

Development and Testing of Seven New Synthetic Coyote Attractants

Bruce A. Kimball*

Analytical Chemistry Project, NWRC, 4101 LaPorte Avenue, Fort Collins, Colorado 80521

J. Russell Mason

Predation Ecology and Behavioral Applications, NWRC, BNR-163, Utah State University, Logan, Utah 84322-5295

F. Sherman Blom

Manager, Pocatello Supply Depot, 238 East Dillon Street, Pocatello, Idaho 83201

John J. Johnston

Analytical Chemistry Project, NWRC, 4101 LaPorte Avenue, Fort Collins, Colorado 80521

Doris E. Zemlicka

Predation Ecology and Behavioral Applications, NWRC, BNR-163, Utah State University, Logan, Utah 84322-5295

Available evidence indicates that effective coyote attractants are blends of volatile substances. Typically, attractants are a combination of biological substances such as fermented glandular materials, urines, and rotted meats. Although effective, these attractants have several distinct disadvantages. Among these is the possibility that they are unnecessarily complex and variable and, thus, difficult to replicate from one batch to the next. Although attractants containing a few reagent grade materials are available, the chemicals selected and their concentrations are not derived from actual attractants. For this reason, commercially available coyote attractants were analyzed with the intention of developing relatively simple synthetic alternatives. Purge and trap headspace analysis with gas chromatography/mass selective detection was employed to identify the volatile components of known conventional and synthetic attractants. All identified compounds were grouped according to chemical functionality, and one compound from each functional group was chosen to represent the group. Using only these representative compounds, seven synthetic attractants were formulated. Bioassays with captive coyotes (*Canis latrans*) were conducted to compare behavioral responses elicited by the seven new attractants, a currently available synthetic attractant, and a control. The results indicated that the attractants elicited significantly different behavioral profiles.

Keywords: *Attractants; bioassay; Canis latrans; coyotes; headspace analysis; volatiles*

INTRODUCTION

Coyote (*Canis latrans*) attractants are used in numerous wildlife management applications. Good attractants are key components of trapping, oral drug and toxicant delivery, and population census methods. Most commercially available formulations are blends of biological fluids (blood, urine, musk), organs (glands), and essential oils that have been brewed and/or fermented (Turkowski et al., 1983). Because of variability in source materials and the fermentation process, attractant effectiveness can be unpredictable. Besides differences

in effectiveness that result from differences in manufacture, attractiveness can be influenced by the changing preferences of targeted wildlife. Coyote preferences for specific attractants vary widely among geographic locations and across seasons (Phillips et al., 1990).

We conducted the present experiments to provide an array of coyote attractants designed to meet changing conditions and circumstances. In particular, we focused on the development of synthetic chemical attractants. This approach is not entirely new. Chemicals such as ammonium or zinc valerate and artificial musks have been used in coyote attractants for over 50 years (Day, 1932; Presnall, 1950), but chemical investigation of attractants and bait formulations did not begin until the early 1970s. This increase in interest reflected improvements in analytical techniques and spin-off from

* Author to whom correspondence should be addressed [telephone (970) 266-6069; fax (970) 266-6063; e-mail bruce.a.kimball@usda.gov].

research in human olfaction (Teranishi et al., 1981). In 1973, a chemical mixture was specifically formulated as a coyote attractant (Savarie, personal communication). Development of this attractant, then known as DRC-6220 or synthetic monkey pheromone (CFA), was based on the fatty acid content of rhesus monkey vaginal secretions (Michael et al., 1971). Attractant research in subsequent years focused on sex odors (Murphy et al., 1978) and food odors (Teranishi et al., 1981). From the chemical analyses of coyote urine, trimethylammonium valerate (TMAV) and TMAV with sulfurous compounds were developed as attractants (Teranishi et al., 1981). Expansion of this work by the Teranishi group led to the development of trimethylammonium decanoate (TMAD) and the W-U lure (U.S. Patent 4,472,377; Fagre et al., 1982).

Fermented egg, originally developed as an insect bait, was investigated as a food-based coyote attractant and received much attention (Bullard et al., 1978a). Synthetic fermented egg (SFE), consisting of a variety of fatty acids, amines, esters, and sulfurous compounds, was developed from the chemical analyses of fermented egg volatiles (Bullard et al., 1978b). An even simpler synthetic attractant was developed from the seven volatile fatty acids found in fermented egg, fatty acid scent (FAS; Roughton, 1982).

Surprisingly, there is no evidence that synthetic alternatives to conventional coyote attractants and baits have been developed through analyses of conventional attractants themselves. We designed the present experiments to address this gap. Rather than analyze commercial products developed for other purposes (such as fermented egg) or focus on only one class of compounds (such as FAS or TMAD) known to be present in food or in sexual or territorial scents, we aimed to identify volatile compounds in conventional coyote attractants. Our plan was then to apply this information to the formulation and behavioral testing of new synthetic attractants.

CHEMICAL ANALYSES METHODOLOGY

Samples. Twenty-four conventional attractants and nine synthetic (chemical) attractants were obtained by the Pocatello Supply Depot of the U.S. Department of Agriculture and delivered to the analytical laboratory of the National Wildlife Research Center for headspace analyses. Included in these was FAS, an attractant manufactured by the USDA.

Sample Analyses. Attractants were sampled immediately prior to analysis by placing a glass Pasteur pipet into the liquid or paste sample so that ~1 cm of the pipet tip was filled. Individual pipets were placed into individual borosilicate glass test tubes. Because the pipet was slightly longer than the test tube, it was necessary to fracture the pipet by pressing the tip firmly into the bottom of the tube. This action also served to distribute the sample throughout the bottom of the tube. The tube was immediately placed on the purge and trap instrument (Tekmar 3000 purge and trap concentrator, Cincinnati, OH) to purge and collect the volatile compounds.

Samples were purged for 10 min at ambient temperature with helium. Volatiles trapped on the Carbopack B/Carboxen 1000 and 1001 trap (Supelco Trap K, Bellefonte, PA) were desorbed at 250 °C with helium onto the gas chromatograph (Hewlett-Packard 5890 series II, Avondale, PA) equipped with a 30 m × 0.25 mm 5% phenyl methylpolysiloxane (0.25 μm film thickness) fused silica capillary column (DB-5.625, J&W Scientific, Folsom, CA) in a split (4:1) injection. The initial oven temperature was maintained at 0 °C for 8 min with cryogenic cooling. The oven temperature was then increased to the final temperature of 300 °C at a rate of 15 °C/min, which was maintained for 2 min. The injection port temperature was 250

Table 1. Representative Compounds for Each Functional Group and Their Correlation Coefficients with the Functional Group Variable

functional group	representative compound	correlation coefficient (<i>r</i>)
esters	ethyl butyrate	0.954
fatty acids	isobutyric acid	0.858
ketones	cyclopentanone	0.961
mercaptans	1-butanethiol	0.894
thiol esters	butylthioacetate	0.952
amines	<i>N</i> -ethylbutylamine	0.780
alkenes	4-octene (<i>trans</i>)	0.973
alkanes	octane	0.999
alcohols	1-butanol	0.911
aldehydes	hexanal	0.971
terpenes	camphene	0.894
furans	2-furaldehyde	0.981
phenols	guaiaicol	0.999
oxygenated aromatics	4-methylanisole	0.980
alkaloids	2,6-dimethylpyrazine	0.985
sulfides	methyl disulfide	0.819
solvents	ethanol	0.886
permanent gases	none	n/a

°C, and the helium carrier gas linear velocity was maintained at 35 cm/s with automated pressure control. Detection was achieved by mass selective detection (Hewlett-Packard 5972, Avondale, PA) in the scan mode (*m/z* 33–500).

Statistical Analyses of Chemical Data. For each sample, normalized peak responses were calculated for each peak. Peaks were identified using a Wiley 138K mass spectral database (John Wiley and Sons, New York). Each compound was classified into one of the following functional group classes: esters, fatty acids, ketones, mercaptans, thiol esters, amines, alkenes, alkanes, alcohols, aldehydes, terpenes, furans, phenols, oxygenated aromatics, permanent gases, alkaloids, and sulfides. An additional category (solvents) was used for all common solvent chemicals (i.e., ethanol, toluene, etc). For those compounds with multiple functional groups, organoleptic properties were used to make category assignments (Arctander, 1969). For example, benzaldehyde could be considered both an aldehyde and an oxygenated aromatic. It was assigned to the oxygenated aromatic category because its organoleptic descriptors of "sweet" and "almond" were similar to those of the other members of this group.

For each attractant analyzed, functional group responses were calculated by summation of the individual responses in each group. Representative compounds were chosen for each functional group by examination of correlations between each individual compound with the appropriate functional group response (Table 1). Commercial availability of the compound was also considered in the choice of a representative compound.

Correlations were also determined among all functional group responses. Thiol esters, alkenes, phenols, and ketones were not used in cluster analysis as they were correlated ($|r| > 0.7$) with mercaptans, alkanes, aldehydes, and furans, respectively. The remaining functional group variables were then subjected to average linkage cluster analysis to cluster the 33 attractants into hierarchical clusters using squared Euclidean distances (CLUS procedure; SAS, 1997). Finally, mean functional group responses (all functional groups) were calculated for all clusters (Table 2).

Synthetic Attractant Formulation. Representative compounds were mixed in various liquid phase proportions in an attempt to produce the desired headspace proportion. Mixtures were formulated on a trial and error basis and analyzed by headspace gas chromatography as described for the attractant samples. Reactivity (neutralization, transesterification, redox, and hydrolysis reactions) among the representative compounds was also evaluated by analyzing mixtures of representative compounds by the described method. This process ultimately resulted in recipes for seven synthetic attractants (Table 3).

Table 2. Mean Functional Group Headspace Compositions (Percent Peak Response) of the Seven Clusters

functional group	cluster 1	cluster 2	cluster 3	cluster 4	cluster 5	cluster 6	cluster 7
esters	4.5	1.0	2.0	13.0	1.0	50.0	1.5
fatty acids	81.0	0.25	1.0	13.0	2.0	8.0	1.0
ketones	0.25	0	0.5	0.25	0.1	0.5	1.5
mercaptans	0	0	2.5	4.0	3.0	0.1	0
thiol esters	0	0	0	2.0	0.25	0	0
amines	1.0	0.5	5.5	2.0	12.0	0	63.0
alkenes	0	0	0	0	0.50	0	0.1
alkanes	0	0	0	0	3.0	0	0
alcohols	0.25	1.0	3.0	8.5	1.5	0.25	0.5
aldehydes	0.25	0.25	0.5	1.0	0.25	0.25	0.5
terpenes	0	1.5	2.0	3.0	0.1	1.0	0.1
furans	0.1	0.1	0.1	0.5	0.25	0.5	3.0
phenols	0	0	0	0.5	0	0	0.5
oxygenated aromatics	0.1	0.1	1.5	0.1	0	0.1	0.1
alkaloids	0	0.25	2.5	0.5	0	0.1	0.1
sulfides	2.0	0.5	1.5	2.0	6.0	11.0	2.5
solvents	10.5	94.5	77.5	49.5	70	28	26

Table 3. Test Attractant Recipes^a

component	test attractant						
	1	2	3	4	5	6	7
ethyl butyrate	0.050	0.025	0.050	0.120	0.110	4.80	0.020
isobutyric acid	10.5	n/a	n/a	4.00	n/a	4.20	n/a
cyclopentanone	0.002	n/a	0.050	0.010	0.020	0.060	0.060
1-butanethiol	n/a	n/a	0.150	0.040	0.200	n/a	n/a
butylthioacetate	n/a	n/a	n/a	0.025	0.030	n/a	n/a
N-ethylbutylamine	n/a	0.750	1.25	n/a	3.00	n/a	10.2
4-octene (<i>trans</i>)	n/a	n/a	n/a	n/a	0.100	n/a	0.006
octane	n/a	n/a	n/a	n/a	0.300	n/a	n/a
1-butanol	0.005	0.075	0.400	1.600	0.120	0.050	0.020
hexanal	0.008	0.030	0.500	0.100	0.120	0.070	0.100
camphene	n/a	0.04 g	0.08 g	0.04 g	0.02 g	0.05 g	0.003 g
2-furaldehyde	0.002	0.010	0.050	0.040	0.0002	0.006	0.450
2,6-dimethylpyrazine	n/a	0.1 g	0.6 g	0.4 g	n/a	0.24 g	0.04 g
methyl disulfide	0.020	0.010	n/a	n/a	n/a	0.500	0.030
ethanol	0.120	10.50	8.50	4.00	11.00	4.50	2.40

^a Volume is milliliters unless otherwise noted.

BIOASSAY METHODOLOGY

Subjects. We tested 14 male–female pairs of adult (9–16 kg) coyotes. All animals were between 2 and 6 years old, individually marked with metal ear tags, and housed in 0.2 ha pens at the Logan Field Station of the National Wildlife Research Center in Millville, UT. A ration of ground meat (~0.6 kg/coyote/day) was provided prior to each daily test. Water was available ad libitum.

Stimuli. Seven synthetic attractants (Table 3), a control (glycerol solution), and FAS were presented to the subjects. Prior to bioassay, 200 μ L of test attractant and 800 μ L of 70% glycerol (in water) were delivered to a 1.5 mL polypropylene microcentrifuge tube with attached cap. The test attractant solutions were thoroughly mixed with a vortex mixture. The FAS attractant was similarly prepared. The control consisted of 1 mL of the glycerol solution.

Apparatus. Attractants were presented in devices especially fabricated for the purpose. Each device consisted of a 1.25 cm copper pipe (length of ~15 cm) fitted with a threaded stainless steel coupler. Microcentrifuge tubes could be placed in the coupler such that the open cap acted as a wedge to hold the tube firmly inside the coupler. The other end of the copper tube was crushed to form a stake. During tests, the stake was hammered into the ground so that only the sleeve was visible. The serum tube was then inserted into the sleeve and pressed far enough into the device that it could not be removed by the coyotes.

Bioassay Procedure. The 14 pairs of coyotes were randomly assigned to two cohorts. Each cohort was then presented with the nine stimuli in a random order (one stimulus per day). The first cohort was tested between June 23 and July 12, 1999, and the second between August 23 and September 9, 1999.

Each stimulus presentation lasted 20 min, and behaviors exhibited by each of the coyote pairs were videotaped for subsequent analysis. On the basis of pilot observations, we selected the following behavioral categories for quantification: rub, roll, scratch, urinate, defecate, sniff, dig, lick, and pull. Video cameras were positioned in observation buildings equipped with one-way glass windows. The apparatus containing the stimuli were placed in the center of the animals' pens, ~5 m from the one-way windows.

Statistical Analyses. The durations of each response in relation to each attractant were evaluated in separate two-factor mixed design analyses of variance. The random independent factor was sex, whereas the fixed repeated factor was stimuli (synthetic attractant, FAS, control). Tukey tests were used to identify significant differences among means after the omnibus procedure ($p < 0.05$).

RESULTS

Chemical Analyses. Chemical analyses of the 33 attractants revealed 319 unique compounds, of which 277 were identified by their mass spectra. All chromatographic peaks measuring >3 times the peak-to-peak noise (height) were evaluated.

Representative Compounds and Cluster Analysis. Representative compounds were chosen by examining individual compound correlations with functional group responses. Commercially available compounds with the highest correlation coefficients were chosen as representative compounds (Table 1). Practical aspects of formulation were also considered in the choice of two

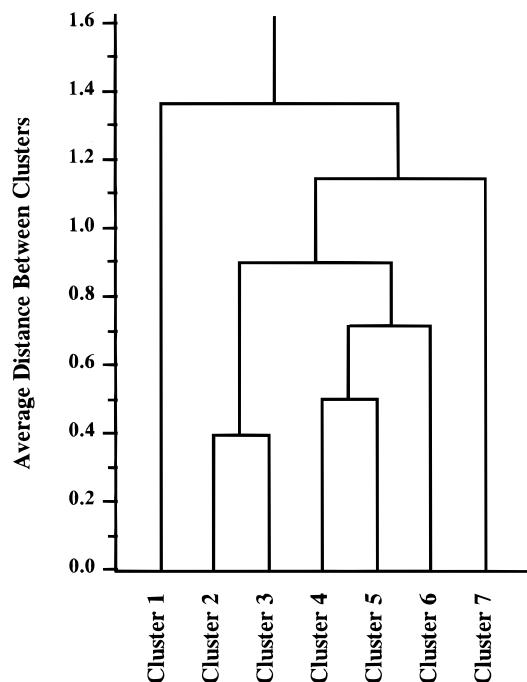


Figure 1. Average linkage cluster analysis dendrogram for 33 attractants. Average distance of 0.3 yielded seven clusters. Mean functional group data for each cluster were used to generate recipes for seven new attractants.

representative compounds. No permanent gases were considered, and ethylbutylamine was selected over the dissolved gas trimethylamine.

An average distance between clusters of 0.3 was chosen to yield seven unique clusters (Figure 1). The mean functional group responses calculated in each cluster were considered to be the target headspace responses for attractant formulation (Table 2).

Synthetic Attractant Formulation. Analyses of the synthetic mixtures identified some incompatibilities that had to be considered in the formulation of the seven new attractants. Because of acid–base neutralization, isobutyric acid and ethylbutylamine were not included in the same synthetic attractant. The choice of using the acid or the base was made on the basis of which constituent was most prominent the appropriate cluster. Furthermore, because losses of several compounds (and formation of new ones) appeared to be related to the presence of the acid or base, isobutyric acid or ethylbutylamine was not added to the formulation until immediately prior to use. Examples of these reactions included hydrolysis of ethyl butyrate, oxidation of furfural and hexanal, and esterification of isobutyric acid.

A redox reaction involving *n*-butyl mercaptan and methyl disulfide was also observed to result in the rapid loss of the mercaptan and formation of methyl butyl disulfide. A similar loss of a mercaptan in coyote attractants was previously reported (Teranishi et al., 1981). To prevent this, only the mercaptan or the disulfide (the most prominent as identified in Table 2) was used in each of the attractants. After these factors had been taken into consideration, recipes were generated that yielded ~10 mL of test attractant (Table 3).

Bioassay Results. Statistical analysis indicated that there was no significant cohort effect ($p > 0.50$); thus, data from both cohorts were combined for the analyses. Separate analyses were conducted for the nine behaviors.

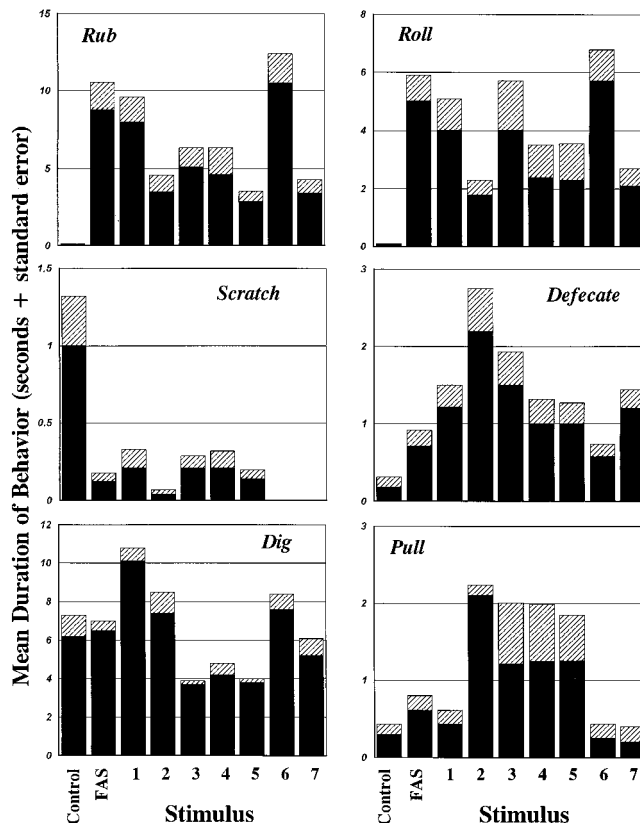


Figure 2. Mean duration of stimulus-induced behavior (seconds + standard errors of the means) by captive coyote pairs (FAS = fatty acid scent).

Rub. There were significant differences between males and females ($F_{1,12} = 35.5$, $p < 0.0002$); females rubbed for longer periods than males (7.2 versus 3.2 s/bout). There were also significant differences among stimuli ($F_{8,96} = 6.4$, $p < 0.00001$). Attractants 1 and 6 and FAS elicited longer bouts of rubbing than the other stimuli (Figure 2). There was no interaction between the factors ($p > 0.50$).

Roll. There were significant differences between males and females ($F_{1,12} = 7.6$, $p < 0.02$); females rolled for longer periods than males (4.1 versus 1.9 s/bout). There were also significant differences among stimuli ($F_{8,96} = 3.1$, $p < 0.004$). Attractants 1 and 6 and FAS elicited longer bouts of rolling than attractants 2, 7, and the control (Figure 2). There was no interaction between the factors ($p > 0.44$).

Scratch. There were no significant differences between males and females ($p > 0.50$). However, there were significant differences among stimuli ($F_{8,96} = 5.3$, $p < 0.0001$). All test attractants and FAS elicited shorter bouts of scratching than the control (Figure 2). There was no interaction between the factors ($p > 0.50$).

Defecate. There were no significant differences between males and females ($p > 0.50$). However, there were significant differences among stimuli ($F_{8,96} = 3.1$, $p < 0.0045$). Attractants 1–5 and 7 elicited longer defecation bouts than the control, FAS, or attractant 6 (Figure 2). There was no interaction between the factors ($p > 0.50$).

Urinate. There were no differences between males and females ($p > 0.25$) or among stimuli ($p > 0.12$), and there was no interaction between the factors ($p > 0.50$).

Sniff. There were significant differences between females and males ($F_{1,12} = 29.5$, $p < 0.0003$); females

showed longer bouts of sniffing than males (9.3 versus 5.6 s/bout). There were no significant differences among attractants ($p > 0.50$), nor was there an interaction between the factors ($p > 0.50$).

Dig. There were no significant differences between males and females ($p > 0.50$). However, there were significant differences among attractants ($F_{8,96} = 2.6$, $p < 0.05$). Attractant 1 elicited longer bouts of digging than any other stimuli (Figure 2). Attractants 3–5 elicited the shortest. There was no interaction between the factors ($p > 0.50$).

Lick. There was a significant difference between males and females ($F_{1,12} = 5.12$, $p < 0.05$); females showed longer bouts of licking than males (4.5 versus 3.1 s/bout). There were no significant differences among attractants ($p > 0.50$), nor was there an interaction between the factors ($p > 0.50$).

Pull. There was a significant difference between males and females ($F_{1,12} = 6.3$, $p < 0.05$); females showed longer bouts of pulling than males (1.9 versus 0.4 s/bout). There were also significant differences among attractants ($F_{8,96} = 3.6$, $p < 0.05$). Attractant 2 was more likely to elicit pulling than attractants 1, 6, and 7, FAS, or the control (Figure 2). There was no interaction between the factors ($p > 0.50$).

DISCUSSION

The volatile components of canid attractants were of utmost interest because olfaction plays a major role in canid food-seeking, reproductive, and territorial behaviors (Phillips et al., 1990). Trapping, oral drug and toxicant delivery, and census-taking applications require attractants that elicit these same behaviors. Although these behaviors may also be vomeronasally mediated by less volatile compounds (such as proteins and larger fatty acids), we chose to focus our analytical efforts on the volatile constituents.

The suite of volatiles found in the sample headspace was extremely complex. The use of the mass selective detector (MSD) allowed for the tentative identification of the very large number of headspace constituents. The percent chromatographic peak area response was used for each compound because it is a relative measure independent of sample amount. Because 277 variables resulted from only 33 observations, we were precluded from employing a statistical approach to variable reduction. Thus, we arbitrarily chose to reduce the large number of variables by classifying each compound according to functionality. The use of functional group classification reduced the number of variables considerably and permitted the application of cluster analysis. We further reduced the number of candidate compounds for attractant formulation by determining which individual compounds best represented each functional group (Table 1).

Cluster analysis of the headspace data allowed us to propose seven different attractant formulations. The first cluster was characterized by high headspace concentrations of fatty acids (Table 2). Past research indicated that fatty acids are important components of effective coyote attractants (Phillips et al., 1990). The second and third clusters were very similar in headspace composition (Table 2). Ethanol dominated the headspace of both clusters, and the minor constituents were similar. The fourth cluster contained a large quantity of other alcohols (not ethanol), esters, and fatty acids (Table 2).

The fifth cluster was found to have relatively high headspace concentrations of amines, as well as mercaptans, thiol esters, and sulfides (Table 2). Sulfides and disulfides comprised the bulk of the volatile constituents in female coyote urine (Schultz et al., 1988) and are a significant portion of bobcat urine (Mattina et al., 1991) and fox urine (Wilson et al., 1978). These same sulfides identified in mammalian urines were identified in the attractants found in cluster 5.

The sixth cluster exhibited high headspace concentrations of esters and sulfides indicative of protein degradation (Wilson et al., 1973; Bullard et al., 1978b). The specific sulfides of this cluster differed markedly from the fifth cluster. For instance, methyl disulfide was the primary sulfide observed in the sixth cluster, yet only a minor constituent of the fifth. This indicates that some sulfur compounds may serve primarily as food odors, whereas those more typically found in urine may have a different semiochemical role. The headspace of the seventh cluster was predominated by amines (Table 2).

Inspection of the dendrogram illustrates that attractants 2 and 3 were chemically very similar (Figure 1). Nevertheless, these formulations elicited different frequencies of digging behavior as evidenced by the behavioral results (Figure 2). This indicates that the choice of 0.3 as the average distance between clusters was not too small to yield significantly different attractants.

The behaviors we selected for examination are representative of the behaviors that coyotes either must exhibit or refrain from displaying for the effective operation of M-44 cyanide ejectors various restraint and capture devices, and the efficient delivery of pharmaceutical-containing baits. Licking and pulling are obviously important behaviors for the ingestion of baits or for the effective operation of M-44s. Sniffing, scratching, digging, and scent marking (urination and defecation) are essential for the activation of restraint and capture devices (traps and power snares). We recorded rolling and rubbing because these behaviors interfere with the effective operation of all capture and control devices and because they are unwanted in the context of bait ingestion.

In general, females were more likely to exhibit behaviors than males. Observation of a sample of videotapes suggested that this difference might reflect that females are often the first in each pair to approach a lure device. This was an unexpected result because males were the dominant member of every pair and there is no evidence in field studies to indicate that females are attracted to synthetic attractants at frequencies greater than males. In a prior study, no relationship between sex and specific attractant was observed (Phillips et al., 1990). Furthermore, a recent study of captive coyotes' responses to visual and olfactory stimuli demonstrated that neophobia was not related to age, sex, or rearing history (Windberg, 1996). Additional research of this phenomenon is warranted.

In addition to sex effects, we identified significant behavioral responses attributed to the stimuli. For example, attractant 2 elicited significantly longer pulling bouts by the subjects than the control. Furthermore, attractant 1 elicited significantly longer periods of digging than the control or the other attractants. These results suggest that attractant 2 may be useful for lures associated with oral delivery devices, whereas attractant 1 may be useful for lures employed with capture devices. Behavioral results also demonstrated that attractants

1–3 and 7 elicited longer bouts of defecation than the control or FAS. In addition to being a desirable behavior in the context of capture devices, attractants that elicit defecation may be useful in ecological studies requiring scat deposition along transects as an indicator of coyote abundance.

An undesirable finding was that attractants 1 and 6 produced longer bouts of rubbing and rolling than several of the other test attractants. The observation that attractant 1 produced longer durations of both desirable and undesirable behaviors in captive coyotes indicates that test attractants may require further refinement. However, altogether these results demonstrate that significantly different behaviors can be produced by varying the presence of relatively few compounds and their concentrations in attractant formulations. This suggests that each attractant mixture may be further manipulated to minimize undesirable behaviors and/or increase the frequency and duration of desirable behaviors.

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