these effects are manifest in quantities that would be obtained in the diets of herbivores. Moreover, animals in environments other than ones where water is limiting could be severely impacted by diuretic compounds through via disruption of sodium balance. Future investigations into the diuretic effects of plant secondary compounds should consider adding plant extracts or specific compounds to the control diet, holding water intake constant and monitoring sodium levels in the urine. It is possible that animals are able to compensate for water lost in the urine by decreasing water lost via other routes such as feces. Thus, studies on the diuretic effects of secondary compounds should take into account the entire water budget of the organism. Lastly, experiments that address whether minimum water requirements are elevated by secondary compounds would be useful in determining whether diuretic effects of secondary compounds limit food intake.

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REFERENCES

- Adams, R. P., T. A. Zanoni, E. V. Rudloff, and L. Hogge. 1981. The south-western USA and northern Mexico one-seeded junipers: Their volatile oils and evolution. *Biochem. System. Ecol.* 9:93–96.
- Adersen, A. and H. Adersen. 1997. Plants from Reunion Island with alleged antihypertensive and diuretic effects—an experimental and ethnobotanical evaluation. *J. Ethnopharmacol.* 58:189–206.
- Beaux, D., J. Fleurentin, and F. Mortier. 1999. Effect of extracts of *Orthosiphon stamineus* Benth, *Hieracium pilosella* L., *Sambucus nigra* L. and *Arctostaphylos uva-ursi* (L.) Spreng. in rats. *Phytother. Res.* 13:222–225.
- Consolini, A. E., O. A. Baldini, and A. G. Amat. 1999. Pharmacological basis for the empirical use of *Eugenia uniflora* L. (Myrtaceae) as antihypertensive. *J. Ethnopharmacol.* 66:33–39.
- Dearing, M. D., A. M. Mangione, and W. H. Karasov. 2000. Diet breadth of mammalian herbivores: Nutrient versus detoxification constraints. *Oecologia* 123:397–405.
- Dearing, M. D., A. M. Mangione, W. H. Karasov, S. Morzunov, E. Otteson, and S. St. Jeor. 1998. Prevalence of hantavirus in four species of *Neotoma* from Arizona and Utah. *J. Mammal.* 79:1254–1259.
- de Medina, F. S., M. J. Gamez, I. Jimenez, J. Jimenez, J. I. Osuna, and A. Zaruelo. 1994. Hypoglycemic activity of juniper “berries.” *Planta Med.* 60:197–200.
- Dial, K. P. 1988. Three sympatric species of *Neotoma*: Dietary specialization and coexistence. *Oecologia* 76:531–537.
- Eriksson, K. and J. O. Levin. 1990. Identification of cis and trans-verbenol in human urine after occupational exposure to terpenes. *Occup. Environ. Health* 62:379–383.
- Foley, W. J. and I. D. Hume. 1987. Digestion and metabolism of high-tannin Eucalyptus foliage by the brushtail possum (*Trichosurus vulpecula*) (Marsupialia: Phalangeridae). *J. Comp. Physiol.* 157: 67–76.
- Foley, W. J. and C. McArthur. 1994. The effects and costs of allelochemicals for mammalian herbivores: An ecological perspective. In D. J. Chivers and P. Langer (eds.), *The digestive system of mammals*, pp. 370–391. Cambridge University Press, Cambridge.
- Foley, W. J., S. McLean, and S. J. Cork. 1995. Consequences of biotransformation of plant secondary metabolites on acid-base metabolism in mammals—a final common pathway? *J. Chem. Ecol.* 21:721–743.
- Hardman, J. G. and L. E. Limbird. 1996. *Goodman & Gilman's, the pharmacological basis of therapeutics*. 9th ed. McGraw Hill, New York.
- Hedenstierna, G., R. Alexanderson, K. Wimander, and G. Rosen. 1983. Exposure to terpenes: Effects on pulmonary function. *Inter. Arch. Occup. Environ. Health* 51:191–198.
- Holchek, J. L., A. V. Munshikpu, G. Nunez-Hernandez, R. Valdez, J. D. Wallace, and M. Cardenas.

1990. Influences of six shrub diets varying in phenol content on intake and nitrogen retention by goats. *Trop. Grasslands* 24:93–98.
- Iason, G. R. and A. H. Murray. 1996. The energy costs of ingestion of naturally occurring nontannin plant phenolics by sheep. *Physiol. Zool.* 69:532–546.
- Iason, G. R. and R. T. Palo. 1991. Effects of birch phenolics on a grazing and browsing mammal: A comparison of hares. *J. Chem. Ecol.* 17:1733–1743.
- Johnson, P. B., E. M. Abdurahman, E. A. Tiam, I. Abdu-Aguye, and I. M. Hussaini. 1999. Euphorbia hirta leaf extracts increase urine output and electrolytes in rats. *J. Ethnopharmacol.* 65:63–69.
- Koppel, C., J. Tenczer, U. Tonnesmann, T. Schirop, and K. Ibe. 1981. Acute poisoning with pine oil—metabolism of monoterpenes. *Arch. Toxicol.* 49:73–78.
- Lindroth, R. L. and G. O. Batzli. 1984. Plant phenolics as chemical defenses: Effects of natural phenolics on survival and growth of prairie voles (*Microtus ochrogaster*). *J. Chem. Ecol.* 10:229–244.
- Lindroth, R. L., G. O. Batzli, and S. I. Avildsen. 1986. *Lespedeza* phenolics and *Penstemon* alkaloids: Effects on digestion efficiencies and growth of voles. *J. Chem. Ecol.* 12:713–728.
- Matsubara, T., T. Bohgaki, M. Watarai, and H. Suzuki. 1999. Antihypertensive actions of methylripariochromene A from *Orthosiphon aristatus*, an Indonesian traditional medicine plant. *Biol. Pharmacol. Bull.* 22:1083–1088.
- McArthur, C. and G. D. Sanson. 1993. Nutritional effects and costs of a tannin in a grazing and browsing macropod. *Func. Ecol.* 7:690–696.
- McLean, S., W. J. Foley, N. W. Davies, S. Brandon, L. Duo, and A. J. Blackman. 1993. Metabolic fate of dietary terpenes from *Eucalyptus radiata* in common ringtail possum (*Pseudocheirus peregrius*). *J. Chem. Ecol.* 19:1625–1643.
- Meyer, M. W. and W. H. Karasov. 1989. Antiherbivore chemistry of *Larrea tridentata*: Effects on woodrat (*Neotoma lepida*) feeding and nutrition. *Ecology* 70:953–961.
- Navarro, E., J. Alonso, R. Rodriguez, J. Trujillo, and J. Boada. 1994. Diuretic action of an aqueous extract of *Lepidium latifolium* L. *J. Ethnopharmacol.* 41:65–69.
- Neuhäuser-Berthold, M., S. Beine, S. Ch. Verweid, and P. M. Luhrmann. 1997. Coffee consumption and total body water homeostasis as measured by fluid balance and bioelectrical impedance analysis. *Ann. Nutr. Metab.* 41:29–36.
- Nyman, U., P. Joshi, L. B. Madsen, T. B. Pedersen, M. Pinstrup, S. Rajasekharan, V. George, and P. Pushpangadan. 1998. Ethnomedical information and *in vitro* screening for angiotensin-converting enzyme inhibition of plants utilized as traditional medicines in Gujarat, Rajasthan and Kerala (India). *J. Ethnopharmacol.* 60:247–263.
- Pass, G. J. and W. J. Foley. 2000. Plant secondary compounds as mammalian feeding deterrents: Separating the effects of the taste of salicin from its post-ingestive consequences in the common brushtail possum, *Trichosurus vulpecula*. *J. Comp. Physiology B* 170:185–192.
- Perhson, A. 1983. Digestibility and retention of food components in caged mountain hares, *Lepus timidus* during the winter. *Hol. Ecol.* 6:395–403.
- Reichardt, P. B., J. P. Bryant, B. J. Andersen, D. Phillips, T. P. Clausen, M. Meyer, and K. Frisby. 1990a. Germacrone defends Labrador tea from browsing by snowshoe hares. *J. Chem. Ecol.* 16:1961–1970.
- Reichardt, P. B., J. P. Bryant, T. P. Clausen, and G. D. Wieland. 1984. Defense of winter-dormant Alaska paper birch against snowshoe hares. *Oecologia* 65:58–69.
- Reichardt, P. B., J. P. Bryant, B. R. Mattes, T. P. Clausen, F. S. Chapin, III, and M. Meyer. 1990b. Winter chemical defense of Alaskan balsam poplar against snowshoe hares. *Oecologia* 16:1941–1959.
- Ribeiro, R. d. A., F. de Barros, M. M. de Melo, C. Muniz, S. Chieia, M. d. G. Wanderley, C. Gomes, and G. Trolin. 1988. Acute diuretic effects in conscious rats produced by some medicinal plants used in the state of Sao Paulo, Brasil. *J. Ethnopharmacol.* 24:19–29.
- Robbins, C. T. 1993. *Wildlife feeding and nutrition*. 2nd ed. Academic Press, San Diego.
- Sinclair, A. R. E., K. J. Madhu, and R. J. Andersen. 1988. Camphor from juvenile white spruce as an antifeedant for snowshoe hares. *J. Chem. Ecol.* 14:1505–1514.
- Snyder, M. A. 1992. Selective herbivory by Abert's squirrel mediated by chemical variability in ponderosa pine. *Ecology* 73:1730–1741.
- Somanadhan, B., G. Varughese, P. Palpu, R. Sreedharan, L. Gudiksen, U. W. Smitt, and U. Nyman. 1999. An ethnopharmacological survey for potential angiotensin converting enzyme inhibitors from Indian medicinal plants. *J. Ethnopharmacol.* 65:103–112.
- Thomas, D. W., C. Samson, and J. Bergeron. 1988. Metabolic costs associated with the ingestion of plant phenolics by *Microtus pennsylvanicus*. *J. Mammal.* 69:512–515.
- Vaughn, T. A. 1982. Stephen's woodrat, a dietary specialist. *J. Mammal.* 63:53–62.
- Vaughn, T. A. and N. J. Czaplewski. 1985. Reproduction in Stephen's woodrat: The wages of folivory. *J. Mammal.* 66:429–443.