# Generalist dietary responses to individual vs combined plant toxin odors: an African elephant study

**Abbreviated:** Individual vs combined toxin odors and generalist herbivory

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## Abstract

The detoxification limitation hypothesis posits that, unless plant defense compounds interact synergistically or additively to increase their harmful effects, generalist herbivores will prefer consuming combinations of these compounds over singular compounds. Monoterpenes are odoriferous defense compounds that may be toxic to mammalian herbivores when ingested in sufficient quantities. Previous research has shown that the addition of individual monoterpenes to food sources reduces consumption by generalist mammalian herbivores. By using African elephants as a case study, we aimed to determine whether odors from monoterpene combinations

(i.e., two or more monoterpenes) also deter generalist mammalian herbivory, and whether generalist herbivores prefer the odors of monoterpene combinations over individual monoterpenes. First, we tested whether the odor of monoterpene combinations that resemble the monoterpene profiles of a high-acceptability, intermediate-acceptability, and low-acceptability plant deter herbivory. We found that elephants preferred plants without the added odors of the monoterpene combinations. Second, we explored how elephants responded to individual monoterpenes found within the combinations compared to the combinations at the same set concentration, and found that the elephants did not always prefer the combinations over the individual monoterpenes. Moreover, the more diverse the combination, the less frequently it was preferred when compared to the individual monoterpene odors. Our results imply that generalist herbivores do not necessarily prefer combinations of plant chemical defenses at comparatively lower concentrations and that, consequently, the composition and diversity of monoterpene profiles in plants likely determine the efficacy of these compounds as an olfactory defense against mammalian herbivory.

**Keywords**: Detoxification limitation hypothesis, foraging behavior, olfaction, pre-ingestive cues, volatile organic compounds


## Introduction

A key way in which plants mediate their interactions with herbivores is through the production and utilization of plant secondary metabolites (PSMs; Fraenkel 1959, Freeland and Janzen 1974, Foley and McArthur 1994). PSMs are products of plant secondary metabolic processes that take place in most vascular plants (Fraenkel 1959, Freeland and Janzen 1974, Metlen et al. 2009). These compounds include polyphenols, alkaloids, and terpenes, and can range from being harmless to fatal when ingested (Acamovic and Brooker 2005, Metlen et al. 2009, Estell 2010). Herbivores have adapted both physiological and behavioral mechanisms to avoid the harmful physiological

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effects of PSMs (Iason 2005, Kohl and Dearing 2011, Schmitt, Shrader, and Ward 2020a, Schmitt, Ward, and Shrader 2020c, Ward etal. 2020). According to the detoxification limitation hypothesis (DLH), the foraging behavior of mammalian herbivores is partially determined by their physiological capacity to metabolize different PSMs (Freeland and Janzen 1974, Marsh et al. 2006). Specialist herbivores often employ metabolic detoxification pathways specific to the PSMs found in their specialized food sources (Freeland and Janzen 1974, Sorensen et al. 2004, Seliskar and Rozman 2007). Increased detoxification rates allow these specialist herbivores to consume higher concentrations before experiencing the adverse effects of the PSMs (Freeland and Janzen 1974, Shipley et al. 2012, Nobler et al. 2018). By contrast, generalist herbivores often have detoxification pathways that may target a variety of PSMs but are slower and more energetically costly than the pathways used by specialist herbivores (Freeland and Janzen 1974, Marsh et al. 2006, Shipley et al. 2012, Nobler et al. 2018). Consequently, generalist herbivores risk overwhelming their detoxification systems if they continually feed on a specific plant species and, by proxy, consume large quantities of individual PSMs (Estell 2010, Nobler et al. 2018).

A behavioral mechanism that generalist herbivores can utilize to circumvent the negative effects of any one specific PSM is dietary mixing (i.e., ingesting a wide range of plant species with different PSMs; Freeland and Janzen 1974). However, the PSMs in certain plants can interact synergistically to increase their adverse effects by, for example, inhibiting detoxification, modifying otherwise inactive compounds to form toxins, or enhancing absorption rate (Berenbaum 1985, Vaaraspan style="font-family:'Times New Roman'"> 1992, Appel 1993, Nelson and Kursar 1999). PSMs can also interact additively by competing for the same detoxification enzymes and acting together to overwhelm the detoxification pathways of herbivores (Freeland and Janzen 1974, Marsh et al. 2006). As such, ingesting a variety of PSMs does not always yield decreased negative physiological responses compared to ingesting individual PSMs, and thus dietary mixing still necessitates a degree of selective foraging (Freeland and Saladin 1989). Herbivores can feed selectively by basing their dietary decisions on

post-ingestive feedback, whereby the animal monitors the physiological effects of a food item after its consumption (Provenza 1995, Ginane et al. 2005), or on pre-ingestive cues, where the animal utilizes gustatory, olfactory, and visual cues of a food item before consuming it (Pass and Foley 2000, Schmitt, Shuttleworth, Shrader et al. 2020b). However, these avenues of information are not necessarily discrete, and mammalian herbivores can learn to associate the pre-ingestive cues of a plant with its consequential post-ingestive feedback (Provenza 1996, Ginane et al. 2005).

The olfactory landscape concept places emphasis on the important role that odors play in animal movement and decision-making (Finnerty et al. 2022). All plants have odors due to their emission of volatile organic compounds (VOCs), which include odoriferous PSMs, that may serve various functions (i.e., defense, communication, protection against abiotic factors; Peñuelas and Llusià 2004). In particular, monoterpenes are VOCs that are present in an extensive range of plant species and can act as a plant chemical defense against a variety of herbivores (Seigler 1998, Ramak et al. 2014, Schmitt, Shuttleworth, Shrader et al. 2020b). For example, α-pinene concentration in Scots pine (*Pinus sylvestris*) is negatively correlated with herbivory by both slugs (*Arion ater*) and capercaillie (*Tetrao urogallus*), whereas β-ocimene concertation is negatively correlated with herbivory by red deer (*Cervus elaphus*) and capercaillie (Iason et al. 2011).

Consumption of these widely-occurring compounds is likely inevitable for most vertebrate and invertebrate herbivores, who both produce detoxification enzymes that metabolize monoterpenes to yield excretable products (Marsh et al. 2006, Cui et al. 2016). However, the process of detoxification itself may contribute to the realized toxicity of these compounds. Monoterpene detoxification is an energetically costly process, which impairs the body condition of vertebrate herbivores by depleting fat reserves (Illius and Jessop 1996). By contrast, monoterpene detoxification is primarily associated with dehydration in invertebrate herbivores, presumably due to the loss in water content when excreting monoterpene metabolites (Reid et al. 2017, Reid and Ahn 2020). Ultimately, although the

mechanism of realized toxicity may differ across taxa, the deleterious effects of monoterpenes likely elicit similar avoidance behaviors from both invertebrate and vertebrate herbivores in relation to their physiological capacity to cope with these compounds (Freeland and Janzen 1974, Boyle et al. 2005).

The toxic effects of monoterpenes do not always necessitate consumption. Indeed, the vapors of monoterpenes themselves have been found to be toxic to a variety of invertebrate herbivores, including fir engravers (*Scolytus ventralis*), spruce spider mites (*Oligonychus ununguis*), and cowpea seed weevils (*Callosobruchus maculatus*; Raffa et al. 1985, Cook 1992, Ajayi et al. 2014). The physiological effects of monoterpene vapors on vertebrate herbivores are less clear. Studies done with rodents have shown that these compounds, especially those found in turpentine, can be absorbed into the bloodstream via inhalation, but that toxicosis and other negative side-effects only occur after long-term exposure (Savolainen and Pfäffli 1978, Jirovetz et al. 1991). Thus, in a real-world setting, monoterpene vapors likely influence vertebrate herbivory through their odor.

Monoterpene vapors may also serve as pre-ingestive deterrents to invertebrate herbivores. For example, the monoterpene vapors of grandisol and *cis*-verbenol, which are aggregation pheromone components of Scots pine and Norway spruce (*Picea abies*), elicit flight avoidance responses in bark beetles (*Pityogenes bidentatus*; Byers 2012). Similarly, the vapors of monoterpenes found in the leaves of citrus fruit (linalool, limonene, and β-pinene) have repellent effects on fruit flies (*Drosophila melanogaster*; Yamasaki et al. 2007). As a result, monoterpenes in the form of essential oils have been considered valid candidates as plant protection products against invertebrate pests (Regnault-Roger et al. 2012).

Monoterpene content in plants have been found to influence vertebrate herbivore diet choice, but it is less established whether this influence is due to the pre-ingestive olfactory cues of monoterpene vapors. For example, Navon et al. 2020 found that intraspecific variation in plant monoterpene content determines the dietary preference of goats (*Capra hircus*) for mastic trees (*Pistacia lentiscus*) with different chemotypes, potentially due to the emission of these

monoterpenes when a leaf is dissected (Navon et al. 2020). The role that odor plays in the influence monoterpenes have on the dietary choices of pygmy rabbits (*Brachylagus idahoensis*) and Eurasian red squirrels (*Sciurus vulgaris*) have, thus far, only been suggested (Rubino et al. 2012, Nobler et al. 2018). Edlich and Stolter (2012) found that low concentrations of limonene and camphene odors do not alter the time that moose (*Alces alces*) spend feeding on food pellets, despite the negative effects of monoterpene consumption on the nutritional condition of moose (Melody 2017). Bedoya-Pérez et al. (2014) found that, at low concentrations, the odor of 1,8-cineole may facilitate swamp wallaby (*Wallabia bicolor*) herbivory, but acts as a deterrent at high concentrations. Given the variety in conclusions drawn by these studies, the extent to which monoterpene odors alone influence mammalian herbivore diet choice remains unclear.

The African elephant (*Loxodonta africana*) has been a focal species in research on herbivore diet selection and the effect that plant odors have on mammalian foraging decisions. According to the Jarman-Bell principle, large herbivores such as elephants are thought to be more tolerant of low-quality foods than small herbivores because of their increased gut volume and, consequentially, longer retention time, which may lead to increased digestive capability (Bell 1971, Jarman 1974). However, despite their large body size and high intake rate, elephants forage selectively (Owen-Smith and Chafota 2012, Shrader et al. 2012, Schmitt et al. 2016). For example, out of 27 woody plant species, more than 90% of the diets of the African elephants in the Chobe National Park consist of only 4-8 species across all seasons (Owen-Smith and Chafota 2012). In general, the diet selection patterns of African elephants are best described using acceptability indices: a measurement of dietary preference that is most applicable to large herbivores situated in areas with patchily distributed vegetation (Owen-Smith and Cooper 1987). Plant-based acceptability indices are calculated by dividing the number of individual plants of a species eaten by the number of individal plants of that species encountered within foraging reach, and compensate for potential sampling errors that may occur in the calculation of other diet selection indices (e.g., measurements of abundance and dietary proportions in foraging rations;

Owen-Smith and Cooper 1987). Previous studies have observed that elephants utilize olfactory cues to inter- and intraspecific dietary choices, and that their dietary preferences are influenced by the volatile PSMs within plants themselves (Schmitt et al. 2018, Nevo et al. 2020, Schmitt, Shuttleworth, Shrader et al. 2020b). In particular, low-acceptability plants such as karee (*Searsia lancea*), pipe-stem fingerleaf (*Vitex rehmannii*), and wild olive trees (*Olea europaea* subsp. Africana) contain high concentrations of diverse monoterpenes (Schmitt, Shuttleworth, Shrader et al. 2020b).

As generalist herbivores, elephants may be less capable of detoxifying high concentrations of individual PSMs (Freeland & Janzen 1974, Marsh et al. 2006). Indeed, the odors of individual monoterpenes deter elephant consumption of blue guarri, with this deterrent effect being more prolific at high concentrations (Bester et al. 2023). However, plants often contain multiple monoterpenes which, in some instances, may interact additively, synergistically, and antagonistically to alter the physiological repercussions of consuming them (e.g., Cox et al. 2001, Pass and McLean 2002, Ouedrhiri et al. 2017). The moose is an example of a large-bodied mammalian generalist whose dietary preference is driven by their avoidance of specific combinations of phenolics, which implies synergistic or additive interactions between these compounds (Stolter et al. 2013). Given that the post-ingestive feedback of consuming compounds is determined by these physiological repercussions, we may observe that the pre-ingestive odor cues of combinations of monoterpenes (i.e., two or more monoterpenes) may not always be preferred over the odors of individual monoterpenes (Provenza 1996, Ginane et al. 2005). Ultimately, how monoterpene diversity affects plant acceptability remains unclear, especially when compared to the established deterrent effect that individual monoterpene odors have on elephant dietary choices (Bester et al. 2023).

In this study, we tested the hypothesis that the added odors of monoterpene combinations deter elephant herbivory, but not to the same degree as the odors of individual monoterpenes. To do so, we first determined the influence that monoterpene combination odors have on elephant diet choice when added to a frequently consumed plant species. Given that

monoterpenes generally act as a defense against herbivory and that the monoterpene combinations in this study lacked the odor of other VOCs that might facilitate elephant herbivory (e.g., green leaf volatiles; McArthur et al. 2019), we predicted that the monoterpene combination odors would decrease elephant preference for the food item. Second, we determined elephant diet choice between monoterpene solutions with a combination of monoterpenes and solutions containing the individual monoterpenes within the combination, both at the same set concentration. Because the monoterpene combination solutions contained comparatively lower concentrations of any single monoterpene, we predicted that the elephants would prefer monoterpene combination odors over individual monoterpenes if the monoterpenes did not alter the pharmacodynamics of each other to increase their post-ingestive costs, or if they were metabolized via separate detoxification pathways (Freeland and Janzen 1974, Pennings et al. 1993). Alternatively, if monoterpenes within these combinations interacted synergistically or additively to increase their post-ingestive cost, we predicted that the elephants would prefer the individual monoterpene odors over the monoterpene combinations (Freeland et al. 1985, Freeland and Saladin 1989). However, terpenes within combinations may interact both synergistically and antagonistically to alter their bioactivity (Ntalli et al. 2010). These interactions could lead to the combinations being more deterrent than some, but not all, individual monoterpenes (Cox et al. 2001). Thus, if the individual monoterpenes within combinations interact in multiple ways (synergistic, additive, and antagonistic) to influence the post-ingestive feedback of the combination, we predicted that there would be no universal response between the monoterpene combinations and their constituents.

To test our predictions, we ran two odor-based choice experiments with three monoterpene combinations representing the monoterpene profiles of woody plants present in the natural vegetation of the elephants' habitat, specifically the jacket plum (high-acceptability species, *Pappea capensis*), common guarri (intermediate-acceptability species; *Combretum zeyheri*), and large-fruited bushwillow (low-acceptability species, *Euclea undulata*; Schmitt, Shuttleworth, Shrader et al. 2020b). Thus, the elephants would already have encountered monoterpene combinations with similar relative concentrations of individual monoterpenes to

those used in this, which ensured that the elephants were familiar with these odors and, if any, the physiological effects of the monoterpene combinations would be recognizable to the elephants. In the first experiment, we tested whether the odor of incresed concentrations of monoterpenes in the form of the three combinations (jacket plum, common guarri, and large-fruited bushwillow) with different monoterpene diversities would influence the acceptability of blue guarri (*Euclea crispa*), which is an intermediate-acceptability plant that is frequently consumed by the elephants (Schmitt, Shuttleworth, Shrader et al. 2020b. In the second experiment, we tested elephant diet choice between the three monoterpene combinations and the individual monoterpenes within the combinations to determine whether the deterrence of the odor of an individual monoterpene changes when presented in a combination.

## Methods

### Sampling

We conducted our study in October 2020 at the Adventures with Elephants facility in Bela-Bela, South Africa (GPS Coordinates: 24°46'53.8"S, 27°57'03.3"E) where we ran odor-based choice experiments on five semi-tame adult elephants (2 males and 3 females). We conducted these experiments each day during three sessions (8h30, 12h00, and 15h00) during which all five elephants participated in five odor-based choice trials per choice pair (see details below). For the safety and comfort of all parties, the experiments were conducted by the elephants' personal handlers. The University of Pretoria Animal Ethics Committee approved all the experimental procedures under the reference number NAS235/2020.

### Experimental procedure

Both of the odor-based choice experiments that we ran consisted of trials where each elephant was presented with a choice between two 25 l opaque plastic buckets (height: 440 mm, top diameter: 300 mm, bottom diameter: 255 mm; Figure 1 ). Inside each bucket, we attached a 2 mL Eppendorf

tubule near the bottom using tape, which, depending on the experiment, would contain either an individual monoterpene solution at 30% concentration, a combination of monoterpenes at 30% concentration, or dipropylene glycol ( Figure 1 ). Due to its largely odorless nature, dipropylene glycol was used as both the control as well as the solvent for the 30% monoterpene solutions (see details below).



**Figure 1**: Bucket with perforated lid (Left). Inside of the bucket containing a branch of *E. crispa* (dry weight= ca. 5 g) and a 2 mL Eppendorf tubule (inside the black circle) taped near the bottom of the bucket (Right).

Along with the Eppendorf tubules, we also placed a branch of blue guarri (dry weight= ca. 5 g) inside each bucket ( Figure 1 ). Blue guarri (intermediate-acceptability species) is a staple food of the elephants used in the study (Schmitt 2017, Schmitt, Shuttleworth, Shrader et al. 2020b) and served as an optional food reward after the elephant chose a bucket. Thus, for both the 2 mL solutions containing the individual monoterpenes as well as those containing the monoterpene combinations, the total amount of monoterpenes (in terms of weight) within these

30% solutions was 10.2±0.3% DW of the blue guarri branch. Although the natural range for monoterpenes as a percentage of dry weight in the vegetation consumed by the elephants is unknown, it is unlikely that the blue guarri branch with the added odor of the 30% monoterpene solutions remained within the natural range. As such, we considered the 30% concentration solution (i.e., monoterpenes added at 10.2±0.3% of the food reward's DW) to be a very high level of added monoterpenes in the choice experiments (see Wiggins et al. 2003, Bester et al. 2023). We used this high level for the monoterpene combinations and individual monoterpenes because it was sufficient to ensure that the elephants could detect the odor of such a small volume (2 mL) of the solution held in the Eppendorf tubule (Bester et al. 2023).

Each bucket had a perforated lid in which we drilled 28 holes that were separated by ca. 3 cm (see Wood et al. (2022) and Bester et al. (2023) for similar procedure; Figure 1 ). This ensured that the elephants could not see the contents of the bucket but could smell what was inside. We marked the buckets with small numbers (c.a. 1 cm high) for easy identification, but when the buckets were presented to the elephants, the buckets were turned such that the small numbers were away from the elephants to prevent them from visually using the marks to make their decisions.

The trials for the odor-based choice experiments followed a smell-smell-choose procedure (as per: Schmitt et al. 2018). Each trial began with the prepared buckets placed on the ground and held by two handlers while the elephant being tested stood roughly 5 m away, facing the opposite direction. A third handler remained at the elephant's side during the trials to issue instructions to the elephant and ensure its comfort. The elephant was first instructed to turn around and walk up to the buckets ( Figure 2 ). Once in position, the elephant was then instructed to smell each of the buckets, tuck its trunk back, and choose which bucket's contents it preferred by placing its trunk on the bucket's lid ( Figure 2 ). The remaining bucket was then taken away while another handler removed the preferred bucket's lid. The elephant was then allowed to reach into the bucket with its trunk to retrieve the blue guarri branch. However, the elephant was not forced to eat the branch and could decide whether to consume it. The procedure of the odor-based choice experiments was specifically designed to enable to elephants to cue off the odor of the

monoterpenes but to prevent them from consuming any of the monoterpenes tested. We did this as the potential post-ingestive repercussions that consuming monoterpenes at the tested volumes have on elephants are unknown. Thus, the dietary choices made by the elephants would have to be based on previously learnt aversions that they may have acquired from feeding in their environment (Provenza 1995, 1996).
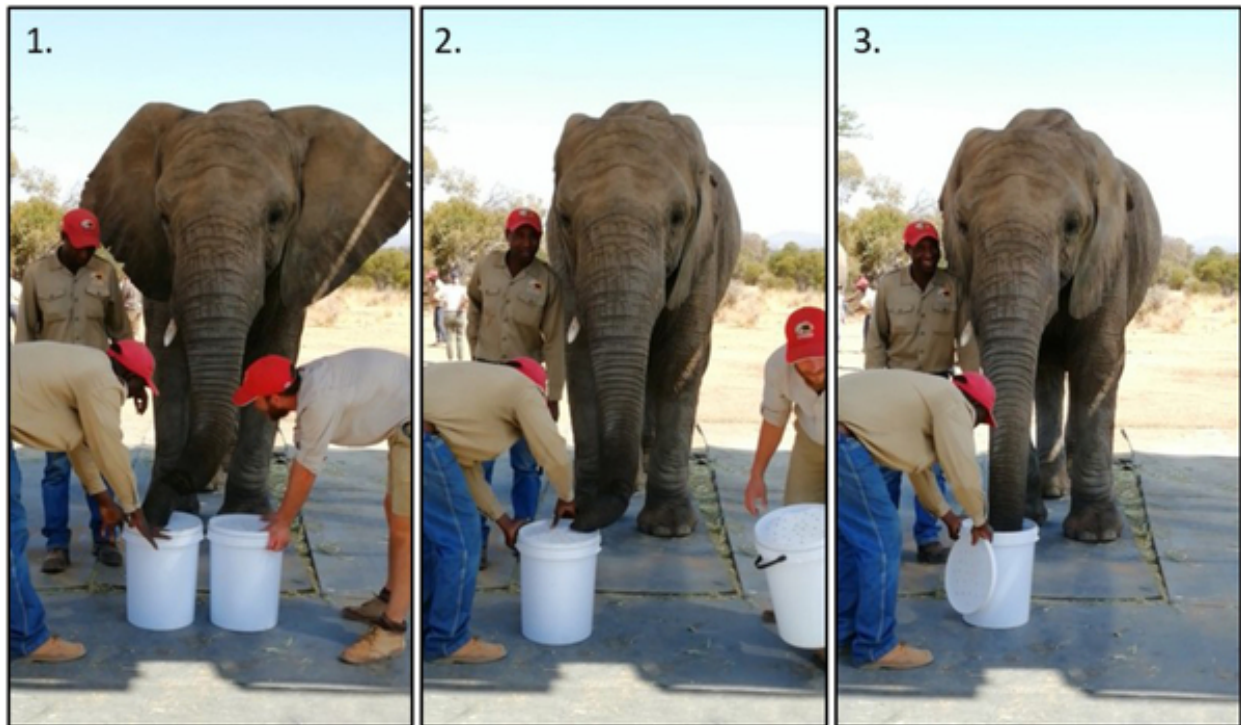


**Figure 2**: The smell-smell-choose procedure followed during the trials: 1. The elephant has turned around and smells both buckets. 2. The elephant has chosen the bucket it prefers, and the unselected bucket is removed. 3. The lid of the chosen bucket is removed, and the elephant is granted access to the buckets' contents.

The position of the buckets and the handlers holding the buckets were randomized for each trial. This ensured that the elephants could only base their choices off the buckets' odors and not the positions of the buckets or handlers. To ensure that the elephants could not cue off mucous deposited in previous trials in the buckets or on the lids, we wiped the lids and the insides of the

buckets between each trial with a damp cloth. We also swapped the lids of the buckets every ca. two trials. After running 5 trials with an elephant and before moving onto the next, we scrubbed the lids of the buckets with water and coarse dirt to remove any lingering odors from the previous elephant. At the end of the sessions, the lids and buckets were washed with 95% ethanol and left to dry in the sun for ca. 3 hours before the next session. This ensured that there were no remaining odors from the monoterpene solutions that were inside of the bucket from the previous session. Elephants were trained on the experimental procedure prior to running trials, which is detailed below.

**Monoterpenes**

The three monoterpene combinations used in both experiments were based on the identification of the VOCs in jacket plum (*P. capensis*), common guarri (*E. undulata*), and large-fruited bushwillow (*C. zeyheri*), which were acquired through GC-MS analyses done by Schmitt, Shuttleworth, Shrader et al. (2020b). However, the GC-MS results did not directly indicate the concentration of each individual monoterpene within these woody plants, but instead provided the relative contribution of each individual monoterpene to the odor profiles of jacket plum, common guarri, and large-fruited bushwillow. As such, to replicate the odor profiles of these plants, we used these relative contributions to determine the proportion and, consequently, total volume of each monoterpene within the three 2 mL monoterpene combination solutions at 30% concentration (Table 1). We chose these three woody plant species because they have varying monoterpene diversities and acceptability indexes (Schmitt, Shuttleworth, Shrader et al. 2020b; Table 1). Of the three species, jacket plum contains seven monoterpenes and is the most preferred by the elephants (i.e., high acceptability index), whereas common guarri, which contains five monoterpenes, is the least preferred (i.e., lowest acceptability index) and is generally neglected by the elephants (i.e., rarely consumed when encountered; Schmitt 2017, Schmitt, Shuttleworth, Shrader et al. 2020b; Table 1). Large-fruited bushwillow contains four monoterpenes and is neither

preferred nor rejected by the elephants (i.e., intermediate-acceptability index; Schmitt 2017, Schmitt, Ward, and Shrader 2020c; Table 1).

**Table 1**

**Average monoterpene proportions of *P. capensis*, *C. zeyheri*, and *E. undulata* combinations obtained from GC-MS analyses done by Schmitt, Shuttleworth, Shrader et al. (2020b).**

| Plant species | Acceptability index | Monoterpene profile | | |
|---|---|---|---|---|
| | | Monoterpene | Proportion | Volume (µL) |
| Jacket plum (*Pappea capensis*) | 0.87 | α-Pinene | 0.078 | 47 |
| | | Sabinene | 0.543 | 326 |
| | | β-Pinene | 0.079 | 47 |
| | | Limonene | 0.051 | 31 |
| | | ɣ-Terpinene | 0.177 | 106 |
| | | Terpinolene | 0.003 | 2 |
| | | Linalool | 0.069 | 41 |
| Large-fruited bushwillow (*Combretum zeyheri*) | 0.65 | β-Pinene | 0.049 | 29 |
| | | Limonene | 0.132 | 79 |
| | | Ocimene | 0.719 | 432 |
| | | Linalool | 0.100 | 60 |
| Common guarri (*Euclea undulata*) | 0.04 | α-Pinene | 0.230 | 138 |
| | | Sabinene | 0.103 | 62 |
| | | β-Pinene | 0.125 | 75 |
| | | Limonene | 0.346 | 207 |
| | | ɣ-Terpinene | 0.196 | 118 |

Total monoterpene content for each combination totaled to 600 µL as to create a combination of 30% concentration. Acceptability index of each plant species are as indicated, which represents the frequency that an elephant will consume the plant per encounter (Schmitt 2017, Schmitt, Shuttleworth, Shrader et al. 2020b). An acceptability index of 1 implies that an elephant consumes the plant 100% of the time that it encounters it, while an acceptability index of 0 indicates that the elephants avoid the plant and do not consume it when encountered.

We prepared the combinations in a 2 mL Eppendorf tubule at 30% concentration by combining individual monoterpenes according to the proportions in which they occur in

the odor profiles of these plants and suspending it in dipropylene glycol (Schmitt, Shuttleworth, Shrader et al. 2020b; Table 1). Thus, at 30% concentration, the total monoterpene content within a 2 mL Eppendorf tubule amounted to 0.6 mL which was suspended in 1.4 mL of dipropylene glycol. Similarly, the individual monoterpene solutions were also prepared at 30% concentration by placing 0.6 mL of the individual monoterpene solution in the 2 mL Eppendorf tubule and suspending it in 1.4 mL of dipropylene glycol.

In the first experiment, we tested elephant diet choice between each of the three monoterpene combinations at a 30% concentration and dipropylene glycol (i.e., the control), which amounted to three different choice pairs. Thus, we ran a total of 75 choice trials in the first experiment where each of the five elephants participated in 15 choice trials. In the second experiment, we tested elephant diet choice between each monoterpene combination and the individual monoterpenes that comprised the combination (Table 1). Thus, the jacket plum combination was tested against of α-pinene, sabinene, β-pinene, limonene, ɣ-terpinene, terpinolene, and linalool (Table 1). The large-fruited bushwillow combination was tested against β-pinene, limonene, ocimene, and linalool (Table 1), while the common guarri combination was tested against α-pinene, sabinene, β-pinene, limonene, and ɣ-terpinene (Table 1). In total, we tested 16 choice pairs in the second experiment (jacket plum combination tested against seven individual monoterpenes, large-fruited bushwillow combination tested against four individual monoterpenes, and common guarri combination tested against five individual monoterpenes). Thus, we ran a total of 400 choice trials where each of the five elephants participated in 80 choice trials in the second experiment. All chemicals (including α-pinene: 98% purity, (-)-β-pinene: 99% purity, linalool: 97% purity, (R)-(+)-limonene: 97% purity, ocimene: ≥90% purity, sabinene: 75% purity, terpinolene: ≥95% purity, and ɣ-terpinene: 97% purity) were produced by Sigma-Aldrich Inc, Johannesburg, South Africa.

**Training**

Before starting with the odour-based choice experiments, we trained the elephants to partake in the smell-smell-choose procedure. The training ensured that the elephants understood the "you get what you choose" reward system of the odour-based choice experiments. Similar to the experiment, we presented the elephants with a choice between the two 25 l opaque plastic buckets with perforated lids. However, one bucket contained a branch of blue guarri (dry weight= ca. 5 g), while the other contained a branch of wild olive (dry weight= ca. 3 g). Elephants reject wild olive (acceptability index=0.00) but will consume blue guarri (acceptability index=0.28; Schmitt 2017, Schmitt et al., 2018). Thus, when the elephants chose the bucket with the blue guarri branch, they gained access to a plant that could act as a food reward (i.e., positive reinforcement). In contrast, if the elephants chose the bucket containing a wild olive branch, they only had access to a plant they did not like to eat and thus did not act as a food reward. After four days, the elephants exhibited a clear understanding of the smell-smell-choose procedure, in that, for all three sessions throughout a full day, they consistently (100% of choices made) chose the bucket containing blue guarri during the trials.

**Data analysis**

Both odor-based choice experiments involved a series of binary choices made by the elephants (i.e., choice between two buckets). We tested the same individual elephant multiple times (five times per choice pair) and consequently treated the individual elephants as subjects for repeated measures in generalized estimating equations (GEEs; See Schmitt et al. (2018), Wood et al. (2022), and Bester et al. (2023) for similar procedure), where the within-subject variable was defined as the combination of choices presented to the elephants. GEEs were used because they support the analysis of repeated measures and compensate for non-independence in the data (i.e., the elephants remembering their previous choices; Ballinger 2004). GEEs also use a population-level approach based on a log quasi-likelihood function and deliver population-averaged parameter estimates. Additionally, the coefficients in GEE regressions are marginal effects that are measured

at population level and average across all subjects in the data. The model incorporated an exchangeable workng correlation matrix and a binomial error distribution with a logit link function. Thus, in this study, the GEEs modelled the proportion of choices made by the elephants between the reference and response variables in a choice pair (i.e., choices between the two buckets) and provided p-values which indicated whether the elephants' preference for the reference/ response variables differed significantly between the different choice pairs in the experiments. We back-transformed the data from the logit-scale for graphical representation and used the estimated marginal means of the proportions of the choices made by the elephants and asymmetrical 95% confidence intervals (CIs) to determine whether the elephants preferred a choice in a choice pair, in which case the 95% CIs would not overlap with the 0.5 proportion line (i.e., the 50% distribution of choices expected under random selection). Alternatively, random selection between the two choices in a choice pair occurred if the 95% CIs of the proportion of choices made by the elephants overlapped with the 0.5 proportion line. Thus, GEEs were utilized to determine (1) elephant dietary preference between the two choices in a choice pair (i.e., proportions of choices made and 95% CIs), and (2) whether the elephants' preference for either choice differed significantly among the choice pairs in the experiments (i.e., significant p-value) or whether their preference for a choice among choice pairs were similar in relation to the 0.5 proportion line (i.e., insignificant p-value).

In our first experiment, we determined the influence that three different monoterpene combinations representing the monoterpene profiles of a high-acceptability (jacket plum), intermediate-acceptability (large-fruited bushwillow), and low-acceptability (common guarri) plant have on elephant diet choice. To do this, we analyzed the proportion of elephants choosing the control bucket with the blue guarri food reward and no added odor over the bucket containing the blue guarri food reward and the odors of the jacket plum, large-fruited bushwillow, or common guarri combinations, respectively. We hypothesized that the odor of the monoterpene combinations would deter elephant consumption and that the elephants would prefer the control bucket. Consequently, we measured the deterrence of the monoterpene combination

odors as the proportion of choices made by the elephants where the control bucket was selected. Thus, the response variable was defined as the selection of the control bucket and the reference variable was defined as the selection of the bucket containing the added odor of a monoterpene combination. We established whether the elephants preferred the control buckets by using the marginal means of the proportion of the choices made by the elephants and 95% CIs and comparing them to the 50% distribution expected under random selection for the two buckets. Marginal means and 95% CIs above the 0.5 proportion line indicated preference for the control bucket. In contrast, marginal means and 95% CIs overlapping or below the 0.5 proportion indicated non-preference for the control bucket (i.e., random selection or preference for the monoterpene combination odors, respectively).

In our second experiment, we analyzed the proportion of choices made by the elephants between monoterpene combinations and the individual monoterpenes. We hypothesized that the odor of the individual monoterpenes would deter elephant consumption compared to the monoterpene combinations, and measured the deterrence of the individual monoterpenes as the proportion of choices made by the elephants where the monoterpene combination was selected. Thus, the response variable was defined as the selection of the bucket containing the odors of the monoterpene combinations and the reference variable was defined as the selection of the bucket containing the odors of individual monoterpenes. Similar to the first experiment, we used the marginal means of choices made by the elephants and their 95% CIs to establish whether the elephants' preference for the monoterpene combinations differed from the expected 50% distribution under random selection. Marginal means and 95% CIs above the 0.5 proportion line indicated preference for the bucket containing the odor of the monoterpene combination. In contrast, marginal means and 95% CIs overlapping or below the 0.5 proportion indicated non-preference for the monoterpene combination bucket (i.e., random selection or preference for the individual monoterpene odors, respectively). All statistical analyses were conducted using IBM SPSS version 26.0 (IBM Corp. 2019).

# Results

## Combinations vs. Dipropylene glycol

When given a choice between a branch of blue guarri with the control odor (dipropylene glycol) added and a branch of blue guarri with the odors of the monoterpene combinations found in jacket plum (*P. capensis*), large-fruit bushwillow (*C. zeyheri*), and common guarri (*E. undulata*) added individually, the elephants preferred the control in all three instances (i.e., the 95% CIs were above the 0.5 proportion line; Figure 3). The elephants' preference for the control did not differ significantly across the three different combinations (GEE: $\chi 2$= 1.779, p= 0.411; Figure 3 , Table 2).

**Table 2**

**Results from GEE analyses ($\chi 2$ and p-value) modelling the proportion of choices made by the elephants selecting the response variables in the choice pairs for the two experiments.**

| Choice pair | GEE results | |
|---|---|---|
| | $\chi 2$ | p-value |
| Combinations vs Control | 1.779 | 0.411 |
| Combinations vs Individual monoterpenes: | | |
| *Pappea capensis* combination | 577.706 | <0.005 |
| *Euclea undulata* combination | 93.564 | <0.005 |
| *Combretum zeyheri* combination | 227.719 | <0.005 |

**Combinations vs. control**
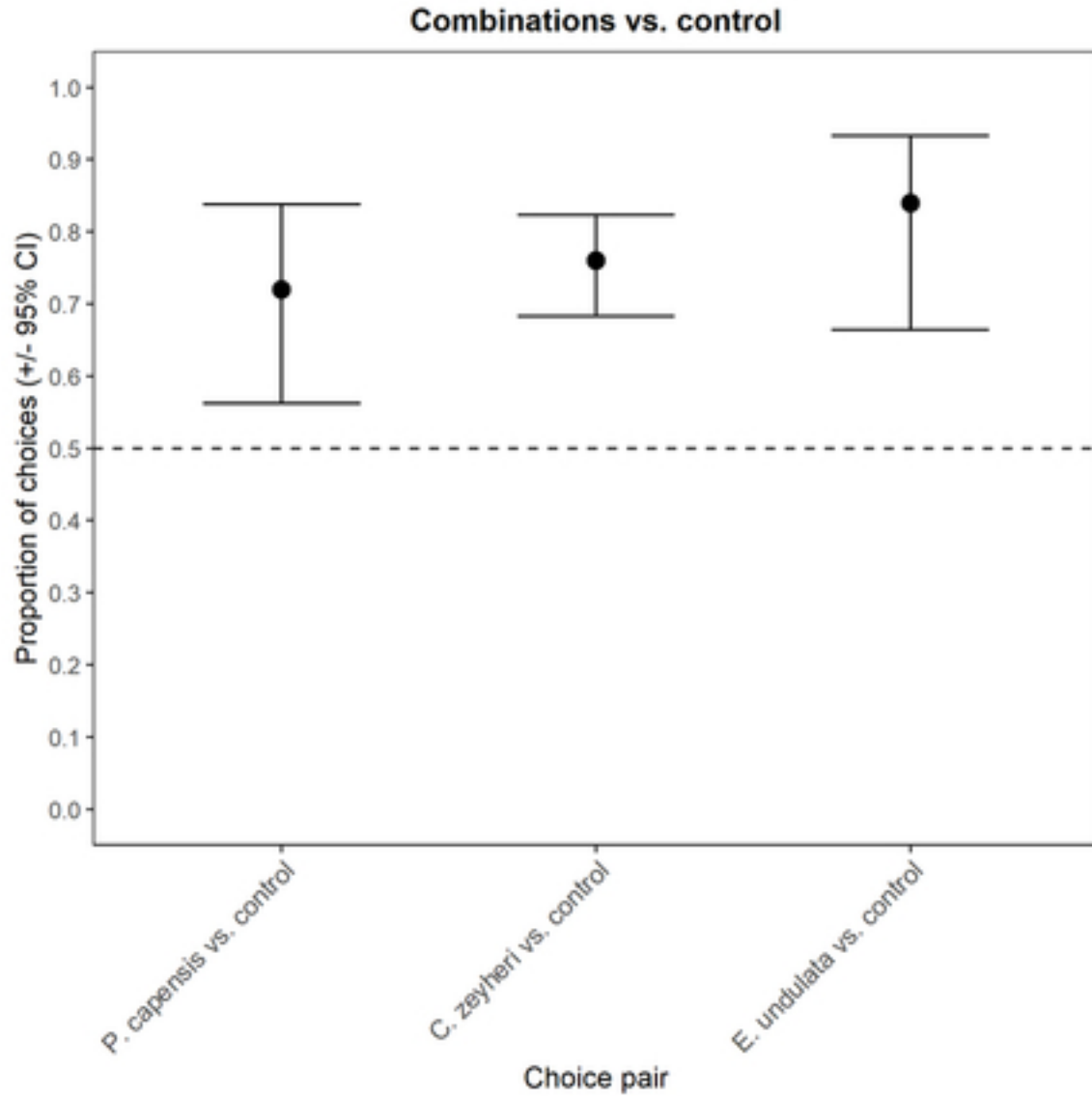
**Figure 3**: The mean proportion of choices made by the elephants (+/- 95% confidence intervals) where the control odor (dipropylene glycol) was selected over the odors of the jacket plum (*P. capensis*), large-fruit bushwillow (*C. zeyheri*), and common guarri (*E. undulata*) monoterpene combinations. Confidence intervals (CIs) that overlap the 0.5 proportion line indicate no difference from random selection. Means and CIs above the 0.5 proportion indicate preference for the control odor.

***Pappea capensis* (jacket plum; high- acceptability) combination vs. Individual monoterpenes**

The elephants' preference for the odor of the jacket plum (*P. capensis*) combination differed significantly when compared to the odors of the seven individual monoterpenes (GEE: $\chi 2$=577.706, p<0.005; Figure 4, Table 2). In particular, the elephants preferred the odors of terpinolene, limonene, and β-pinene when compared to the jacket plum combination (i.e., the 95% CIs were below the 0.5 proportion line). By contrast, they showed random selection between the jacket plum combination and the odors of sabinene, linalool, ɣ -terpinene, and α-pinene (i.e., the 95% CIs overlapped with the 0.5 proportion line). The elephants did not prefer the combination when compared to any of the individual monoterpenes.
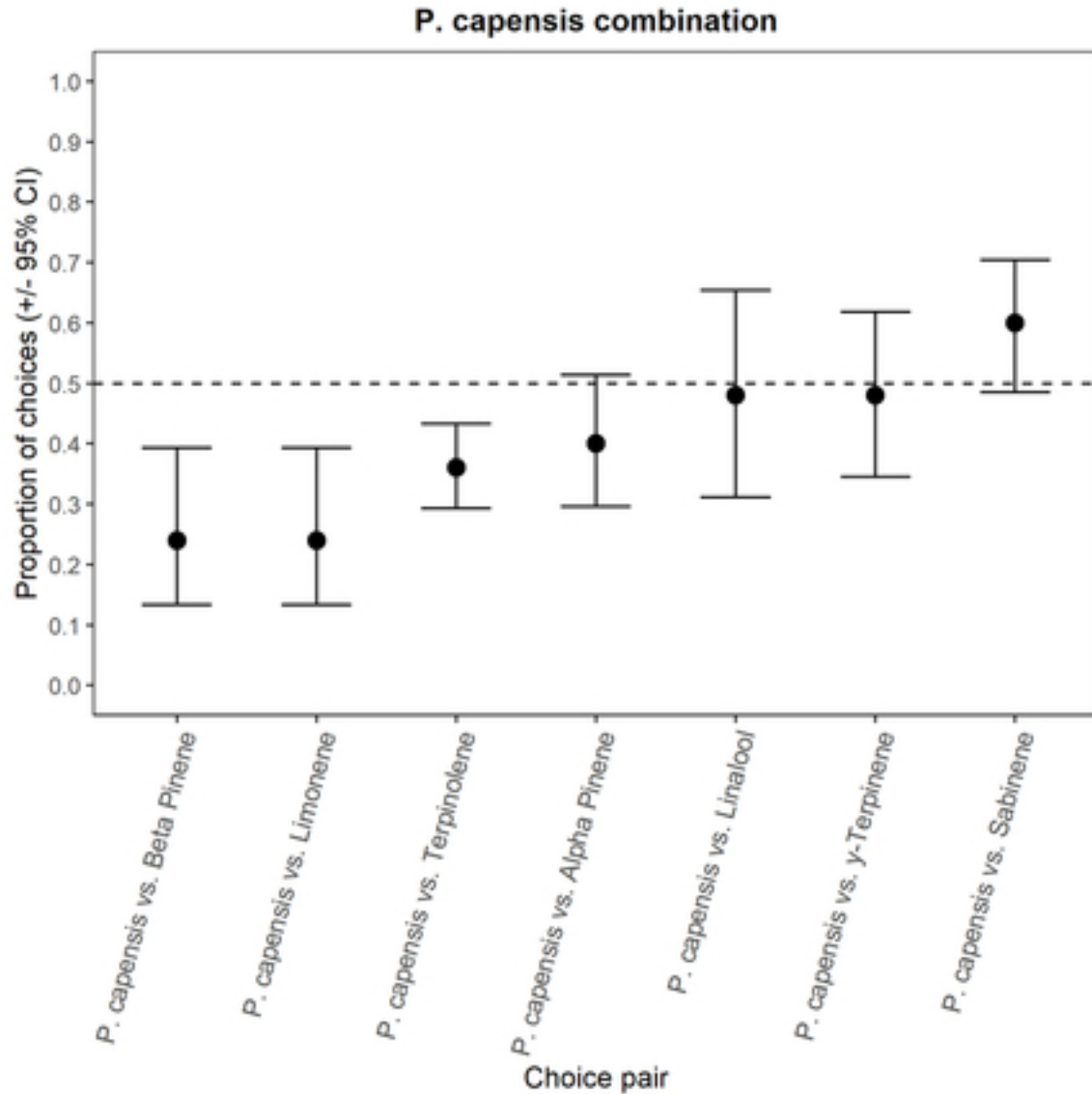
**Figure 4**: The mean proportion of choices made by the elephants (+/- 95% confidence intervals) where the odor of the jacket plum (*P. capensis*) monoterpene combination was selected over the odors of the seven individual monoterpenes (sabinene, linalool, ɣ-terpinene, α-pinene, terpinolene, limonene, and β-pinene) within the combination. CIs that overlap the 0.5 proportion line indicate no difference from random selection. Means and CIs below the 0.5 proportion indicate preference for the individual monoterpenes. Alternatively, means and CIs above the 0.5 proportion line indicate preference for the jacket plum (*P. capensis*) combination.

**E. undulata combination**
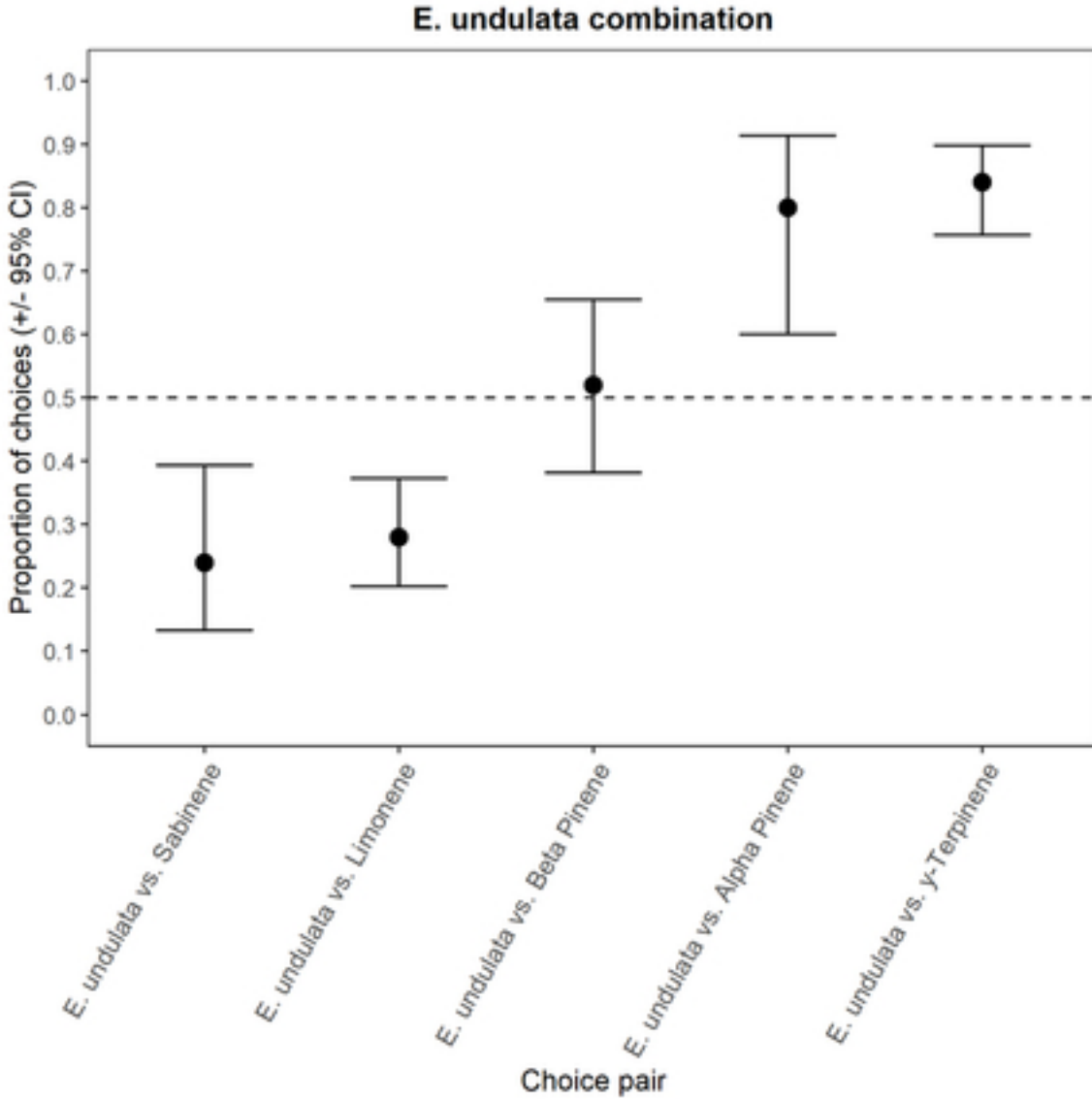
**Figure 5**: The mean proportion of choices made by the elephants (+/- 95% confidence intervals) where the odor of the common guarri (*E. undulata*) monoterpene combination was selected over the odors of the five individual monoterpenes (ɣ-terpinene, α-pinene, β-pinene, limonene, and sabinene) within the combination. CIs that overlap the 0.5 proportion line indicate no difference from random selection. Means and CIs below the 0.5 proportion indicate preference for the individual monoterpenes. Alternatively, means and CIs above the 0.5 proportion line indicate preference for the common guarri (*E. undulata*) combination.

*Euclea undulata* **(common guarri; low-acceptability) combination vs. Individual monoterpenes**

The elephants' preference for the odor of the common guarri (*E. undulata*) combination differed significantly when compared to the odors of the five individual monoterpenes (GEE: $\chi 2=93.564$, $p<0.005$; <u>Figure 5</u>, Table 2). The elephants preferred the common guarri combination when compared to both ɣ-terpinene and α-pinene (i.e., the 95% CIs were above the 0.5 proportion line). By contrast, they preferred the odors of limonene or sabinene over the common guarri combination (i.e., 95% CIs below the 0.5 proportion lie), and showed random selection between the common guarri combination odor and the odor of β-pinene (i.e., 95% CIs overlap 0.5 proportion line).

*Combretum zeyheri* **(large-fruit bushwillow; intermediate-acceptability) combination vs. Individual monoterpenes**

The preference that the elephants showed towards the large-fruited bushwillow (*C. zeyheri*) combination odor differed significantly across the four choice pairings (GEE: $\chi 2=227.719$, $p<0.005$; <u>Figure 6</u>, Table 2). The elephants preferred the large-fruited bushwillow combination odor compared to ocimene and limonene, respectively (i.e., 95% CIs above 0.5 proportion line), but preferred the odor of β-pinene over the combination (95% CIs below 0.5 proportion line). When given a choice between odor of the combination and linalool, they did not show a preference for either odor (i.e., 95% CIs overlap 0.5 proportion line, indicating random selection).

**C. zeyheri combination**
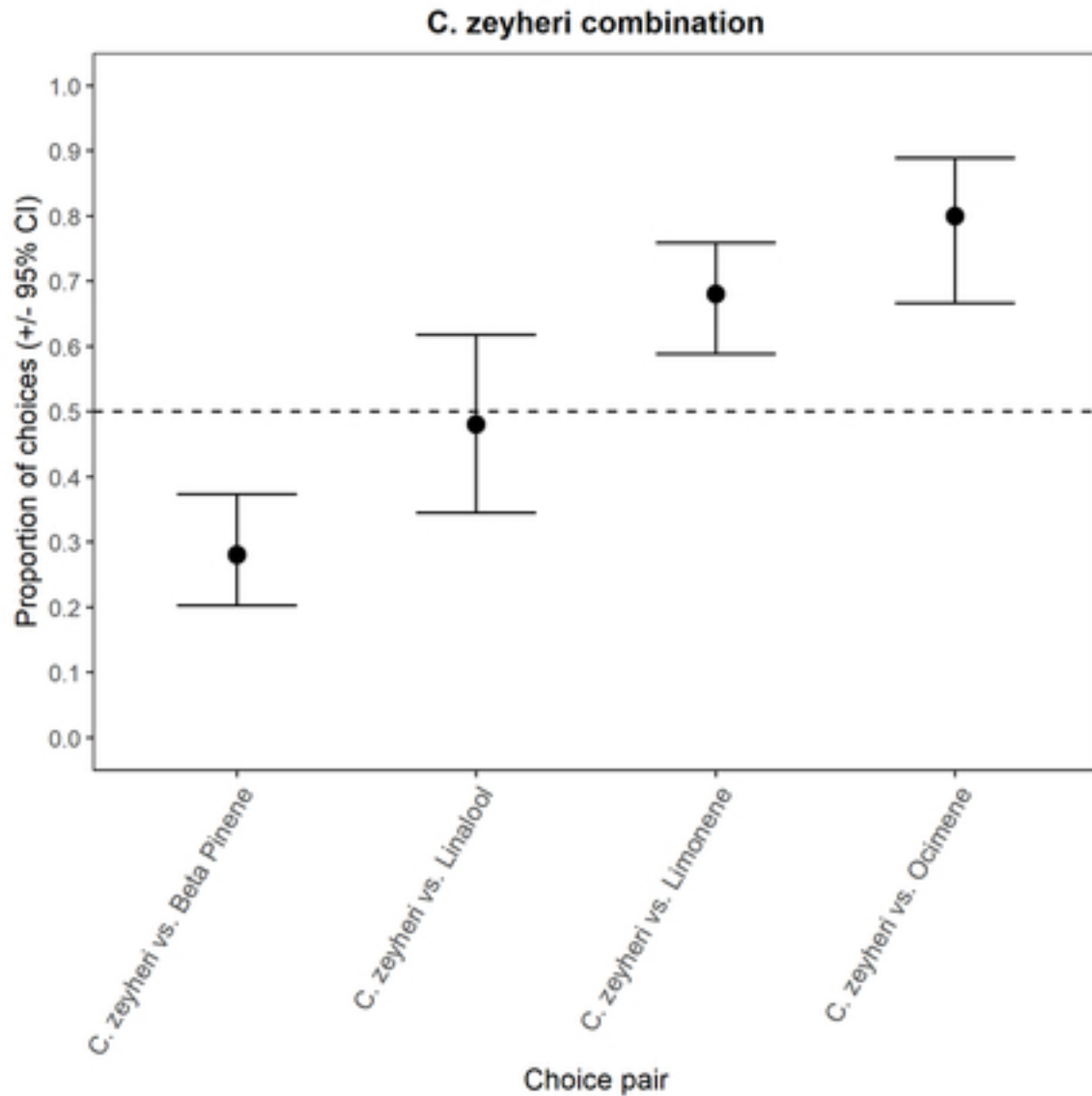
**Figure**

**6**: The mean proportion of choices made by the elephants (+/- 95% confidence intervals) where the odor of the large-fruit bushwillow (*C. zeyheri*) monoterpene combination was selected over the odors of the four individual monoterpenes (ocimene, limonene, linalool, and β-pinene) within the combination. CIs that overlap the 0.5 proportion line indicate no difference from random selection. Means and CIs below the 0.5 proportion indicate preference for the individual monoterpenes. Alternatively, means and CIs above the 0.5 proportion line indicate preference for the large-fruit bushwillow (*C. zeyheri*) combination.

# Discussion

We found that the odors of the three monoterpene combinations decreased African elephant preference for an intermediate-acceptability plant species (i.e., blue guarri). We also found that elephants showed varying degrees of preference towards the odor of monoterpene combinations when compared to individual monoterpenes, despite the combinations containing comparatively lower concentrations of any single monoterpene. In particular, the elephants did not prefer the odor of the combination of monoterpenes found in the high-acceptability species (i.e., jacket plum) when compared to any of the individual monoterpenes found within the combination. In contrast, the elephants displayed mixed responses towards the odors of the monoterpenes in the common guarri and large-fruit bushwillow (low- and intermediate-acceptability species) combinations when compared to individual monoterpenes (i.e., preference for the combination or individual monoterpene, random selection). Thus, the production of multiple specific monoterpenes at lower concentration may sometimes be a more effective defense against elephant herbivory for plants than producing high concentrations of an individual monoterpene.

Generalist herbivores are thought to increase their diet breadth to incorporate a variety of PSMs at lower relative concentration and limit the negative effects on any single PSM (i.e., dietary mixing; Freeland and Janzen 1974, Dearing and Cork 1999). Although dietary mixing may prove particularly beneficial in environments that contain heterogeneously distributed resources, herbivores often display food-switching behaviors even when monospecific diets provide similar, or greater, advantages (Parsons et al. 1994). In grasshoppers (*Taeniopoda eques*), a polyphagous insect, food-switching may be a "compulsive" behavior, whereby the rate of switching does not only depend on the previously-consumed food item itself, but also the length of the herbivore's previous feeding bout and the novelty and variety of alternative items (Bernays et al. 1992). Consequently, dietary mixing may be the result of several behavioral mechanisms, with the reduction in total amount of individual PSMs consumed being a byproduct, and not a

driver, of food-switching decisions (Bernays et al. 1992, Bernays et al. 1994, Provenza 1996). However, food-switching behavior may also be attributed to learnt aversions triggered by the continuous consumption of the initial plant species (Provenza 1996). Thus, even preferred food items may decrease in acceptability following consumption (i.e., the herbivore becomes "satiated"), which may be attributed to learnt aversions to overstimulation of certain pre-ingestive cues (sensory-specific aversions) or to post-ingestive feedback (nutrient- and toxin-specific aversions; "satiety hypothesis"; Provenza 1996).  As a driver for food-switching, learnt aversions could be directly linked to nutrient balancing and toxin dilution, as observed in both vertebrate and invertebrate generalist herbivores (Raubenheimer and Simpson 1997, Singer et al. 2002, Wiggins et al. 2006).

Previous studies have found that African elephants use odor as a pre-ingestive cue to make within- and between-patch foraging decisions (Schmitt et al. 2018, Nevo et al. 2020). Schmitt, Shuttleworth, Shrader et al. (2020b) found that the monoterpene content of plants plays a large role in the dietary choices made by elephants. Specifically, low-acceptability plat species had higher monoterpene concentrations and contained more than twice the number of monoterpenes than preferred plant species (Schmitt, Shuttleworth, Shrader et al. 2020b). Furthermore, individual monoterpene odors alter elephant diet preference between identical food choices (Bester et al. 2023). Thus, dietary mixing behavior in African elephants is likely driven by sensory-specific aversions towards monoterpene odors due to the post-ingestive costs of these compounds themselves (Provenza 1995, 1996). Compared to dietary mixing, which occurs over the course of multiple bites, herbivores can also dilute toxins through selectively feeding on plants that contain lower total concentrations of specific PSMs (Freeland and Janzen 1974).

In this study, consuming a variety of monoterpenes could only be effective if the combinations incur less post-ingestive costs than individual monoterpenes, which may not be the case if individual monoterpenes within the combinations interact additively or synergistically (Freeland et al. 1985, Marsh et al. 2006). In our first experiment, the addition of the odor from the three  monoterpene combinations (jacket  plum, common  guarri,  and large-fruited  bushwillow)

artificially increased both the observed number and concentration of monoterpenes within the food presented to the elephants (i.e., a branch of blue guarri) and, subsequently, decreased the elephants' preference for this intermediate-acceptability species ( Figure 3 ). Thus, the odor of monoterpene combinations elicits similar food aversion responses from elephants as the odor of individual monoterpenes at high concentrations (Bester et al. 2023). In particular, the odors of β-pinene, linalool, limonene, ocimene, sabinene, terpinolene, and ɣ-terpinene also deterred elephant preference for blue guarri at 30% concentration (i.e., 10.2±0.26% dry weight of food reward; Bester et al. 2023). Monoterpenes occupy a subset of VOCs that contribute to the odour profiles of plants (Peñuelas and Llusià 2004), and the degree to which elephants utilize the ratios (i.e., relative concentrations) of these potentially toxic compounds remains unknown. However, if elephants use relative concentrations of monoterpenes to identify plant species, and the monoterpene profiles of high-acceptability (jacket plum) and intermediate-acceptability (large-fruited bushwillow) plants facilitate elephant herbivory, these monoterpenes may still deter elephants at the high concentrations used in this study because of the increased post-ingestive costs associated with consuming them (Bedoya-Pérez et al. 2014).

Another explanation for the results in our first experiment could be that the added odors of the monoterpene combinations may have created a novel scent that repelled the elephants ( Figure 3 ). Indeed, some herbivores frequently avoid foods with novel tastes or smells when given an alternative, familiar food (Provenza 1995). This would mean that the preference shown toward the control odor was due to indirect, neophobic reactions towards the monoterpene combinations rather than the direct physiological implications of consuming the monoterpenes themselves (Provenza and Balph 1988, Squibb et al. 1990). However, research conducted on roe deer (*Capreolus capreolus*) and sika deer (*Cervus nippon*) suggest that, in large herbivores that are prone to crop-raiding, the degree of neophobia/neophilia exists on a spectrum and differs between individuals (i.e., the species itself cannot be classified as either neophobic or neophilic; Debeffe et al. 2014, Honda 2020).

In the second experiment, the elephants did have definite preferences for or against the monoterpene combinations when compared to certain individual monoterpenes (Figure 4-6). These preferences did not align with novelty (e.g., limonene, which is already present in blue guarri, was still not preferred when compared to the large-fruit bushwillow combination; Figure 6 ). Bester et al. (2023) also found that the novelty of individual monoterpene odors (i.e., whether these monoterpenes are naturally present in the food reward) did not influence their deterrent efects on elephant diet choice. Given that we did not find instances in our study where the results of the choices tests were primarily driven by the choices by a single elephant, we believe that the interindividual differences in neophobia did not skew our results, and that the elephants' reactions to monoterpenes cannot entirely be attributed to the avoidance of novel odors.

Alternatively, at 30% concentration (i.e., 10.2±0.3% DW), the 2 mL combination solutions may have masked the odor of the blue guarri branch (Bedoya-Pérez et al. 2014), limiting the elephants' ability to detect or identify the food reward within the buckets containing the monoterpenes. However, even at low concentrations, certain individual monoterpene odors still deter elephant herbivory (Bester et al. 2023). Furthermore, in our second experiment, the 2 mL solutions of the individual monoterpenes and the combinations had the same total amount of monoterpenes, which would most likely lead to the odor of the food reward being masked in both buckets. As such, the dietary choices that the elephants made between the combinations and individual monoterpenes were likely still driven by the deterrent effects of the odors, and not the degree to which they masked the odor of the food reward.

Because elephants are generalist herbivores (Codron et al. 2011, Owen-Smith and Chafota 2012), we predicted that, if the individual monoterpenes within the combinations did not interact to increase their post-ingestive costs when consumed together, or if the monoterpenes were metabolized via separate detoxification pathways, the elephants would prefer the monoterpene combinations over the individual monoterpenes (Freeland and Janzen 1974). However, this was not the case, and the elephants did not consistently prefer the monoterpene combinations over individual monoterpenes (Figures 4-6). Furthermore,

the elephants did not consistently prefer the individual monoterpenes over the combinations either, which implies that the interactions between individual monoterpenes in combinations are not always synergistic or additive (Figures 4-6). Instead, depending on the two choices presented to them, the elephants showed preference and non-preference (i.e., preference against or random selection) towards the odors of the individual monoterpenes and monoterpene combinations (Figures 4-6). According to our predictions, this could indicate that, due to varied interactions between individual monoterpenes (not just additive or synergistic, but also antagonistic), the monoterpene compositions of each combination led to the combinations differing in post-ingestive costs and, consequently, deterrence when compared to different individual monoterpenes (Provenza 1996, Dziba and Provenza 2008, Nobler et al. 2018). For example, the main bioactive bactericidal component in the essential oil of *Melaleuca alternifolia* (tea tree oil) is the monoterpene, terpinen-4-ol (Southwell et al. 1993). However, ɣ-terpinene and ρ-cymene, which are also monoterpenes within tea tree oil, decrease the solubility of terpinen-4-ol, which consequently interact antagonistically with terpinen-4-ol by decreasing its absorption rate (Cox et al. 2001). Thus, in theoretical terms regarding herbivore behavior, this could lead to the avoidance of terpinen-4-ol compared to a combination of terpinen-4-ol, ɣ-terpinene, and ρ-cymene. However, due to the presence of the bioactive terpinen-4-ol within the combination, a herbivore may avoid the combination when compared to either ɣ-terpinene or ρ-cymene. Ultimately, the interactions between monoterpenes themselves remain a largely unexplored field of study, especially regarding mammalian herbivores, and requires further research.

Another pattern observed in our results was that the combinations with higher monoterpene diversity were proportionately less frequently preferred when compared to their constituents. For example, the jacket plum combination, which is a high-acceptability plant species, but also had the highest monoterpene diversity of the three tested combinations, was not preferred when compared to any of the seven individual monoterpenes ( Figure 4 ). The common guarri combination, which is low-acceptability plant species and had intermediate monoterpene diversity, was preferred as

frequently as the individual monoterpenes within the combination ( Figure 5 ), while the large-fruited bushwillow combination (an intermediately-acceptability species), which had the lowest monoterpene diversity of the three combinations, was preferred more frequently over the individual monoterpenes ( Figure 6 ). These results could imply that increased diversity of monoterpenes in combinations also increases the likelihood of harmful interactions (i.e., additive and synergistic interactions) occurring, and the elephants could have likely learnt to associate more complex odors from diverse monoterpene combinations with higher post-ingestive costs (Provenza 1996). The potential synergistic or additive interactions between individual monoterpenes would also explain why α-pinene was not preferred over the jacket plum or common guarri combinations, despite that fact that α-pinene does not deter elephant herbivory at 30% concentration (Bester et al. 2023).

Previous studies have found that herbivores from various taxa (e.g., mollusks, grasshoppers, rats) are more likely to develop learnt aversions towards unpalatable plants with added detrimental compounds than palatable plants (Gelperin 1975, Bernays and Lee 1988, Bernstein 1999). Similarly, elephants may rather avoid plants on the basis of palatability compared to PSM content, instead of PSM content alone (Shrader et al. 2012). Consuming plants with high nutritional quality increases the toxin-threshold of herbivores by providing the resources required to metabolize and, consequently, excrete monoterpenes (Illius and Jessop 1996, Villalba et al. 2006). The food reward offered in our study, *E. crispa*, has a low nutritional value (0.01 grams N/g dry matter; Schmitt et al. 2016), which likely did not offset the post-ingestive costs of diverse monoterpene combinations and led to the elephants preferring these combinations less frequently. Thus, using a different food reward with a different monoterpene profile and higher nutritional value than *E. crispa* may have resulted in the elephants reacting differently towards the monoterpene combinations.

Despite having relatively low nutritional value, blue guarri is encountered and consumed more frequently than woody plants preferred by the elephants that participated in this study, i.e., high-acceptability species such as jacket plum (P. capensis), South African wild pear (Dombeya

rotundifolia), and silver cluster-leaf (Terminalia sericea; Schmitt et al. 2016). Like most woody plants, blue guarri already contains monoterpenes (Bryant et al. 1991, Schmitt, Shuttleworth, Shrader et al. 2020b). However, because blue guarri is an intermediate-acceptability species that is necessary for the elephants' survival, changing the observed toxin content of E. crispa reflects more accurately on the diet choices made by the elephants (Shrader et al. 2012, Schmitt et al. 2016). During our experiments, the elephants received the same food reward for both choices and did not experience the post-ingestive costs that consuming the monoterpenes would entail. The total amount of food that the elephants received during our experimentation (~75 gDW) equated to a very small portion of their daily intake (Rees 1982, Ruggiero et al. 1992), and the elephants would immediately return to feeding in their environment after the trials. As such, the preference that the elephants showed against the monoterpene combinations, especially when compared to that of the combinations' constituents, could be based on previously learnt aversions (i.e., associating the odor of the combinations at the concentration at which it is presented with its potential post-ingestive feedback) that were likely maintained and reinforced during their foraging bouts in their environment (Provenza 1995, 1996).

This study focused on the small-scale, instantaneous dietary choices of elephants. Thus, the pattern that we observed is possibly only applicable when monoterpenes are ingested simultaneously (i.e., a single bite of a single plant species). Currently, it is unknown whether monoterpenes that are not simultaneously ingested (i.e., consumed over the course of several bites, feeding bouts, or days) can still interact to alter the effect that monoterpene diversity has on elephant diet choice. The answer to this question may rely on the rate at which herbivores detoxify different individual monoterpenes (McLean and Duncan 2006). Monoterpenes with fast detoxification rates could be excreted before a potential synergistic, additive, or antagonistic monoterpene is consumed. If, hypothetically, elephants are able to effectively and quickly detoxify all individual monoterpenes, and the metabolic products of these detoxification processes remain inactive, then the influence that monoterpene diversity has on their foraging behavior would be limited to the instantaneous choices exemplified in this study. However, because these processes

typically involve a series of rate-limited chemical reactions, near-instantaneous detoxification and excretion of all monoterpenes is unlikely (Freeland and Janzen 1974, Marsh et al. 2006). Thus, monoterpenes with slow detoxification rates may remain in herbivores' bodies and be available to react with subsequently consumed monoterpenes (McLean and Duncan 2006), which would influence foraging decisions over a wider timespan than was tested in this study.

According to Freeland and Janzen (1974), physiological changes occur more rapidly for hindgut fermenters because they detoxify PSMs via microsomal enzymes after it has been absorbed in the gastrointestinal tract, which makes these herbivores more likely to utilize post-ingestive feedback to make dietary decisions. However, despite being hindgut fermenters, elephant feeding choices are better explained by pre-ingestive cues than post-ingestive feedback measures (Schmitt et al. 2018), which is further highlighted by the results of our study. Furthermore, our study also highlights that, despite being large-bodied generalist herbivores, elephants remain highly selective feeders that may, in some instances, prefer plants with high concentrations of individual chemical defense compounds compared to combinations with relatively lower concentrations. Current literature is limited to the influence that the odors of individual monoterpenes have on a select few species (e.g., Elliott and Loudon 1987, Vourc'h et al. 2002, Bedoya-Pérez et al. 2014). In turn, this limits our understanding of how plant VOCs shape the olfactory landscape of herbivores. We propose that the influence that odoriferous plant chemical defenses in plants have on herbivore diet choice can not necessarily be predicted by certain morphological or behavioral traits, but that it is rather reliant on multiple factors (i.e., the ability of herbivore species to detect pre-ingestive olfactory cues, associate these cues with post-ingestive feedback, and to detoxify the defense compounds within the plant).

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