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Authors

Kimball, Bruce A.
Johnston, John J.
Mason, J. Russell
et al.

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DEVELOPMENT OF CHEMICAL COYOTE ATTRACTANTS FOR WILDLIFE MANAGEMENT APPLICATIONS

BRUCE A. KIMBALL, and JOHN J. JOHNSTON, USDA-APHIS-WS-NWRC, Analytical Chemistry Project, 4101 LaPorte Avenue, Fort Collins, Colorado 80521.

J. RUSSELL MASON, and DORIS E. ZEMLICKA, USDA-APHIS-WS-NWRC, Predation Ecology and Behavioral Applications, BNR-163, Utah State University, Logan, Utah 84322-5295.

F. SHERMAN BLOM, USDA-APHIS-WS, Pocatello Supply Depot, 238 E. Dillon Street, Pocatello, Idaho 83201.

ABSTRACT: Coyote attractants are inherently variable because they are usually derived by mixing and fermenting complex biologically derived substances. We designed the present study to address this problem. We collected volatiles by purge and trap headspace analyses from 33 commercially available attractants, and analyzed the trapped odors by gas chromatography with mass selective detection. We then statistically evaluated chromatographic peak area data to produce recipes for seven new chemical attractants. We presented these attractants to coyotes in one-choice tests at the Predation Ecology and Behavioral Applications Field Station of the USDA-APHIS-WS National Wildlife Research Center near Logan, Utah. Our results indicated that there were both seasonal and sexual differences in stimulus attractiveness. We also found that several attractants were more effective than Fatty Acid Scent (FAS), a commonly employed coyote attractant. A field trial to evaluate the effectiveness of new candidate attractants is planned.

KEY WORDS: attractants, behavior, bioassay, *Canis latrans*, coyotes, synthetic, volatiles

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INTRODUCTION

Typical coyote attractants are a mixture of fermented glandular materials, urines, rotted meats, and other biological substances. While effective, these preparations have several distinct disadvantages. Chief among these is that attractants are unnecessarily complex and difficult to replicate from one batch to the next. Not surprisingly, effectiveness can be highly variable. This variability has stimulated attempts to develop synthetic attractants for more than 80 years. For example, ammonium and zinc valerate, and various artificial musks have been used in coyote attractants since the 1920s (Day 1932; Presnall 1950).

DRC-6220, or synthetic monkey pheromone (CFA), was the first true synthetic coyote attractant, appearing in 1973 (Savarie, pers. comm.). CFA is comprised of fatty acids present in rhesus monkey vaginal secretions (Michael et al. 1971). Subsequently, Teranishi et al. (1981) developed a synthetic attractant containing trimethylammonium valerate (TMAV), or TMAV in combination with various sulfur-containing volatiles. Expansion of this work led to the development of trimethylammonium decanoate (TMAD) and the TMAD plus sulfides (W-U lure; U.S. Patent No. 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 (fatty acid scent, FAS) was developed

from the seven volatile fatty acids found in fermented egg (Roughton 1982).

To date, there has been no systematic chemical evaluation of actual coyote attractants. We designed the present studies to identify the volatile compounds present in effective commercial attractants, prepare new candidate attractants on the basis of our chemical analyses, and test these new attractants with captive coyotes. We also evaluated the behaviors elicited by these new formulations during both summer and winter to discover whether seasonal differences in effectiveness might exist. We evaluated pairs of coyotes in an attempt to address possible sexual differences in the attractiveness of our candidate substances. The results of the chemical analyses and the bioassays conducted in the summer have recently been reported (Kimball et al. 2000). Here, we report the results of the winter bioassays and compare these results with the results obtained in our summer tests.

METHODS

Chemical Analyses

Attractants were analyzed according to the methods of Kimball et al. (2000). Briefly, we collected headspace odors above 33 commercially available attractants using a Tekmar 3000 purge and trap instrument, equipped with Carboxen B/Carboxen 1000 & 1001 traps (Supelco Trap K). We desorbed volatiles at 250°C with helium onto a gas chromatograph (Hewlett Packard 5890) equipped with a 30 m x 0.25 mm DB-5.625 fused silica capillary column (J&W Scientific). We isolated 319 unique compounds using mass selective detection, scanning m/z 33 to 500. Of these, we were able to identify 277 substances on the basis of their mass spectra.

Synthetic Attractant Formulation

To evaluate chromatographic data, we calculated the normalized peak response for each substance (Kimball et al. 2000). Next, we classified compounds by functional-group (eg., esters, fatty acids, ketones, mercaptans) and calculated the total response for each group across all 33 attractants. We chose representative compounds for each functional group by examination of correlations between each individual compound and the appropriate functional-group response. We considered commercial

availability of the compound when choosing representative materials.

We subjected non-collinear functional-group variables to average linkage cluster analysis. This clustered the 33 attractants into hierarchical clusters on the basis of squared Euclidean distances (CLUS procedure; SAS 1997). We calculated mean functional-group responses for all clusters, and then combined representative compounds to produce seven mixtures with desired headspace concentrations (Kimball et al. 2000; Table 1).

Table 1. Recipes of attractants offered to captive coyotes in bioassays (units are milliliters unless otherwise noted).

Component	Test Attract 1	Test Attract 2	Test Attract 3	Test Attract 4	Test Attract 5	Test Attract 6	Test Attract 7
Ethyl butyrate	0.050	0.025	0.050	0.120	0.110	4.80	0.020
Isobutyric acid	10.50	none	none	4.00	none	4.20	none
Cyclopentanone	0.002	none	0.050	0.010	0.020	0.060	0.060
1-Butanethiol	none	none	0.150	0.040	0.200	none	none
Butylthioacetate	none	none	none	0.025	0.030	none	none
N-Ethyl butylamine	none	0.750	1.25	none	3.000	none	10.20
4-Octene (trans)	none	none	none	none	0.100	none	0.006
Octane	none	none	none	none	0.300	none	none
1-Butanol	0.005	0.075	0.400	1.600	0.250	0.050	0.020
Hexanal	0.008	0.030	0.500	0.100	0.120	0.070	0.100
Camphene	none	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	0.200	0.060	0.450
Guaiaicol	none	none	none	0.400	none	none	none
2,6-Dimethyl pyrazine	none	0.1 g	0.6 g	0.4 g	none	0.24 g	0.04 g
4-Methyl anisole	0.001	0.005	0.070	0.001	none	0.002	0.006
Methyl disulfide	0.020	0.010	none	none	none	0.500	0.300
Ethanol	0.120	10.50	8.50	4.00	11.00	4.50	2.40

Behavioral Assays

Bioassays were conducted according to the methods of Kimball et al. (2000). Briefly, we tested 14 male-female pairs of adult (9 to 16 kg) coyotes in two cohorts (7 pairs/cohort) during two bioassay periods. During each period, each attractant, a negative control (70% glycerol solution), and a positive control (FAS), were presented individually to each pair in both cohorts in 20 minute one-choice tests (1 test/day). We presented 1.00 mL (200 μ L attractant + 800 μ L glycerol solution) of each stimulus in serum tubes placed inside a stainless steel coupler fitted to a 15 cm length of copper pipe. We drove the pipe into the ground so that only the coupler showed above the surface. Serum tubes were placed below the lip of the coupler so that they could not be removed by the subjects.

In the summer bioassay, we tested the first cohort in June and July 1999, and the second, between August and September 1999. For the winter bioassay, we tested both cohorts simultaneously during November and December 1999. All tests occurred in 0.5 ha observation pens adjacent to observation buildings equipped with one-way

glass windows. We used a video camera to record behavior, and subsequently scored each test session for the frequencies of the following behaviors: walk (not oriented toward the device), approach (change of direction in walk with focus on device), sniff, lick, pull (attempt to remove device with mouth), dig (removal of soil around device), scratch (pawing or touching of device), urinate, defecate, rub (neck area in contact with device), and roll (back or side of subject in contact with device). We selected these behaviors because they could aid or hinder the effectiveness of various capture devices, as well as the ingestion of baits.

Statistical Analyses

We evaluated the frequency of each behavior in separate three factor mixed design analyses of variance (ANOVA). We treated sex as a random independent factor and season and stimuli as repeated factors. Tukey tests were used to identify significant differences among means subsequent to the omnibus procedure ($p < 0.05$).

RESULTS

Walk

We found significant differences in frequencies of this behavior between seasons ($F_{1,12}=61.5$; $p<0.00001$). Coyotes were more likely to walk by stimulus devices in summer (0.98 ± 0.08) than in winter (0.00 ± 0.0). Otherwise, there were no significant effects.

Approach

We found a significant difference between seasons in stimulus approaches ($F_{1,12}=5.68$; $p<0.03$). Coyotes were more likely to approach attractants in winter (3.98 ± 0.21) than in summer (3.16 ± 0.17). We also found a significant interaction between seasons and stimuli ($F_{8,96}=3.2$; $p<0.003$). The frequency of approaches was significantly higher in the winter for attractants 3, 4, 6, and 7 (Figure 1). Conversely, the frequency of approaches for attractants 1 and 2 decreased in the winter tests. There were no other significant effects.

Figure 1. Approach frequency exhibited by coyote pairs produced by the interaction of season and stimulus (*indicates no significant difference between seasons for that stimulus).

Sniff

We found a significant difference between males and females in stimulus sniffing ($F_{1,12}=9.98$; $p<0.009$). Females (11.14 ± 0.63) were more likely to sniff stimulus devices than were males (6.61 ± 0.64). Also, we detected a significant difference between seasons ($F_{1,12}=9.98$; $p<0.03$). The overall frequency of sniffing was relatively higher in winter (10.3 ± 0.8) than in summer (7.4 ± 0.4). Differences among stimuli were significantly different ($F_{8,96}=4.3$; $p<0.0004$). Sniffing frequencies were highest for attractants 6 and 7, and lowest for the glycerol control. Finally, we found a significant interaction between seasons and stimuli ($F_{8,96}=4.93$; $p<0.0001$). Sniffing increased significantly in the winter in response to attractants 4, 6, and 7 (Figure 2). Decreased frequencies were noted for the control and attractant 1, but were not significant.

Legend:

Summer

Winter

Figure 2. Sniffing frequency exhibited by coyote pairs produced by the interaction of season and stimulus (*indicates no significant difference between seasons for that stimulus).

Lick

We found a significant difference between males and females in the overall frequency of stimulus licking ($F_{1,12}=7.3$; $p<0.02$). Females (4.24 ± 0.51) licked all stimuli more frequently than males (1.95 ± 0.28). Otherwise, there were no significant effects.

Pull

We found a significant difference between seasons in the overall frequency of stimulus pulling ($F_{1,12}=4.4$; $p<0.05$). Although frequencies of pulling were low in general, pulling was more common in summer (0.689 ± 0.18) than in winter (0.087 ± 0.034). Otherwise, there were no significant effects.

Dig

We found a significant difference between seasons in the frequency of digging around stimulus devices ($F_{1,12}=15.2$; $p<0.003$). Overall, digging was more common in summer (6.1 ± 0.58) than in winter (2.6 ± 0.38). Otherwise, there were no significant effects.

Scratch

We found a significant difference between seasons in the overall frequency of scratching at stimulus devices ($F_{1,12}=36.9$; $p<0.0002$). Scratching bouts were observed more often in winter (1.82 ± 0.23) than in summer (0.21 ± 0.05). We also detected a significant interaction between seasons and stimuli ($F_{8,96}=1.94$; $p<0.07$). Although scratching at the glycerol control did not change between seasons, scratching towards FAS and all attractants increased in the winter (Figure 3). The largest change was for attractant 7.

Figure 3. Scratching frequency exhibited by coyote pairs produced by the interaction of season and stimulus (*indicates no significant difference between seasons for that stimulus).

Urinate

We found a significant interaction between seasons and stimuli in the frequency of urination bouts directed at the stimulus device ($F_{8,96}=4.02$; $p<0.0006$). While the frequency of urination bouts directed towards the control, FAS, and attractants 1 and 5 did not change between seasons, urination frequencies were higher in the summer for attractants 2 and 3 (Figure 4). Conversely, urination was more common in the winter in response to attractants 4, 6, and 7. There were no other significant effects.

Figure 4. Urination frequency exhibited by coyote pairs produced by the interaction of season and stimulus (*indicates no significant difference between seasons for that stimulus).

Defecate

We found a significant difference between seasons ($F_{1,12}=66.14$; $p<0.00001$) and among stimuli ($F_{8,96}=3.0$; $p<0.005$) in the frequency of defecation bouts directed towards the stimulus devices. Defecation bouts were more common in summer (1.06 ± 0.11) than winter (0.03 ± 0.05). Among stimuli, defecation bouts were most common towards attractant 2, and least common towards the glycerol control. We also detected a significant interaction between season and stimuli ($F_{8,96}=3.01$; $p<0.005$). Although the frequencies of defecation bouts were generally low, the difference in the frequencies of bouts between seasons was greatest in response to attractants 2, 3, and 7 and least towards the glycerol control (Figure 5). There were no other significant differences.

Figure 5. Defecation frequency exhibited by coyote pairs produced by the interaction of season and stimulus (*indicates no significant difference between seasons for that stimulus).

Rub

We found a significant difference between sexes ($F_{1,12}=15.4$; $p<0.003$). Females (9.65 ± 0.89) rubbed more often than males (4.5 ± 0.57). Also, we detected a significant difference between seasons ($F_{1,12}=6.6$; $p<0.03$). Rubbing was more frequent in winter (8.9 ± 0.93) than in summer (5.21 ± 0.52). Finally, we found a significant difference among stimuli ($F_{8,96}=8.3$; $p<0.00001$). Rubbing bouts were least often seen in response to the glycerol control and most often seen in response to attractant 6 (Figure 6). Otherwise, there were no significant differences.

Roll

We found a significant difference between sexes ($F_{1,12}=5.4$; $p<0.04$), and among stimuli ($F_{8,96}=5.36$; $p<0.0001$). Females (5.48 ± 0.58) showed more bouts of rolling than males (2.73 ± 0.4). Among stimuli, the fewest bouts of rolling were observed towards the glycerol control and the most towards attractant 6 (Figure 7). There were no other significant differences, although

the difference between seasons approached statistical significance ($F_{1,12}=4.1$; $p<0.07$); the mean frequency of rolling during winter was 5.2 ± 0.4 , while the mean frequency of rolling during summer was 3.0 ± 0.6 .

Figure 6. Rubbing frequency produced by coyote pair exposure to the control, fatty acid scent (FAS), and seven test attractants (mean + standard error).

Figure 7. Rolling frequency produced by coyote pair exposure to the control, fatty acid scent (FAS), and seven test attractants (mean + standard error).

DISCUSSION

The behaviors we selected for examination in this study are indicators of the value of each attractant for use with M-44 cyanide ejectors, various restraint and capture devices, and pharmaceutical-containing bait delivery devices. Licking and pulling are important behaviors for the ingestion of baits or for the effective operation of M-44s. Sniffing, scratching, digging, urinating, or defecating are essential behaviors for the activation of restraint and capture devices. Rub and roll are deleterious behaviors that may interfere with the effective operation of capture devices and are unwanted in the context of bait ingestion.

Females elicited sniff, lick, rub, and roll behaviors more frequently than males. There were no sex x stimuli interactions. Our previous analyses of summer data also suggested that females may have been more likely to exhibit longer bouts of rubbing, rolling, sniffing, licking, and pulling (Kimball et al. 2000). We were surprised by these differences between sexes because at least one previous study of attractant effectiveness reported no sex effects (Phillips et al. 1990), and a recent study of coyotes' responsiveness to novel visual and olfactory stimuli indicated no age, sex, or rearing history effects (Windberg 1996). Furthermore, there is no published evidence that free-ranging females are relatively more susceptible to capture than males.

Pull, walk, dig, and defecate behaviors were more frequent in the summer versus winter. Conversely, scratch, rub, and sniff behaviors were more frequently displayed in the winter. Because we classified walking past stimuli as a reflection of indifference, it is not surprising that this behavior was more common in summer. A common anecdotal observation is that attractants are uniformly more effective during winter and early spring, times when food is scarce and sexually significant chemicals are increasing in biological significance.

Because coyote attractants may well be less effective in summer than winter, we were especially encouraged that several of our candidate attractants elicited relevant behaviors in both summer and winter. We plan to test these materials with free-ranging coyotes to evaluate their potential utility with various capture devices. Along these lines, there were significant interactions between season and stimuli for several behaviors: approach, sniff, defecate, and urinate. These interactions are consistent with our hypothesis that different odor mixtures might be differentially effective during winter versus summer months. Specifically, we found that attractants 3, 4, 6, and 7 were more likely to elicit approach responses during winter than were our other stimuli (Figure 1). Approaches to FAS, and test attractants 1 and 2 were more frequent in the summer but not significant ($\alpha=0.05$). Likewise, we observed that attractants 4, 6, and 7 were more likely to elicit urination in the winter while test attractants 2 and 3 were more likely to elicit this response in the summer (Figure 4).

Our results suggest that different candidate attractants may be more useful in baits or on M-44 lures, while others may be more useful as trap attractants. For example, all seven of our synthetic attractants were more likely to elicit sniffing than the control. Similarly, all stimuli were more likely to provoke defecation by the test subjects than the control.

One disappointing and undesirable finding was that all attractants and FAS were more likely to elicit rubbing and rolling than was the glycerol control (Figures 6 and 7). We interpret this result as an indication that our attractants require further refinement to diminish the occurrence of these behaviors. For example, attractant 6 (which was most likely to produce these behaviors) contained high quantities of ethyl butyrate and methyl disulfide, relative to the levels of these chemicals in the other attractants. These compounds are associated with fermentation (Bullard et al. 1978b) and protein

degradation (Wilson et al. 1973), respectively. By minimizing substances suggestive of rotting meat, we may be able to reduce the occurrence of rubbing and rolling in our attractant formulations. Conversely, attractants 2 and 5 elicited fewer rolling bouts than the other test attractants or FAS, suggesting that certain characteristics of these mixtures might be emphasized to minimize the occurrence of these undesirable behaviors.

Broadly, our results suggest that attractants 1, 2, 3, 4, 5, and 7 may have some practical utility, and may be more effective than commonly used materials such as FAS. Apart from usefulness in coyote management, these substances may also have use in predator census operations. For instance, all six of these test attractants were relatively likely to provoke defecation. Therefore, they could be incorporated into basic ecological studies that rely on scat depositions along transects as an indicator of coyote presence and abundance. Already, we have planned field tests of our candidate attractants to evaluate their practical utility with free-ranging coyote populations.

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Mention of specific products is for identification purposes only and does not constitute endorsement by the U.S. Department of Agriculture.

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